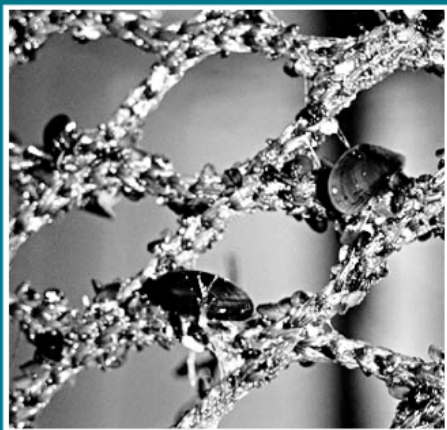


WOODHEAD PUBLISHING IN MATERIALS



Advances in marine antifouling coatings and technologies

Edited by Claire Hellio and Diego Yebra



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CRC Press
Boca Raton Boston New York Washington, DC

WOODHEAD PUBLISHING LIMITED

Oxford Cambridge New Delhi

Published by Woodhead Publishing Limited, Abington Hall, Granta Park, Great Abington, Cambridge CB21 6AH, UK
www.woodheadpublishing.com

Woodhead Publishing India Private Limited, G-2, Vardaan House, 7/28 Ansari Road, Daryaganj, New Delhi-110002, India

Published in North America by CRC Press LLC, 6000 Broken Sound Parkway, NW, Suite 300, Boca Raton, FL 33487, USA

First published 2009, Woodhead Publishing Limited and CRC Press LLC

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British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library.

Library of Congress Cataloging in Publication Data

A catalog record for this book is available from the Library of Congress.

Woodhead Publishing ISBN 978-1-84569-386-2 (book)

Woodhead Publishing ISBN 978-1-84569-631-3 (e-book)

CRC Press ISBN 978-1-4200-9490-9

CRC Press order number: WP9490

The publishers' policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufactured from pulp which is processed using acid-free and elemental chlorine-free practices. Furthermore, the publishers ensure that the text paper and cover board used have met acceptable environmental accreditation standards.

Typeset by SNP Best-set Typesetter Ltd., Hong Kong

Printed by TJ International Limited, Padstow, Cornwall, UK

C HELLIO, University of Portsmouth, UK and
D M YEBRA, Pinturas Hempel S.A., Spain

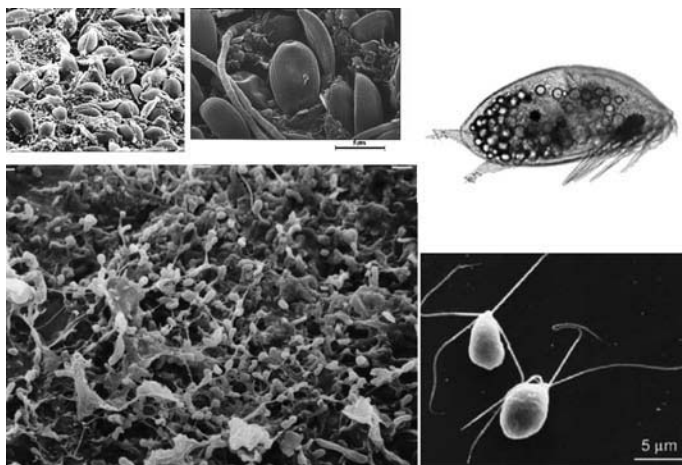
1.1 Marine biofouling

From the dawn of navigation, the growth of aquatic organisms on the underwater part of vessels has been regarded as a serious problem constraining growth and progress. The early Mediterranean cultures, the medieval Vikings, the trans-oceanic European empires from the modern era and even the contemporary trading and war fleets: no one has ever found a totally satisfactory solution to this problem.

Battle of Trafalgar (1805). '[...] all ships in the Royal Navy were copper bottomed [...] increasing sailing speed by 20% due to reduced bottom fouling [...]. Nelson's [...] victory at Trafalgar was partly due to the superior speed of his clean-hulled ships.] Richard Holdsworth 'Victory and the road to Trafalgar'

'The earliest official mention of keelhauling is a Dutch ordinance of 1560, and the practice was not formally abolished until 1853. The sailor was tied to a rope that looped beneath the vessel, thrown overboard on one side of the ship, and dragged under the ship's keel to the other side. As the hull was often covered in barnacles and other marine growth, this could result in cuts and other injuries.'

Marine biofouling can be defined as the undesirable accumulation of microorganisms, algae and animals on structures submerged in seawater. Even though the interest in the fouling process mainly originates from its detrimental effects on man-made structures, it can also occur on the surfaces of living marine organisms (epibiosis) and lead to problems in the aquaculture of seaweeds and shellfish. Biofoulers are roughly divided into microfoulers (bacterial and diatomic biofilms) and macrofoulers (e.g., macroalgae, barnacles, mussels, tubeworms, bryozoans) which live all together forming a fouling community (see Chapters 2–4, Figs 1.1 and 1.2). A simplistic overview of the fouling process is given in Fig. 1.3 (see also, e.g., Yebra *et al.*, 2004). Regardless of the location and the season, every immersed surface will be covered in a matter of seconds by a layer of adsorbed organic compounds (e.g., polysaccharides, lipids and proteins). Less than 24 hours after the formation of this 'conditioning layer', the biofouling process starts. Primary colonizers mainly consist of bacteria, yeasts and diatoms which establish themselves within protective biofilm structures (see Chapter 17 and

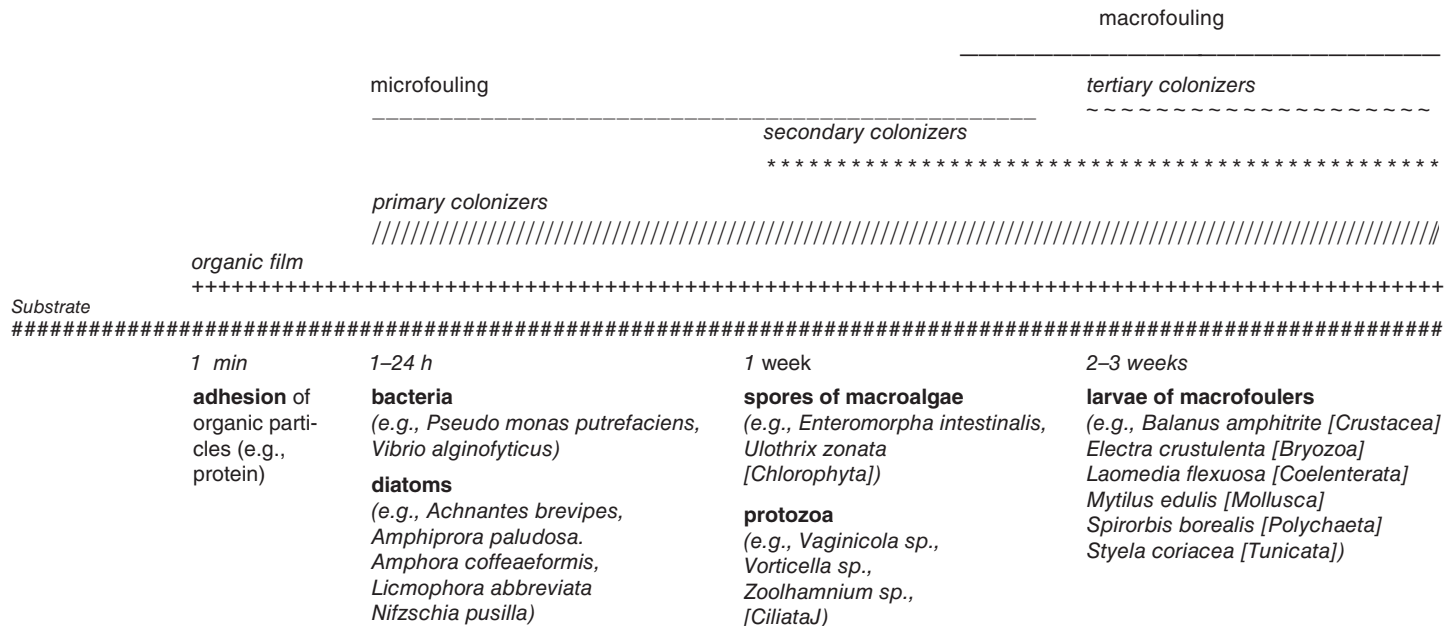


1.1 Microscopic foulers, including diatoms (top left pictures), bacteria (bottom left) and *Ulva* zoospores (bottom right). A *Balanus amphitrite* cyprid larva (about 300–500 μm in size) is also shown (top right).



1.2 Various common macrofoulers, including macroalgae (top pictures), adults of *Balanus amphitrite* (bottom left), serpulid worm (*Hydroides elegans*) (bottom middle). On the bottom right, a picture showing a heavily fouled ship's hull.

Yebera *et al.*, 2006). Secondary colonizers comprise the spores of macroalgae, fungi and protozoa which, according to the literature, settle roughly about 1 week after immersion when the environmental conditions are favourable. Invertebrate larvae are often regarded as the last stage of the marine biofouling process, their arrival to the surface taking place on average after 2–3 weeks of immersion during the spawning season. It must be noted, though, that despite such a successional description of the fouling process



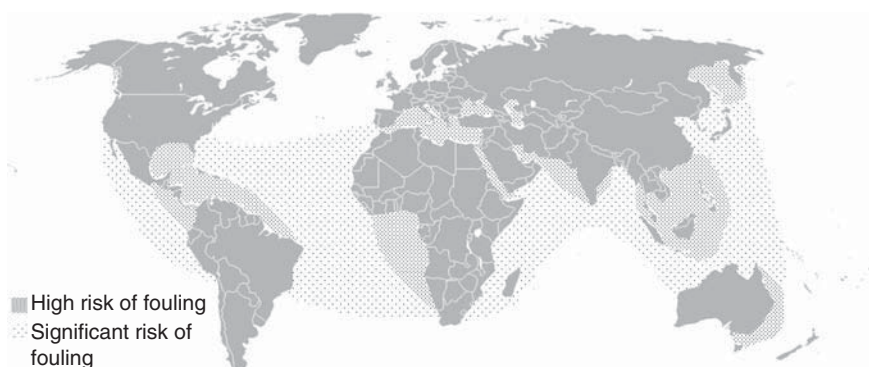
1.3 Simplified temporal structure of biofouling settlement. The linear successional process depicted above has been demonstrated to be oversimplifying the enormous complexity and variability of the biofouling process (from Yebra *et al.*, 2004; with permission from Elsevier).

being suitable for didactic purposes, more complex patterns are often found in nature (Chapter 2).

Biofouling is governed by several factors including: salinity, temperature, nutrient levels, flow rates and the intensity of solar radiation. These factors vary seasonally, spatially and with depth. Colonization and succession in biofouling communities are highly affected by seasonality in temperate regions, with less fouling development in winter (due to the reductions in light levels, water temperature and the numbers of spores and larvae). Tropical and sub-tropical regions show less variations in the biofouling communities, due to the consistency of water temperature and light levels at these latitudes which contribute to a continuous breeding and settlement of some macrofouling species. Even so, fouling in tropical environments is still a very complex and changing phenomenon.

Fouling species vary with geographical location (Fig. 1.4): intense fouling pressure has been recorded in sub-tropical and tropical regions due to preferable physical and chemical conditions in comparison with the temperate, cold and polar regions. Generally, the same major groups of organisms are responsible for fouling worldwide, but the individual dominant species involved tend to vary (e.g., for barnacles, *Balanus amphitrite* is the dominant species in the Mediterranean Sea, while *Semibalanus balanoides* is in north of France and south of UK).

The influence of depth on the marine biofouling process has remained largely uncharacterized due to its limited influence on the shipping industry. Nevertheless, the development of the deep-water oil and gas industry has led to increased interest in deep biofouling communities and on the development of suitable mitigation methods. Despite deep waters having often been regarded as strongly unfavourable for the development of life, it has been found that organisms in deep water have developed adaptation mechanisms for lower light levels, water temperature and nutrients levels combined with



1.4 World map showing those areas with higher fouling risk.

very high hydrostatic pressures (Chapter 6). Even if fouling does occur at a slower pace in deep water, the lack of hard substrates makes man-made structures very attractive to colonizing organisms and can still be a significant problem on long term subsea structures. Hence, one can conclude that biofouling is a truly ubiquitous problem in the marine environment.

1.1.1 Economical and environmental effects

According to the International Maritime Organisation (IMO), the world trading fleet, responsible for about 90% of the global trade of goods and large contributor to the so-called ‘welfare society’, will be burning about half a billion tonnes of fuel per year by 2020 (Table 1.1). According to some estimations (Schultz, 2007), the potential absence of fouling protection on ship hulls may roughly add up to about 70% in propulsion power compared to a largely fouling-free hull (see also Chapter 7). When this is translated into easily understandable units, a highly efficient antifouling (AF) protection will be saving globally over 150 billion USD per year¹ in 2020 (see Table 1.2), excluding indirect costs resulting from transport delays, hull repairs, sunk vessels due to biocorroded hulls, etc. Perhaps even worse than that are the hundreds of millions of additional tonnes of CO₂ and significant amounts of other contaminant gases and harmful particles that would be released to the atmosphere (see Chapter 9). And all that just because thousands of marine species find attachment to immersed surfaces favourable to complete their life cycle. In addition to ship hulls, biofouling can lead to major economic costs when organisms settle on immersed man-made surfaces such as buoys, membrane bioreactors and desalination units, in power plants’ cooling water systems and seawater pipelines. Biofouling is also a significant problem for all aquaculture industries, with the broadest and

Table 1.1 Estimated global fuel consumption and gas emissions from the global trading fleet for 2007 and projected figures for 2020. Review of MARPOL Annex VI, IMO, Sub-Committee on Bulk Liquids and Gases 2007

Calculation assessment	Result 2007 mill. tonnes	Result 2020 mill. tonnes
Total fuel consumption by ships	369	486
CO ₂ emissions from ships	1120	1475
Total SO _x emission from ships	16.2	22.7
NO _x emissions from ships	25.8	34.2
PM ₁₀ emissions from ships	1.8	2.4

¹ \$500/ton.

Table 1.2 Estimated effect of the choice of fouling control method on annual fuel consumption and CO₂ emissions. It is assumed that IMO estimations for 2020 correspond to a fleet featuring hydrodynamically smooth hulls. The increased shaft power as a function of the fouling degree is obtained from Schultz (2007) based on his calculations for an Oliver Hazard Perry class frigate sailing at 15 knots

	Additional shaft power %	Fuel savings 2020 million tonnes	ΔCO ₂ emissions million tonnes	Money savings \$billions
Freshly applied coating	0	0	0	0
Deteriorated coating or thin slime	9	44	134	22
Heavy slime	19	92	279	46
Small calcareous fouling or macroalgae	33	160	486	80
Medium calcareous fouling	52	253	768	127
Heavy calcareous fouling	84	408	1238	204

most well-documented impact being in marine finfish aquaculture, in particular sea cage-based aquaculture (Chapter 8). The accumulation of large amounts of biomass results in creation of microenvironments that may encourage corrosion, physical obstructions (e.g., seawater intakes), additions to weight loading and increased hydrodynamic loading. Each of these factors leads to huge cost implications in relation to inspection, maintenance and repair of immersed surfaces.

Unfortunately, increased economic costs and the emission of greenhouse and acid rain gases are not the only undesirable effects of fouling. It is now clear that one of the most common vectors of marine species to become introduced or 'alien' in non-native environments is through ship transport. Fouled vessels can be considered as 'biological islands' harbouring biofouling communities within their hulls (planktonic and adult stages), ballast waters (planktonic, adult and resting stages) and in sediments (adult and resting stages), which could potentially infest any waters where the ship will travel. Ballast waters are a good example: many flora and fauna groups are drawn into the tanks during ballasting operations. Despite the unfavourable conditions for survival in ballast tanks, some organisms do manage to survive and are subsequently released into new habitats, where they generally find a niche and become integrated into the local biological community. A few of such invasive species have life cycle related qualities which, given the right environmental conditions, can make them a nuisance (Fig. 1.5). The impacts of such phenomena can be on the one hand, ecological and evolutionary (direct and indirect competition with native biota, effect on



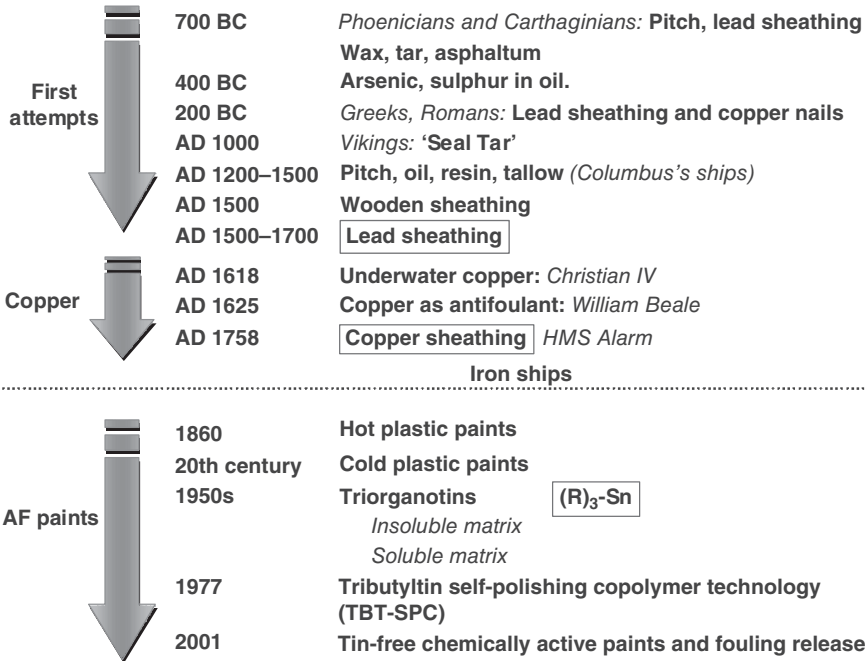
1.5 Zebra mussels (*Dreissena polymorpha*), native to the Black, Caspian and Azov Seas, were introduced into the Great Lakes in the mid-1980s through the ballast water of vessels from Europe, and have subsequently become one of the most injurious invasive species to affect the US.

higher trophic levels, habitat change, change of ecosystem processes) and, on the other hand, economic and societal (management costs, costs of the loss of ecosystem functions or values, impact on human health, costs for eradication and control measures). Examples of exotic species becoming a ‘pest’ in a new environment are toxin-releasing dinoflagellates in Australian waters (and others) and comb jelly (*Mnemiopsis leidyi*) in the Black Sea.

1.2 Historical development of AF protection technologies

The development of fouling control solutions has taken place in parallel with the history of navigation. Some of the disadvantages of marine biofouling have been recognized and combated for more than 2000 years (see Chapter 2; Fig. 1.6).

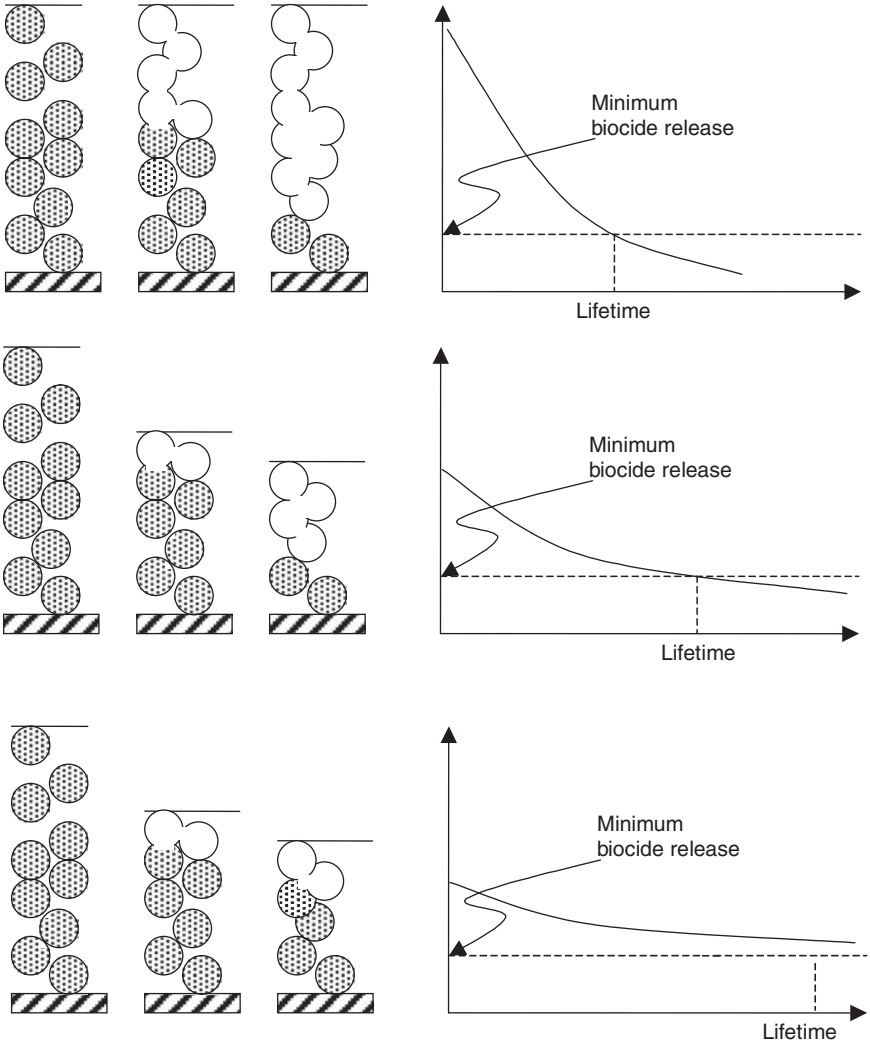
After the Second World War, important changes took place in the AF paint industry. The appearance of new synthetic petroleum-based resins featuring improved mechanical characteristics, the increased concern about safety and health (causing the abandonment of organo-mercurials and organo-arsenicals) and the introduction of airless spraying are examples of these changes. Also during this period, the addition of extremely toxic organotins (e.g., tributyltin oxide; TBTO) to rosin-based soluble matrix paints (see Yebra *et al.*, 2005; Fig. 1.7) constituted a major step forward in terms of AF performance. This performance was improved even further



1.6 Chronogram of the development of antifouling technologies.

when the release rate of organotins could be finally controlled thanks to the so-called tributyltin self-polishing copolymer paints (Chapter 18 and Yebra *et al.*, 2004). Surprisingly, in spite of the paramount importance of a proper release of the active compounds through the carrier system, this topic is largely absent from current scientific discussions. In fact, only one talk out of more than 150 abstracts dealt with controlled-release polymers during the 14th International Congress on Marine Corrosion and Fouling that took place 27–30 July 2008 in Kobe (in this book, see Chapters 13, 14 and 18).

The deleterious effects of tributyltin (TBT) released by AF paints were first documented in Arcachon Bay (France) at the end of the 1970s. Organotin belong to the most toxic pollutants known so far for aquatic life. TBT's extreme toxicity to marine biota has been well documented for a variety of organisms *in vivo* and *in vitro*. Ecotoxicological effects are dependent on the bioavailable fraction of pollutants, and concentrations at the target sites, that induce molecular effects that propagate to a variety of toxic manifestations in organisms. The main perturbations which were highlighted to TBT were: dysfunction of calcium homeostasis, inhibition of mitochondrial oxidative phosphorylation and ATP synthesis, inhibition of photophosphorylation in chloroplast and of ATPases and cytochrome P450 monooxygenases. TBT can be incorporated into the tissues of filter-feeding zooplankton, grazing



1.7 Schematic representation of the classical biocide delivery mechanisms (Yebara *et al.*, 2004). In insoluble matrix antifouling paints (top), the matrix was a hard insoluble resin (e.g., vinyls, chlorinated rubber) heavily loaded with biocides showing purely diffusion dependent biocide release. Soluble matrix paints (middle) incorporated rosin-type resins as a relatively seawater soluble binder ingredient. The paints showed some surface erosion, but the biocide release rate was too high initially and uncontrolled, hence insufficient eventually. Self-polishing paints (bottom) are characterized by a controlled surface polishing and a constant biocide depleted layer thickness which translates into virtually constant biocide release rates over the paint's lifetime.

invertebrates, and, eventually higher organisms such as fish, water birds and mammals, where it accumulates. Thus, the consumption of marine foods has been reported as an important route of human exposure. Marine fishery products may contain high TBT concentrations and different diets are expected to result in different organotin loads in human tissues and blood. A Tolerable Daily Intake (TDI) value for TBT of $0.25 \mu\text{g (kg bw)}^{-1} \text{ day}^{-1}$ was established (Penninks, 1993). In many countries, surveys conducted using commercial species showed that organotin levels were generally low, and it was then suggested that they did not present a risk to health. However, a potential risk may exist for high consumers and persons weighing less, e.g. children. Despite the recent entry-into-force of the worldwide ban on TBT (see Chapter 10), the risks associated with organotins will still persist during the coming decades. Organotins did accumulate in sediments without any significant anoxic degradation, so contaminated sediments can act as a long-term source of dissolved-phase contamination to the overlaying water column.

1.3 Current biocide-based AF protection technologies

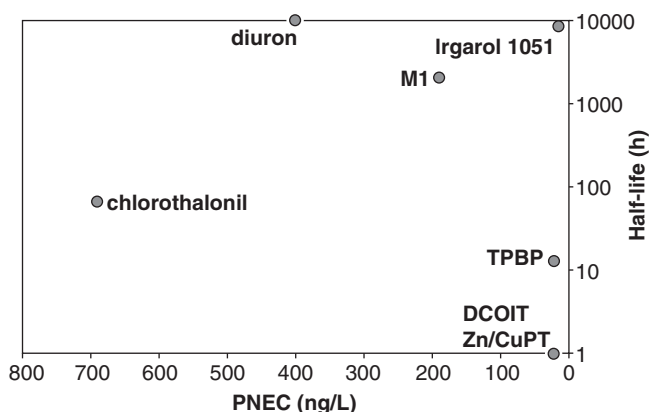
Since the abandonment of tin-containing paints by the major AF paint producers in 2003, tin-free technologies dominate the market. Those vessels with dry docking intervals up to 36-month AF systems usually rely on economical rosin-based control depletion paints (CDPs) and hybrid ablative-self-polishing systems. These paints are typically loaded with high amounts of Cu_2O and algacide co-biocides, such as, e.g., Irgarol 1051, diuron, chlorothalonil, dichlorofuanid and zineb. These paints can be considered evolutions over the classical soluble matrix paints commercialized during the second half of the 20th century. The current 60-month technologies are mainly based on three patented self-polishing technologies (Chapter 18):

- silylated acrylate technologies
- metallic acrylate technologies
- acrylic nano capsule technology.

Non-stick/fouling release silicone elastomers containing incompatible silicone or fluoropolymer oils are also gaining market acceptance for fast ships having relatively short and infrequent idle periods (see Chapter 26).

The above tin-free self-polishing paints ultimately rely on the controlled formation of seawater soluble sodium-acrylate salts within the paint matrix which allows fine-tuning the surface erosion of the paint even under static conditions. This controlled polishing is key for the attainment of a constant and sufficient release of biocides throughout the service life of the paint (see Chapters 14 and 18). Again, these top-tier self-polishing paints employ significant amounts of algacides (e.g., Cu/Zn -pyrithiones and DCOIT) and, especially, Cu_2O (see Chapter 19).

All in all, around 18 compounds are currently used as AF biocides and nine of them, chlorothalonil, dichlofluanid, diuron, Irgarol, Sea-Nine 211, TCMS pyridine, TCMTB, zinc pyrithione and zineb, are very widespread in many countries (Europe, North America and Japan; see Chapter 20). Important coastal concentrations are already being detected in areas of high yachting and shipping activity, particularly in marinas and harbours. Although the concentration levels of some biocides are usually not high enough to have direct acute toxic effects on higher species, their sub-lethal chronic effects at lower concentrations are largely unknown. Hence, significant efforts are being devoted to the characterization of the fate and effects of these biocides in the marine environment and their associated toxicities to non-target organisms throughout their degradation pathway. Reliable estimates of their release rates into the marine environment (see, e.g., Finnie, 2006), coupled with advanced hydrodynamic modelling and simulations of their degradation process allows estimation of the predicted environmental concentrations (PECs) of these substances for any real-life scenario (see, e.g., the MAMPEC model). These PECs values can then be compared to the predicted no effect concentration (PNEC) values determined from ecotoxicological studies to assess the risks associated with the use of a specific product incorporated into a specific paint under a given exposure scenario. Fig. 1.8 shows an overview of the information available for some typical biocides in terms of toxicity and degradability.



1.8 Classification of some commonly used biocides based on their toxicity and degradation times (Kevin Thomas, personal communication). The bottom left corner means low toxicity and short lifetimes in the environment (i.e., 'safe' compound). Biocides having the opposite behaviour are found in the top right corner. For a complete risk assessment, the main degradation products (see, e.g., M1 for Irgarol 1051) should be included, too.

1.4 Antifouling legislation and non-toxic technologies

Before the commencement of the discussions to ban tin-based coatings, the research on alternative more environmentally friendly solutions was very limited and had little chance of commercial success. Tributyltin self-polishing copolymer paints were extremely effective and relatively cheap, hence saving large amounts of money for ship owners. Promising research lines, such as those on very low surface energy coatings combined (or not) with elastomeric fouling release properties were largely abandoned due to the unmatched cost-efficiency of organotins. The awakening of the global environmental awareness in the form of legislative measures (Chapter 10) has completely changed the way we do antifouling research nowadays. Partly because the increased costs associated with the use of biocides, the research interest and commercial feasibility of non-toxic approaches has increased drastically (Chapter 24–28). The relative success of fouling release coatings based on silicone matrixes combined with silicon or fluoropolymer oils is a reference in the research on passive surface approaches to prevent fouling. Modern techniques can be utilized in order to bioassay and screen vast amounts of materials in the search for non-stick and fouling release properties (Chapters 12 and 15). Long-term ageing tests are subsequently used to confirm whether short-term screening tests and bioassays translate into appropriate performance in presence of a complex fouling community for extended periods of time (Chapters 16 and 17). Also, the use of surface nano, micro and macro topographies is being explored together with extreme wettabilities (super-hydrophilic and super-hydrophobic surfaces; see Chapters 24 and 25).

In spite of this wealth of non-toxic research lines, it is still widely accepted that ‘passive’ solutions will probably be insufficient to deter all sorts of fouling species throughout drydocking intervals of 7 and even 12 years. Hence, a combination of surface fouling control approaches and periodic hull cleaning mimicking the grooming behaviour of some marine species may need to be considered. Most likely, the final solution will at least partly depend on the incorporation of active compounds capable of inhibiting the attachment, metamorphosis or growth of fouling species very much like current broad-spectrum biocides but featuring:

- toxicities far away from those concentrations where they can exert antifouling activity and
- degradation rates in the marine environment so that toxic concentrations to non-target organisms are never built up.

In this respect, novel safer synthetic biocides (Chapters 20 and 21), antifouling secondary metabolites from marine species (Chapter 22), enzymes (Chapter 23) and pharmaceuticals (Chapters 11 and 28) are promising candidates.

1.5 Multidisciplinary collaborative research towards a sustainable future

Back in 1952, the Woods Hole Oceanographic Institute published the best compendium on marine biofouling published to date titled '*Marine Fouling and its Protection*'. For the first time ever, the most detailed review of fouling organisms was presented together with state-of-the-art knowledge about protection mechanisms and technologies. Perhaps the major novelty of this compendium was actually visualizing the continuous interaction and feedback between all these separate fields of study as the only way forward. Unfortunately, such learnings have been largely forgotten and AF research is still taking place, to a large extent, in separate compartments.

The 14th International Congress on Marine Corrosion and Fouling held in Kobe (Japan) 27–30 July 2008 and sponsored by the Comité International Permanent pour la Recherche sur la Préservation des Matériaux en Milieu Marin (COIPM, Chapter 2) has highlighted once more the diversity associated with fouling control research. Researchers from 29 countries gathered to exchange the recent developments in their respective areas of expertise. Biologists unveiling the mysteries associated with the settling, adhesion, metamorphosis and growth of the thousands of sessile species inhabiting the oceans. Biochemists describing their efforts towards the screening and bioassaying of many novel natural substances believed to deter epibiosis in natural marine communities. Organic chemists and biotechnologists working on the inexpensive synthesis and large-scale production of novel AF substances. Environmental chemists studying the fate of such substances when exposed to natural seawater, key to the estimation of their predicted environmental concentrations as a function of biodegradation and photodegradation rates, partition to sediments, chelation processes, etc. Ecotoxicologists studying the potential toxicity of those compounds and their degradation products to relevant non-target species. Legislators deciding upon what is admissible and sustainable and what is not. Polymer chemists engineering controlled-release systems key to the long-term efficiency of an AF coating. Coating technicians who can turn a concept into a cost-efficient, stable, robust and practical coating, which can be applied virtually at any shipyard in the world. And finally, ship owners, who have in their decision the key to saving tons of CO₂ and other harmful gases, while preventing the translocation of non-native species, or avoiding/minimizing the release of tons of broad-spectrum toxins into the environment.

In front of such an overwhelming diversity and complexity, the choice of isolating within each one's field of expertise is very tempting. What do I care about polymers if I am studying metabolites from a local species of

red algae? ‘Let others invent!’², but we cannot innovate efficiently without each others’ contribution. The American ONR and the European AMBIO (Chapter 24) research programmes are two admirable attempts at bringing together scientists from multiple disciplines towards a common goal. In a similar fashion, this book has tried to combine academic research and industrial knowledge in a search for their mutual synergy. Academia has been showing industry better ways of doing things for the last many decades, but industry must also make an effort to train academia in how to carry out their research in a way that is fully compatible with business models and, hence, useful from the practical point of view in as short a time as possible (see Chapters 11 and 28). All this aiming towards leaving the future generations a legacy of respect for the environment and the lesson that human progress cannot be built on the basis of unsustainable exploitation of the earth’s natural resources for the economic benefit of (a small part of) humanity.

1.6 Advances in marine antifouling coatings and technologies

This book is divided into 4 parts:

- I Marine fouling organisms and their impact
- II Testing and development of antifouling coatings
- III Chemically active marine antifouling technologies
- IV Surface approaches to the control of marine biofouling.

Throughout the book, we have tried to deliver an overview of the recent advances in the biology of fouling organisms, the latest developments in antifouling screening techniques both in the field and in the laboratory, the research status on safer active compounds and the progress on nontoxic coatings with tailor-made surface properties. The aim is to offer the researcher an overview of the different fields involved in antifouling research, hoping it can help in making faster progress towards a holistically viable solution to the marine biofouling problem.

1.7 Acknowledgements

Above all, we would like to warmly thank all the authors for their hard work getting their outstanding manuscripts ready on time in spite of their very busy agendas. We are also grateful to Woodhead Publishing Ltd for inviting us to be editors.

²Quoting Miguel de Unamuno (1864–1936).

On a personal note, C Hellio is very happy to thank Prof. Y. Le Gal (College de France, Marine Station of Concarneau, France), who has inspired her work since the beginning and who has always offered his continual support over the years. Similarly, D.M. Yebra would like to express his most sincere gratitude to all his colleagues at Hempel R&D, especially those at the Fouling Control department, for all the hard work and good times shared together.

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Part I

Marine fouling organisms and their impact

The battle against marine biofouling: a historical review

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Abstract: This chapter reviews scientific milestones in the study of marine biofouling from early wooden shipping to modern maritime installations, such as offshore platforms and sonic devices. It focuses on stages in the settlement and biofouling of surfaces, from primary film formation to macrofouling, strength of attachment and methods of control.

Key words: macrofouling, microfouling, historical aspects, biocontrol, bacteria, diatoms, seaweeds.

2.1 Introduction

The battle to control biofouling goes back to the dawn of civilization when man constructed boats and began his ocean travels. Earliest documented accounts date from the Greek and Roman civilizations when copper or lead sheathing was used to protect wooden boats (Anon, 1952). In this chapter I shall only give an overview of the key developments in our search for a better understanding of fouling organisms and their prevention. Greater detail of specific topics will be found in appropriate chapters in this volume.

Any non-toxic material exposed in the marine environment represents a potential surface for colonization by a variety of organisms. When this occurs on man-made structures, such as ships, buoys, sonar devices, pontoons, it is referred to as marine fouling or 'biofouling'. The term biodeterioration can also be used, as it refers to the undesirable effects marine organisms cause to economically and commercially important man-made structures immersed in the sea. The result of biofouling is economically great, especially with fuel costs of large container boats and naval and ocean going ships, resulting in reduction of speed of the vessel and the extra frictional resistance created (Anon, 1952; Houghton, 1970; Ralph and Goodman, 1979; Townsin *et al.*, 1980; Terry and Edyvean, 1981). In recent times, the protection of offshore structures, oil installations and platforms has been logistically difficult and expensive because of the cost of monitoring such

operations and in the development of long-lasting coatings (Eikers, 1978; Gray, 1978; Hamilton and Sanders, 1986; Hill *et al.*, 1987; Edyvean *et al.*, 1988).

Marine biofouling is ubiquitous throughout the world and is of greatest concern in ports and inshore waters, although in more recent times it has had important implications in the off shore industry (oil and gas platforms, navigation buoys) (Gray, 1978; Heaf, 1979; Hamilton and Sanders, 1986). Biofouling is widespread as marine organisms produce prolific spores, or larvae, that ensure their survival on any intertidal and subtidal substrata. Aquatic environments offer ideal conditions for their growth, such as hydration of the organisms, and water movement that brings a constant supply of nutrients and carries away metabolic wastes (Crisp, 1973). Shipping is not the only industry affected by biofouling; other examples include marinas, seawater cooling systems, underwater acoustic instruments, and underwater cables.

Various estimates have been made of the losses incurred due to biofouling, and include a wide range of parameters that have to be considered: reduction in ships' speed because of extra frictional resistance leading to increased fuel consumption and additional stress to engines to maintain cruising speeds (see Chapter 7 Kane); docking charges for ship servicing; loss in income due to time ships are not operational, labour costs, and cost of paints/coatings (Banfield, 1980; Townsin *et al.*, 1980). On fixed and gas platforms (see Chapter 6 Apolinario), there is increased structural and hydrodynamic loading (Ralph and Goodman, 1979), metal fatigue (Terry and Edyvean, 1981), coating breakdown and cost of regular inspections and maintenance (Hardy, 1981). For example, the cost of cleaning fixed platforms in UK waters alone exceeded £17.3 million (data for 1985). In 1974 the US Navy spent US\$ 200 million on the maintenance of shipping and related marine biodeterioration (Baciocco, 1984). Baciocco draws attention to the spiralling cost of energy (1981) to the US Navy and the problems this causes, and this issue is now exacerbated with the current increase in the cost of fuel. The fuel costs to the US Navy in 1981 was 16 million barrels, but it was estimated that effective antifouling bottom coatings would save 3.3 million barrels annually, or US\$ 180 million. Worldwide commercial shipping use some 300 million tonnes of fuel annually, but this would be 120 tonnes higher if there were no adequate antifouling measures (Champ, 2000). In 1996, it was estimated that the global cost for antifouling ships and pleasure craft was of the order US\$ 700 million annually (Callow, 1996).

Traditionally biofouling of structures in the sea has been divided into a number of major phases: molecular fouling; primary film formation, slime layer by microorganisms (bacteria, diatoms, blue green algae, fungi, actinomycetes, protozoa and algal spores); secondary (macro algae, barnacles, hydroids, serpulids); and tertiary (mussels, ascidians, sponges). These are

arbitrary sequences which can vary dramatically seasonally and with geographical location. The once held view that they were sequential has been well disproved. However, there are reports that selected macroorganisms will only settle if a primary film is present on the surface to be colonized (Wilson, 1955; Ryland, 1974; Mitchell and Maki, 1988). The toxicity of the surface also plays a role in the sequence of colonization by fouling organisms; however, antifouling formulations may only delay the initial development of the fouling communities (Fletcher and Chamberlain, 1975). This may be due to insufficient release of biocide, some organisms may be tolerant of the biocide, e.g. *Ectocarpus* (brown filamentous alga) on copper based paints, or insufficient water movement in marinas for the effective release of biocide.

2.2 Timber ships

Ever since man built boats/ships he has encountered problems with marine occurring organisms. Initially, such ships were made of wood and prone to decay by marine borers: molluscs, such as *Teredo*, *Bankia* and *Lyrodus* (Clapp, 1951; Turner, 1966, 1971), Crustaceans, such as *Limnoria*, and *Sphaeroma* (Clapp and Kenk, 1963; Becker, 1971; Kühne, 1971). Then in the early 1950s the role of fungi in the decay of wood in the sea was recognized (Barghoorn and Linder, 1944; Meyers, 1971; Jones, 1971). The role of bacteria in timber decay has received less attention for their ability to penetrate deeply within wood is minimal (Floodgate, 1971; Holt *et al.*, 1980; Mouzouras *et al.*, 1988; Venkatasamy *et al.*, 1990). Ray (1959) and Ray and Stuntz (1959) were of the opinion that marine fungi were implicated in wood digestion by the Crustacean wood borer *Limnoria*, while Geyer (1980) and Geyer and Becker (1980) also noted the attractive effects of marine fungi to *Limnoria tripunctata*. However, we were not able to prove this in our studies (Jones, personal observation). Cutter and Rosenberg (1972), and Boyle and Mitchell (1984) examined the role of bacteria in the digestive processes of woodborers. Although bacteria were present on the exoskeleton of *Limnoria lignorum*, none were found in the gut.

Protection of wooden vessels against marine borers and fouling has a long history dating back before 200 BC, when hot pitch, tars, greases and other materials were used as antifouling coatings. Atheneus (200 BC) reported that lead sheathing, held on by copper nails, was used to protect the ships of Archimedes (Visscher, 1928). In Roman times, lead and copper sheathing was to protect wooden ships from ship worm and gribble (Anon, 1952). Losses caused by timber decay of ships have not been widely documented, but Pepys (1660s) reported that HMS ships often rotted away and sank before being launched! This subject is dealt with in greater detail by Yebra *et al.* (2004).

2.3 Iron clad ships

Decay of ships' timbers by marine borers and the limits on the length (*circa* 80 meters) and strength of wooden ships led to the construction of ships in iron in the early 19th century. Then around 1865, ships were built of steel. The first British Royal Navy iron clad ship was the Warrior launched on 29 December 1860, saw service for a decade, and was then relegated to the Reserve Fleet in 1883. Copper sheathing of iron ships could not be used because electrolytic action corroded the hull. This gave rise to the need for alternative methods to protect ships, and the dawn of modern paint systems (Anon, 1952). Young (1867) reported that in 1865 there were no fewer than 300 patents issued for antifouling compositions in England, most of no use!

2.4 Documentation of macrofouling organisms

The earliest scientific documentation of ship fouling is in various reports of naval departments (Adamson, 1937; Fitzgerald *et al.*, 1947) and from the famous Clapp laboratory (Clapp, 1951; Clapp and Kenk, 1963) and the Woods Hole Oceanographic Institute (Hutchins and Deevey, 1944). In 1952 the United States Naval Institute published the biofouling 'bible' '*Marine Fouling and its Prevention*' prepared by the Bureau of Ships, Navy Department and the Woods Hope Oceanographic Institution (Anon, 1952). This dealt with all aspects of marine fouling: fouling communities, seasonal sequence, geographical distribution, history of the prevention of fouling, to the effects of fouling on ships and other maritime structures. In the chapter on marine fouling organisms they list 37 bacteria, 14 fungi, 111 diatoms, 452 seaweeds, 33 sponges, 120 barnacles, 116 holothuroidea, to list but a few, giving a total of 1964 marine fouling organisms (current estimates put this as nearer 10000). This has never been updated, but emphasizes the problem facing those in the shipping industry to develop antifouling coatings to counteract this rich biodiversity.

The biodeterioration of materials and equipment during the Second World War led to intensive research to protect military structures. In the early 1950s, NATO countries initiated a number of committees to examine various aspects of material failure under the aegis of the Organisation de Cooperation et de Developpement Economiques (OECD). They included the following:

1. Comité sur la Préservation du bois en milieu marin, launched in 1963.
2. Group of Experts on the Preservation of Materials in the Marine Environment, launched in 1955.
3. Biological Deterioration of Materials Research Group.
4. Group of Experts on the Preservation of Wood.

These committees greatly developed the study of the biodeterioration, prevention and control of materials both terrestrially and in the sea. All set up international conferences where results of work carried out in collaboration with scientists from member countries were presented and frequently resulting in published proceedings.

This impetus led to a number of journals devoted to these topics: *Biodeterioration Journal* (later the *International Biodeterioration*, then *International Biodeterioration and Biodegradation*; *Bulletin de Liaison* and later the journal *Biofouling* launched in 1989). Subsequently a wide range books have been published devoted to biofouling and corrosion, and in particular biofilms and adhesion (Jones and Eltringham, 1971; Berkeley *et al.*, 1980; Costlow and Tipper, 1984; Thompson *et al.*, 1988; Flemming and Geesey, 1991; Sleight, 1987; Campbell *et al.*, 1998; Railkin, 2004).

Group 1 was set up in 1955 with Dr D.L. Ray as chairman of the Ecology Commission of the OECD group of experts on the Fouling and Corrosion of Ships' Hulls. Groups 1 and 2, listed above, eventually amalgamated to form the Comité International Permanent pour la Recherche sur la Préservation de Matériaux en Milieu Marin in 1966 (COIPM) chaired by Professor V. Romanovsky and later by Dr John de Palma (to 1995), Prof. Gareth Jones (1995 to 2006) and Dr. Andy Jacobson (2006 to present).

Over the years COIPM has done much to promote the study of marine biofouling with many subcommittees (period 1976–1985): Cathodic protection: Dr de la Court; Methods of testing antifouling paints: Prof. E. Mor; Biology: Prof. G. Relini; Wood in the marine environment: Prof. G. Jones; Offshore: Dr J. de Palma; Surface conditions before application of paints: O. Hansen; Corrosion in the sea: G. Dechaux; Marine pollution: Dr N.A. Ghanem and Concrete in the marine environment: Prof. Th. Skoulikidis.

Every four years COIPM organized international conferences (Cannes, 1964; Athens, 1968; Washington, 1972; Antibes, 1976; Barcelona, 1980; Athens, 1984; Valencia, 1988; Tarranto, 1993; Portsmouth, 1995; Melbourne, 1999; San Diego, 2002; Southampton, 2004; Rio de Janeiro, 2006; Japan, 2008). The topics covered at these conferences changed as our knowledge of biofouling evolved. In the early days (1968–1992) great attention was given to corrosion and macrofouling organisms, the latter documenting biodiversity at various geographical locations, weight of the biomass, studies of the effect of depth of exposure of panels and the biofouling organisms recovered (Fletcher, 1974; Lovegrove, 1978; Ghobashy and Hamada, 1984; Greene and Schoener, 1984; DePalma, 1968, 1984; Haderlie and Elphick, 1968; Haderlie, 1984; Schoener, 1984 (Table 2.1). Subsequently attention focused on microfouling and their mode of attachment: bacteria (Characklis, 1981; Dempsey, 1981a,b; Hamilton, 1987), protozoa (Brown *et al.*, 1986; Jackson and Jones, 1988, 1991), diatoms (Daniel *et al.*, 1980; Cooksey *et al.*, 1984), strength of attachment (Duddridge *et al.*, 1982; Gunn *et al.*, 1984; Woods

Table 2.1 Topics covered at COIPM congresses from 1968 to 2002

Subject	1968	1976	1980	1984	1992	1995	1999	2002
Corrosion	23	10	0	3	16	0	0	1
Surface preparation	1	2	2	0	3	0	0	0
Ship cleaning	1	2	2	0	0	0	0	0
Cathodic protection	7	13	1	1	10	0	0	0
Bacterial corrosion	3	0	1	5	10	2	2	1
Wood decay: borers	4	4	4	1	2	1	3	2
Balanus studies	2	1	0	0	0	1	0	0
Biofouling	12	10	14	16	19	6	7	12
Deep ocean	1	1	0	0	0	0	0	0
Microbial biofilms	0	2	1	3	0	6	2	0
Leaching rate	0	1	0	1	0	0	0	1
Rapid cleaning	0	2	0	0	1	2	1	3
Surface chemistry	0	3	1	2	1	0	3	1
Attachment	0	2	1	0	2	4	5	0

and Fletcher, 1991) and chemical composition of microbial adhesives (Callow and Evans, 1974; Daniel *et al.*, 1987). In more recent years attention has turned to natural antifouling chemical defence by marine animals and seaweeds (Pawlik, 1992; Steinberg *et al.*, 1992; Fusetani, 2004; De Gama *et al.*, 2008; Plouguerné *et al.*, 2008), invasive marine organisms and marine pollution, some 175 introduced as biofouling organisms into Australia (Noonsite.com).

Workshops were also held on specific topics and COIPM also published a journal, the *Bulletin de Liaison*. Manuals on various aspects of biofouling and a number of Catalogues of the Main Marine Fouling Organisms have also been produced by COIPM: Barnacles (Southward and Crisp, 1963), Polyzoa (Ryland, 1965), Serpulids (Nelson-Smith, 1967), Ascidians (Millar, 1969), Marine Sponges (Sará, 1974), Algae (Fletcher, 1980), Hydroids (Morri and Boero, 1980) and Bivalves (Knudsen, 1997). COIPM set up a number of collaborative programmes with the aim to document test sites within member countries, test procedures and evaluation of selected marine paints (e.g., Fletcher, 1974; Jones *et al.*, 1977).

The period 1950 to circa 1985 focused on documenting marine macrofouling, primarily animal taxa, with research devoted to methods of assessment: weight of biomass (Relini, 1977; Rao and Balaji, 1988), diversity of species (Pyefinch, 1951; Stubbings and Houghton, 1964; Bruce, 1976; Fletcher, 1981), and dominant taxa. Other aspects studied included: seasonality in the occurrence of fouling species (Ghobashy, 1976), sequential colonization of exposed panels (Relini, 1983), geographical distribution (DePalma, 1984), occurrence at different depths (Relini, 1976; Houghton

and Stubbings, 1963) and colonization of different materials and surfaces (Crisp and Barnes, 1954). These studies greatly advanced our knowledge of the diversity of macrofouling organisms, but did not necessarily advance what control measurements should be used.

2.4.1 Barnacles and ship fouling

In the period 1950 to 1970s, the greatest concern with ship fouling was with barnacle settlement, especially the annual scrubbing of small boats and yachts with inadequate antifouling protection. Even at the start of the Second World War, UK naval ships, although treated with antifouling compositions, were only protected for a few months (Houghton, 1968). Experience with the sheathing of ships proved that copper was a vital component and research focussed on developing antifouling paints with copper. This proved effective in the control of hard fouling but less so with algae. So up to about 1966, animals dominated fouling communities on ships (Crisp, 1965; Berry, 1966), but by 1968 Pearson regarded algae as the dominant fouling organisms. Many reasons were advanced for this: better antifouling coatings, higher ship operational speeds and shorter docking times, larger hulls that were well illuminated thus favouring settlement by algae.

Early studies on barnacles focussed on documenting the species (Anon, 1952; Geraci *et al.*, 1984), their identification and provision of keys (Southward and Crisp, 1963). Subsequent research was concerned with barnacle larval stages (Costlow and Bookout, 1958; Geraci and Romairone, 1982; Scheltema and Williams, 1982; Crisp, 1984), attachment and exploration of surfaces leading to larval settlement (Nott, 1969; Nott and Foster, 1969; Lindner, 1984; Holm, 1990; Mollineaux and Buttman, 1991), larval sense organs (Walker, 1974; Crisp, 1976), nature of the adhesive (Lindner *et al.*, 1972; Lindner and Dooley, 1976a,b; Fyhn and Costlow, 1976; Naldrett and Kaplan, 1997; Smith and Callow, 2006), surface preference for larval settlement (Crisp, 1984) and preconditioning of surfaces and the role of biofilms in larval settlement (Knight-Jones, 1953; Crisp and Meadows, 1963; Gomez *et al.*, 1973).

With the development of new techniques and equipment, research into barnacle fouling was able to seek answers to more critical questions, for example the nano-scale properties of adhesives in a hydrated natural condition (Phang *et al.*, 2007). Topics under investigation in the period 1990 to 2007 include: inhibitory effect of bacteria on larval settlement (Maki *et al.*, 1992; Kon-ya *et al.*, 1995), force required to detach settled larvae (Crisp *et al.*, 1985; Ina *et al.*, 1989; Swain *et al.*, 1992; Becker, 1993; Anon, 1997; Schultz *et al.*, 1999), atomic force microscopy to image polymer surfaces, to improve details of structure and function of adhesive proteins (Phang *et al.*, 2006, 2007), signal transduction processes (Morse, 1988, 1990; Rittschof

et al., 1986; Clare *et al.*, 1995; Clare, 1996; Clapham, 1995; Yamamoto *et al.*, 1995), settlement of barnacles on different surfaces, such as, hydrogels (Rasmussen *et al.*, 2002), chemicals involved in larval settlement (Dreanno *et al.*, 2006; Vogan *et al.*, 2003), and effect of enzymes on settlement (Pettitt *et al.*, 2004). While barnacles are the major animal fouling of ships, serpulids and mussels cause fouling of cooling water pipes to power stations (Relini, 1988), marine platforms (Forteath *et al.*, 1984) and buoys (Selin, 1980).

2.4.2 Algal fouling

The study of animal macrofouling became somewhat academic with development of marine coatings with copper, and later tributyltin, and attention focused on algal fouling, 1970 to 1980 (Russell, 1971). These studies focused on the green alga *Enteromorpha* (Christie, 1973, 1974; Evans, 1981) and the brown alga *Ectocarpus siliculosus* (Russell, 1971; Baker and Evans, 1973; Russell and Morris, 1974), the latter showing tolerance to copper based paints. Besides documenting the diversity of algal fouling, studies also concentrated on sequential colonization of test panels (Fletcher and Chamberlain, 1975; Jones *et al.*, 1982; Fletcher, 1976, 1977; Fletcher *et al.*, 1984a) and their mode of attachment (Evans and Christie, 1973; Chamberlain and Evans, 1973; Christie, 1973; Chamberlain, 1976; Fletcher and Callow, 1992). Algal settlement and attachment was studied at the ultrastructural level focussing on the production of adhesives (Chamberlain, 1976; Fletcher and Chamberlain, 1975), cytochemistry of the adhesive (Baker and Evans, 1973; Callow and Evans, 1974), carpospore polarization (Jones and Jones, 1986) and the effect of different enzymes on adhesion (Christie *et al.*, 1970). Marine algae are extremely adaptable to antifouling paints, as demonstrated by the crustose brown algae *Hecatonema*, *Myrionema* and *Stebulonema oligosporum* and their tolerance of heavy metals (Fletcher, personal communication).

2.5 Bio-films

One of the earliest accounts of bacteria in ship biofouling was by Zobell and Allen (1935) and Zobell (1938, 1939) who cited their importance in causing increased hydrodynamic resistance in shipping. An example of how microfouling affects the efficiency of a ship is given by Lewthwaite *et al.* (1984). An Admiralty fleet tender coated with an insoluble matrix antifouling coating over a 2-year period had an 80% increased frictional resistance with an associated 15% loss in ship speed. The tender was covered in a layer of slime 1 mm thick, but once this was removed the skin frictional resistance returned to that of a clean ship. Biofilms have been shown to contribute most to frictional resistance at the front quarter of the hull (Baba and Tokunaga, 1980) while Woods *et al.* (1986) showed that biofilms were often

reduced in the aft region of the hull and usually composed of the smaller, firmly addressed diatom species. The effect of drag on the efficiency of ships is discussed in greater detail in Chapter 7.

The 1970s to 1990s was an active period in determining the role of microorganisms in biofouling and they were a precursor in the attachment of macrofouling organisms (Wilson, 1955; Ryland, 1974; Mitchell and Maki, 1988; White, 1988). The study of microfouling was greatly enhanced by the use of scanning and electron microscopy (SEM, TEM) which revealed stages in the settlement and internal organization of these organisms (Daniel *et al.*, 1980; Jones *et al.*, 1982; Jones, 1995b). The scanning probe microscopes (SPM) offer even greater tools for observing microfouling. Scanning tunnelling microscopy (STM), atomic force microscopy (AFM) and confocal laser scanning microscopy (CLSM) all have potential for better resolution and quantification of biofilm components (Beech, 1994; Beech *et al.*, 1996; Denault *et al.*, 1998; Radford *et al.*, 1998; Sekar *et al.*, 2002).

Although a number of studies documented microbial diversity (Russell, 1971; Fletcher, 1981; Brown *et al.*, 1986; Gunn *et al.*, 1987; Woods *et al.*, 1988) greater attention was devoted to the formation of primary films (Marszalek *et al.*, 1979; Characklis, 1981; Dempsey, 1981a; Characklis and Cooksey, 1983), mode of attachment (Marshall, 1973, 1981; Chamberlain, 1976; Dempsey, 1981b; Jones *et al.*, 1982) and cytochemical characterization of the adhesives (Baker and Evans, 1973; Callow and Evans, 1974; Daniel *et al.*, 1987). The major marine microbial taxa include: bacteria (Corpe, 1973, 1977), diatoms (Daniel, 1983; Cooksey *et al.*, 1984; Pyne *et al.*, 1984), protozoa (Brown *et al.*, 1984, 1986) and fungi (Jones, 1973, 1995a,b).

The nature of the surface greatly influences the settlement of microorganisms (Baier, 1973; Corpe *et al.*, 1976; Davies *et al.*, 1984; Gerchakov *et al.*, 1977), toxic nature of the surface (Russell and Morris, 1974; Blunn, 1986; Pyne *et al.*, 1987; Woods *et al.*, 1988), substrate wettability (Dexter *et al.*, 1975; Dexter, 1977), and surface energy (Fletcher *et al.*, 1984b).

Attached bacteria can also increase the rate of corrosion of metal surfaces (Gerchakov *et al.*, 1977; Characklis, 1981; Gaylarde and Johnson, 1980; Little *et al.*, 1984; Mihm and Loeb, 1988) and in the immobilization of copper in copper and copper-nickel alloys (Blunn and Jones, 1985, 1988).

Although the Woods Hole list of fouling organisms listed 37 bacterial species isolated from test surfaces, their importance in biofouling was not seriously studied until the 1970s with early observations by Corpe (1973, 1977; Corpe *et al.*, 1976; Marshall, 1973, 1981; Dexter, 1977; Dexter *et al.*, 1975). Studies of bacterial attachment, observed at the ultrastructural level followed (Fletcher and Floodgate, 1973; Dempsey, 1981a,b).

The study of diatoms as fouling organisms has been more widely studied, initially on non-toxic surfaces (Pyefinch, 1951; Skerman, 1956; O'Neill and

Wilcox, 1971). Subsequent studies have followed colonization of toxic surfaces, anti-fouling coatings and copper-nickel alloys (Blunn and Jones, 1985, 1988; Daniel, 1983). Various aspects of diatom fouling and adhesion have been investigated: Copper immobilization (Daniel and Chamberlain, 1981), ultrastructural studies of diatom settlement and attachment (Cooksey and Cooksey, 1980; Daniel *et al.*, 1980; Cooksey *et al.*, 1984; Pyne *et al.*, 1984), cytochemical characterization diatom adhesive (Daniel *et al.*, 1987; Smith and Callow, 2006), and role of calcium in diatom adhesion (Cooksey and Cooksey, 1980).

Although marine fungi do not play a major role in biofouling they have unique ways of securing attachment to wood and other substrata (Hyde *et al.*, 1986a,b, 1989; Jones, 1995b).

Of the marine fouling microorganisms, the least investigated groups are the protozoa and choanoflagellates (Jackson, 1988). Although protozoa have been noted as fouling organisms (Anon, 1952), there have been few specific studies. Brown *et al.* (1984, 1986) documented the settlement, attachment and growth of sessile, fouling peritich protozoan, *Carchesium polypinum*. Jackson and Jones (1991) also explored the dynamics of marine primary films, demonstrating that they were periodically 'sloughed off' followed by further colonization. Amoeboid protozoa were implicated in this process, by tunnelling through the primary film, causing its disruption and ultimate breakdown. The only published account of choanoflagellates occurring as primary colonizers was that of Marszalek *et al.* (1979). Jackson (1988) documented the settlement of choanoflagellates on a wide range of substrata: antifouling paints, glass cover slips, thermanox discs and stainless steel stubs.

2.6 Strength and mode of attachment

Most studies of marine fouling organisms have concentrated on their mode of attachment, chemistry of the attachment adhesive or their tolerance to antifouling paints. However, the strength of the attachment of these organisms is also significant, for they are subject to strong currents on marine structures, especially boats. Once fouling organisms have secured spore attachment, further development of the thalli is required to maintain their attachment (Fletcher, 1976, 1977; Fletcher *et al.*, 1984a). Few studies have been concerned with measuring the strength of adhesion and factors affecting it (Charters *et al.*, 1973; Christie *et al.*, 1970; Houghton *et al.*, 1973; Norton, 1983; Schultz *et al.*, 2003).

The major problem in tackling this topic is the availability of suitable equipment to measure the forces that operate on settled spores. Early studies employed a water broom method (Charters *et al.*, 1973; Norton, 1983), a water jet technique (Christie *et al.*, 1970; Rees and Jones, 1984),

a water velocity procedure (Houghton *et al.*, 1973), and a turbulent channel flow apparatus (Schultz *et al.*, 2000). Fowler and McKay (1979) developed the Radial Flow Chamber (RFC) which was used to measure strength of attachment (as shear stress) of bacteria (Duddridge *et al.*, 1982), diatoms (Pyne *et al.*, 1984; Woods and Fletcher, 1991), *Enteromorpha* spores (Gunn *et al.*, 1984) and marine fungi (Hyde *et al.*, 1989; Read *et al.*, 1991). The highest shear stress at which diatoms remained attached to the disc was 4.4 N/m^2 (approximately 0.25 knots) after settlement for 120 hours (Pyne *et al.*, 1984). Unpublished data show that diatoms allowed to settle to discs for 2–3 weeks were able to withstand shear stresses of 120.4 N/m^2 , which is equivalent to 10 knots, while diatoms tolerate much higher shear stress on ships in service (Daniel, 1983). Different methods have been used to measure strength of adhesion in marine animal fouling organisms and the adhesives produced differ significantly from those of algae and other micro-organisms (Lindner and Dooley, 1976a,b; DeVore *et al.*, 1984). Several barnacle adhesion tests have been proposed and generally measure shear stress, although tension tests have also been tried (Anon, 1997; Schultz *et al.*, 2000, 2003; Kavanagh *et al.*, 2001; Wendt *et al.*, 2006; Beigbeder *et al.*, 2008; Holm *et al.*, 2006). These tests have been applied to other macrofouling organisms, especially their settlement on silicone elastomers (fouling release coatings, FR) (Holm *et al.*, 2006). The removal stresses for *Crepidula fornicata* were very low (0.009–0.042 MPa), while for *Balanus eburneus* shear stresses of 0.240 to 0.088 MPa were recorded (Holm *et al.*, 2006).

2.7 Development of antifouling coatings

Antifouling paints have undergone dramatic changes since 1952, when the active life might be as low as a few months to one year. These have involved insoluble or soluble matrix paints, the cooperation of biocides, generally heavy metals, self-polishing copolymer paints, tin-free self-polishing copolymers with the use of booster biocides, non-stick surfaces to hydrogels (Yebra *et al.*, 2004).

The development of antifouling coatings is a complex process involving consideration of many factors: choice of the metallic toxin (copper, lead, tributyltin, zinc, see Chapter 19 Brooks) or organic poisons (e.g., isothiazolones, pyrithiones, see Chapter 20 Thomas); selection of the carrier or matrix (rosin, silyl acrylates, metal acrylates, see Chapter 18 Bressy) and pigments (cuprous oxide, zinc oxide, iron oxide, see Chapter 13 Yebra); and leaching rate of active ingredients (Anon, 1952; Relini, 1977, 1988; Chapter 17 Howell). With the move away from metallic ions and the prohibition of organotin-based paints such as tributyl tin, the industry has had to look at alternative systems (Hunter and Anderson, 2000; Stupak *et al.*, 2003). Omae (2003) discusses the use of tin-free paints containing copper compounds

with the addition of booster biocides. This has led to research into the natural resistance of selected marine plants and animals to biofouling, the mechanisms they employ and their potential for use in the shipping industry (Sjögren, 2006; Sjögren *et al.*, 2004; Plouguere *et al.*, 2008). More detailed accounts of coatings, ingredients, use of biocide boosters and mode of release of biocide are reviewed by Yebra *et al.* (2004) and Chapter 13 and 14.

Other considerations include longevity of the paint; mode of application to vessels and self polishing paints (Christie, 1974). All of these systems require long-term evaluation requiring different exposure test methods: exposure of panels from rafts, different types of rafts and laboratory testing with rotating disc devices (see Chapter 16 Sanchez). The toxic effects of the selected biocides on different fouling organisms and non-target species, and their mode of action are all necessary in the evaluation of materials used in antifouling paints.

Ships' surfaces must also be prepared before application of antifouling paints, with consideration given to corrosion by bacteria. The development of self-polishing antifouling paints containing organotin derivatives was a major breakthrough in the protection of ships from marine fouling organisms. However, their harmful effect to the marine environment was not established for a decade or more (Evans *et al.*, 2000). The International Maritime Organization therefore agreed to withdrawal of tributyltin (TBT) in a phased out operation from 2003 to 2008. Withdrawal of the use of tributyltin coatings has focused research on development of non-toxic surfaces: hydrogels and low energy surfaces (Rasmussen *et al.*, 2002). Research into understanding how marine animals and seaweeds remain free of fouling organisms is in progress to try to mimic these and their application to the shipping industry (Pawlik and Faulkner, 1988; Paul, 1992; Pawlik, 1992; De Gama *et al.*, 2002; Sjögren, 2006; Jones, 2008). While these often work in the laboratory, their application for use on ships is a major challenge.

The recognition that metals and their alloys are subject to corrosion gave rise to considerable research effort to document and understand the processes involved (Kik, 1980; Iverson, 1981; Hamilton, 1987; Campbell *et al.*, 1998). In particular, microbial corrosion is a serious problem to the offshore oil industry, due to the high costs of regular inspections (Edyvean, 1984). Cathodic protection offers a control mechanism, but additionally biocides may have to be used in power station inlets. However, as this chapter is devoted to biofouling, this aspect is not discussed further here.

2.8 Conclusions

Since the publication of the Woods Hole Oceanographic Institution Book (Anon, 1952) on marine biofouling organisms and the problems they cause, immense strides have been made in our understanding of settlement,

attachment, strength of attachment and chemical nature of the adhesives involved. Similarly, our knowledge of surface chemistry and physical characteristics of substrata has advanced and how this affects the attachment of fouling organisms. Antifouling paint technology has progressed, both in the use of biocides, their incorporation into coatings and their release. The challenge for the future is the production of coatings that are environmentally acceptable and efficient.

2.9 Acknowledgements

I would particularly like to pay tribute to my dear friend Dr David Houghton who first got me interested (sweet talk, coax, cajole, liberal supply of coffee!) in marine microfouling, and for all his work with the biology group in COIPM and who did so much in promoting the subject in the UK. My warm thanks to Dr Bob Fletcher for all his support and friendship over many years, to my COIPM colleagues for their tolerance and understanding when chairman of the wood group, and as president, and to my many students who worked on various aspects of microfouling organisms, in my Portsmouth years, and Prof. Tony Clare for assistance with the barnacle literature. Finally, my thanks to Prof. Morakot Tantcharoen and Dr Kanyawim Kirtikara, Thailand, for their continued interest and support.

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Surface colonisation by marine organisms and its impact on antifouling research

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Abstract: Most contemporary antifouling coatings release biocides that are potentially polluting. Non-polluting antifouling requires either the use of biocides that degrade rapidly or a nontoxic approach. One of the most promising methods of achieving nontoxic antifouling is by interfering with the adhesion of fouling organisms. The present generation of coatings that operate in this way – so-called fouling-release coatings – have focussed attention on adhesion of adult forms of fouling organisms because the coatings foul. The targets of any preventive technology, however, are the colonising stages. Barnacles, as major fouling species, are the focus of this chapter. The main nontoxic antifouling approaches are introduced, highlighting the need for an understanding of cyprid adhesion and surface properties that discourage settlement. The current status of knowledge in these areas is then reviewed and prospects for future research are discussed.

Key words: barnacle, adhesion, cyprid cement, temporary adhesive, cyprid settlement, fouling-release, antifouling.

3.1 Introduction

Marine biofouling is an age-old problem (Callow 1990) and the colonisation stages of fouling organisms have traditionally been a target for control (e.g., Houghton 1970). The state-of-the-art for antifouling¹ technology still relies on this strategy through the use of biocides to deter or kill colonising forms (see Anderson *et al.* 2003; Yebra *et al.* 2004; Chambers *et al.* 2006; Almeida *et al.* 2007 for recent reviews of antifouling technology). The well publicised effects of tributyltin on non-target organisms (e.g., Fent 1996; Matthiessen and Gibbs 1998) and subsequent legislation (Champ 2003) have focussed attention on other antifouling biocides, in particular regarding their environmental fate and effects (e.g., Thomas *et al.* 2001; Konstantinou and Albanis 2004; Bellas 2006; Cresswell *et al.* 2006; Gatidou *et al.* 2007; Jones and Bolam 2007; Sapozhnikova *et al.* 2007; Srinivasan and Swain 2007). Nevertheless, biocides are likely to remain the mainstay of antifouling

¹The term antifouling is used here for any strategy that mitigates colonisation of an artificial surface.

strategies for the foreseeable future (Anderson *et al.* 2003). If a nontoxic antifouling coating that was comparable in efficacy, though not necessarily cost, to biocidal coatings were to be developed, however, it would undoubtedly find a market. To this end, a number of strategies have been suggested, which have proved more or less successful. Research to underpin the development of these various nontoxic approaches is the focus of major international research programmes such as EC AMBIO (see Chapter 24 Callow and Callow) and the US Office of Naval Research Biofouling Control Coatings Research and Development Program.

The aim of this review is to first briefly introduce some of the nontoxic approaches that are being pursued and then to attempt to identify critical areas for research on surface colonisation by fouling organisms, focussing by way of example on barnacle adhesion. Progress with respect to the application of nanotechnology to non-toxic antifouling in the EC AMBIO programme is described by Callow and Callow (Chapter 24).

3.2 Nontoxic marine antifouling approaches

3.2.1 Fouling-release coatings (FR)

So-called FR coatings comprise a nontoxic solution to control fouling that has been commercialised but has yet to find wide application. These coatings operate on the principle of interfering with the normal adhesion of fouling organisms so that they self-clean when exposed to hydrodynamic forces. For the most part, silicone elastomers are used, which are relatively expensive and are easily damaged (Yebra *et al.* 2004). Although FR coatings have inherent antifouling properties, perhaps by affecting the behaviour of larvae or through physical entrapment (Asfar *et al.* 2003), they do foul (Swain and Schultz 1996). Diatom slime is a particular problem in this regard (Holland *et al.* 2004). Current research is directed at improving the antifouling and release performance, and longevity of FR coatings, through, for example, modification of surface architecture (Carman *et al.* 2006; Schumacher *et al.* 2007a; 2007b), and increasing their toughness, through, e.g., incorporation of nanofillers such as nanotubes (see Callow and Callow Chapter 24; Beigbeder *et al.* 2008). Because FR coatings foul, there has been a focus on understanding the nature of the adhesives and release mechanics of adult fouling organisms (e.g., Kavanagh *et al.* 2005). The corresponding settlement stages have received little attention from a FR perspective (cf. Gray *et al.* 2002), but if antifouling performance of these coatings is to be improved, settlement comes sharply into focus. Improving the antifouling performance of FR coatings will require knowledge of the surface and perhaps bulk properties of FR coatings that are important to deterring attachment and/or interfering with the adhesion of colonisers. Moreover,

understanding the nature of the adhesives and mechanisms of adhesion of these early life-cycle stages would be beneficial to this effort.

3.2.2 Enzymes as antifoulants

Another strategy that holds promise for its ability to interfere with adhesion is the use of enzymes – direct enzymatic antifouling in the terminology of Olsen *et al.* (2007). This is not a new concept (see, e.g., Houghton 1970; Christie 1973). Serine proteases that are capable of degrading the proteinaceous adhesives that characterise many fouling organisms (e.g., Callow and Callow 2002; Wiegemann 2005a; Khandeparker and Anil 2007; Kamino 2008) have received most attention recently (Pettitt *et al.* 2004; Olsen *et al.* 2007; Aldred *et al.* 2008; Leroy *et al.* 2008), though other enzymes have been studied (e.g., Rittschof *et al.* 1991; Dobretsov *et al.* 2007). A combined nanotube-enzyme approach to improve the antifouling performance of a poly(methyl methacrylate) coating (Asuri *et al.* 2006) has proved effective against model proteins, but to the author's knowledge has yet to be investigated for marine antifouling.

The use of enzymes was dismissed as being only of academic interest by Christie and Dalley (1987) on the basis of cost, but this should be less of an issue since the advent of commercial-scale production of bacterial enzymes for biological detergents. Attaining broad-spectrum activity against the myriad of adhesives used by marine organisms (perhaps requiring a mix of enzymes as is common in detergents) and engineering of coatings to retain enzyme activity in the marine environment (see Callow and Callow Chapter 24 for progress in AMBIO; Huijs *et al.* 2004) are the main issues that need to be addressed before the concept can be tested rigorously. Here again, knowledge of the adhesive systems of colonising forms of fouling organisms, as these are the likely target (Olsen *et al.* 2007), to complement the greater understanding of adult adhesives, is needed. As reversible adhesion is a common strategy in early colonisation of surfaces by fouling organisms (Crisp 1984), this process should be particularly susceptible to enzymic degradation as the temporary or 'first-kiss' (Wetherbee *et al.* 1998) adhesives employed do not 'cure' and presumably would not become refractory to enzyme action as do the permanent adhesives employed later in development (e.g., Pettitt *et al.* 2004).

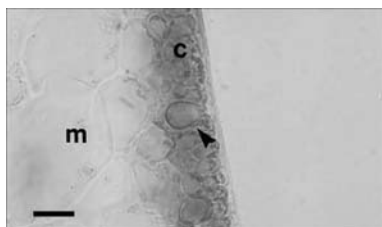
3.2.3 Natural product antifoulants (NPAs)

NPAs have attracted considerable attention over the last 30 years as a possible nontoxic, or at least environmentally benign solution to fouling control (e.g., Clare 1996; Rittschof 2001; Hellio *et al.* Chapter 22). The rationale that natural compounds that defend organisms against epibiosis can be exploited

in antifouling appears sound but has yet to lead to the development of a commercial product. The best characterised NPAs are the halogenated furanones (e.g., Dworjanyn *et al.* 2006; de Nys *et al.* 2006), which afford protection to the red alga *Delisea pulchra* against a broad spectrum of potential epibionts. In laboratory assays, the furanones are able to inhibit settlement of ecologically relevant epibiont species at concentrations that are not overtly toxic (de Nys *et al.* 1995) and which are environmentally relevant (Dworjanyn *et al.* 2006; Nylund *et al.* 2007). Furanones are perhaps most significant for their ability to interfere with acylated homoserine lactone (AHL)-regulated quorum sensing systems of bacteria (see de Nys *et al.* 2006 for a review). Whilst this mechanism helps to explain the inhibitory action against potentially colonising bacteria – though *Delisea pulchra* is not sterile (Maximilien *et al.* 1998; de Nys *et al.* 2006) – it is far less clear how other colonising forms are inhibited. The lactone moiety is, however, a common feature in NPAs, and is presumably important to broad-spectrum activity (Targett and Stochaj 1994; Clare 1996; Rittschof 2001; de Nys *et al.* 2006).

Nylund *et al.* (2007) provide a useful cautionary note to NPA research, most of which still relies on whole extracts of tissues and cells (e.g., Hellio *et al.* 2005; Qi *et al.* 2008). By comparing laboratory assays of non-destructive surface extracts containing non-polar compounds with whole-cell extracts, it was concluded that the latter are a poor predictor of ecological function. In this context, knowledge of the chemical ecology of putative natural antifoulants can help to identify those that hold most promise as chemical leads for antifouling applications. The targets for NPAs are surface colonisers, so the compounds should be expressed or secreted externally (see, e.g., Dworjanyn *et al.* 1999; Steinberg *et al.* 2001). Extracting whole organisms or tissues is arguably an unnecessary complication. The counter argument would be that chemicals that do not function as antifoulants *in vivo* may nevertheless be potent inhibitors of settlement. Too little is known, unfortunately, about the natural roles of most of the NPAs that have been identified through biological assays (Clare 1996; Rittschof 2000, 2001) to comment more definitively on this topic.

In most instances, the source of the NPA is not identified in initial biological screens. Furanones are an exception as they have clearly been shown to be secreted to the algal surface by gland cells in the cortex of the thallus (Fig. 3.1). In most other cases, however, microbial symbionts cannot be ruled out as the source (e.g., Holmström and Kjelleberg 1999; Steinberg *et al.* 2001; Liebezeit 2005). Evidence of a microbial origin makes exploitation of NPAs potentially more tractable, particularly if the organism grows and produces the compound of interest in culture (Clare 1996). Evidence of bacterial symbionts providing protection to the host against epibionts would place greater emphasis on the need to understand signalling in prokaryote-eukaryote interactions (Holmström and Kjelleberg 1994).



3.1 Light micrograph of a transverse section of *Delisea pulchra* showing medullary (m) and cortical cells (c), and a gland cell (arrowed) that secrete furanones to the surface. Scale bar is 10 μm . Reproduced with permission from Dworjanyn *et al.* (1999).

The potential for exploiting bacterial products (e.g., Burgess *et al.* 2003; Eguía and Trueba 2007) and even live bacteria (Gatenholm *et al.* 1995; Holmström *et al.* 2000; Yee *et al.* 2007), has been demonstrated using prototype coatings in field trials. The deterrent bacteria or 'living paint' approach is particularly intriguing for its potential to provide a long-lived, broad-spectrum solution to fouling control. By immobilising a species well known for its antifouling potential (e.g., Holmström *et al.* 1998) – *Pseudoaltermonas tunicata* – in κ -carrageenan beads, Yee *et al.* (2007) obtained promising short-term antifouling performance in a field trial, while highlighting the need for further development of the immobilising matrix to improve performance.

3.2.4 Impediments to realising nontoxic control of fouling

Under the EC Biocidal Products Directive (BPD; 98/8/EC) (see Callow and Callow Chapter 24), the NPA (1.2.1) and the direct enzymatic antifouling (1.2.3) approaches would be classed as biocidal and thus subject to regulatory approval. While there may be a market in other parts of the world for the time being (Olsen *et al.* 2007), trade with Europe, requiring entry into European ports, would require coatings to comply with the BPD. To date there are no NPAs or enzymes in the authorised list of biocides in Annex 1 of the BPD for future use as antifouling agents. Unless relevant product evaluation has been done for another purpose (more likely for enzymes than NPAs, though furanones are being considered for various applications; de Nys *et al.* 2006), registration is a protracted and costly process (Rittschof 2001), in the region of €7–9 million per product (Pereira 2006) and likely to be prohibitive.

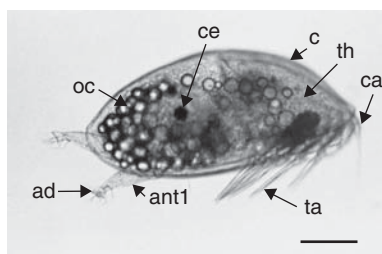
Another impediment to progress is the relative paucity of knowledge on surface colonisation, including how settlement stages of fouling organisms perceive surfaces, what physical and chemical characteristics of surfaces are

important to surface selection, how these cues (facilitatory and inhibitory) are processed to effect surface rejection or settlement and how adhesion is manifested. For example, while the identification of inhibitory cues may directly inspire new solutions to fouling control (Clare 1996), the characterisation of facilitatory cues may highlight ways to interfere with the normal process of colonisation, for example, through the development of antagonists.

Interference with adhesion is viewed by the authors as the most promising avenue to pursue for nontoxic antifouling, as this is likely to involve surface-active technologies that do not release potentially polluting agents to the water column. What follows is a brief review of recent progress in addressing adhesion for one of the best studied colonising forms – the cypris larva of barnacles. The reader who is interested in progress in the other aforementioned areas of marine natural products (including bacterial metabolites) and chemical cues (including those of microbial origin) and their perception, is directed to the following recent reviews: Holmström and Kjelleberg (2000); Rittschof (2001); Hadfield and Paul (2001); Steinberg *et al.* (2001); Tait (2003); Callow and Callow (2006); Krug (2006); Dahlström and Elwing (2006); Qian *et al.* (2007); Hellio *et al.* Chapter 22.

3.3 The barnacle model

Ever since Darwin published his series of monographs on fossil and extant barnacles (Darwin 1851; 1852; 1854; 1855), this crustacean sub-class (Cirripedia) has been a focus of attention for, e.g., evolutionary biologists (e.g., Raimondi 1992; Pérez-Losada *et al.* 2008), ecologists (e.g., Connell 1961; Appelbaum *et al.* 2002), and physiologists (Masonsharp and Bittar 1981; Simpfendorfer *et al.* 2006). Barnacles comprise over 1200 species and occupy habitats as varied as hydrothermal vents (Watanabe *et al.* 2004) and whale skin (Nogota and Matsumura 2006). They are arguably most notorious, however, for their ability to attach to immersed artificial structures, including ships' hulls where they are among the most prominent and troublesome of fouling organisms (Visscher 1927; Christie and Dalley 1987). Their relatively large size incurs a significant drag and thus economic penalty (Christie and Dalley 1987; Thomason *et al.* 1998; Townsin 2003; Schultz 2007). Moreover, the growth form of the hard calcareous shell plates (Bourget 1987) can damage coatings by cutting into them, which may lead to corrosion of the underlying metal (Haderlie 1984). The cosmopolitan distribution, high fecundity and capacity to colonise most hard surfaces predisposes certain shallow-water species, such as *Balanus amphitrite*, to fouling (Visscher 1927, 1928). Ultimately, however, this ability is attributable to the final larval stage in the life cycle, the cypris larva (Fig. 3.2). This 'pinnacle of sessile evolution' (Crisp 1984) is highly conserved in form and



3.2 Light micrograph of the cyprid of *Balanus amphitrite* showing the adhesive (antennular) disc (ad); 1st antennular segment (ant1); carapace (c); caudal appendage (ca); compound eye (ce); oil cell (oc); thoracic appendages (ta); and thorax (th). Scale bar is 100 μm .

function (Walker 1995) and is generally regarded as having the sole purpose of finding a suitable place to settle (Crisp 1984). As has already been mentioned, a greater understanding of settlement could highlight ways to interfere with the process by repellence and/or by presenting a surface that is difficult for the cyprid to adhere to.

Despite their importance, it is worth emphasising that barnacles are only part of the fouling problem and studies of settlement, and the adhesives of the colonising stages of other fouling species are also needed.

3.4 The nature of barnacle cyprid adhesives and mechanisms of adhesion

In stark contrast to work on adult barnacle cement (see, e.g., Walker 1981; 1987; Kamino and Shizuri 1998; Wiegemann 2005a; 2005b; Kamino 2006; 2008 for reviews), the adhesives employed by cyprids to explore and permanently attach to a substratum have received scant attention (see Walker 1981; Crisp 1984; Crisp *et al.* 1985; Walker 1987; Walker 1995; Aldred and Clare 2008a, 2008b for reviews). Interest in the efficacy and mechanism of action of fouling-release coatings has been a driver of recent research on adult cement. To re-iterate, prevention of fouling, however, requires that the cyprid is targeted (e.g., Visscher 1927; Clare 1995). One way to achieve this is to interfere with attachment of the cyprid, either as the cyprid attempts to explore substrata, or at the point of permanent attachment (syn. fixation).

3.4.1 The cyprid temporary adhesive

When a surface is encountered, the cyprid uses its paired antennules to 'walk' in a stilt-like fashion over the surface (Walker 1981; see supplement-

tary material to Phang *et al.* 2008 for a movie). This exploratory behaviour of cyprids has been described in some detail (e.g., Darwin 1854; Visscher 1928; Doochin 1951; Barnes 1955; Crisp 1976; Crisp 1984; Walters *et al.* 1999; Lagersson and Høeg 2002). Distinct phases of barnacle settlement – wide exploration, close exploration and inspection – were first described by Crisp (1976). Wide exploration is essentially straight-line walking and most detachment events take place during this phase (Lagersson and Høeg 2002). When a favourable surface is encountered, cyprid behaviour shifts to close exploration, which is characterised by frequent turns and tight changes in direction. The latter behaviour was termed inspection by Crisp (1976), who reasoned (Crisp 1984) that ‘the optimal strategy [for a larva] is first to move over broad areas but, on encountering a favourable stimulus, to make frequent turns in order to remain in the same general vicinity while exploring further’. In contrast, Hills *et al.* (1998), who used freshly-collected *S. balanoides* cyprids in laboratory assays, found that cyprids settled rapidly with little searching behaviour. A possible explanation for the apparent differences between the results obtained by Crisp (1976) and Hills *et al.* (1998) is that the wild cyprids used by each laboratory differed in their overall physiological age (*sensu* Visscher 1928; Miron *et al.* 2000; Thiyagarajan *et al.* 2002). Laboratory observations made with wild-caught *S. balanoides* cyprids suggest that the tendency of cyprids to explore surfaces decreases with increasing physiological age (Neal and Yule 1992).

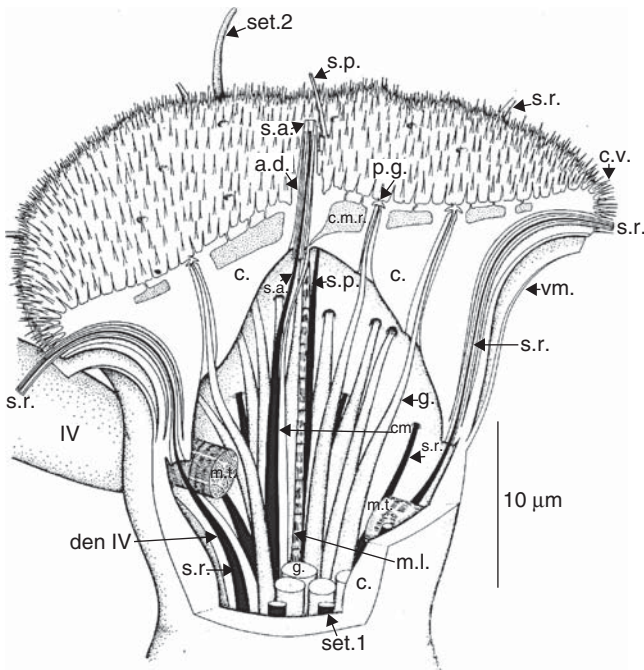
Neal and Yule (1992) found that *S. balanoides* cyprids that do engage in searching behaviour have an increased propensity to settle. However, Hills *et al.* (2000), who studied settlement behaviour of wild *S. balanoides* cyprids in the field, did not detect a link between searching and settlement, possibly because the observations, which were made over a 30-minute timeframe, were too short in duration. Instead, almost all the cyprids alighted from the surface after searching.

Crisp (1955) used rotating plates (see also Walton Smith 1946) and tubes (see also Wethey *et al.* 1988) to examine the effect of shear stress on cyprid exploration and attachment. The pipe flow experiments have subsequently been criticised (Barnes 1970; Mullineaux and Butman 1991), but Crisps’ studies did highlight the remarkable tenacity of cyprids and their ability to reversibly attach as they walk over a surface. Empirical measurements under tension, applied perpendicular to the surface, have recorded values in the range 0.06–0.30 MPa for *S. balanoides* cyprid tenacity to slate, a natural but complex surface (Yule and Crisp 1983; Yule and Walker 1984; Neal and Yule 1992). Tenacity to glass, a surface frequently used as a reference surface in evaluations for antifouling/fouling-release surfaces, was ~0.07–0.10 MPa for *S. balanoides* (Crisp *et al.* 1985; Yule and Walker 1987) and ~0.12 MPa for *Balanus amphitrite* (Maki *et al.* 1994), though it must be mentioned that different types of glass were used in these studies. Measures

of adhesion in shear are more typical for evaluating fouling-release surfaces (e.g., Swain and Schultz 1996). In the only study to have approximated this measure, Eckman *et al.* (1990) calculated that the mean force required to detach *B. amphitrite* cyprids (estimated from shear forces) from poly(methyl methacrylate) (PMMA) that had been coated with a crude extract of a settlement inducer – an adult glycoprotein (Rittschof *et al.* 1984) – was ~0.013 MPa. This value did not consider lift forces on a cyprid, but even when these are factored in, the vector sum of lift and drag forces gives a value of only ~0.019 MPa. This is an order of magnitude less than reported for the tenacity (measured under tension) of *S. balanoides* cyprids to adult-extract coated PMMA (Yule and Walker 1984) and *B. amphitrite* cyprids to borosilicate glass (Maki *et al.* 1994). Even accounting for differences in methodology (measures by Yule and colleagues also determined whether the cyprid was held by one or both antennules) and a greater elevation of the cyprid above the surface (Eckman *et al.* 1990), the maximal force arrived at is 0.031 MPa. Clearly species differences in the cyprid and adult extract may be important, but it is more likely that the lower force measured by Eckman *et al.* (1990) is due to the lesser resistance of the cyprid temporary adhesion to shear than tension (see Baier 1970).

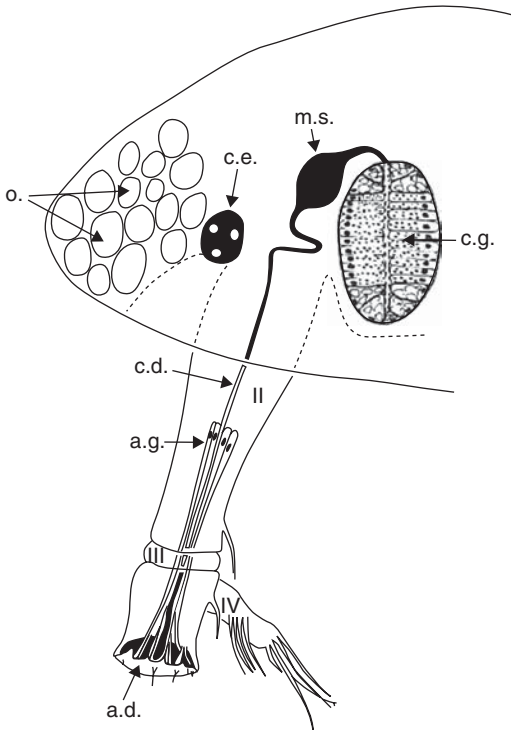
The cyprid attachment organs are the antennular discs, which are the third article of the paired antennules (Fig. 3.3). Nott (1969) showed, using scanning electron microscopy, that the surface of each disc is covered with cuticular villi. The role of these villi is not understood. They may serve to increase the surface area of the disc for adhesion (Nott 1969), as species that are subject to greater hydrodynamic forces tend to have higher densities of villi (Moyse *et al.* 1995). Adhesion may be facilitated by a proteinaceous secretion (Walker and Yule 1984) produced by unicellular glands in the 2nd antennular segment (Fig. 3.4) and released to the disc's surface via two concentric rings of pores (Nott and Foster 1969; Walker and Yule 1984). One function of the villi may be to increase the capacity of the disc to retain this secretion (Nott 1969). As cyprids exploring a surface also have to be capable of detaching, the secretion is referred to as a 'temporary adhesive' (e.g., Barnes 1970). Ideally, the disc should be able to resist much larger detachment energies while it is attached than are required to voluntarily detach the disc from a surface (see Gravish *et al.* 2007).

Depending on the surface that is being explored by the cyprid, temporary adhesive is left behind as 'footprints' which can be visualised with protein dye reagent (Walker and Yule 1984; Clare *et al.* 1994). The arrangement of the unicellular gland openings on the disc is reflected in the staining pattern of the adhesive, at least for *S. balanoides* (Walker 1987). Footprints are hard to detect on glass and polystyrene, but are observed in large numbers on nitrocellulose membrane (Matsumura *et al.* 1998a), perhaps faithfully recording cyprid steps (Fig. 3.5). The superior protein-binding characteris-

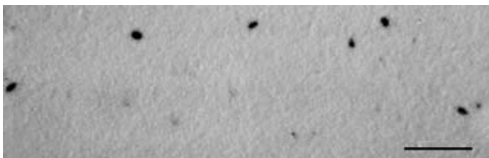


3.3 The attachment disc (3rd antennular segment) of *Semibalanus balanoides*. Internal structures are visible in cutaway section from the preaxial side. The internal sensory organs are shown in black. Axial dome (a.d.); cuticle (c.); cement gland duct (cm.); radial canals of cement gland duct (cm.r.); cuticular villi (c.v.); dendrites going to fourth segment (den. IV); fourth segment (IV); antennular glands and ducts (some severed by cutaway) (g.); longitudinal muscle (m.l.); transverse muscle (m.t.); pore of antennular gland (p.g.); axial sense organ (s.a.); post-axial sense organ (s.p.); radial sense organ (s.r.); pre-axial seta (set. 1); post-axial seta (set. 2); and velum (vm.). Reproduced from original artwork (see Fig. 2 of Nott and Foster 1969).

tics of nitrocellulose membrane (it is used to blot proteins) presumably account for the higher number of footprints. Adhesive may be retained on nitrocellulose because the adhesive forces between the membrane and adhesive are stronger than between the adhesive and the attachment disc or the cohesive forces in the adhesive. The same reasoning may account for the greater quantity of footprint adhesive that is deposited on R-NH₂-compared CH₃-terminated glass (Phang *et al.* 2008). Nevertheless, footprints could be deposited in much higher numbers than are detected on unmodified glass and plastic if they are removed during processing for staining. Whether or not footprints are deposited on natural settlement surfaces, which unless recently immersed will be biofilmed (Loeb and Neihof 1975; Callow and Callow 2006), is unknown.



3.4 Diagrammatic sagittal section to show the arrangement of the cyprid cement apparatus. Adhesive disc (a.d.); antennular glands (a.g.); cement duct (c.d.); compound eye (c.e.); cement gland (c.g.); muscular sac (m.s.); oil droplets (o.); and segments of the antennule (II, III, IV). Reproduced with permission from Walker (1981).



3.5 Footprints of *Balanus amphitrite* temporary adhesive deposited on nitrocellulose membrane and immunostained (see Dreanno *et al.* 2006a for methodology). Scale bar is 200 μm .

Aldred and Clare (2008a, b) likened the surface morphology of the antennular disc with contact splitting (Autumn *et al.* 2002) observed in other organisms with 'hairy' adhesive appendages such as gecko toe pads (see, e.g., Autumn 2006) and fly pulvilli (e.g., Gorb *et al.* 2001). The morphological

similarity has been noted before (Yule and Crisp 1983; Crisp 1984; Walker *et al.* 1985). Crisp (1984) also remarked that the mechanism of adhesion likely involves molecular forces between the cuticular villi and the substratum or viscous forces of a secretion. The gecko adhesive system does not employ glandular secretions, while insect systems may (Walker *et al.* 1985; Langer *et al.* 2004). The lipid secretion of insects is thought to enhance 'wet' adhesion through capillary and viscous forces (Gorb *et al.* 2002). Neither system, of course, operates underwater but the effect of water cannot be ignored. In the 'dry' adhesive system of the gecko, humidity increases adhesion. There is some debate about whether capillary forces (Huber *et al.* 2005; Sun *et al.* 2005) or van der Waals forces (Autumn 2006; Gravish *et al.* 2007) dominate. Nevertheless, adsorbed water is thought to increase contact between the gecko nanoscale setae and the substratum (Huber *et al.* 2005).

Underwater, there is presumably no need for a gap-filling secretion unless the surface is periodically emersed and topographical features trap air. Under this scenario, which of course can apply to the intertidal that many barnacle species habit, the temporary adhesive may have an important function in increasing the contact fraction of the cuticular villi. The villi themselves may increase contact area, depending on how flexible they are. Another possible function of temporary adhesive is to displace water from the surface that could otherwise dampen adhesive interactions between the cuticular villi and the surface (see Smith and Callow 2006; Varenberg and Gorb 2007). The ability of a biological adhesive to improve the adhesive performance of an engineered dry adhesive system was admirably demonstrated by Lee *et al.* (2007), in what may be an analogous mechanism to cyprid temporary adhesion. A nanofabricated array of pillars cast from polydimethylsiloxane (PDMS) was shown to have greater adhesion strength, both in air and underwater, when coated with a thin layer of synthetic, mussel adhesive-inspired polymer – poly(dopamine methacrylamide-comethoxyethyl acetate). Moreover, the system maintained its adhesive performance for more than a thousand contacts with the substratum, as would be required of a temporary adhesive.

Another common feature of adhesive mechanisms involved in locomotion, which may be shared by cyprids, is directionality of the adhesive structures. There is a tendency for structures to attach by being pulled proximally, while detachment involves a distal push (e.g., Autumn *et al.* 2000; Federle *et al.* 2002; Clemente and Federle 2008). Other than noting the flexible nature of the cyprid attachment disc and that it detaches by peeling (Yule and Walker 1987), no comparable observations have been made for cyprid attachment and detachment and there is no obvious directionality in the arrangement of cuticular villi. This may be advantageous when forces are unpredictable as they are in the turbulent benthic environment that

cyprids attach in (e.g., Koehl 2007). Nevertheless, a common observation made in the earlier studies of cyprid searching behaviour is that they engage in tugging on their antennules (Crisp and Meadows 1963; Barnes 1970). Could this be a mechanism to maximise the adhesion forces?

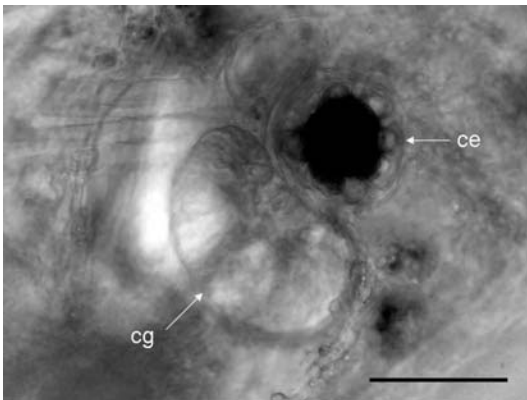
Two pieces of evidence support the contention that the temporary 'adhesive' is indeed an adhesive. First is the result of direct measurement of the interaction forces between an atomic force microscope (AFM) cantilever tip and the footprint material. Assuming a porosity of 50% from AFM images of a fibrillar deposit, Phang *et al.* (2008) calculated a theoretical tenacity of 0.026 MPa. As this was approximately a third, or less, of the empirical value determined for *S. balanoides* cyprids attached to glass (see above), the cuticular villi presumably also contribute to adhesion in a hybrid wet/dry adhesive mechanism. Secondly, Alcalase[®] (a commercial preparation of the serine endopeptidase subtilisin) prevented cyprid searching behaviour. The hypothesis that the mechanism of action is to degrade the temporary adhesive was supported by direct AFM observations of footprints when exposed to enzyme (Aldred *et al.* 2008; see Section 3.4.3).

Future progress on elucidating the mechanism of temporary adhesion would undoubtedly benefit from a full characterisation of the footprint material, not least because protein adhesion is strongly dependent on protein structure (Sagvolden 1999), as well as precise measurements of cyprid temporary adhesion in shear. A partial characterisation has been arrived at indirectly from clues gleaned from settlement assays. After demonstrating the existence of footprints and suggesting an origin from modified hypodermal cells, Walker and Yule (1984) speculated that the protein acts as a chemical stimulus to cyprid gregarious settlement. Confirmatory evidence that cyprid footprint protein is a settlement pheromone was subsequently obtained for both *Semibalanus balanoides* (Yule and Walker 1984) and *Balanus amphitrite* (Clare *et al.* 1994). As the adult glycoprotein – the settlement-inducing protein complex (SIPC) – (syn. arthropodin) that induces gregarious settlement (Matsumura *et al.* 1998b; Clare and Matsumura 2000) has long been thought to be of cuticular origin (Knight-Jones 1953), presumably synthesised by hypodermal cells, it seemed reasonable to hypothesise a link between SIPC and temporary adhesive. Evidence of such a link was obtained using antibodies to SIPC, which were found to stain footprint protein that had been deposited onto nitrocellulose membrane (Fig. 3.5) (Matsumura *et al.* 1998a; Dreanno *et al.* 2006a). Evidence that the SIPC is a cuticular, α_2 -macroglobulin-like protein was obtained subsequently (Dreanno *et al.* 2006b; Dreanno *et al.* 2006c). It was concluded that footprint protein contains SIPC, or at least a major portion of the molecule (Dreanno *et al.* 2006a). Species differences in the SIPC (e.g., Kato-Yoshinaga *et al.* 2000; Dreanno *et al.* 2007) are presumably reflected in the nature of the temporary adhesive, which in turn may contribute to species

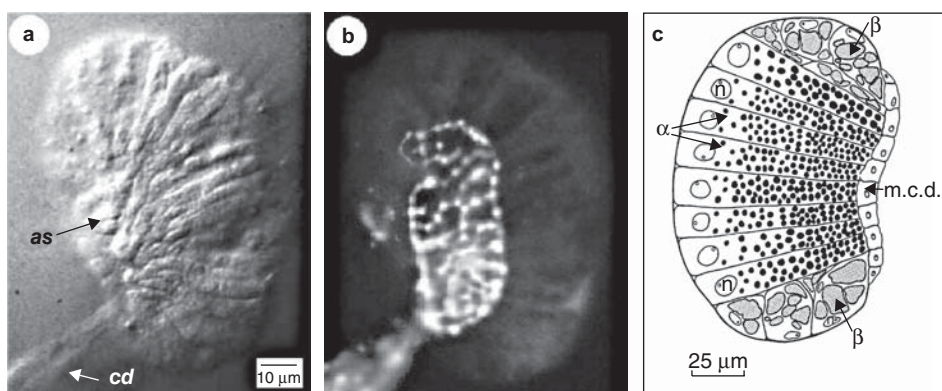
'preferences' with regard to surface wettability (see, e.g., O'Connor and Richardson 1994; Dahlström *et al.* 2004; section 3.5).

3.4.2 The cyprid permanent cement

Once a cyprid has located a suitable place to settle, it secretes a cement to anchor itself permanently to the substratum (Yule and Walker 1987; Walker 1981; Walker 1987). The cement is produced by a pair of cement glands located behind the compound eyes (Fig. 3.6). Walker (1971) described two types of secretory cell in the glands – α and β cells. In *S. balanoides*, the α cells contained numerous round granules of 3–4 μm diameter, which gave positive staining reactions for proteins, phenols and the enzyme polyphenol oxidase. The β cells, which were located at the dorsal and ventral margins of the gland, bore large but irregular-sized, membrane-bound secretory granules. Their content was less electron dense than that of α cell granules and stained for protein. Both cell types were isolated from cement glands of *Megabalanus rosa* by trypsin digestion (Okano *et al.* 1998). Approximately 70% of the isolated cells were comparatively large α cells of $22.4 \pm 1.8 \mu\text{m}$ (average length of the long and short axis), with granules of $1.8 \pm 0.8 \mu\text{m}$ diameter. Corresponding dimensions for the β cells were $16.4 \pm 1.4 \mu\text{m}$ and $3.1 \pm 0.8 \mu\text{m}$ respectively. Pronounced swelling of α - but not the β -cell granules, occurred in low osmolarity water. The magnitude of swelling was inversely correlated with sodium chloride concentration, which suggested that the granule content was similar to a hydrophilic polymer gel. The granule matrix was also sticky but did not harden over a 24-hour period unlike the extruded cement. Presumably, therefore, the content of the β



3.6 Light micrograph of a cement gland (cg) of *Balanus amphitrite* adjacent to a compound eye (ce). Scale bar is 20 μm .



3.7 (A) Light micrograph (Nomarski optics) showing an apical view of a cement gland isolated from *Megabalanus rosa* and treated by the glyoxylic staining method for catecholamines. Apical surface of cement gland (as); cement duct (cd). (B) The same gland viewed by fluorescence microscopy showing catecholaminergic innervation associated with many varicosities. (C) Diagram of a median transverse section through a cement gland of *Semibalanus balanoides* showing the positions of the two cell types (α and β) and also the cells of the median collecting duct (m.c.d.). Nucleus (n.). A and B from Okano *et al.* (1996) reproduced with permission of the Company of Biologists. C reproduced with permission from Walker (1971).

cells is important to the polymerisation process, consistent with the two-component system proposed by Walker (1981).

Granules are released to the median collecting duct of the cement gland (Fig. 3.7) by exocytosis (Okano *et al.* 1996). This process is under catecholaminergic innervation which may modulate an influx of calcium ions through voltage-gated calcium channels (Okano *et al.* 1998). Pronounced changes in granule morphology, including swelling, were noted in the cement cells of *Balanus improvisus* following catecholaminergic stimulation (Ödling *et al.* 2006). It is not known what controls the explosive release of cement, via the single cement duct, through pores on the antennular attachment disc's surface (Walker 1981, 1987). Based on histological observations, Walley (1969) proposed that the cement is expelled by muscular sacs (see Fig. 3.4), possibly via a valve at the distal end of the cement duct (Nott and Foster 1969). Further work is clearly needed to elucidate the mechanism of cement release and thus, possibly the means to prevent cyprid permanent attachment to surfaces.

Once expelled to the surface, the cement forms a domed mass that embeds the third and fourth antennular segments (Walker 1971, 1981). The diameter of the mass was $\sim 100\ \mu\text{m}$ for *B. amphitrite* (Phang *et al.* 2006) and $\sim 150\ \mu\text{m}$ for *S. balanoides* (Walker 1987). Histological stains and transmis-

sion electron micrographs revealed distinct zones, which may be related to polymerisation of the cement (Walker 1971). As cement presumably does not polymerise in the cement ducts, contact with seawater is likely required for molecular cross linking. Based on positive staining reactions for proteins, phenols and polyphenyloxidase (Knight-Jones and Crisp 1953; Saroyan *et al.* 1970; Walker 1971; cf. Hillman and Nace 1970), cement polymerisation by quinone tanning has been proposed, consistent with earlier suggestions of this mechanism (Harris 1946; Knight-Jones 1953; Knight-Jones and Crisp 1953). Quinone tanning was suggested for adult cement (e.g., Lindner and Dooley 1973) but this hypothesis was dismissed by Naldrett (1993) on the basis that quinones were not identified in the cement. Instead, a mix of hydrophobic interactions and sulphur cross links were proposed. More recent studies have confirmed the importance of hydrophobic residues and their possible involvement in intermolecular interactions in the assembly of the bulk cement. In contrast, intermolecular cross-linking is thought less likely to contribute to cement curing than noncovalent molecular interactions (Wiegemann *et al.* 2006; Kamino 2008). In comparing barnacle cement with the mussel byssal plaque, Kamino (2008) conjectured that rapid curing, as occurs for the byssus, may not be required for adult barnacle cement as the already affixed basis may provide sufficient adhesion to resist hydrodynamic dislodgement. Extending this analogy, rapid curing is more likely to be required of the cyprid cement than the adult cement, as it is relatively exposed and has to resist dislodgement of the cyprid during metamorphosis; a period when the barnacle becomes less streamlined in shape. Interestingly, the observed mobility of balanomorph barnacles with a membranous basis (Crisp 1960) and of lepadomorphs (Kugele and Yule 1993) could be construed as evidence of the absence or limited curing of adult cement beneath the basis (see Crisp 1973 for mention of barnacle viscous adhesion). Confirmatory evidence would require comparable experimentation on barnacles with a calcified basis, where potential basal membrane tearing is not an issue. Detailed observations of detachment of membranous-based barnacles using video recordings should help to resolve whether the cement is viscous or solid (see Kavanagh *et al.* 2005).

Recent studies of expressed cyprid cement using AFM in force mode (Phang *et al.* 2006) have obtained evidence supporting a curing process. Significantly, the frequency of successful force curves acquired through contact of the cantilever tip and the cement, decreased over time. Moreover, shorter pull-off lengths were measured over time indicative of proteins that have become constrained within a network. If it is accepted that this mechanical property equates with 'curing' of the outer layer of cement as depicted by Walker (1971), then the process occurs within ~2 hours. This figure is in close agreement with the estimate arrived at by Yule and Walker (1987) based on measures of tenacity of cyprid adhesion to slate over time

Table 3.1 Tenacity of barnacle adhesives, used at different periods of the life cycle, to glass and slate ^aData from Walker (1987); ^bdata from Yule and Walker (1987)

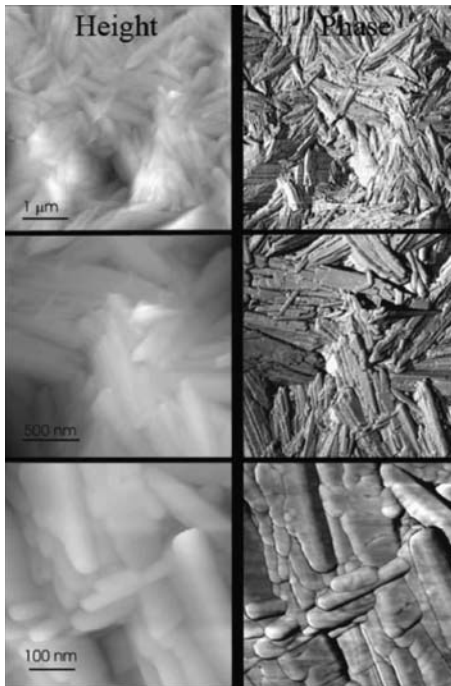
	Adhesive/surface	Tenacity (MPa)
Exploring cyprid ^a	Temporary adhesive/glass	0.098
Settled cyprid ^a	Permanent cement/glass	1.25
Adult (7 mo old) ^a	Adult cement/glass	0.23
Exploring cyprid ^b	Temporary adhesive/slate	0.015–0.03
Settled cyprid ^b	Permanent cement/slate	0.097
Adult (<19 mo old) ^b	Adult cement/slate	0.093

(see their Fig. 3.1). However it is at odds with the findings of Pettit *et al.* (2004) and Aldred *et al.* (2008) who estimated a curing time in excess of 10 h. The difference between studies is likely to relate to the extent of curing. Initial curing of the outer layer of cement suggests catalysis by a component(s) of seawater (Walker 1971).

The tenacity of permanently attached cyprids of *S. balanoides* to glass was much higher than the comparable values for adults and for cyprid temporary adhesion. In contrast, little difference was seen between adults and permanently attached cyprids removed from slate (Table 3.1). Nevertheless, the pronounced differences for glass suggest that the cements differ in composition in line with recent molecular evidence of a lack of expression of adult cement protein genes in the cyprid (Kamino 2006). More recently, Aldred *et al.* (2008) presented AFM images of ‘cured’ cyprid cement (Fig. 3.8) showing a crystalline structure quite different to previously published images of adult cement (Berglin and Gatenholm 2003; Sun *et al.* 2004). The opposing view has also been expressed (see Walker 1981).

3.4.3 Effects of proteolytic enzymes on cyprid adhesives

Both the temporary adhesive and the permanent cement of the cyprid are susceptible to degradation by proteolytic enzymes (Pettitt *et al.* 2004; Aldred *et al.* 2008). Exposure to commercial serine proteases in solution prevented cyprid settlement, with minimum inhibitory concentrations for Alcalase, Savinase and Esperase of 1.1 µg ml⁻¹, 0.8 µg ml⁻¹ and 4.0 µg ml⁻¹ respectively (Pettitt *et al.* 2004). A failure to detect cyprid footprints when cyprids were exposed to active solutions of these enzymes and the presence of footprints in heat-inactivated enzyme solutions, suggested that the mode of action of the enzymes was either degradation of the cyprid temporary adhesive or inhibition of its secretion. The latter hypothesis was not supported by the results of pre-exposing cyprids to enzymes. Direct evidence of proteolytic



3.8 AFM contact mode images of cured (3-day-old) and hydrated *Balanus amphitrite* cyprid permanent cement. Reproduced with permission from Aldred *et al.* (2006).

digestion of footprint adhesive was obtained by AFM imaging (Aldred *et al.* 2008). When exposed to a 1:400 dilution of Alcalase solution (equivalent to $100 \mu\text{g ml}^{-1}$ of pure Subtilisin), footprints deposited on glass were completely removed within 20–25 minutes. Interestingly, overall behaviour of cyprids, as measured by video tracking, was not affected by the same concentration of Alcalase. Thus, details of the mechanism of settlement inhibition have yet to be resolved.

Exposing permanently attached cyprids to Alcalase, at set intervals post settlement, provided further evidence that the cyprid cement undergoes ‘curing’. The metamorphosing cyprids became resistant to detachment by the enzyme after about 11 hours. It is likely that detachment corresponded to proteolysis by the enzyme, as Aldred *et al.* (2008) presented visual evidence of this process for recently secreted cement (~1 hour old). Two-day-old, cured cement was refractory to Alcalase.

The results obtained with Alcalase are promising. If nontoxic antifouling by means of proteolysis of biological adhesives is to be realised, however, an effective means of presenting active enzyme at the surface of a coating

is required. Moreover, the enzyme needs to retain activity to afford long-term antifouling protection. Recent progress in this area has been made in the EC AMBIO project (see Callow and Callow Chapter 24).

3.5 Effects of surface properties on cyprid settlement

A detailed review of the surface physico-chemical factors that modulate barnacle settlement is beyond the scope of this review. Rather, the effectiveness of modulating surface wettability, topography and colour will be considered here. The first two are of course interrelated; altering texture alters wettability (see e.g., Genzer and Efimenko 2006), as well as bulk properties (Assender *et al.* 2002), so any conclusions are undoubtedly premature and warrant further investigation. Colour has long been known to affect barnacle settlement, and this literature will also be reviewed briefly.

3.5.1 Wettability

Species differences in settlement with respect to surface wettability have already been mentioned. For example, *Balanus improvisus* settles more readily on relatively hydrophobic surfaces (O'Connor and Richardson 1994; Dahlström *et al.* 2004). *B. amphitrite*, on the other hand, settles at a higher rate on hydrophilic surfaces (Rittschof and Costlow 1989; O'Connor and Richardson 1994; Roberts *et al.* 1991). A possible criticism of these short-term experiments is that different surface chemistries (e.g., glass modified by different silanising agents) and in some cases different materials (glass versus polystyrene), which would also differ with respect to, e.g., modulus and glass transition temperature, were used to vary wettability. It is thus not possible to assign effects to any one variable. The importance of controlling for wettability alone has been demonstrated in studies of its effect on adhesive spreading (Callow *et al.* 2005; Aldred *et al.* 2006). Effects of wettability on macrofouling seen in the laboratory and in short-term field assays are likely to be masked in longer term exposures (Becker *et al.* 1997; Holm *et al.* 1997; Faimali *et al.* 2004), probably reflecting a convergence of surface wettability as biofilm develops (see Holm *et al.* 1997 for a discussion).

3.5.2 Topography

The importance of surface topography² to larval settlement is well known. In general, invertebrate larvae have a predilection for rough as opposed to

²At the scale of the larva, surface topography has been classified as *texture* if the scale is below that of the larva and *contour* if the scale is above that of the larva (e.g., Le Tourneux & Bourget, 1988).

smooth surfaces (Crisp, 1974). Conveniently, most work has addressed barnacle settlement, but the literature does not reveal a consistent picture with respect to what scale of topography is important. For *B. crenatus*, texture seems more important than contour (Hudon *et al.*, 1983). Likewise, Hills and Thomason (1998) found that *S. balanoides* cyprids prefer to settle on a surface texture in the range 0–0.5 mm, i.e. below the 1 mm length of the cyprid. Wetthey (1986), on the other hand, concluded that surface contour was most important to *S. balanoides* settlement. Intraspecific differences have, however, been reported for *S. balanoides*. In the Gulf of St Lawrence, Chabot and Bourget (1988) found that cyprids of this species prefer to settle in natural crevices, consistent with other reports. On the Atlantic coast, however, cyprids preferred to settle on horizontal surfaces rather than in crevices. Unless this difference in behaviour is specific to the region, it may go some way to explaining the conflicting results. The effect of local hydrodynamics might also have contributed to differences observed (see, e.g., Abelson and Denny 1997). Yet, Crisp and Barnes (1954) remarked that cyprids showed the same settlement tendencies with respect to topography whether observed under static or field conditions.

If it is accepted that there are scales of topography that are attractive to cyprids, then an obvious question to ask is: are there scales of topography that are also inhibitory to settlement? This question has been examined for *B. improvisus*, which bucks the aforementioned trend by preferring to settle on smooth compared to rough surfaces. Laboratory and field studies with moulded surfaces (riblets and ridges) revealed that a roughness height within the range 30–45 μm , an average roughness of 5–10 μm and a roughness width of 150–200 μm were strongly inhibitory to cyprid settlement (Berntsson *et al.*, 2000). Moreover, evidence of behavioural rejection of the surfaces was obtained (Berntsson *et al.*, 2004).

There has recently been considerable interest in how some natural (biobiont) surfaces inhibit epibiosis (see Scardino *et al.* 2008). Attachment point theory basically states that the fewer points of attachment the lower the settlement and vice versa. Thus if the relevant surface fits tightly into a depression, it will have more potential attachment points than a larger surface that straddles the depression (see Fig. 2 of Scardino *et al.* 2006). With the notable exceptions mentioned above, the ability of the whole cyprid to fit into a depression would appear to be important to surface 'attractiveness'. When considering deterrence, however, it is not clear what the relevant surface is – is it the whole cyprid, the antennules or the micro/nanotextured surface of the attachment discs or multiple scales? For *B. amphitrite* at least, the dimensions of the attachment disc, which measures $\sim 25\text{--}30 \times 15 \mu\text{m}$ (Clare and Nott 1994), would seem to be important. Assays of PDMS with topographical features of 20 μm width and spacing inhibited settlement compared to smooth PDMS (Schumacher *et al.* 2007a). Moreover,

a trend of lower settlement with increasing aspect ratio (feature height : feature width) suggests that perceived stability of the elastomeric surface may be important to settlement inhibition. As previously mentioned, other surface properties that change in concert with topographical changes, e.g. wettability, complicate the interpretation.

Of course, there is a practical challenge for an antifouling strategy based on manipulating topographic features, namely, what may be inhibitory for one species may be stimulatory for another (e.g., Berntsson *et al.* 2000). It will thus be difficult if not impossible to develop an environmentally benign coating that is universally antifouling (Genzer and Efimenko 2006). As can be seen from the above discussion, this point even applies to closely related species. A predicted consequence of using a single topographic feature dimension is that it will foul and, unless the incumbents are chemically defended, become overlaid by organisms, including those that the feature was originally effective against. For example, as diatoms adhere tenaciously to PDMS (Terlizzi *et al.* 2000), microscale features in this material would presumably fill and be overwritten, unless a feature deterrent to diatoms were also present. A hierarchy of features, directed at the different, relevant scales of fouling, may overcome this outcome (Schumacher *et al.* 2007a).

The above discussion has emphasised PDMS. Other materials are also being actively researched for their fouling-release capabilities (see e.g. Callow and Callow Chapter 24).

3.5.3 Colour

In view of recent interest in the effect of coating colour on biofouling (Swain *et al.* 2006; Finlay *et al.* 2008), it is worth giving brief mention to the fact that barnacle settlement has long been known to be affected by light (Visscher 1927; 1928; Visscher and Luce 1928). As noted for a number of invertebrate species, the early literature states consistently that barnacles prefer dark backgrounds (Visscher 1927; Pomerat and Reiner 1942; Gregg 1945; Walton Smith 1948; Wisely 1959). These observations accorded with existing knowledge that fouling tended to be greater on the shaded areas of ships' bottoms (Visscher 1928). The mechanism behind this surface selection by barnacles appears to be based on the positive response of cypris larvae to 'general light flux or intensity of diffuse light' (Walton Smith 1948). Negative phototaxis of cyprids observed in the laboratory (e.g., Visscher 1928) could not explain the results of short-term field experiments performed by Walton Smith (1948) and instead an optimum light intensity for settlement was claimed. Cyprids of *Balanus amphitrite* are maximally sensitive to light of 530–545 nm wavelength (Visscher and Luce 1928), perhaps mediated by the median nauplius eye rather than the paired compound eyes

(Stratten and Ogden 1971). The spectral sensitivity of the cyprids of *Semibalanus balanoides* is thought to be broader, extending towards 600 nm. This conclusion was based on observations of cyprid searching behaviour and adhesion strength to Perspex panels which had been painted different colours on their reverse sides (Yule and Walker 1984). Cyprids explored the darker coloured panels (black and red) and avoided those of lighter colour (blue, yellow and white). The finding that colour also affected cyprid adhesion strength is surprising. Presumably it was the 'willingness' of the cyprid to detach that was affected rather than adhesion per se (Yule and Walker 1984).

The effect of coating colour is, of course, not only of academic interest. Although long-term coating performance may not be influenced by background colour (Edmondson and Ingram 1939), the interpretation of short-term field assays is potentially confounded by this factor if appropriate controls are not used (Swain *et al.* 2006; Finlay *et al.* 2008).

3.6 Future trends

Starting from the premise that interference with adhesion of barnacle cyprids is a promising approach to nontoxic antifouling, this chapter has reviewed the extent of our knowledge of the nature of cyprid adhesives and the mechanics of cyprid adhesion. In the context of developing a surface that prevents barnacle colonisation, it would clearly be advantageous to know the protein composition of cyprid adhesives. The primary obstacle to obtaining such information by traditional biochemical means is procuring enough starting material for characterisation. Significant progress has been made, in this regard, with adult cement because secondary cement can be sampled readily (Walker 1981; Kamino 2006). Progress for cyprid adhesives will likely depend on generating genome sequence (there is surely a case for sequencing a barnacle genome) sufficient for techniques like peptide mass fingerprinting. In situ micro-analytical chemical methods, such as Raman and infrared spectroscopies, also have potential to provide qualitative information on the molecular composition of freshly deposited adhesives and to probe the chemistry of cyprid cement during curing. Preliminary AFM studies have provided important information on the nanomechanical properties of cyprid adhesives (Phang *et al.* 2006; 2008). There is also scope to use AFM with functionalised cantilever tips to probe surface interactions and to couple this approach to direct measures of cyprid permanent adhesion to the same surfaces. In situ measures of cyprid temporary adhesion are complicated by the behavioural component to release from the surface. Accordingly, measures of cyprid behaviour will continue to be important to the research on nontoxic antifouling coatings, including progress with engineered topographies and immobilised enzymes.

3.7 Acknowledgements

ASC would like to thank Dr Jim Nott for the original artwork for Fig. 3.3. Work on barnacle adhesives in our laboratory has been supported by awards from the Natural Environment Research Council (GST/02/1436; NER/A/S/2001/00532), the US Office of Naval Research (N00014-05-1-0767 and N00014-08-1-1240) and the AMBIO project (NMP-CT-2005-011827) to ASC. AMBIO is a European Commission 6th Framework Programme. Views expressed in this publication reflect only the views of the authors and the Commission is not liable for any use that may be made of information contained therein.

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Algae as marine fouling organisms: adhesion damage and prevention

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Abstract: Human impacts on coastal waters, through eutrophication and overfishing, associated with the global change phenomenon are leading to a proliferation of both micro- and macroalgae. Algae can settle and develop on a wide range of surfaces including both natural and man-made structures. They are fast colonisers and can out-compete numerous species. The fixation of marine organisms on immersed man-made structures is responsible for major economic costs and the recent recrudescence of algae in the environment might increase the fouling phenomenon observed: the resistance penalty for ships is known to be increased by 11% in presence of a light microalgal slime to 34% in case of colonisation by macroalgae (Schultz, 2007). The paints used nowadays often contain biocides, are non selective against target organisms and are not environmentally friendly. The development of new antifouling paints requires a better understanding of both the mechanisms of fixation and colonisation of the fouler organisms. This chapter focuses on the fixation of algae (both micro and macro), the damages they cause and the method of prevention currently used.

Key words: adhesion, algae, *Ulva*, diatoms, fouling, settlement.

4.1 Introduction

Colonisation, which is one of the most fundamental and precarious processes in the life history of a benthic marine algae, regulates the distribution and abundance of organisms and therefore determines the community structure (Fletcher and Baier, 1984, Fletcher and Callow, 1992). Some algae species can allocate between 4–50% of their annual biomass production to their reproductive effort (Maggs and Callow, 2003). Algae are very successful in settling on a wide range of substrata such as oil and gas platforms, pilings, wrecks, mariculture facilities (nets and cages), ships, buoys and pontoons (Fletcher, 1981). The colonisation of immersed structures can cause significant damage such as the corrosion of steel surfaces, the decrease of the

ship's speed as the consequence of increased drag, technical problems in aquaculture systems, fish nets and power plant cooling system (Yebra *et al.*, 2004). Schultz (2007) calculated the effect of algal fouling on ships' hulls and demonstrated that the resistance penalty was respectively increased by 11%, 20% and 34% in presence of a light slime, heavy slime and macroalgae. Environmental damages such as the introduction of new species which could become invasive (Lewis *et al.*, 2003) and the increase of CO₂, NO_x and SO₂ production (linked to the increase of fuel consumption) have been reported too. Antifouling (AF) solutions are often based on toxic paints that prevent the settlement and/or development of organisms on man-made structures. However, these paints are often non selective and act against non-targeted species when not degraded sufficiently fast. The development of new AF formulations requires being more specific by acting only on the species involved in fouling. Knowing and understanding the colonisation process of the main algal species involved in man-made fouling and biodeterioration could allow for the development of products specifically acting against the fixation events. The study of adhesion process is important to develop new AF products; however, the induction of algal settlement (micro and macro) has an economic interest for aquaculture farms (algae and shellfish) and rehabilitation of marine habitats.

This chapter presents general characteristics about algae, and then focuses on the adhesion processes of selected micro- and macroalgae, the damage caused to man-made structures by algal fouling, and the prevention strategies developed.

4.2 General characteristics of algae

4.2.1 Definition

Algae are thallophytes (plants without roots, stems and leaves), generally aquatic, photosynthetic (with numerous exceptions), and oxygenic autotroph. This definition encompasses the cyanobacteria (Chloroxybacteria) which are photosynthetic prokaryotes closely related to the bacteria (Graham and Wilcox, 2000; Lee, 1999). The microalgae include all the algae that can be seen only with a microscope due to their sizes (from 2 µm for *Nannochloropsis* sp. (Ochrophytes) to 100 µm for *Ceratium* sp. (dinoflagellates)). However, colonies of *Volvox* sp. (Chlorophyta) can measure up to 2 mm (Graham and Wilcox, 2000). In contrast, macroalgae encompass all the species which can be observed with a naked eye. They range from a few centimetres, such as *Polysiphonia lanosa* (Rhodophyte) to a few metres, thus *Macrocystis pyrifera* (Ochrophyte) can measure up to 50 m (Graham and Wilcox, 2000).

4.2.2 Classification

The classification of algae is principally based on three main characteristics: pigment composition, nature of the storage products, and composition of the cell walls (Table 4.1). Owing to the topic of this book, we focus specifically on marine ecosystems, where 7 main phyla of algae have been described (Table 4.1). The evaluated number of algal species is still debated and is estimated from 36000 to 50000, and possibly over 10 million depending on the source (Graham and Wilcox, 2000).

4.2.3 Ecology and physiology

The algae are at the basis of the food web and are key components of marine ecosystems. Their productivity influences the density of herbivores and, indirectly, of all the organisms of the food web. The majority of microalgae are planktonic, in contrast to macroalgae which are mainly benthic (Graham and Wilcox, 2000). Only few species, such as *Sargassum muticum* and *S. fluitans*, are known to be able to live floating in the water. Competition for finding a free suitable substratum is highly important among macroalgae communities and could explain in part the greater diversity of macroalgae involved in biofouling in comparison with microalgae. Algae with fast growth rate will have a considerable advantage for the surface colonisation competition. Growth rates can vary greatly among different species, (e.g., $0.89 \text{ cell.day}^{-1}$ for *Oscillatoria* sp., $1 \text{ g}^{-1} \text{ dry weight hr}^{-1}$ for *Sargassum polyceratum* and $11 \text{ g}^{-1} \text{ dry weight hr}^{-1}$ for *Chaetomorpha linum*) and explain in part the speed of development of fouling organisms on a new substratum (Graham and Wilcox, 2000).

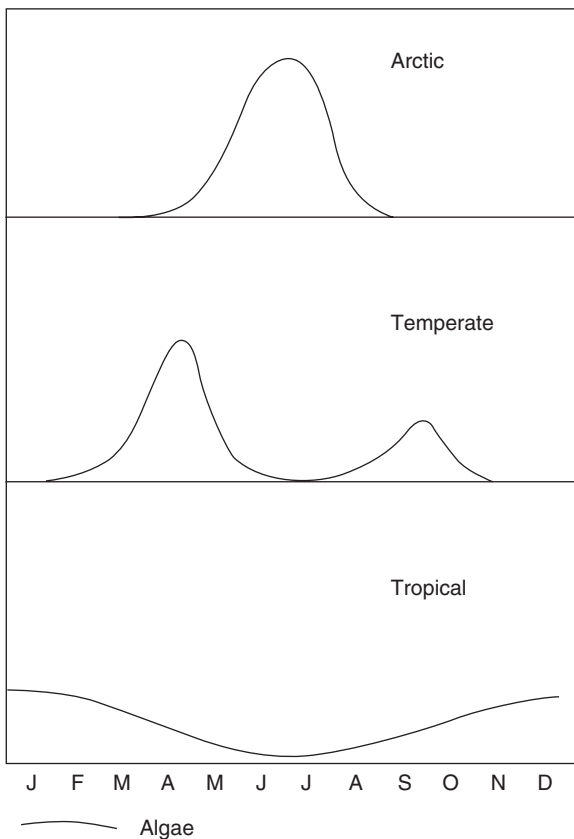
The main factors affecting algal distribution are depth (linked to the light intensity) and nutrient availability.

Algae, relying on photosynthetic metabolism, have an absolute need for light and as a consequence can only be found in the euphotic zone (limited by the depth where the light cannot penetrate, around 200 m deep depending on the turbidity) (except very few exceptions with heterotrophic or mixotrophic species) (Lee, 1999). Algae's sensibility to light varies concomitantly with their pigment composition. This characteristic, associated with the resistance to desiccation and salinity variations, is responsible for the zonation of algae observed on the littoral. Thus, usually Rhodophyceae are found deeper than the Phaeophyceae and the Chlorophyceae (Graham and Wilcox, 2000). For example, *Fucus serratus* requires light level of $150 \mu\text{Em}^{-2}\text{s}^{-1}$ for maximum growth rates, while *Chondrus crispus* will thrive at $94 \mu\text{Em}^{-2}\text{s}^{-1}$ (Lee, 1999). This variation of species biodiversity versus the depth has to be taken into account for paint design, especially for fixed structures.

Table 4.1 Principal characteristics of the main marine algal phyla (Graham and Wilcox, 2000; Lee, 1999)

Phylum	Thalli	Pigments	Storage products	Cell wall	Number of species
Cyanobacteria (Chloroxybacteria)	Unicellular or filamentous forms	Chlorophyll <i>a</i> , phycobilins, carotenoids, some with chlorophyll <i>b</i>	α -1,4-glucan (cyanophytan starch)		Undetermined
Cryptophyta	Unicellular with unequal flagellates	Chlorophyll <i>a</i> , <i>c</i> ; carotenoids; phycobilins; alloxanthin	Starch	No typical cell wall	200
Haptophyta	Unicellular, flagellates or not, or colonies	Chlorophyll <i>a</i> , <i>c</i> carotenoids (fucoxanthin)	β -1,3-glucan	Many species (Coccolithophorids) produce Calcium carbonate rich scales (= coccoliths)	300
Dinophyta	Unicellular with 2 dissimilar flagella	Chlorophyll <i>a</i> , <i>c</i> , carotenoids, xanthophylls	Starch granules in the cytoplasm	Membrane bound vesicles in many cases enclose cellulosic plates	2000–4000
Ochrophyta (Diatoms, Rhaphidophyceans, Chrysophyceans, Phaeophyceans . . .)	Unicellular or multicellular	Chlorophyll <i>a</i> , <i>c</i> , carotenoids (fucoxanthin, vaucheriaxanthin)	Cytoplasmic lipids, soluble carbohydrate (β -1,3-glucan, chrysolaminarin, laminarin)	Variable	10,000 species of diatoms only
Rhodophyta	Unicellular, simple filaments or complex filamentous aggregations	Chlorophyll <i>a</i> , phycobilins, carotenoids	Granular floridean starch (α -1,4-glucan)	Cellulose and sulphated polygalactan some with Calcium carbonate	4000–6000
Chlorophyta	Unicellular or multicellular thalli	Chlorophyll <i>a</i> , <i>b</i> , β -carotene	Starch	Cellulosic, some are calcified	17,000

Nutrient availability is also a key factor regulating algal growth. Nitrogen (constituent of amino acids, nucleotides, and chlorophyll), carbon (major cellular constituent), and phosphate (constituent of ATP, ADP, and phospholipids) are essential nutrients for algal growth. In addition, some other elements are necessary for some groups of algae, such as silica (frustul component of diatoms) or calcium (for coccolithophorids and coralline algae). Some secondary elements are required for the growth of algae, such as iron (involved in the composition of antimicrobial and anti-herbivore compounds), cobalt (including in vitamin B12), magnesium (component of chlorophyll) and manganese (component of the photosystem II). The variations of the concentrations of these elements strongly influence the algal growth and community biodiversity (Graham and Wilcox, 2000). Amsler and Neushul (1990) highlighted that for *Pterygophora californica* and *Macrocystis pyrifera* spore settlement was stimulated concomitantly to the increase in nutrient concentrations.



4.1 Representation of algal density cycle in three different systems: arctic, temperate, and tropical (adapted from Cushing, 1975).

In oceanic waters, the most limited component for algal growth is usually nitrogen. However, increasing human industrial and agricultural activities result in enhanced discharges of nitrate and phosphate in coastal waters, which have been correlated with strong algal growth (Van Dolah, 2000).

The availability of nutrients associated with the temperature influences the production patterns of algae and determines bloom cycles in the different latitudes (Fig. 4.1). Algal production is generally homogenous all year

Table 4.2 List of the main algae involved in marine fouling

Size range	Phylum	Species
Microalgae	Ochrophyta (Diatoms)	<i>Achnanthes brevipes</i>
		<i>Achnanthes longipes</i>
		<i>Amphiprora paludosa</i>
		<i>Amphora coffeaeformis</i>
		<i>Licmophora abbreviata</i>
Macroalgae	Cyanobacteria	<i>Navicula</i> sp.
		<i>Nitzschia pusilla</i>
		<i>Oscillatoria</i> sp.
		<i>Phormidium</i> sp.
		<i>Chaetomorpha fibrosa</i>
	Chlorophyta	<i>Cladophora</i> sp.
		<i>Codium fragile</i>
		<i>Fucus vesiculosus</i>
		<i>Rhizoclonium</i> sp.
		<i>Siphonales</i>
		<i>Stigeoclonium</i> sp.
		<i>Ulothrix zonata</i>
		<i>Ulva intestinalis</i>
		<i>Ulva lactuca</i>
		<i>Urospora penicilliformis</i>
	Phaeophyta	<i>Desmarestia</i> sp.
		<i>Ectocarpus</i> sp.
		<i>Laminaria</i> sp.
		<i>Petalonia fascia</i>
		<i>Pilayella littoralis</i>
		<i>Sargassum muticum</i>
		<i>Scytosiphon lomentaria</i>
		<i>Undaria pinnatifida</i>
		<i>Vaucheria</i> sp.
		<i>Acrochaetium</i> sp.
	Rhodophyta	<i>Antithamnion</i> sp.
		<i>Bangia fuscopurpurea</i>
		<i>Ceramium rubrum</i>
		<i>Furcellaria lumbricalis</i>
		<i>Hildenbrandia</i> sp.
		<i>Polysiphonia</i> sp.
		<i>Rhodomela confervoides</i>

long within tropical zones, due to the low variations of temperature and light. In temperate oceanic waters, two bloom events can be generally observed, in March–April and in September–October. The first bloom is dominated by diatoms, possibly due to the high availability of silica, favourable external conditions, and their high competition capacity. The second bloom event is associated with other algal groups (due to the lack of silica after over-consumption during the first diatom bloom) and the favourable external conditions (temperature, nutrients concentrations). In arctic waters, only one peak of algal production is generally observed due to the short duration of favourable conditions (Cushing, 1975; Graham and Wilcox, 2000).

4.2.4 Algae involved in fouling

The main algal species involved in colonisation of man-made surfaces are listed in Table 4.2. Despite the large variety of algae involved, only a few species are used in order to assess the efficacy of AF products on adhesion mechanisms in the laboratory (see Chapter 12 on bioassays). Regarding microalgae, the most studied species in laboratory is *Amphora* sp., which is a dominant species in marine fouling communities (Daniel *et al.*, 1980); for macroalgae, research efforts have been focused on *Ulva* sp. (formerly *Enteromorpha* sp.), *Cladophora* sp., and *Sargassum muticum* (Callow *et al.*, 1997; Finlay *et al.*, 2002a).

4.3 Adhesion of microalgae

Microalgae are constituents of marine biofilm (a microbial community with a structure maintained by the extracellular polymeric substances (EPS) matrix produced by the cells) (Decho, 2000). The biofilm formation on immersed substrates is an early step of the fouling process, and is followed by colonisation of macroorganisms such as macroalgae and invertebrates. Biofilm research studies have essentially focused on prokaryotes and still little is known on the adhesion mechanisms of microalgae, their EPS production schemes and their exact role within the biofilm community (Cooksey 1981; Domozych *et al.*, 2007; Wetherbee *et al.*, 1998). In marine benthic ecosystems, raphid diatoms, such as *Amphora* sp., *Navicula* sp., and *Nitzschia* sp. are the most abundant microalgae to colonise new substratum in the early stages of colonisation (Anil *et al.*, 2006; Beech *et al.*, 2005; Mitbavkar and Anil, 2000; Wetherbee *et al.*, 1998).

For aquatic microorganisms, the adhesion to a substratum is essential in order to benefit from soluble nutrients and light availability (Wetherbee *et al.*, 1998). As soon as a surface is immersed in the water, it will be subjected to biofilm formation within a few seconds or minutes. The bacteria, their secretions and the macromolecules all form together a microbial

biofilm which is almost always required for the last two stages. Then, the next stage starts with the settlement of yeasts, protozoa and diatoms. From the literature, it appeared that some diatoms had the ability to adhere to glass surfaces deprived of bacteria, suggesting that the conditioning phase by bacteria is not always essential for their fixation (Cooksey, 1981). An explanation could be that thanks to high production of adhesive mucilage, their first stage of adhesion is significantly facilitated (Wetherbee *et al.*, 1998; Higgins *et al.*, 2002). In most cases, the adhesion of microalgae is composed of three successive stages: a) location of the surface, b) initial contact and c) secondary adhesion for the final settlement.

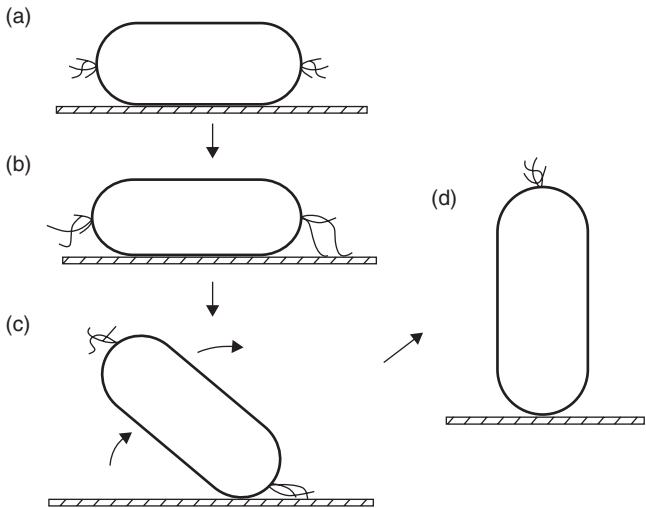
4.3.1 Location of the surface

The first step in the colonisation process consists of finding a suitable substrate. The dimorphic life cycle of some benthic microalgae, with a free-floating stage prior to a benthic phase, facilitates the propagation and colonisation of new surfaces (Fattom and Shilo, 1984). Motile algae (with flagella for example) can use their capacity to find a new surface, but it is restricted to very small distances. Raphid diatoms, without flagella, find a place to colonise by chance only (Wetherbee *et al.*, 1998).

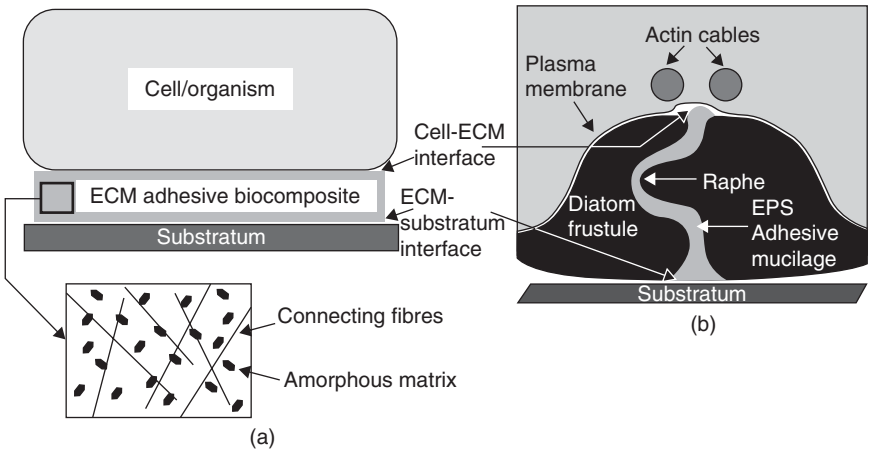
4.3.2 Initial contact

Transitory chemical attraction between the cells and the surface may be involved in the initial contact. During this phase, raphid diatoms activate adhesion mechanisms using energy to allow a subsequent binding to the substratum. The first contact phase is reversible; cells can be released if the conditions are not favorable and are able to adjust their position if necessary, by proper motility named 'gliding', which involves the secretion of mucilage (Wetherbee *et al.*, 1998).

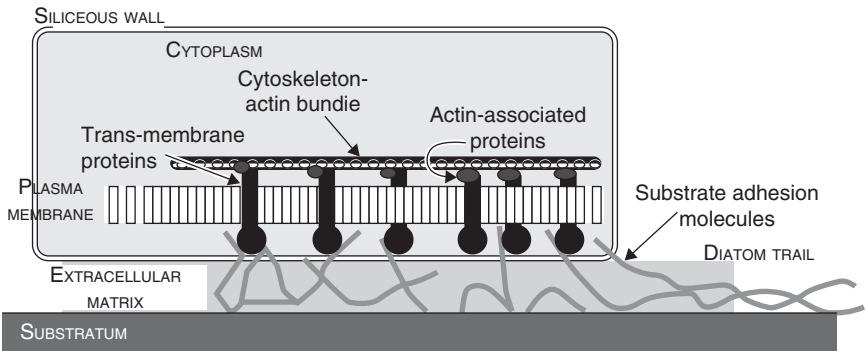
The process of initial adhesion described for the raphid diatom *Stauroneis decipiens* (Lind *et al.*, 1997) illustrates the interaction between adhesion and motility that would seemingly necessitate the kind of 'continuum' provided by an adhesion complex (AC). This complex is composed of connector molecules extending from the actin cytoskeleton through trans-membrane connections to the extracellular matrix and surface adhesive, resulting in diatom-substratum adhesion (Fig. 4.2). Over time, the nature of the AC composition is modified by addition of new molecules such as connector molecules and enzymes (Wetherbee *et al.*, 1998). *S. decipiens* firstly contact the flat substratum surfaces with its girdle region and not its raphe, thus a series of manoeuvres are necessary to take position on the raphe and for the activation of the AC (Fig. 4.3). The diatoms can easily be dislodged



4.2 Diagram showing the initial adhesion process of the diatom *Stauroneis decipiens*. (a) = landing on the girdle or the side; (b) = contact and interaction between the motility and the AC; (c) and (d) = pull of the cell onto the raphe (from Wetherbee *et al.*, 1998).



4.3 Diagram showing the position of the adhesion complex (AC) of the raphid diatom *Stauroneis decipiens*. (a) interaction cell with the substratum, (b) the raphe of *S. decipiens* involved in the adhesion complex (from Wetherbee *et al.*, 1998).



4.4 Diagram showing the AC of a raphid diatom (from Wetherbee *et al.*, 1998).

during the first attachment process and become firmly attached within 30–90s (Wetherbee *et al.*, 1998).

For raphid diatoms, the raphe is involved in the adhesion (Wetherbee *et al.*, 1998). For the diatoms without raphe, the adhesion probably takes place in several points of the frustules, with extracellular secretion of mucilage including adhesive molecules. The adhesion of these diatoms may result in different structures such as stalks and pads (Wetherbee *et al.*, 1998).

The adhesion of benthic microalgae involves EPS within the extracellular matrix (ECM), which correspond to the organic matter outside the plasma membrane of the cells. EPS are secreted from the raphe or from a thin layer between the cell and the substratum (Wetherbee *et al.*, 1998). Anil *et al.* (2006) stated that the mucilage of *Amphora* was composed of polysaccharides with uronic acids and sulfate substituents. The adhesion process to a substratum involves two adhesive interfaces: cell-ECM interface and the ECM-substratum interface (Fig. 4.4), connected by the AC (Wetherbee *et al.*, 1998).

The AC may be involved in the adhesion and in the release of the diatom cell, during the primary adhesion. Cooksey (1981) showed that calcium is an important factor in both adhesion and motility. The calcium, positively charged, may be a link between both negatively charged diatom frustul and substratum. The charge of the diatom frustul which is expected to be negative can be modified by the presence of an organic coat on the cell wall (Cooksey, 1981).

4.3.3 Secondary adhesion

After the initial contact, cells can activate a second stage, named secondary adhesion, which involves permanent adhesive structures (Wetherbee *et al.*,

1998). Due to a stronger adhesion of the cells to the surface, the algae expend less energy than in the previous phase, but are still able to release if deterioration of external conditions appears (Wetherbee *et al.*, 1998). The EPS are important components of the diatom adhesion system, but their nature is still not well known (Higgins *et al.*, 2002).

For some benthic organisms, such as cyanobacteria, the hydrophobicity of the cell wall is an important factor in the adhesion process. Fattom and Shilo (1984) showed that benthic species of the cyanobacteria *Phormidium* sp. present a more hydrophobic cell wall than the planktonic species. The presence of divalent cations (calcium or magnesium) is required by the cyanobacteria for hydrophobicity. These cations reduce the electrostatic repulsion between the both negatively charged cell wall and substratum. This phenomenon was also observed in the diatom adhesion process by Cooksey (1981).

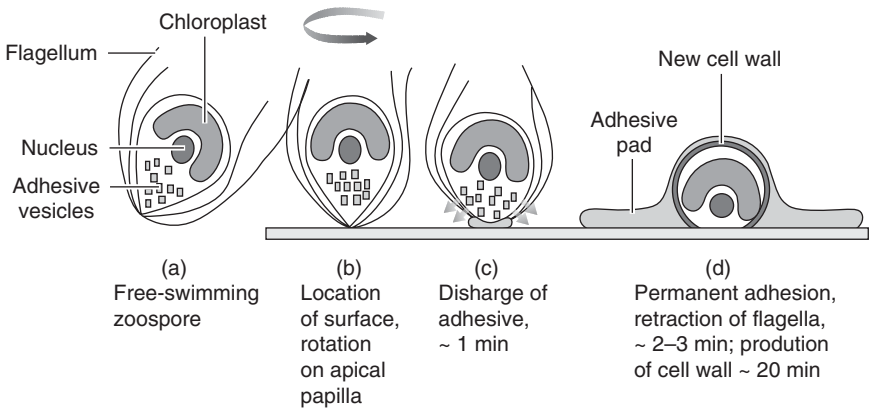
4.4 Adhesion of macroalgae

After the colonisation of a natural or man-made substratum by microorganisms such as microalgae, macroorganisms such as macroalgae are able to bind to these surfaces. This phenomenon occurs usually within a week after the deposition of the organic film (Abarzua and Jakubowski, 1995). The colonisation of a new substratum by macroalgae is a precarious stage in their life history which involves several phases. The following paragraph describes the colonisation of surfaces by marine macroalgae. The example of *Ulva* sp. which is a main species responsible for fouling and the most studied, has been taken.

4.4.1 Strategies of colonisation

Several strategies, most often including spreading of propagules (material required for the reproduction, i.e.: spores, gametes, zygotes), a part of the thallus, or lateral outgrowth of the rhizoids (external 'root-like' part of the algae) were developed by algae to colonise new substrata. Colonisation involves several stages including a pelagic phase (dispersal in the water column) and a benthic phase (location of a suitable substratum and attachment to a surface) (Fletcher and Callow, 1992). The latest can be responsible for the fixation and the development of organisms on man-made structures, process which involves three main stages (described for *Ulva* by Callow *et al.*, 1997):

- 1) The settlement corresponds to both the location of the surface and the establishment of a contact (Fig. 4.5). It is a selective process regulated by numerous factors including the spore biological properties



4.5 Diagram of the three main stages involved in algal fixation (from Maggs and Callow, 2003).

(presence of flagella or not) and the substratum physical and chemical properties.

- 2) The adhesion corresponds to both the primary attachment and the secondary permanent attachment.
- 3) The establishment phase involves the formation of a cell wall, the acquisition of polarity by the cell and the germination process.

4.4.2 Settlement phase

Spore morphology, environmental conditions and substratum properties are of great importance concerning the location of a substratum (Lobban and Harrison, 1994). Depending on the algal species, spores possess (or not) one to multiple flagella (e.g., rhodophyta and chlorophyta and phaeophyta algal aplanospores and multicellular propagules do not possess any flagella; zooids of some phaeophyta and of five classes of chlorophyta are motile) (Maggs and Callow, 2003; Lobban and Harrison, 1994). Non-motile spores reach the seabed through physical forces only (the gravity being the most important), while motile cells can actively discriminate the surface to colonise. The viscosity of the water column does not allow spores to use their flagella during the dispersal phase. However, it can be of crucial importance for the choice of a substratum and the establishment of a contact with the surface to colonise (Maggs and Callow, 2003). Many zoospores swim randomly (Lobban and Harrison, 1994). However, some of them have developed some phototactic, thigmotactic and chemotactic behaviours (Fletcher and Callow, 1992; Callow and Callow, 1998). It has been recently demonstrated that some algal spores are able to sense chemical cues produced by microorganisms present within a biofilm. Thus,

Wheeler *et al.* (2006), highlighted that zoospores of *Ulva intestinalis* were able to use the acyl-homoserine lactone (AHL) quorum sensing system to detect bacterial biofilms, and thus a binding place. The zoospores showed a decrease of the swimming speed and a chemotactic orientation to the AHL that allow a preferential settlement where a bacterial biofilm is present. Some algal spores have a negative phototaxis, allowing them to be attracted by a low irradiance area and thus establish a contact with the substratum rapidly, while others, such as *Ulva* exhibit a positive phototaxis (Lobban and Harrison, 1994). This property is thought to aid the dispersal into the water column (Maggs and Callow, 2003).

Surface characteristics of substrata, such as colour, roughness, hardness and surface energy, can influence the colonisation process (Fletcher and Baier, 1984; Callow *et al.*, 2000b; Callow *et al.*, 2002). Some algae are mainly found on hard substrata whereas others colonise smooth surfaces. Nienhuis, 1969, showed *Pelvetia canaliculata* to be more abundant on limestone than on basalt, and *Entophyalis*, *Ulothrix* and *Fucus spiralis* belts present on limestone to be absent from granite. The size and location of the belts of *Pelvetia canaliculata*, *Fucus spiralis* and *Ascophyllum nodosum* change with the substratum (Nienhuis, 1969; Hartog, 1959). Hardy and Moss (1979) demonstrated *Fucus vesiculosus* germling rhizoids to be long and thin on smooth hard surfaces, while they were branched on more porous substrates.

The microtopography of the substratum to colonise is important (Callow *et al.*, 2002): both field and laboratory experiments showed spores likely to colonise roughened surfaces which provide a safe environment for the development of the algae by reducing the effect of the water current, desiccation and the grazing on spores (Fletcher and Callow, 1992). Although not all the algae develop on roughened surfaces, there are some exceptions such as *Sargassum* sp. (Maggs and Callow, 2003). The substratum surface energy also influences the settlement of several algae such as *Ulva* by modifying the morphogenetic development of the rhizoids (Fletcher and Baier, 1984). The surface free energy is the energy produced by atoms, or molecules, at the surface of a substratum which is available to create interaction between organisms, molecules or atoms and this surface (Callow and Fletcher, 1994). The interactions between the substratum and an organism (or molecule) can be of different types, such as the Van der Waals force, polar, electrostatic and hydrophobic interactions. The lower is the surface free energy the weaker is the adhesion of the organisms (Callow and Fletcher, 1994). The strength of attachment of *Ulva* spores is greater on hydrophilic surfaces (Finlay *et al.*, 2002b). However, a higher number of *Ulva* spores were demonstrated to settle on hydrophobic surfaces (Callow *et al.*, 2000b) and spores clusters were larger on these surfaces. Chaudhury *et al.* (2006) also showed a similar pattern on homogenous surfaces while observing *Ulva* sp. settle-

ment; however, an opposite pattern was observed when testing surfaces possessing a gradient from hydrophobic to hydrophilic. This could possibly be due to the migration of spore during the surface probing phase. Moreover, organisms present on the surface to colonise can also influence the settlement process. It is the case of bacteria which can modify both the microtopography and the physicochemical properties of the substratum (Maggs and Callow, 2003).

The settlement phase is ended by the establishment of a contact. Establishment of a contact is achieved when the spore goes from a random swim on the surface to localised searching behaviour. Once the spore has found a suitable substratum it presents a spinning behaviour (Callow *et al.*, 1997) with the flagella acting as a propeller (Callow *et al.*, 2000b).

4.4.3 Adhesion to the surface

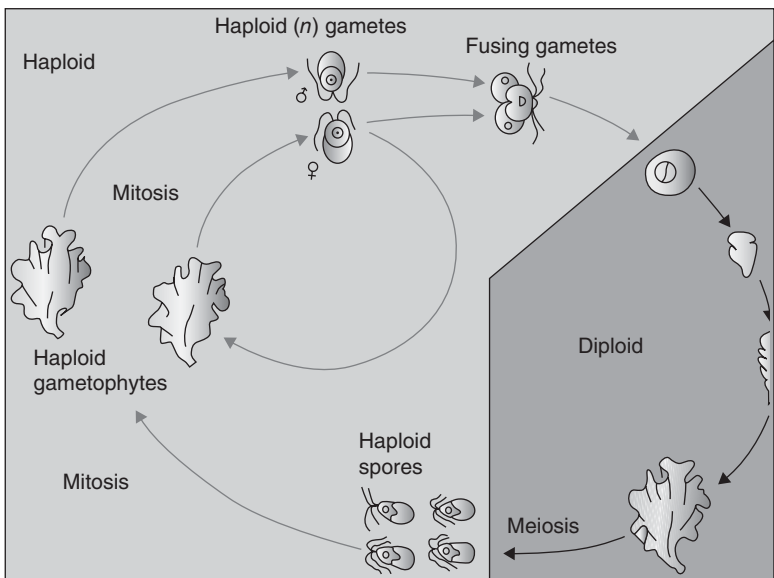
Adhesion to a new surface is controlled by numerous factors for both motile and non-motile spores. Physical and chemical properties of the substratum as well as biological communities present on the substratum are of great importance in the adhesion phenomenon and thus the development of algal community. The adhesion process is a fast phenomenon (30 to 60s) (Callow and Callow 2006) which can be divided into two main phases: the primary attachment and the permanent attachment.

1. Temporary (or primary) attachment: studies conducted on *Ulva* propagules showed the zoospores to possess a unique chloroplast in the anterior part of the cell, a nucleus in the centre and numerous vesicles at the apex of the cell. Once the contact is established between the zoospore and the surface, the apical region of the cell is flattened and the cytoplasmic activity increases before the discharge of the content of adhesive vesicles occurs (Callow and Callow, 2006).
2. Permanent (or secondary) attachment is obtained within 1 minute for *Ulva* species (Callow *et al.*, 1997). During this process, the flagella are withdrawn, the spore contracts and flattens against the surface to maximise the area of adhesion, and secretes an adhesive substance (Callow *et al.*, 1997). The exocytosis of the adhesive vesicles occurs within a minute (Callow and Callow, 2006) and does not induce an increase of the membrane surface. The fusion of the vesicles to the membrane is regulated by endocytosis of part of the plasma membrane (Thompson *et al.*, 2007). The material contained within the vesicles is an adhesive made of glycoproteins and comes from golgi-derived vesicles (Evans and Christie, 1970; Stanley *et al.*, 1999; Callow *et al.*, 2000a in Callow *et al.*, 2002). Once

secreted, the gel forms an adhesive pad-like material which can be circular to very irregular (Callow *et al.*, 2003). This swollen structure allows a strong anchorage for the spore (Callow *et al.*, 2000a). Freshly released pads have a multilayer aspect before hardening an hour after the settlement occurs (Walker *et al.*, 2005). Its average thickness is $1\ \mu\text{m}$ and its adhesive strength (when freshly released) is of $173 \pm 1.7\ \text{mN.m}^{-1}$ suggesting a very strong adhesive (Callow *et al.*, 2000a). This adhesive was demonstrated to possess very hygroscopic character (Callow *et al.*, 2003). It is of high importance to inhibit this phase of attachment as it costs lots of energy to remove the algae with water-jets when they are firmly attached.

4.4.4 Establishment

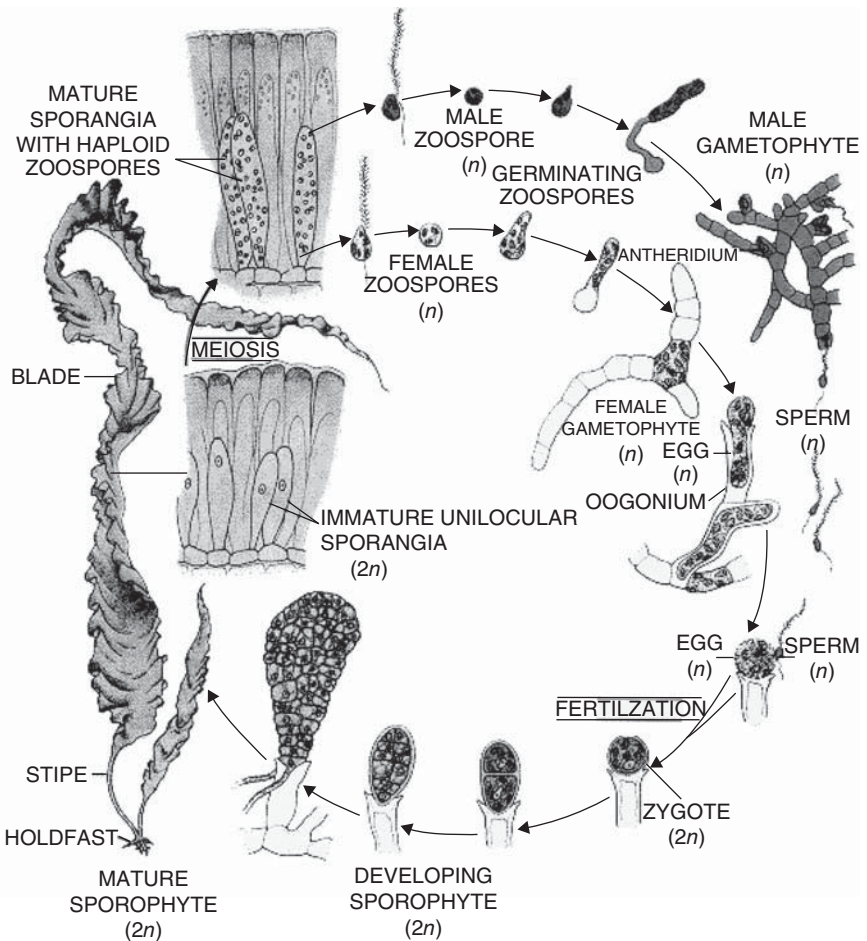
Establishment phase occurs after the production of the adhesive and corresponds to the creation of a cell wall as well as the loss of the flagella (Callow *et al.*, 1997). Once this process is achieved, the cell can germinate to form either a gametophyte or a sporophyte. Algae life cycle varies greatly between species and can be complex; it is often constituted of two generations (with the exception of rhodophyta that possess three, i.e.: gametophyte, sporophyte and carposporophyte). The gametophyte and sporophyte can have similar form and size (for example, *Ulva* sp.) (Fig. 4.6), or can be com-



4.6 Life history cycle of the chlorophyta *Ulva* sp. (from Purves *et al.*, 2004).

pletely different and easily distinguished from each other (for example *Laminaria* sp.) (Fig. 4.7). The gametophyte generation is haploid and produces male or female gametes. Once the fecundation occurs and if the zygote can settle and develop, the germination will produce a sporophyte. This generation is diploid and produces spores which will develop into a gametophyte.

Most of the publications concerning the colonisation of a substratum by macroalgae, such as *Ulva* sp., focus on the settlement and adhesion phase. This is probably due to the fact that such studies focus on the parameters



4.7 Life cycle of the phaeophyta *Laminaria* sp. (from Raven *et al.*, 1992). Copyright 1992 by Warth Publication, Inc.

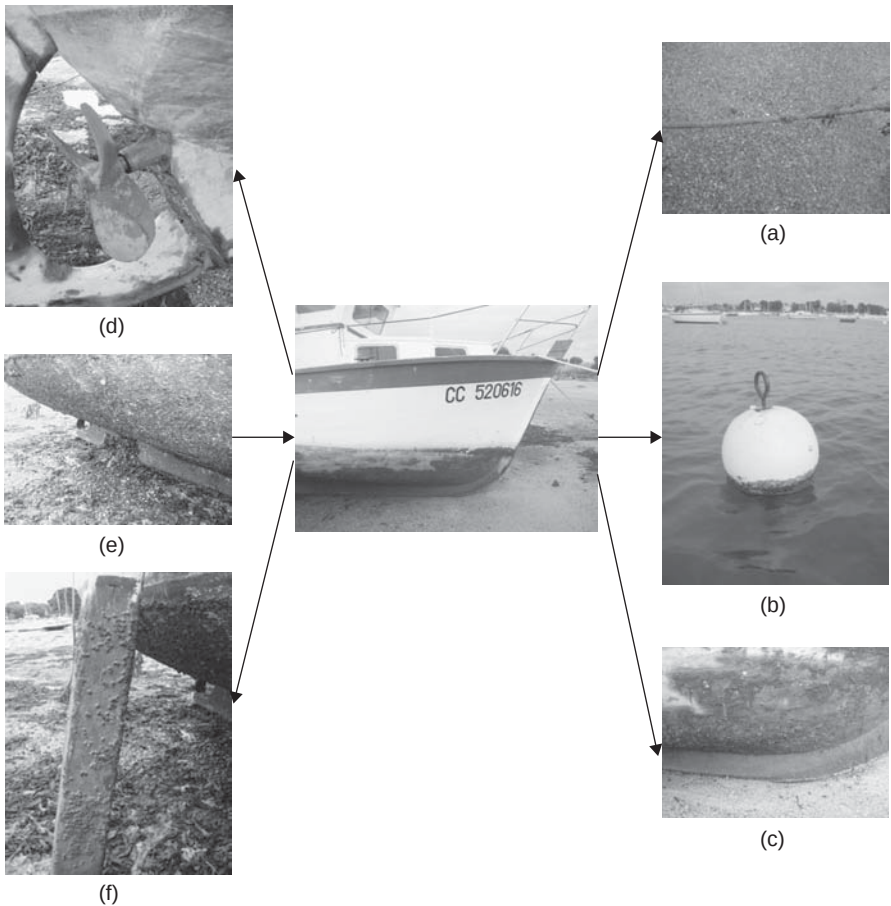
that could be useful for the production of new AF paints (properties of the substratum preferred by spores, secretion and properties of adhesive material).

4.5 Algae and chemical cues for invertebrates

Algae are able to produce compounds that can influence positively or negatively the settlement of other algae and/or invertebrates, and act as chemical cues. In order to be efficient, these products have to be released on the substratum or into the surrounding water (Walters *et al.*, 1996). The chemical cues produced by algae vary within the species: *Dictyota acudloba*, *D. sandvicensis*, *Sargassum echinocarpum*, *S. polyphyllum* and *Sphacelaria tribuloides* produce negative signal against invertebrate larvae settlement, while *Padina australis* produce stimulatory signals for larval settlement (Walters *et al.*, 1996). Some algae, such as crustose coralline algae, can be a substratum for invertebrate. e.g. *Agaricia humilis* larvae recognise the sulphating polysaccharides present in the cell wall of the algae which induce their settlement (Rodríguez *et al.*, 1993). Owing to competition for space in the environment, the colonisation of living surfaces is a common phenomenon (Steinberg and De Nys, 2002). However, some organisms are never epiphytised in the environment (Wahl, 1989). One of the most common defence mechanisms used by sessile organisms to prevent the settlement of organisms on their surface is the production of biogenic compounds. The defence molecules synthesised have several methods of action such as the inhibition of the bacteria settlement, the inhibition of the fixation and the development of fungi, algae, larvae (Abarzua and Jakubowski, 1995; Clare, 1996; Da Gama *et al.*, 2002; Hellio *et al.*, 2000b, 2002, 2004). Moreover, some algae and cyanobacteria produce AF and anti-herbivory products (toxic secondary compounds) to reduce the competition (Kuffner and Paul, 2004; Hay 1996; Hay *et al.*, 1987; Hay and Fenical 1988; Hay 1997). Thus, *Dictyota menstrualis* is known to produce an AF component that limit its surface colonisation and herbivory pressure (Schmitt *et al.*, 1995). These products can be studied to develop new environmentally friendly anti-fouling products for ship paints (see Chapter 22 on marine natural antifoulants).

4.6 Damage caused by algal fouling

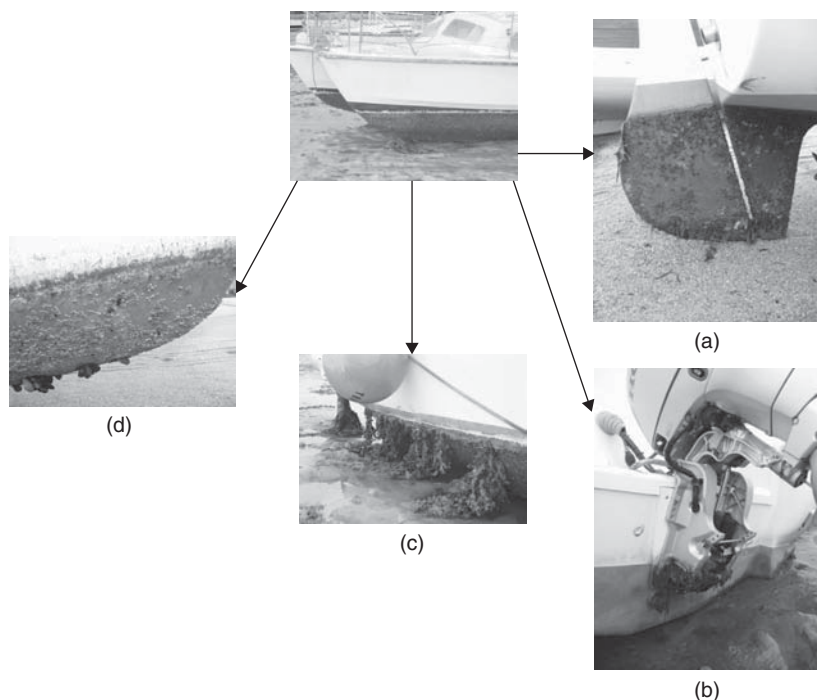
Significant technical and environmental damage of man-made structures is linked to algal fouling (see Figs 4.8–4.12). By settling and developing on immersed metallic structures, organisms within a biofilm can lead to the



4.8 Algal fouling damages on boat and examples of colonisation of rope (a), buoy (b), stem (c), propeller (d), hull (e) and stabilising leg (f) (courtesy of B Hellio).

biological corrosion of the substrates. Even if this phenomenon is mainly linked to the presence of sulfo-reducing and acid-producing bacteria, however, the production of EPS as well as the respiration and emission of O_2 by the photosynthetic organisms can be in part responsible for this phenomenon (De Brito *et al.*, 2007).

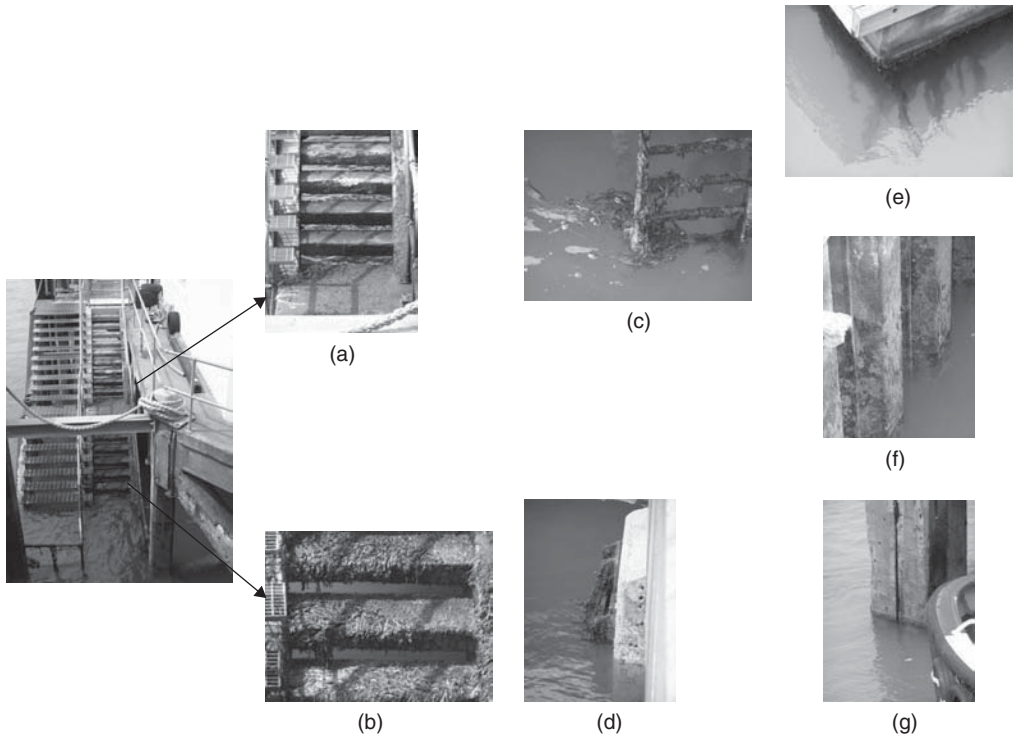
Heat exchanger systems can be fouled by microorganisms and when light is available by algae too. This settlement leads to a decrease in system efficiency by inhibiting the convective transport and increasing the energy consumption (reviewed by Flemming, 2002). Desalination water process



4.9 Algal fouling damage on boat and examples of colonisation of rudder blade (a), outboard motor (b), water-line (c) and stem (d) (courtesy of B Hellio).

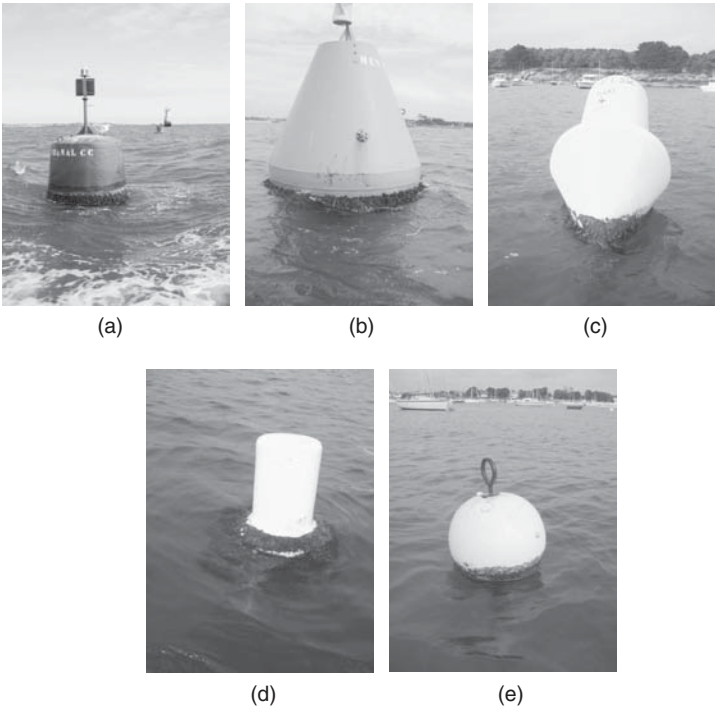
is also affected by biofilm development which can represent a hazard when pathogenic microorganisms are present (Flemming, 2002). Algal development on structures such as aquaculture nets, buoys and marine blazes can result in such a weight increase that they can subsequently sink. Another serious problem linked to algal fouling is the development of a slippery covering of structures, such as slipway and marinas, which necessitate regular and intensive cleaning.

Algal fouling on hulls is very abundant because ships move between different areas with different biological, physical and chemical properties and are always in the photic zone (Chambers *et al.*, 2006), and is responsible for increased dragging on ship hulls. Algal fouling develops mostly in well-aerated areas such as stem, waterline, propeller and rudder blade. A biofilm of 100 μm thickness enhances the friction resistance of 5–15% (Flemming, 2002), leading to an increase in the production of greenhouse gases (due to an increase of fuel consumption) and implications



4.10 Algal fouling damage to harbour infrastructures and examples of colonisation of underwater stairs (a, b), ladder (c, d), pontoons (e), concrete pillar (f) and wood pillar (g) (courtesy of K Lebre).

in the phenomenon of global warming. Moreover, the colonisation of ship hulls and ballast waters has been linked to the introduction of new species which can potentially become invasive (Gollasch, 2006) (see Lewis, Chapter 27). Williams and Smith (2007) estimated that 277 species of algae have been introduced in new environment with a total of 408 introductions (some species can be introduced several times to a new environment). Among these, only 60% of the introduction vectors are known and 77 species were reported to be introduced by ship hull transport (Williams and Smith, 2007). Marine macroalgae are a significant component of marine alien taxa (Schaffelke *et al.*, 2006) and invasions can result in an alteration of the environment through modification of the habitat, or competition with indigenous species, resulting in important ecological, evolutionary, economic and societal impacts (Table 4.3).



4.11 Algal fouling damage on buoys and examples of colonisation of signalling (a, b, c) and mooring buoys (d, e) (courtesy of B Hellio).



4.12 Example of algal hull fouling on ship's hull.

Table 4.3 Impact of introduced algae (from Schaffelke and Hewitt, 2007)

Ecological impact	Evolutionary impact	Economic and societal impact
Direct and indirect competition with native biota (space monopolisation; change in community composition)	Change of ecosystem processes	Direct: Cost of loss of ecosystem functions or values Impact on environmental amenity Impact on human health
Effects on higher trophic levels	Genetic effects (within and/or between species)	Indirect: Management costs Costs for the research into introduced species Costs for eradication and control measures
Habitat change		

4.7 Prevention of algal fouling

To limit the economic and environmental costs linked to algal fouling, prevention systems have been developed. The first attempt to prevent biofouling occurred centuries ago when copper, lead, arsenic and mercury were used on wooden sailing vessels hulls (Yebra *et al.*, 2004). Nowadays, new technologies, including biocidal (banned or still actually used), and non-biocidal techniques such as fouling-release surfaces, natural AF compounds and physical systems are under constant development to prevent this phenomenon.

4.7.1 Biocidal products

Usually, the active AF compounds are non-target specific and will inhibit a wide range of organisms from settling on the surfaces. But some herbicides molecules added to some formulations act specifically against the development of algae. TBT was the main toxicant in use since 1960 but due to its high toxicity potential on non-targeted organisms, these products were recently banned and new compounds more environmentally friendly are developed (Evans and Clarkson, 1993; Chambers *et al.*, 2006).

Banned products

Until recently AF paints containing organotin such as TBT were widely used. TBT acts on the chloroplasts and mitochondria, and interferes with energy metabolism (Table 4.4). It also interacts and binds with proteins and

Table 4.4 List of AF products against algae and their action level

AF compounds	Chemical name	Mode of action	Legislation
Arsenic trioxide			
Benzothiazole zinc pyrrithione	(bis(1-hydroxy-2(1H)-pyridethiomato-o,s)		
Chlorocresol	4-chloro-meta-cresol		
CTAC	cis 1-(3-chloroallyl)-3,5,7-triaza-1-azonia adamantane chloride		
Cuprous thiocyanate			
Diuron	(3-(3,4-dichlorophenyl)-1,1-dimethyl-uree)	Herbicide that blocks electron transport at photosystem II and thus inhibits photosynthesis	Banned
Irgarol 1051	(2-methylthio-4-tertiarybutylamino-6-cyclopropylamino-s-triazine)	Inhibits the photosystem II by binding to the D1 protein (Hall <i>et al.</i> 1999 in Arrhenius <i>et al.</i> , 2006), and inhibition of the electron transport for the photosynthesis (van Wezel and van Vlaardingen, 2004)	Restricted to vessels more than 25 m
Kathon 5287 (or Sea Nine 211)	(4,5-dichloro-2-n-octyl-4-isothiazolin-3-one)	Microbiocide that affects several enzymes in the Krebs' cycle of microbial organisms (Assessment of Antifouling Agents in the Coastal Environments (ACE)).	Restricted to vessels more than 25 m
Oxy tetracycline hydrochloride			
TBT	Bis(tributyltin)oxide	Acts in the chloroplasts and mitochondries, interfering with energy metabolism. It also interacts with proteins and membrane and can bind or interact with proteins containing free sulfhydryl groups (Fent 1996 in Arrhenius <i>et al.</i> , 2006).	Banned since January 2008
Zinc pyrrithione	2-mercaptopyridine-1-oxide	Algaecide (Bao <i>et al.</i> , 2008)	
Ziram	Zinc dimethyldithiocarbamate	Growth inhibition of algae (van Wezel and van Vlaardingen, 2004)	

membrane containing free sulfhydryl groups (Fent, 1996 in Arrhenius *et al.*, 2006). TBT is well known to inhibit the growth of some microalgae such as *Nannochloropsis oculata* and *Skeletonema costatum*, by affecting the protein metabolism (Sidharthan *et al.*, 2002). TBT has been prohibited worldwide since 2008 due to its high toxicity. Diuron, a substituted urea herbicide (Gatidou and Thomaidis, 2007) that inhibits the photosynthesis, is now banned from use. Depending on the legislation of each country, different AF formulations may be used to prevent biofouling.

Authorised and restricted products

As replacements for banned compounds, new paint formulations have been developed and are now available on the market (Thomas, 1998). However, even if these paints were stated to be environmentally friendly when launched, it has been now proved that some formulations are linked to the diffusion of toxic molecules in the surrounding environment and do not target only the species settled on the immersed structures (Beaumont and Budd in Cho *et al.*, 2001). As a consequence, the use of some of these biocides has already been restricted by the UK Health and Safety Executive (Konstantinou and Albanis, 2004; Chambers *et al.*, 2006), e.g. Irgarol 1051, and Sea-Nine 211 use has been authorized only to vessels measuring more than 25 m. The biocides added into paints can act specifically against algae or can be less specific with a broad range of actions (and act on fungi and invertebrates). The biocides acting on macroalgae have different mechanisms of action. Irgarol 1051 (highly toxic for algae) mainly used coupled with copper, inhibits the photosystem II by binding to the D1 protein, thus affecting the photosynthesis (Hall *et al.*, 1999 in Arrhenius *et al.*, 2006). Sea-Nine, which belongs to the group of Kathon, has a mechanism of action not clearly established (Arrhenius *et al.*, 2006). However, some Kathon were demonstrated to quickly penetrate the cell membrane and to react with intracellular thiols thus inhibiting specific enzymes (Collier *et al.*, 1990a and b, 1991 in Arrhenius *et al.*, 2006). Sea-Nine also seems to be able to affect more than one thiol group by generating a cascade of intracellular radicals (Diehl *et al.*, 1999 in Arrhenius *et al.*, 2006).

4.7.2 Non-biocidal solutions

Fouling-release surfaces

The composition and the physical characteristics of the surfaces (hulls, pontoons, for example) can be designed to inhibit the adhesion of algae. New surfaces, including silicone, less adhesive, have been developed to limit the binding of fouling organisms. Macroalgae which do not adhere strongly

on these surfaces are easily removed. The wettability of a surface is an important factor for algae adhesion. Spores of *Ulva* sp. were demonstrated to settle less easily on hydrophilic surfaces (Finlay *et al.*, 2002a). However, diatoms still showed a great ability to adhere on silicon surfaces (Callow and Callow, 2002). This technology cannot be applied to all the fouling problems due to its high cost, and is only used for limited applications (high speed ships).

Natural antifouling

Many benthic organisms produce secondary metabolites to limit the settlement of other organisms, to reduce the competition for the substrate, nutrients or light and the fouling on their surfaces (Hellio *et al.*, 2002; Amsler *et al.*, 2005). These compounds can be used to produce new natural AF products (see Chapter 22 on natural additives). Some compounds or extracts specifically active towards microalgae and/or macroalgae have been described by Hellio *et al.* (2002) and Plouguerné *et al.* (2008). The development of AF including natural products can be an alternative to the toxic chemical AF compounds against algae. Nevertheless, despite the good levels of activity in the laboratory, when extracts are formulated in paint matrix, the activity is generally lost after a few months. Working on encapsulating the extracts could be a way forward.

Physical systems

Technologies without chemical compounds can be foreseen as an environmentally friendly AF solution for closed-looped systems only.

An innovative European project CHEM-FREE tested the efficiency on microorganism (bacteria and microalgae) removal of a new system integrating ultrasound (US), filters and UV-C in closed-loop systems. US waves generate the release of the biofilm from the surfaces (Oulahal *et al.*, 2004) and are responsible for alteration of algae vesicles (Hao *et al.*, 2004). This technology is environmentally friendly (US are inaudible to humans and no threat to people, animals or fish) and cost effective. The filters have the ability to retain physically the organisms released in the water and especially diatoms, and larger microalgal species or cell clusters (Cangelosi *et al.*, 2007). The UV-C allows a good level of disinfection by altering the DNA of the exposed organisms, thus limiting their multiplications (Bin Alam *et al.*, 2001). Experiments done within this EU project showed encouraging results for reduction of biofilms and of microalgal growth on immersed surfaces, and this technology could be potentially further developed for closed-loop systems such as sea-water swimming pool, aquaculture, or ballast waters. More information can be found on www.chem-free.eu.

4.8 Conclusions and future trends

Algal development on immersed structures can lead to major economic and environmental damages. This is particularly true for estuaries and coastal areas where the algal fouling pressure is highest in comparison to the open ocean. Government and environmental agencies should emphasise actions to reduce drastically the factors responsible for the algae proliferation in order to limit their recrudescence and thus their potential as fouling organisms. Over the last decades, the occurrence and intensity of algal blooms has increased significantly due to anthropogenic activities and the global climate changes (Van Dolah, 2000). The nutrient release in the coastal environment is responsible in part for the increase of algae biomass and needs better control through wastewater treatment and management policies. The phenomenon of global warming could deeply influence the algal biomass enhancement in future. Indeed, the increase of temperature associated with the decrease of pH and carbonate ion concentrations could be responsible for important modifications of marine benthic and pelagic ecosystems (Hoegh-Guldberg *et al.*, 2007).

Concerning the evolution of fouling communities, a potential impact would be a decrease of calciferous species (e.g., corals, barnacles, oysters) in favour of the soft species, e.g. sponges or algae. Coral species have been proven to be affected by both the temperature variations and reduction in carbonate ion concentrations through an inhibition of aragonite formation (Kleypas *et al.*, 2006) and an augmentation of the occurrence of mass-bleaching events (Hoegh-Guldberg, 1999). As a result, free space linked to the death of calcareous organisms will be available for colonisation by fast colonisers such as algae (Hoegh-Guldberg *et al.*, 2007). This increase in algae (micro and macro) populations will inevitably increase the number of propagules and spores spread in the environment and thus their probability to settle and develop on man-made structures.

The pressure of algal fouling may be very high in future; all these facts corroborate the idea that the research of inhibitors of algal fixation and adhesion should be a priority in order to be proactive regarding the environmental changes. So far, in AF research, the priority has been given to organisms such as *Balanus amphitrite* and only few species of algae (*Ulva* and *Amphora*) have been studied in comparison with the large biodiversity of this group. The literature reporting algal fouling species at various times and location is still scarce, and more effort should be spent in this direction in order to highlight new biological models representative of the major groups of benthic algae (macro and micro) in the different geographical areas (cold, temperate and tropical waters). The crucial life stage is the fixation and new AF tests should be developed specifically toward this stage. The main problem regarding AF assays using algae is that they are highly

time consuming (see Chapter 12 on bioassays): microalgae need to be collected, identified (or purchased from a strains bank), cultivated (solid and liquid media) and maintained on a regular basis, involving special growth room and lights; some macroalgae can be cultivated, but usually for AF screening they are collected in the field and spores or zygotes production are artificially induced in the laboratory; the reproductive season is limited in cold and temperate areas, thus leading to a significant time constraint for researcher, moreover the spores or zygotes releases need usually to be performed by experienced scientists.

Another restriction is that only teams working not too far from the sea can be involved in this research topic. In order to open this field of research to worldwide scientists and to skip the seasonal and/or cultivation constraints, new solutions for AF testing against algae should be conceived: a promising area is the development of biochemical assays. Interestingly, it has been stated that the use of oxidases (enzymes) is one of the opportunistic strategies which has been developed by numerous marine organisms, whose marine micro- and macroalgae, for adhesion strategies (Vreeland *et al.*, 1998). Adhesion usually requires an oxidase mediated polymerisation of quinine (Vreeland *et al.*, 1998; Hellio *et al.*, 2000a). There are some common adhesion mechanisms among micro and macroalgae, such as the involvement of bromide for stalk assembly and adhesion (Johnson *et al.*, 1995; Wustman *et al.*, 1997; Passow and Alldredge, 1995; Waite *et al.*, 1997) or of vanadium for the cross-linking of polyphenols to extracellular carbohydrate fibers (Vreeland and Epstein, 1996). Testing new AF compounds for their inhibitory activities towards various oxidases such as haloperoxidase, bromoperoxidase or similar oxidase-activated mechanisms could be the next breakthrough for the development of fast, reliable, targeted and non-expensive anti-algal assays.

4.9 Acknowledgements

Bernard Hellio and Karen Lebret are kindly acknowledged for providing Figs 4.8–4.12.

4.10 References

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Bacterial adhesion and marine fouling

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Abstract: Early biofilms composed of bacteria and organic matter on submerged material surfaces are the key drivers for subsequent attachment of notorious fouling organisms, such as mussels, barnacles and algae. Bacterial adhesion on material surfaces is a highly complex phenomenon that is not only governed by properties of the material surface, but more importantly by surface properties of the bacterium itself. The design of anti-adhesive materials is challenged by the fact that the chemical diversity of extracellular bacterial polymers results in numerous possible types of adhesion that additionally may vary with respect to environmental parameters. This chapter reviews the different engineering approaches of material surfaces in the light of an extreme adaptability of bacterial adhesion mechanics.

Key words: bacteria, adhesion, biofilm, microfouling, marine sessile organisms.

5.1 Introduction

A large variety of marine phyla has adopted the sessile mode of life for at least one developmental stage. The distinctive role of water as a food vector for sessile organisms is the main reason why adhesion and growth on solid substrata is so widespread among aquatic organisms. For most planktonic larvae of sessile invertebrates, settlement on solid substrates is the ultimate prerequisite for successful metamorphosis into sessile juveniles (Hadfield and Paul, 2001). The colonization pressure exerted by planktonic dispersal stages (in seawater these are: 10^1 to 10^2 microscopic larvae and spores, 10^3 fungal cells, 10^6 bacteria per ml) can be immense on immersed surfaces (Davis *et al.*, 1989) with severe consequences for the substratum itself. Consequently, adhesion, metamorphosis and growth of marine invertebrates, plants and microorganisms on solid substrata generally result in spatially close associations of a variety of organisms. If these agglomerates appear on inanimate material surfaces, then this phenomenon is generally termed as 'fouling'.

The chronology of the fouling procedure has been described in successional and stochastic models and is broadly reviewed in (Clare *et al.*, 1992, Maki and Mitchell, 2002). Biofilm formation generally commences with the

rapid adsorption of dissolved organic molecules, such as polysaccharides, proteins, lipids, humic acids, nucleic and amino acids, resulting in a conditioning film on the submerged substratum (Loeb and Neihof, 1975). Estimates of dissolved organic carbon are in the range of 0.5 to 1.0 mg l⁻¹ in the open sea and can be ten times higher in coastal waters (Benner *et al.*, 1992). Given the long exposure times of material surfaces to seawater these relatively dilute solutions will eventually saturate all the binding sites on material surfaces. Early bacterial colonizers preferentially adhere to such preconditioned substrates as they can utilize these concentrated carbon sources (Bakker *et al.*, 2003b). Microcolonizers, such as bacteria, benthic diatoms and algal spores often proliferate more rapidly or sometimes exclusively once attached to a solid substratum (Grossart *et al.*, 2007, Vanloosdrecht *et al.*, 1990). Already in 1935, Zobell and Allen concluded 'that bacteria and, to a lesser extent, other microorganisms are the primary filmformers on submerged glass slides, and that such films favour the subsequent attachment of the larger and more inimical fouling organisms' (Zobell and Allen, 1935). As bacteria sensitively respond to changes in environmental parameters the composition and physiology of bacterial communities in marine biofilms reflects local environmental conditions in close proximity to a substratum (Anderson, 1995, Olivier *et al.*, 2000, Wieczorek *et al.*, 1995). Invertebrate larval settlement has been suggested to be a function of the biofilm community's bacterial species composition (Lau *et al.*, 2003, 2005). As such, bacterial biofilms, and especially the composition of bacterial extracellular polymers, are informative signposts and often a prerequisite for attachment and metamorphosis of many fouling organisms (Harder *et al.*, 2002).

Given the pivotal role of organic conditioning films and bacteria as main constituents of early marine biofilms and modulators of subsequent invertebrate larval fouling, in this chapter we will focus our attention to the diverse mechanisms of bacterial adhesion in order to guide feasible design strategies for coatings with potent antiadhesion/fouling properties.

5.2 Sequential steps of bacterial adhesion

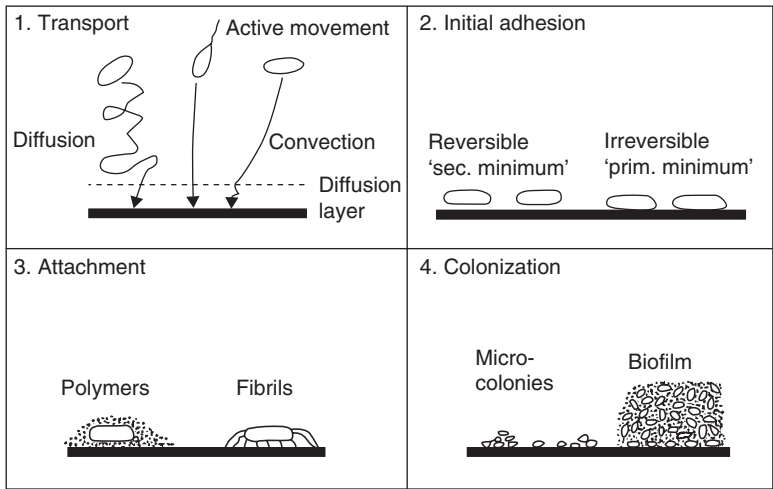
Bacterial adhesion to a substratum is generally described as a multi-stage process comprising the transport of cells to the material surface, initial adhesion of cells, followed by irreversible attachment and surface colonization of cells (Marshall, 1985). The formation of an organic conditioning layer precedes the formation of an initial bacterial film on surfaces submerged in seawater in the strict sense as the transport of molecules to the surface is faster than for bacteria. The conditioning film of adsorbed organic molecules strongly influences the substratum surface properties including surface charge, wettability and surface free energy, which play an important

role in subsequent colonization by microorganisms, especially bacteria (Baier, 1980, Bakker *et al.*, 2003a, Baty *et al.*, 1996, Salerno *et al.*, 2004, Schneider, 1997).

5.2.1 Thermodynamics of bacterial adhesion

In the absence of flow, bacterial cells are passively transported by Brownian motion, which allows their passage through the diffusion layer beyond which no convection exists. Under hydrodynamic conditions, cells are passively transported by convection, a process which is several orders of magnitude faster than diffusion. Many cells possess flagella that allow active movement on small spatial scales, especially when cells respond chemotactically towards a gradient of chemical cues in the interfacial region. Upon closer contact (< 20 nm), the mutual forces between the material surface and the cell surface have classically been described in a first approximation with a thermodynamic model named after Derjaguin, Landau, Verwey, and Overbeek (DLVO approach) based on the decay of the so-called free energy of adhesion with separation distance of cells and material surface (Rutter and Vincent, 1980, Tadros, 1980). This model describes the change in Gibbs energy as a function of the distance between two bodies. In the absence of steric hindrance, the Gibbs energy results from the sum of the van der Waals and the electrostatic interactions. While the van der Waals interaction is usually attractive, the electrostatic interaction can be repulsive due to the fact that both bacteria and material surfaces covered with a conditioning film are predominantly negatively charged. More recently, novel conceptual models derived from the DLVO approach have been introduced that describe the interactions between cell surface structures and material surfaces more realistically (Ginn *et al.*, 2002, Salerno *et al.*, 2004, Bayoudh *et al.*, 2006).

The Gibbs interaction energy between a bacterium and a material surface largely depends on the ionic strength of the surrounding medium. At intermediate values of ionic strength, as given in seawater, it has a minimum at approximately 10 nm distance from the material surface. Due to this minimum bacteria initially adhere without direct contact to the surface (Vanloosdrecht *et al.*, 1989, 1990). As such initial adhesion is reversible implying continuous fluctuation of planktonic and adhered cells (Power *et al.*, 2007, Wiencek and Fletcher, 1995), the transition from initial, reversible adhesion to more permanent adhesion requires cells to pass an electrostatic energy barrier in order to come into close contact ($< 1\text{--}2$ nm) with the surface. As the electrostatic repulsion forces are low in seawater this barrier is generally passed by the cells' thermal energy. Once cells are within less than 1 nm distance to the material surface, passive short range interactions, such as hydrogen bonding, ionic and dipole interactions as well as



5.1 Schematic representation of the sequential steps in the colonization of surfaces by microorganisms (From Vanloosdrecht *et al.*, 1990).

hydrophobic interactions come into effect that bind the cell to the surface. While up to this point the bacterial adhesion process is largely driven by passive forces, molecular reactions between bacterial surface structures, such as capsules, slime, and fimbriae, and the material surface increasingly lead to firmer adhesion of cells (see Fig. 5.1) (Ward and Berkeley, 1980).

5.2.2 The bacterial surface

Considering a material surface with given physico-chemical characteristics, the magnitude of bacterial adhesion differs among bacterial species (Fletcher and Pringle, 1985). This is mainly due to the fact that different bacterial strains vary enormously with respect to their surface properties (i.e., hydrophobicity and surface charge) and hence attachment abilities. Bacteria can be generally classified on the basis of their cell envelope structure and composition. The Gram-negative envelope contains proteins, lipids, peptidoglycan, and on the outer surface, lipopolysaccharides. The chain length of these extracellular polymers varies considerably and largely determines the degree of hydrophobicity on the cell surface. The Gram-positive envelope is simpler and contains primarily peptidoglycan with small amounts of teichoic and teichuronic acids, polysaccharides and proteins. Not only are bacterial envelopes chemically complex and structurally diverse, the cells may also have associated capsules or slimes and filamentous appendages (fimbria, pili) that can extend beyond the outer membrane by considerable distances of up to two microns (Ward and Berkeley, 1980).

Therefore, the bacterial cell surface is a rather diffuse boundary of loose hydrogels than a finite plane. The chemical diversity of the extracellular hydrophilic polymers results in numerous possible types of adhesion among different bacteria. In addition to this wide range of inherent surface properties in bacteria, the cell surface properties of a given bacterium can change with regard to environmental or growth conditions, thus providing bacteria with a dynamic range of attachment options (Fletcher and Pringle, 1985).

5.2.3 Bacterial adhesives

The apparent involvement of extracellular polymers in non-specific adhesion of bacteria to solid surfaces was firstly suggested by Zobell (Zobell, 1943), and has since been supported by many studies (Costerton *et al.*, 1985, Fletcher and Pringle, 1985).

Many analyses of bacterial adhesion and the nature of microbial extracellular polymers support that polysaccharides indeed act as adhesives for cells, but detailed knowledge about the nature of the polymers involved and their interactions with material surfaces is scarce (Callow and Callow, 2002). The polar nature of carbohydrates implies strong potential for short range forces, such as dipole-dipole and ion-dipole interactions and most likely hydrogen bonding (Christensen, 1989). Further, the large number of segments in a polymer chain can lead to strong, irreversible adsorption of the molecule even if the segment-surface energy is very low (Robb, 1984). It has also been suggested that polymers can penetrate the repulsion barrier that otherwise exists between the cell envelope and the material surface (Robb, 1984).

Given that extracellular polymers function as bacterial 'glues', the question arises as to whether specialized polymers are required for firm attachment. In other words, do microorganisms produce a special 'glue' for the sole purpose to attach to surfaces, and are certain extracellular polysaccharides produced mainly for this purpose? The nature of these putative polymers is largely unknown, since they have usually not been isolated and analyzed separately. However, sophisticated labelling techniques and modern surface spectroscopy and microscopy have been used to provide more specific molecular information of bacterial glue chemistry (Abu-Lail and Camesano, 2003, Burks *et al.*, 2003, Camesano *et al.*, 2007, Mantus *et al.*, 1993, Neu, 1992). A few studies have provided evidence that specialized biomolecules have evolved to serve as primary bacterial adhesins (Barnes *et al.*, 1988, Frolund *et al.*, 1996, Rosenberg *et al.*, 1982, Yun *et al.*, 1994) and that molecular architecture of extracellular components may be fashioned for adhesion to inert material surfaces (Shea and Smithsomerville, 1994). As these studies were performed with single isolates or mutants thereof, there is still a great deal of uncertainty whether specialised bacterial adhesins are common and widespread in the marine environment.

5.3 Biofilm growth

Since many excellent reviews have covered the biofilm matrix, its composition and architecture, as well as the numerous intra- and intercellular processes within this matrix (Givskov and Kjelleberg, 2007, Stoodley *et al.*, 2002, Sutherland, 2001) we will only give a brief account of this subject. Whereas adhesion determines the initial rate of accumulation of cells on the material surface, the main process leading to a biofilm is usually maturation of attached cells and their production of an extracellular, hydrated matrix. From an antifouling perspective, this additional production and secretion of extracellular polymers that embeds and glues the cells more firmly to the surface, is probably most crucial. However, no system or surface has apparently been described in which the number of attached cells can be reduced to a level which is not compensated for by the growth of surface-bound bacteria after a few generations. As Christensen argued, 'reducing attachment by [...] 90% may appear significant, but has practical importance only in systems with very low growth rates' (Christensen, 1989). Therefore, reducing adhesion alone in order to control and suppress the total biofilm accumulation seems to be impractical.

5.4 The material surface

Whilst bacterial surface structures involved in adhesion are diverse, adaptable and impossible to generalize, the characteristics of material surfaces are mostly static and can be accurately described in terms of their physico-chemical properties. The physical molecular topography and chemical composition of a material surface is expressed in properties such as surface charge and energy, hydrophilicity or hydrophobicity, wettability and roughness. These properties partially overlap and relate with each other. An excellent in-depth review of material surface properties, their definition and relevance to marine fouling has recently been presented by (Genzer and Efimenko, 2006).

It has long been recognized that material surface properties seem to be critical for microbial adhesion (Baier, 1970, Fletcher and Pringle, 1985) and that the modification of these properties alters the bacterial propensity to adhere (Bos *et al.*, 1999). The surface energy of a material has most frequently been correlated with resistance to bioadhesion. A generalized relationship between the surface tension of a polymeric material surface and the relative amount of bioadhered material, known as the 'Baier curve' (Baier, 1970), has since been validated in further marine exposure studies (Brady and Singer, 2000). The main characteristic of this curve is a narrow window of surface energy wherein adhesion is minimal. Others have stressed the importance that the amount of bioadhesion on material surfaces results

from the combined interaction of surface energies at all the participating interfaces, i.e. the surface energy of the bacteria, the surface energy of the material surface and the surface tension of the surrounding medium (e.g., seawater) (Absolom *et al.*, 1983). The notion of this complex interplay of incremental energies coming into play at different locales of the adhesion site was further elaborated by (Fletcher and Pringle, 1985). These authors argue that the energy contribution due to the liquid medium is greatly affected by dissolved macromolecules and other surface active agents that themselves influence the surface tension of the liquid and hence the magnitude of adhesion.

In conclusion, the thermodynamics of bioadhesion with respect to the properties of material surfaces are complex, and generalizations of surface properties regarding their effect on bioadhesion have to be judged cautiously in the context of the environmental parameters in close proximity to the surface and the type of potential bacterial surface colonizers.

5.4.1 Engineered material surfaces with antifouling properties

To date there have been various proofs of concept to reduce fouling on engineered material surfaces, which broadly fall into two major categories based on their mode of action: Passive surfaces; Active surfaces.

The range of passive material surfaces encompasses three sub categories:

- a) Passive physical control – strategies that tailor the physical properties of the material, such as erodability, microtopography, hydrophobicity and hydrophilicity.
- b) Passive chemical control – strategies that employ chemical additives, leaching from the surface.
- c) Combination of passive physical and chemical control.

a) Passive physical control

Passive coatings refer to all the technologies where physical or chemical modifications of surfaces are performed at the point of manufacture and product completion. Once in the marine environment, these coatings are left to their own devices and do not contain any means or possess any ways of adjusting, changing or altering the physical or chemical properties of the coating in response to the fouling pressure.

The strategy of a passive physical antifouling surface is the modification of physical shape as a means of controlling bioadhesion. Technologies that exemplify this approach include the erosion of the surface (self-polishing paints), microtopographical design of the surface and finally surface energy control.

The principle of eroding surfaces relies on frequently producing new surfaces whilst eroded paint polymers carry off adhered fouling organisms. Moreover, the principle of erodible paints has been used as a vehicle for exposing chemical additives that are present throughout the depth of the erodible surfaces (Howell and Behrends, 2006, Löschau and Krätke, 2005, Thouvenin *et al.*, 2003, Valkirs *et al.*, 2003, Candries *et al.*, 2003). Hence, although the name suggests a physical approach to antifouling, in reality this strategy represents a combined chemical and physical approach to control bioadhesion. An example of the latest findings in this area points to coatings that attempt to emulate hydrolysable ester bond linkages that made TBT paints so famous (Fay *et al.*, 2007). This combined strategy towards antifouling is comprehensively covered in Chapter 18.

Another strategy to physically disrupt adhesion is the use of microtopography as it is employed by natural organisms in their defence against bioadhesion (Bers *et al.*, 2006, Bers and Wahl, 2004). Such surfaces (e.g. mussel shells) are designed with micro-patterns that minimise the total surface area available to microorganisms for a firm adhesion base. Surface topography has a pronounced effect on bacterial biofouling. Studies conducted on optical sensors in a marine estuarine environment showed fouling build-up was greatest at ~10 nm rms roughness value than at either 5 or 15 nm (Kerr and Cowling, 2003). Both stainless steel (316L) and polymethyl methacrylate were tested and produced similar results for surface roughness value suggesting the topographical effect to indeed be physical rather than chemical. Although the microtopographical control of bioadhesion is at an early stage (de Nys and Steinberg, 2002), there is a clear advantage in the combined and simultaneous mode of action these patterns have on both wettability and cell attachment (Carman *et al.*, 2006, Granhag *et al.*, 2004, Schumacher *et al.*, 2008). More detail on microtopographical control over antifouling can be found in Chapters 24 and 25.

Lowering the material surface energy is another strategy of passive physical control of the surface. An example of such control is the use of silicone-based foul-release coatings. These do not contain biocides and hence do not prevent fouling chemically but rather reduce the adhesion of organisms where fouling can be removed mechanically with a brush, water jet < 50 bar and or hydrodynamic vessel speeds of > 15 knots at > 70% of the vessel's activity. However, these coatings do leach silicone oils and poly di-methyl siloxanes (PDMS) into the environment as a course of their mechanism and the toxicity of these seemingly inert molecules comes into question (Nendza, 2007). The subject of silicone based coatings will be covered in Chapter 26.

b) Passive (non-toxic) chemical control

A wide range of bioactive compounds, both synthetically produced or isolated from the natural environment, have been employed in passive antifouling coatings.

This section highlights the use of enzymes as non-toxic disruptors of bioadhesion. The use of enzymes in the form of protein-polymer biocomposites was identified recently as a non-toxic coating strategy to address fouling through resistance of lipids, proteins, oligosaccharides, microbes and invertebrates in aquatic and marine environments (Gill and Ballesteros, 2000). Historically, in 1976, albumin, gelatin, fibrinogen and pepsin were shown to inhibit the attachment of a marine pseudomonad to polystyrene (Fletcher, 1976). More recently, serine-proteases have been identified to reduce the adhesion of spores and sporelings of *Ulva linza*, cells of *Navicula perminuta* as well as cypris larvae of *Balanus amphitrite* (Pettitt *et al.*, 2004). However, the performance was deemed to be too species-specific, lacking the broad spectrum approach required for an enzyme strategy to be useful in the marine environment (Chambers *et al.*, 2006). Very recently, a broad spectrum protease, subtilisin, has been identified from extracellular proteins produced by *Pseudoalteromonas* D41 and proven to be an effective means of reducing bacterial adhesion in laboratory experiments (Leroy *et al.*, 2008).

The mechanism by which 2,4-dinitrophenol (DNP) is able to reduce adhesion of bacteria on marine surfaces has been studied by Jain and co-authors (Jain *et al.*, 2007). The major findings of this study were how DNP is capable of non-lethally reducing the attachment of mixed bacterial communities (isolated from stainless steel, titanium and copper) to polystyrene and glass. DNP appears to be a non-toxic solution as the effect on bacterial growth is minimal (10–30% reduction in growth).

Marine antifouling coatings that are enzyme-based will be thoroughly covered in Chapter 23.

c) Combined passive physical and chemical control

Evidently, a more efficient strategy to passively control bioadhesion combines the physical and chemical strategies simultaneously. Different forms of combinatorial approaches and even high-throughput screening methods to determine the optimum antifouling performance of the combined application of surface properties and chemical additives in the marine environment have been comprehensively reviewed by Webster and co-authors (Webster *et al.*, 2007).

Stafslie and coworkers, for instance, employed two series of biocides containing coatings: i) a commercially available poly(methyl methacrylate) (PMMA) and a silicone elastomer (DC) blended with an organic antifouling biocide Sea-Nine 211, and ii) a silanol-terminated polydimethylsiloxane (PDMS-OH) reacted with an alkoxy silane-modified polyethylenimine containing bound ammonium salt (PEI-AmCl). When compared to PMMA, DC consistently showed an equal or greater percentage reduction in biofilm retention as the level of Sea-Nine 211 increased. Evaluations of the PEI-AmCl/PDMS-OH coatings with *Cytophaga lytica* showed that all PEI-AmCl concentrations significantly reduced biofilm retention (Stafslie *et al.*, 2007).

Active surfaces

As outlined earlier in this chapter, bacterial adhesion is a natural dynamic phenomenon. It stands to reason that material surfaces, in order to compete against this ever changing fouling pressure, themselves need to be dynamic and capable of change over time. Surfaces with such properties are referred to as 'active surfaces'. The driving force for change may be frequent human intervention via remote control; for example, by dynamically influencing the conditioning film through cyclically altering the material surface temperature (Balamurugan *et al.*, 2005). Briefly, by altering the temperature of mixed self-assembled monolayers (SAMs) of oligo(ethylene glycol) (OEG) and methyl-terminated alkanethiolates above and below 32 °C the SAM changed its hydrophobicity, and thus affected protein adsorption and cellular attachment.

Another example of human intervention is the use of electrons as the non-toxic mediator that produces anti-adhesion effects and is particularly relevant on electrically conductive surfaces. Hong and co-authors (Hong *et al.*, 2008) discuss many examples of anodic and cathodic approaches. What appears to be most effective for electro-assisted antifouling is to have a surface that is able to vary the flow of electrons, i.e. change from anode to cathode in a cyclical fashion.

The ultimate active control of a material surface is probably a 'living paint'. This coating example contains viable, immobilized bacteria capable of not only producing a range of secondary metabolites targeting different fouling organisms but also being able to change phenotype and gene expression patterns as a response to changing environmental conditions such as fouling organism diversity and abundance, temperature and nutrients. This concept stems from the observation that certain marine surfaces (for example the green alga *Ulva lactuca*) are relatively free from fouling (Rao *et al.*, 2005).

The concept of a living paint was introduced by (Goupil *et al.*, 1973) and requires both a suitable bacterium or bacterial community capable of producing antifouling metabolites (Holmstrom *et al.*, 2000) and a suitable polymeric delivery system (Gatenholm *et al.*, 1992, 1993, 1995, 1996). A proof of concept model was achieved under field conditions for a period of up to 8 weeks in Sydney Harbour (Yee *et al.*, 2007).

5.5 Conclusions

In the light of the various designs and modes of action of material surfaces with antifouling properties presented in this chapter, naturally the question arises what should be 'the ideal' antifouling surface? Unfortunately, there is no straightforward answer to this question, as we are faced with a highly

complex, diverse and plastic phenomenon of bacterial adhesion. The challenge in addressing the microbial antifouling problem from a surface-engineering angle is the sheer multitude of adhesives utilized by different organisms, which themselves actively respond and tailor their glue chemistry according to the surface type encountered. In other words, the natural propensity of planktonic bacteria to enter the-biofilm-mode-of-life relies on many adhesion mechanisms, all of which must be defeated simultaneously by a 'universal' antifouling surface.

As if this is not difficult enough to achieve for a diverse microbial community at one specific location, the challenge is amplified by the fact that, for instance, container vessels frequently cover different fouling zones in the world's oceans, which vary in salinity, clarity, temperature, micronutrients and thus, in the types of native fouling organisms in each of these zones (WHO Institution, 1952).

Coming back to the original question of the nature of an ideal antifouling surface, it seems logical to attack the problem at the common denominator among different bacteria and environments, i.e. the prerequisite of surface contact, by minimizing the contact area between the material surface and seawater, and thus reducing the chance of bacteria reaching that surface. This approach has been tested with so-called superhydrophobic polymers engineered in different laboratories (Genzer and Efimenko, 2000). Contrary to this approach, others have tried exactly the opposite, by tailoring superhydrophilic surfaces that prefer contact with water rather than dissolved molecules, thus preventing the formation of a conditioning layer (e.g., Ista *et al.*, 2004). While superhydrophilic surfaces based on polyethylene glycol derivatized polymers have been successfully used in the medical sector to avoid protein adsorption (Kingshott *et al.*, 2003), so far they have yet to be tested in the marine environment. Even though these extreme surface properties have the potential to reduce microbial fouling to a large extent, their performance is undermined by the fact that certain bacteria and proteins adhere to superhydrophobic and superhydrophilic surfaces, respectively (Ista *et al.*, 1999, Lord *et al.*, 2006), and form a pioneer film with surface properties that support further bacterial attachment.

The observation that various bacteria may tune their adhesive mechanisms differentially with respect to a surface with static physical properties has stimulated research targeted at the design of multifunctional surfaces. This approach has been experimentally addressed with co-polymers of different properties resulting in compositional and topographical structures that address different adhesion strategies of organisms and change properties with regard to the polarity of the immersive fluid (Gudipati *et al.*, 2005). A similar approach was taken by Krishnan and coworkers who designed amphiphilic copolymers, i.e. a surface with both hydrophobic and hydrophilic properties (Krishnan *et al.*, 2006). In order to resist multiple

mechanisms of adhesion, this approach is promising; however, none of these surfaces has been tested in the marine setting so far.

Cyclically changing surfaces comprising more than one physical property, and 'living paints', are just a few examples of addressing antifouling in a pro-active, almost pre-emptive strike fashion. These technologies represent a promising opportunity to bridge the chasm between the highly diverse nature of microbial adhesion versus the passively designed surface approaches to date.

5.6 Future trends

Only recently, through the discovery of the organization of multicellular behaviour in bacteria through cell-to-cell signals (quorum sensing) (Fuqua *et al.*, 1994), a novel antifouling approach has been considered, following first experimental evidence that bacterial biofilm formation and dispersal involves cell-to-cell signals (Koutsoudis *et al.*, 2006, McDougald *et al.*, 2006). Utilizing individual strains of bacteria and mutants thereof, several studies have clearly demonstrated that quorum sensing signals are involved in the formation of bacterial films on material surfaces and that quorum sensing inhibitors do interfere with the formation of bacterial films (Manefield *et al.*, 1999, 2002, Tait *et al.*, 2005). In a study utilizing a natural, complex bacterial consortium harvested in the field, Dobretsov and coworkers (Dobretsov *et al.*, 2007) demonstrated that the presence of quorum sensing inhibitors at fairly high concentrations did reduce the bacterial abundance and diversity under laboratory conditions. Natural biofilm communities treated in this manner had a significantly weaker effect on subsequent stimulation of larval settlement of a polychaete and a bryozoan species. While bacterial adhesion was not eradicated by the presence of these cellular infochemicals, this result may provide a promising avenue to a completely different antifouling strategy. This particularly holds true since bacterial quorum sensing is not directly involved in processes essential for bacterial growth, hence its inhibition does not impose a strong selective pressure for the development of resistance (Rasmussen and Givskov, 2006). Many chemical libraries of both natural and synthetic origin have been screened so far and several quorum sensing inhibitory compounds have been identified (Smith *et al.*, 2003). Their suite as potentially promising additives in coatings that otherwise combine different surface properties is a possible avenue in the design of novel antifouling strategies. The prospect of novel antifouling strategies against bacterial adhesion that circumvent the development of bacterial resistance against biocides is promising in the light that a variety of bacteria resistant or being able to degrade organotin compounds have been identified so far (reviewed in Dubey and Roy, 2003).

5.7 Acknowledgements

We thank Staffan Kjelleberg, Emma Johnston and Stanley Lau for providing comments and suggestions on this chapter.

5.8 References

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Understanding the biofouling of offshore and deep-sea structures

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Abstract: Marine growth on offshore platforms has many implications for the oil and gas industry. First, there are engineering aspects such as increase in structural loading of the structure, affecting the dynamic response for the drag forces, calculated by naval engineers. Second, there are fouling problems at the heating exchange mechanisms and other pipes collecting sea water (problems similar to power plants located on the coast using sea water for cooling). Finally, we have the transport of fouling from one biogeographical region to another. The rapidly rising world energy requirement, with platforms being built in different shipyards of the world, creates an artificial situation of a sessile benthic organism (biofouling) being transported from one ocean to another. These unintentional introductions of non-native aquatic organisms have resulted in the establishment of many species beyond their native ranges (Gollasch, 2002).

Also, with the oil and gas industry going deeper, we have a completely new situation, which consists in artificial structures being placed in the deep blue. Normally, we would expect not much biofouling in the middle of the ocean, on the continental slope (deep sea); however, recent results have been showing that there is great growth of marine fouling attaching to the platforms and risers in the deep sea.

In this chapter, we will discuss this new situation of offshore and deep-sea biofouling and its implications on operational and environmental aspects for the oil and gas industry.

Key words: barnacles, biofouling, deep-sea, recruitment, offshore.

6.1 Introduction

‘Marine biofouling’ has been a major problem for man since the first navigations and the first interventions of man in the sea with his artificial structures. ‘Biofouling’ consists, mostly, in marine organisms with two distinct phases in their complex life cycles: a larval phase, which is planktonic, and juvenile/adult phase, when, after the metamorphosis of the larva, the specimen becomes benthonic and fixed on a natural surface (rocky slopes) or on artificial surfaces (ships and platforms) (Apolinário, 2003, Ferreira *et al.*, 2004). Most of the biologic groups that form marine biofouling, also known

as 'fouling community' are composed of algae, barnacles, mollusks, bryozoans, polychaetes, tunicates and hydrozoans.

Among these, the barnacles and the mollusks present very rigid calcareous structures, which are difficult to remove and are referred to, along with some species of calcareous algae, polychaetes and bryozoans, as 'hard fouling'. The sequence of the establishment of the fouling community follows a classic pattern described in an important publication of the 'Woods Hole Oceanographic Institution' (1952) and better detailed from the ecologic point of view by Railkin (2004).

Nevertheless, there has been evidence indicating a more dynamic model of the biologic encrusting process, in which the process is considerably more complex, where the various encrusting organisms interact among themselves, and the colonization of a surface depends not only on a directional succession process, but also on the availability of each of these organisms at a certain moment (e.g., availability of the methods of propagation on the plankton). These organisms, when not established on a surface, agglomerate and slowly precipitate towards the seabed in the form of 'marine snow'. This model has been gaining growing support in various recent studies that include tropical systems in which the succession processes do not always follow the classic models elaborated for temperate regions.

The negative effects of the marine biofouling are felt not only in navigation, when the encrusted ship hulls lose speed and therefore spend more fuel to move, but also in the refrigeration systems on ships, platforms and even at hydroelectric power plants and water catching systems (Nandakumor & Yano, 2003). In the petroleum industry, the rigs and platforms that operate offshore suffer with long stoppages for maintenance, difficulties in the cooling systems with seawater, as well as with weight and drag on their structures. Anti-fouling coatings for deep-sea risers cost about US\$ 22,000,00 per riser. Considering that a producing platform has an average of 50 risers, we could assume a cost of US\$ 1,100,000,00 to cover only the first 100 m depth of each riser.

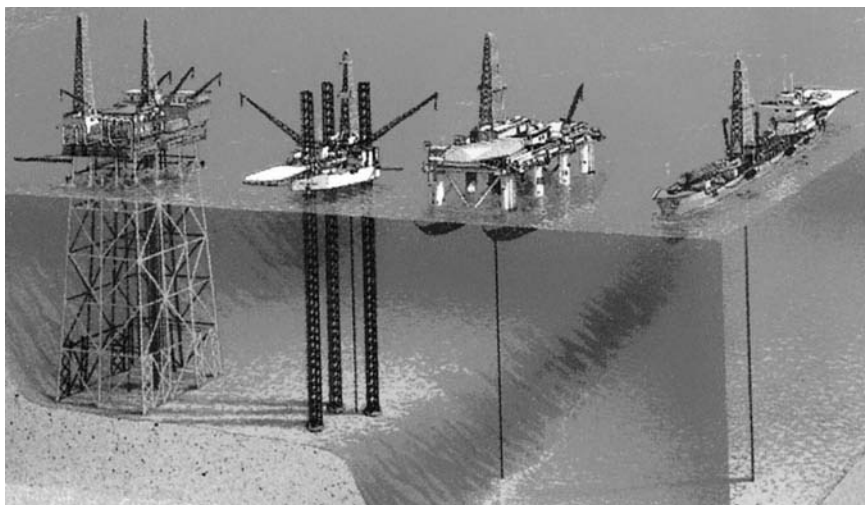
In this chapter we are going to approach every aspect of biofouling in the open and deep sea: the operating and structural aspects in the open sea, environmental and biodiversity considerations, as well as the recruitment and composition of the communities. In the deep sea environment (more than 200 meters deep) research is very scarce and there is little information in this area and it is considered one of the new frontiers in marine biofouling studies.

6.2 Operational and structural aspects

With the advance of the oil and gas industry into the offshore environment and deep waters, new artificial structures were inserted in this environment, thus creating a new possibility for the formation of biofouling communities.

In addition to the large ships that transport products across the oceans, supporting vessels and other smaller vessels that move between the ports and operate in areas in the open sea, there are structures specific of the gas and oil industry, which are the exploration and production platforms and rigs, installed on fixed and mobile structures, or on ships, described below (Corrêa, 2003):

- **Barges:** are used on shallow waters and in swampy regions. Of little importance for the offshore biofouling problems.
- **Jack-up platforms:** are movable units destined to operate in water depths of about 100 meters. They have legs seated on the bottom of the sea, enabling the formation of biofouling communities from the surface all the way down to the seabed. They may be towed from one location to another with their legs raised, which remain very close to the hull. When they arrive at the new location, the legs are lowered to the bottom of the ocean, providing stability to the unit. Most of the jack-up units have 3 or 4 legs positioned vertically or slightly inclined. There is also a slot on one of the sides of the deck, where the drilling rigs are positioned. Almost half of the fleet of platforms currently in operation in the world are of the jack-up type, and the most recent units are self-propelled, increasing their movement capacity (Corrêa, 2003). This type of unit creates countless possibilities for the establishment of fouling communities in water depths of 130 meters (Thomas, 2004).
- **Fixed platforms:** they are basically made of steel or concrete. The steel unit consists in a steel jacket fixed on the ocean floor by steel pillars. They are used in water depths of 300 meters. The steel mesh forms a true reef structure in the new marine environment. The concrete platforms have an aspect quite different from that of the steel units, but with similar operating purposes. They are made of reinforced concrete, towed to the operation location and flooded inside, being fixed by their own weight. These concrete structures form true 'walls' all the way to the ocean floor, and are usually in very shallow waters – down to 40 meters deep. They enable the formation of a continuous community from the surface to the bottom of the sea with the concrete acting as the substratum.
- **Semi-submersible platforms:** these are floating units, self-propelled or not, with great movement capacity and the possibility to act in water depths of nearly 500 meters. When they arrive at the operating location, their pontoons are partially flooded to reach a safe depth for operation and they are then anchored, and this is the contact of the unit with the bottom, in addition to the risers, which are pipes connecting the unit to the wells. The biofouling communities attach to the surface structures and also along the risers, constituting a serious problem in the operation.



6.1 Types of units and their structures used as surfaces for offshore biofouling. (Reproduction of Figure 4.51 of the book *Fundamentos de Engenharia de Petróleo*, José Eduardo Thomas – Organizer – Editora Interciência, Rio de Janeiro, Brazil, with permission.)

- **Submersible platforms:** these are basically used in shallow waters and moved with the help of tugboats. At the operation site they are ballasted until their lower hulls sit on the seabed, which is generally composed of sand and mud (Thomas, 2004). These units are not a concern, as far as the biofouling communities are concerned.
- **Floating platforms:** these may be semi-submersible platforms or drill ships. Ship hulls are adapted or specially constructed to operate in deep waters (more than 1000 meters deep). They are usually anchored with anchors or use a dynamic positioning system (DP) to be fixed at a spot. Since they operate in deep waters, they create a particular environment for the appearance of biofouling communities. Currently, offshore biofouling studies focus on this type of unit to understand the communities that develop on the hulls and on the risers, which extend more than 1000 meters down to the ocean floor.

Figure 6.1 shows examples of platforms.

6.3 Environmental aspects

The artificial structures in the marine environment have different sizes, shapes, materials and textures and may be in shallow waters or in great depths of more than 1000 meters. These structures create real-life ‘islands’

in an area of the ocean where these communities would not be able to develop naturally. The offshore colonization process can be defined as the occupation of a new territory or environment. Biofouling is a special case of colonization of artificial substrata consolidated by organisms that are in the water depth (Railkin, 2004).

The entire process initiates with the transport of molecules and bacteria to meet a submerged surface. In this initial phase there is the formation of the biofilm, which is a layer of bacteria and micro-algae that makes the surfaces more favorable for the arrival of other organisms. Wahl (1989) considers this biofilm formation phase important for the sequence of the process of colonization by eukaryote cells. Thus, the colonization is a sequence of accumulation and growth, in which accumulation is understood as the construction of the fouling community on surfaces consolidated by the result of the transport by marine currents, attachment and recruitment variables (Cairns and Henebry 1982; Caldwell, 1984; Stevenson, 1986).

Biofouling on artificial surfaces represents the possibility of the appearance of a complex biologic community in the offshore and deep water environments, where, without the presence of the substratum, the organisms would not be attached and established to complete their life cycles. Both the biological and the physical aspects control the growth of these communities. The main biological factor could be considered as the intra and inter-specific competition among the organisms and the influence of the predators (Sebens, 1986). The competition occurs basically for space, taking account that at a moment of great concentration of larvae in the environment, the substratum becomes scarce and a very small number of organisms are able to establish (Apolinário, 1999). This aspect generates the formation of conglomerates where the species with calcareous carapaces or shells serve as substrata for other species, completely altering the characteristics of the surfaces, which become more irregular and complex, in addition to the very alteration of the artificial substratum to a substratum of living and dead organisms (e.g., barnacle shells without the organisms) (Apolinário, 2003). The physical aspects that regulate the growth of the marine biofouling are the seawater temperature, salinity, turbidity and actions of the waves on the surfaces.

The appearance of a complex community in the middle of the ocean attracts the presence of fish schools that start to 'visit' this area in search of food and protection, as well as other organisms with reef characteristics that effectively establish in this new community and complete their life cycles in this environment, spawning and increasing their local populations. These communities are perennial in tropical environments, usually presenting small biomass and high diversity, whereas in sub-tropical and temperate regions, they suffer great seasonal influences with the spawning peak of most species occurring in the spring (Smedes, 1984). The annual cycle of the

biofouling communities varies with the reproductive cycles of the organisms. Also very significant is the contribution of the particulate material in suspension in the water depth that interferes directly in the maintenance of filtrating specimens (Abelson and Denny, 1997).

6.4 Biodiversity and bioinvasion

Migration and dispersion mechanisms are characteristics that enable the perpetuation of the species. A group of organisms may disperse into new areas and be able to establish at these new locations. Thus, they expand the area of occurrence of the species until they find a physical or biological barrier, which prevents the expansion of their distribution, and they achieve a state of balance that tends to remain stable for long periods (Shigesada and Kawasaki, 1997). However, in a geological scale of time, the geographic distribution of the species also changes according to the large-scale variations of the climatic and geomorphologic phenomena (Cox and Moore, 1993).

The invasion of a species occurs when it colonizes and remains in an area that it previously didn't inhabit. An invasion generally creates competition or predation relationships between the invading species and one or more native species (Shigesada and Kawasaki, 1997). The competition between native species and the invading species may result in local extinction (competitive exclusion) or in the utilization of a vacant niche, which maintains a balance between the invading species and the native species.

The correct interpretation of the invasions with the distinction between natural dispersive and migratory phenomena, with eventual establishment of populations at new locations (herein referred to as invading species), as opposed to abrupt entries of populations enabled through the human interference (introduced species), is fundamental for the comprehension of the invasion mechanisms of the species. The flow of airplanes and ships made the international boundaries and the areas of natural occurrences of the species easily transposable. The problem of the biological invasions transcends the academic interests, and is also a matter of public health. In the past few years there has been a substantial increase in the number of studies about this theme, and basically the three most relevant and most frequently approached topics are the following: (1) the necessary conditions for an invasion to occur, both on the part of the invading species, and on the part of the invaded ecosystem (Marco *et al.*, 2002); (2) the way an invasion propagates in space (Cannas *et al.*, 2003); and (3) the characteristics of the communities after the invasion (Roughgarden, 1968; Williamson, 1989; Hengeveld, 1994). Relatively few studies, however, examined thoroughly the interaction between invading species and native species (Marco *et al.*, 2002; Cannas *et al.*, 2003).

An important aspect in studies about ecology and dynamics of populations involving invading species is to relate them to the pre-existing ecosystem. The invading species will always interact with the native species (or cryptogenic), either through predation or competition, resulting or not in local extinction. There are three strategies (Shigesada and Kawasaki, 1997) for the success of an invasion with competition between the invading species and native species and the persistence of the invader in the new environment: 1) an invading species takes over the habitat of the native species by direct aggressive competition, excluding the native species; 2) the competition is not so aggressive, or there is possibility to share niches, causing the coexistence between invaders and native species; 3) even being competitively weaker, the invading species keeps making use of spaces and small niches that appear occasionally or periodically. Thus, one can consider that an aggressive invading species tends to occupy its space expelling or eliminating local species (Marco *et al.*, 2002; Cannas *et al.*, 2003). A non-aggressive invading species tends to find a point of balance with the local species and generates coexistence or also adopts a strategy of a fugitive species, occupying the few spaces available, which happens more frequently with the annual plants (Shigesada and Kawasaki, 1997).

On the other hand, defining invading species as threats or pests (Lodge, 1993) may be only a simplistic vision of a greater process (Gherardi, 2000). According to Williamson (1996), one invading species with great potential to establish in the new habitat appears in every ten other invading species with low potential to establish in the natural means, one in every ten of these species with high potential effectively establishes, and one in every ten of the established species becomes a pest. In a timescale of decades, many populations float for years as a result of alterations in the climate and biological interaction (Vermeij, 1991). Following the various different trends, may see the presence of invading species in an environment as extinction agents (Lodge and Hill, 1994), while others understand them as components of a global environmental change, which is not necessarily harmful (Williamson and Fitter, 1996; Daehler and Gordon, 1997).

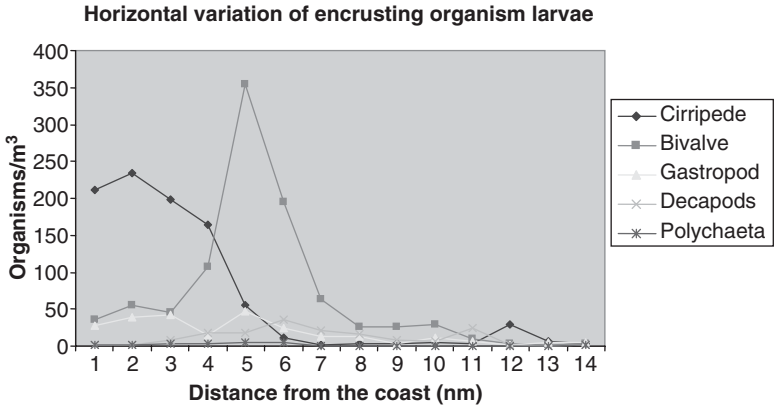
There are two major possibilities for the entrance and establishment of benthonic marine species with larval phases in their life cycles. The first is by means of planktonic larvae, whose main vector would be the ballast waters of the large merchant ships; and the second is the invasion of the organisms in their adult phase, whose vectors would be hulls of ships. The ballast waters have been used since 1880 by large-size vessels and are useful to ensure more stability to the vessel during its journeys across the oceans. These waters are obtained at the port of origin of the vessel and carry native planktonic organisms of the port environment, which are discharged many thousand miles away in the waters of the port of destination. The current era of transportation of large volumes of waters among the oceans is more

than a century old and is modifying the patterns of distribution and dispersion of many benthonic marine organisms. A classic example of a marine species introduced via ballast water is the zebra mussel (*Dreissena polymorpha*), which was taken from the Caspian Sea and Black Sea to other locations in western Europe and later crossed the North Atlantic, probably via ballast water, and established in the Great American Lakes. The cost of the attempt to remove this species was estimated as US\$ 3.1 billion in ten years (Vitousek *et al.*, 1996).

6.5 Offshore colonization and metapopulation

The colonization of new substrata by larvae, spores or propagules of marine specimens depends on the availability of these different methods of dispersion in the water. In the coastal regions, the main contribution seems to occur by the arrival of the bottom communities, whereas offshore the role of the distance from the coast and of the organisms encrusted on floating objects and artificial oceanic structures seem to be preponderant (Railkin, 2004). The marine environment is normally divided into benthonic environment and pelagic environment, and the plankton is part of the pelagic environment (Boero *et al.*, 1996). Most of the encrusting organisms belong, in the first stages of their life cycles, to the dominion of the plankton and only after metamorphosis and recruitment do they become benthonic (Apolinário, 1999). Different studies focused on the aspects of the dispersion of the marine invertebrate larvae rescued. A classic study accomplished by Thorson (1950) considered the supply of larvae and mortality post-recruitment as strong determining factors of marine benthonic communities (Gaines *et al.*, 1985; Roughgarden *et al.*, 1987; Underwood and Fairweather, 1989).

The growth of biological encrustations in oceanic regions is limited by the availability of larvae. Taking into account that the larvae of these organisms are produced on the coastal rocky slopes, the more we move away from the coast, the smaller is the presence of these reproductive structures, and theoretically the biological encrustation would be smaller. A study carried out near the Cabo Frio Island, Rio de Janeiro, Brazil revealed a significant reduction in the number of larvae of encrusting organisms, from sites situated near the coastal slopes to nearly 14 miles from the coast (Fig. 6.2). The regions situated near the coast were dominated by the cirripede larvae (barnacle larvae) followed by the bivalve and gastropod larvae. Beyond 4 miles from the coast, the bivalve larvae achieved their highest values (5 miles). The abundance of decapod larvae also increased beyond 4 miles, however maintaining low densities as far as 11 miles from the coast. Beyond 12 miles, there was a great reduction of the encrusting organism larvae.



6.2 Horizontal variation of the larvae of encrusting organisms between the East End of the Cabo Frio Island and 14 miles to the east (R. Coutinho, non-published data).

The fact that the reduction is not continuous is due to the different water masses present from the coast towards the offshore region, which may have specific compositions. Based on these results, if we place an inert substratum, i.e. without any encrustation, 100 miles away from the coast, it will take a much longer period for it to be colonized by encrusting organisms than one situated 1 mile away from the coast, where the presence of the larvae is stronger.

Nevertheless, in the case of a petroleum platform situated 100 miles away from the coast, the result may be different, taking into account that normally when the platform arrives at its destination site, it already brings a great diversity of encrusting organisms attached to its structure, and very often the very populations present furnish enough larvae to enable a greater growth of the encrustations. Furthermore, a large part of the encrusting organisms, such as sponges, ascidia, corals, etc., have the capability to grow by vegetative propagation and therefore do not need reproductive structures for their increment.

For the offshore and deep water environments, the most realistic approach to the dynamics of the populations that compose the biofouling communities must assume that the local populations recently recruited come from various other geographically distant regions, which compose an offshore metapopulation. This metapopulation is characterized by its vulnerability to the variations in the quantity and composition of the larvae that arrive to recruit along the different seasons of the year. For the maintenance of the life of the meroplankton (temporary life as part of the plankton), some strategies are necessary for dispersion and recruitment feasibility, and the

behavior of the larvae in relation to luminosity, pressure and their capacity to remain in the water are fundamental (Railkin, 2004).

Thorson (1950, 1964), suggested that the vertical distribution of larvae that belong to the biofouling communities is divided into more than 100 species into groups according to their responses to the light factor during their first stages of life in the plankton. The first and largest group, with almost 80% of the species, is photopositive and includes some species of cirripedes, hydroids and polychaetes, as well as some bivalve mollusks. Almost all of these organisms attach to consolidated substrata and this group includes not only planktotrophic larvae but also lecithotrophic larvae (Crisp, 1984). These organisms of planktotrophic and some lecithotrophic characteristics are basically concentrated in the surface waters of the oceans, including offshore regions, with almost the entire meroplankton concentrated in the first 10 meters of the water depth (Maximovich and Shilin, 1993). Both on the continental shelf and offshore, the distribution of the meroplankton is similar, and is only different in the quantitative aspect, with the first 300 meters of the water depth being rich in larvae of mollusks, cirripedes, equinoderms and bryozoans, which are important components of the biofouling communities (Mileikovsky, 1968).

Results obtained in the Campos Basin, Brazil, for example, revealed the presence of cirripede larvae (barnacles) at the depth of 100 meters. Other organisms (alevin, copepodites, etc.) 'reappeared' at 400 meters, which corresponded to the presence of a warmer water mass on the bottom, which was evidenced by the temperature, oxygen and nutrient showing a water mass quite typical from 200 to 300 meters in the water depth. This water with temperature and high levels of nutrient is, according to studies made on the Brazilian coast, of the Falkland Islands Current (Central Waters of the South Atlantic – ACAS), which is the same water observed in the Cabo Frio resurgence.

These results demonstrate that besides the reduction of the encrusting organism larvae towards the offshore regions, there is also a significant reduction towards the bottom, which limits substantially the attachment, recruitment and development of the encrusting communities at great depths (Table 6.1).

In the offshore region the larvae disperse beyond the coastal lines and their major currents. These currents can carry them along considerable distances of up to hundreds of kilometers (Crisp, 1958), until this larvae find propitious substrata for recruitment. On this transport along the currents the larvae derive from coastal regions to the open sea and encounter artificial surfaces where they recruit and form islands of encrusting populations, which in turn will disperse new larvae and reach new distribution limits through the years. A typical example is the report of Mileikovsky (1971), which describes the process of new occurrences of the barnacle named

Table 6.1 Density of zooplankton in the water (number of individuals/10 liters) collected at different depths (in meters) in the Campos Basin, Brazil, on Feb/08/1998, (R. Coutinho, non-published data)

DEPTHS	1 m	10 m	20 m	30 m	50 m	100 m	200 m	300 m	400 m
<i>Nauplii</i>	4		4	3		2			4
<i>Copepodite</i>	49	15	9	7	14	9			12
<i>Salpa</i>	4								
<i>Paracalamus quasimodo</i>	2	1	2			1			
<i>Decapod larva</i>	3								
<i>Limacina inflata</i>	1				2				
<i>Oikopleura longicauda</i>	1		1	3	2				1
<i>Oithona plumifera</i>		1							
<i>Oithona setigera</i>									1
<i>Oncaea media</i>		3	1		3	1			1
<i>Oncaea subtilis</i>		1	1		3				1
<i>Evadne Tergestina</i>		1		1					
<i>Tornaria larva</i> (polychaete)		1	1						
<i>Creseis acicula</i>			1		1				
<i>Doliolidae</i>					1				
<i>Cirripede larva</i>						1			
<i>Microsetela rosea</i>						1			
<i>Alevino</i>									1
<i>Corycaeus typicus</i>									2
<i>Mecynocera clausi</i>									1

Elminius modestus in the northwest region of Europe. This species that migrated from the west coast of Europe to the British Isles expanded along the Welsh coast at a speed of 20 to 30 km per year (Railkin, 2004). This example demonstrates the important role of the benthonic populations on natural or artificial substrata dispersing their larvae in the water, exercising a fundamental function in the dispersion of biofouling communities in wide oceanic areas.

The larvae have the capacity to form complex metapopulations and disperse along hundreds and even thousands of kilometers in the oceans, finding new surfaces in order to establish new populations. As a result we have new colonized territories, genic flow along great distances and among the metapopulations of different organisms (particularly barnacles) and formation of new biogeographic frontiers, mixing natural dispersion and distribution processes, with the presence of artificial offshore structures enabling the formation of metapopulations very distant from the coastal regions.

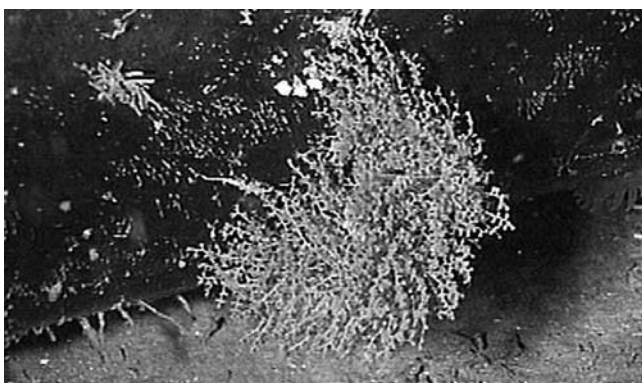
6.6 Observing biofouling on underwater structures

Photographs (Figs 6.3 and 6.4) taken on platform structures in the Campos Basin reveal a rich and dense fauna of encrusting organisms even at great depths.

At the depth of 45 meters, we could observe the presence of anemones, with their tentacles open, with a density of approximately 35 individuals per 100 cm², covering approximately 80% of the available substrata. These soft body organisms feed on live or dead material in suspension, which is grabbed or stuck on their tentacles. Among the anemones we notice the presence of orange-colored encrustations (sponges) on the substratum. The



6.3 Vehicle used for hull structural inspection at FPSO/FSO unities passing over the biofouling. (Photograph from PETROBRAS files, with permission.)



6.4 Biofouling at a 745 meter depth pipeline in Campos Basin, Rio de Janeiro, Brazil. (Photograph from PETROBRAS files, with permission.)

presence of small shells of dead cirripedes (barnacles) was also observed. The presence of anemones may be related to the fact that the observed jacket has a horizontal section. On the vertical sections the presence of these organisms is not observed.

At the depth of 55 meters, we could observe a reduction of the density of anemones in relation to the previous depth, but a conspicuous presence of tubes of white polychaetes extending on the substratum.

At the depth of 66 meters, we observed a great quantity of tree-like tube corals (gender *Carijoa*), of calcareous formation, which may be up to 10 centimeters thick.

The inspection of the cooling water intake tube accomplished by a robot (ROV) evidenced the total absence of encrustations, both on the rigid tubes and on the neoprene pipes, from the collecting depth (344 meters) down to the depth of 175 meters, when small colonies of polychaetes began to appear. These results corroborate the field data obtained concerning the hydrologic conditions and density of zooplankton, which verified the presence of a cold water mass, deprived of zooplankton, between the depths of 200 and 300 meters.

In the film that was shown we could observe the start of the formation of a biofilm at the depth of 175 meters. At some locations we could also observe structures that were reminiscent of polychaetes and at 164 meters we observed a probable presence of hydrozoans. At 122 meters we saw an invertebrate and after 109 meters we observed the presence of polychaetes, hydrozoans and barnacles, and generally, the presence of the encrustations becomes more evident above the depth of 100 meters.

Our observations accomplished based on films and photographs of jackets and underwater cables demonstrated that most of the communities present on them are composed of colonial organisms whose growth occurs by sprouting or vegetative propagation, which reduces their dependence on the meroplanktonic larvae.

It is obvious that even with the arrival of few larvae at deeper locations the species that depend on these reproductive structures can, in the long run, establish populations with relatively high density. This is the case of petroleum platforms, which in a period of time of 10–20 years can accumulate larvae and so develop their populations. However, these aspects will be chiefly due to the patterns of the local currents that will transport the larvae from the surface or even re-suspend or deepen the larvae by means of internal waves.

Another important aspect to be considered is the density of the encrusting organisms. Denser organisms offer more frictional resistance and are also heavier on the structures. Among the various species whose densities were estimated, we can highlight the mussels and the barnacles, which are dominant organisms on most of the underwater structures. This high density

associated with the formation of rigid structures makes these communities able to achieve great encrustation thickness. In the studies that we made on steel plates without treatment we obtained 11.3 cm in a period of two years, basically formed by these two groups of organisms.

6.7 New frontiers in fouling studies

The importance of the naval industry and the new offshore enterprises related to the gas and oil industry in the past few years and in different seas of the world has been creating new fixed substrata (platforms) and mobile substrata (vessels) that enable the establishment of new biofouling communities and a new biofouling dynamics appears as a challenge for the researchers. Older studies concentrated on biofouling communities on fixed petroleum platforms, mostly situated in shallow waters, as well as on navigation signaling and marking buoys (De Palma, 1972; Ralph and Troake, 1980; Hardy, 1981). More recent studies made by Yan and Yan, (2003) and Yan *et al.*, (2004) are focused on deeper environments and on non-fixed platforms, which are typical in the exploration and production of oil and gas in the China Sea.

Brazil has a condition similar to that of China in the exploration and production of petroleum in deep waters, and experiments on platforms and anchoring lines are being planned to understand the offshore biofouling phenomenon in the western south Atlantic.

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The effects of corrosion and fouling on the performance of ocean-going vessels: a naval architectural perspective

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Abstract: The primary role of antifouling precautions on ship hulls is to avoid the tremendous increase in drag resistance caused by the settlement of fouling species. In this chapter, basic hydrodynamic concepts needed to fully understand the effect of fouling species on the propulsion requirements of a ship will be introduced. Subsequently, studies on the drag resistance associated with various coating types and fouling species are reviewed. Finally, the main topic of this chapter is to discuss how to monitor the hydrodynamic performance of an antifouling coating applied to an actual ship during service through appropriate data logging and mathematical models.

Key words: drag resistance, boundary layer, hull roughness, hydrodynamics, ship powering.

7.1 Ship hydrodynamics basics

The primary role of a ship antifouling coating is to limit the increase in frictional drag as a result of surface deterioration and biofouling accumulation. Frictional drag alone can account for as much as 90% of the total drag on some hull types, even when the hull is relatively smooth and unfouled (Schultz, 2007). Hence, for a given ship design, the coating condition is crucial to the performance of ships. Frictional drag in a ship is directly linked to the interaction between the moving hull and the surrounding seawater. As the ship moves, a significant mass of water, sometimes reaching 1/4 or even 1/3 of the total mass of the ship, is accelerated to a speed close to that of the ship. The consequence of this is that the engine must deliver additional power to keep constant speed.

The boundary layer is the area closest to the hull in which the fluid is impeded as a result of its viscosity. The relative velocity at the surface of an object is zero (no-slip condition) and, at some distance away from the object, the velocity is the freestream value. In the layer between the two, there must

be a velocity gradient. The thickness of the boundary layer, δ , increases down the length of the hull and may reach a meter or more in thickness on a large ship (Schultz and Swain, 2000). In a boundary layer developing over a surface, the flow remains laminar for a distance downstream. After some development, instabilities arise, and the flow begins to transition to turbulence. The transition for the case of a smooth flat plate occurs approximately when:

$$\text{Re}_x = \frac{U_e \cdot x}{\nu} > 1 \cdot 10^6 \quad 7.1$$

Re_x is the Reynolds number at point x placed x meters downstream.

U_e is the freestream fluid velocity and ν is the kinematic viscosity of the fluid.

For a ship moving at 10 m/s (almost 20 knots), transition takes place at about 10 cm downstream of the bow. We will therefore focus on turbulent boundary layers, which cover the majority of the hull. Further downstream, the boundary layer may detach from the hull, and flow reversal may occur (point of separation).

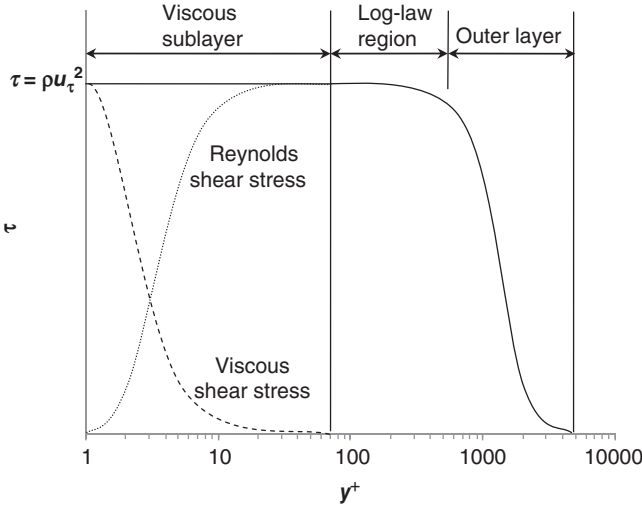
Ship powering requirements are directly linked to the shear stress along this boundary layer. In the boundary layer, the total shear stress is made up of both viscous ('laminar') and Reynolds ('turbulent') stresses.

$$\tau = \mu \cdot \left(\frac{\partial U}{\partial y} \right) - \rho \cdot \overline{u'v'} \quad 7.2$$

To understand the meaning of viscous drag, one needs to imagine the fluid as layers move smoothly over each other without macroscopic mixing. The layers close to the hull have higher velocity and therefore there is a shear stress between these and the upper layers further away from the hull surface. In turbulent flow the particles move both horizontally and vertically and there is a continuous 'mixing of particles' causing continuous exchange of momentum.

Figure 7.1 shows a typical shear stress profile as a function of the normalized distance from the wall (y^+).

Wall roughness, in the form of, e.g. fouling or coating defects, leads to increased turbulence and fluid mixing in the boundary layer. This manifests itself as increased turbulent and wall shear stress (i.e., increased powering requirements). Before discussing the effect of fouling on ship drag, we will introduce some basic boundary layer concepts.



7.1 Turbulent boundary shear stress profile as a function of dimensionless distance from the hull. Adapted from (Schultz and Swain, 2000). ' y^+ ' equals $y \cdot U_\tau \cdot \nu^{-1}$. U_τ is the friction velocity and equals $(\tau_w/\rho)^{1/2}$, with τ_w being the wall shear stress and ρ the seawater density. ν is kinematic viscosity of the fluid and y is the distance from the hull.

7.2 Turbulent boundary layer basics

The turbulent boundary layer is considered to consist of several regions characterized by their water velocity profile. These regions include the viscous sublayer, the log-law region, and the outer region (Fig. 7.2).

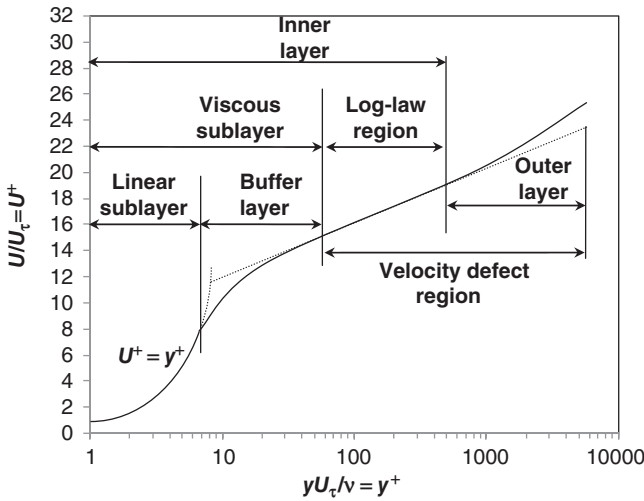
1. The viscous sublayer covers the innermost 10–20% of the turbulent boundary layer $y/\delta = 0.1\text{--}0.2$. Despite its low thickness, about 70% of the velocity gradient is found in this region. The local mean velocity in this region is a function of the wall shear stress, fluid density, kinematic viscosity, and distance from the wall. The law of the wall is expressed as:

$$\frac{U}{U_\tau} = f\left[\frac{y \cdot U_\tau}{\nu}\right] \Rightarrow U^+ = f[y^+] \quad 7.3$$

U_τ is the friction velocity and is expressed as the square root of the ratio between the wall shear stress τ_w and the fluid density.

$$U_\tau = \sqrt{\frac{\tau_w}{\rho}} \text{ with } \tau_w = \mu \cdot \left(\frac{\partial U}{\partial y}\right)_{y=0} \quad 7.4$$

The viscous sublayer consists of two parts, the linear sublayer and the buffer layer.



7.2 Law of the wall plot for a turbulent boundary layer (from Schultz and Swain, 2000).

- i. Linear sublayer: $y^+ \leq 7$, $U^+ = y^+$. Across this layer the total shear stress is almost constant and equal to the wall shear stress (Fig. 7.1). The wall shear stress is often normalized with the dynamic pressure to form the skin friction coefficient:

$$C_f = \frac{\tau_w}{1/2 \cdot \rho \cdot U_e^2} \quad 7.5$$

where U_e is the mean axial velocity at the edge of the boundary layer.

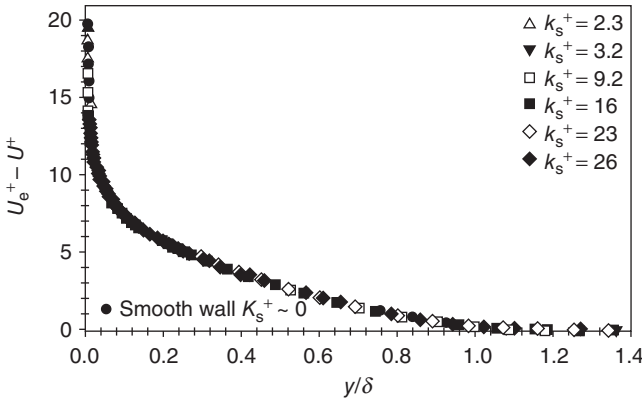
- ii. Buffer layer: $7 < y^+ < 40$. The velocity profile departs from linearity and shows high turbulence.
2. Log-law region: the flow just outside the viscous sublayer ($y^+ > 40$) and with $y/\delta > 0.15$ is also highly turbulent in nature. In this region, also called the log-law region, we find that:

$$U^+ = A \cdot \log(y^+) + B \quad 7.6$$

3. Outer region: in this layer the difference between the freestream velocity and the local mean velocity U at a distance y from the wall is determined by the boundary layer thickness δ and the friction velocity U_τ .

$$\frac{U_e - U}{U_\tau} = g \cdot \left(\frac{y}{\delta} \right) \quad 7.7$$

where $g(y/\delta)$ is a universal function (Schetz, 1993)



7.3 The velocity profiles in the outer layer are shown to be largely insensitive to the surface roughness (Schultz, 2007). Reproduced with permission from Taylor & Francis.

This region is thought to be independent of surface roughness, except for its influence on setting the velocity (U_τ) and length (δ) scales. Figure 7.3 depicts the turbulent boundary layer mean velocity profiles throughout the boundary layer showing the insensitivity of the outer layer to the surface roughness. In this plot, the roughness height, k , is assumed to be equal to the equivalent sand roughness height, k_s , for the case of uniform, tightly packed sand, which gives the same roughness function as the roughness of interest in the fully rough flow regime (Schultz, 2007).

7.2.1 Wall roughness

Wall roughness leads to increased turbulence and fluid mixing in the boundary layer. This phenomenon manifests itself as increased turbulent and wall shear stress. For most surfaces the increased skin friction depends somehow on ' k ,' the roughness height. Three distinct flow regimes can be identified for this type of roughness depending on the value of the roughness Reynolds number:

$$k^+ = \frac{k \cdot u_\tau}{\nu} \quad 7.8$$

1. Hydrodynamically smooth regime: $k^+ < 5$ (or 3, according to Schultz and Flack, 2007) and the roughness elements are sufficiently small to be completely submerged in the linear sublayer. In this case, the skin friction is equal to a smooth surface and is dominated by the viscous component.
2. Intermediate regime: $5 < k^+ < 70$ (25, according to Schultz and Flack's tests), the skin friction is increased, and τ_w depends on both viscous and

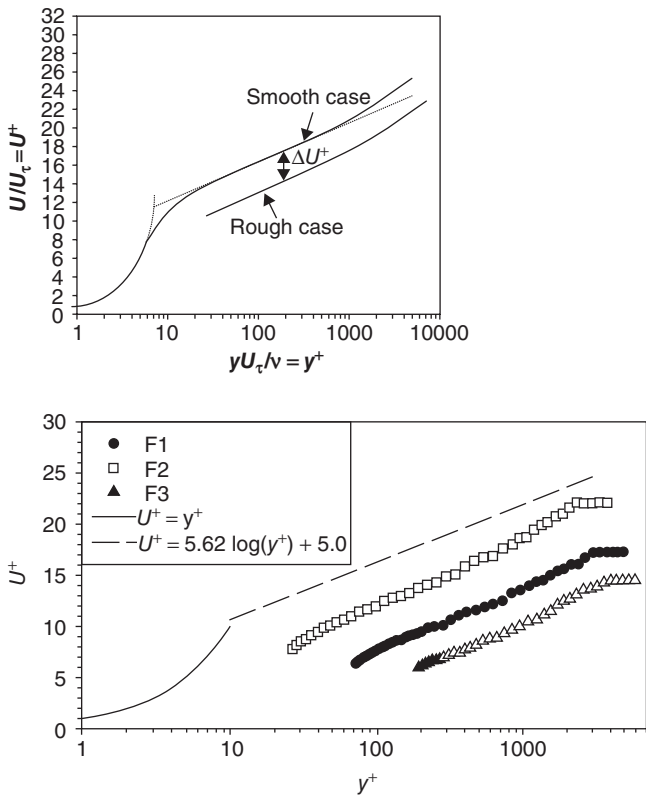
turbulent components. This is the most significant for the majority of practical full-ship conditions.

3. Fully rough regime: $k^+ > 70$, the linear sublayer is completely destroyed, and τ_w is dominated by the turbulent components.

If we look at the velocity profile within the boundary layer, the effect of roughness can be expressed as:

$$U^+ = \frac{1}{\kappa} \cdot \log(y^+) + B - \Delta U^+ \quad 7.9$$

where κ is the von Karman constant ($= 0.41$), B is the smooth wall log-law intercept ($= 5$), and ΔU^+ is the roughness function.



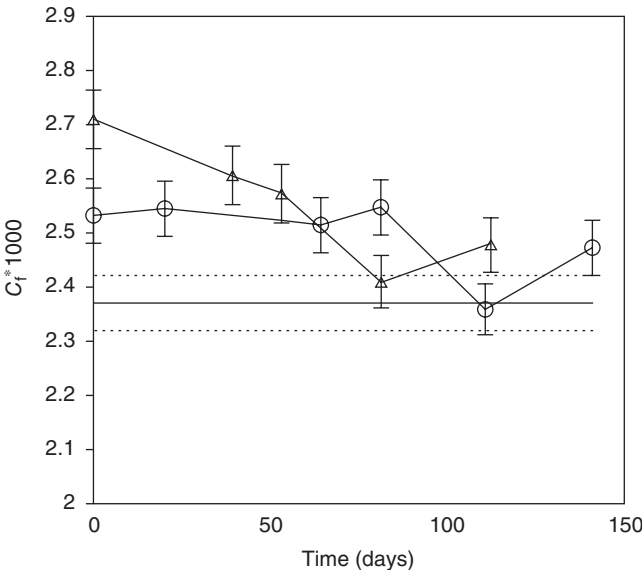
7.4 The theoretical effect of roughness on the law of the wall (top). On the bottom, experimental measurements showing the effect of biofilms on the law of the wall as measured by Schultz and Swain (2000) are shown. Reproduced with permission from Taylor & Francis.

ΔU^+ expresses a downward shift of the velocity profile of the log-law region and is directly related to the increase in frictional drag of the surface (momentum deficit). Unfortunately, a general correlation between ΔU^+ and measurable surface parameters (represented as k^+) does not exist, despite several attempts to find one are reported in the literature (Schultz and Swain, 2000). The relationship between different fouling types and roughness is elaborated further in Section 7.4.

An example of the effect of increased roughness (slime buildup, in this case) on the velocity gradient across the boundary layer is shown in Fig. 7.4 (Schultz and Swain, 2000).

7.3 Coating type and associated drag

Soon after the introduction of foul release coatings in the mid 1990s, it became evident that the choice of coating type does influence a ship's hull resistance right after application. Long-term fouling deterrence is the most important feature when discussing the drag performance of an antifouling coating, as stated by Weinell *et al.* (2003) (Fig. 7.5). In other words, any initial positive influence that coatings have on ship drag are meaningless unless such effects can be maintained during immersion time until the next dry-



7.5 Decrease in the friction coefficient with dynamic exposure time as a result of self-smoothing of the topcoat. From Weinell *et al.* (2003). Reproduced with permission from Taylor & Francis.

docking operation (as much as 60 months later in many cases). In spite of this, a number of tests have been carried out in the last few years comparing the drag resistance of freshly applied fouling-free silicon coatings to that of traditional self-polishing paints in fouling-free waters (Table 7.1). In all studies, silicone topcoats demonstrate improved hydrodynamic behavior compared to eroding-type paints. This difference is hypothesized to result from differences in surface texture, as reported in Candries *et al.* (2003). While tin-free SP coatings presented a spiky ‘closed’ texture, the silicon topcoat featured a wavy ‘open’ texture with a smaller proportion of short-wavelength roughness (Anderson *et al.*, 2003). On top of this, current fouling release coatings are usually applied after a full blasting of the hull, which is also expected to smooth out hull roughness inherent to old hulls hence providing additional fuel savings (see, e.g., Section 7.10). Only the study by Holm *et al.* (2004) reports higher drag resistance for a silicone topcoat compared to an eroding-type coating, but those measurements were performed after cleaning the fouling settled on the different topcoats after three weeks of static exposure in Chesapeake Bay. Hence, small-scale surface damage may be responsible for such results.

In Table 7.1, it is also important to note that self-polishing topcoats do smooth out during service, as demonstrated by Weinell *et al.* (2003), which is not reflected in the above studies. To the author’s knowledge, there exists no systematic study comparing drag behavior of fouling control coating families with ageing time in the presence of fouling species under realistic speed-activity conditions. Schultz (2004), as an example, compares self-polishing and fouling release topcoats after static exposure to natural seawater, which are not the most relevant conditions for fouling release topcoats.

Table 7.1 Representative differences in friction coefficient when comparing clean fouling release coatings to self-polishing type ones. Non-fouled silicone topcoats are reported to consistently decrease the drag resistance of a hull compared to eroding-type paints

Source	$\Delta C_F\%$	Remarks
Weinell <i>et al.</i> (2003)	6.1%	Rotary study. Topcoat on smooth PVC
Candries <i>et al.</i> (2003)	3.5%	Rotary study. Full system on smooth PVC
Schultz (2004)	3.0–3.8%	Full system on 304SS. No sandpaper strip
Holm <i>et al.</i> (2004)	–2.5%	Friction disk machine. After biofilm removal. Potential surface damage
Candries and Atlar (2005)	5.3%	Topcoat on smooth steel. Turbulent boundary layer measurements
Anon (2008)	1.4%	Towing test. Full system on smooth Al/ smooth undercoats

7.4 Fouling species and related drag

As mentioned before, it would be quite convenient to be able to relate ΔU^+ (i.e. indirectly C_f) to measurable surface parameters (represented as k^+). Ideally, such a correlation would also be applicable to fouling species, so that we could estimate increased hull resistance for almost any hull condition. Unfortunately, the latter is quite a difficult task, especially given the enormous complexity and variability of fouling communities.

Townsin (2003) provided a summary of reported drag increase associated with different fouling species from real-life ship measurements (Table 7.2). It is important to note that this type of measurement has a very high associated uncertainty, as discussed in the second part of this chapter.

Schultz and Swain (2000) carried out laser Doppler velocimeter (LDV) boundary layer measurements on heavily slimed specimens in a water channel. Large scattering in the increased C_f as a result of the growth of biofilms on the acrylic panels was reported, with values reaching 370% in specific cases. Holm *et al.* (2004) reported a drag increase of 9–29% from friction disk machine measurements of slimed, fouled topcoats. The disks had been exposed under static conditions for three weeks in Chesapeake Bay. Schultz (2004) carried out towing tank tests with panels immersed for more than nine months at Severn River (Annapolis). For the silicone plates (worst case), the C_f increased 300–400%, while 4% barnacle fouling on an ablative copper coating increased the C_f by 138%. In the same study, a thin slime film on a tributyltin self-polishing copolymer paint was reported to increase drag by 58–68%.

Perhaps the best summary of all of the above information comes from the attempt by Schultz (2007) to relate the hull fouling condition to equivalent sand roughness height k_s and the average coating roughness (Rt_{50} ; Table 7.3).

Naval Ships' Technical Manual (NSTM) ratings below 30 are used to denote coverage of soft fouling only. A rating of 40 indicates the initial

Table 7.2 Reported effect of fouling on the hull's frictional resistance (see Townsin, 2003 and Schultz and Swain, 1999 for full reference list)

Slime	5%	Conn <i>et al.</i> (1953)
	8–14%	Watanabe <i>et al.</i> (1969)
	18%	Lewkowicz and Das (1986)
	10–20%	Loeb <i>et al.</i> (1984)
	25%*	Lewthwaite <i>et al.</i> (1985)
	8–18%*	Bohlander (1991)
Shell and Weed	85%	Kempf (1937) 75% coverage shell 4.5 mm.

* Also some hard fouling and/or macroalgae.

Table 7.3 Reported effect of different hull conditions on the total resistance and shaft power for the case of US Navy Oliver Hazard Perry class frigate. Increased fouling severity, represented as increased average coating roughness (Rt_{50}) and equivalent sand roughness height (k_s) values, involves higher frictional resistance and, consequently, higher powering demands to keep a speed of 15 knots (Schultz, 2007).

Description of condition	NSTM rating	k_s (μm)	Rt_{50} (μm)	ΔR_t (%)	ΔSP (%)
Hydraulically smooth surface	0	0	0	–	–
Typical as applied AF coating	0	30	150	2	2
Deteriorated coating or light slime	10–20	100	300	11	11
Heavy slime	30	300	600	20	21
Small calcareous fouling or weed	40–60	1000	1000	34	35
Medium calcareous fouling	70–80	3000	3000	52	54
Heavy calcareous fouling	90–100	10000	10000	80	86

presence of calcareous fouling. As the degree of shell fouling increases, the rating rises to a value of 100, which is given for heavy, large-size shell fouling. The increase in C_f as a result of increased surface roughness is estimated using the similarity law scaling procedure for the case of the US Navy Oliver Hazard Perry class frigate. This frigate has a waterline length of 124.4 m with a beam of 14.3 and displaces 3779 tonnes. Different results would be obtained for larger trading ships such as tankers and container vessels. The increased total resistance and shaft power for the above-mentioned ship sailing at 15 knots are given in Table 7.3.

7.5 Introduction: fouling and ship powering

Marine biofouling begins to accumulate on the submerged portion of an oceangoing vessel within minutes of making contact with the water. Over time, this accumulation increases the drag of the vessel, causing the physical resistance of the vessel to increase. As a result of fouling drag on the vessel, higher fuel consumption to maintain a given speed or lower speeds at a maintained power will occur.

Owners of oceangoing vessels spend considerable time and money to mitigate the effects of fouling on vessel performance. Mitigation can be accomplished by several means, including pre-treating the surface of the hull in drydock, as well as increasing the frequency of vessel drydocking, which usually takes place every 2½ to 3 years or 5 years, depending on the age of the vessel. In addition, a wide variety of coating manufacturers and antifouling technologies are available, depending on vessel type, speed, trading area, activity rate (time away from ports), and geographic voyage pattern.

Typical costs of hull treatment during a drydocking can range from tens of thousands of dollars to several million dollars depending on vessel size, the type of coating system applied, and the pretreatment of the hull prior to the coating application. Between drydockings, the vessel operator may undertake hull cleanings or propeller polishing in order to regain some of the vessel performance losses resulting from fouling and corrosion. Hull cleanings cost between 10 000 and 30 000 USD depending on the portion of the underwater hull that is cleaned and the cleaning technique employed, and may require between one and three days in port. Propeller polishing costs range from 2000 to 5000 USD and can be done in one day.

TBT coatings were developed in the 1970s, and over a period of 20 years have proven to exhibit an excellent ability to prevent fouling accumulation, including slime (mainly bacterial; see Chapter 17 for more details.) The ban on TBT coatings enacted by the International Maritime Organization in 2003 spurred the development of many new coating systems. These modern alternatives contain less toxic biocides; however, as a result of the presence of less effective – or, in some cases, no – biocides in the coating, the rate of fouling on vessel hulls is in general higher than with the good-quality TBT predecessors. Over the period of a three- or five-year drydocking interval, the advantages of efficient pretreatment and good-quality coating systems become more important, given higher fuel costs and governmental interests in mitigating the biorisk associated with heavily fouled hulls.

The interest in sustaining high hull and propeller efficiency in the total picture of economic and environmental efficiencies has increased substantially over the past few years, mainly due to higher fuel prices, as well as pressures to reduce marine vessel emissions and mitigate the translocation of marine biofouling pests attached to the underwater portion of vessel hulls. The following section will examine in more detail vessel performance and the effect of fouling on the speed–power relationship for typical ocean-going vessels with single displacement hulls.

It should be kept in mind that the overall CO₂ and GHG emissions from oceangoing vessels are extremely low (emissions per ton-mile of goods moved) within the context of all modes of transportation (Swedish Network for Transport and the Environment). Nevertheless, more efficient, non-toxic antifoulant systems for the underwater portion of the hull will still benefit the environment greatly.

7.6 Background on vessel performance

Many vessel owners are not aware of the true impact that fouling has on vessel performance, owing to the inherent limitations of performance monitoring systems. Nowadays, methods for more precise analysis of vessel performance, based on the standard measuring equipment onboard, are

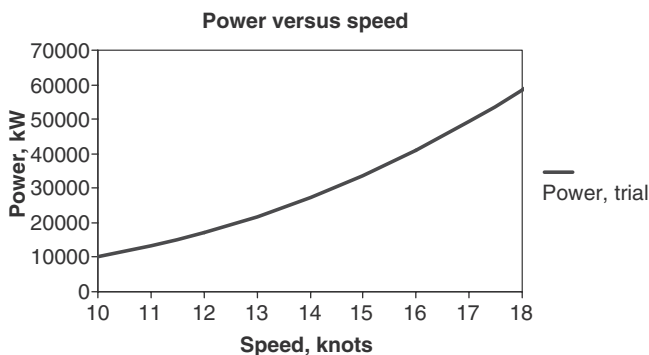
available, as will be described in this chapter together with some examples of the results that have been achieved.

7.6.1 How vessel performance is measured

For most vessels delivered from a shipyard there is a diagram showing the relation between speed and required power for one or more loading conditions, as shown in Fig. 7.6. This diagram has been prepared based on theoretical calculations and in most cases has been confirmed by towing tank tests. The towing tank is a basin, several meters wide and hundreds of meters long, equipped with a towing carriage that runs on two rails. The towing carriage can either tow the model or follow the self-propelled model, and is equipped with computers and devices to register or control, respectively, variables such as speed, propeller thrust and torque, rudder angle, and so on. The towing tank serves, in resistance and propulsion tests with towed and self-propelled ship models, to determine how much power the engine will have to provide to achieve the speed laid down in the contract between shipyard and ship owner. Figure 7.6 is an example of the power versus speed diagram derived from the towing tank and/or from sea trials.

7.6.2 Sea trials

Formal speed trials are a necessary procedure for fulfilling contract terms between the shipyard and ship owner. Contract terms usually require that the speed be achieved under specified conditions of draft and deadweight, a requirement met by runs made over a measured ocean course. This speed trial is a complicated and time-consuming procedure. The vessel must be loaded correctly, the weather must be reasonably good, and the trial must take place in a test area with deep water at a time when there is no other



7.6 Theoretical power versus speed for an oceangoing vessel.

immediate traffic. Time must be given to accelerate the vessel up to a constant steady-speed and, as a sea current may be present, each speed run has to be made twice in opposite directions to compensate for this. Consequently, only a limited number of draft/speed combinations are tested, so the achieved speed/power results, properly adjusted for wind, waves, temperature, salinity, and draft differences, are used merely to confirm or adjust the already existing diagram.

7.6.3 Sea trials: power versus speed

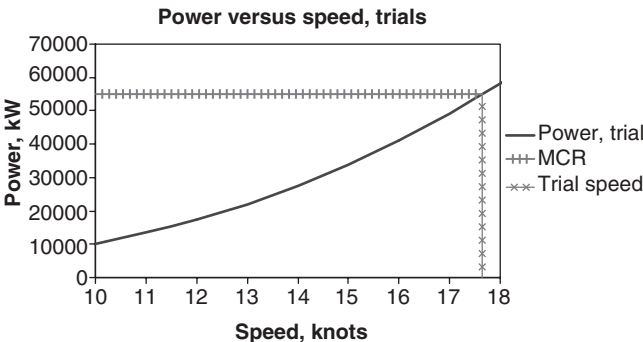
If the engine's maximum continuous rating (MCR) is plotted in this diagram, the maximum speed for the ship may be found, as illustrated in Fig. 7.7.

Vessel owners know that this is not the speed they can expect in daily operation; for commercial consideration, they define a so-called 'service speed.'

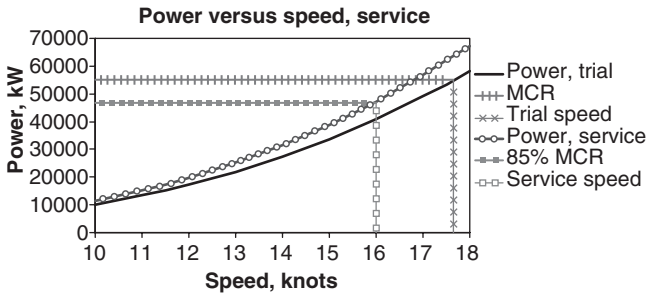
7.6.4 Vessels in service: power versus speed

This service speed is traditionally found by adding 15% to the power curve and subtracting 15% from the engine power line, as shown in Fig. 7.8. The 15% added power is expected to consist of 5% for weather losses and 10% for losses due to hull and propeller surface roughness caused by marine growth and corrosion. For a well-organized introduction to ship propulsion, see Man (2004).

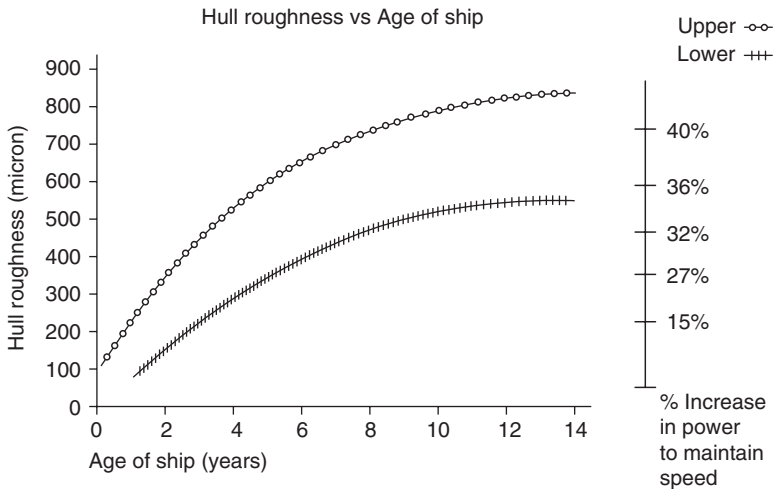
The actual situation with respect to marine fouling for any particular vessel may be worse. This situation will only be discovered if the fouling is significant, because it is very difficult in practice to obtain a reliable and accurate picture of the speed/power performance of a vessel in service.



7.7 Actual power versus speed for an oceangoing vessel confirmed by sea trials.



7.8 Service speed calculation for an oceangoing vessel.



7.9 Increased average hull roughness with ship age and effect on powering.

Estimates of increases in fuel consumption from biofilm attached to the hull alone range from 8% to 12%, and from normal propeller fouling range from 6% to 14% (Haslbeck, 2003). Hull and propeller fouling are the topic of increased focus as causes of increased fuel consumption and corresponding increases in GHG emissions from oceangoing vessels (Buhaug, 2005).

7.7 Degradation of vessel performance

The main reason for performance degradation is marine growth and roughness on the vessel's hull. Figure 7.9 shows the hull roughness increasing over time and the increased power needed to maintain speed (or speed losses at

a maintained power; Swain, 2007). This is because the ship's viscous resistance increases markedly with increasing roughness (IMO, 2000). More careful hull pre-treatment and coating application work during drydock stopovers (at 5, 10 and 15 years) would halt or reduce this hull roughness increase.

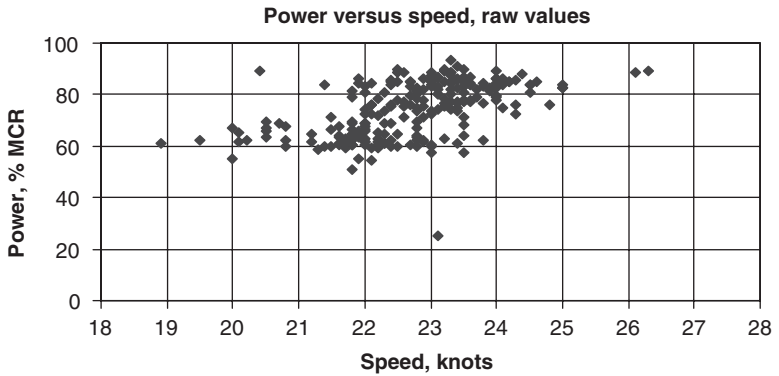
Vessel managers devote resources of time and money to prevent or mitigate the degradation. The main remedies are various types of hull surface preparation in drydock, coatings applied to the underwater portion of the hull at regular intervals (see, e.g., Chapter 18), and in some cases, in-water cleaning of the hull and/or polishing of the propeller. Many factors are emerging in the shipping industry that make the investigation of hull fouling as a mode of invasive species translocation more important. These factors include larger vessels that travel farther and faster, as well as the ban on TBT hull-coating systems, considered the most successful antifouling coatings ever developed (Swain, 2007).

Altogether, the total costs of all vessel owners' antifouling precautions are of the order of 1.5 billion USD per year, or approximately 5% of total marine fuel oil costs. Unfortunately, it is difficult for an owner/operator to determine whether this money is optimally invested. There are many different types of hull treatments, and the price for the coatings varies greatly. In addition, each vessel owner has his or her own way of handling coating selection and directing efforts to control marine fouling. Furthermore, it is difficult to evaluate and compare the effect of the different hull treatments, unless reliable methods of performance analysis are available.

7.7.1 Monitoring of vessel performance

Most vessel operators have established a procedure for speed/power monitoring, for instance by measuring the daily fuel consumption and the daily distance covered at noon-time when nice weather prevails. In this way, the daily mean power and mean speed may be calculated, and the result may be plotted in the speed/power diagram for comparison with the trial trip results. Unfortunately, results achieved in this way usually scatter so much that it is impossible to conclude anything directly from such a diagram, as may be seen from Fig. 7.10, which is a plot for a well-maintained containership.

Procedures may also have been established for more precise measurements at longer intervals – for instance, once a month. A day with good weather may then be chosen. In such cases, and where the prime mover is a slow-running diesel engine, the power may be measured more accurately by cylinder indication, and speed may be measured over a period of time (for instance, two hours) at constant power on a constant course. The result of such an exercise will be more accurate than one based on 'noon data;' however, even such monthly results scatter to an extent that an accurate service speed prediction may be difficult or impossible to obtain.



7.10 Power versus speed: raw performance data for an oceangoing vessel.

Underwater inspections of the hull as a supplement to speed and power measurements are of course useful; however, they do not provide a meaningful metric between fouling and impact on vessel performance.

7.7.2 Factors influencing speed/power monitoring

There are many reasons why the directly obtained speed/power values are scattered as in the above illustration. The main factors that need to be taken into account are:

1. Drafts. Mean draft and trim has a great influence on the vessel resistance. It is reasonably easy to adjust the results for differences in mean draft, but differences in trim are more difficult to deal with, especially when most ships today are equipped with a bulbous bow.
2. Weather. Wind and waves can seldom be ignored; therefore, the results will need to be corrected accordingly. It is not that difficult to measure and make corrections for the wind, but waves can neither be measured (by instruments) nor be easily corrected for.
3. Sea current. Today the speed over ground may be measured with great accuracy by means of the DGPS; however, this speed will not be the true speed due to the presence of sea current. The true speed, the speed through the water, is more difficult to measure. The problem is that there is no assurance that the speed-log is measuring speed outside of the ships boundary layer. Normally, it will not be possible to correct the speed for sea current unless a reciprocal run is performed, and this extra step is too time-consuming to be done during commercial operation.

4. Temperature and salinity. These two factors do have some influence on analysis results, but are seldom taken into account in performance analysis by vessel owners/operators.
5. Last but most importantly: the lack of a method for interpreting results. Even if reliable speed/power values, corrected for all the above-mentioned factors, are obtained and plotted in the speed trial speed/power diagram, it may be difficult accurately to describe the degradation of the performance, because the ship's resistance may be roughly divided into frictional resistance and wave-making resistance. Fouling only influences the frictional resistance, and as the frictional resistance fraction of the total resistance depends on the speed and the draft, the additional power demand, expressed as percentage of the total power requirement, will not be the same for different loading conditions and different speeds.

Note: other causes of changes in resistance of a vessel involve loss in efficiency of the engine; however, engine/bearings/propeller shaft degradation will not manifest itself in the same way as hull or propeller fouling, but in other ways – for example, as a high-exhaust gas temperature of one or more cylinders.

7.8 Methods for measuring vessel performance losses due to fouling and corrosion

The effect of hull resistance on propulsion performance is complicated to describe in an unambiguous way. The primary effect is that more water is dragged forward along with the vessel, and this phenomenon will of course increase the vessel resistance. The increased forward velocity of the water in the vessel's boundary layer will also cause the inflow velocity to the propeller to be reduced. This reduced inflow has several effects. On one hand, the efficiency of the propeller will decrease; on the other hand, some of the power lost in the boundary layer will be regained. Altogether, the required power will increase, though not quite as much as the resistance. Since it is not possible to state a fixed relation between added resistance and added power, for simplicity it is proposed to use the 'added resistance' as a measure for degradation and *not* the added power. A vessel's added resistance may be helpful in establishing a CO₂ Operational Index for oceangoing vessels, since specific ship resistance affects fuel consumption (Delft, 2006).

Even describing hull degradation in the form of the added resistance as a percentage of the total resistance is ambiguous, unless it is specifically designated for which speed and which loading condition (draft) this percentage is valid. Therefore, it is further proposed to refer the added resistance to 'the design speed and the design draft.' This is not a precise

reference, but it works in practice and is quite useful not only for evaluating the condition of a single vessel, but also for comparing several vessels, which do not need to be of the same shape and size. At deep draft and low speed the power increase will be more than the added resistance, and in ballast condition at full speed it may be less than half of the added resistance (Schultz, 2007). Note that it is always possible to calculate the actual power increase for any draft/speed from the found added resistance. The implication here is that the fouling factor for different coating systems may be compared, even if they are applied to vessels of different size or hull form.

7.8.1 Collection of performance data

As mentioned above, performance data may be collected daily or, in a more detailed form, at an interval of a month or so. Some vessels have an automatic data logging system that files performance observations continuously.

In principle, any of these methods may be relevant and useful, as long as the observations are made carefully. These different methods do have their advantages and disadvantages:

1. Continuous data logging excludes all human errors, but some data, for instance wave data, are normally not available in this way. Furthermore, this method produces a lot of data, which means that some kind of data reduction or data selection needs to be introduced together with the system. Even if wave data are recorded in some automatic fashion, it remains difficult to assure that only data for valid navigation conditions are further processed.
2. Daily performance observations, the so-called 'noon-data,' are useful for some purposes if carefully dealt with. Daily reports can only be used for reliable performance analysis if all conditions have remained unchanged during the 24-hour noon-to-noon period, but this is seldom the case.
3. Monthly, detailed observations over a time interval of a couple of hours are normally as reliable as such observations can be and quite useful. It will be described later that these observations cannot stand alone, but need to be treated together. Twelve sets of performance observations a year are therefore too few to establish a reliable 'time history' for the development of the added resistance for a ship.
4. A reasonable solution seems to be a procedure in which observations are made once a week. This interval is so short that the routines are not forgotten, but on the other hand is long enough that careful attention can be placed on this new dataset. In addition, it is usually possible to find a two-hour period with constant navigation conditions within a time interval of a week, and ± 50 observations per year is adequate for a detailed time history of the propulsion efficiency.

7.8.2 Processing of performance data

One way of processing the performance data is to compare the observed power and RPM values to those found for similar weather and loading conditions from a mathematical model of the vessel's propulsion performance. It can then be determined at which speed through the water and with which added resistance the calculated values match the measured values, and both speed through water and added resistance are then determined.

This method requires that such a mathematical model be available or be possible to establish, but creating such a model is not as easy as it sounds (Townsin, 2003). There are complicated theoretical methods for the calculation of resistance, propulsion system performance, weather resistance, and influence of hull resistance for a specific vessel, but in practice a robust general mathematical model that can easily be adapted to any vessel is needed. Such a model may be established by means of a combination of theoretical considerations and approximation formulas with empirical constants.

The number of empirical constants in a model developed in this way is quite high, but fortunately, some of these values are valid for all vessels or for large groups of similar vessels. Other constants are specific to individual vessels. The value of some of these latter constants may be found by careful analysis of the tank test and/or trial trip results, whereas other constants can only be found by statistical analysis of a sufficient number of performance observations for the vessel in service.

As an example, CASPER[®] is based on a general mathematic model; it is a build up by well-known, state-of the art elements for the calculation of vessel resistance, propeller performance, weather resistance, and so on. The general model, based on the type and main dimensions of vessel and propeller, may stand alone and may be used directly for comparison to actual performance data, but a more reliable model can easily be established by adjusting the general model, considering tank test/trial data. Even this model will not normally reflect all changes in the operational conditions, and the model is therefore not used for performance evaluation until it has been adjusted further by means of a statistical analysis of a number of performance observations. In general, 10–12 sets of performance observations are required (in some cases, the standard noon reports can be utilized) for this purpose, and the model will then be used for performance analysis and predicting speed/fuel penalties due to fouling. The adjustment of the model continues weekly, as more observation data are received. Normally, the basic constants of the model will remain unchanged after 30–40 sets of observations, but the constants describing the condition of the hull and propeller resistance are updated in real time as service performance data from the vessel is acquired.

7.8.3 Accuracy of the analysis

In practice, the accuracy of the analysis results depends more on the accuracy of the performance observation data than of the mathematical model itself. Experience shows that the actual added resistance as earlier described may be found with an accuracy of approximately 1%, and that the result from a single set of observations normally will not deviate more than 3% from the mean value. The actual speed/power diagrams that may be produced from the adjusted mathematical model are therefore fully valid for all practical purposes (transport cost calculations, cost-benefit decisions for coating selection, optimal maintenance intervals, etc.).

7.9 Added resistance diagrams and their use

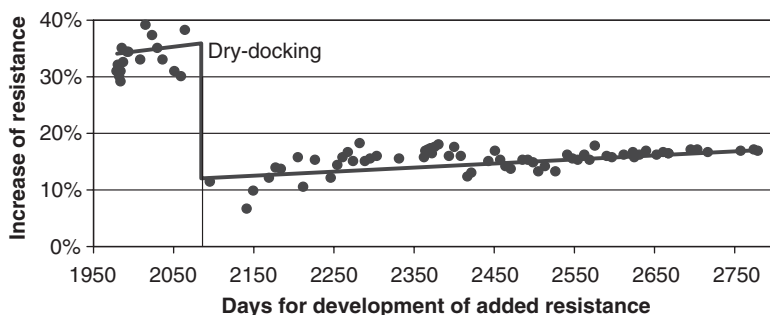
In the following, an added resistance diagram is shown for three vessel types in order to illustrate the described method. The individual analysis results are shown, and a first-order curve (a straight line) is faired through the points in order to show the development. For each vessel, there is a direct relationship between added resistance (fouling factor) and speed/fuel penalties.

7.9.1 Tankers

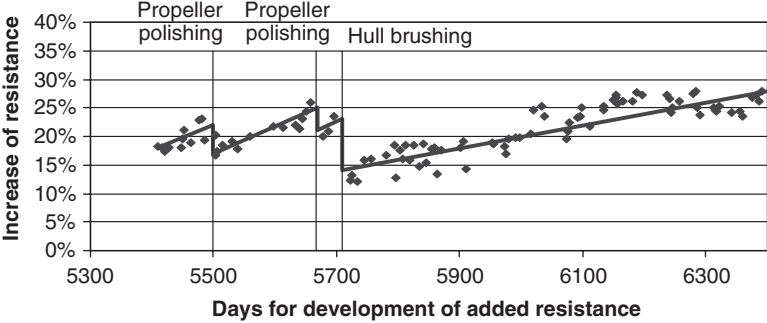
1. A typical example (see Fig. 7.11) of development of added resistance. It is seen that the added resistance of the hull and propeller in this case develops very slowly, less than 0.5% per month.

7.9.2 Containerships

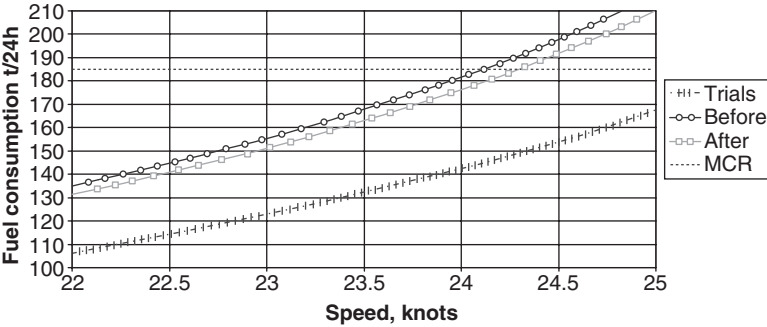
2. An example (see Figs 7.12, 7.13 and 7.14) of a more pronounced development of added resistance. At 24 knots, the propeller polishing at



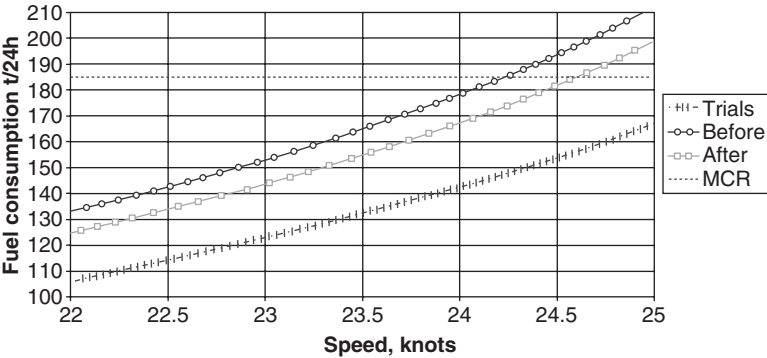
7.11 Added resistance diagram illustrating the decrease in resistance due to drydocking hull treatment.



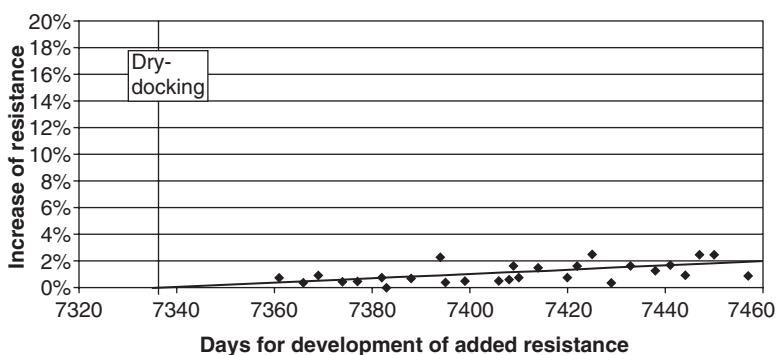
7.12 Added resistance diagram illustrating the decrease in resistance due to two propeller polishings and hull cleaning.



7.13 Fuel consumption versus speed diagram, before and after propeller polishing.



7.14 Fuel consumption versus speed diagram before and after hull brushing.



7.15 Added resistance diagram for a 20-year-old dry cargo vessel, illustrating the very low resistance after drydocking attributed to a fully blasted hull pretreatment.

six-month intervals resulted in a fuel savings of five tons per day for each propeller polish, and the hull cleaning resulted in a fuel savings of approximately 12 tons per day. The corresponding speed/power curves for one of the propeller polishes and the hull cleaning are shown in Figs 7.12–7.14. The overall development of resistance of the hull and propeller is 0.7–1% per month. Note that the slope of the line following the hull cleaning is stable and of a similar magnitude as before the hull cleaning, indicating that the hull cleaning was done in time so that a light cleaning removed fouling but did not alter the integrity of the hull coating system. Propeller polishing is a basic, low-cost strategy that saves fuel (Grigson, 1983; NEAA, 2007).

7.9.3 Bulkers

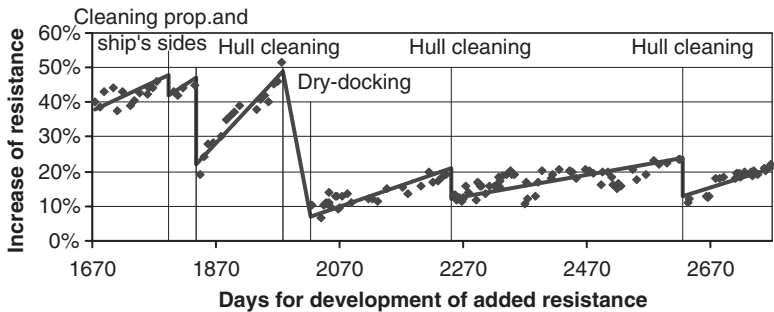
This 20-year-old bulker (see Fig. 7.15) had a very thorough treatment in the drydock, prior to application of a new hull coating system, including straightening of warped hull plates along with a full blast down to bare metal. The results of this drydock treatment are phenomenal, as the added resistance at outdocking was the same as the trial trip. In addition, the resistance is developing quite slowly, with only a 2% increase in resistance appearing after 120 days.

7.10 Benchmarking hull pretreatment in drydock and hull coating systems performance

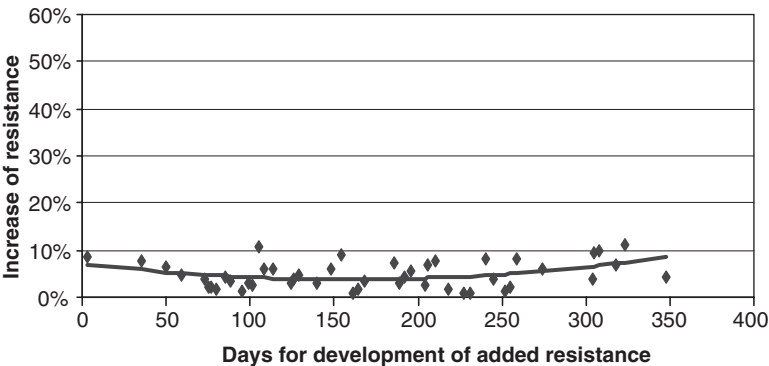
In the following, four more in-depth examples of the use of the diagrams will be shown as an important tool in vessel performance analysis.

7.10.1 Hull cleanings and drydocking with spot blast

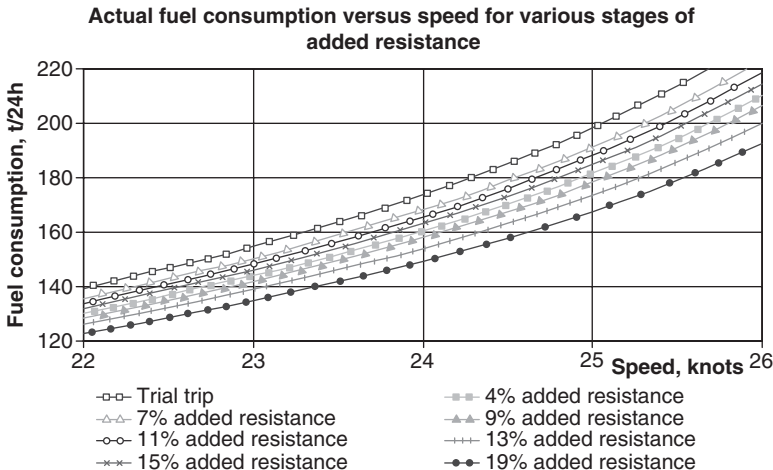
This vessel (see Fig. 7.16) initially had a high added resistance of approximately 50%. When the situation was discovered, the propeller was polished and the ship's sides were brushed. It is seen that the effect of this treatment was marginal. The operator was advised to have the ship drydocked, but as drydocking was inconvenient at that time he decided to clean the sides and bottom of the hull thoroughly a few weeks later. The result of this cleaning was remarkable, but as the antifouling was apparently depleted, the result did not last long (as indicated by the steep slope of the line), and the ship was drydocked on schedule. Subsequent to the drydocking, the hull was cleaned in-water when the added resistance exceeded 20%.



7.16 Added resistance diagram illustrating the changes in resistance due to hull cleanings, drydocking, propeller cleaning, and changes in development due to fouling.



7.17 Added resistance diagram for initial port stay with decreasing resistance due to fouling removal and slow increase in resistance due to fouling.



7.18 Fuel consumption versus speed for a fleet of seven similar vessels with the only difference being the added resistance, compared to trial trip vessel performance.

7.10.2 Long port stays

This tanker (see Fig. 7.17) sat for one month prior to its maiden voyage, and it is clear that the vessel accumulated fouling while sitting in port. Upon sailing, the self-polishing copolymer coating was activated, and the fouling and corresponding resistance reduced over the course of one year. The resistance is now developing along normal lines.

7.11 Added resistance as a metric to compare hull and propeller condition of similar vessels

7.11.1 Fleet monitoring

The graph (see Fig. 7.18) shows a fleet of seven vessels of similar design, plotting the actual performance due to their present state of corrosion and fouling, with all other variables corrected. This graph illustrates that performance losses due to fouling are seen as an increase in consumption to maintain a speed or as an incremental speed loss at a maintained power (with no change in fuel consumption; Delft, 2006). Note that in this speed window, the fleet of seven ships varies in fuel consumption from 156 tons per day to 174 tons per day (at 24 knots) due solely to fouling and type of treatment in drydock. Alternatively, the 'speed loss' due to fouling can be measured as a speed loss percentage of the total distance traveled. Significant

differences in vessel performance can be attributed to hull and propeller fouling due to different hull coating formulations in connection with vessel age and operational patterns (such as vessel speed, time in port, geographic region of operation, and drydocking time interval; Prochaska, 1981; Townsin and Wynne, 1976).

Please keep in mind that the added resistance percentage is not always equal to the percentage increase in fuel use or the percentage speed loss.

7.11.2 Lessons learned

Analysis over the past ten years on vessels in service has provided some general conclusions that are noteworthy.

1. The added resistance (due to fouling of the hull and propeller) varies from around 6% to 80% in the worst cases. On average, the added resistance for a single displacement hull oceangoing vessel is approximately 30%, if no special attention has been paid to the vessel. A 30% added resistance on an Aframax tanker equates a speed penalty of 1.0 knots – or an increase in fuel use of 12 tons per day at design speed. Thirty per cent added resistance on a high-speed containership equates to a speed loss of 1.8 knots or an increase in fuel use of 70 tons per day at a design speed of 25 knots @ 195 tons per day.
 - a) Roughly one-third of all vessels are in good condition with added resistance less than 20%.
 - b) Half of all vessels are in reasonable condition, but in a condition that could easily be improved, showing an added resistance of between 20% and 40% but exhibiting no unusual fouling pattern. For these vessels, improvement in performance can be achieved by some standard maintenance procedures without interfering with the normal course of operations.
 - c) The remainder of the world fleet (over 10000 dwt) is in poor condition, showing an added resistance of over 50% (with a good likelihood of biorisk from high levels of hull fouling).
2. The development of added resistance normally follows a curve. The increase will normally be between 0.5% and 2% per month at the beginning of a drydocking period. In some cases, increases of 5%–6% per month for a limited duration have been seen. Later in the period, when the added resistance has reached a certain level, its development may be more restricted. The slope of the lines for development of resistance are a good business tool in determining future performance penalties due to fouling.
3. The basic hull treatment in drydock has a pronounced influence on added resistance after drydocking. In the best cases, the baseline added

resistance will only be 0%–4%. A partial hull blasting treatment with new coating system has been seen to result in an added resistance of 5%–20%, while in the worst cases there is no benefit at all from drydocking.

4. The type of hull coating has a pronounced influence on the development of added resistance. It is also important that the coating be applied to the correct thickness, and that the dissolution speed – or, for self-polishing paint, the polishing speed – be carefully adjusted to the service speed and operational patterns of the vessel. As regards the performance of silicon coatings (see Chapter 26), the treatment in drydock is even more critical than with paint systems.
5. Hull cleaning between drydockings may have a remarkable effect, especially if one of the less active types of antifoulants has been used. Hull cleaning may to a certain degree compensate for an antifoulant's low efficiency.

It is advisable to clean the hull *before* the slimy layer of bacteria and algae has turned into a layer of seaweed. In that case, very soft brushes (for example, softer than the bristles of a toothbrush) can be used, and the antifouling system will not be damaged. This stage corresponds to approximately 12% of resistance added to the resistance after drydocking. At a later stage, harder brushes are required, and though they can easily remove seaweed, they will most probably remove some of the antifoulant as well; this removal may result in an increased development of added resistance after the cleaning. The slime associated with foul-release systems has been shown to remain on ship hulls even at 30 knots (Candries *et al.*, 2001)

7.12 Conclusions

Economically optimal precautions can only be taken if the propulsion condition of the vessel is well defined, which requires not only a reliable performance monitoring system, but also rigorous methods of analysis. Any vessel owner/operator may establish such a system; however, significant hydrodynamic and statistical expertise are required in order to develop and extract actionable information for prudent business decisions:

- To evaluate drydocking treatment such as water-blasting, robotic systems, and other emerging technologies.
- To follow the development of hull and propeller resistance for individual vessels and to take action, when economically justified, on a vessel-to-vessel basis. Such action includes evaluating the before-and-after effect of hull cleanings, by divers with brushing machines, underwater robotic hull cleaning and propeller polishing, as well as the mitigation of invasive species introduced through the vessel hull.

- To benchmark the efficiency (total ownership cost) of any coating system by comparing vessels with different coating systems; vessels need not be identical in hull form.

Experience has shown that at least 10% may be saved on average on fuel costs for lightly fouled hulls, and up to 35% may be saved when hulls are heavily fouled. For a ship that burns 100 tons of fuel per day, such as a very large crude carrier or medium-size containership, at least 10 tons per day may be saved, corresponding to 31.9 tons of CO₂ emissions reduction. With bunker fuel prices at 300 USD per ton, that represents a value of approximately 3000 USD per day. Some naval vessels exhibit a 15% decrease in fuel consumption attributable to hull and propeller cleanings (US Navy and EPA, 2003).

7.13 Sources of further information and advice

Ship trials with biocide-free antifouling paints in Australian waters (12:15–12:45) **J. Lewis** (Defence Science & Technology Organisation, Melbourne) <http://www.limnomar.de/download/05-Workshop-A-Lewis.pdf>

Outcome of the research project ‘Performance of biocide-free antifouling paints for deep-sea going vessels’ (11:45–12:15) **B. Daehne** (LimnoMar) <http://www.limnomar.de/download/04-Workshop-A-Daehne.pdf>

The Development of Foul Release Coatings for Ocean Going Vessels http://www.imarest.org/proceedings/samples/candries_coatings.pdf

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The impact and control of biofouling in marine finfish aquaculture

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Abstract: We review the impact and control of fouling of netting and cages in finfish aquaculture. The large surface area and structure of netting material, particularly multifilament mesh, is highly suitable for colonisation and growth of fouling. Furthermore, fouling growth is often rapid because the waters surrounding aquaculture operations are enriched by organic and inorganic wastes (uneaten food, faecal and excretory material) generated by high-density fish populations. Biofouling of fish-cage netting is a significant operational problem to aquaculture. The occlusion of mesh and the resulting restriction in water exchange adversely affects fish health by the reduction in dissolved oxygen (DO) and the accumulation of metabolic ammonia. Fouling is of further concern because it significantly decreases cage flotation, increases structural fatigue and cage deformation, and may act as a reservoir for pathogens. The impacts of fouling vary dramatically depending on season and location, and are also influenced by farming methods and practices. The impacts of these factors are reviewed and highlighted. The overall outcome is that there are few comprehensive quantitative studies of fouling or its impacts on sea-cage aquaculture, and this impairs the ability to develop the most appropriate mitigation strategies to control fouling.

Effective fouling control is particularly difficult, given the high species diversity and spatial variation typical of many fouling communities on cages. However, the continual expansion of finfish aquaculture, in particular cage aquaculture into tropical regions where fouling is highly diverse with rapid year-round growth rates, is increasing demand for fish-cage antifouling technologies. At the same time, the control and regulation of products available for use in aquaculture, and the phasing out of many metal-based products, mean that there are fewer antifouling products available than there were a decade ago. We review the range of antifouling technologies currently available, including mechanical cleaning, coatings incorporating biocides, and their non-release alternatives. Recommendations for effective biofouling control and directions for future research are identified given the need to develop non-toxic coatings specifically suited for aquaculture applications.

Key words: aquaculture, finfish, salmon, biofouling impacts, biofouling control.

8.1 Introduction

8.1.1 Biofouling and finfish aquaculture

Biofouling is a significant problem for all aquaculture industries with the broadest and most well documented impact being in marine finfish aquaculture, in particular sea cage-based aquaculture, hence the focus of this review. This is due to the broad phylogenetic diversity of fouling species, the ideal conditions of large uncolonised surface areas provided by net sea cages, and an abundance of nutrients that cage-based aquaculture provides. Over half of the total world aquaculture production value of \$US78.8 billion in 2006 was attributable to finfish (32.6 million tonnes, US\$47.3 billion) (FAO, 2008). In 2006, freshwater fish production (27.9 million tonnes) was valued at US\$29.2 billion, diadromous fish production (3.1 million tonnes) was valued at US\$11.8 billion, and marine fish production (1.6 million tonnes) was valued at US\$6.3 billion (FAO, 2008). While cages are used to some extent within all of these industry sectors, the production of diadromous and marine fish is dominated by the use of sophisticated cage aquaculture systems. This is particularly true for marine salmonoid aquaculture, which is dominated by Norway, Chile, United Kingdom (especially Scotland), and Canada. The industry is also valuable in Ireland, United States, Japan, and New Zealand. The principal salmonoid species reared in marine aquaculture (mariculture) are Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), and Chinook salmon (*Oncorhynchus tshawytscha*). Given the strong science base associated with salmon production, the vast majority of peer-reviewed information on the impact of biofouling in aquaculture targets marine salmonoid culture. However, given the importance of the aquaculture industry on a global scale there is only a sparse and often disparate literature on the quantitative impact of biofouling in all forms of aquaculture.

Similarly, there is a scarcity of peer-reviewed information on the efficacy of methods to control biofouling in aquaculture (although see Braithwaite and McEvoy, 2005). The vast majority of literature on biofouling research focuses on the fouling of ship hulls, oil platforms and other marine industries (Evans, 1981; Yebra *et al.*, 2004; Bram *et al.*, 2005; Chambers *et al.*, 2006). A rigorous assessment of the implementation and efficacy of biofouling control in finfish mariculture has been largely neglected in the peer-reviewed literature. Some large studies specifically aimed at fouling in the aquaculture industry have been conducted by research students and are not widely available (Wee, 1979; Mak, 1982; Gormican, 1989; Cronin, 1995) while commercial research and development is rarely published. Given the significant impacts of biofouling on aquaculture operations, and the disparate information on the effective control methods available, we review the current state

of knowledge on the impact and control of biofouling in the finfish mariculture industry.

8.1.2 Finfish mariculture – farm practices

Mariculture is undertaken in enclosed natural lochs, fjords or bays (enclosures), in pens (man-made structures enclosed on all sides with the bottom formed by the sea-bed) or cages (man-made structures enclosing all submerged surfaces) (Beveridge, 2004). The size of facilities ranges enormously, but enclosures and pens are larger (0.1 ha to > 1000 ha) compared to cages (1 m² to 1000 m² in surface area), and come in four basic designs: fixed, floating, submersible and submerged (reviewed in Huguenin and Ansuini, 1978; Beveridge, 2004). There is, however, a move to develop offshore aquaculture with increasingly larger cages.

Cages for intensive commercial finfish culture are typically multifilament netting-bags suspended from a floating frame. Circular cages of 40 m to 70 m circumference are the most common design (Beveridge, 2004), but there is a trend in the fish farming industry to increase the size of fish cages. Larger 80 m to 120 m cages are used for salmon culture in Australia (Isles, 1998; Douglas-Helders *et al.*, 2003), 90 m to 160 m cages for salmon culture in Norway (Sunde *et al.*, 2003; Neyts and Sunde, 2005; Hansen and Windsor, 2006), and 125 m to 160 m cages for tuna culture in Australia (Cronin *et al.*, 1999). Square cages are also frequently used, and are produced commercially in a range of sizes from 6 m² to 25 m² in area. The depth of cages is limited by cage diameter, depth of the farm site, and ease of maintenance. The depth of fish cages ranges from just 2 m (Lee *et al.*, 1985) to 20 m (Hodson and Burke, 1994). Deeper cages (10–15 m) are typical for large-scale finfish culture.

The stocking density of cages is dependent on the cultured species, cage size and environmental conditions. In Australia, for example, Atlantic salmon are cultured at 10–15 kg/m³ (e.g., 12000 × 2.5 kg salmon in a 65 m cage) and bluefin tuna at 4 kg/m³ (e.g., 2000 × 23 kg tuna in a 125–160 m cage), while the maximum allowed stocking density for salmon in Norway is 25 kg/m³ (Lovdata, 2004). Given the intensity of these aquaculture practices, it is evident that farms using a high number of cages are required to manage a significant volume of enclosed water and large populations of fish. Good husbandry techniques are required to maintain optimum culture conditions, and protect such a sizeable monetary investment. In particular, a high standard of water quality must be maintained by water exchange, which is dependent on water current, and which in turn is influenced by salinity, temperature and topography of the site. The critical nature of water exchange makes the impact of biofouling so significant for all forms of aquaculture, but in particular cage-based aquaculture. The principal effect

of biofouling on cages is to restrict water flow, increasing its effect as the biofouling communities develop from a biofilm to a complex three dimensional climax community dominated by sessile invertebrates.

8.2 The ecology of biofouling in mariculture

8.2.1 Community composition and temporal variation

The ecological progression of biofouling in marine environments is broadly applicable to submerged surfaces including aquaculture infrastructure, and is well understood (reviewed in Little, 1984; Wahl, 1989; Maki, 1999; Holmström and Kjelleberg, 1999; Maki and Mitchell, 2002). An organic conditioning film composed of proteins, proteoglycans and polysaccharide compounds precedes bacterial adsorption (Loeb and Neihof, 1975; Lewin, 1984). Within hours, bacteria settle and irreversible adhesion and growth occurs on the solid surface, which ultimately leads to the formation of a macroscopic slime film (Wahl, 1989). Within days or weeks, diatoms and spores of macroalgae and protozoa colonise the surface, and subsequently larvae of macrofoulers including tunicates, coelenterates, bryozoans, barnacles, mussels, and polychaetes settle and metamorphose (reviewed in Holmström and Kjelleberg, 1999). Biofouling involves organisms from nearly every invertebrate phylum.

The development and composition of fouling communities on fish cages has been described for many types of mariculture in a number of countries, including Scotland (Milne, 1975a, b), Australia (Cronin, 1995; Hodson and Burke, 1994; Cronin *et al.*, 1999), China (Chengxing, 1990), India (Santhaman *et al.*, 1983), Japan (Kuwa, 1984), Malaysia (Cheah and Chua, 1979; Lee *et al.*, 1985), Tanzania (Bwathondi and Ngoile, 1982) and the USA (Moring, 1973; Moring and Moring, 1975; Greene and Grizzle, 2007). There are also some studies of cage fouling in freshwater ponds and lakes (Pantastico and Baldia, 1981; Greenland *et al.*, 1988; Dubost *et al.*, 1996).

Multi-filament netting material is an ideal surface for fouling, and the succession of organisms that colonise aquaculture netting has been evaluated specifically (Milne, 1975a, b; Hodson and Burke, 1994; Svane *et al.*, 2006). Generally, macroalgae are the most serious type of fouling on cages immersed for short periods (< 1 month) (Milne, 1975a, b; Hodson and Burke, 1994). The dominant macroalgae reported on fish cages include *Gracilaria* sp. (Cheah and Chua, 1979), *Ulva* spp. (Moring and Moring, 1975; Cronin, 1995; Cronin *et al.*, 1999; Svane *et al.*, 2006), *Antithamnion* sp. (Hunter and Farr, 1970), *Enteromorpha* spp. and *Ectocarpus* spp. (Milne, 1975a, b; Wee, 1979). It is important that both *Ulva* and *Enteromorpha* species are now considered to be within the genus *Ulva* based on a molecular revision of both genera (Hayden *et al.*, 2003).

In contrast, bivalves, ascidians and hydroids are the predominant fouling types on cages immersed for longer periods (Milne, 1975a, b), but can also cause significant fouling in short periods, particularly during times of high larval settlement (Sutterlin and Merrill, 1978; Greene and Grizzle, 2007). Bivalves reported as major net-cage foulers include the wing shell *Electroma georgiana* (Cronin *et al.*, 1999), the mussels *Mytilus edulis* (Koops, 1971; Milne, 1975a, b; Moring and Moring, 1975; Paclibare *et al.*, 1994; Braithwaite *et al.*, 2007; Greene and Grizzle, 2007), *Modiolus* sp. and *Perna viridis* (Cheah and Chua, 1979; Lee *et al.*, 1985), and the oysters *Crassostrea* spp. and *Pinctada* spp. (Cheah and Chua, 1979; Bwathondi and Ngoile, 1982).

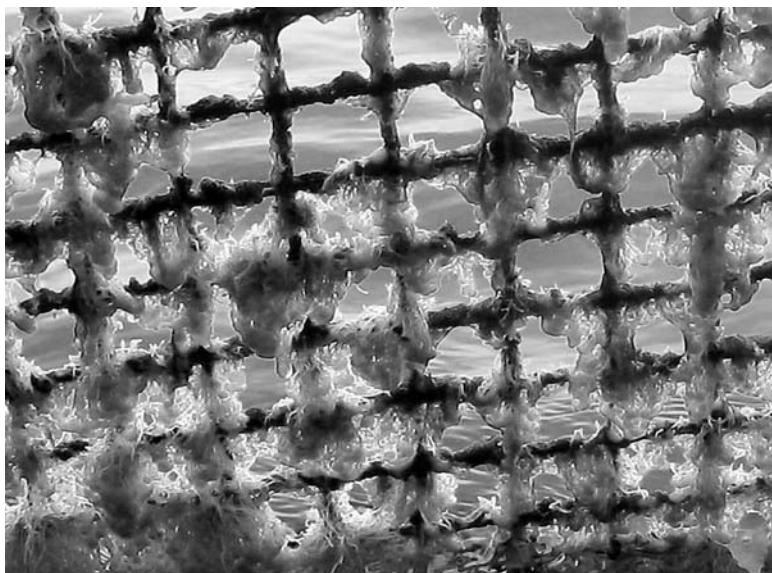
The major fouling ascidians include solitary species such as *Styela picata* (Chengxing, 1990), *Ascidiella aspersa* and *Ciona intestinalis* (Milne, 1975b; Braithwaite *et al.*, 2007), and colonial genera including *Botryllus*, *Botrylloides*, *Symplegma* and *Trididemnum* (Cheah and Chua, 1979). The most problematic hydroids are *Ectopleura larynx* (Fig. 8.1) (Swift *et al.*, 2006) while significant mesh occlusion by filamentous (tube-dwelling) diatoms has also been reported (Moring and Moring, 1975; Hodson and Burke, 1994).

8.2.2 Spatial variation between sites

There are obvious differences in biofouling between sites separated over large spatial scales such as between temperate and tropical waters (> 1000 km); however, there is also spatial variation within smaller scales (< 100 km). Studies of fouling on mariculture netting reveal spatial variation over a wide geographical range, with test sites located in Scotland (Milne and Powell, 1967; Milne, 1969, 1970, 1975a, b), Hawaii (Rothwell and Nash, 1977), Hong Kong (Tseng and Yuen, 1979; Mak, 1982), Maine and Massachusetts (Huguenin and Ansuini, 1975, 1978). Spatial variation may represent differences in environmental conditions (Santhanam *et al.*, 1983) or the abundance of larval stages (Bwathondi and Ngoile, 1982).

For example, fouling communities on polyethylene netting differ between cages immersed in brackish and marine waters (Santhanam *et al.*, 1983). Cages in brackish water (24.5–33.8%) were colonised by the algal genera *Enteromorpha* and *Ectocarpus*. However, cages in marine conditions (36%) were colonised by a more diverse community including bivalves (*Avicula vexillum*, *Dasychone* sp., *Crassostrea madrasensis*, and *Pinctada* sp.), sea anemone, solitary and colonial ascidians, algae (*Caulerpa* spp., *Codium* sp. and *Gracilaria* sp.), amphipods (*Corophium* spp.), barnacles (*Balanus amphitrite variegatus*), and polychaetes (*Serpula* sp.) (Santhanam *et al.*, 1983).

In terms of the abundance of larval stages Bwathondi and Ngoile (1982) found different age classes of bivalves fouling fish cages, and identified the frequency and time of settlement of different species. They identified eight



8.1 The hydroid *Ectopleura larynx* fouling a salmon net in Norway during late summer. Hydroids are common and problematic fouling organisms that are difficult to remove and re-grow rapidly. They cause decreased flow and a decrease in oxygen levels within cages and require constant management to prevent impact on growth and survival of salmon. Photograph by Leif Magne Sunde-SINTEF.

age groups of an *Ostrea* sp., four groups of a *Pinctada* sp. and three groups of *Pinctada vulgaris* on cages immersed for 103 days. The number of individuals per age group was dependent on environmental conditions, and greater settlement of *Ostrea* sp. occurred during spring tides (the time of greatest plankton abundance), and greater settlement of *Pinctada* spp. occurred at high rainfall. As an example of the variance both between and within sites Haegele *et al.* (1991) recorded the abundance of fouling invertebrates at numerous sites, and at various depths within sites, at salmon farms in British Columbia. Mussels, isopods and pycnogonids were frequently observed, but their abundance varied greatly between sites on different sampling dates. Further, species such as polychaetes that occurred in low abundance were only found at a few sites.

8.2.3 Spatial variation within sites

Variation in biofouling within sites is predominantly driven by the availability of light and water flow, and these relate to the depth and orientation of cages. Fouling mass and species diversity can vary between sides of cages

at the same depth, and this microenvironment difference is directly related to light intensity. The southern side (which received direct sunlight) of a tuna cage had a greater photosynthetic biomass than other sides, and the highest total biomass over most depths (Cronin *et al.*, 1999). Variation between cage sides is only detectable near the surface where light intensity differences are most pronounced, to the extent that variation in fouling that was significant at 0.5 m was not significant at a depth of 2.0 m (Moring and Moring, 1975).

Significant differences between sides were also noted for specific organisms. Ascidians comprised a greater proportion of the community on the sunny southern side than any other side, and red algae were most abundant on the southern side (Cronin 1995; Cronin *et al.* 1999). In contrast, bryozoans were least abundant on the southern and western sides (Cronin 1995; Cronin *et al.*, 1999). Lee *et al.* (1985) observed significant differences in the mass of algae and invertebrates between cage sides, and the two faces that had the greatest mass of bivalves (*Modiolus* spp. and *Perna viridis*) had the lowest mass of marine worms and algae.

A reduction in light intensity also causes significant variation in species diversity and abundance between depths. Overall, fouling mass decreases significantly with increasing depth (Moring and Moring, 1975). The upper portion of fish cages are fouled with *Ectocarpus* spp., *Enteromorpha* spp. and other algae, whilst bivalves including *Mytilus edulis*, *Electroma georgiana*, oysters, hydroids and amphipods are predominant at lower depths (Wee, 1979; Santhanam *et al.*, 1983; Cronin, 1995; Cronin *et al.*, 1999).

Increased fouling growth around the top of cages, particularly of algae, is also shown by measurements of mesh occlusion. Fukuda (1965) reported fouling growth and mesh occlusion increased with distance above the base of a cage, while Haegele *et al.* (1991) reported a gradual decrease in mesh occlusion from 50% to 10%, over 0.3 to 9.1 m depth. Consequently, restriction in water exchange and the associated degradation in water quality are also likely to vary with depth, and could result in aggregation of the fish at specific depths to avoid unfavourable conditions (Gormican, 1989).

The orientation of submerged surfaces affects fouling development, and significant differences occur between vertical and horizontal substrates. For example, Lee *et al.* (1985) found a greater mass of bivalves on the bases, rather than the walls, of 2 m deep cages. To some extent these observations reflect a change in fouling with depth, but they also represent an orientation effect. This was demonstrated in a comparison of vertically and horizontally mounted net panels. The vertical panels were fouled more rapidly, developed a greater mass of fouling, and had increased abundance of compound ascidians and tubeworms (Cheah and Chua, 1983). However, barnacles and oysters were more abundant on the horizontal frame (Cheah and Chua, 1983). The increased mass on the vertical panel was thought to reflect a

greater interception of horizontally moving planktonic larvae thus increasing larval settlement. However, it is also likely that an increase in collisions with suspended material would increase nutrition of filter-feeding organisms. Communities on horizontal surfaces are subject to greater siltation and predation than vertical surfaces, and upright or mounding species are favoured. However, colonial growth is more effective on vertical surfaces where competition for space is critical and predation pressure is less (Harris and Irons, 1982).

Variation in fouling composition has also been observed between cages and between the outer and inner surfaces of cages (Bwathondi and Ngoile, 1982). After 103 days immersion of two adjacent 0.5 m³ cages, 9 groups of bivalve species were identified, with 672 bivalves on one cage and only 315 on the other. In addition, the relative abundance in numbers of individual bivalve was recorded, and more individuals were found growing on the outer (503) than inner surfaces (169) of a cage. This effect was principally due to the preferential settlement of *Ostrea* spp. on the outside of the cage, but the significance of both observations is impossible to evaluate given the limited (n = 1) sampling design (Bwathondi and Ngoile, 1982).

8.3 The dynamics of biofouling

8.3.1 Water quality and nutrients

Fouling growth is often rapid because the waters surrounding mariculture operations are enriched by organic and inorganic wastes (uneaten food, faecal and excretory material) generated by the high-density fish populations (Gowen and Bradbury, 1987; GESAMP, 1991; Cook *et al.*, 2006; Sanderson *et al.*, 2008; Navarro *et al.*, 2008). The increased carbon, nitrogen and phosphorus levels in the waters immediately surrounding mariculture farms favour the growth of annual filamentous algae (Rothwell and Nash, 1977; Ruokolahti, 1988). The rapid fouling growth in the nutrient enriched waters of Pearl Harbour resulted in the complete blockage of netting mesh within 2 months, whereas the majority of panels immersed at two sites with minimal nutrient enrichment had only 0–10% blockage after 3 months immersion (Rothwell and Nash, 1977). In fact, the growth of algae around fish farms has spurred the commercial integration of seaweed culture in marine aquaculture systems (reviewed in Chopin *et al.*, 2001), and this development has the potential to mitigate many of the environmental impacts caused by mariculture operations (Neori *et al.*, 2004; CRAB, 2007).

8.3.2 Netting characteristics

Fouling of mariculture structures differs from that of many other marine industries, in particular marine transport, in terms of surface characteristics,

as they are rough and consequently have a high surface area. They are also not subject to the high water velocities and shear forces associated with ship hulls or the internal surfaces of pipes. Early studies of fouling on mariculture netting showed netting material and mesh size to significantly affect fouling rate, mesh occlusion, and density and abundance of fouling species (Milne and Powell, 1967; Rothwell and Nash, 1977). From this data, and observations of mesh deterioration, materials were rated for their suitability in the construction and maintenance of fish cages. More recently, the effects of net angle (Cheah and Chua, 1983) and of microfouling development on multi-filament mesh (Hodson and Burke, 1994; Corner *et al.*, 2007) have also been investigated.

8.3.3 Effect of mesh size

A variety of mesh sizes are employed for commercial finfish culture, ranging from 12–40 mm for salmon cages, 60–90 mm for Bluefin tuna cages to 100–150 mm for predator fences. The larger meshes are often of thicker gauge, but generally the smaller the mesh size the greater the surface area per m². Consequently, smaller meshes typically support a greater number of fouling organisms and total biomass (Milne, 1975a; Cheah and Chua, 1983). Cheah and Chua (1983) found the rate of fouling, mass of fouling, species diversity and species abundance to increase with a decrease in mesh size. For example, mesh sizes of 38 mm, 25 mm and 13 mm were fouled by 1, 3 and 5 species of colonial ascidian respectively. Small mesh sizes are also blocked by a relatively low mass of fouling, whereas larger mesh material (> 50 mm) can support large fouling communities but maintain a significant open area (Milne and Powell, 1967). Consequently, to maintain acceptable water exchange, small mesh nets must be cleaned far more frequently than larger meshes (Cheah and Chua, 1983).

Small mesh netting (≤ 15 mm) is particularly prone to accumulation of suspended sediment, and often has significant decrease in fouling for this reason alone (Mak, 1982; Lai *et al.*, 1993). In contrast, Cheah and Chua (1979) found high silt loadings on nets provided an excellent substrate for settlement and growth of fouling, particularly species of the red alga *Gracilaria*. The accumulation of sediment due to the size of the netting is exacerbated by the rough surface of multifilament mesh.

Comparisons between different mesh sizes are affected by twine thickness because this changes the total surface area. Mak (1982) quantified fouling on mesh panels after 3, 6 and 9 months immersion, and found 25 mm and 50 mm multifilament meshes supported a greater biomass than 9 mm, 63 mm and 88 mm single-filament meshes. Tseng and Yuen (1979) found no significant difference in fouling mass on 50 mm, 38 mm, 20 mm and 19 mm mesh nets, which were woven from 36, 27, 9 and 4 filaments,

respectively. Thus, mesh size and total surface area interact to influence biofouling development.

Whilst short-term fouling development (< 3 months) appears dependent on available surface area, long-term fouling mass (particularly of filter-feeding invertebrates) is dependent on the area in which the organisms can expand and feed. That is, smaller meshes supported the greatest fouling biomass after 3 months immersion, but larger meshes supported the greatest biomass after 9 months immersion (Mak, 1982). These communities are dominated by invertebrates, and more than 75% of the 9-month community was composed of solitary ascidians. Similarly, Milne (1975a) found large mesh sizes eventually developed mussels of a greater size than small mesh, and suggested that the water flow through larger mesh improved feeding. Thus, large mesh netting ultimately has a larger carrying capacity for biofouling communities.

8.3.4 Effect of mesh structure

The microtopography of multi-filament netting affects the distribution and type of initial fouling (Hodson and Burke, 1994). The cylindrical shape of mesh bars leads to differences in light intensity between the upper and lower surfaces of bars immersed horizontally. Consequently, horizontal bars develop a community dominated by phototrophs (e.g., diatoms) on the upper surfaces, and heterotrophic protozoan communities on the lower surfaces (Hodson and Burke, 1994).

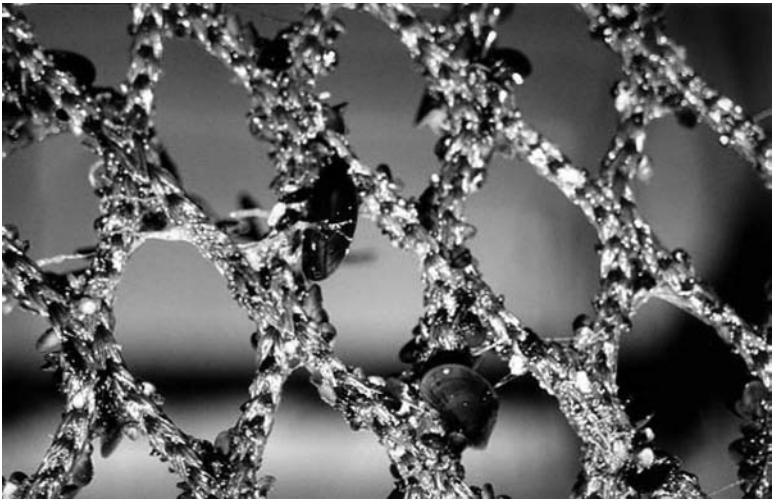
The large crevices and many filaments of the netting are likely to aid colonisation, either through entrapment of suspended material or because larvae of some fouling invertebrates, and spores of common fouling organisms such as *Ectocarpus* spp. and *Enteromorpha* spp., preferentially settle in small depressions (Crisp, 1984). There is also a preferred settlement size for many organisms and this may affect the community composition for both micro (Scardino *et al.*, 2006) and macro fouling (Callow *et al.*, 2002, Hills *et al.*, 1999, Scardino *et al.*, 2008). The use of monofilament netting would obviously reduce problems associated with crevices, but it has significantly less strength than multifilament mesh. Furthermore, monofilament nets must be constructed with knots at the mesh intersections, which results in increased abrasion damage to nets during on-shore handling and increased abrasion of fish during culture.

Fouling development on netting is influenced by the 3-dimensional structure of mesh. Preferential colonisation at mesh intersections has been noted in many studies (e.g., Milne, 1975a, b; Rothwell and Nash, 1977; Tseng and Yuen, 1979). Milne (1975a, b) observed that mussels developed large aggregations at intersections, and Tseng and Yuen (1979) reported bryozoans, barnacles, and green algae primarily occurred at knotted intersections. This

preferential settlement presumably results from the greater surface area and changes in turbulence at these regions. Milne (1975b) also noted that the netting structure led to entanglement of drifting algae. This type of fouling can quickly block netting, because it is entangled rather than directly attached to the surface.

8.3.5 Effect of mesh material

A number of materials are suitable for the construction of fish cages, and these have varying degrees of fouling resistance. In this regard, several studies have demonstrated the relative performance of many types of netting: multifilament-polymer mesh, extruded polymer mesh, metallic hardware cloth, and extruded metallic mesh (e.g., Milne and Powell, 1967; Milne, 1969; Rothwell and Nash, 1977). Milne and Powell (1967, 1970) compared 10 mesh types at 4 sites in Scotland, and found polymer-fibre nets were the most susceptible to fouling and galvanised meshes the least. After 4 months immersion, mussel growth (*Mytilus edulis*) completely blocked polymer-fibre netting (Fig. 8.2), and the weight of test panels (0.4 m²) had increased from 5.5 kg (clean) to more than 15.5 kg. In comparison, reasonable water flow still occurred through galvanised materials, and panel weight had increased from approximately 7 kg to 9 kg.



8.2 The recent settlement of the blue mussel *Mytilus edulis* on a small mesh salmon net in Norway during late summer. Mussels often foul as a mono-specific cohort, rapidly increasing the weight of the net and in particular occluding smaller mesh size nets. Photograph by Rocky de Nys-JCU.

Nine types of netting were assessed in a 6-month trial at three locations in Hawaii (Rothwell and Nash, 1977). Netting panels were compared to determine time interval before cleaning, and by the total fouling mass after 5 months. Nylon and polyethylene meshes were found to foul at a significantly greater rate than metal meshes, and after 5 months polyethylene mesh had the greatest fouling and galvanised mesh the least (Rothwell and Nash, 1977). The composition of the fouling community also differed between mesh types. Initially algae colonised the majority of net types, but became most abundant on nylon netting and netting with an ineffective antifouling paint. After 5 months, serpulid tubeworms were abundant on all panels, but were least prevalent on extruded polymer mesh and PVC-coated chain-link, on which barnacles were abundant (Rothwell and Nash, 1977).

The colour of the mesh netting strongly impacts biofouling dynamics. Previous studies have demonstrated that the presence of fouling organisms is generally greater on darker surfaces compared to light surfaces (Hodson *et al.*, 2000; Swain *et al.*, 2006). For example, significantly less fouling occurred on white silicone-coated netting compared to uncoated white and black netting after 163 days of immersion in Tasmania, Australia (Hodson *et al.*, 2000). Furthermore, Swain *et al.* (2006) also tested the effect of substrate colour (acrylic overlying white and black background) on the settlement of fouling organisms in the field and demonstrated that the settlement of both the alga *Ulva* sp. and the calcareous tubeworm *Spirorbis* sp. was greater on acrylic overlying the black background than the white background.

8.3.6 Fouling composition and biomass

Fouling communities on cages are often characterised by a large biomass. For example, a 4-month old fouling community had an almost identical species composition to a 2-month old community, but had double its weight (Cheah and Chua, 1979). Wee (1979) quantified biomass change over time, and found an increase from 1.85 kg/m² to 2.84 kg/m² and 4.98 kg/m² after 52, 77, and 106 days immersion respectively. Biomass in the range of 1–5 kg/m² (wet weight) is typically reported (Lee *et al.*, 1985; Chengxing, 1990; Cronin, 1995; Braithwaite *et al.*, 2007), although one study showed that 58% of the total fouling mass of 4.5 kg/m² was silt (Lee *et al.*, 1985). This degree of fouling constitutes a significant load since a mean biomass of 4–5 kg/m² on 90 m circumference net tuna cage would equate to a total mass of 6.5 tonnes (Cronin, 1995).

Atypical and very large values for biomass production have also been reported. Rothwell and Nash (1977) reported a total fouling mass of 13 kg/m² on nylon netting after 1 month in Pearl Harbour, but in excess of 80 kg/m² after 3 months. Similarly, Milne (1975a) found that 25 mm nylon mesh could support a mussel biomass of up to 140 kg/m².

8.4 The impact of biofouling on finfish culture

8.4.1 Restriction of water exchange

The predominant concern with fouling of fish cages is the occlusion of netting mesh and the resulting changes in water quality from restriction of water flow. A number of studies have demonstrated the extent of flow restriction through clean and fouled mesh (Hisaoka *et al.*, 1966 (in Japanese); Wee, 1979). The flow of water through cages is generally measured as transmission: the current speed inside the cage expressed as a percentage of the current outside the cage. The transmission of clean nets is related to mesh size, but typically varies from 50% to 80%. Transmission is also affected by the external current velocity (Edwards and Edelsten, 1976), and by the angle of the mesh to the current flow (Gularte and Huguenin, 1984). Differences in measurement of transmission may arise from the method used to quantify current, the stocking density of the cage and circulating currents created by the fish (Inoue, 1972; Wee, 1979).

Transmission is significantly reduced with fouling of mesh and grouping of cages. Transmission for clean 13 mm mesh (57.5%) was reduced to 23.4%, 18.7% and 13.1% after 52, 80 and 120 days in the sea, corresponding to fouling weights of 1.85 kg/m², 2.84 kg/m² and 4.98 kg/m², respectively (Wee, 1979). Similarly, Gormican (1989) measured current speed inside and outside a salmon cage and found a 65% transmission decrease at depths of significant fouling. The significant flow restriction through clean nets necessitates good fouling control in order to maintain adequate water exchange.

Flow decreases serially when cages are grouped in a row parallel to the current. Across three 9 mm mesh cages, the transmission dropped from 70% in the first cage to 35% and 18% in the second and third cages, respectively (Inoue, 1972). Across three 24 mm mesh cages, the transmission dropped from 80% in the first cage to 50% and 35% in the second and third cages, respectively (Inoue, 1972). When cages are aligned in a series, and when netting becomes fouled, the effects combine to reduce water exchange (Aarsnes *et al.*, 1990). Beveridge (2004) thus recommended that although groups of 8–10 cages may be oriented perpendicular to the current, there should be no more than two or three cages in a series parallel to the current.

There is a trend throughout the fish farming industry to increase the size of net pens and to hold the large nets in the sea for the whole production cycle (Sunde *et al.*, 2003). Therefore, the effects of biofouling on water exchange and net deformations are expected to become more severe, because larger cages and nets have a smaller surface area to volume ratio and hence reduced rates of water exchange compared to smaller nets (Sunde *et al.*, 2003; Lader *et al.*, 2008).

8.4.2 Water quality

Water exchange is critical for the replenishment of dissolved oxygen and the removal of excess feed and waste products. A reduction in oxygen concentration from the outside to the inside of cages, and a relationship between oxygen reduction and short-term water exchange, has been demonstrated in many studies (Hisaoka *et al.*, 1966; Inoue, 1972; Wee, 1979). In addition, increasing stocking density increases the rate of oxygen consumption in cages (Kadowaki *et al.*, 1978). Consequently, with a combination of low current flow and significant mesh occlusion, and a high stocking density of fish, dissolved oxygen may be reduced rapidly to critical levels (Edwards and Edelsten, 1976).

Kennedy *et al.* (1977) reported fish mortality due to anoxia in a heavily fouled cage in which the dissolved oxygen (DO) concentration fell below 4.0 mg/l. This low DO concentration was directly attributed to poor water exchange, and was increased to 8.25 mg/l after installation of a clean net. Oxygen concentrations of > 7 mg/l are recommended for salmon farming, whilst concentrations of < 5 mg/l negatively impact on fish growth and respiration, and levels of < 2 mg/l can result in mortality (Boyd, 1982).

A number of factors contribute to total supply and consumption of dissolved oxygen within sea cages, and the relative importance of these has been calculated through modelling (Edwards and Edelsten, 1976; Silvert, 1992; Løland, 1993; Silvert, 1994; Cronin, 1995). Oxygen supply is largely through water exchange, but also from photosynthetic fouling communities and atmospheric diffusion. Oxygen is primarily consumed by the fish, but to some extent also by the biochemical oxygen demand of the immediate environment and the fouling communities. The model identifies the most important factors as the respiratory demands of the fish and the mass of water exchanged.

The maximum stocking density of fish is almost completely dependent on water exchange and can be calculated based on the rates of oxygen consumption and supply. The model also allows calculation of tolerable mesh occlusion levels for existing stocking densities. For example, Cronin (1995) found that commercial tuna cages (30 m diameter, 15 m deep, 800 mm mesh, 840 × 25 kg tuna) require a transmission of at least 42% in spring (15°C water) and 80% in summer (22°C water) to maintain satisfactory oxygen levels. These latter figures also demonstrate the effect of decreased oxygen solubility with increased water temperature. However, this data is species-specific to some extent, and in Cronin's (1995) model respiration rates were based on salmonoids, which are significantly lower than for tuna.

Whilst oxygen levels within cages are primarily controlled by water exchange, oxygen production or consumption by fouling communities can

affect oxygen concentration (Wildish *et al.*, 1993; Cronin, 1995; Cronin *et al.*, 1999). Diurnal changes in oxygen concentrations at salmon farms suggest that respiratory activity of phytoplankton and fouling macroalgae significantly affected cage oxygen concentration (Gormican, 1989; Wildish *et al.*, 1993). Cronin (1995) found fouling communities on tuna cages to be net consumers of oxygen, because of a greater proportion of non-photosynthetic to photosynthetic biomass. However, Cronin *et al.* (1999) stated that the fouling community had minimal impact on the cage oxygen levels (less than 3% of the total oxygen exchange) relative to the processes of fish and sediment respiration and of mass water exchange.

A reduction in water exchange may also impact on fish health because increased levels of ammonia have been found within cages, compared to surrounding waters (Gormican, 1989; Wildish *et al.*, 1993). Detrimental levels of ammonia have not yet been reported in sea-cages because of sufficient water exchange (Gormican, 1989; Wildish *et al.*, 1993), but this is potentially a problem, and acute ammonia toxicity has caused mortality in salmonoids farmed in ponds (Lumsden *et al.*, 1993). Gowen and Bradbury (1987) estimated that 78% of nitrogen consumed by salmon is lost as faecal and excretory nitrogen, which equated to 32 kg of ammonium produced per tonne of fish food consumed. A 450 m³ cage, holding 8 t of fish, would produce 1120 mg ammonia/m³/h over an average 8-month growing season (Wildish *et al.*, 1993).

8.4.3 Disease risk

Fouling communities may present a health risk to cultured species because they can act as reservoirs for pathogenic microorganisms harboured by macrofouling species or existing in the extensive microbial communities on cage netting. Viral pathogens of finfish may accumulate and persist for long periods within shellfish. The viruses, identified as finfish pathogens, isolated from bivalves included 13p2 reovirus and the related chum salmon virus, JOV-1 Japanese oyster virus, infectious pancreatic necrosis strains and infectious hematopoietic necrosis virus (Leong and Turner, 1979; Meyers, 1984). In addition, a number of bacterial agents that cause disease in finfish are also common to bivalve tissues (e.g., *Vibrio* spp.).

Marine aquaculture may lead to infections with unusual parasites, either due to culture in new geographical areas, or in net pen environments (Kent, 2000). Amoebic gill disease, affects Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), and causes significant mortality in Ireland (Rodger and McArdle, 1996), Chile, France, New Zealand and Tasmania aquaculture industries, particularly in summer (Clark and Nowak, 1999). Atlantic salmon and coho salmon (*O. kisutch*) have also been affected in the USA (Kent *et al.*, 1988). Biofouling was reported to be a risk factor

for amoebic gill disease outbreaks, based on the premise that *N. pemaquidensis* was detected on macrofouling species (especially the bryozoan *Scupocellaria bertholetti* and the ascidian *Ciona intestinalis*), in the microbial biofilm layer and in the water column (Tan *et al.*, 2002). However, the causative agent of AGD, the new species of amoeba *N. perurans* (Young *et al.*, 2007, 2008), has not yet been associated with biofouling, potentially due to its recent discovery.

The occurrence of disease in caged fish has also been linked to the consumption of fouling organisms by the cultured species (Kent, 1990; Andersen *et al.*, 1993). Netpen liver disease (NLD) was thought to be caused by a hepatotoxin that may be produced by algae, during summer (Kent, 1990). The toxin isolated from affected liver has been identified as microcystin-LR, a protein phosphatase inhibitor (Andersen *et al.*, 1993). In addition, injection of microcystin-LR is sufficient to re-create the pathologic changes of the disease in Atlantic salmon (Andersen *et al.*, 1993). Furthermore, the fouling biota of the salmon cage is a reservoir of the microcystin (Andersen *et al.*, 1993), and the disease is likely to be contracted by feeding on net biota. The organism responsible for producing microcystins has not been identified, but the toxin is produced by freshwater cyanobacteria, and it has been detected in mussels collected near a NLD outbreak (Chen *et al.*, 1993).

Fish farms can also disrupt the parasite life cycle, by increasing the host density and promoting transmission from wild to cultured stocks and *vice versa*. Infection by *Gilquinia squali* metacestodes has been implicated in the deaths of Chinook salmon smolts of fish farms in British Columbia, where 10% mortality was associated with the eye disease at a particular site (Kent *et al.*, 1991). The definitive host for the parasite is the spiny dogfish *Squalus acanthias*, which were prevalent in and around the affected net pen sites (Kent *et al.*, 1991). It is likely that, during one of its life-stages, an unidentified crustacean acts as an intermediate host, and that transfer to the definitive host (or the farmed salmon) occurs directly through ingestion (Kent *et al.*, 1991). For salmon, therefore, the crustaceans within the fouling biota are a reservoir of the parasite. However, it is not known if the parasite is sufficient to cause the observed morbidity and mortality associated with the eye disease.

Fouling communities may directly impact on fish by causing physical damage to cultured species. Gill lesions and mortality caused by the spines of diatoms in dense mixed algal blooms have been recorded for pen-reared Atlantic salmon in British Columbia (Kent *et al.* 1995). Heavily fouled nets can also support the existence of free-swimming stages of sea lice (*Lepeophtheirus salmonis*).

However, biofouling may also have some positive effects on disease risk. The potential for mussels *Mytilus edulis* to harbour the bacterial kidney

disease bacterium *Renibacterium salmoninarum* has been ruled unlikely (Paclibare *et al.*, 1994). *R. salmoninarum* is shed in the faeces of infected salmon, and it was considered possible that the pathogen may be concentrated in the filter-feeder, which fouls the net cages and acts as a continuous source of re-infection for salmon. However, the mussels killed the majority of *R. salmoninarum* during digestion, and in fact are likely to reduce the levels of the pathogen in the cage environment (Paclibare *et al.*, 1994).

8.4.4 Cage deformation and structural fatigue

Exposure to currents causes net cages to change their shape by deflection and deformation (Fredheim, 2005). The extent of the change in shape depends on current velocity, original shape and construction of the cage, placement weights, type of netting, and level of biofouling (Fredheim, 2005; Lader *et al.*, 2008). An increase in mesh occlusion will significantly increase drag forces on netting. Milne (1970) determined current forces on clean and fouled nets at various current velocities, and showed that forces on a fouled net may be 12.5 times that of a clean net. Consequently, unless cages are heavily weighted, the shape of the cage may be severely deformed by current flow (Osawa *et al.*, 1985). Aarsnes *et al.* (1990) calculated deformation rates for a 12000 m³ cage (with 400 kg of bottom weight) and found that the cage volume was reduced by 45% (to 6600 m³) under a current velocity of 0.5 m/s (1 kn), and by 80% (to 2300 m³) under a velocity of 1 m/s (2 kn). Wee (1979) observed a 50% reduction in volume of a heavily fouled in use cage. Reduced cage volume is likely to impact on fish health because oxygen consumption and ammonia production will increase per unit volume, and crowding is likely to stress the cultured fish.

Lader *et al.* (2008) analysed the effect of incoming currents of varying velocities on net cage deformations and volume reductions of gravity cages in two working Atlantic salmon farms in Norway and Faroe Islands. At Varaldsøy, Norway, current speeds of 0.13 m/s caused a cage volume reduction of 20%, whereas at Hestur, Faroe Islands, current speeds of 0.35 m/s caused a cage volume reduction of 40%. Highly deformed nets increase the structural stress of the cage and, although increasing cage weight will reduce deformation, this adds to the structural stress (Anon, 1993). Tomi *et al.* (1979) reported that weight added to cage corners resulted in a two- to six-fold increase in horizontal forces on the cage. With heavy weighting, waves will cause the floating frame to move upward whilst the weights pull the netting downward. Structural loadings and fatigue are likely to increase further when predator netting is attached to cages.

The static load of the net is also directly impacted on by the biomass of the fouling community, which may increase the weight of the net up to 200-fold (Milne, 1972 in Beveridge, 2004). This increased load must be taken

into account when designing the floatation and mooring systems. Failure to do so can result in net failures, which have been devastating in commercial enterprises (Huguenin and Ansuini, 1978; Huguenin, 1997).

Furthermore, Swift *et al.* (2006) measured the increase in fluid drag on fish cage netting due to biofouling, including the skeleton shrimp *Caprella* sp., the hydroid *Tubularia* sp., the blue mussel *Mytilus edulis* and the clam *Hiatella actica*. Biofouled drag coefficients generally increased with solidity (the ratio of the projected area to the circumscribed area) and biomass, and drag of fouled nets may be over three times that of clean nets (Swift *et al.*, 2006).

8.5 Summary of impacts

Biofouling on sea cages causes mesh occlusion and a resultant decrease in productivity and fish health, as well as structural fatigue and cage deformation. Consequently, biofouling is a significant management issue resulting in increased operational expenses. What is surprising, for an issue with such high impact, is the sparse information on the effects of fouling, which is only now beginning to be addressed in detail. Given the limited choice of products available to control fouling in aquaculture, quantitative studies on the spatial and temporal variation in fouling communities at the level of species, and the effects of animal husbandry and farm management on fouling development, will assist industries choose the most cost-effective method for fouling control taking into account regional and seasonal variation. To date the focus on biofouling has been heavily skewed to more traditional aquaculture industries, such as the northern hemisphere salmon industry, with little quantitative information on fouling in new aquaculture regions and in the tropics where fouling is most rapid. The development of cage aquaculture in tropical regions, and the move to offshore cage culture where nearshore space is limiting, presents new challenges in understanding the impacts of fouling, and in particular in implementing successful strategy for biofouling control.

8.6 The control of biofouling

Given the severe impacts of biofouling in cage culture commercial fish farms, operations usually employ a multifaceted approach to control net fouling, which typically involves utilisation of farm management techniques of frequent net changing and cleaning, and the use antifouling coatings. These methods are reviewed, as are developing options for the non-biocidal control of biofouling in finfish mariculture. This is particularly critical as restrictions on the application of traditional metal-based coatings are applied to the broader aquaculture industry.

8.6.1 Net changing

Fouling develops very rapidly on cages in nearly all regions of the world at some stage of their use, and frequent changing and cleaning of nets is critical to maintain water exchange within cages. For example, nets must be changed every 5–8 days in summer in Australia (Hodson and Burke, 1994), every 8–14 days in Japan (Milne, 1979) every 14 days in Malaysia (Lee *et al.*, 1985), and every 3–4 weeks in Canada (Menton and Allen, 1991). Large mesh cages are also changed less frequently because of the considerable amount of fouling required to significantly occlude the mesh. In Australia predator fences (100–150 mm mesh) are changed every 3–6 months, and tuna cages (60–90 mm mesh) are cleaned every 6 months (Cronin *et al.*, 1999). Some delay in the frequency of cleaning may be achieved by raising the top few metres of the cage out of the water (Needham, 1988), but this is only applicable where the fouling is restricted to the upper area of the cage. Whilst frequent net changing is common in temperate and tropical regions, cages immersed at off-shore sites and in very cold water can remain immersed for long periods without cleaning. For example, cages in northern Norway are changed only once per year, usually in July after the period of maximum ascidian and mussel settlement (Sutterlin and Merrill, 1978).

Net changing incurs a major cost to the industry, necessitating the purchase of a large number of nets and provision of dedicated net-changing and cleaning teams. Moreover, frequent net changing also risks damage or loss of stock, and disturbs feeding regimes which lowers growth rates. However, the extent of the economic consequences of fouling and fouling control to the aquaculture sector are largely unquantified.

8.6.2 Shore-based net cleaning

The removal of fouling communities from cages is generally achieved by replacing the fouled net, and transporting it to shore for manual or semi-automated cleaning (Lewis, 1994a). However, the frequent changing of netting on a standard floating cage is labour and capital-intensive, and boat-mounted hydraulic cranes are needed for large cages. During changing, the fouled net is partially raised, and a clean net is peeled underneath and attached to the collar. The fouled net is then untied and removed, with the fish released into the clean cage. Fouled netting is usually left to compost for 1–2 weeks on-shore, followed by cleaning with high-pressure water hoses or automated washing machines (Sutterlin and Merrill, 1978; Lewis, 1994a; Cronin *et al.*, 1999).

Unfortunately, washing procedures and net handling frequently cause damage to netting and reduce its life-span. Consequently, after cleaning nets are laid out for mending and replacement of damaged sections. At some

farms nets are dropped to the seabed after removal from the cage, and the fouling is degraded biologically over a period of weeks (Sutterlin and Merrill, 1978). However, this latter technique is unsuitable when clean nets must be available within short time periods, and the practice is also likely to increase benthic pollution.

8.6.3 Underwater net cleaning

Given the large expense involved in frequent net changing, it is surprising that little information is available on underwater cleaning of cages. There are few published reports on the success of underwater cleaning, but there are commercial cleaners available and in use. The Tasmanian Atlantic salmon industry trialled the efficacy of an underwater net cleaner, which prevented fouling over a 10-week period during summer (Hodson *et al.*, 1997). However, fouling was not removed from the netting bars or crevices due to physical constraints, which led to rapid recolonisation and regrowth of fouling.

Simpler forms of underwater cleaning are also practised, but often require SCUBA diving and are therefore more expensive and dangerous than shore-based cleaning. High pressure water hoses have been used to clean tuna cages in South Australia (Cronin *et al.*, 1999), and vacuum cleaning equipment has been used for salmon cages in Tasmania (Doedens, 1992). However, the latter technique was only effective on painted nets (because the fouling attached poorly), and was eventually abandoned because of the considerable amount of time required to clean an entire cage. In general, *in situ* cleaning is unlikely to be viable unless fully automated; any fouling remnants left after cleaning are likely to regrow quickly and underwater cleaning may therefore be required at a high frequency (Moss and Marsland, 1976). Geffen (1979) suggested that brushing increases fouling problems because it scratches the mesh and encourages rapid recolonisation.

In Norway, which has the most well-reported cleaning practices, three main strategies are used for the handling of biofouling on nets and they include cleaning of nets both onshore and *in-situ*. Copper-based coatings are used on nets and this is combined with regular washing *in-situ*. Copper-based coatings on nets are also combined with regular drying to remove fouling. Finally, uncoated nets are used with frequent cleaning (Olafsen, 2006). The use of copper-based coatings on nets combined with regular washing in the sea is employed by approximately half of Norway's 1500 fish farms (Olafsen, 2006). Regular cleaning of nets is primarily done with cleaning discs on remote operating vehicles, or manually by divers. Net cleaners in Norway have two, four or six rotating discs with a diameter of 400 or 500 mm each. The smooth discs have a stainless steel curved front and rotate between 750 and 1500 rpm, depending on water pressure, water flow and

diameter on the cleaning disc. Although frequent mechanical cleaning can be an expensive strategy, the combination of with other strategies, such as air drying, can reduce the costs by up to 50% per m² of netting (CRAB 2004–2007).

8.7 Biological control

An increase in profitability and sustainability could be achieved by use of herbivorous fish or invertebrates to control fouling (Beveridge, 2004), and benthic/detritus feeders to remove uneaten food (Angel *et al.*, 2002). The biological control concept is constrained by the great variation in types of algal and invertebrate fouling, which suggests that only herbivores and omnivores with a broad dietary range will be successful control agents. Furthermore, it is likely that continuous grazing will provide an environment *selective* for inedible species, and thus polyculture may only reduce the frequency of net changing.

Nevertheless, biological control using sea urchins and hermit crabs has proved effective for controlling fouling of suspended shellfish systems (Hasse, 1974; Littlewood and Marsbe, 1990; Lodeiros and Garcia, 2004; Ross *et al.*, 2004). For finfish, biological control of fouling has been successful with co-culture of other finfish. These include mullet (*Mugil cephalus* at 0.5–0.78 kg/m³) in small cages of pompano (Swingle, 1972), rabbit fish (*Siganid* sp.) in cages of grouper and carangids (Chua and Teng, 1977, 1980), rohu (*Labeo rohita*) in cages of carp (Sharma, 1979), and knifejaws (*Oplegnathus* spp.) in cages of yellow tail (Kuwa, 1984). The stocking density of the added herbivorous fish varies greatly, from 3–9% of the total cage biomass (Kuwa, 1984; Li, 1994) to densities of only 1 fish/5 m³ (Sharma, 1979). However, there are potential negative impacts where knifejaws prey on the tail and fins of sick yellow tail (Kuwa, 1984), and there may be a number of risks to the primary culture species, such as greater disease potential and increased demands on dissolved oxygen.

Detritivores like the red sea cucumber *Parastichopus californicus* have proved effective in significantly reducing fouling in salmon mariculture. One hundred sea cucumbers placed inside an 18 m diameter, 7.5 m deep, 5 mm mesh pen containing one million salmon maintained 58% of the transect line completely clean, whereas control pens were uniformly fouled (Ahlgren, 1998). However, sea cucumbers were negatively affected by wave-generated undulation and were unable to maintain their positions on the sides of cages suspended with buoys, although they were able to maintain positions throughout in rigid frame pens (Ahlgren, 1998). The advantage of polyculture with sea cucumbers is that they are a commercially important aquaculture crop in their own right, with strong demand for the product in Asia (Conand and Sloan, 1989).

8.7.1 Alternative cage designs

An alternative to both frequent net changing and underwater cleaning is the use of fully-enclosed rotating cages (Geffen, 1979; Blair *et al.*, 1982; Menton and Allen, 1991; Willinsky *et al.*, 1991). These have either been horizontally-mounted cylinders which rotate on a central axle (Menton and Allen, 1991) or rectangular boxes with inflatable buoyancy devices in each corner. The rectangular cages are gradually rotated by sequentially changing the buoyancy of the corners (either by inflation and deflation, or displacement and filling with water). With rotatable cages no area of netting needs to be left submerged for long periods, and netting can be brought to the surface to air dry and hence kill attached fouling. Furthermore, the cage is easily accessible for fouling removal and netting repair, and by keeping the net immersed for short periods significant fouling growth can be avoided. Blair *et al.* (1982) found that a cage rotation of 90 degrees per week was sufficient to keep cages essentially free of fouling, and Geffen (1979) reported that cage rotation at 3-day intervals kept cages completely clean.

Despite other benefits of completely enclosed cages, such as prevention of bird predation and avoidance of storms and ice through cage submergence, rotating cages are not widely used. It would be necessary to construct very large rotating cages if they were to hold volumes of fish comparable to conventional floating collars of > 90 m circumference. Moreover, commercially available rotating cages are more expensive than conventional designs and continued exposure to direct sunlight can increase netting degradation (Beveridge, 2004). A measure of success for many of these early technologies is their uptake by industry and clearly this has not been the case with rotating cages where they are not used in large scale commercial production.

8.7.2 Chemical antifoulants and paints

Chemical antifoulants in paints prevent the establishment of a marine biofilm through leaching of a biocide that produces a thin layer of toxic solution around the net. During the past 50 years antifouling paints and coatings have been intensively studied. Some of the earliest published attempts at antifouling of fish cages were conducted in the western Baltic Sea by the Institute for Coastal and Freshwater Fisheries and showed an antifoulant kept nets completely clean for 5 months, during which time untreated nets became totally occluded (Koops, 1971).

However, products designed specifically for fish cages are scarce, and the industry has historically borrowed antifouling technologies from other

marine industries, particularly shipping. Consequently, chemicals and heavy metals that are now clearly recognised as dangerous in the environment have been used in the aquaculture industry. There is well documented public concern about the use of chemicals in the aquaculture industry, in particular antibiotics and antifoulants (Costello *et al.*, 2001). Therefore the broader aquaculture industry including producers, regulators and research providers need to develop a safe industry, in both perception and reality.

8.7.3 Tin

The organotin antifoulant tributyltin (TBT) is a broad-spectrum algicide, fungicide, insecticide and miticide and was one of the most widespread antifoulants used on ship hulls from the 1960s (Yebra *et al.*, 2004). Because of its antifouling efficacy, TBT was also used extensively on the netting of sea cages in mariculture of salmon. For example, fouling on cages ($2 \times 2 \times 2$ m; 13 mm, 9-ply mesh) coated with an organotin antifoulant was reduced to 1 kg/net after 2 months submersion, whereas 91 kg/net was present on untreated cages (Lee *et al.*, 1985).

However, the use of tributyltin (TBT) antifoulants has exemplified the hazards of toxic coatings in mariculture (Ellis, 1991; Alzieu, 1998; Terlizzi *et al.*, 2001). TBT leaches out of impregnated nets and has been recorded in the waters around treated fish cages (Balls, 1987). Balls (1987) measured TBT release in newly painted salmon cages, and recorded 1 mg/m^3 (pg/l as Sn), 0.1 mg/m^3 , and 0.005 mg/m^3 after 1 day, 2 weeks and 5 months, respectively. Similarly, Short and Thrower (1986) reported TBT concentrations from 0.007 to 0.026 mg/m^3 Sn in treated salmon pens in the USA.

The use of TBT impregnated nets in salmonid aquaculture can induce histopathological effects (Bruno and Ellis, 1988) and mortality (Lee *et al.*, 1985). Short and Thrower (1986) reported acute intoxication with a 96-h LC_{50} of 1.5 pg/l for Chinook salmon. Behavioural abnormalities and pathological changes occurred in farmed Atlantic salmon (*Salmo salar*) that were transferred to a newly antifouled cage and feeding responses were dramatically reduced after 4 days (Bruno and Ellis, 1988). Salmon show lifting of the gill epithelium, an increase in the number of leucocytes in the retina, and a lens that was opaque inferring blindness. After 7 weeks exposure hyperplasia was observed in the dermal layers of the skin, resulting in protruding scales, especially along the lateral line (Bruno and Ellis, 1988). These observations were interpreted as TBT interfering directly with the normal growth of salmon.

Salmon raised in treated nets also rapidly bioaccumulate TBT. Short and Thrower (1986) reported bioaccumulation after 3–4 days exposure to 1.5 µg/l. They recorded levels of 6.4, 1.9 and 0.3 µg TBT/g wet weight of liver, brain and muscle respectively. Similarly, Atlantic salmon exposed to 0.1–1.0 µg/l TBT for 26 days had bioaccumulation in tissues with the highest concentration found in the liver (Davies and McKie, 1987). Bruno and Ellis (1988) reported that after 7 weeks exposure, TBT had bioaccumulated in the flesh, liver, gills and caeca. Given its ability to bioaccumulate TBT was able to enter the human food chain, where it is toxic to humans. The WHO has set a limit of 3.2 µg/kg body weight for tin in humans, which corresponds to a daily consumption of 150 g salmon for a 70 kg person (WHO, 1999).

TBT also affects non-target organisms, particularly bivalves (Paul and Davies, 1986). In the early 1970s, the deleterious effects of TBT in the environment were observed through shell malformations and reduced growth in the Pacific oyster, *Crassostrea gigas* (Alzieu *et al.*, 1981, 1986). The serious problems encountered in commercial oyster cultures in France were soon followed by reports of similar problems in the UK (Waldock and Miller, 1983). TBT also induces imposex in gastropods (Gibbs *et al.*, 1991), and has since been found in fish, seabirds and marine mammals (reviewed in Terlizzi *et al.*, 2001). Clearly, TBT antifouling products pose an unacceptable risk to non-target species that was unidentified when introduced into the market.

The adverse effects resulting from widespread use of TBT has led to a ban on its use (Alzieu, 1991; Evans, 1999). In 1986, the National Farmers' Union in Scotland introduced a voluntary ban on its use in fish cages, and in 1987 its retail sale was prohibited by the Scottish government (Balls, 1987). In Australia, TBT antifouling is presently restricted to vessels greater than 25 metres in length, and in New Zealand there has been a complete ban on all TBT sales and use since December 1993 (ANZECC, 1995). The International Maritime Organisation banned the use of TBT in paints from 2003 (Julian, 1999) and concordantly many governments have prohibited organotins in antifouling paints (Bell and Chadwick, 1994; Costello *et al.*, 2001).

The challenge for the aquaculture industry is not to repeat the TBT scenario in the future (Ellis, 1991). In the wake of the TBT ban, Lewis (1994b) recommended six criteria for antifouling strategies in the aquaculture industry. They should:

- (1) be effective against a broad range of fouling taxa
- (2) be environmentally benign
- (3) have no negative effects on the cultured species
- (4) leave no residues in the cultured species
- (5) be able to withstand on-shore handling and cleaning
- (6) be economically viable.

8.7.4 Copper

In the void left by the ban on TBT, attention soon re-focused on copper and copper-containing coatings which have a long history of use in shipping and mariculture (Lewis and Metaxas, 1991; Lewis, 1994b, Chapter 19). For example, in 2005 a total of 261 tonnes of copper were sold to the aquaculture industry in Norway (Skolbekken, 2007). Copper-based antifoulants are currently approved for use in aquaculture in Europe and North America, and have also been used in Australia (Hodson and Burke, 1994; Douglas-Helders *et al.*, 2003). Copper adds approximately 20–25% to the cost of a knotless nylon cage (Beveridge, 2004), but it is a very effective antifoulant. In temperate regions, nets must be coated each year, but antifouling with copper gives good protection for 6 months and is effective during summer when fouling is worst (Beveridge, 2004).

The major active ingredient in copper-based antifoulants is cuprous oxide and copper. Copper leaches out of impregnated nets into the water column. The leaching rate of copper in paints is increased by the presence of zinc, usually in the form of zinc oxide (French *et al.*, 1984). Leaching rates of 10 and 20 $\mu\text{g}/\text{cm}^2/\text{day}$ are required to prevent the settlement of barnacles and diatoms, respectively (Callow, 1999). In Jervis Inlet, British Columbia, the concentration of copper inside a treated salmon net pen was 0.54 $\mu\text{g}/\text{l}$ 2 days after net installation, and this concentration was present one month later (Lewis and Metaxas, 1991). About 700 m away from the nets, the copper concentration was 0.38 $\mu\text{g}/\text{l}$, but the difference was not statistically significant (Lewis and Metaxas, 1991). Other studies demonstrate that copper from nets treated with Flexgard XI[®] was released into the environment at an exponential rate of 155 $\mu\text{g Cu}/\text{cm}^2$ until reaching a long-term rate loss of 37.6 $\mu\text{g Cu}/\text{cm}^2$ (Brooks, 2000; Brooks and Mahnken, 2003). Industry best practice is to introduce fish into nets one month after newly coated nets are in position, to minimise the potential for bioaccumulation.

The reason for this caution is that copper is highly toxic to many marine organisms, but particularly to the larval stages of invertebrates (Mance, 1987). Relatively low concentrations of copper are known to be harmful to fish and diverse effects have been reported from toxicity studies (Chapman, 1978; Chapman and Stevens, 1978; reviewed in Peterson *et al.*, 1991; reviewed in Brooks and Mahnken, 2003). Acute copper intoxication 96-h LC_{50} occurs in adult salmonid fish at 60–680 $\mu\text{g Cu}/\text{l}$ (Sorensen, 1991), and the USA EPA chronic marine standard of 3.1 $\mu\text{g Cu}/\text{l}$ is a 4-day average that must not be exceeded more than once every 3 years. The UK environmental quality standard for dissolved copper in seawater is 5 $\mu\text{g Cu}/\text{l}$ (Voulvoulis *et al.*, 1999a), but in practice this value is often exceeded and may be having a detrimental ecological effect (Matthiessen *et al.*, 1999).

Copper in antifouling paints may also have negative impacts on non-target organisms. For example, copper reduces the germination of the alga

Fucus vesiculosus (Andersson and Kautsky, 1996) and *Fucus spiralis* (Bond *et al.*, 1999). Copper oxide also induces changes in growth, chlorophyll, carbohydrate and protein compositions in the marine microalgae *Tetraselmis suecica* and *Dunaliella tertiolecta*, and has 96 h EC₅₀ concentrations of 1.3 mg/l for both species (Lim *et al.*, 2006). Copper also reduces the growth of the clam *Tapes philippinarum* (Munari and Mistri, 2007), and damages gill filaments of sea bream *Pagrus major* (Mochida *et al.*, 2006).

However, whether copper bioaccumulates as a consequence of aquaculture is unresolved. Voulvoulis *et al.* (1999a) regard copper as showing 'only a slight tendency for bioaccumulation'. There are reports that salmon raised in copper-treated nets do not bioaccumulate copper in muscle or liver tissue (Petersen *et al.*, 1991; Solberg *et al.*, 2002), and there was no detectable bioaccumulation of copper in the brown seaweed *Ascophyllum nodosum*, the blue mussel *Mytilus edulis*, or the saithe *Pollacius virens* from fish farms (Solberg *et al.*, 2002). In contrast, intestinal copper levels in the green sea urchin *Strongylocentrotus droebrachiensis* were elevated at salmon aquaculture sites (Chou *et al.*, 2003), and copper has been shown to bioaccumulate in the hepatopancreas of lobsters sampled near salmon farms (Chou *et al.*, 2000). Furthermore, oysters growing around marinas have elevated levels of copper, which may be due to antifouling paints (Claisse and Alzieu, 1993).

In addition, there are environmental concerns from the elevated concentrations of copper found in sediments around salmon farms (Miller, 1998). Copper accumulation in sediments is highly dependent on physical characteristics and sediment chemistry. An increase in average copper concentrations in the sediment, from 21 mg/kg at a reference site, to 49–430 mg/kg was found for four out of five farms using copper-treated nets (Solberg *et al.*, 2002). However, owing to high variance within sites the differences were not statistically significant. In another study, an approximate two-fold increase in copper in the sediments was found in 117 farms using copper-treated nets (48.24 ± 27.00 $\mu\text{g Cu/g}$) compared to 39 not using copper-treated nets (26.27 ± 2.77 $\mu\text{g Cu/g}$), but again the difference was not statistically significant (Brooks and Mahnken, 2003). Given recent evidence on the effects of dissolved organic carbon (DOC) on the bioavailability of copper, and therefore its impact on non-target species, the use of copper in areas with high levels of DOC may not be as detrimental as perceived and is an area of on-going and topical research (Chapter 19).

Importantly, simply the perception of using copper as an antifouling compound has impacts. Fish may be exposed to antifoulants for long periods, up to months and the use of toxic metal-based antifouling is therefore an undesirable aspect in an industry selling a food product from a 'clean and green' marketing perspective. Most countries are now working towards a reduction in the use of copper-based antifouling in the short-term. The

European Commission is proposing to give copper a R50/R53 classification, based on the 67/548/EEC directive on dangerous substances, which recognises that copper is toxic to aquatic organisms and may cause long-term adverse effects in the environment. The Norwegian aquaculture industry is moving towards a reduction of copper based on public perception of copper treatment as a negative environmental impact (Sandberg and Olafsen, 2006).

8.7.5 Booster biocides

Worldwide, there are a number of other biocides currently being used as antifoulants, albeit not necessarily in mariculture (Callow, 1999; Konstantinou and Albanis, 2004, reviewed by Konstantinou, 2006), which are potential candidates to supplement or replace the use of copper as an antifoulant (Chapter 21). The most commonly used biocides include Irgarol 1051, Diuron, Sea-Nine 211, Dichlofluanid, Chlorothalonil, Zinc pyrithione, TCMS (2,3,5,6-tetrachloro-4-methylsulfonyl) pyridine, TCMTB (2-thiocyanomethylthiobenzo-thiazole), and Zineb (Callow, 1999; Konstantinou and Albanis, 2004, Yebra *et al.*, 2004, Konstantinou, 2006). Products based on cuprous oxide containing chlorothalonil, and dichlofluanid, have been used in aquaculture in the UK. However, these are now being phased out due to the minimisation of biocide use for aquaculture. Coatings using isothiazolinones as the sole biocide class have been successfully tested in Australia (Svane *et al.*, 2006) but there is little peer-reviewed literature on the efficacy of other biocides trialled in an aquaculture context.

There is a potential danger that the biocides listed, which are in some cases largely untested, may be less efficient and/or more harmful to the environment than either TBT or copper (Evans, 1999). The known chemical and physical properties of the common biocides vary widely, and their properties, toxicity, environmental fate and gaps in knowledge in the aquatic environment have been extensively reviewed (Callow, 1999; Thomas *et al.*, 1999; Voulvoulis *et al.*, 1999a, 1999b; Thomas *et al.*, 2000; Thomas, 2001; Thomas *et al.*, 2001; Okamura *et al.*, 2002; Thomas *et al.*, 2002; Voulvoulis *et al.*, 2002a; Voulvoulis *et al.*, 2002b; Thomas *et al.*, 2003; Konstantinou and Albanis, 2004, Konstantinou, 2006). It is also clear that biocides will persist in the environment when associated with paint particles, released particularly during cleaning procedures (Thomas *et al.*, 2003). With many gaps in our knowledge of the longer-term effects of biocides, it is difficult to evaluate impacts and risks on the aquatic environment, and hence good environmental policy must be formulated according to the precautionary principle. A summary of key data on each biocide follows, and the reader is directed to a comparative environmental assessment of relevant biocides for detailed information (Voulvoulis *et al.*, 2002a). There is also a detailed review on the

photochemical fate of these booster biocides on their release (Sakkas *et al.*, 2006; Chapter 21).

In summary, the herbicides Irgarol 1051 and Diuron persist in the water column, whereas Sea-Nine 211, Dichlofluanid, Zinc pyrithione and Chlorothalonil disappear quickly (Thomas, 2001; Thomas *et al.*, 2002; Thomas *et al.*, 2003; Konstantinou and Albanis, 2004). Diuron, Sea-Nine 211, Zineb and Thiram do not bioaccumulate appreciably, whereas Irgarol does (reviewed in Konstantinou and Albanis, 2004). Diuron and Irgarol 1051 show the least toxicity to Chinook salmon *Oncorhynchus tshawytscha*, while pyrithiones showed high levels of toxicity (Okamura *et al.*, 2002). Overall, Zinc pyrithione and Zineb perform the best for environmental parameters, then Irgarol, Chlorothalonil, Sea-Nine 211 and Diuron. Dichlofluanid, TCMTB, TCMS pyridine and TBT perform poorly, with TCMS pyridine and TCMTB demonstrating environmental characteristics similar to TBT (Voulvoulis *et al.*, 2002a). Clearly, there are impacts on the aquatic environment with all booster biocides, and no ideal replacement for either TBT or copper has been developed.

In the current regulatory environment, development and registration of toxic biocides is extremely expensive. For example, Rohm and Hass spent 10 years and 10 million dollars registering Sea-Nine 211 in the USA (Bingaman and Willingham, 1994; Rittschof, 2000). It is considered uneconomical to develop future toxic booster biocides for biofouling control (Bingaman and Willingham, 1994). The focus in research and development has shifted to antifouling agents that are both effective and environmentally benign as a consequence of their chemistry (non-toxic coatings) or their physical properties (foul-release coatings and non-leaching biocides) (Yebra *et al.*, 2004).

8.8 Non-toxic coatings

8.8.1 Natural products

Natural products have a long history in aquaculture. Prior to use of modern polymer-based netting, farmers in Malaysia soaked cotton nets with tannins extracted from the bark of mangrove trees (*Rhizophora* sp.) (Lai *et al.*, 1993). Tannins are toxic and act as natural biocides, but whilst these absorb well to traditional fibre nets, the absorbancy to synthetic materials is poor and effectiveness is short-term (Lai *et al.*, 1993). However, the potential for natural antifoulants is very limited as they are simply another chemical entity that requires a large commercial development time in a regulatory and commercial environment moving towards surface-effect coatings.

The identification of non-toxic antifoulants derived from natural products is only the first stage in developing a commercial product. The

compound must be synthesised in large quantities at reasonable cost, incorporated into the paint matrix, and undergo the same regulatory evaluation by environmental agencies that biocides go through. The lengthy time-frames and the cost incurred in this process may be prohibitive (Yebra *et al.*, 2004), and these products are unlikely to be viable commercial alternatives to currently registered biocides. This realisation has led to recent investigations demonstrating the effects of well-known pharmaceuticals (Rittschof *et al.*, 2003; Dahlström and Elwing, 2006) and commercially available enzymes (Pettitt *et al.*, 2004; Aldred *et al.*, 2008) as antifoulants, and this approach may prove productive in the future. A comprehensive review of antifouling compounds of natural origin is provided by Fusetani and Clare (2006) including the potential of pharmacoactive compounds. Notably studies in the context of antifouling in aquaculture are not included and this can be expected given larger markets such as shipping where these technologies are targeted.

8.8.2 Foul-release coatings

Biocide-free low surface energy siloxane elastomers and fluoropolymers may provide a non-toxic alternative to control biofouling (reviewed in Callow and Fletcher, 1994; Yebra *et al.*, 2004; Chambers *et al.*, 2006). These 'foul-release' coatings aim at reducing or preventing the adhesion of fouling. Silicone-based paints are not toxic to any organisms tested (Karlsson and Eklund, 2004). They are presently seen as an alternative to toxic paints for ship hulls, where the speed of the vessel produces the hydrodynamic shear needed for the loosely attached fouling to fall off (reviewed in Yebra *et al.*, 2004).

The hydrodynamic forces and hence the efficacy of 'foul-release' or self-polishing coatings should be much reduced in a 'stationary' aquaculture net. Nevertheless, nets and panels coated with non-toxic silicone coatings effectively reduced the initial stages of fouling development and make it easier to clean the net of fouling that does accumulate (Rittschof *et al.*, 1992; Swain *et al.*, 1992; Edwards *et al.*, 1994; Hodson *et al.*, 2000; Terlizzi *et al.*, 2000). A number of commercial products are available for aquaculture and this area is likely to spurn commercial outcomes of great interest to the aquaculture industry in the medium to short term as practical issues such as cost-effective coating of aquaculture nets are addressed. There are also further developments in the field of foul release technologies using the principles of super-hydrophobicity (Genzer and Efimenko, 2006; Marmur, 2006). However, the commercial development of these technologies and their application to stationary aquaculture infrastructure is over an even longer time frame.

8.8.3 Non-leaching biocides

Biocides irreversibly bound to the antifouling coating surface or net are known as non-leaching biocides (Clarkson and Evans, 1993, 1995). While this approach offers advantages in terms of limitation of environmental contamination it has not been successfully pursued, presumably due to technical issues and the broad range of fouling organisms, many of which may not respond to bound biocides. The techniques have been used effectively against bacterial biofouling on biomedical devices (Hume *et al.*, 2004; Zhu *et al.*, 2008) and this is an area of technical promise with the move towards legislation restricting antifouling technologies to non-release mechanisms.

8.8.4 Microtexturing of surfaces

Research identifying physical defences used to combat fouling in specific plants and animals may also have commercial application to antifouling technology. This approach characterises topography and microtextured surfaces that prevent settlement of common fouling organisms. For example, a natural regular rippled surface has been characterised on the blue mussel *Mytilus edulis* (Wahl *et al.*, 1998) and *Mytilus galloprovincialis* (Scardino *et al.*, 2003) and this structure significantly inhibits the development of fouling (Wahl *et al.*, 1998; Scardino *et al.*, 2003; Scardino and de Nys, 2004). Surface microtopographies, many with a bio-inspired design, inhibit the settlement and growth of specific fouling organisms, and also facilitate their release (Callow *et al.*, 2002; Carman *et al.*, 2006; Scardino *et al.*, 2006, 2008; Schumacher *et al.*, 2007a, b). In many cases the efficacy is dependent on scale of the topography relative to the size of the settling larvae or propagule and this limits the effectiveness of surfaces to a restricted range of fouling organisms. However, surfaces are now being developed with multiple scales of topography in order to broaden their deterrent effects (Schumacher *et al.*, 2007a). This non-leaching surface-effect technology has application in conjunction with foul release coatings, and may also prove effective against fouling in aquaculture where a single species which dominates the fouling community can be targeted.

8.9 Conclusions

The methods for controlling fouling on nets and other aquaculture structures are restricted to a limited range of products based on the release of copper and zinc with the addition of booster biocides. This limited range of products is also likely to be reduced as copper and possibly zinc are phased out through legislation, and booster biocides become restricted in their use.

This will leave specific biocides with lower impact environmental profiles as the only mechanism of fouling control. There will, however, be the development of new products with a focus on foul-release antifouling technologies based on low-surface energy (foul-release) coatings, texturing and surface-bound compounds. Foul-release technologies rely on hydrodynamic force to remove fouling organisms with poor adhesion on the foul-release surface making them less suitable for aquaculture. However, as the technology for vessels improves, the transfer (trickle-down) of technology to aquaculture industries will become more feasible with product development targeted at larger aquaculture industries. Another alternative to metal and biocide-based technologies, that has yet to be demonstrated as having broad-spectrum efficacy in controlling fouling, is biological control. Although this will be industry and site specific, and it is difficult to envisage its broad application, it may offer significant benefits to some industry sectors. Alternatively, as metal and biocide based technologies are removed from the market, the aquaculture industry may have to return to the traditional methods of net changes and shore-based cleaning.

8.10 Acknowledgements

Thanks to Dr Stephen Hodson and Dr Bronwyn Houlden for their contribution to the primary reports underpinning the development of this manuscript. Thanks also to the Australia Tuna Boat Owners Association (TBOA), the Aquafin Cooperative Research Centre (Aquafin CRC), and the Australian Fisheries Research and Development Corporation (FRDC) for their financial and intellectual contributions to this manuscript and the broader biofouling research program at James Cook University.

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Expected effect of climate change on fouling communities and its impact on antifouling research

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Abstract: In the coming decades, the marine environment will be subject to profound changes, such as elevation of water temperature and ultraviolet radiation, changes in salinity, and decreases of pH due to acidification. These changes will affect not only the survival of dispersal stages and their development but also their recruitment, which among other factors is mediated by the quality and quantity of settlement cues. Climate change can also modify characteristics of microbial communities and the production of metabolites, which in turn, can change settlement of dispersal stages and development of macrofouling communities. In this chapter, I review the possible effects of climate change on microbial and macrofouling communities, performance of antifouling coatings and highlight future research perspectives.

Key words: climate change, biofouling, larvae, settlement, biofilms, antifouling.

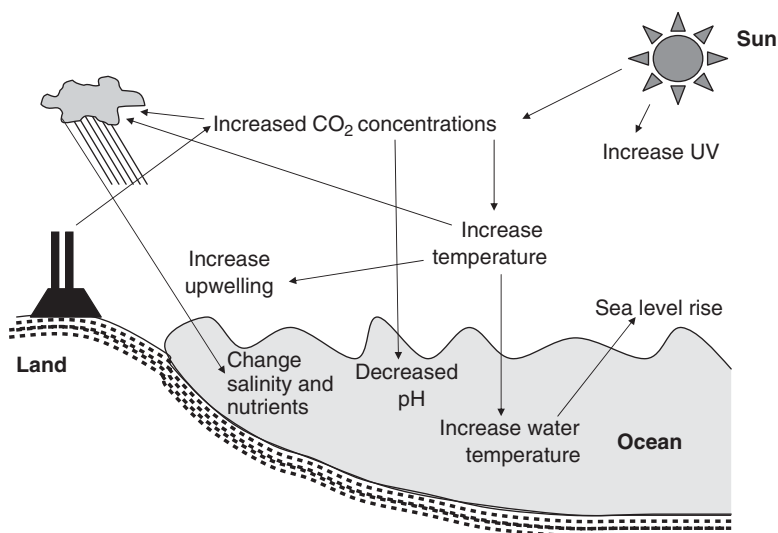
9.1 Introduction

Any natural and man-made substrates in the marine environment are immediately subjected to biofouling by different species of micro- and macroorganisms (Railkin, 2004). In the process of marine biofouling microorganisms play an important role by inducing or inhibiting settlement and metamorphosis of invertebrate larvae and algal spores (see reviews: Wieczorek and Todd, 1998; Maki, 2002; Dobretsov *et al.*, 2006; Qian *et al.*, 2007). Both micro- and macrofouling in the world's oceans cause huge economic losses in the maintenance of mariculture, shipping facilities, vessels, and seawater pipelines (reviewed by Wahl, 1997; Clare, 1998; Fusetani, 2004; Yebra *et al.*, 2004). Moreover, a 5% increase in biofouling subsequently increases ships' fuel consumption by 17% (Evans *et al.*, 2000) and causes a 14% increase in emission of CO₂, NO_x and SO₂ gases (Townsin, 2003) that damage our environment considerably and caused climate changes (Lin and Chen, 2006).

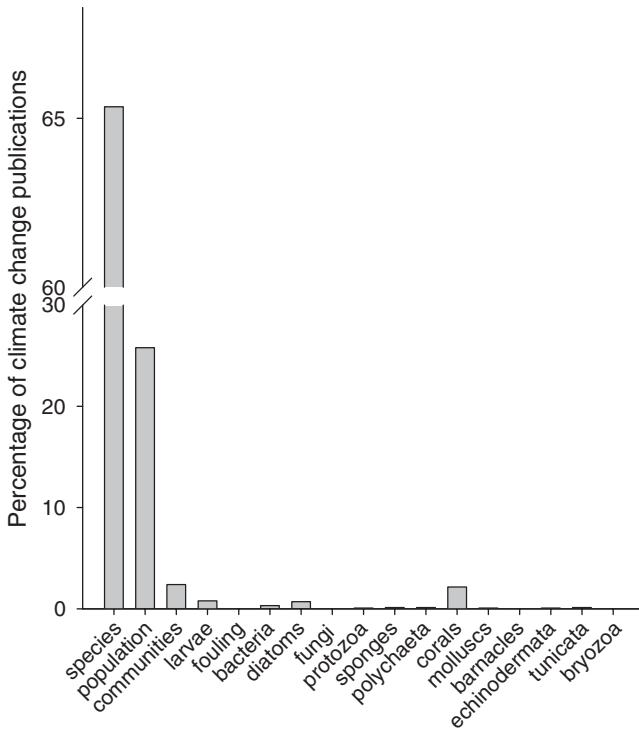
Recent anthropogenically caused climate changes, which are only a fraction of predicted changes in the coming decades, have already triggered

significant responses in the Earth's biota (IPCC, 2001). These climatic changes are mediated primarily by greenhouse gas emissions. Atmospheric greenhouse gas traps some of the heat energy that would otherwise re-radiate to space and heat up the planet (Fig. 9.1). In the past century, global air and sea surface temperatures have risen by $0.6\text{--}0.8^{\circ}\text{C}$ (IPCC, 2001). In this century, the average global temperature is expected to increase by $1.4\text{--}5.8^{\circ}\text{C}$. Ocean warming results in ice melting and increase of freshwater input which causes sea level rise around 2 mm year^{-1} and the global mean level is projected to rise by 0.09 to 0.88 m during this century (IPCC, 2001). Input of freshwater affects nutrient input and decrease of salinity in coastal regions (Fig. 9.1). Increasing temperatures affect atmospheric circulation, which results in increased upwelling and storm intensity and influences precipitation (Goreau *et al.*, 2005; Fig. 9.1). Finally, future warming is predicted to lead to more El-Niño and La-Niña-like conditions (a global ocean-atmosphere phenomenon resulted temperature fluctuations in the Pacific Ocean) that have tremendous effects on marine communities (Timmermann *et al.*, 1999), global climate and socio-economy (Keister *et al.*, 2005).

An increase in atmospheric carbon dioxide (CO_2) leads to an increase in its concentration in the oceans. It is estimated that about 30% modern CO_2 emissions are stored in the ocean (Feely *et al.*, 2004). Continuous uptake of CO_2 is expected to decrease oceanic pH (Fig. 9.1), which will decrease by 0.3–0.5 units over the next 100 years and by 0.3–1.4 units over the next 300 years (IPCC, 2001). In 1996, the observed accumulation of CO_2 in surface



9.1 Abiotic changes associated with climate change.



9.2 Climate change-related publication trends in the scientific literature. The search was performed on the Web of Science (Science Citation Index) for the period from 1980 to 2006. Our search terms were 'climate change' plus one of the terms. Because some articles considered multiple variables, the bars in sum to more than 100%.

water had already caused a decrease of water pH by 0.1 units (Pörtner *et al.*, 2004). Decrease in pH will have a striking effect on marine calcifying organisms, while soft-bodied organisms may take an advantage of such changes. Additionally, it is predicted that an increase in atmospheric CO₂ will deplete the ozone layer which will elevate UV radiation fluxes (Fig. 9.1).

The number of publications that investigate the effect of global climate change on marine communities is limited. According to the literature, most publications about global climate changes are dealing with corals and study single species, while population and community-level studies receive less attention (Fig. 9.2). Since factors associated with climate change affect whole communities, this simplistic approach is likely to give inadequate results in predicting the impact of future climate change. There are

no publications about the expected effect of global climate change on fouling (Fig. 9.2), this is why mostly examples used are from benthic studies for this review.

In this chapter, I summarize the response of biofouling organisms and communities to global changes in environmental conditions with special emphasis on the:

- impact of global climate changes on invertebrates, algae and microbes
- influence of climate change on biofouling communities
- effect of climate change on biological invasion
- directions of future investigations.

9.2 Impact of global climate change on biofouling species

9.2.1 Response to temperature and salinity

Response to elevated temperatures is different between different species (Goreau *et al.*, 2005). Stenseng and co-workers (2005) investigated the thermal limits of two congeneric species of the marine snail *Tegula* that inhabit low and mid-intertidal zones in laboratory experiments. Surprisingly, the investigators found that the warm-adapted, intertidal species of the snail are more impacted by global warming than the congeneric subtidal species that are less tolerant to heat. Similar results were found for upper- and mid-intertidal mollusc species by Przeslawski *et al.* (2005).

It is suggested that organisms that live close to their thermal tolerance level will be negatively impacted by rising temperatures in the future and may disappear (Grainger, 1992; Ayre and Hughes, 2004). For example, stressed, overheated corals expel most of their pigmented endosymbionts (zooxanthellae), become white and eventually die (Hughes, 2003). In 1998, bleaching of corals resulted in a 79–99% loss of living corals in the Indian Ocean (Hughes, 2003). There is not much information about the effect of heat on biofouling species but it is likely that species that are living close to their thermal limit will be stressed and eventually die due to global warming (Bhaud *et al.*, 1995). Additionally, it is likely that cold-water biofouling species will be replaced by warm-water species (Grainger, 1992).

Sensitivity to heat of different ontogenetic stages of organisms is different. For example, certain planktonic larval stages are susceptible to thermal stress (Pechenik *et al.*, 1998) and young benthic stages of many organisms are more susceptible to stress than their adults (Edmunds *et al.*, 2001; Harley *et al.*, 2006). An increase of temperature may cause early release of gametes and propagules, which might then suffer from the absence of particular settlement cues and substrata, predation and low food supply. For

example, recent warming trends in north-western Europe led to an earlier spawning of *Macoma balthica* (Harley *et al.*, 2006). This resulted in a mismatch between larval production and phytoplankton blooms and increased intensity of larval predation. Global warming, in turn, may increase the survival of larvae of invasive species, such as *Dreissena polymorpha* (Wilhelm and Adrian, 2007). Additionally, recent mild winters and elevated water temperatures increased the abundance and the length of plankton peak occurrence of echinoderm larvae in the North Sea (Kirby and Lindley, 2005). Since elevated temperatures may be harmful and beneficial to larvae of different biofouling species, more investigations are needed in order to study this phenomenon.

Global warming intensifies the frequency of El-Niño and La-Niña-like conditions, which have a tremendous effect on marine ecosystems. Elevated temperatures during El-Niño events severely affect coral reefs and cause massive coral bleaching and mortality (Hughes, 2003). On the contrary, other species, such as shrimps and scallops, reproduce and survive better during El-Niño events (Barber and Chavez, 1983). The flow anomalies during El-Niño events have resulted in anomalous in northward transport of plankton and fishes by as much as 350 km/month (Keister *et al.*, 2005). This may increase the amount of successful biological invasions of warm water species (see Section 9.4) and decrease the amount of cold-water species. From one side, elevated temperatures increase growth and photosynthesis rate of some harmful phytoplankton species (Hayes *et al.*, 2004; Peperzak, 2003). From the other side, El-Niño events result in a critical reduction of surface nutrients that are necessary for the phytoplankton growth and subsequently affect the growth and reproductive success of many marine invertebrates and fishes (Barber and Chavez, 1983). Therefore, high intensity of El-Niño-like conditions in future will significantly change diversity and composition of marine communities.

Elevated temperatures can accelerate the transmittance of diseases between species and increase the competition between species. For example, the coral pathogen *Vibrio shiloi* invades the tissues of the host corals *Oculina patagonica* and causes their bleaching (Israely *et al.*, 2001). Relatively higher water temperatures (about 28°C) increase the infection rate by the pathogen, while low temperatures (about 16°C) prevent bacterial growth. Thus, global warming will promote the infection of *O. patagonica* by *V. shiloi* which may result in the disappearance of this coral. Recent population declines and local extinction of the mussels *Mytilus trossilus* (Geller, 1999) and *Haliotis cracherodii* (Raimondi *et al.*, 2002) along the California coast might have been driven by the increasing competition between species and a high abundance of parasites.

Global climate change resulting ice melting and increase of freshwater input in coastal areas will lead to decrease of salinity in the shallow waters

and can affect larval settlement of biofouling species. For example, it has been shown that low salinity decreased the survival, increased the duration of the development of the tube worm *Hydroides elegans* larvae, and reduced its larval settlement rate in laboratory experiments (Qiu and Qian, 1997). Similarly, both temperature and salinity affected the development and attachment of the barnacle *Balanus trigonus* (Thiyagarajan *et al.*, 2005). At high temperature and low salinity, larvae quickly developed into cyprids while less than one third of them attached. All these data suggest that future global climate changes may be critical for survival of both larvae and adults.

9.2.2 Response to CO₂ and acidification

Due to anthropogenic activity CO₂ concentrations in the atmosphere are increasing. Because the oceans are in the equilibrium with the atmosphere, the predicted increase of CO₂ atmospheric concentrations of 140–253% by the year 2100 (IPCC, 2001) is expected to increase CO₂ concentrations in the oceans despite the fact that elevated temperature and low pH (associated with increase of atmospheric CO₂) decrease solubility of CO₂ in the oceans. In any case, it is expected that over the next millennium the oceans will absorb approximately 90% of the CO₂ emitted in the atmosphere (IPCC, 2001). Increasing CO₂ concentrations in the marine environment are expected to increase the photosynthetic rates of sea grasses, which evolved during the Cretaceous period, when atmospheric CO₂ concentrations were much higher than now (Harley *et al.*, 2006). On the contrary, marine algae are carbon-saturated and elevated CO₂ concentrations will not enhance their growth (Beardal *et al.*, 1998).

CO₂ dissolved in the ocean reacts with water to form carbonic acid resulting in the ocean acidification. Reduction of pH due to an increase of CO₂ concentrations will have a profound effect on the physiological reactions of marine organisms. Experiments suggested that short-term elevations of CO₂ resulted in reductions of the protein synthesis and the ion exchange in invertebrate cells (Pörtner *et al.*, 2004). It is predicted that elevated concentrations of CO₂ in seawater will fertilize marine phytoplankton depending on other nutrients and will affect the growth of many invertebrates and algae with carbonate structures (Pörtner *et al.*, 2004).

Information about long term climatically realistic effects of elevated CO₂ on marine organisms is rare and only a few long-term studies are available (Harley *et al.*, 2006). Elevated CO₂ that reduced pH by 0.7 units for 3 months resulted in a slow growth of mussels (Michaelidis *et al.*, 2005). In a similar study, pH changes as low as 0.03 units significantly reduced growth and survival of gastropods and sea urchins (Shirayama and Thornton, 2005). Low pH decreases the size of invertebrate eggs, affects their fertilization

and delays their development (Pörtner *et al.*, 2004). Reduction of more than 30% calcification rates in response to elevated CO₂ concentrations and lower pH values has been shown for coralline algae, scleractinian corals and molluscs (Feely *et al.*, 2004; Pörtner *et al.*, 2004; Harley *et al.*, 2006). Decreased pH causes weakening of coral skeletons and reduces the accretion of coral reefs (Hughes, 2003).

Calciferous structures are usually used as skeleton structures for the protection from predators or grazers in invertebrates and coralline algae. Therefore, animals and plants with reduced calcification rates might become an easy food for other marine organisms, which might have strong population and community-level impacts. It is proposed that some animal groups are more resistant to an elevation of CO₂ than others (Pörtner *et al.*, 2004). Molluscs, arthropods and chordates are to a greater extent resistant to pH changes than corals, brachiopods, bryozoans and echinoderms. Alongside with the prediction that all marine organisms with carbonate structures will be affected by oceanic acidification it is possible to hypothesize that biofouling communities, usually dominated by mussels and barnacles (Railkin, 2004), will face dramatic changes and might be replaced by tunicate- and algal-dominated communities. In this case more investigation on the impact of acidification on biofouling organisms and communities due to elevated CO₂ are urgently needed.

9.2.3 Response to sea level rise and water turbulence

Rising sea level will affect only slow-growing, long-lived species, such as corals (Scavia *et al.*, 2002). According to the prediction, intertidal habitat areas may be reduced by 20–70% over the next 100 years due to increased anthropogenic activity and because of decreased habitat availability (Harley *et al.*, 2006). Since most biofouling species are fast growing on any anthropogenic structures, these organisms will not suffer much from predicted sea level rises.

Increase of water temperature and water vapour over the oceans due to global warming intensifies hurricanes and leads for the development of tropical cyclones with intensive precipitation (Trenberth, 2005). According to the National Oceanic and Atmospheric Administration (NOAA) from 1995 to 2004 there was a 1.6 fold increase in tropical storms and hurricanes compared to the previous 25-year period (Trenberth, 2005). Increased intensity of tropical storms and hurricanes will have a dramatic effect on intertidal and subtidal communities. Caribbean corals require over 8 years to recover from damage occurred by storms (Gardner *et al.*, 2005). When these disturbance events will happen frequently in future it will reduce the odds of recovery and seriously affect slow growing communities (Harley *et al.*, 2006).

Increased upwelling is going to shift nutrient supplies in the oceans and fuel growth and reproduction of planktonic and benthic algae; these might

affect productivity of marine ecosystems in the future (Harley *et al.*, 2006). Organisms with planktonic life are sensitive to upwelling and offshore advection. It has been suggested that strong upwelling could prevent larval dispersal and settlement and affect larval supplies. Changes of larval supplies with increased adult mortality due to high storm and cyclone activity may lead to local population extinctions (Svensson *et al.*, 2005). For some species, though, increased water movement may increase their recruitment success and synchronize their spawning. Spawning of the abalone *Haliotis diversicolor* in Japan occurs only after typhoon events (Onitsuka *et al.*, 2007). Larval settlement of the blue mussel *Mytilus edulis* is enhanced by water agitation (Bayne, 1964; Eyster and Pechenik, 1987). Similarly, barnacle species preferred to settle at sites with increased water turbulence (Hills and Thomason, 1996). Both negative and positive effects of sea level rise and water turbulence will affect the recruitment and survival of species and will have a dramatic effect on biofouling communities.

9.2.4 Response to UV

Increase of CO₂ concentrations will likely lead to the depletion of the ozone layer (Austin *et al.*, 1992; Fig. 9.1). Knowledge about impact of ultraviolet radiation (UVR) on benthic communities in the oceans is scant. UVR affects primary production via damaging organic molecules (i.e., DNA, RNA) and inhibition of photosynthesis (Franklin and Foster, 1997; Wiencke *et al.*, 2000). UVR has a negative impact on invertebrate larvae (Chalker-Scott *et al.*, 1992; Bingham and Reitzel, 2000; Kuffner, 2001) and algal spores (Santas *et al.*, 1998; Wiencke *et al.*, 2000), which are more sensitive to UVR than adults. Additionally, UVR inhibits recruitment of larvae and germination of algal spores. In laboratory experiments, a UV-B radiation dose of 28 kJ m⁻² reduced the recruitment of an intertidal fouler, the barnacle *B. amphitrite* (Chiang *et al.*, 2003; Hoag, 2003). Similarly, UVR significantly decreased settlement of the polychaete *H. elegans* in outdoor experiments (Dobretsov *et al.*, 2005). Additionally, UVR modifies bacterial communities, which in turn affects larval settlement (Unabia and Hadfield, 1999; Hung *et al.*, 2005). Inhibition of larval settlement and recruitment, as well as poor survival of juveniles, caused by an increase of UVR due to global climate change might ultimately alter species composition of biofouling communities.

9.3 Impact of climate change on biofouling communities

Organisms and their various life stages (adults, juveniles and propagules) respond to global climate change differently (Harley *et al.*, 2006). This will lead to changes in the composition of species in biofouling communities

Table 9.1 Predicted impact of global warming on biofouling communities

Factors associated with climate change	Their effect on communities
Increase of the water temperature	Dominance of warm-water non-native biofouling species. High competition between warm-water non-native and cold-water native biofouling species. Change larval development, growth and recruitment.
Increase of CO ₂ concentrations and ocean acidification	Replacement of species with calcium structures by non-calcareous species. Possible dominance of Tunicata and algae in biofouling communities.
Decrease of salinity in coastal waters	Species that tolerate low salinity dominate in biofouling communities. Changes in biofilm composition possibly affect propagules settlement.
Increase of UV radiation	Dominance of species resistant to UV radiation in shallow water biofouling communities. Negative impact of UVR on species with planktotrophic larvae and species that require biofilms for their settlement.
Increased water turbulence and increased storm activity	Possible negative effect on species with planktotrophic larvae. Changes in larval supply of adult populations.
Combined effect of all factors	Rather unpredictable and differ from the effect of the single factor

Although these predictions are speculative, they are based on publications cited in the text.

(Table 9.1). It is predicted that elevated temperatures will lead to the replacement of cold-water biofouling species by warm-water species (Harley *et al.*, 2006). Additionally, it is possible to expect an increase in the introduction of invasive species from warm waters into temperate and cold waters (see below). At the same time, a study of Schiel *et al.* (2004) suggested that the effect of elevated temperature on benthic communities from the California coastline is rather unpredictable. There was no trend towards warm-water species replacing cold-water species over a 10-year sampling programme near a power plant. Instead, the communities were rather unaltered with the exception of taxa sensitive to elevated temperatures, such as kelps and foliose red algae (Schiel *et al.*, 2004). Additionally, high temperatures increased the amount of invertebrate grazers. This study indicated that ocean warming can have a direct effect on habitat-forming taxa as well as an indirect effect operating through different ecological interactions.

Population-level effects of global climate changes on biofouling communities occur via changes in transport processes that influence dispersal and

recruitment of organisms. Additionally, climate change may affect the interactions between biofouling species by altering predator-prey and competitor interactions. This includes climate driven changes in abundance of species, which affects species distribution, biodiversity, productivity and their microevolution.

Global climate change can affect biofouling communities indirectly via the modification of chemical cues that are necessary for successful larval and spore settlement. It has been shown that biofilms are the major mediators of invertebrate larvae and algal spore settlement (Qian *et al.*, 2007). Different species of bacteria, diatoms and fungi in biofilms can induce, inhibit or have no effect on larval and algal settlement (Dahms *et al.*, 2006; Dobretsov *et al.*, 2006). The resulting effect of multi-species biofilms on larval settlement depends on species composition of biofilms and densities of different groups of microorganisms (Lau and Qian, 1997; Dahms *et al.*, 2004). Under different culture conditions the same microorganisms are able to produce different secondary metabolites. For example, bacteria were inhibitive to the larvae of *B. amphitrite* at sea water salinity 35 and 45‰ but induced their settlement at 15 and 25‰ (Khandeparker *et al.*, 2006). Similarly, production of the antibacterial compounds [3-methyl-N-(2-phenylethyl) butanamide and cyclo(D-Pro-D-Phe)] from the sponge-associated fungus *Letendraea helminthicola* was different at different temperature, salinity, pH, and carbon and nitrogen regimes (Miao *et al.*, 2006). Additionally, environmental changes can modify the density and the composition of microbial communities, which in turn change larval settlement (Lau *et al.*, 2005). In the laboratory, larvae of different species responded differently to biofilms developed at different environmental conditions. Biofilms that were developed at a high temperature stimulated the settlement of the barnacles *B. amphitrite* and *B. trigonus*. In contrast, the same biofilms had no effect on larval settlement of the polychaete *Hydroides elegans* (Lau *et al.*, 2005). These examples indicate that climate change may affect biofilm density, composition, production of metabolites and their effect on the recruitment of propagules, which will finally change the composition of biofouling communities.

9.4 Effect of climate change on biological invasions

It is recognized that biological invasions are associated with global climate change (Dukes and Mooney, 1999). Modification of the climate will favour invasive species and exacerbate the impacts of invasions on ecosystems (Harley *et al.*, 2006). These impacts include competitive effects, whereby an invading species competes with resources of indigenous species, and ecosystem effects, whereby invaders alter fundamental ecosystem properties.

Recently, many shallow water hard substrata in Massachusetts, USA have been invaded by five non-indigenous species of tunicates (Agius, 2007).

Usually, these species occupy about 80% of primary hard substrata and account for the majority of species richness in biofouling communities. Increased seawater temperatures and moderation of winter temperatures facilitate the establishment and persistence of warm-water and tropical invaders all over the world (Stachowicz *et al.*, 2002). It was found that the recruitment of the introduced tunicates *Asidiella aspersa*, *Botrylloides violaceus* and *Diplosoma listerianum* was shifted in time and more abundant in warmer years (Stachowicz *et al.*, 2002). There was no correlation between water temperature and recruitment of native species. Laboratory experiments demonstrated that the growth rate of invasive species was higher than the native ones at elevated water temperatures. These data suggest that rising seawater temperatures will provide alien species from warmer waters an early start and increase their growth and recruitment compared to native species, which may facilitate a shift to dominance of non-native species in biofouling communities (Table 9.1).

9.5 Conclusions and directions for future research

The data presented in this review clearly demonstrate that biofouling communities and other marine communities are going to face dramatic modifications in response to future climate changes. Analysis of the literature suggests that only few investigators studied the effect of factors associated with global climate change on biofouling species. Studies about the impact of climate change on benthic and biofouling communities are even rarer (Fig. 9.2). Thus, it is necessary in future studies to investigate and predict possible effects of climate change on biofouling communities.

Elevated temperatures, low salinity, high water turbulence, and low pH due to increased CO₂ concentrations are main factors associated with global climate change. All of them, separate and in combination, will affect the development of biofouling communities (Table 9.1). Based on the analysis of available literature we predict that the most drastic changes in biofouling communities are going to happen because of elevated water temperature and a decrease of water pH. Possibly, in polar and temperate regions, cold-water biofouling species will be replaced by alien warm-water species. Additionally, species that have calciferous structures, like barnacles, mussels, polychaetes and bryozoans, may disappear from biofouling communities or become less dominant than soft-bodied species like tunicates and algae. Most recent biocides and antifouling compounds have been tested on single species of biofoulers, usually barnacles or molluscs (Dobretsov *et al.*, 2006). Therefore, predicted changes in biofouling communities will require the development not only of new antifouling compositions but also of new bioassay techniques that include several target species from different phylogenetic groups (Dahms and Hellio Chapter 12).

Replacement of cold-water species and an increase of invasive warm-water species due to global climate change will possibly affect the aquaculture of marine species. Aquaculture of mussels, such as oysters and abalones, will possibly face huge problems as these species will be affected by low water pH, high water temperatures and might compete for resources with newcomers. Additionally, elevated temperatures, UV radiation, acidification and water agitation might affect larval survival, supply and settlement of aquaculture species. Such effects should be properly investigated and future predictions and recommendations need to be made.

Marine organisms can respond differently to multiple stressors and the combined effect of two and more variables cannot be predicted from an individual effect (Harley *et al.*, 2006). This is because the impact of one factor can either be strengthened or weakened by another factor and the combined effect of two and more stressors may push an individual beyond a critical threshold that would not be reached by a single stressor. For example, high levels of UV radiation had no effect on the survival of algal spores in warm water, while spores died in treatments with high levels of UV radiation in cold water (Hoffman *et al.*, 2003). Since the majority of biofouling studies are dealing with a single stressor, either temperature or salinity, future work is necessary to determine an effect of multiple stressors associated with global climate change on biofouling communities.

Factors associated with global climate change may affect not only biofouling organisms but also the performance of antifouling coatings (Table 9.2). Elevated sea water temperature will affect biocide leaching rate,

Table 9.2 The effect of factors associated with global climate change on performance of antifouling coatings

Factors associated with climate change	Potential consequences on paint performance
Sea water temperature rise	• Increased polishing and biocide leaching rates • Earlier paint exhaustion • Paint efficiency and the leached layer thickness
Sea water pH decrease	• Decreased hydrolysis reaction rate for both acrylate- and rosin-based binders with decrease of polishing rates • Potentially lower dissolution rates for hydrolyzing particulate organic biocides • Increased Cu₂O dissolution rates • Increased biocide-leached layer thickness, with negative effects on paint performance
Sea water salinity decrease	• Lower Cu₂O dissolution rates • Potential changes on the hydrolysis rate of specific binders

diffusion rates, erosion rates and performance of antifouling coatings (Kiil *et al.*, 2001). For example, it has been predicted that elevation of the seawater temperature decrease leached layer thicknesses for TBT-SPC systems and efficiency of the paint (Kiil *et al.*, 2001). Additionally, intensive growth of microorganisms on antifouling coatings operating at elevated temperature will affect their performance and biocide leaching (Yebra *et al.* 2006; see Chapter 29). Acidification of the oceans will decrease hydrolysis reaction rate for acrylate (Kiil *et al.*, 2001) and rosin-based binders (Yebra *et al.*, 2005) which will decrease polishing rate of antifouling coatings, change dissolution rate for organic biocides and Cu_2O and affect paint performance (Table 9.2). Decrease of sea water salinity due to global climate change will result in low Cu_2O dissolution rate as it is correlated with chloride concentration in sea water (Kiil *et al.*, 2001). Additionally, ice melting will open up the possibility of new sailing routes around the North Pole, which will require good antifouling performance in cold waters, as well as additional mechanical strength of antifouling paints. These examples suggest that antifouling paints require some reformulations due to global climate change.

Susceptibility of biofouling organisms to biocides may vary due to climate change. For example, exposure of crab larvae to non-toxic concentrations of aromatic hydrocarbons in the presence of UV significantly increased their larval mortality (Peachey, 2005). Elevated CO_2 reduces the tolerance limits of marine organisms to certain biocides *via* the depression of important physiological pathways (Pörtner *et al.*, 2004). Elevation by 5–10°C of water temperatures increases the respiration of zebra mussels and their sensitivity to copper (Rao and Khan, 2000). These examples demonstrate that concentrations of antifouling agents in coatings and their performance at future climate scenarios should be subjects of intensive future investigations.

In addition, this review showed that global climate change can have both direct and indirect impacts on biofouling communities. Composition of microbial biofilms and their production of chemical cues can vary at different environmental conditions (Dobretsov *et al.*, 2006; examples in this chapter). Therefore, larval and algal spore settlement on biofilms will be affected by global climate change. Due to the lack of appropriate cues, the density of some biofouling species will become low, while other species will dominate in biofouling communities. Changes in microbial biofilms may finally affect the whole composition of biofouling communities.

Overall, the data presented in this review suggest that global climate change can seriously affect the productivity, development, dynamics, and composition of biofouling communities. Future studies should focus on the impact of climate change on biofouling species, populations and communities and require multidisciplinary approaches. Additionally, composition of

antifouling coatings should be re-formulated and development of novel antifouling coatings that perform well at altered climate conditions should be an important future direction.

9.6 Acknowledgements

The author acknowledges Dr H-U Dahms and Dr Y M Yebra for helpful comments and suggestions. This study was supported by an Alexander von Humboldt Fellowship (Germany), SQU grant IG/AGR/FISH/09/03 and by a George E. Burch Fellowship (USA). I am grateful to His Majesty Sultan Qaboos for the chance to utilize time, space and facilities of the Sultan Qaboos University during my work on this manuscript.

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Legislation affecting antifouling products

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Abstract: Antifouling products legislation has been developed by several countries and regions to control the use and marketing of antifouling paints in order to protect human health and the environment. The chapter begins by discussing the IMO International Convention for the Control of Harmful Anti-Fouling Systems on Ships. It then describes the EU Biocidal Products Directive (BPD) and mentions the legislation on tributyltin in EU as examples of regional schemes. Further, an overview of the various types of legislation in a number of countries worldwide is given to illustrate national antifouling product legislation schemes. Finally examples of two pieces of legislation which regulates chemical preparations in general are described. The chapter includes a list of sources for further information.

Key words: IMO AFS Convention, EU Biocidal Products Directive, tributyltin legislation, REACH, Dangerous Substance Directive.

10.1 Introduction

Legislation is a practical instrument used by politicians to bring into life the aims and objectives of their political strategies. It is crafted with the objective of creating a balance between the need for a set of rules, the beneficial effects of them and the weight of requirements imposed on those within scope. The balance is normally found taking into account the benefits and costs to the industry, the consumers, the environment and the social standards. Legislation can be drafted with the purpose of applying to a specific country, a region of countries or in some instances even as a legislative framework on a global scale. Antifouling products with biocides are products intended to render harmless organisms that may attach to surfaces submerged into the aquatic environment. Political attention is given to antifouling products since these, by their way of functioning, release substances potentially affecting the environment adversely, if they are released in uncontrolled amounts. Legislation therefore has been developed by several countries and regions to control the use and marketing of antifouling products in order to protect the environment. Countries in particular

with massive ship traffic and/or large areas of water bodies have implemented legislation specifically regulating antifouling products.

The Biocidal Products Directive (EU, 1998) emerged in the 1990s covering non-agricultural pesticides in the European Union. The directive covers 23 different biocidal product types, antifouling products being one of these and defined as: 'products used to control the growth and settlement of fouling organisms (microbes and higher forms of plant or animal species) on vessels, aquaculture equipment or other structures used in water.' Thus the Biocidal Products Directive is a class example of a regulation applying to a region of countries. Please refer to Section 10.3 for more information about regional legislation.

Some countries enforce their own national legislation specifically applying to the marketing and use of antifouling products. The requirements from country to country, however, are very diverse, ranging from having to notify to the authorities basic information about the composition of the paint, to the production and submission of an extensive data package easily covering several hundreds of pages. In Section 10.4, covering national legislation, you will find more information about the different schemes applying to antifouling paints.

The use of antifouling products is also a matter of global concern, since many vessels applied with antifouling paints trade internationally. A ship painted in one country may have a trade pattern causing the substances in the paint to be released in other parts of the world. The International Maritime Organization (IMO) is a political body establishing the framework to regulate the use of substances in antifouling paints globally. Please refer to Section 10.2.

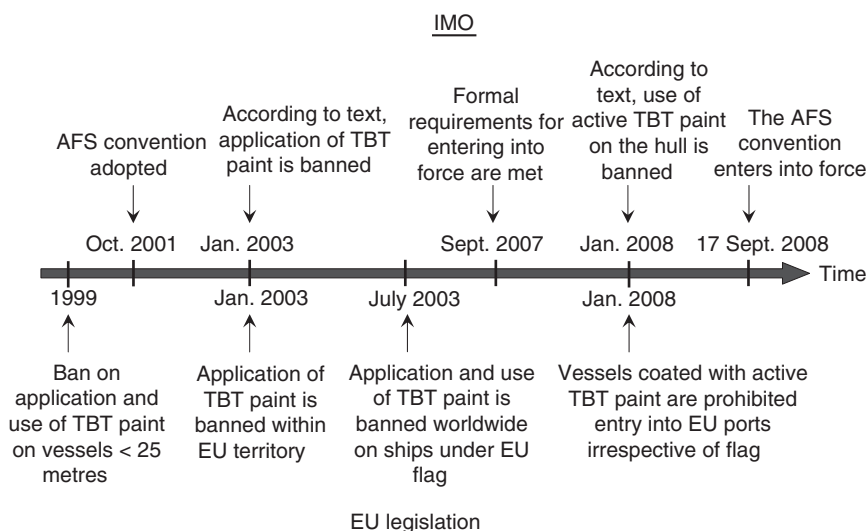
Finally it should be noted that although a country may not have rules specifically regulating antifouling products, it normally still enforces legislation regulating chemical products in general. The rules for classification and labelling (EU, 1967) and the Global Harmonization System (GHS) for instance would also apply to biocidal products as these are considered chemical preparations. Furthermore it would be important to take REACH, the new European Chemicals legislation into regard since this may very well have an impact on constituents in an antifouling product other than the active ingredients. Please refer to Section 10.5.

10.2 Global antifouling products legislation

The International Maritime Organization (IMO) is a specialized agency under the United Nations with the purpose of developing international conventions and guidelines to regulate shipping between nations. One of their objectives is to prevent pollution from ships.

For a convention to enter into force, ratification by a specified number of the 17 member states is needed. In October 2001 IMO adopted the International Convention for the Control of Harmful Anti-Fouling Systems on Ships (AFS Convention) (IMO, 2001). According to the agreed text, the AFS Convention will enter into force 12 months after 25 member states representing min 25% of the world's merchant shipping tonnages have ratified the text. On 18 September 2007 with Panama's ratification, the entry-into-force requirement was reached with the effect that the AFS Convention entered into force 17 September 2008. According to the text in Annex 1 to the convention, application of organotin compounds, e.g. TBT-based paints, has been banned since 1 January 2003.

Furthermore sailing with a ship with active TBT-based paint was banned from 1 January 2008. As the convention entered into force after both these dates, the effective dates of 1 January 2003 and 1 January 2008 in Annex 1 have been replaced by the entry into force date which was 17 September 2008 (IMO, 2007). Figure 10.1 illustrates the important dates in the IMO AFS Convention. However, a number of member states indicated during the development and negotiations of the AFS Convention text in IMO that when the convention enters into force after these dates, they will enforce the application date retroactively with the effect that ships with TBT-based paint applied after 1 January 2003 will be considered illegal. However the major antifouling paint suppliers phased out their TBT-based assortment during 2002 and in the beginning of 2003 thereby considerably decreasing the number of underwater hull square meters applied with these



10.1 Important dates in TBT regulations.

types of paints. The AFS Convention has not only an effect for the ships flying a flag of the ratifying Flag States, but also for all ships irrespective of flag entering their ports. Hence this convention has an effect on worldwide shipping activities. Also important is that the convention covers not only ships engaged in international voyages but also ships engaged in local trading activities. However, it is up to each country how strictly they may enforce the AFS Convention in that respect. The AFS Convention includes a list of banned or regulated antifouling systems which could be, e.g., a coating, a paint, a surface treatment, an antifouling system containing organotin compounds acting as biocides being the first on that list. Following the described methodology in the AFS Convention other antifouling systems may be added to the list based upon an evaluation of data on active substances submitted by the active substance manufacturers. The North Sea States have already proposed that any biocides failing to be included in Annex I/IA of the Biocidal Products Directive (see Section 10.3.1) will be proposed for banning through the AFS Convention (North Sea Conference, 2006).

For the purpose of demonstrating compliance with the AFS Convention, ships need to carry official certificates containing information pertaining to the applied antifouling system. IMO has therefore subsequently developed a set of guidelines to be followed by the maritime authorities on survey and certification of ships (IMO, 2002), on sampling of antifouling paint from ships (IMO, 2003a), and on inspection of antifouling systems on ships (IMO, 2003b).

10.3 Regional antifouling products legislation

Regional legislation relates to the case when a collective of countries agrees mutually on a set of harmonized rules to apply for every country within the collective. This has several benefits as is given below and is becoming a more common practice, especially within the field of environmental legislation. The most heavily regulated region in the world is the European Union. In the following, the most renowned piece of regional antifouling legislation, the Biocidal Products Directive (BPD) (EU, 1998), is described in detail. However, the principles of the BPD are also readily applicable to the understanding of other antifouling regulations applied regionally. Another regional legislation regulating the marketing and use of antifouling products is the European regulation restricting the use of organic tin compounds.

10.3.1 The Biocidal Products Directive (BPD)

The BPD entered into force 14 May 2000 and intends to regulate the production, marketing and use of non-agricultural products intended for

biocidal purposes. It covers not only antifouling products but biocidal products in general such as wood preservatives, drinking water disinfectants, rodenticides plus an additional 19 product types.

The BPD has a dual regulatory impact on the development, marketing and use of biocidal products. Firstly it regulates biocidal products placed on the market since these will need to obtain an authorization before being marketed. Secondly it regulates and approves active substances allowed for use in such products. Approved active substances are included into a positive list. This list is an integral part of the directive and is commonly referred to as Annex I/IA¹.

Within the context of the BPD, an antifouling paint is therefore defined as a biocidal product that should be authorized, whereas the substances giving the product its biocidal effect are defined as the active substances.

Whereas the approval and inclusion of substances into Annex I/IA is coordinated centrally by the European Commission the authorization of products takes place at member state level.

Aims and objectives

The main objectives of the BPD are to:

- secure a high level of protection of man and the environment,
- harmonize legislation concerning the placing of biocidal products on the market in the European Union, and
- remove barriers of trade within the European Union.

Historically, antifouling products have been dealt with as a specific regulatory issue under national legislation. In the European Union this has, however, only been the case in some member states.² Some countries have no regulation, others have a notification system in force and yet other countries have extended regulatory schemes whereby products need to be authorized before placing them on the market (see also Section 10.4.1). One of the aims of the BPD is to harmonize the legislation concerning placing of biocidal products on the market in the European Union. This should result in antifouling legislation being the same in all countries whether it is Finland, United Kingdom or Greece. Furthermore, the aim is to secure a high protection of humans and the environment and to remove barriers of trade within the European Union. Barriers of trade are some of the negative side effects seen when some countries have harsh

¹Annex IA is the annex listing the active ingredients satisfying the criteria for being of low risk while Annex I includes the remaining approved active ingredients.

²As of January 2008 these countries are Finland, Sweden, United Kingdom, Ireland, Belgium, The Netherlands and Malta.

regulatory requirements while other member states have very limited or no requirements.

Annex I/IA entry procedure

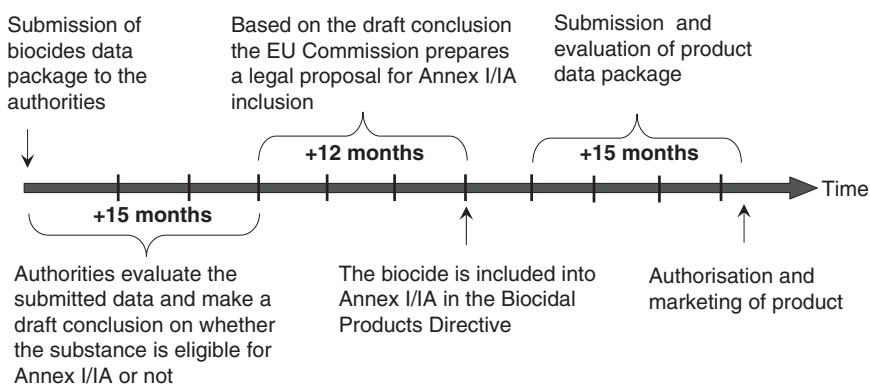
An antifouling product containing biocides can only receive an authorization if these biocides are included in Annex I/IA. It is upon the initiative of the manufacturer of the biocides that a substance enters into the process of gaining Annex I/IA entry and it is the authorities who review the application and evaluate whether the submitted data satisfies the criteria for Annex I/IA entry.

In order to obtain annex I/IA entry, the applicant has to present a data package demonstrating that the substance, when used as intended, does not present any unacceptable risk to man or the environment. Furthermore, the dossier must provide evidence of the efficacy of the biocide and show that it is actually efficacious in a realistic biocidal product. In this regard, manufacturers of the products containing the biocides are often involved, since they have more thorough knowledge of the products. Thus, in the process of preparing the data package, the biocides supplier depends on detailed information related to the exposure of the active substance in the various use scenarios. This is, for example, information relating to the direct exposure from applying the product by the professional and the non-professional user but also indirect exposure to bystanders. Also information about emissions to the various environmental compartments is imperative in order to make an accurate risk assessment. This includes information about the use areas of the product, e.g. number and size of commercial ships and/or recreational boats painted, maximum active ingredient content, the emission rate of the biocides from the product, how the product degrades in the water, etc.

The information is used to prepare a human and environmental risk characterization and the results will indicate if the risk is acceptable or needs to be refined, utilizing further more detailed data.

The data package is submitted to the competent authority that has been appointed the rapporteur member state (RMS) of that particular biocide. It is the European Commission who decides whom to appoint as rapporteur member state. The RMS evaluates the data and makes a proposal for Annex I/IA entry. Once the biocide has entered Annex I/IA, applications for products containing that biocide shall be submitted for authorization in countries in the European Union where marketing is expected. Figure 10.2 illustrates the process from submitting a dossier for inclusion of a biocide on Annex I/IA until the authorization of the products containing the biocide. In Table 10.1 the existing biocides applying for Annex I/IA listing for use in antifouling products are listed.

BPD Timeline



10.2 BPD timeline.

Table 10.1 Anti-fouling biocides applying for annex I/IA entry as of January 2008

Biocide	CAS number	Rapporteur member state
Copper thioyanate	1111-67-7	France
Dicopper oxide	1317-39-1	France
Copper	7440-50-8	France
N'-tert-butyl-N-cyclopropyl-6-(methylthio)-1,3,5-triazine-2,4-diamine	28159-98-0	The Netherlands
Dichlofluanid	1085-98-9	United Kingdom
Dichloro-N-[(dimethylamino)sulphonyl] fluoro-N-(p-tolyl)methanesulphenamide	731-27-1	Finland
bis(1-hydroxy-1H-pyridine-2-thionato-O,S) copper	14915-37-8	Sweden
Zinc Pyrithione	13463-41-7	Sweden
4,5-dichloro-2-octyl-2H-isothiazol-3-one	64359-81-5	Norway
Zineb	12122-67-7	Ireland
Tralopyril	122454-29-9	United Kingdom

Product authorisation procedure

Once a biocide is granted Annex I/IA listing, all antifouling products in the European Union containing that biocide need an authorization in order to stay on the market.

Albeit being of a much lesser extent, the principles of generating and submitting an application for product authorization is a process comparable to the process of including an active ingredient into Annex I/IA. The product data must be submitted to the authorities in the country where the product is intended to be marketed. Utilizing relevant data the dossier shall

demonstrate that the product can be used without an unacceptable risk to man or the environment. Furthermore, the dossier shall show that the product is efficacious and meeting its claim. If, for example, the marketing material for an antifouling paint is claiming that the hull can be kept clean from hard fouling such as barnacles, a study should be provided demonstrating that this is actually the case.

Frame formulation

Instead of applying for an authorization for a single product, an authorization may be given to a group of products of similar composition, risk and efficacy. In such an instance the applicant can make use of the frame formulation concept, making it possible to apply for an authorization for a number of similar products within one application. This means that the product's use area, e.g. the painting of commercial ships, recreational boats, fish nets, and the user group (professional, non-professional) should be the same. Furthermore the level of efficacy should be similar. An example of a frame formulation is an antifouling product marketed in different colours. Utilizing the concept of frame formulation makes it possible to apply with one application for the whole range of shades. By introducing the frame formulation concept, authorization of closely related products should be easier, less time consuming for both the applicant and the member states and should reduce costs for the authorization process.

Mutual recognition

Once a product has received an authorization by the member state where it was firstly applied for, other member states must, by the principles of mutual recognition, also grant an authorization through a simplified procedure. This means that if one member state grants an authorization there shall be a very well justified reason from other member states not to grant it also.

Both the authorities and the industry believe that the BPD being a regional regulation offers many advantages compared to antifouling products being regulated on a national level. It is simpler to develop a marketing strategy when it can cover a whole range of countries instead of having to tailor-make a strategy for every country having their own regulatory system. Having to consider regions instead of individual countries also offers paint companies the opportunity to streamline the assortment, thus doing business more efficiently both in terms of understanding the markets and managing the practical aspects of for instance logistics and procurement.

10.3.2 Tributyltin legislation

Following the detection of the impact of the increased concentration of TBT in the Bay of Arcachon in France on the oyster population and other wild mollusc species in the 1980s the European Union introduced legislation to restrict the use of these compounds in antifouling paints.

Through the 'Marketing and Use Directive' (EU, 1976) products containing organic tin compounds such as tributyltin (TBT) have since the 1980s been banned for use on pleasure crafts in many countries. In 1999 the European Union adopted a change to the Directive whereby marketing and application of TBT-based paints on vessels less than 25 metres was banned together with other restrictions on use (EU, 1999).

After the adoption of the AFS Convention, this directive was further amended in order to make the 1 January 2003 ban effective within the European Union for *all* ships irrespective of size (EU, 2002).

Furthermore, an EU Council Regulation was implemented in 2003 banning the application of TBT-based paint on any member state vessel from 1 July 2003 (EU, 2003). Consequently an EU-flagged vessel was not allowed to be applied with any TBT-containing paint even outside the borders of the European Union. At the same time, this regulation bans the use of (active) TBT-based paint on EU-flagged ships after 1 January 2008 and also bans the entry into European harbours of any ships carrying an active TBT-based paint on the outside of the hull irrespective of flag. Figure 10.1 illustrates the important dates in the EU legislation concerning TBT-containing paints.

The general idea was that the EU Council Regulation would place the member states in a better position to ratify the AFS Convention. However, this has proven not to be the case as only 16 out of 27 member states in the European Union had ratified the AFS Convention by 1 March 2009.

10.4 National antifouling products legislation

The following sections give a general overview of the types of legislation in a number of countries worldwide. It is not the intention to go into details, as this would be outside the scope of this book. Additional information may be obtained from the authorities' official websites (see Section 10.7.1).

Several terms are used to identify the various types of requirements imposed by the authorities prior to placing antifouling products on the market. The definitions below apply to the following sections describing the various national legislative approaches:

- *Notification* is a process whereby the authorities are notified about the product in the market. Mostly it consists of product safety data sheet and in certain cases detailed formulation information. As opposed to

the registration procedure, standard practice is that the antifouling product can be introduced to the market before and while it is being notified.

- *Registration* followed by authorization to sell the product in the specific market. A registration process includes the submission by the manufacturer of a detailed data package which is more than just the product formulation, safety data sheet, and label. The package furthermore includes a human health and environmental risk assessment. The data package is evaluated by the authorities, who decide if the risk from normal use of the product is acceptable. Following the evaluation a marketing approval and often an approval number are normally issued for the product. In countries with registration schemes antifouling products cannot be introduced to the market until they have been evaluated and granted an approval by the authorities.

10.4.1 European countries

Until 2014 (this date may be postponed), member states in the European Union may continue to apply their current system of practice applying specifically to antifouling products on their markets. This implies that currently an associated member of the European Union may have either implemented the Biocidal Products Directive (BPD) or is continuing with current practice. The picture, however, is not clear, as various degrees of requirements now exist. Some countries have their own system of practice applying specifically to antifouling products where some incorporates a registration scheme, while others use a notification scheme only. Furthermore some countries have never had a current legislation applying specifically to antifouling products but are in the process of implementing some or all parts of the BPD.

Three different regulatory systems are employed within the European Union:

1. *No current practice requiring registration of antifouling products specifically.*

Some parts of the requirements in the BPD are implemented such as requirements on labelling and notification to poison centres.

These countries will continue without requirements until the Annex I/IIA inclusion of active substances for use in antifouling products has been decided.

As of January 2008 this applies to Austria, Bulgaria, Czech Republic, Cyprus, Denmark, Estonia, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, Norway (non-EU), Poland, Portugal, Slovakia, Slovenia, and Spain.

2. *National antifouling products legislation applies*

Some BPD antifouling requirements have entered into force thereby altering existing antifouling legislation. Member states with existing registration procedures for antifouling products will continue as previously, although the data requirements may have been changed. As of January 2008 this applies to Belgium, Ireland, Malta, The Netherlands, Sweden, and the United Kingdom. Switzerland (non-EU) issues a permit to market antifouling paints based on detailed information on the product.

3. *All parts of the BPD have been implemented into national antifouling legislation so that it is fully aligned with the BPD requirements.*

One member state, Finland, has decided to implement the full system of the BPD.

The picture within the European Union is at present also diverse when it comes to the approval of active substances for use in antifouling products, in various countries. Some countries only allow certain substances to be used in antifouling products, whereas other countries have banned the same active substances for use in antifouling paints. Being allowed to use an active substance may also depend upon the leaching rate from the antifouling product. Denmark has no legislation concerning registration of antifouling paints but a ban on the presence of certain active substances in pleasure craft paints and in addition leaching rate limits of copper from antifouling paints for use on pleasure crafts has been issued (DK-EPA, 2008). Sweden has banned the use of any biocidal antifouling paints for pleasure craft on the east coast of Sweden. With the BPD in force more uniform conditions will be found in all EU member states.

10.4.2 USA

In USA antifouling paints are considered to be an antimicrobial pesticide product and are governed by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 2004). Before selling or applying antifouling paints on the US market, approval of the products must be granted by the United States Environmental Protection Agency (US-EPA). When an approval has been granted at federal level by US-EPA products can subsequently be registered at state level, which in some instances may introduce further stringent measures before the product is allowed to be marketed in that particular state. States may even choose not to register an antifouling product. Registering a product with the US-EPA is a tiered process where more data is needed if the level of concern increases. So if little but very conservative data in tier one may demonstrate that the level of concern is acceptable no further data is needed to be produced and submitted. Conversely if

the first tier encompassing conservative data gives rise to concern, more data is required on a higher tier level in order to refine the risk assessment. This is different from the BPD scheme in the European Union, which requires more extensive data sets. Required for registration purposes are data on toxicology, human exposure, environmental exposure, environmental fate, product chemistry, residue chemistry, and efficacy. Both generic data supporting the active ingredient and specific data supporting the product are needed. The evaluation of the submitted data establishes whether there is an unacceptable risk towards workers, human health, and the environment when the product is used as dictated on the label.

At present a re-registration program is taking place whereby chemicals registered before 1 Nov 1984 must be re-evaluated and re-registered to comply with contemporary safety standards and data requirements. Cuprous oxide is in this program and the process is scheduled to be finalised in 2010. Further, a registration review programme has been initiated covering all approved pesticides ensuring a periodical re-evaluation to establish that the products are still safe to use.

10.4.3 Canada

As in the USA, antifouling paints are considered to be antimicrobial pesticide products and are regulated under the Pest Control Products Act (PCPA, 2002), which is administered by Health Canada's Pest Management Regulatory Agency (PMRA). Antifouling paints need an approval before they can be imported, manufactured, sold, and used in Canada. Companies must submit a data package similar to the data required by US-EPA. PMRA carefully review the submitted data to determine if there is evidence to show that the product does not pose an unacceptable risk to human health and the environment. All pest control products, hereunder antifouling products, must be re-evaluated on a 15-year cycle to ensure the acceptability for continued use is examined using current standards and scientific approaches. Canada has established a maximum copper release rate limit for antifouling paints of 40 microgram/cm² painted surface per day.

10.4.4 Australia

In Australia antifouling paints are regulated as agricultural chemicals. The manufacture, supply, and sale of antifouling paints are regulated through the Agricultural and Veterinary Chemicals Code Act 1994 (the Agvet code) (Agvet, 1994) which is administered by the Australian Pesticides and Veterinary Medicines Authority (APVMA). To register an antifouling paint, a comprehensive set of scientific data (details depending upon the category of the application) must be submitted for evaluation by APVMA. Based

upon this information, the APVMA ensures that the products are properly labelled and that the registered products are safe to human health and the environment when used according to the directions on the label. A very important issue in Australia is the efficacy of the products. Depending on the type of efficacy data produced in non-Australian waters, the APVMA may require that the efficacy is also demonstrated in Australian waters and according to a specific Australian guideline.

10.4.5 New Zealand

Previously antifouling paints were registered/approved under the Pesticides Act, which was replaced by the Hazardous Substances and New Organisms Act 1996 (HSNO Act) (HSNO, 1996). Already registered antifouling paints have been transferred to the HSNO Act in 2004. Paints introduced into New Zealand after 2004 have been approved under the HSNO Act. The Environmental Risk Management Authority New Zealand (ERMA) evaluates and approves hazardous substances under the HSNO Act and places controls or conditions on the substance to manage its risk to the environment. These conditions must be complied with by the users. An approval of an antifouling paint under the HSNO Act as a 'hazardous substance' then interfaces with a registration under the Agricultural Compounds and Veterinary Medicines Act 1997 (ACVM Act) (ACVM, 1997). This act controls the import, manufacture, sale, and use of agricultural compounds and veterinary medicine. The ACVM Act is presently under revision and antifouling paints are part of the group defined as agricultural chemicals. When applying for an approval of an antifouling paint under ACVM data requirements are very similar to the requirements for registration in Australia and should be submitted under the ACVM Act. The approval under the ACVM Act is given to each trade name applying for one.

10.4.6 Hong Kong, China

In Hong Kong antifouling paints are considered as pesticides and are controlled under the Pesticides Ordinance Cap. 133 (PO, 1991), which is administered by the Agriculture, Fisheries and Conservation Department. Before an antifouling product is imported, supplied and sold for use in Hong Kong, it must obtain a registration. Details of registered antifouling products on active ingredients and concentration limits are found in a register. As long as a product conforms to the specified maximum concentration of registered active substances in antifouling formulations, an individual product registration is not needed but a permit must be obtained.

10.4.7 Japan

There is no requirement for registration of antifouling products in Japan. However only paint containing biocides which are 'approved' by the Ministry of Economy, Trade and Industry (METI) and the Shipbuilding Industry may be marketed in Japan ('the MITI list'). However the Japan Paint Manufacturers' Association (JPMA) has developed a self-regulatory management programme 'to provide IMO Convention compliance information and related information to ship owners, ship operators, government authorities in charge and other related bodies by examining paints manufactured/distributed by JPMA Regular members, Supporting members or non-membership companies'. (JPMA, 2007). Legislative initiatives concerning antifouling paint and the biocides used are in the development process involving both government and industry.

10.5 Other legislation affecting antifouling products

Legislation specifically addressing the marketing and use of antifouling paints is the focal point of attention to the regulatory people working with this class of products. However, a number of other legislative instruments exist that regulate chemical preparations in general. Such legislation is relevant not because the products are intended to be used for antifouling purposes but because they are chemical preparations.

It would be too extensive to go into details with the various requirements of these legislations. Instead the reader is advised to explore the contents of specific legislation if more exhaustive knowledge is sought. In the following, some examples of European legislation are given, which must be taken into account during the development and marketing of an antifouling product. Although the following are examples from European legislation, there is a trend that countries worldwide are implementing legislation similar in principles and objectives.

10.5.1 Dangerous Substance Directive

The Dangerous Substance Directive (EU, 1967) applies to pure chemicals and to mixtures of chemicals (preparations), which are placed on the market in the European Union. The Directive lists the classes of substances or preparations which are considered to be dangerous. Some, but not all, of these classes are associated with a chemical hazard symbol and/or a code indicating whether the substance is liable to give adverse effects to man or the environment. Substances or preparations falling into one or more of these classes are listed in Annex I of the Directive, which is regularly updated. A public database of substances listed in Annex I of the Directive is maintained by the European Commission.

Since antifouling paints are chemical preparations, they fall within scope of the Dangerous Substance Directive. Therefore it is important to take note of the classification of the preparation in order to determine whether this is acceptable. It is for example illegal to market to the general public chemical preparations which are classified as toxic or very toxic. The classification and labelling provisions in this directive are currently being taken over by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). The latter system is the result of a multilateral global agreement on the harmonization of the various classification and labelling rules around the world.

10.5.2 REACH

REACH (EU, 2006) is a new European Community Regulation on chemicals and their safe use. It deals with the **R**egistration, **E**valuation, **A**uthorization and **R**estriction of **C**hemical substances. This new law entered into force on 1 June 2007 and applies to all actors on the European Market using chemical substances within their line of business.

The aim of REACH is to improve the protection of human health and the environment through better and earlier identification of the intrinsic properties of chemical substances. At the same time, innovative capability and competitiveness of the EU chemicals industry should be enhanced. As opposed to earlier chemicals legislation, REACH gives greater responsibility to industry to manage the risks from chemicals and to provide safety information on the substances. Manufacturers and importers will be required to gather information on the properties of their chemical substances, which will allow their safe handling, and to register the information in a central database run by the European Chemicals Agency (ECHA) in Helsinki, Finland. The Regulation also calls for the progressive substitution of the most dangerous chemicals when suitable alternatives have been identified. Starting 1 June 2007 the REACH provisions will be phased-in over an 11-year transitional period.

Other relevant directives in EU such as those setting the rules for the development of safety data sheets (EU, 1991) and the directive restricting marketing and use of certain substances (EU, 1976) are being repealed and their rules are incorporated into the provisions in REACH.

10.6 Future trends

As shipping is an increasing worldwide activity, the use of antifouling paints is also a worldwide need. Today the major new building markets are located in Asia and, as the risk to man and the environment from use of chemical products including antifouling paints is attracting an increasing global

attention, it is expected that more countries in Asia will regulate antifouling paints in the future. Furthermore Resolution 3 in the AFS Convention (IMO, 2001) 'invites' member states to 'approve, register or license' antifouling paints used in their territories. Today most existing legislation regulating the use of antifouling products, such as the Biocidal Products Directive or the Federal Insecticide, Fungicide, and Rodenticide Act in the US, is of such complexity that they form a natural part of any new development of antifouling products containing biocides. It has no meaning to go through a full development project of a new paint only to discover at the time of market launch that the biocides used in the paint are illegal to market and use. Consequently any new development project of an antifouling paint should not only take the technology and market into regard but the legislative requirements as well. The market, the technology and the legislation are all factors to be considered equally when setting the limits of freedom within which to develop a sustainable product satisfying the market in all its aspects.

10.7 Sources of further information and advice

Several Internet sites contain further detailed information about legislation regulating the marketing and use of antifouling paints. In order to obtain advice on specific products or substances one should contact the local authorities directly. The central national authority enforcing antifouling legislation is typically the ministry or agency dealing with environmental policies.

10.7.1 Antifouling Legislation

International Maritime Organisation: www.imo.org

Regulatory information relating to TBT-free antifouling paints and legislation relation to AFS Convention: www.antifoulingpaint.com

US: <http://www.epa.gov/pesticides/>

Canada: <http://www.pmr-arla.gc.ca/english/aboutpmra/about-e.htm>

Australia: <http://www.apvma.gov.au/index.asp>

New Zealand: <http://ermanz.govt.nz/hs/index.html>

Hong Kong, China: http://www.afcd.gov.hk/english/quarantine/qua_pesticide/qua_pes_pes/qua_pes_pes_prc.html

Japan: <http://www.toryo.or.jp/eng/imo-e/imo-eng.html>

The following link contains a general description of the national legislation in some of the European countries in addition to countries outside Europe. The page is not fully updated: http://www.fouling-atlas.org/index.php?option=com_content&task=view&id=23&Itemid=52

10.7.2 The Biocidal Products Directive and guidance to the Directive

European Union Commission – DG environment: ec.europa.eu/environment/biocides/index.htm

The European Chemicals Bureau: ecb.jrc.it/biocides

OECD – Environment Directorate: www.oecd.org/departement/0,2688,en_2649_32159259_1_1_1_1_1,00.html

10.7.3 Tools for risk assessment

Compliance with antifouling legislation of today requires a risk assessment to be produced. The following links refer to sites where risk assessment tools can be found. Note that the tools are all accepted in regards of submission of data under the Biocidal Products Directive.

MAMPEC is used to assess the environmental concentration of active ingredients following the use of antifouling paints. The tool can be found on both the following sites:

www.cepe.org

<https://delftsoftware-wldelft.nl> and go to downloads

EUSES is used to perform risk assessments in general and can be found on ECB's website: ecb.jrc.it/euses

Standard emission scenarios are used in the environmental risk assessments.

Of obvious interest to the manufacturers of antifouling products and suppliers of active substances for antifouling products is the emission scenario for Product Type 21. This scenario can be found on ECB's website: ecb.jrc.it/biocides/documents

10.7.4 General legislation

Through the following link you are able to search on any legislation which has been or is in force in the European Union: <http://eur-lex.europa.eu/en/index.htm>

REACH

Extensive information about the new chemicals legislation in the European Union, REACH, can be found on the following website: <http://echa.europa.eu>

GHS

Find more information about the Globally Harmonized System of Classification and Labelling of Chemicals using the following link: http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html

10.8 Abbreviations

ACVM Act	Agricultural Compounds and Veterinary Medicines Act 1997
AFS	Anti-Fouling System
Agvet code	Agricultural and Veterinary Chemicals Code Act 1994
APVMA	Australian Pesticides and Veterinary Medicines Authority
BPD	Biocidal Products Directive
CEPE	The European Paint, Printing Ink and Artists' Colours Industry
DK-EPA	Danish Environmental Protection Agency
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
ERMA	Environmental Risk Management Authority New Zealand
EU	The European Union
EUSES	The European Union System for the Evaluation of Substances
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GHS	Globally Harmonized System of Classification and Labelling
HSNO Act	Hazardous Substances and New Organisms Act 1996
IMO	The International Maritime Organization
JPMA	Japan Paint Manufacturers' Association
MAMPEC	Marine Antifoulant Model to Predict Environmental Concentrations
METI	Ministry of Economy, Trade and Industry
PCPA	Pest Control Products Act
PMRA	Pest Management Regulatory Agency
PO	Pesticide Ordinance Cap. 133
REACH	Registration, Evaluation and Authorisation of Chemicals
RMS	Rapporteur Member State
TBT	Tri-Butyl Tin
US-EPA	United States Environmental Protection Agency

10.9 References

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Part II

Testing and development of
antifouling coatings

Developing new marine antifouling substances: learning from the pharmaceutical industry

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Abstract: The search for the ideal antifouling substance has a long history. In times before occupational health and environmental concerns, efficacy was the only parameter that was taken into account. However, that has dramatically changed during the last thirty years. It is now necessary to understand the regulatory framework of the area to ensure safety, and combine that with an understanding of the economic realities that in the end have to pay for the new developments. A sector that has successfully implemented efficacy, safety and market economy is the pharmaceutical industry. Scientific efforts, industrial development and regulatory framework have together created novel science as well as new and safer products. Today, the search for new antifouling substances shares many of the features experienced by the pharmaceutical industry. For example, scientific knowledge in biology, development of a control release system, production costs and how to prove the product safe for the end consumer, independent of man or nature.

Key words: product development, regulatory framework, antifouling substances, pharmaceuticals, bioassays, neurotransmitter receptors.

11.1 Introduction

As long as mankind has used the sea for transportation, marine biofouling has been an issue. When the seafarers were few and the ocean infinite and when there was no occupational health care, the biological growth on a ship hull could be avoided by using such toxic compounds as lead, mercury or arsenic. Compounds with high general toxicity could kill off all adhered organisms, regardless of whether they were algae, bacteria or invertebrates. With the increased awareness that non-target organisms as well as humans can be affected by antifouling substances, new regulatory demands have been introduced as well as an increased scientific effort to find new substances that will satisfy new demands. The strategy in how to find those substances was phrased 50 years ago.

While interest in the biological aspects of fouling may appear to end with the discovery of toxic coatings capable of preventing the growth, it should be remembered that new protective devices can not very well be developed without a fundamental understanding of the fouling populations.

Marine Fouling and Its Prevention. Contribution No 580 from Woods Hole Oceanographic Institute 1952.

However, toxic coatings, especially regarding the tributyltin (TBT) era, were not without complications, of which we are now well aware. But do we have new solutions today or are we re-using the same knowledge in a different way?

The first intuitive answer is that yes, of course we have come up with new solutions, but the second thought is, no, we are still using copper and to some extent, less favourable biocides on most ship hulls or other marine constructions. A huge effort is now available resulting in a vast range of modern coatings with much higher performance and efficiency, but the biology part of the two-side problem is dragging behind. Why so? One may speculate. A contributing factor may be that biofouling is a truly multidisciplinary research and as such, it is not suitable in the academic tradition and outside the scope of the coating industry. However, the fact that biofouling cannot be solved without different area of competence was also recognized in 1952:

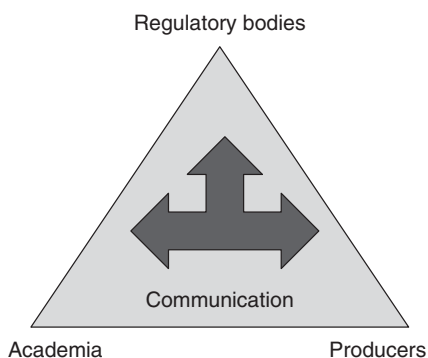
Fouling is, however, a biological phenomenon. If it is to be dealt with effectively from an engineering point of view, it is important that the biological principles which determine its development be understood.

In the world of today, if one addresses biofouling in a structural way, competences are needed not only in general marine biology or paint formulation, but in subdisciplines such as ecotoxicology, molecular biology and surface chemistry, but even more important an understanding of regulatory science. A contributing triad has to be constructed (Fig. 11.1).

11.2 The communication triad

The triad is built on three main players and three different components. Most of the regulatory framework is set by political decisions to protect citizens and the environment, with authorities to implement those decisions. Paint formulation skills and knowledge is mostly associated with paint companies, and more explorative research is performed by different academic groups.

The major difference from the 1952 report is development within environmental sciences and as a result thereof, the upcoming of regulatory science seen as new regulations and legislations and the implementation thereof. The triad is rather new within the area of marine biofouling, but as



11.1 The communication triad. Three main players are involved in the development of new antifouling substances. The regulatory body has as its purpose the protection of man and the environment. Their demands are directed towards the producers that need to fulfil the regulatory demand of information. A third player, the concept providers, academia, are traditionally less aware of market and regulatory demands, thereby reducing the ability to choose the right strategy and substances for product development. With a more interactive discussion between different components of the triad, a better innovation climate can be created.

such, it is a well-developed model in other areas such as the pharmaceutical industry. Paint formulation has a contra part in pharmaceutical formulation, since the task is the same: controlling release of potent chemicals over time. Another similarity between the two areas seems to be an incidence of harmful side effects as a wake up and starting point in developing regulatory science. The pharmaceutical industry had its thalimodide (Neurosedyne) incident in the late 50s and regarding antifoulants, TBT in the 80s.

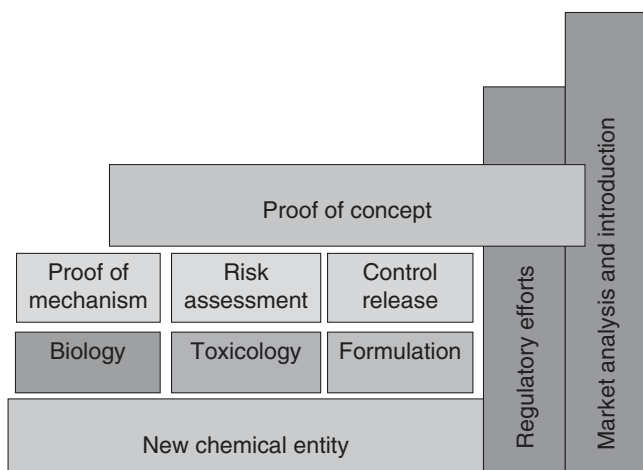
A defined goal for all parts of the triad is to develop new products, with less risk, with effective substances that can be incorporated into a final product that has market compliance. We all want to find solutions for the market that are in every respect better than the older ones: safer for the environment and with higher efficacy, giving better performance. The different positions in the communication triad are all equally necessary for developmental work but it is also necessary to understand the different roles. Since it is a communication triad, for success it points towards the fact that within such a multidiscipline area as antifouling, I as a scientist have to orientate myself toward understanding at least parts of the regulatory framework as well as market demands regarding products and relate that back to the latest developments within science.

As we stand now, the market does not have unlimited resources nor does science, while society demands increasing assurance of safe, and more

effective, products. Neither the paint industry nor academia are capable of finding resources demanded by regulatory bodies for new substances. A new product as it is now, is associated with risks higher than the market may be willing to take or pay. To be able to push the area forward, e.g. coming up with new substances, it is vital that all parts are willing to agree on risks and share the burden of risk assessment.

11.3 From concepts to products

Many concepts are not fruitful, lacking components for successful market introduction or perhaps even an insight that a substance (concept) is not immediately a product. As a multidisciplinary research area, a developmental model is composed of different interactive boxes with different expertise (Fig. 11.2). At certain times, one speciality may be of more importance but none can be missing. As the demands are increasing in all aspects, a more structural analysis of resources and competences needs to be performed in projects regarding biofouling control. A key issue in the developmental work is how to transfer concepts to market acceptable products. Scientists are often the concept providers, and the paint (or chemical) industry the product producers and market providers. Proof of concept is the bridge



11.2 A development model. All horizontal boxes represent concepts and the critical process when concepts must be transferred into products. Within that process, horizontal boxes must relate to the vertical boxes complying with regulatory and market demands in an interactive way. That process is best described as 'proof of concept' and it is critical in the development process. A hindrance may be different opinions in what is needed and what has to be proven. That may hamper, or in the worst case, put an end to the process.

between the two. It should contain field efficacy and a basic set of toxicological testing, preferably at least degradation, bioaccumulation, cell toxicity and assurance that the suggested substance does not induce mutagenicity or bind to DNA-molecules. From my point of view, being a concept provider also includes assurances that the new substance may be suitable as a new antifoulant, and also have no mutagenic or bioaccumulative properties. Proof of concept is the most critical step associated with high risks in the innovation process and is not regularly discussed, and in some cases is referred to 'not being my problem'. A functional communication triad is necessary to overcome problems associated with proofs of concept; to build collaboration linking concepts into products.

11.3.1 There is no single solution

The biological complexity of the phenomenon we call biofouling is enormous. It is an ecological community with entities originating from all that we call life. Also, each organism has its own solution for how to find and stay firm on a surface, evolved during millions of years. In my view, it is impossible to invent new antifouling substances without restricting the problem, meaning that new molecules have to be part of a bigger solution. Any new substance will have an efficacy profile that differs from barnacles compared to algae (Konstantinou and Albanis, 2004). Therefore, it seems to be more logical and rational to use a combination of different substances to reduce the overall usage of marine biocides. By being more precise in mode of action, identifying key biological components in the biology of surface attachment, it is possible to reduce the amount of the biocide.

11.3.2 Rational development needs multidisciplinary action: the barnacle example

As all organisms have their own solutions in adhering, we may also find several new ways to combat marine fouling and it is likely to be driven forward by increased knowledge in relevant sciences. There is a need to understand the general fouling biology, an increased knowledge in the area between traditional marine biology and surface chemistry. In our experience, based on cyprid larvae of the species *Balanus improvisus*, it is not difficult to demonstrate antifouling effects in laboratory of most substances in concentrations about 1 μM and above (Rittschof *et al.*, 2003; Dobretsov and Qian, 2003; Dahlström *et al.*, 2005). Without confirming a mode of action, this is not satisfactory from a scientific point of view. It points towards the fact that to understand attachment mechanisms, it is not enough to perform experiments with pharmacological molecules, without knowing if the biological targets exist in barnacles or other fouling organisms. It has

to be combined with a number of different assays and experimental approaches (see Chapter 12: Dahms and Hellio).

11.4 Learning from the pharmaceutical industry

A focus within the pharmaceutical industry has always been to know the physiology as well as pathology within a chosen area. To be able to develop a pharmaceutical against high blood pressure, it is essential to identify hormones and neurotransmitters that contribute to blood pressure regulation and their specific receptors. Especially the receptors have proved to be fruitful targets in the development of small molecules that could alter the physiological output in a more favourable way. When a receptor, or ion channel or enzyme, has been identified as a key target for pharmaceutical treatment, it is then possible to screen for new substances in a systematic way. With molecular techniques, it is possible to isolate and develop biological assay with the target protein, a receptor of a specific hormone or neurotransmitter. With the isolated protein, expressed in a bioassay, the chemists will be able to create a vast number of derivate molecules which become a chemical library that can be tested against the target protein within an appropriate bioassay. In such a way, it is possible to create a high throughput system that combines knowledge and serendipity. The development that has made this possible is most of all knowledge in basic physiology and the ability to adopt modern technology within test systems that could become new bioassays.

This methodology is also a possibility within antifouling research. An objection is that there are too many and too different types of organisms regarding marine biofouling so that it would be impossible to introduce such systematic research routes. However, the most basal physiological systems are similar between species. It is possible to identify targets and perform high throughput systematic research using general systems such as neurotransmitter receptors.

Barnacles are no different from most other organisms. They do have neurotransmitters such as dopamine (Okano *et al.*, 1996), serotonin (Yamamoto *et al.*, 1996; Yamamoto *et al.*, 1999), histamine (Callaway and Stuart, 1998), acetylcholine (Faimali *et al.*, 2003) and probably also octopamine. Barnacle octopamine receptors have been identified and the receptor is known from other crustaceans. However, usually noradrenaline or adrenaline are not thought to act as neurotransmitters within invertebrates but are exclusive for vertebrate species (Roeder, 2005).

There are some indications that noradrenaline may affect settling and metamorphosis (Yamamoto *et al.*, 1996; Okano *et al.*, 1996) in higher concentrations, further supported by antissettling effects seen with phentolamine (Yamamoto *et al.*, 1998). However, phentolamine and yohimbine are also

known as octopamine antagonists (Evans and Robb, 1993; Roeder, 2005; Maqueira and Chatwin, 2005) and noradrenaline binds as well towards dopamine receptors (Degen *et al.*, 2000). Any results suggesting receptor specificity using the settling assay must be regarded with some care. The term specificity is a relative term based on mammalian pharmacology where the minutiae differences between receptor subtypes have been studied. Claiming specificity needs comparative studies between different receptor types. There are few such studies regarding invertebrate receptors and none within the area of antifouling. Instead, several adrenergic compounds bind to octopamine receptors (Evans and Robb, 1993; Howell and Evans, 1998) and dopamine receptor pharmacology within invertebrates is different compared to what is known from vertebrate, especially mammalian, studies. (Degen *et al.*, 2000; Ödling *et al.*, 2007; Zega *et al.*, 2007).

Any claim of specificity must be carefully evaluated on its own merits. What is lacking is knowledge regarding receptor biology and pharmacology, especially when looking at future rational development within the area. Only two receptors have been cloned (Isoai *et al.*, 1996; Kawahara *et al.*, 1997), both octopamine receptors (GPR18_BALAM (Q93127) and GPR9_BALAM (Q93126); www.expasy.org). A successful pharmacological approach must include detailed knowledge regarding receptor biology in terms of affinity, efficacy and specificity. This does not mean that we need to characterize every species. There are similarities between species even if science tends to emphasize the differences. As seen above, dopamine is a ubiquitous neurotransmitter and substances that have been developed for human diseases do affect barnacle cement secretion (Ödling *et al.*, 2006). But to ensure that the right receptor protein is targeted, more biological assays need to be developed as well as molecular biology tools need to be applied in future developmental work.

Regarding medetomidine as an example of a new antifoulant, the goal has been to find a reversible mechanism that does not necessary kill the cyprid larva, only prevent settling. We know that medetomidine binds to a receptor; the effect is reversible and specific antagonists have been identified (Dahlström *et al.*, 2000; 2005). The mode of action is activating swimming movements that will short cut the surface behaviour (Hasselberg Frank, in manuscript). At the same time, the surface is nevertheless of great importance. Medetomidine is a surface active molecule (Dahlström *et al.*, 2000; 2004).

The receptor responsible for medetomidine activity within barnacles has now been cloned and been transferred into a yeast and been expressed within yeast cells (see Lind; 14th ICMCF conference, Kobe, Japan). This opens up the possibility to find and explore future substances that can be found within a chemical library that has the right biological and chemical properties that could either activate or block the receptor. In similar way,

any identified receptor, for example a dopamine receptor, can be transferred over into an expression system and become the target in evaluating a chemical library designed for invertebrate dopaminergic receptors.

The differences between pharmaceutical and antifouling development is the life cycle analysis of the biological active substance. To be included within a paint matrix or absorbed in the gastrointestinal system are two different means of distribution. A paint system may want a more lipophilic substance, whereas a more hydrophilic may be advantageous regarding a pharmaceutical. Degradability is a key issue within antifouling to avoid bioaccumulation, whereas a high degradation rate might not be favourable regarding pharmaceuticals, since it might mean a more complicated dosing regimen. If the biological target is identified and high throughput screening methodology implemented, it is possible to find substances that could meet biological efficacy criteria as well as having acceptable chemical properties. However, the ideal antifouling (or pharmaceutical substance) will never be found. There will always be compromises between the biological, chemical and environmental properties to decide between. Whether those decisions are right or wrong, that is the task for the regulatory system.

11.5 The importance of formulations

The paint industry has for centuries provided the market with different products and solutions. Most of the knowledge is empirical. Paint formulation is in many respects a difficult task, since small amounts of a new ingredient may totally change the physical and chemical properties. Rationally, it would be easier if incorporated biocides were a minor part of the formulation. At the same time, no one can foresee how mixtures may interact with each other and the formulation *per se*.

The paint formulation is at the same time a storage and release system for the antifouling substance. As such, it plays a key role regarding efficacy and performance. In laboratory tests, medetomidine and a sister compound, clonidine are almost equally effective (Dahlström *et al.*, 2000), but in field they differ totally. While medetomidine will stay completely effective for over two years, clonidine does not last for a single season on the Swedish west coast (unpublished data). This difference in efficacy is likely to be associated with surface behaviour of the molecules.

Leakage of a new substance is one key issue when assessing environmental risks. Models like MAMPEC (free download from www.cepe.org) used to predict Predicted Environmental Concentration (PEC) is balancing between degradability, toxicity and leakage. A substance with low leakage rate is less sensitive to factors such as non-target toxicity or degradability. Leakage is also a parameter that can be refined by providing a control release system. A new tendency within paint formulation is to use

microcapsule techniques also known from the pharmaceutical industry (Zhang *et al.*, 2007).

11.6 Side effects and regulation

Dealing with such complex biological mechanisms, claiming efficacy and not expecting to find any side effect is a bit naïve. The question is more whether the side effects are acceptable and what the risks are with a specific substance. We may all conclude that every new antifouling substance will be hazardous, but the question we ought to answer is whether the risk is acceptable. In those terms, it is a great advantage if the mode of action is known. Is it a common biological mechanism that is a target and does it act through toxicity alone? Then the degradation data will become more critical, since general mechanisms are generally more hazardous. This is seen with substances such as Sea-Nine which has a high degree of toxicity but is quickly degradable and not bioaccumulative (Willingham and Jacobson, 1996; Jacobson and Willingham, 2000). The risks are therefore acceptable. Medetomidine is an example of the opposite way of dealing with side effects. It is specific and is fully reversible (Dahlström *et al.*, 2000). Tests can be set up to predict foreseeable side effects from what is already known regarding its mode of action. The hazard is less, but still risks need to be quantified. Even though medetomidine is not hydrolyzed and degraded as quickly as Sea-Nine 211, it is not bioaccumulative (Hilvarsson, 2007) and its release rate is in ng/cm²/day (Dahlström, 2004; Borgert, 2004). Those features allow medetomidine to be used as an antifouling substance.

11.6.1 The regulatory process

A way of handling risks from the society perspective is to have a regulatory procedure. Regarding Europe, this is now being transformed from the national level towards a European legislation. This is summarized in the Biocidal Product Directive (BPD) 98/8. For many European countries it changes the legislation regarding antifouling products. In most European countries notification of antifouling substances has been necessary but not a regulatory process. These issues are discussed in Chapter 10.

11.7 Marketing a new product

No development efforts are without a price, and antifouling development must in the end be economically sound. The product *per se* must be priced such that it may enter the market – but still not be underfinanced, since that will stop any further development. No one will ever say:

We are prepared to take the costs for a new antifoulant.

In a broader perspective, this means that for a successful and a more dynamic market, we as scientists must learn more about issues beyond science if we are to be realistic in our hopes for our results and inventions. We must learn that to make a compound a success, it means more than efficacy in lab and field; it also means dealing with and judging the outcome from much more difficult aspects such as formulation, toxicological risks, as well as keeping on an eye on the market. In that process we need to interact with other experts, regulatory authorities as well as industrial competences. Once again, probably the most successful industry in terms of profit, the pharmaceutical sector, learned this lesson much earlier than the biocide sector. The sooner we create a more dynamic viewpoint, the more successfully will science come up with new possible and realistic alternatives that could interact with authorities and industry. As part of a three communication triad, industry and authorities need to have the ability and willingness to interact with new dynamic approaches and possibilities within science.

When comparing the antifouling sciences with the pharmaceutical industry, one must also be aware of the differences between those industry sectors. Most of all, the pharmaceutical industry market is, in terms of money, much bigger than the antifouling paint market. It means that the driving forces and the risks that venture capitalists are willing to take are also higher. Therefore, pharmaceuticals are subsidized in many societies and therefore not comparable with the antifoulant market. It means that the innovation rate within the pharmaceutical industry can be faster due both to market size as well as society contribution measured as efforts and resources invested in science. Efforts are also driven by emotional factors since pharmaceuticals concern us as human beings. This is not the case regarding antifouling, although the increased awareness of the marine environment has been raised, also seen as new regulatory standpoints.

Scientifically, the pharmaceutical and antifouling development processes are similar; find a substance, formulate and register. Although the overall biological complexity within the area of marine biofouling is overwhelming, especially knowing the lack of background information that exists in biomedicine and clinical sciences, as a matured industrial sector, pharmaceutical development serves as an example to reflect upon.

11.8 Summary

In many ways, antifouling research is still a very young scientific area, although efforts to protect marine surfaces have been an issue for mankind as long as the sea has been used for transportation. Our main and most

used antifouling substance is still copper, used as an antifouling substance in paints since the 19th century. However, with the increased awareness of the marine environment and the new regulatory situation, this might change. There is an expectation that more substances will be banned and therefore create a demand for new ones with fewer risks, developmental work including academia, industry and society. Although, the trend can be the complete opposite due to the costs associated with the regulatory demands, causing new innovations to become economically impossible and therefore damage future opportunities for a better marine environment. In the long run, it might also be a dead end for market providers in that the alternatives will become fewer, reduced to few products with many producers. This is more of a political issue, but if we are serious in wanting new environmentally friendly products, we need all parts of the communication triad to share risks and development costs. In my view, new products are necessary to prove success for all parts involved, regardless of being concept or market providers.

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Laboratory bioassays for screening marine antifouling compounds

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Abstract: This overview surveys the widely scattered and disparate literature on laboratory bioassays available for the screening of activity and toxicity of potential antifouling compounds. The laboratory bioassays discussed herein particularly target antifouling compounds that may interfere with the fouling process by either attracting or deterring micro- and/or macroorganisms. Biological assays are deployed to direct the purification, identification, and, possibly judgements on the effectiveness of new products. This is essential for any statements on substance-effectiveness as well as for environmental considerations about ecological side-effects of compounds to be used in fouling treatments. Although it is desirable to use a large number of ecologically relevant test organisms, economic constraints may restrict most bioassay objects to key-taxa.

Key words: bioassays, fouling, marine biotechnology, toxicity, activity evaluation.

12.1 Introduction

The biotechnological potential of bioassays lies in the screening of bioactive substance effectiveness (e.g., as antifouling (AF) agents) as well as in the monitoring of their side-effects. Bioassay screening for fouling applications provides information for the development of AF coatings and other biotechnological appliances such as for the food and drinking water industries, mariculture and conservation management (His *et al.*, 1996; Fusetani, 2004). Bioassays can be static or dynamic, consider physiological or behavioral traits, and can comprise (multiple-) choice tests. The design of semiochemical bioassays (i.e., bioassays monitoring chemicals with intra- and interspecific bioactivity) must be governed by the often conflicting objectives of ecological relevance and the need for simplicity.

Experiments performed with natural assemblages of fouling organisms may provide a more realistic picture of the ecological effects of bioactive

substances than do controlled laboratory experiments (see chapter 16 on Field trials). Although laboratory bioassays can neither mimic the complexity of the natural environment (especially water flow patterns, hydrostatic pressure, and substrate- and substance-dependent diffusibility), they still provide the bulk of assays screening for activities of new AF compounds. There are many uncertainties involved in the design of suitable experiments that verify AF chemical interactions at surfaces in an ecologically meaningful context (Clare, 1996). Any potential biotechnological application of bioactive compounds would require conclusive bioassay-derived evidence that the components are not active against non-target species, are harmless to humans, non-polluting, and biodegradable (Clare, 1998).

It is unclear for most AF studies, whether the methods, concentrations, and organisms used in the bioassays are ecologically relevant. To be that, assay systems have to consider the character and functionality of the active molecules under *in situ* conditions (Steinberg *et al.*, 2002). Parameters that are particularly difficult to imitate realistically under experimental conditions are substrate- and substance-dependent diffusibility, as well as flow conditions and hydrostatic pressure. The choice of test organisms is important for the interpretation of bioassays. Because of the wide range of species and chemicals, and the diversity of responses to those chemicals (Hellio *et al.*, 2000a, 2001), it is not possible to consider all of the potential means of bioassaying all species for all interactions.

Since the first attempt to develop bioassays (Rittschof *et al.*, 1992), there has been little change in the approach. The first step usually is to show a biological effect of a chemical substance, since the primary role of bioassays is to target fouling organisms to direct the purification, identification, and development of antifoulants. It may generally be assumed that the larger the number of ecologically relevant test organisms to be employed, the more detailed the information about the effects of the AF compounds under consideration will be.

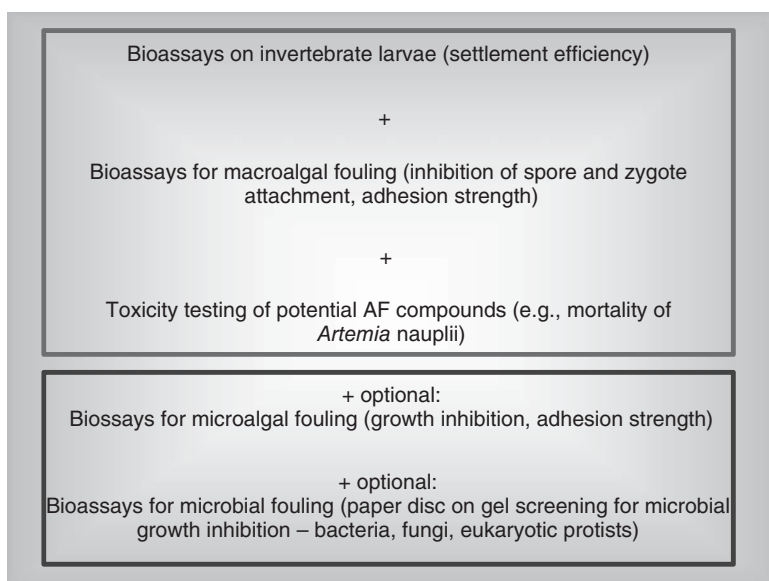
There is increasing evidence that bioactive substances can have significant effects on organisms at non-toxic concentrations (Maximilien *et al.*, 1998). Thus, assays that measure sublethal or particularly behavioral responses such as the ability to swim, attach, and swarm will provide more information in assessing the spectrum of possible ecological effects than assays that simply measure toxicity. Sublethal effects are also reflected in differences of life-history parameters. The susceptibility of younger stages to bioactive substances may be different from those of advanced stages. This also holds for differently aged microorganisms when used in assays (Lau *et al.*, 2003). Behavioral assays have to take into account that reactions of settlers usually result from the biological and individual predisposition and a multiple sensory input to potential settlers (Dahms *et al.*, 2006a).

De Nys *et al.* (1995) were the first to combine several different assays in a multiparametric screening procedure in order to provide more detailed information about the role of AF compounds in the fouling process and they showed that no single compound was most active in all tests and some metabolites that were effective against one organism showed little or no activity against the others. Hence, appropriate bioassays rely on the knowledge of the effectivities of chemical compounds on target organisms. The number of organisms used in fouling assays is increasing, as is our understanding of the complexity of the biological processes that are involved in settlement processes. It is the choice of test organisms for a screening assay which is of predominant importance for the interpretation of substance effectivity. Ideally, potential new AF compounds should be tested against models organisms involved in each stage of a surface colonization: bacteria, fungi, microalgae, macroalgae and invertebrates in order to provide the best picture of the new compound activity (Table 12.1). Active compounds are simply not detected because they are not tested against appropriate target

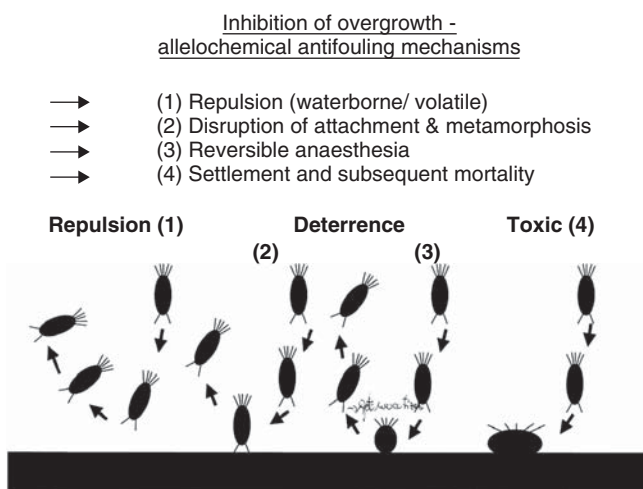
Table 12.1 Bioassays that are currently in use in antifouling studies

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1. Bioassays for microbial fouling
 - Bioassays with bacteria
 - using gels (paper disc method, glass ring method, spectrophotometric chemotaxis assays, hydrogel)
 - using broth (turbidity, bioluminescence)
 - using biochemistry and settlement (flow cytometry, ATP measurements, settlement-slide bioassay)
 2. Bioassays for microalgal fouling
 - inhibiting growth
 - showing adhesion strength of diatom
 3. Bioassays for fungal fouling
 - using agar
 - using broth
 4. Bioassays for macrofouling
 - Bioassays with macroalgae
 - inhibition of the attachment of spores and zygotes
 - settlement and adhesion strength
 - monitoring brown algal spore swimming behaviour
 5. Bioassays on invertebrate larvae
 - Cirripedia
 - Bryozoa
 - Teredinidae
 - Polychaeta
 - Bivalvia
 6. Toxicity testing of antifoulants (using: microalgae, sea urchins, *Artemia*, oysters, barnacles, mussels, ascidians, fish, mammals)
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organisms. The choice of the target organisms will vary within the potential paint application, e.g. cold model species will be chosen for cold waters formulated paints, and tropical species for the other paint formulations. Usually, when a screening of new compounds is run, in order to obtain a comprehensive study, assays are conducted on both cold and warm water species. Macroalgae and invertebrates colonization on surfaces will cause a significant increase of the frictional drags (>10% and >40% respectively) (Callow, 1996) and as a consequence potent compounds against these organisms will be searched uppermost (Fig. 12.1). Owing to the new legislation, AF compounds had to be active at non-toxic concentrations against non-target species (Fig. 12.2), as a result, ecotoxicity evaluation is now a top list priority in the screening process. Biofilm formation results in a slightest increase of frictional drags (1–2%), this is why bioassays on microalgae and bacteria can be considered as optional. Nevertheless, they should not be neglected as these organisms have been linked with biocorrosion and the mediation of macrofouling. An ideal AF compound should be active at non toxic concentration: $EC_{50} < LC_{50}$ and should be stable in a paint formulation. Owing to financial constraints, only the compounds active at low concentrations (ng/ml) will be potentially useful for an industrial scale-up.



12.1 Array of most feasible bioassays for initial screening.



12.2 Diagram of allelochemical antifouling mechanisms: (1) repulsion (by waterborne, volatile chemicals), deterrence (by (2) disruption of attachment and metamorphosis, or (3) reversible anaesthesia), (4) toxic effects (settlement and subsequent mortality).

Einhellig (1996) has shown that the effect of compounds from different chemical classes can be cumulative. In addition, many compounds degrade into products that can intensify the activity of those already present. Active compounds may not be detected because they degrade during collection, storage, and extraction, or because they are not tested against appropriate target organisms (Clare, 1996). It remains unknown in most cases where compounds act, and after what time lags they reach target tissues or become active.

Concentration is a confounding factor in all AF fouling assays. At different exposure levels, the same substance may be attractive, repellent, or even toxic (see Fig. 12.2). Hence, it is crucial to relate the response to a biologically relevant concentration. Among the most pressing issues for an effective chemical evaluation is the estimation of the concentrations of bioactive substances experienced by target organisms at interfaces.

Because compounds that differ only slightly in chemical structure can vary greatly in their deterrent effects, the same metabolites may show pronounced differences in their effects even in closely related species (Paul and Pennings, 1991). It is desirable to link specific variation in the structure of these metabolites with variation in activity against different fouling organisms. According to de Nys *et al.* (1995) there are some patterns that become emergent.

The purpose of this overview is to provide an introduction to the principles and problems involved in bioassays that have been used to study the antifouling and toxicity effects of bioactive substances on marine biota. The examples chosen should demonstrate a diverse set of assay realizations and should provide the reader with an overview of approaches that are utilized in the screening of bioactive chemicals under laboratory conditions.

12.2 Bioassays for microbial fouling

Microbial biofilm development is an important step in biofouling processes (Holmstrøm and Kjelleberg, 1994) and frequently provides a supporting substrate for the subsequent attachment of other fouling organisms (Qian *et al.*, 2007). The deterrence of the formation of this initial biofilm layer has been suggested to be fundamental to effectively control further large scale biofouling (Holmstrøm and Kjelleberg, 1999). However, Maki *et al.* (1998) controversially discussed this issue, stating that bacterial films are not a necessary prerequisite for subsequent macrofouling. Most microbial biofouling studies have focused on bacteria rather than on other taxa (fungi and microalgae) (Olgoi-Oribe *et al.*, 1997).

12.2.1 Bioassays with bacteria

Target microorganism selection remains a significant challenge. It is important that the bacteria used for various bioassays meet a number of criteria, including motility and the ability to settle quickly on an agar surface. Provided a large number of ecologically relevant bacteria are employed, valuable information about potential semiochemical effects of bioactive compounds may be deduced. Owing to their isolation history *via* standard enrichment media, they represent only an insignificant proportion of the natural diversity in the natural habitat (Eilers *et al.*, 2000). Besides the disadvantage of a culture-dependent technique, it remains unclear which bacteria are in fact ecologically relevant for fouling processes. Three main groups of bioassays are widely used and are described in the section below: bioassays using gels, broth, or biochemical tools.

12.2.2 Bioassays using gels

Commonly, assays for antibacterial activity are performed using inhibition zone tests named disc diffusion assays (DDA). These assays are widely in use applying the method of Beer and Sherwood (1945) as modified by Hellio *et al.* (2000a). Briefly, a lawn of microorganisms is prepared by pipetting and evenly spreading bacterial cultures (1 ml of a 5.10^4 CFU/ml culture) onto agar set in Petri dishes, or, depending on the strains used, the plate

containing a first layer (10 ml) of medium agar 12% (w/v) were overlaid with a second layer of 5 ml of medium agar 6% seeded with the target organism.

Four methods have been developed using respectively: (a) paper discs, (b) glass ring, (c) a spectrophotometric chemotaxis assay and (d) hydrogel.

a) Paper disc method

Sterile filter paper discs are placed at the center of this agar plate to which the test compound dissolved in solvent is added. Six treatments per dish can be done simultaneously. The plates are inverted and after incubation (length depending on the strain and the temperature of incubation) the diameter of the inhibition zone around the disc is measured. Control experiments are performed where only equivalent volumes of solvents without test compounds are added and applied to the paper discs. Positive controls consist of commercially available antibiotics or biocides used in paint formulation. The antibacterial activities are expressed as minimum inhibitory concentration (MICs) values corresponding to the lowest concentration of the compound that produced a measurable zone of inhibition (Bauer *et al.*, 1966).

b) Glass ring method

Another method consisted of placing a sterile glass ring (4 mm internal diameter) on the bilayered agar. AF compounds to test were placed in the glass ring and allowed to diffuse for 2 h at 4°C. Six treatments per dish can be done simultaneously. After incubation, the activity is evaluated by measuring the diameter of the inhibition zones around the rings (Hellio *et al.*, 2000a).

Several problems are associated with these diffusion methods comparing the relative antimicrobial activity of different compounds. The time taken for incubation may not allow testing for volatile or unstable antimicrobial agents. The zones of inhibition may only be compared among antimicrobial agents with similar physical properties such as diffusion rates in agar, volatility or solubility in aqueous solution.

The variability inherent to the disc diffusion assay has to be considered, and Jensen *et al.* (1996) estimated at least ± 1.0 mm variability of the same treatments. This emphasizes the importance of replicate sampling, and makes it difficult to determine the accuracy of small zones if they are outside the detection limits of the assay. Non-reproducible zones of bacterial inhibition should be considered with caution as indicative of an antimicrobial chemical defense. The level of activity that is measured in the

diffusion assay is dependent on both the rate of diffusion of extract into agar and the potency of the compound. An extremely potent compound with a slow diffusion rate will appear to have a low level of activity in the bioassay.

c) *Spectrophotometric chemotaxis assays*

Boyd *et al.* (1999) used 10 × 10-mm cuvettes with round openings for testing aqueous extracts by incorporating them into an agar solution that was used to form a solid plug at the bottom of the cuvette. The volume of agar used (450 µg) was such that the light beam of the spectrophotometer passed through the cuvette 4 mm above the surface of the agar.

The target strain was suspended in semisolid agar which was placed on top of the solid agar surface so as to fill the cuvette. The cuvette was then stoppered with a rubber bung, with care to eliminate any air bubbles, and sealed with parafilm. A second cuvette was prepared as described above containing semisolid agar without the addition of an AF compound. Before and after each assay, a 10 µL portion of the cell suspension was examined under the microscope to ensure the cells were motile throughout the assay.

Optical density (OD) measurements were made using a computer-controlled Shimadzu UV-1601 UV-visible spectrophotometer. The cell free cuvette was used as a blank when recording the OD of the bacterial suspension. As a control, the experiment was carried out with the substance to be screened incorporated into the agar plug in place of the aqueous extract.

d) *Hydrogel*

Henrikson and Pawlik (1995) developed a technique where compounds were incorporated into hard, stable hydrogels, testing originally for macrofouling settling stages. Harder *et al.* (2004) utilized these gels as substrata for attachment and colonization by natural bacterial populations, either in laboratory wells or in the field. The main advantages of a gel-immobilized assay of tissue extracts over conventional antibacterial assays were:

- (1) its culture-independent approach (no bias with respect to the choice of ecologically relevant test bacteria because the gels were exposed to a (semi-) natural assemblage of microbial colonizers),
- (2) no restriction to particular modes of inhibitory microbial colonization (antibiotic and/or repellent),
- (3) AF compounds within the gel matrix do not alter the physical characteristics of the settlement surface, and
- (4) the ability to perform this assay under still water and flow conditions.

12.2.3 Bioassays using broth

The bioassays using broth have the advantage of being more rapid and of using minor quantity of compounds. These assays can be run at minor economic costs and are based on (a) turbidity and (b) bioluminescence.

a) *Turbidity*

Determination of the MICs can be carried out for bacteria (NCCLS, 1998) by macrodilution methods for testing the AF activity of various compounds. A dilution series of the compounds to be tested is prepared and then the bacterial solutions are added (2×10^8 CFU/ml). These experiments are run in 96 well plates. After incubation (usually 20 °C, 24 h for marine bacteria, Tsoukatou *et al.*, 2002), results are read visually or by using a spectrophotometer (630 nm). MIC represents the lowest concentration that inhibits the organism's growth.

Arikan *et al.* (2002) calculated the geometric mean (GM) and range of the MICs and MECs (minimum effective concentration) and the arithmetic mean and range of the IZ (inhibition zone) diameters for each genus/species combination, applying the broth microdilution and disc diffusion method. They demonstrated that the relatively high MECs obtained by the microdilution method correlate well with the absence of inhibition zones on disc diffusion agar plates. Being less time-consuming and less labor-intensive, the disc diffusion method is preferable to the microdilution method. However, the inability to determine the susceptibility test result at 24 h for an individual isolate, appears to be a notable limitation of the disc diffusion assay.

b) *Bioluminescence*

FDA (fluorescein diacetate) assays were run in 96 well ELISA trays by Adam and Duncan (2001). A solution of each concentration of the test substance, or the appropriate solvent was added as a control. ELISA trays were incubated before FDA was added. Incubation was continued until green color resulting from the hydrolysis of FDA was measured at 490 nm (referenced to 630 nm) and blanked against control wells containing microbial cultures only, using a MR7000 automatic ELISA tray reader from Dynatech Laboratories.

To determine the percentage of cells killed under particular assay conditions, cultures were serially diluted on both sides of the MIC that had been treated with varying amounts of compounds. The diluted cultures were plated on agar and after incubation, counts of visible growing colonies (CFU) were performed.

The FDA (fluorescein diacetate) method can be used with a wide range of different microorganisms since most microorganisms will hydrolyse FDA. Liquid sample preparations allow for maximum exposure of bacteria to both nutrients and test compound(s). According to Toama *et al.* (1979) the FDA method displays greater reproducibility than the disc diffusion assay method where there is always a danger of flooding the paper disc, or compounds evaporating or diffusing at different rates. In addition, the FDA hydrolysis assay technique can be adjusted for a microassay when very little of the compounds are available or large numbers of samples need to be screened. This method is rapid and led in 2 h to accurate MIC values of pure isolated compounds that are comparable to other methods could also be established by this method.

c) *Flow cytometry and ATP measurements*

Beside the typical microbiological techniques, effects of AF compounds can be measured using biochemical bioassays such as flow cytometry and measurement of ATP and settlement bioassays.

When the target of antimicrobial activity is the cell membrane, methods based on flow cytometry or measurements of ATP can be used. This method is very sensitive but requires expensive materials and equipment. Flow cytometric analysis is based on a non-polar fluorogenic substrate, carboxy-fluorescein diacetate (cFDA) (Budde and Rasch, 2001). When antimicrobial substances are membrane active, they disrupt the proton motive force (PMF). Disturbance of PMF depletes the amount of intracellular ATP and this can be measured using bioluminescence assay based on the eukaryotic luciferase reaction: $\text{ATP} + \text{O}_2 + \text{D-luciferin} \rightarrow \text{AMP} + \text{PPi} + \text{CO}_2 + \text{oxyluciferin} + \text{light (f560 nm)}$ (Ennahar *et al.*, 2000).

d) *Settlement-slide bioassay*

This assay was designed to determine the effects of compounds on bacterial attachment to an agar matrix (Wahl *et al.*, 1994). This assay can detect substances that interfere with any number of behavioral or physiological processes associated with the attachment of bacteria to a surface. AF compounds are added to molten agar at concentrations near or below those estimated to occur in the test organism. This process ensures that, regardless of the compound tested, the bacteria encounter the same topographically homogeneous surface. Consequently, the observed effects on bacterial attachment to the treatment *versus* control surfaces should only represent chemical modulations of the attachment process.

The 'settlement-slide bioassay' has the advantage of applying extracts not within an agar matrix but directly onto glass slides. However, when the

extracts are less evenly distributed, the extract concentration cannot be calculated. Physical properties of the extract (e.g., hydrophobicity) may contribute to settlement effects, and water-soluble materials may be lost during the immersion phase. Conversely, this method requires much smaller quantities of extract, and is beneficial for the identification of settlement-inhibiting fractions during chemical isolation, when the quantity of material is limited.

12.3 Anti-microalgal bioassay

Microalgae are important components of biofilms and play an active role in submerged surface deterioration. The range of microalgae involved is still poorly known due to the large biodiversity of organisms within this group. Microalgal strains for AF tests can be either bought from bank (algbank or CCAP, for example) or be directly isolated from the natural environment (Dahms *et al.*, 2006b). Two main methods are available to evaluate the efficiency of potential antimicroalgal activities of AF substances, respectively based on the observation of growth inhibition and on adhesion of cells.

12.3.1 Inhibition of growth

Tsoukatou *et al.* (2002) cultivated microalgal strains (*Tetraselmis levis*, *Micromonas pusilla*, *Pyramimonas amyliifera*, *Rhodosorus marinus*, *Porphyridium purpureum*, *Rhodella* sp., *Dunaliella tertiolecta*, *Chlorococcum submarinum*, *Cylindrotheca closterium*, *Phaeodactylum tricornutum* and *Amphora coffeaeformis*) under continuous illumination ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$, white fluorescent lamps) at 18°C in Guillard's F2 medium (Guillard and Ryther, 1962). Then, 15 ml aliquots of F2 medium were introduced into sterile conical flasks and inoculated with 5×10^5 cells/ml⁻¹ of cultivated microalgae in exponential growth phase. The compounds to test were then introduced into the flask. Positive controls used were solutions of any biocides used in the formulation of AF paints. A standard, containing no biocides, was also set up. Cell growth was estimated daily, over 5 d, by direct counting of the cells in a Malassez haematocytometer (Hellio & Le Gal, 1998) and the lowest extract concentration leading to total absence of cellular growth was recorded. It is also possible to determine the chlorophyll *a* concentration and use the value as a probe for growth rate evaluation (Tsoukatou *et al.*, 2002).

In order to determine whether the AF compounds killed the algae or merely inhibited their growth, complementary experiments were carried out. Flasks containing 5×10^5 cells/ml were incubated with compounds at concentrations which stopped microalgal growth, as previously determined.

After 5 d incubation, the cells were rinsed and maintained in plain medium for further 7 d and the cell number was assessed daily.

When a limited amount of compounds are available, this assay can be run using 96 well plates (Tsoukatou *et al.*, 2007). After incubation, results are read using a fluorometer plate reader (200 nm).

12.3.2 Settlement and adhesion strength of diatoms

In a method developed by Statz *et al.* (2006), *Navicula* sp. was cultured in F2 medium for 3 d until log-phase growth was achieved. Cells were washed in fresh medium before harvesting and diluting to give a suspension with a chlorophyll *a* content of approximately 0.3 mg/ml. Cells were settled on 6 slides of each treatment in individual dishes containing 10 ml of suspension. Cells settle in the water column by gravity, thus a comparable number of cells will settle on all surfaces. After 2 h the slides were gently washed in seawater to remove cells that had not been properly attached. Three slides of each treatment were fixed in 2.5% glutaraldehyde in seawater, desalted by washing in 50:50 seawater:distilled water, followed by distilled water and dried before counting. The density of cells attached to the surface was counted on each of three replicate slides using a fluorescent microscope. On each slide, 30 fields of view (0.17 mm²) taken at 1 mm intervals along the centre were counted to provide cell attachment data.

The remaining slides were used to evaluate the strength of diatom attachment. This was achieved by exposure to a shear stress of 20 Pa in a specially designed water channel that has been modified by Finlay *et al.* (2002a). After exposure to flow, the slides were fixed in glutaraldehyde and processed for counting as described above. The number of cells with AF properties of bio-inspired polymer coated surfaces remaining attached was compared with unexposed control slides to determine the percentage removal under flow.

12.4 Anti-fungal assays

Fungi are major constituents of marine biofilms and have been linked with biodeterioration of wood structures (Kohlmeyer *et al.*, 1995). Their importance in marine biofouling has been for a long time under-estimated. Two bioassays are used to test the antifungal potency of AF compounds: a) agar-based bioassay and b) a broth-based bioassay.

12.4.1 a) Agar-based bioassay

Since many fungal isolates do not sporulate at culture conditions, common antimicrobial assays, such as the determination of MIC and the standard

disc diffusion assay, are possibly not suitable (Koh *et al.*, 2002). Compounds to tests can be diluted in DMSO (5%), filtered and then incorporated into maize meal agar (12%), pH 6 (Sigma) (Hellio *et al.*, 2000a). Following closely the method described by Holmstrøm *et al.* (2002) in a modified assay by Hadacek and Greger (2000) inoculate targeted fungal strains in the middle of an agar plate and then inoculate the bacterial test strain around the fungal colony. This method is much easier to manipulate and avoids contamination. After incubation (1 week at 25 °C), the activity was evaluated by measuring the diameter (mm) of the fungal colonies.

12.4.2 b) Broth-based bioassay

Arikan *et al.* (2002) performed microdilution tests in accordance with the NCCLS guidelines for conidium-forming filamentous fungi (NCCLS, 1998). Antibiotic medium 3 was used as the test medium. Serial two-fold dilutions of compounds to test over a range of 16 to 0.05 g/mL were prepared in microdilution plates. The results were read after several incubation periods by using two different parameters: the visual MIC of compound that provided a *ca.* 50% reduction in growth compared to the growth in the control well and the microscopic MEC that results in the formation of abnormal hyphal growth with short, abundant branchings.

12.5 Macrofouling

Most sessile marine organisms produce planktonic propagules referred to as larvae for invertebrates and spores for algae. The planktonic phase lasts for minutes, hours, days, weeks, or even months while the propagules grow, develop, and drift for various distances in the water column before they settle in new habitats (Levin, 2006). The settlement process is characterized by habitat exploration, which is often followed by a cascade of ontogenetic events and the planktonic existence is terminated when propagules finally attach to substrates.

Settlement and metamorphosis are sequential critical events in the life cycle of marine macroorganisms. These are also crucial events in the fouling process. Although various physical factors have been documented that regulate invertebrate larval settlement and metamorphosis or algal zoospore attachment, chemical cues are believed to be most important in the mediation of fouling by macroorganisms (Qian *et al.*, 2003). A variety of chemical compounds have been proposed as larval settlement cues (Hadfield and Paul, 2001). However, only a few cueing substances have been isolated from natural habitats or natural biofilms and tested in the field.

12.5.1 Macroalgal assays

Macroalgal AF assays particularly include those of their spores and zygotes, their behavior, attachment, germination and growth. Members of Rhodophyta, Heterokontophyta, Chlorophyta and Chlorarachniophyta produce spores which affect human activities by settling on artificial surfaces and causing biofouling that is a menace on many technical surfaces. Algae and their spores exhibit enormous diversity (Callow *et al.*, 2000, see chapter 4). Several environmental factors may induce spore formation, such as photoperiod and temperature. Enormous numbers of spores can be produced, e.g. 5.3×10^5 zoospores per *Ulva linza* per plant and per day (Maggs and Callow, 2002). For more details, see the chapter on algae (Chapter 4).

Inhibition of the attachment of spores and zygotes of macroalgae

An attachment inhibition assay can be carried out using various species of macroalgae, such as *Ulva intestinalis*, *Sargassum muticum*, *Polysiphonia lanosa* and *Undaria pinnatifida* (Fletcher and Callow, 1992; Finlay *et al.*, 2002b; Hellio *et al.*, 2002; Plouguerné *et al.*, 2008). Plastic Petri dishes (35 mm in diameter) are used throughout the experiment as the substrate for settlement of spores (Hattori and Shizuri, 1996). Compounds to test were dissolved in methanol and spread on the inner surface of a Petri dish and dried at room temperature. Each Petri dish containing 5 mL of F₂ medium was inoculated with approximately 3000 spores. Dishes were placed in the dark for 2 h to allow for even settlement of gametes. Biocides were used as positive controls. After incubation for 5 days at 20°C with 24 hours light ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$ white fluorescent lamps), the attached and unattached spores were counted on 1 cm² areas of each Petri dish using an inverted binocular microscope. The attachment rate was calculated (Hattori and Shizuri, 1996). All experiments were replicated three times.

Settlement and adhesion strength of Ulva sp.

In an assay by Chaudhury *et al.* (2005), 10 ml of a zoospore suspension containing 16,106 spores ml⁻¹ were added to individual compartments of Quadriperm dishes, each containing one slide. The slides were incubated in darkness for 1 h, and then gently washed in seawater to remove zoospores that were still motile and unattached. The density of zoospores attached was counted on each of three replicate slides using an image analysis system attached to a fluorescent microscope.

Slides settled with zoospores for 1 h were subsequently exposed to a shear stress of 53 Pa in the water channel. The number of spores remaining

attached was compared with unexposed control slides. Sporelings were cultured in enriched seawater medium in individual (10 ml) wells in polystyrene dishes under illuminated conditions inside a growth cabinet at 18°C with a 16:8 light:dark cycle (photon flux density 330 μmol). The medium was refreshed every 2 d. After an 8 d culture period, the sporeling biomass on half of each slide was removed by scraping with a razor blade, and the chlorophyll was extracted in dimethyl sulphoxide. The amount of chl *a* present was determined spectrophotometrically using the equations of Jeffrey and Humphrey (1975). The remaining half slides of biomass (from above) were exposed to a shear stress of 53 Pa in the water channel as for the spore test. The biomass remaining in the samples was analysed for chl *a* content as described above.

Monitoring brown algal spore swimming behavior

Iken *et al.* (2003) monitored the swimming behavior of zoospores of the brown alga *Hincksia irregularis* to develop a new laboratory AF bioassay. Computer-assisted motion analysis was used to distinguish between the straight and fast swimming movements of undisturbed spores (controls) and the helical and erratic swimming patterns of chemically irritated spores, using the quantitative parameters: rate of direction change (RCD) and swimming speed (SPD). The ratio RCD/SPD of spore swimming paths at AF compound treatments compared to controls is used to quantify the detrimental effect of echinoderm extracts. Comparative studies on spore settlement and germination under similar treatment conditions show that changes in spore swimming behavior reflect decreased fitness and survivorship of algal spores. This bioassay can be used to screen potential AF compounds at very low concentrations, making this assay particularly suitable for detection of concentration dependent effects.

12.5.2 Bioassays on invertebrates

Invertebrate larval fouling bioassays include larval swimming, behavior, surface investigation responses in flow and attachment assays. The power of laboratory assays is in the rapid and highly sensitive screening of potential AF compound effectiveness.

There are different types of assays like full dish *versus* half dish assays, still water *versus* assays in flow through systems. To use competent larvae in assay wells of settlement assays is an essential prerequisite for appropriate testing and the interpretation of results (Rittschoff *et al.*, 1992). Available larval bioassays provide different types of information. LC50 (lethal compound concentration where 50% of the targeted organisms die within a given period of time: 24 h or 72 h) are data that are used to identify toxic

compounds. Toxic effects are not differentiated from narcotic compounds. When this is of interest, organisms at the lowest concentration showing a '100% dead' result are placed in seawater to see if they can recover. EC50 data quantify biological effects – so by settlement inhibition in AF screening tests. This is the most sensitive point in time during the assay and enables identification of inhibitory compounds (Tsoukatou *et al.*, 2007). The therapeutic ratio, LC50/EC50 is a way of expressing the effectiveness of the compound in relation to its toxicity. From the perspective of potency for use in an AF coating, the desired target ratio should be much greater than 1.0 (Rittschof *et al.*, 2003). Compounds of low or negligible toxicity that still inhibit settlement, represent a more environmentally acceptable solution for inhibition.

In laboratory assays where multiple batches of larvae are utilized, non-toxic antifoulants appear to be more variable than toxic agents in their ability to inhibit settlement of invertebrate larvae (Clare, 1998). This is reasonable, given the potential for variability based on genetic differences, physiological conditions, and sensory perception between larval batches.

Target taxa for larval fouling assays are particularly: Cirripedia, Bryozoa, Teredinidae, Polychaeta and Bivalvia. They are mostly characterized by rapid larval development, the ease of raising synchronous mass cultures, and the predictable settlement at static conditions (see Rittschof *et al.*, 1992). Particular examples are provided here.

Cirripedia

Barnacles are dominant fouling organisms worldwide and have been most studied target organisms. Some species turned out to be easy to raise under laboratory conditions (*Balanus amphitrite*, for example) or larvae can be easily collected from the field (*Balanus improvisus* and *Semibalanus balanoides*), providing suitable experimental models for AF studies. Settlement bioassays are widely used tools for the screening of AF substances.

a) Bioassays using B. amphitrite

Adult barnacles of *B. amphitrite* can be collected from the coast and be maintained at 22°C in an aerated aquarium and fed on a daily diet of *Artemia* sp. nauplii (7 nauplii/ml) (Hellio *et al.*, 2004). Induction of spawning can be performed using two methods: a) by changing the water and providing light-shock (Qiu and Qian, 1997) or b) by removing them from the water for 12 h (Hellio *et al.*, 2004). The non-feeding nauplius I moulted into nauplius II within 3 h. Planktotrophic nauplius II develop through planktotrophic nauplius III, IV, V, and VI to a lecithotrophic cypris stage. Newly moulted nauplius II larvae are attracted to a point-source light and

collected using a pipette under a dissecting microscope. The diatom *Skeletonema costatum* was fed to the nauplii at late log growth phase at concentrations of about 2×10^6 cells ml^{-1} . Crystamycin (22.5 mg l^{-1} Penicillin G and 37.5 mg l^{-1} Streptomycin sulfate) was added to inhibit bacterial growth. The culture was maintained at a photoperiod of 12:12 h light:dark. After 4 days, most of the larvae had metamorphosed, cyprids were collected by filtration (250 μm) and allowed to age at 6°C for 3 days, before being used for the settlement experiments (Maréchal *et al.*, 2004). The settlement of barnacle larvae in laboratory assays is highly variable and the variation has been ascribed to different putative factors affecting larval performance, such as physiological condition (Miron *et al.*, 2000) or inherent genetic variation (Holm *et al.*, 2000). As yet, there is no uniform account for the variability in settlement assays. The gregarious settlement of most balanomorph barnacles has raised the question whether this behavior influences the outcome of assay experiments, and alternative settlement assays have been proposed (Kawahara *et al.*, 1999) where only one larva is used per test container. However, if experimental assays include adequate controls, this should be a minor problem when working with inhibitory substances. Another concern is the age of the larvae used in settlement assays. Newly hatched cyprids (day 0) are much less prone to settle than day 4 larvae. In the classic procedure for AF assays, settlement tests were conducted by adding 10 cyprids to each well of an Iwaki microplate (24 wells) filled with 2 ml of a solution containing the AF compounds to test in filtered seawater (0.45 μm) (Hellio *et al.*, 2005). Experiments are performed in 6 replicates and using 2 batches of larvae. Test plates were incubated in the dark at 28°C and results were recorded after 24 h. The physical state of each larva was examined under a dissecting microscope. Cyprids with extended thoracopods that did not move and did not respond after a light touch by a metal probe were regarded as dead (Lau and Qian, 2000; Rittschof *et al.*, 1992). Permanently attached and metamorphosed individuals were counted as settled. All others were counted as swimmers. For each extract, EC50 value (concentration of extract which results in a 50% inhibition of settlement compared with the seawater control) was calculated.

b) Bioassays using *B. improvisus* and *S. balanoides*

Balanus improvisus and *Semibalanus balanoides* cyprids can be collected from the wild using a plankton net. The cyprids should be kept for 24 h at 14°C and fed with *Skeletonema costatum* before being used for settlement experiments. Settlement assays can be carried out in 24-well Iwaki microplates (5 cyprids per well containing 2 ml of the extracts in 0.45 μm -filtered seawater). Six replicates for each AF compound concentration are performed. Test plates were incubated in the dark at 14°C and results were

recorded after 48 h. For each extract, EC50 value, percentage of attached cyprids, swimmers and dead were calculated as above.

A more qualitative approach can be used where effects on behavior can be evaluated (Yamamoto *et al.*, 1999), however, behavioral effects are probably best studied with video monitoring as has been described in several cases (Hills *et al.*, 1999; Berntsson *et al.*, 2000; Maréchal *et al.*, 2004).

Bryozoa

More than 100 of about 5000 bryozoan species are known worldwide that grow on ship hulls, causing drag and thus reducing efficiency and maneuverability of ships (Burgess *et al.*, 2003). Bryozoans also grow on other man-made structures like piers, pipelines, and docks (Wahl, 1989).

Broodstocks of adult bryozoa can be collected by hand, by wading or snorkelling at low tide, from exposed bedrock, boulders, jetties, and mooring ropes and from the lower stipes and holdfasts of macroalgae. Bryozoa are reported to be voracious feeders and colonies maintained in open systems are dependent for food on plankton, particularly nanoplankton brought in through intake pipes if they are not provided as supplements. Bryozoans feed on microorganisms, including diatoms and other protists.

During the period of larval release, larvae increase in volume approximately 500-fold, and any change in maternal resources has the potential to influence the provisioning of those offspring whilst being brooded (Marshall and Keough, 2004). Larvae eventually settle on suitable substrata and metamorphose into new sessile bryozoans (or ancestrulae). Unlike most other marine invertebrate larvae, non-feeding larvae of *B. neritina* have a brief pelagic phase that lasts for minutes to a few hours at most (Wendt, 2000).

For routine bioassay surveys, larvae were obtained according to Bryan *et al.* (1997, 1998) and Dahms *et al.* (2004a,b). Only newly (within 30 min) released larvae were used for larval settlement bioassays. To collect larvae for the experimental treatments, the colonial material from the experimental setups was placed inside glass aquaria that were filled with 4.5 L of filtered seawater (FSW). These aquaria were placed in parallel, with one corner exposed to a strong artificial light beam. Due to the positive phototactic behavior of the bryozoan larvae, these swam towards the light source and were collected from the illuminated corner of the aquarium by pipetting, not later than 30 min after broodstock exposure to light. Thereafter, larvae were enumerated under a dissecting microscope and/or subjected to larval settlement assays. The number of settled larvae was enumerated after

24 h (or after 1 h and 24 h in experiment 5), as the counts of attached and metamorphosed juveniles.

Teredinidae

Wooden structures in temperate and tropical seawater are subject to severe biodegradation by isopod crustaceans (*Limnoriidae* and *Sphaeromatidae*). An experimental approach to assess feeding rates of *Limnoria* by measuring faecal pellet production has been recently developed by Borges *et al.* (2008). This method has the advantage of monitoring individuals and then avoiding the confounding factor of population variation. Moreover, the short exposure period and ease of replication enables rapid production of statistically robust measures of AF activity against *Limnoria*.

Limnoria can be maintained in culture in blocks of the pine *Pinus sylvestris* immersed in running seawater under laboratory conditions. For the experiment, blocks were transferred to a tank with seawater kept at $20 \pm 2^\circ\text{C}$ to allow acclimation during one week, before extraction for experimentation. Wood sticks (20 mm $\times$ 2.5 mm $\times$ 4 mm) (10 replicates) can be impregnated with AF compounds to test and placed one per well in cell culture boxes with 12 wells measuring 20 mm in diameter. One animal was placed into seawater (32%) in each well.

Experiments can be run in three different situations:

- a) *static seawater* – with 4 ml of filtered seawater per well, which was replaced every third day to avoid the build up of leachate from the test wood stick;
- b) *leachate* – with 4 ml of seawater leachate from a test wood species replaced every third day;
- c) *running seawater* – cell culture boxes with 5 mm diameter holes drilled in the lids above each well were immersed in a tank of slowly running seawater; fecal pellets and animals were retained by a fine mesh placed over the top of the wells.

The cell culture boxes with animals, seawater and sticks were kept in the laboratory under fluorescent light ($19.9 \mu\text{mol s}^{-1} \text{m}^{-2}$) at $20 \pm 2^\circ\text{C}$. After 3 days, 1 week and 2 weeks, the numbers of fecal pellets produced by the animals were counted. A record was kept of moulting or dead animals. The lengths of experimental animals were measured to the nearest 0.1 mm at the end of the experiments with a dissecting microscope. The width and length of ten randomly selected fecal pellets in each of 3 wells per wood species were measured by means of a microscope eyepiece graticule. The volume of each pellet was calculated with the volume formula = $0.75 \pi ((\text{width}/2)^2) \times \text{length}$. Pellets can be fixed in 0.2 M cacodylate-buffered

3% glutaraldehyde then dehydrated through a series of aqueous ethanol solutions before transferring to hexamethyldisilazane and drying by evaporation before being transferred to adhesive carbon mounts, sputter coated with gold for SEM examination.

Polychaeta

Polychaete larvae show a considerable diversity in form, developmental patterns, behavior, nutritional characteristics, and ecology. Metamorphosis includes the loss of larva-specific organs and the development of juvenile or adult-specific organs (Giangrande, 1997; Qian and Dahms, 2005).

The serpulid polychaete *Hydroides elegans* provides a good example. Bryan *et al.* (1997) obtained *H. elegans* from submerged rafts of a fish farm in Hong Kong. They placed individual females and males in sterile Petri dishes containing 20 ml of 0.22 μm FSW. Tubes of adult *H. elegans* were broken and if gametes were released after a few minutes, eggs (pink in color) and sperm (milky white) were put together from 2 or 3 individuals of each gender and agitated. Usually, more than 95% of eggs were fertilized and developed. Excess sperm was removed by filtration and the eggs were transferred to a 4 L Nalgene beaker containing FSW. Larvae were fed *Isochrysis galbana* at 1×10^5 cells ml^{-1} for 6 to 7 d until they reached a competent state to settle and metamorphose. Competent larvae had elongated tails and the ciliary ring became reduced and migrated close to the head. Cultures were maintained at 24°C at a 15:9 h light:dark photoperiod. Culture beakers were aerated at a rate of 2 bubbles s^{-1} . Larvae were fed every 2 d. Water was changed on days 3 and 5 of the larval culture period.

Settlement experiments were performed on larvae that were 6 to 7 d post-fertilization. Approximately 20 larvae were placed in Petri dishes (5 cm diameter), containing 5 ml of FSW, and incubated at 24°C at a 15:9 h light:dark photoperiod. The status of larvae was monitored 2 and 4 d after initiation of the assay. Larvae attached to the dish, with a tube and tentacles were considered to have undergone normal metamorphosis. Several types of abnormal metamorphosis were classified: a) attached, with tentacles but no tube; b) not attached, with tentacles but no tube; c) deformed development involving elongation of larvae and crawling behavior, but no tube or tentacle developed.

Bivalvia

Mussels are among the major fouling macroorganisms which cause serious problems by settling on man-made surfaces. They attach to the substratum by means of adhesive plaques connected to a stem of byssus. These

plaques are thought to be produced by the action of a phenoloxidase on a protein precursor by oxidising phenols to catechols and catechols to o-quinones from covalent bonding with amines. The plaque adheres to a variety of substrata including rocky surfaces, slates, iron or polystyrene and even on Teflon plates in seawater.

The antifouling effect on mussel often is determined by recording a) the attachment ability of juveniles or b) by measuring the effects of AF compounds on the activity of the phenoloxidase (enzyme involved in the synthesis of the byssus).

a) *Attachment ability of juveniles of Perna perna*

Da Gama *et al.* (2003) set-up a bioassay using juveniles of *Perna perna*. Juveniles were collected during low tide from the field and kept in a 230 L recirculating laboratory aquarium at 20 °C, salinity of 35‰ and aeration for 12 h. Individuals (ranging from 2.0 to 3.0 cm) exhibiting substrate exploring behavior (actively exposing their foot and crawling) were selected. Water-resistant filter paper was cut into 9 cm diameter circles and soaked in solvent (control filter) or in a solution containing the AF compounds to test. A positive control consisted of a filter impregnated with a 15 mM solution of CuSO₄. After air drying, the entire filter circles were then placed in the bottom of sterile polystyrene Petri dishes. Dishes were filled with 8 ml of seawater and three mussel specimens were added. Ten replicates of each treatment were used and were incubated in total darkness for 12 h. Mussel activities (substratum exploring behavior, gamete release and number of byssal threads attached to each substratum) were recorded immediately after the start of the experiment, after 2 h, and after 12 h. After the 12 h period, mussels were placed in a plastic mesh and suspended in a seawater aquarium for 24 h to check for possible mortality due to exposure to the test substances.

b) *Assay of the inhibition of the phenoloxidase activity of Mytilus edulis*

The previous method may have the inconvenience of requiring a large quantity of specimens for testing and being time consuming. A study by Hellio *et al.* (2000b) showed that AF activity can be correlated with a high level of inhibition of the phenoloxidase activity (enzyme involved in the byssus formation). This new assay technique led to a similar result as the previous methods in use (plate assay and foot retraction) (Vanelle and Le Gal, 1995; Hayashi and Miki, 1996) with the advantage of being rapid, repetitive and using only little amounts of AF products. These characteristics make it suitable for screening naturally occurring AF compounds. The assay can be performed using commercial tyrosinase or

phenoloxidase purified from *M. edulis* (Hellio *et al.*, 2000b). The enzymatic activity is measured spectrophotometrically at 475 nm in the presence of L-Dopa as a substrate (Pomerantz, 1963).

12.6 Toxicity testing

After the definitive ban of tin-based AF substances, new organic compounds have recently been introduced in AF paint formulations, as either principal or booster biocides. Toxicity bioassays are needed to get information on the toxicological potential of AF chemicals. Alternative compounds should be as effective as conventional paints but of lower toxicity. Zinc pyrithione (ZPT), Copper pyrithione (CPT), Chlorothalonil and Diuron are four of the most widely used AF biocides in boat paints as alternatives to tributyltin (TBT). In most cases, previous risk assessment of these biocides has been inadequate so that their possible effects on aquatic ecosystems is a matter of great concern since most previous laboratory bioassays for these biocides have been conducted solely based on acute tests with a single compound; information on the possible combined toxicity of these common biocides to marine organisms are limited. Observed synergistic interactions underline the requirement to review water quality guidelines, which are likely underestimating the adverse combined effects of these chemicals.

Toxicity tests of AF compounds are usually performed towards a wide range of organisms: 1) microlagae, 2) sea urchins, 3) *Artemia* sp., 4) oysters, 5) barnacles, 6) mussels, 7) ascidians, 8) fish and 9) cells of mammals.

12.6.1 Toxicity tests towards microalgae

Gatidou and Thomaidis (2007) set up an experiment where 2 planktonic algae, *Dunaliella tertiolecta* and *Navicula forcipata*, were exposed during 96 h to various concentrations of single and binary mixtures of AF booster biocides and their metabolites. Estimations of EC₅₀ values were obtained by counting cell numbers of the tested microorganisms daily. According to OECD (1981) at least 5 concentrations should be tested in order to determine the concentration range in which the effects of a compound will occur. The highest concentration should completely inhibit the growth (or at least at a 50% level) and the lowest concentration should result in no effect compared to the control. The 96-h EC₅₀ values in the different bioassays were calculated. Interaction and combined effects of chemicals resulted in

synergism in most cases, for both species of phytoplankton. Antagonistic effects were observed owing to the joint action of copper with either diuron or one of its metabolites.

12.6.2 Toxicity tests towards sea urchins

Sea urchins can be collected by scuba-diving and kept in the laboratory. Toxicity tests for AF compounds can be performed according to the methods described by Hellio *et al.* (2000a) and Fernandez and Beiras (2001). Individuals were dissected and sperm and eggs directly pipetted from the gonads. Eggs from a female with optimal condition were collected in a 100 ml measuring cylinder containing natural seawater filtered through a 0.2 μm pore filter. A few microliters of undiluted sperm from one male were added to the egg suspension and carefully stirred to allow fertilization. Fertilization success and egg density were determined by counting the fertilized eggs in four 10 μl aliquots.

Fertilized eggs were exposed to AF compounds in 24-well plates, for a 48 h period and at 20°C. After incubation, the solutions were fixed with 40% formalin. Three replicates of each experimental treatment were done. Embryogenesis success was evaluated by measuring the percentage of pluteus larvae and larval length.

12.6.3 Toxicity tests with *Artemia*

The brineshrimp *Artemia* can be used to evaluate the toxicity of AF formulations. Koutsaftis & Aoyama (2007) evaluated the toxicity of binary, ternary and quaternary mixtures using the brine shrimp *Artemia* sp. as a test organism. Mixture toxicities were studied using a concentration addition model (isobolograms and toxic unit summation), and the mixture toxicity index (MTI). The different types of combined effects (interactive and synergistic) subsequent to proportion variations of binary mixtures underline the importance of the combined toxicity characterization for several concentration ratios.

Two different types of test containers have been used by Castritsi-Catharios *et al.* (2007): 50 ml modified syringes and 7 ml multiwells. The results of the toxicity experiments follow a normal distribution around an average value which allows one to consider these values as reliable for comparison of the level of toxic effects detected with the two types of test containers. The mean lethal concentration L(S/V) 50 in the test series conducted in the multiwells did not differ significantly from that obtained in the test series using modified syringes.

12.6.4 Toxicity tests towards oysters

The bioassay of AF compounds on oysters followed the method reported by His (1997) and Hellio *et al.* (2000a). Mature adults of *Crassostrea gigas* were induced to spawn by thermal stimulation (alternating immersion in seawater of 15°C and 28°C for 30 min each time). Spawning males and females were individually isolated in beakers with 0.2 µm natural filtered seawater. The oocytes and sperm of different oysters were observed under an inverted microscope, and the best reproductive pair (regular oocytes and very mobile spermatozoa) was selected for the experiment. Oocytes and sperm solutions, respectively, were sieved through a 100 µm and a 32 µm mesh to remove debris. The oocytes were fertilized using a few milliliters of the sperm-dense solution. Fifteen minutes after fertilization, the embryos were counted and placed in 30 mL transparent polypropylene vessels filled with the different media to be tested (1000 eggs; three replicates per treatment). The embryos were incubated at 24°C for 22 h until D-larvae stages were obtained. After incubation, 0.5 mL of 8% buffered formalin was added to each vessel, and abnormalities were determined by direct observation of 100 individuals (chosen at random from the 1000 in each vessel). According to His (1997), the categories of abnormal larvae included: segmented eggs, normal or malformed embryos that had not reached the D-larval stage, and D-larvae with shell abnormalities (convex hinge, indented shell margins, incomplete shell) or protruded mantle.

12.6.5 Toxicity tests towards barnacles

Toxicity tests were conducted according to Hellio *et al.* (2004). Briefly, stage I-II nauplii, actively swimming towards a light source, were collected by pipette. For the test, 10–15 nauplii were added to 2 mL solution in the wells of a 24-well (Iwaki) plate. Compounds dissolved in seawater (and prepared from the same stocks as for the settlement assays) were tested at the same concentrations as for the settlement assays, with six replicates of each treatment and the control (filtered seawater). The number of swimming, and dead nauplii was recorded after 24 and 48 h exposure to the compounds. For the purposes of the analysis, non-swimming larvae were regarded as dead (Rittschof *et al.*, 1992) and the data are expressed as a 24 or 48 h LC₅₀ with a 95% confidence interval. The LC₅₀ was determined using Sigma Plot 8.0.

12.6.6 Toxicity tests towards mussels

The AF toxicity bioassay towards mussels were based on the methods described by His (1997). *Mytilus edulis* were collected at low tide and

cleaned and scrubbed in natural filtered seawater. Spawning was induced by raising the temperature to 22 °C. Freshly collected oocytes and sperm were suspended in a 100 ml measuring cylinder containing natural seawater filtered through a 0.2 µm pore filter to achieve fertilization, facilitated by gentle stirring. The density of fertilized eggs was assessed by counting the fertilized eggs in four 10 µl aliquots. Some 30 min-old fertilized eggs were incubated in the test solutions at a concentration of 20 eggs/ml. Incubation was conducted for 48 h at 20 °C. Results were recorded as percentage of embryos that reached the D-larva stage.

12.6.7 Toxicity tests towards ascidians

Cimaa *et al.* (2008) studied the effects of 2 new organic biocides often associated in paint formulations, Sea-Nine 211TM (4,5 dichloro-2-*n*-octyl-4-isothiazoline-3-one) and chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), on haemocytes of the compound ascidian *Botryllus schlosseri* exposed for 60 min to various concentrations (from 0.1 to 10 µM) of these xenobiotics. This species had previously proved to be a good bioindicator of organotin compounds. Results indicate that short-term *in vitro* exposure of haemocytes to high concentrations of Sea-Nine 211TM and chlorothalonil provokes a marked reduction in haemocyte functionality, higher than or comparable to that of TBT. These assays of acute toxicity stress the immunosuppressive potential of these compounds, which, although counterbalanced by their short half-life in the marine environment, can lead to biocoenosis dismantling through rapid bioaccumulation by filter-feeding non-target benthic organisms.

12.6.8 Toxicity tests towards fish

A rapid straightforward toxicity test was developed by Okamura *et al.* (2002) using the suspension-cultured fish cell line CHSE-sp derived from Chinook salmon *Oncorhynchus tshawytscha* embryos in order to assess the toxicity of new marine AF compounds. The *in vitro* acute toxicity (24-h EC50) of compounds to these fish cells was evaluated using the dye Alamar Blue to determine cell viability, which was then correlated with the results of *in vivo* chronic toxicities (28-day LC50) to juvenile rainbow trout *Oncorhynchus mykiss*. The suspension-cultured fish cells were found to be suitable for preliminary testing before performing an *in vivo* test.

Tiano *et al.* (2003) investigated the mitochondrial toxicity and proapoptotic activity of tributyltin chloride (TBTC) in teleost leukocytes and nucleated erythrocytes, by means of electron microscopy and mitochondrial membrane potential evaluation. Leukocytes and erythrocytes were obtained from an inbred strain (*O. mykiss*). Transmission

electronic micrographs of trout red blood cells (RBC) incubated in the presence of TBTC for 60 min showed remarkable mitochondrial morphological changes. TBTC-mediated toxicity involved alterations of the cristae ultrastructure and mitochondrial swelling, in a dose-dependent manner. Both erythrocytes and leukocytes displayed a consistent drop in mitochondrial membrane potential following TBTC exposure at concentrations >1 AM. The proapoptotic effect of TBTC on fish blood cells, and involvement of mitochondrial pathways was also investigated by verifying the release of cytochrome c, activation of caspase-3 and the presence of 'DNA laddering'. Although mitochondrial activity was stronger affected in erythrocytes, leukocytes incubated in the presence of TBTC showed the characteristic features of apoptosis after only 1 h of incubation. Longer exposures of up to 12 h were required to trigger an apoptotic response in erythrocytes.

Grinwis *et al.* (1998) exposed flounders (*Platichthys flesus*) to bis(tri-*n* butyltin)oxide (TBTO). The effects on several organs (gills, skin, eye, liver, mesonephros, ovary/testis, spleen, and gastrointestinal tract) were examined using histopathology, and morphometric analysis of the thymus to assess the target organ(s) for TBTO in this fish species. Also, the function of the non-specific and specific resistance was studied using *ex vivo:in vitro* immune function tests.

12.6.9 Toxicity tests towards cells of mammals

De Sousa *et al.* (1998) compared the toxicity of several currently used paint lixiviats in rat hepatocytes, human HepG2 and HaCaT cells. Acute toxicity was assessed by the Neutral Red and MTT assays. Chronic effects were tested using induction of the 7-ethoxyresorufin-*O*-deethylase (EROD) activity as a marker. Large variations were observed among the various cell types or the AF formulations, both in terms of LC50 values (from 0.5 to 10%, v:v) and EROD induction (from 1 to 10-fold over control). These differences appear to be related to variable biocide (copper compounds, organotins, etc.) concentrations in the different paint formulations, or to the specific metabolic capabilities of the cell system used.

12.7 Conclusions

Bioassays should consider that fouling can either be facilitated or inhibited by environmental cues, with the response often specific to the cue and the fouling organism investigated. If either the recipient is not motivated to settle or the surface does not provide appropriate physical or chemical cues, invertebrate larvae can arrest developmentally, for example, and remain in the water column for extended periods of time (Pechenik 1999). Hence,

bioassays in fouling studies have to consider that fouling also depends on the perceptive potential and the motivation of colonizing organisms.

Bioactive substances are not isolated from their physical, chemical and biological environment. All aspects of biofouling should, therefore, be better approached holistically, considering various levels of integration, particularly in the design of bioassays. Recent studies showed how chemically mediated interactions themselves are affected by physical factors such as water movements, nutrient availability, electromagnetic radiation, or, by biological factors (e.g., microbial symbionts, microbial infections, fouling organisms, interactions with neighbors, competition, predation, or parasitism). Badly neglected are studies on ontogenetic susceptibility shifts. Considering the possibility of multifunctional effects of bioactive substances interacting with fouling processes, compounds should be tested against several different naturally co-occurring organisms. The use of field assays in addition to laboratory assays will be indispensable at a final stage in order to determine how effective (specific or broad) bioactive substances are in the targeted context and whether they are environmentally acceptable.

12.8 References and further reading

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Key issues in the formulation of marine antifouling paints

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Abstract: Even though an overwhelming percent of antifouling solutions used worldwide are commercialized in the form of coatings, it is indeed likely that many readers, somehow related to antifouling (AF) research, are not familiar with basic paint technology concepts. This may become a serious problem because, at some point, the success of their research will inevitably require scaling-up their lab experiments to an actual coating capable of withstanding extensive long-term field testing. The aim of this chapter is to provide part of the theoretical basis on paint technology to apply new AF concepts in the most efficient manner. In other words, no single promising AF concept should be abandoned because of unsatisfactory field results attributed to an improper paint formulation, fabrication or application.

Key words: critical pigment volume concentration, paint ingredients, pigment dispersion, mechanical paint test methods, exposure paint test methods.

13.1 Introduction

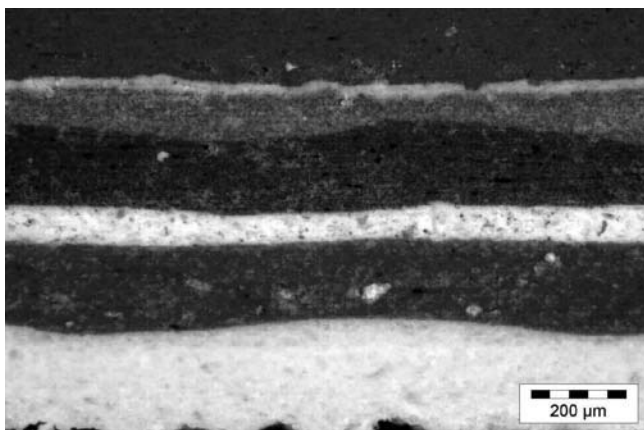
The *Encyclopaedia Britannica* defines the term *paint* as ‘decorative and protective coating commonly applied to rigid surfaces as a liquid consisting of a pigment suspended in a vehicle, or binder. The vehicle, usually a resin dissolved in a solvent, dries to a tough film, binding the pigment to the surface’. This definition outlines the two-fold purpose of paints: solution of aesthetic or protective problems, or both. In the case of antifouling (AF) paints, which spend most of their service life underwater, it is clear that the aesthetic aspect is secondary, except for, perhaps, new ships and yachts. Once in service, AF paints are one of the few paint products in which a large percentage of their ingredients are expected to dissolve away or degrade during service (except for fouling release products; see Chapter 26). The latter, together with the high risk of suffering mechanical damages and the ubiquitous presence of marine biofilms (Chapter 17), ends up in fairly ‘ugly’ paint surfaces under service.

Painting a vessel's underwater hull serves two main purposes:

- protection of the construction steel
- prevention of undue hull roughness.

This book mainly deals with this second point, i.e., prevention of fouling settlement and its subsequent hydrodynamic penalty. Regarding corrosion prevention in ship hulls, it is important to stress that salt-water immersion or partial immersion with spray followed by drying winds are extremely aggressive environments (C5M for non-immersed areas and Im2 for the ship hull according to ISO 12944). Antifouling paints are, by definition, fairly water permeable and most of them experience an important thickness loss during service. Their barrier properties are, thus, very poor. Furthermore, the use of Cu_2O entails the risk of galvanic corrosion. Hence, corrosion protection of the steel hull requires the use of a full paint system, an example of which is presented in Fig. 13.1.

The anticorrosive (AC) system basically relies on creating a physical barrier that keeps out ions and retards the penetration of water and oxygen. A well-adhering, highly impermeable anticorrosive barrier coat or primer is the first coat to be applied on the steel substrate. In the case of new build-ings, a shopprimer may, however, partly precede such a coat. A shopprimer is a thin anti corrosive coat (15–30 μm) which provides a temporary protec-tion to the steel plates during ship building. In the final hull surface, a barrier effect is obtained by applying thick coatings of paints with very low water permeability. These paints are nowadays usually based on epoxy binders and they are typically applied in 2 or even 3 turns to obtain a final thickness



13.1 An example of an old paint system used on the underwater hull of a ship. Usually the system applied to a new ship consists of 5–6 layers (2 AC coats, 1 link coat, 2–3 AF coats).

of 300–500 μm . The choice and amount of pigments and fillers significantly contribute to the barrier properties. However, if a barrier-type anticorrosive coating is damaged, the area of damage lies open for corrosion, which can proceed both down into the steel and outwards under the intact coating (under rusting or rust creep). Thus, where there is a risk of mechanical damage, additional protection, in the form of cathodic protection is often provided. Cathodic protection basically consists of assuring that the metallic substrate to be protected behaves at all times as the cathode of the corrosion cell. The latter can be done either by connecting the hull electrically with less noble materials, which become the anode (e.g., zinc-rich paints and sacrificial anodes), or by connecting the steel to an external power supply which ‘saturates’ the metal with electrons (i.e., it is polarized) and hinders the metal oxidation (impressed currents). Because of the risk of osmotic blistering and their poor mechanical properties, zinc-rich paints must be used with caution as cathodic protection in continuously immersed areas.

In order to secure a proper adhesion between the antifouling topcoats and the epoxy undercoat, even after relatively long recoating intervals (i.e., days), a so-called tie-(or link-)-coat usually precedes the antifouling system. The tie-coat is typically based on a technology with properties which are compatible with both the chemically curing epoxy films and the physically drying antifouling topcoats (see later on for further explanations). The need for adhesion is, perhaps, the first paint property which is often overlooked by scientists developing AF solutions. However, good adhesion to underlying coats is a requirement *sine qua non* for any paint system. A recent example can be found with the fouling release technology. The successful worldwide commercialization of this technology was only possible after a consistently strong adhesion between the silicone topcoats and the remaining hull coating system under real-life application conditions was achieved.

13.2 The components of antifouling paint

Table 13.1 summarizes the role of the main components found in a paint formulation. It has to be noted that not all of these components are necessarily present in a specific paint.

13.2.1 Binders

The resin or binder is the essence of the coating and establishes most of the chemical and physical properties of the paint. Very simplistically, the role of the binder is limited to carrying and binding any particulate component together, thus providing the continuous film-forming portion of the coating. Most often, however, this binder should also be able to withstand the exposure conditions to which it is exposed and protect the substrate from

Table 13.1 Paint constituents and their role (after Lambourne and Strivens, 1999)

	Components	Typical function
Vehicle (continuous phase)	Polymer or resin (Binder)	Provides the basis of continuous film, sealing or otherwise protecting the surface to which the paint is applied. Varies in chemical composition according to the end use.
	Solvent or diluent	The means by which the paint may be applied. Avoided in a small number of compositions such as powder coatings and 100% polymerizable systems.
	Additives	Minor components, wide in variety and effect, e.g. catalysts, driers, flow agents.
Pigment (discontinuous phase)	Primary pigment	Usually, it provides opacity, colour, and other optical or visual effects. In anticorrosive paints, it may have barrier, corrosion inhibition or sacrificial anode properties, while in antifouling paints it may have a biocidal effect.
	Extender	Used for a wide range of purposes including opacity/obliteration (as an adjunct to primary pigment); to facilitate sanding, e.g. in primer surfaces.

Table 13.2 Methods of film formation for typical polymer systems (Lambourne and Strivens, 1999)

Method	External agent	Typical polymer system
Solvent evaporation	None or heat	Lacquer systems
Environmental cure	Oxygen	Oil-modified alkyd
	Moisture	Moisture-curing urethane
Vapour phase curing	Amine	Hydroxy acrylic/isocyanate blend
2-pack	None or heat	2-pack epoxy/amine
Radiation	Infrared/ultraviolet/	Photocuring unsaturated
	electron beam cure	polyester
Thermosetting	Stoving oven	Alkyd/nitrogen resin blend
		Thermosetting acrylic

degrading. Once the paint is applied on a surface, the binder will need to 'dry' or 'cure' by any of the methods described on Table 13.2 and secure adhesion. An external agent may be used to evaporate the solvent or to induce chemical reactions such as free radical or condensation polymerization, converting liquid polymers into highly cross-linked solids.

Binders can be firstly classified according to their molecular weight into low molecular weight film formers and high molecular weight film formers. The former class will not form solid films normally without further chemical reaction (e.g., epoxy resins). The final cross-linked matrix has extremely high molecular weight and usually possesses superior chemical resistance. On the contrary, high molecular weight polymers can form useful films without further chemical reaction (e.g., acrylics), but they will always be sensitive to strong solvents (e.g., those in which they were originally dissolved in the can). Other classifications can divide resins according to their origin (natural, natural modified and fully synthetic), and polymeric binders into addition and condensation types according to the polymerization mechanism (Lambourne and Strivens, 1999).

The special case of antifouling coatings

While these classifications apply for most types of coatings, the range of binders available for fouling control coatings is much more limited. Except for fouling release coatings (typically 2- or 3-component polysiloxanes), which are not designed to degrade during service, all (i.e., soluble matrix, controlled-depletion and self-polishing; see Yebra *et al.*, 2004) copper-based modern antifouling paints dry through a solvent evaporation mechanism. This is because these paints must react with seawater at a tightly controlled rate (see Chapter 14) so the polymer composition in the dry binder phase must be carefully engineered (i.e., it cannot depend on 'uncontrolled' curing reactions). Hence, self-polishing polymers are polymerized following very narrow structure and composition specifications and then dissolved as such into the one-component liquid paint.

As Yebra *et al.* (2004) review, after the ban of tin-containing copolymer paints, there are only three main binder families currently used in antifouling coatings for ocean-going vessels (Chapter 18):

- rosin-based systems with various co-binders (see Yebra *et al.*, 2005)
- silyl acrylate polymers
- copper and zinc acrylate polymers.

Recently, a new commercially successful binder system has been developed by Hempel A/S under the name NCT (nanocapsule technology), as reviewed in Chapter 18. This shortage of good performing binders highlights the difficulties of designing antifouling controlled-release systems. Since the global ban on tin-containing paints, most research efforts dealing with chemically-active antifouling coatings are focused on finding cleaner and more efficient active agents. However, the extreme difficulties in finding suitable carrier systems for such novel antifouling agents should not be overlooked (see Chapter 18).

Fouling release coatings

Fouling release coatings are mostly based on silicone elastomers that are very different from other products typically used in the industry (see also Chapter 26).

Silicone elastomers have very specific properties in terms of low surface tension, low roughness and high flexibility (low E-modulus; see Yebra *et al.*, 2004). This will ensure a weak attachment of marine fouling organisms and their easy removal when the vessel is moving through the water. This term is described as ‘self-cleanability’ or ‘fouling release’ properties.

The formulation of fouling release coatings is based on similar principles to other coatings, even though there is an even more critical focus on maintaining the above-mentioned properties throughout their service-life. Any loss of any of them will irreversibly trigger the fouling process. To ensure a proper film formation, the silicone polymers have to be cross-linked. For evident practical reasons, the most desirable cross-linking method is a RTV-type (room temperature vulcanization) that can be formulated either as one- or two-component compositions, and which allows the application of the coating onto large structures such as ships.

The ‘non-stick’ properties of silicones pose some adhesion problems with the underlying substrate, since it is difficult to make the product adhere easily to any undercoat without compromising the fouling release performance. For this purpose, special ‘tie-coatings’ have been developed to provide good adhesion between the two different chemistries (e.g., Grønlund *et al.*, 2005).

13.2.2 Pigments

Primary pigments

Pigments are defined as coloured, black, white or fluorescent particulate organic or inorganic solids, which are insoluble in, and essentially physically and chemically unaffected by, the vehicle or substrate in which they are incorporated. Contrarily to supplementary pigments, extenders, fillers, etc., primary pigments contribute to a large extent to one or more of the principal functions, namely colour, opacification, antifouling and anticorrosive properties. Colouring pigments can be classified into inorganic and organic pigments as seen in Table 13.3.

The colour of a pigment is mainly dependent on its chemical structure. The selective absorption and reflection of various wavelengths of light that impinge on the pigmented surface determines its hue. Other qualities of pigments often required are tinctorial strength (determining the amount of pigment necessary), insolubility, fastness to solvents, durability, price, dispersibility, flocculation resistance and chemical and heat stability. Some of

Table 13.3 Typical primary pigments (after Lambourne and Strivens, 1999)

Colour	Inorganic	Organic
Black	Carbon black Copper carbonate Manganese dioxide	Aniline black
Yellow	Lead, zinc, and barium chromates Cadmium sulphide Iron oxides	Nickel azo yellow
Blue/violet	Ultramarine Prussian blue Cobalt blue	Phthalocyanin blue Indanthrone blue Carbazol violet
Green	Chromium oxide	Phthalocyanin green
Red	Red iron oxide Cadmium selenide Red lead Chrome red	Toluidine red Quinacridones
White	Titanium dioxide Zinc oxide Antimony oxide Lead carbonate (basic)	

these properties are also affected by variables related to the particulate nature of pigments and the dispersion process. In this way, the crystalline structure of the pigment and the particle shape and size play an important role in the final properties of the pigments. Dispersion is further analyzed in a different section.

Primary pigments in AF paints

Again, antifouling paints are a very particular case. In copper-based technologies, which are clearly dominating the AF market, a large percent of the dry film corresponds to Cu_2O pigments, which are meant to dissolve in seawater and impart AF protection mainly against animal foulers (see Yebra *et al.*, 2004). Indirectly, Cu_2O is also important for the performance of AF paints since its dissolution creates a pore network at the paint surface exposing a large surface area of the binder system, and allowing seawater attack to the reactive groups. In other words, Cu_2O and soluble pigments in general (see Kiil *et al.*, 2002), play a crucial role in the paint polishing process (see Chapter 31) and, thereby, in the long-term efficacy of the paint. Having said this, the addition of inert colouring pigments (such as, e.g., Fe_2O_3) and/or fillers will decrease the polishing rate by mechanically stabilising the paint

surface (Yebra *et al.*, 2006a). Hence, their pigment volume concentration (PVC) within the formulation is usually kept to the minimum value, which assures satisfactory paint appearance upon atmospheric and seawater exposure. It is important to realize that the initial coating colour will be strongly influenced by the presence of the copper pigments while the in-service colour is determined by the non-soluble pigments dispersed into the paint.

In any case, the extensive amounts of Cu_2O used within AF films do not leave room for the addition of significant amounts of other pigments/fillers, provided that the paint is to be maintained sufficiently far away from the critical PVC value (CPVC; see discussion later on). Cuprous thiocyanate (white) is the substitute of red copper oxide (I) when vivid bright colours are desired. Cuprous thiocyanate is, in this sense, more appropriate than cuprous bromide (too soluble), cuprous iodine (too expensive) and cuprous cyanide (too insoluble and toxic).

Regarding fouling release coatings, the pigments and fillers used to provide colour and mechanical strength to the film must be carefully selected in order not to have any detrimental effect on the fouling release properties. Compared to biocide-based AF topcoats, fouling release coatings have a much lower pigment content so as to maximize the fouling release properties of the polymeric system.

Extenders, fillers, and supplementary pigments

All three names have been applied to a wide range of materials that have been incorporated into paints for a variety of purposes (e.g., add mechanical strength, reduce gloss). They tend to be relatively cheap and for this reason may be used in conjunction with primary pigments to achieve a specific type of paint. Extenders do not normally contribute significantly to colour, and in many cases it is essential that they are colourless. Usually, they are used to adjust the total volume of pigment to the required level (see PVC/CPVC ratio later on) without excessive cost. Many of the extenders in common use are naturally occurring materials that are refined to varying extents according to the use to which they are put. A list of typical inorganic extenders is given in Table 13.4.

Zinc oxide is a commonly used filler in chemically active fouling protection coatings, especially in yacht paints, due to a certain solubility in seawater (Yebra *et al.*, 2006b). Hence, it can be used as a polishing control filler (see Chapter 14).

Pigment volume concentration: how much pigment should I add?

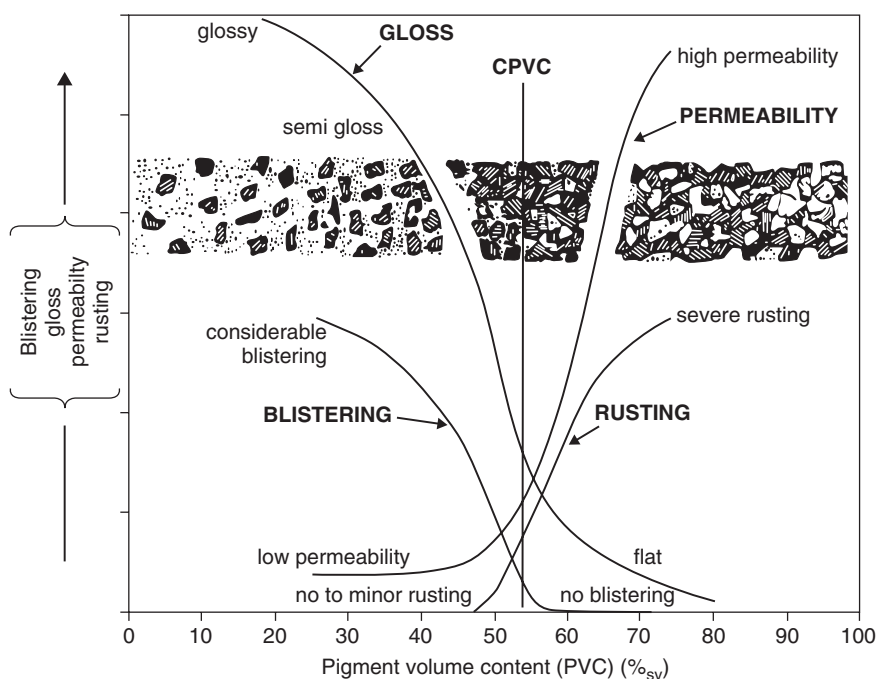
When formulating paint, the pigment/binder ratio used plays a central role. This ratio is accounted for by means of the pigment volume concentration

Table 13.4 Most typical extenders (after Lambourne and Strivens, 1999)

Chemical nature	Type
Barium sulphate	Barytes Blanc fixe
Calcium carbonate	Chalk Ground calcite Precipitated calcium carbonate
Calcium sulphate	Gypsum Anhydrite Precipitated calcium sulphate
Silicate	Silica Diatomaceous silica Clay Talc Mica Microfibres (Yebra <i>et al.</i> 2004)

(PVC), calculated as pigment volume divided by the total dry film volume, and it is key to paint's aesthetics and physical properties. Using the correct pigment PVC is an essential consideration in designing coatings of all kinds, but it becomes critical when considering specialized coatings (e.g., Zn-rich primers). Unfortunately, it is not possible to state which the optimum PVC in a coating is, since that depends greatly on the choice of binders, pigments and the final purpose of the paint. There is, however, one critical point which should be always kept in mind when formulating (see Fig. 13.2).

In this figure, it is easy to appreciate the critical coating behaviour achieved at a certain value of the PVC. This value is reached when the amount of binder added is the minimum to fill the voids between the pigment particles and is named the critical pigment volume concentration (CPVC). The exact value of this parameter for a given pigment dispersed in a given resin is a complex function of a number of parameters including the particle size distribution, particle shape, surface morphology and particle surface–resin physicochemical affinity. Since obtaining exact CPVC values for each combination of paint ingredients is clearly impractical, some assumptions must be made. A common way of estimating the approximate value of the CPVC for a single pigment is to add linseed oil to the dry pigment until a coherent mass is formed (the so-called spatula rub-out oil absorption (OA) method; see ASTM D281-95(2007)). One can assume that at that point, all the pigment surface and the interstices between particles at the maximum packing density are occupied by linseed oil, and that such



13.2 Effect of varying PVC on several paint properties (Meyer *et al.*, 1997).

oil represents the binder of interest. For paints containing multiple pigment types a simple extrapolation can be used as a coarse approximation:

$$\text{CPVC} = \frac{1}{1 + \frac{\sum_{i=1}^n V_i \cdot \rho_i \cdot OA_i}{100 \cdot \rho_L}} \quad 13.1$$

Where OA is given in grams of linseed oil per gram of pigment, V_i is the fraction of each pigment in the total pigment volume, ρ_i is the pigment density and ρ_L is the density of linseed oil (0.93 g/cm^3). This equation is, however, generally misleading, especially when the various pigments have significantly different particle size distributions (PSD). If that is the case, the packing characteristics of the pigment mixture can be significantly different from those of the single pigment due to smaller particles filling the interstices created by the larger particles. Hence, more elaborate algorithms using the PSD of the individual pigments, their respective OA value and their density have been developed (Bierwagen, 1972).

Table 13.5 Simple table summarizing the ways in which the PVC and the CPVC (and their ratio) can be modified so as to attain certain final properties

Goal	Effect	Action
Increase PVC	Reduce the relative amount of binder in the dry film	Substitute resin by pigments
Increase CPVC	Allow the addition of larger amounts of pigment particles without modifying the amount of 'free binder' in the dry film	Use pigments of lower oil absorption (and similar density) Using pigment of lower density (and similar oil absorption) Use pigments/fillers of selected PSD

Many additional negative effects will arise of PVC higher than the CPVC in an antifouling paint, such as the possibility of water penetration into the voids, loss of mechanical strength, high surface roughness, etc. Thus, the CPVC value is a crucial parameter in the design of paints in general and antifouling paints in particular.

Table 13.5 summarizes the formulation tools available for the paint formulator to modify the PVC and CPVC values in their coatings.

13.2.3 Solvents

Solvents enable the paint both to be made and to be applied according to the selected method of application. As it can be deduced from its name, a solvent dissolves the binder phase of the paint. In case that the resulting solution does not meet specific application requirements (e.g., temperature, application method), other substances named thinners and diluents may be added *in-situ* by the painter to further reduce the viscosity of the mixture (their efficiency depending on their ability to 'truly' dissolve the binder system). Thus, a better control of the flow of wet paint on the substrate and a satisfactory, smooth, even thin film, which dries in a predetermined time can be achieved. Drying time of ship bottom hull paints during dry dock operations is critical to avoid mechanical damage due to, e.g., docking blocks. In this respect, final coating hardness (partially influenced by retained solvents) and impact resistance properties (e.g., those provided by the addition of mineral microfibres; Yebra *et al.*, 2004) are also very important paint parameters.

Solvents are rarely used singly, as the dual requirements of solvency and evaporation rate usually cannot be obtained from the use of one solvent alone. Other factors affecting the choice of solvents and the use of solvent

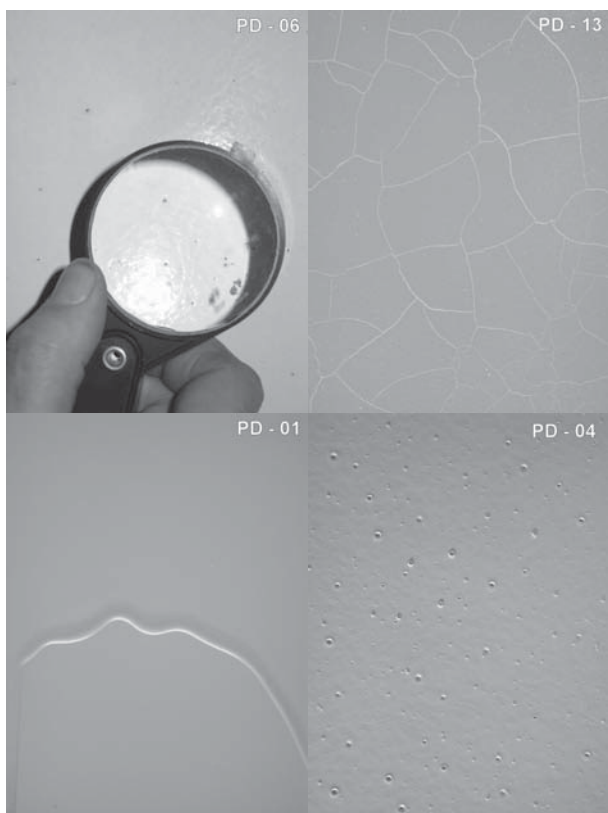
mixtures are their viscosity, boiling point (or range), flash point, chemical nature, odour, toxicity, and cost. Typical solvents for antifouling paints include aliphatic and aromatic hydrocarbons (e.g., white spirit and xylene respectively), ketones (e.g., methyl isobutyl ketone), esters (e.g., butyl acetate) and alcohols (e.g., n-butanol).

The use of water in the continuous phase of paint systems is increasing markedly due to legislative efforts (see, e.g., Chapter 10) to improve the safety in application by controlling the flammability, odour and toxicity of paint solvents establishing threshold limit values (TLV), a value that refers to airborne concentrations of substances and represents conditions under which it is believed that nearly all workers may be repeatedly exposed day after day, without adverse health effects. However, water-based AF paints, although existing, present an added difficulty compared to solvent borne products. The seawater sensitive, controlled-release, self-polishing resins must be stabilized in the aqueous media by water-soluble additives which tend to result in elevated water absorption upon immersion. The latter usually results in deficient controlled release properties. As further drawbacks, water-based coatings feature strongly water-dependent film formation properties and their drying time in those humid conditions typical of ship-yards are significantly slower compared to solvent-borne systems.

13.2.4 Paint additives

The simplest paint composition comprising a pigment dispersed in a binder, carried in a solvent (or non-solvent liquid phase) is rarely satisfactory in practice. Defects are readily observed in a number of characteristics of the liquid paint and in the dry film. These defects arise through a number of limitations both in the chemical and physical terms, and they must be eliminated or at least mitigated in some way before the paint can be considered a satisfactory article of commerce. Some of the main defects worth mentioning are (Fig. 13.3):

- Settlement of pigment during storage if the pigment dispersion is not sufficiently stabilized. At a microscale, Cu_2O settling after application due to, e.g., too low paint viscosity could lead to early fouling (Iselin, 1952).
- Aeration and bubble retention on application. If craters are formed after bubble rupture, the resulting macrorough surfaces would most probably enhance fouling (see Chapter 25). Also, bubbles may weaken the mechanical properties of the coating and influence the permeability of the film to both seawater and dissolved paint ingredients.
- Sagging: development of an uneven coating as the result of excessive flow of the paint on a vertical surface. If that happens on a ship, areas



13.3 Examples of paint defects. From top to bottom and from left to right: cratering, pinholes, sagging and cracking.

with a low coating thickness would be exhausted very soon, leading to early fouling.

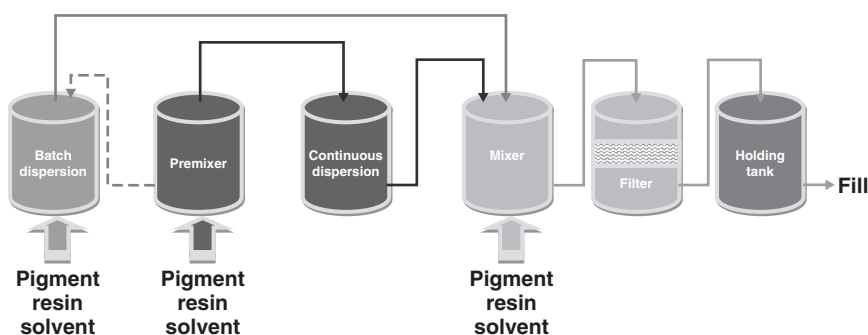
- **Cracking:** in brittle binders (e.g., rosin compounds), plasticizers are required in order to add flexibility to the film (see Yebra *et al.*, 2005). Cracking in a paint under service would enhance water attack to the AF coating markedly, decrease its mechanical properties and may also result in film detachment.
- **Cold-flow:** excessive solvent retention (e.g., use of high boiling point solvents) may lead to soft films which deform as a result of friction with seawater.

When simple reformulation of the paint is no sufficient to overcome any of these problems, and generally there is no such thing as ‘simple reformulation’ in antifouling technology, the use of additives will be necessary.

Additives are substances included in a paint formulation at a low level and which have a marked effect on the properties of the paint. The book by Lambourne and Strivens (1999) cites a large number of additives classified according to their function as: anti-corrosive, antifoaming, antisetling, anti-skinning, can corrosion inhibitors, dehydrators/antigassing, dispersion aids, driers, modifiers of electrical properties, flash corrosion inhibitors, floating and flooding additives, in-can preservatives, in-film preservatives, insecticidal, optical whiteners, reodorants, and UV absorbers. Additive suppliers such as, e.g., Byk Chemie, Air Products, Dow Chemical, Wacker Chemie, Elementis, Ciba, Tego Chemie, or Evonik Degussa, have broad expertise and an extensive product portfolio which will most likely fine tune the properties of your experimental paint.

13.3 Paint making

A simplified flow diagram of the paint making process is shown in Fig. 13.4. For high quality coatings and topcoats, the required degree of pigment dispersion and fineness of grinding is very high, so high-shear continuous dispersion mills must be used (e.g., pearl mills). The so-called millbase (typically all particulate ingredients and part of the continuous phase) is passed through the mill iteratively until the strict quality requirements are fulfilled ('continuous dispersion'). The remaining paint ingredients are added to the millbase in a subsequent stage prior to the final paint quality control. In those cases in which the grinding requirements are not as strict, the millbase can be sufficiently dispersed in a high speed disperser ('batch dispersion'; see further info below). Often, both processes are combined. For a thorough review of the physics of the dispersion process and machinery, refer to Lambourne and Strivens (1999).



13.4 Block diagram of a generic paint-making process (after Lambourne and Strivens, 1999). Note that not all steps are always required. 3000 litres and 12000 litres are typical sizes for 'batch dispersion' and 'mixer' tanks respectively.

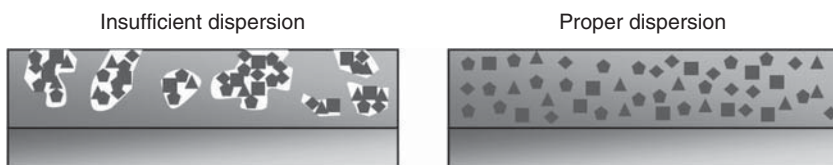
13.3.1 Dispersion

Dispersion of pigments is one of the important factors determining the performance of a coating. When developing novel antifouling coatings, it is crucial that good pigment dispersion is achieved if the true potential of the novel formulations is to be assessed through, e.g., field tests.

Dispersion refers to the complete process of incorporation of powdered pigments and fillers into the liquid medium so that the final product consists of fine pigment particle distribution throughout the medium (Fig. 13.5). The state of pigment dispersion can affect the optical properties, flow properties, durability, opacity, gloss, barrier properties (porosity) and storage stability. In a way, poor pigment dispersion results in localised film regions with PVC values over the CPVC. Although sometimes occurring simultaneously, separate operations in which the dispersion can be subdivided are:

- immersion and wetting
- deagglomeration
- distribution and colloidal stabilization of the pigment.

Wetting refers to the displacement of gases (such as air) or other contaminants (such as water), adsorbed at the pigment surface, with the dispersing medium. The wetting efficiency of the dispersant is strong enough to overcome, or at least reduce, the cohesive forces within the liquid and the surface tension between the solid/liquid interface, leading to ‘adhesion’ of the wetting groups of the dispersant onto the pigment surface. The majority of the vehicles used for paint-making can be considered as dispersants, their wetting efficiency depending on their molecular weight, structure, and the presence of substituent groups (e.g., carboxyl, hydroxyl, etc.). Since the electrical charges in the molecules of liquid and pigment are responsible for the wetting process, the polarity of ingredients, resins, solvents, and solid particles plays an important part in the dispersion stage. Dispersants and paint media adsorb onto pigment surfaces by means of a wetting group (anchor group), leaving the non-polar part, which is soluble with the liquid phase extended. The solvent balance, the compatibility of subsequent resin constituents, and the order of addition are of great importance to maintain stability.



13.5 Inadequate particle dispersion leads to heterogeneous pigment/filler distribution along the paint film and the presence of air filled pores jeopardizing, e.g., the barrier properties.

A large variety of wetting agents (surfactants) are being used in the paint industry to reduce surface tension at the solid/liquid interface favouring adsorption of paint media. The surfactants may be cationic (adsorbable ions positively charged), anionic (adsorbable ions negatively charged) or non-ionic, the activity of which may be due to polar and non-polar groups in the molecule. Typical wetting agents are salts of organic amines, alkali soaps, sulphated oils, glycol ethers, etc.

13.3.2 Grinding or breakdown of particle clusters

Pigment particles do not usually exist as primary particles but instead as aggregates or agglomerates. Reduction of these clusters to primary particles is necessary in order to obtain optimum visual, economic and performance properties of pigments. Grinding refers to mechanical breakdown and separation of particle clusters to primary particles, and follows generally after the pigment has been wetted. The effectiveness of grinding depends on the magnitude of forces holding the individual particles together in clusters. The exact breakdown mechanisms of clusters are not clear, however, but are generally brought about either by smearing (shearing), or smashing (impact) types of dispersion equipment. Some of the most typical machinery for dispersion are reviewed in Lambourne and Strivens (1999). For laboratory made paints, such as experimental novel antifouling formulations small scale high-speed dispersers and mechanical shakers can be used.

In the former, the most common processing equipment, the operating principle is a free rotating disc of 'limited' design (saw-type blades) in an open vessel. Since there is no grinding medium present, the pigment disperses on itself if the millbase is formulated properly (see Lambourne and Strivens, 1999). The impeller diameter in relation to the vessel (usually 1:3), the impeller's peripheral speed and the height of the paint charge (1.5 to 2 times the diameter of the impeller). If the millbase is correctly formulated and the dispersion parameters are correct, the paint will acquire a characteristic 'doughnut' shape during dispersion. Heat will inevitably evolve due to friction, which might actually serve to develop the properties of some additives, but evaporation losses must be corrected after dispersion. Average dispersion time is 30 minutes.

After paint manufacture, it is mandatory to check the fineness of grinding to assess whether such pigment agglomerates have been effectively broken down. Optimally, particle size distribution (PSD) instruments could be used for such purpose (see Yebra *et al.*, 2006c), even though it is a relatively slow analysis and it is not available in every laboratory. Most often grindometers, stainless steel blocks with one or two channels with increasing depths from zero to, e.g., 100 μm , are used for a rapid assessment of the maximum

agglomerate size. A sample of liquid paint is distributed with a scraper along the channel length, and those particles with a size larger than the depth of the channel will protrude. The transition from a smooth to a 'rough' liquid paint surface indicates the maximum agglomerate size.

13.3.3 Paint application: surface preparation

Good surface preparations are the key to obtaining good protection by surface coatings. The method to be used depends on the nature of the substrate to be coated, the types of contaminants commonly encountered (dust, chemisorbed fluids, residual oil and grease, residual metal powders, oxide scales, etc.) and sometimes on the type of coating used. The preparation techniques used can be divided into (Paul, 1985):

- solvent cleaning
- alkali cleaning
- acid pickling
- mechanical methods.

For experimental AF testing, it is recommended to first assure a good anchoring to the substrate. That is typically assured by priming the substrate with a coating with excellent adhesion to both the substrate and the topcoat (usually, the latter requires good surface cleaning and some surface abrasion to provide surface roughness and increase contact area). If the substrate is steel, priming is also necessary for anticorrosive purposes. If the substrate is plastic (e.g., acrylic panels) priming might not be necessary provided that good adhesion between the AF topcoat and the substrate has been previously demonstrated.

13.3.4 Application method

There are four main methods of applying paint:

- By spreading, e.g. by brush, roller or doctor blade.
- By spraying, e.g. air-spray and airless spray.
- By flow coating, e.g. dipping, curtain coating, roller coating, and reverse roller coating.
- By electrodeposition.

The methods adopted depend on the market in which the paint is used, each type of paint being formulated to meet the needs of the application method. Airless spraying is the most widely applicable method in general industrial paints. A novel paint which cannot be sprayed will have very limited chances of success for large ocean-going vessels and off-shore structures, for example. However, for static testing of experimental formulations,

paint drawdowns, by e.g. Dr Blade applicators (metal bars containing a gap of known depth or clearance on one or more faces), can be used.

13.3.5 Simple formulation tools

Table 13.6 may summarize the main raw material properties a formulator needs to take into account when formulating a paint. Basically, it is important to differentiate between the 'wet' paint (i.e., with solvents) and the 'dry' coating. This last one is, obviously, responsible for the final paint properties although, as emphasized earlier on, the choice of solvents may also affect those greatly. Once we know the solvent content of our raw materials (0% in the raw materials chosen in Table 13.6), it is possible to analyze the dry film composition. Such composition can be expressed in weight units or in volume units. The latter one is preferred, since it tells us about the spatial distribution of the paint ingredients into the coating. Imagine that we have a paint product formulated at a PVC/CPVC ratio of 0.8, and we decide to substitute a given pigment by the same weight of a different pigment with a much lower density. Obviously, the new pigment will occupy a larger volume (higher PVC, higher surface area, potentially higher OA) than the original pigment, approaching or even surpassing the new CPVC value, with the subsequent effects on the final paint properties.

Table 13.6 Example of a formulation sheet for a model antifouling formulation

	Liquid paint			Solid paint			Solid volume composition	Oil absorption g/g
	weight	S.G.	volume	weight	S.G. sol	Volume		
Gum rosin	17.3	1.1	16.1	17.3	1.1	16.1	58.0	
ZnO	7.6	5.5	1.4	7.6	5.5	1.4	5.0	14.0
Cu ₂ O	41.7	6.0	7.0	41.7	6.0	7.0	25.0	12.0
Fe ₂ O ₃	6.0	4.3	1.4	6.0	4.3	1.4	5.0	19.5
Biocide	1.4	1.8	0.8	1.4	1.8	0.8	3.0	40.0
Thix. Agent	2.0	1.7	1.1	2.0	1.7	1.1	4.0	60.0
Xylene	24.1	0.9	27.8	0.0		0.0	0.0	
	100.0		55.6	76.0		27.8	100.0	
Solids volume ratio			50.0					
Solids weight ratio			76.0					
PVC			42.0					
CPVC			60.2					
PVC/CPVC			0.77					

13.4 Testing general paint properties

In addition to outstanding antifouling properties, antifouling paints also need to fulfil a series of other 'general' paint properties before commercialization. More specifically, the mechanical integrity of the antifouling paint system should be adequate to stand up to the harsh environment these paints are subjected to. Salt-water exposure (may be even fresh water exposure), partial immersion, drying out periods and changing temperatures are some conditions which pose stress on an antifouling paint system having an enormous effect on the durability of the paint. Keel block impact during dry dock, risk of 'cold flow' if the ship is launched too early after painting and fender impact during service are examples of potential scenarios to be considered.

Therefore, when developing and monitoring the performance of paints, test methods which simulate the actual usage are always adopted. In the paint industry, test method specifications have been drawn up not only by a number of national and international organizations such as the International Standards Organization (ISO) and the American Society for Testing Materials (ASTM) but also specific test methods are used among coating suppliers to evaluate, e.g. hardness, cold flow tendency and fresh water resistance. In the following, some of the test methods of special interest for antifouling systems are described. The test methods all include full painting system on steel substrate, say anticorrosive paint, tie (or link) coat and antifouling top coat. It is also important to note that the desired paint properties must be stable even after prolonged storage under extreme conditions (e.g., very high and very low temperatures).

13.4.1 Applicability

A novel paint product must possess good sprayability over a wide range of application conditions (temperature, humidity, etc.). Technologies too sensitive to application conditions will have significantly higher probabilities of early failure. Once on the substrate, the liquid paint must have sufficiently good flow and levelling properties to develop a smooth final surface which minimizes the risk of fouling and maximizes its aesthetic properties. However, very 'liquid' paints onto the substrate rapidly develop sagging defects. Sagging should only happen at relatively high wet film thicknesses (e.g., 500 μm). Only then high dry film thicknesses (i.e., long active lifetimes) can be achieved with as few layers as possible (shorter docking time). Adequate viscosity during application coupled with high viscosity once on the final surface is generally achieved via addition of thixotropic and other rheological additives.

Sagging is tested by using a notched bar with indentations of specified sizes. The thixotropy of the paint is first 'broken' through high speed

dispersion and then the paint is drawn through a substrate by means of the mentioned bar, so that a series of paint 'stripes' of known wet thickness are created. The substrate is placed vertically during drying and the sag resistance of the coating is measured as the highest thickness of coating at which sagging does not occur during the drying process.

13.4.2 Exposure, mechanical testing and evaluation

In this section, methods involving impact, indentation, bending and adhesion are described. Several of the standard methods include damage of the steel substrate as well as the paint, and ideally a coating system should be able to absorb this deformation without failures such as peeling and cracking. However, in real life, compromise between hardness and flexibility is always necessary to meet the satisfactory properties. The tests are either applied to freshly applied paint systems or to systems aged in exposure equipment which simulate accelerated weather situation (e.g., elevated temperature, alternating dry and wet conditions).

13.4.3 Blister box test

Blister box test, according to ASTM D 4585 (ISO 6270) evaluates the water resistance of a coat by condensation of water vapour. The panel surface with the coating system is exposed to 40 °C, saturated water vapour, at an angle of 15° to the horizontal. The reverse side of the panel is exposed to room temperature. At each inspection, blisters and rust are evaluated according to ASTM D 714 (ISO 4628-2) and ASTM D 610 (ISO 4628-3) respectively. Cracking is evaluated according to ISO 4628-4. When the test is stopped, adhesion is evaluated according to ASTM D 3359, tape test (ISO 2409) or ASTM D 4541 (ISO 4624), pull-off test (see below).

13.4.4 Cyclic blister box test

According to ASTM D 4585, this test evaluates the water resistance of a coat by condensation of water vapour. The panel surface with the coating system is exposed to saturated water vapour (e.g., 40 °C) at an angle of 60° to the horizontal. The reverse side of the panel is exposed to room temperature. The apparatus can be set to continuous condensation or run in a cyclic-condition, changing between condensation and drying. The evaluation is the same as with standard blister box test.

13.4.5 Immersion

Half the panel is immersed in fresh water and half the panel exposed to vapour. Possible weak adhesion is hereby provoked. The panels are applied,

cured for 7 days and immersed in potable water for 2 weeks. After exposure the panels are immediately examined for blistering and adhesion (Knife test, X-cut, #-cut; see below).

13.4.6 Atmospheric exposure

The AF topcoat is applied onto acrylic panels (about 100 cm²) and exposed to atmospheric weathering (i.e., UV radiation, rain, etc.). Depending on the angle of exposure, the relative importance of UV radiation and rain water can be varied (the latter influencing the most in panels exposed horizontally). At each inspection, the potential presence of aesthetic and mechanical defects such as, e.g., discolouration, yellowing/whitening and cracking (ISO 4628-4), is evaluated. A spectrophotometer can be used to quantify some of these variables. Typical exposure time is about one year with weekly inspections during the first month and monthly inspections the remaining experimental period.

13.5 Mechanical testing

13.5.1 Adhesion test

Adhesion test is used to evaluate the adhesion of a paint system to the substrate and between coats (layers). The test can be performed by one or a combination of four methods.

X-cut

According to ASTM D 3359, method A, an X is cut into the film to the substrate, pressure tape (TESAPACK 4287) is applied over the X and then removed, and adhesion is evaluated by comparison with descriptions and pictures. The method is used to identify the weakest point of the system, which can be found between the steel and the primer or between consecutive coats (adhesive break) or in the coating (cohesive break).

Cross-cut (#-cut)

According to ASTM D 3359, method B (ISO 2409), a lattice with 6 cuts in each direction is made in the film to the substrate. Then, pressure tape (TESAPACK 4287) is applied over the lattice and then removed, and adhesion is evaluated by comparison with descriptions and pictures.

The spacing of cuts depends on the thickness of the coating and of the type of substrate.

- 2 mm spacing: not suitable for dry films thicker than 125 microns.
- 3 mm spacing: not suitable for dry films thicker than 250 microns.

Knife test (KNF)

The test is done by making two intersecting scratches through the paint film to the substrate with a sharp steel knife. Adhesive or cohesive failures are evaluated by peeling the coating from the intersection point and outwards. Common for the three adhesion evaluation methods are that the test is performed on both immersed and non-immersed panel-halves (referred to as respectively 'wet' and 'dry' adhesion). The type of rupture is reported, and the severity is judged on a scale from 5 (perfect) to 0 (poor).

Pull-off test

Pull-off test according to ISO 4624 (ASTM D 4541), with PAT hydraulic adhesion tester. The standard conditions are 1.58 cm² dolls and Standard Araldite glue, cured for 24 hours. This test covers the determination of the pull-off strength of a coating or coating system, by determining the greatest perpendicular force (in tension) that a surface area can bear, before a plug of material is detached. Failure will occur along the weakest plane within the system comprising the test fixture, adhesive coating system and substrate. After the proper curing time of the glue, the paint film is cut free around the dolls down to the substrate and the dolls are pulled off. The pull-off value (tensile strength) is registered, and converted in relation to the area of the doll in MPa. The type of rupture is also noted (cohesive/adhesive).

13.5.2 Impact

Impact (effect of rapid deformation), according to ISO 6272-1 is a falling-weight test, large-area indenter using an Erichsen impact tester. This test method covers a procedure for rapidly deforming by impact a coating film and its substrate and guidelines on how to evaluate the effect of such deformation. The test is performed on 1.5 mm panels. After the coatings have been cured, a falling-weight of 1 kg, with an indenter-head of 20 mm Ø, is dropped a distance, in metres, onto the test panel. The panel is supported by a steel fixture, with a hole of 27 mm Ø, centered under the indenter. When the indenter strikes the panel, it deforms the coating and the substrate. By gradually increasing the distance the weight drops, the point at which failure usually occurs can be determined. The impact value is reported as the highest impact, reproduced five times, which results in no visible cracks and no adhesion failure in the paint film. The impact value is stated as kg-m. A possible rupture is evaluated as cohesive or adhesive.

13.5.3 Mandrel bending test

Mandrel bending test according to ISO 1519-1973 (ASTM D 522-88 method B) is a test method covering the procedure for assessing the resistance of a coating of paint, varnish or related product to cracking and/or detachment from a metal substrate (with a thickness of 250 microns) when subjected to bending round a cylindrical mandrel under standard condition. When the panel has been coated, cured for 4 weeks and cut to size, the test panel is placed over a mandrel with the uncoated side in contact and with at least 50 mm overhang on either side. Using a steady pressure of the fingers, bend the panel approximately 180° around the mandrel. Remove and examine the panel immediately for cracking visible to the unaided eye. If cracking has not occurred, repeat the procedure using a smaller diameter on untested panels of a specimen until failure occurs or until the smallest diameter mandrel has been used.

13.5.4 MAN-H – hydraulic mandrel bending test

This mandrel bend test is a slight modification of ISO 1519-1973 (ASTM D 522-88 method B), allowing the paint systems to be applied on thicker steel panels which can be prepared in accordance to the product data sheet. The test method covers the procedure for assessing the resistance of a coating of paint, varnish or related product to cracking and/or detachment from a metal substrate when subjected to bending round a cylindrical mandrel under standard condition. When the panel has been coated and cured for 4 weeks, the test panel is placed over a mandrel with the uncoated side in contact and with at least 50 mm overhang on either side. Using a hydraulic pressure with constant velocity, bend the panel around the mandrel. Remove and examine the panel immediately for cracking. If cracking has not occurred, repeat the procedure using a smaller diameter on untested panels of a specimen until failure occurs or until the smallest diameter mandrel has been used. Record the diameter of the smallest mandrel at which the coating does not crack.

13.5.5 Wooden block settings (keel block impact)

When ships are in dry dock, they are supported by wooden blocks. After the antifouling system is applied, these wooden blocks are sometimes moved in order to apply antifouling on the areas where the wooden blocks originally were. The blocks are moved to areas with freshly painted antifouling. The pressure underneath the wooden blocks is around 70 kg/cm², which means that if the paint is not dry enough, the paint will ooze out from underneath the wooden blocks. This test determines how quickly the blocks

can be moved without oozing. The paint system to be tested is affected by specified pressure in 3 minutes. If pressure is not specified, the test pressure is 70 kg/cm² (a 3 cm² wooden block is used) The test is repeated 24 hrs, 48 hrs, 72 hrs, etc., after application of the last coat until an acceptable level of deformation is obtained.

13.6 Conclusions

This chapter has been intended as a help for antifouling researchers willing to scale-up their ideas for novel AF coatings from the lab to a panel immersed at a sea site. Once the types and amounts of controlled-release binder(s) and the active agent(s) have been chosen, the next formulation parameter to be considered is the pigment volume concentration (PVC). Such parameter, and, more specifically its ratio to the critical PVC (CPVC), is key to the final paint performance, and can determine the success of the formulation. The same applies to correct pigment dispersion procedures. The potential presence of unbroken pigment clusters, only partially wetted by the binder phase will also influence the final paint properties and potentially yield a good formulation into a failing coating. Similarly, the addition of suitable amount and types of rheological additives is a must in order to avoid heterogeneous dry film thicknesses along the test panel (because of, e.g., sagging) or heterogeneous paint composition along the paint film (due to, e.g., pigment settling during solvent evaporation).

Antifouling paints are extremely sensitive paint systems, in which all the paint working mechanisms are tightly coupled and must be finely synchronized so as to develop the full potential of the formulation. Even though there is no doubt that finding a correct combination of controlled-release binder and active substances is the most difficult part when developing novel antifouling coatings, it is not difficult at all to develop failing coatings out of promising raw materials. As a clear example, paint companies spent many years of research to optimize copper-containing tributyl tin based coatings, in spite of the recognized AF efficiency of both agents.

Antifouling paint research will therefore strongly benefit from correct use of coating formulation principles to fully evaluate the potential of a new concept. Research projects involving an important investment in time and resources could upgrade from promising ideas to promising products, too often the most difficult step for academic research, with the subsequent benefit to the marine environment.

13.7 Sources of further information and advice

Books on general coatings technology and including formulation principles are listed below:

- Hare, C.H. (1994). *Protective Coatings – Fundamentals of Chemistry and Composition*. Technology Publishing Company, Pittsburgh, PA (USA).
- Lambourne, R., Strivens, T.A. (1999) *Paint and Surface Coatings*. 2nd Edition. Woodhead Publishing Limited, Cambridge (UK).
- Müller, B., Poth, U. (2006). *Coatings formulation*. Vincetz Network GmbH, Hannover (Germany)

On antifouling paints, the outstanding book published back in 1952 by WHOI is still an excellent source of information to understand fouling control coatings:

- Iselin, C.O.D. (ed.) (1952) *Marine Fouling and its Prevention*. Woods Hole Oceanographic Institution, US Naval Institute, Annapolis.

13.8 Acknowledgements

Sincere thanks to Antoni Sánchez for thoroughly reviewing the manuscript at its different stages. Lars Thorslund Pedersen and Anders Blom must also be acknowledged for their insightful comments to different sections of the chapter.

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- Yebra, D.M., Kiil, S., Weinell, C., Dam-Johansen, K. (2006b) Dissolution Rate Measurements of Seawater Soluble Pigments for Antifouling Paints: ZnO. *Prog. Org. Coat.* 56(4), 327–337.
- Yebra, D.M., Kiil, S., Weinell, C., Dam-Johansen, K. (2006c) Parametric Study of Tin-Free Antifouling Model Paint Behaviour Using Rotary Experiments. *Industrial and Engineering Chemistry Research* 45, 1636–1649.

Modelling the design and optimization of chemically active marine antifouling coatings

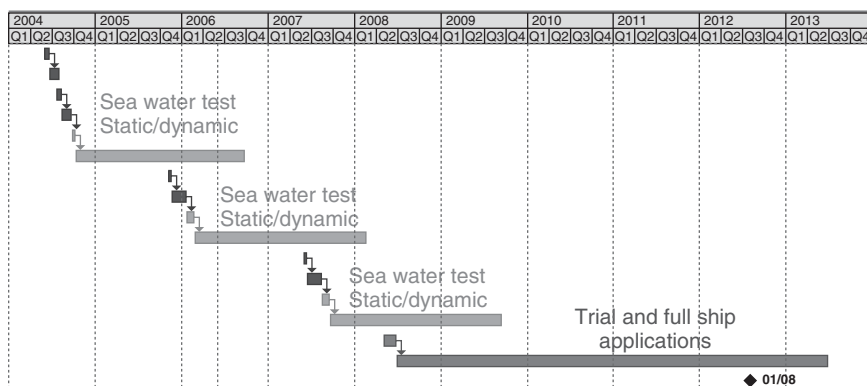
S KIIL, Technical University of Denmark, Denmark and
D M YEBRA, Pinturas Hempel S.A., Spain

Abstract: Current chemically active antifouling (AF) coating development strongly relies on long-term, empirical field tests. The latter fact is not only a consequence of the high complexity of such multicomponent, reactive products, but also because no less than 36–60 months sustained activity against biofouling must be guaranteed to the customer. In recent years, several studies have been aimed at characterizing the mechanisms and quantifying the main physico-chemical phenomena associated with the working mechanisms of chemically active antifouling coatings. When such knowledge is utilized in the form of mathematical coating models, reliable estimates of the coating performance under any exposure conditions and, more importantly, information about optimization routes, can be obtained. The outcome can be more qualified coating design and optimization and thereby a faster switch to more efficient and environmentally friendly products. This chapter summarizes such AF coating mathematical modelling attempts and the experimental methods used for the quantification of the relevant coating mechanisms.

Key words: mathematical coating models, coating polishing, biocide leaching rate, binder reaction rate, pigment dissolution rate, cuprous oxide, simulation tools, rosin-based tin-free antifouling coatings.

14.1 Introduction

Historically, the design and development of chemically active antifouling (AF) coatings has been based on an empirical approach. Most formulation changes, from small adjustments to innovative solutions, basically rely on experimental inputs from systematic raft (non-moving) and dynamic tests (e.g., rotating cylinders) conducted in the laboratory or at selected sea sites (see also Chapter 16). The duration of such tests typically exceeds one year; 36–60 month real-life testing on ship hulls using small test areas first and, eventually, full-scale paint jobs are mandatory to prove the long-term effect and stability of promising products before worldwide commercialization. Such time-consuming testing, already from the early screening stages, owes to the fact that a sustained and sufficient paint activity during prolonged exposure periods (i.e., years) is, without any doubt, the most difficult



14.1 Timeline for a typical development process of a new AF technology.

requirement a successful AF coating must fulfil. Success does not solely depend on the biocide package incorporated, which must be effective enough, but, most importantly, on the way the paint system releases it to the environment over time. This is why short term tests without sufficient paint ageing, including bioassay testing (see Chapter 12), are insufficient to fully evaluate a chemically active AF paint.

In light of the above, overall product development times longer than 10 years are not at all unusual (Fig. 14.1). As an example, the copper-acrylate tin-free technology has been subjected to patent-protected improvements from 1986 to 2005 (Solomon *et al.*, 2005, Yebra *et al.*, 2004) without 60-month performance data on ship hulls for the most recent version of the technology.

14.2 Empirical versus model-based screening and optimization

The empirical approach is, most likely, the most efficient way of assessing the effect of various obvious formulation parameters on the paint performance: e.g., testing various concentrations of Cu_2O and co-biocide or testing small variations of the main binder system. That is especially true when statistical design of experiment (DoE) and advanced computer data analysis tools are utilized. Commercial examples of successful empirical developments are the identification of the tetrahydrogenated version of abietic acid as the most suitable derivative of natural rosin for antifouling purposes as well as the beneficial effects of pre-reacting such resin with Zn^{2+} prior to pigment dispersion (Yebra *et al.*, 2004, 2005).

However, further optimizations and the efficient development of novel functionalities require an understanding on how an AF paint works and

what the weak points are for a given formulation. Such knowledge would open the door for faster screening stages, in which short-term paint characterization tests can be used to assess the long-term potential of the product. Otherwise, the development process inevitably becomes a long and frustrating one in which success is largely dependent upon a stroke of luck.

In the past 35 years, and especially with the growing environmental concern experienced in the last 8–10 years, there have been various attempts to provide part of a theoretical basis for the polishing and biocide leaching behaviour of different types of antifouling coatings. The early studies focused on insoluble matrix coatings, where the binder is insoluble in seawater, while the more recent publications treat advanced self-polishing coatings, where the binder reacts or dissolves in seawater. A detailed knowledge of how the various ingredients in a coating behave and interact during service life can help in generating ideas for new products and ways of tackling coating deficiencies. Simulations, based on a properly verified mathematical model, can provide “what if” studies to evaluate innovations and lifetime estimations under selected conditions. Optimization of biocide release rates (to reduce cost and environmental pollution) is also an important option with the increased focus on copper and organic biocides.

The development of AF paint simulation tools basically relies on:

- Identifying the working mechanisms of a chemically active antifouling paint. Most often, these are believed to be biocide leaching and paint polishing (via binder seawater reaction). Other mechanisms have been suggested, such as, e.g., biocide surface affinity (Dahlström *et al.*, 2004).
- Characterizing mathematically each of the individual mechanisms which are believed to determine the paint behaviour.
- Characterizing quantitatively each of the above processes through suitable experimental procedures.
- Building up a mathematical model incorporating such individual processes and their interactions.
- Verification of the model through lab and/or field tests.

This chapter will provide an overview of the various simulation tools developed as well as examples of experimental techniques that can be used to measure/estimate the required model inputs. Directions of future work are suggested at the end.

14.3 Previous modelling work on classical AF paints

14.3.1 Insoluble matrix coatings

The insoluble matrix technology, also referred to as continuous contact or contact leaching, is based on seawater-insoluble, mechanically tough binders

from which soluble pigment particles and other soluble material leach, whereby a porous matrix is formed. As one particle dissolves, another in contact with it becomes exposed to seawater. Binders traditionally used are vinyl and chlorinated rubber. The effect against biofouling is due to the dissolving components being biocidal. Reports on the use of insoluble matrix type coatings goes back a long way (see e.g., Iselin (WHOI), 1952) and the products are rarely used today on trading vessels. The main reason for this is that the leaching rate of the biocides decreases exponentially with time as a result of a growing biocide particle-paint surface diffusion path. The latter eventually results in the biocide surface concentration falling below the critical value needed for fouling control. Several publications are available on the mathematical modelling of these systems (reviewed in Kiil *et al.*, 2003). The models developed are very simple and they all need some kind of ‘calibration’ against experimental data to work well. There are no reports of the models actually being used in subsequent formulation work.

14.3.2 Soluble matrix coatings

Another type of antifouling coating, still used today for dry-docking intervals of about 36 months, is the soluble matrix type (sometimes also called ablative or controlled depletion type; Yebra *et al.*, 2004). In principle, these paints are designed so that the release rate of biocides remains constant until the paints have completely dissolved. That is because, contrarily to insoluble-matrix paints, the biocide is dispersed into a seawater soluble binder, typically containing free acidic groups as in, e.g., unreacted natural gum rosin (see Yebra *et al.*, 2005). The presence of such resins facilitates surface polishing aiming at stabilizing the distance between the dissolving biocide particles and the paint surface (stable diffusion path lengths). However, the leaching rate of toxicants out of these vehicles is often too high and uncontrolled, which leads to either too early paint exhaustion (if the polishing rate is designed to be high), or to thick biocide-depleted layers (i.e., approaching insoluble-matrix paints if the polishing rate is chosen to be low). In the latter case, structurally weak films can occur which jeopardize subsequent recoating actions. No publications exist on modelling of the polishing and leaching behaviour of traditional soluble matrix paints (Kiil *et al.*, 2003).

14.4 Modelling self-polishing antifouling coatings

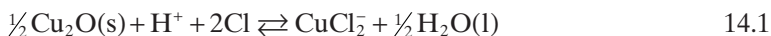
Advanced chemically active antifouling coatings rely on a self-polishing mechanism. The exact definition of a self-polishing mechanism is in dispute as discussed in Kiil *et al.* (2003) and Yebra *et al.* (2004), but most authors seem to agree that the (now banned) TBT-SPC technology is the coating

type which the word was first associated with back in the beginning of the 1970s. With tin-free coatings, there is a tendency to associate the 'true self-polishing' behaviour to acrylate-based coatings because of its 'similar' chemical structure. As elaborated in, e.g., Yebra *et al.* (2004), the term self-polishing should perhaps define an outstanding AF paint performance, namely 60+ months of fouling free and hydrodynamically smooth hull surface, rather than a given (mostly uncharacterized) chemistry. With respect to mathematical modelling, this has been done for TBT-based and selected tin-free coatings. Some of these models are now used in formulation work.

14.4.1 Working mechanism of self-polishing chemically active antifouling paints

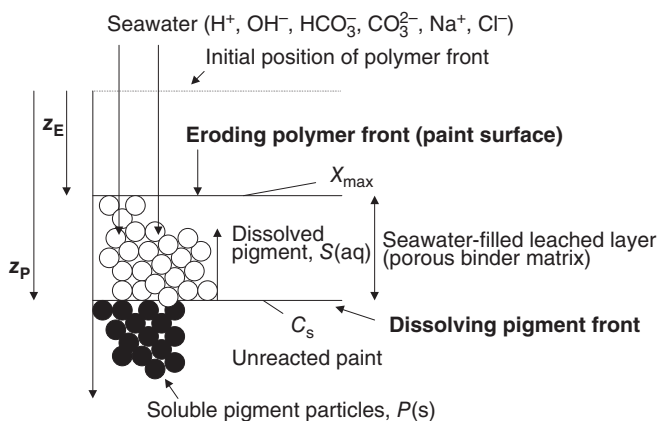
To illustrate the underlying working mechanisms of self-polishing coatings the well-known tin-based technology will be used. This coating type has been thoroughly described over the years (Kiil *et al.*, 2001, 2002a,b,c, 2003). Other modern antifouling coatings appear to have similar, but not identical, working mechanisms (Yebra *et al.*, 2004; 2006b).

Seawater-soluble pigment particles, such as Cu_2O and ZnO , dissolve near the surface of the coating. As an example, the reactions of Cu_2O to form CuCl_2^- and CuCl_3^{2-} are provided (Ferry and Carritt, 1946):



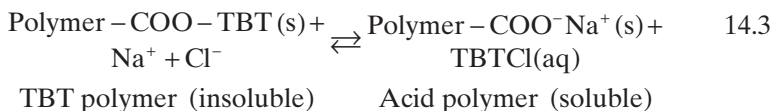
The continuous removal of such soluble pigments from the solid pigment phase creates voids in the uppermost paint layer (see Fig. 14.2). The distance from this dissolving front (typically the Cu_2O one) to the paint surface is termed the 'leached layer', which can be described as a porous, Cu_2O -depleted, binder matrix. For an active AF protection, seawater must diffuse into the porous polymer matrix (think of the structure of a traditional catalyst), and dissolve biocidal compounds dispersed into the paint. Dissolved compounds diffuse out through the seawater-filled leached layer and across the external solid-liquid boundary layer into the bulk seawater. Dissolution and mass transport rates determine the velocity of biocide exhaustion and the concentration of bioactive compounds at the paint surface.

The fact that some binder components are susceptible to seawater reaction means that the leached layer pore walls are also exposed to chemical attack. In the TBT-SPC technology, the copolymer used in the binder phase, which can undergo hydrolysis in seawater, is based on tributyltin methacrylate and methyl methacrylate. Often, another copolymer, a retarder, is present in smaller amounts to increase the film hydrophobicity and to give



14.2 Self-polishing antifouling coating with soluble Cu_2O particles exposed to seawater. Notice the pigment-leached layer and the two moving fronts (eroding polymer front, z_E , and dissolving pigment front, z_P). Reprinted from Computer Aided Chemical Engineering, vol. 23, Kiil *et al.*, 'Marine biofouling protection: design of controlled release antifouling paints', Chapter 7 in *Chemical Product Design: toward a perspective through case stories*, 181–239, 2006, with permission from Elsevier.

strength to the polymer matrix, thereby tuning the polishing rate to the desired value. The self-polishing mechanism of the polymer begins with the following reversible reaction in seawater (Kiil *et al.*, 2001, 2006)



As the polymer hydrolysis proceeds, leading to Na^+ -carboxylate groups and releasing the TBT-groups as TBTCl, the partially reacted outer layer of the polymer film loses mechanical strength. At the surface, the binder reaches a certain conversion termed $X_{\max}$, the conversion at which the polymer begins to erode by moving seawater (self-polishing effect; Kiil *et al.*, 2001), exposing a fresh layer of acrylate polymer.



Consequently, two moving fronts, the dissolving pigment front and the eroding polymer front, develop (note that the presence of more than one seawater soluble pigment, e.g., ZnO and Cu_2O , may result in the formation of more than two moving fronts; Yebra *et al.*, 2006e). After some time, due to the strong coupling of the rate of movements of the different fronts (Kiil *et al.*, 2002b), the thickness of the leached layer becomes stable.

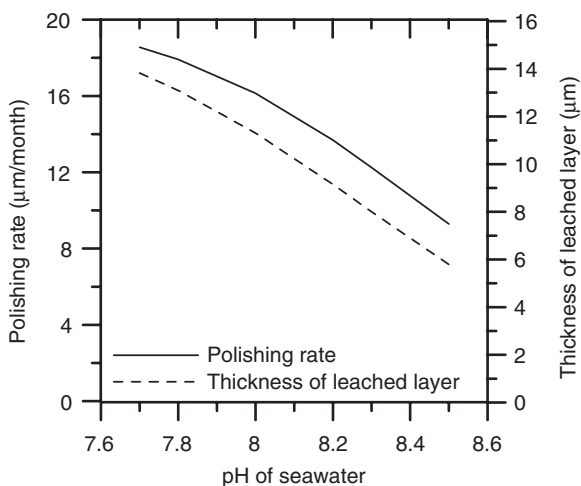
This mechanism basically holds for the case of rosin-derived self-polishing AF paints too, but with a few modifications. In case of tin-free paints containing gum rosin derivatives (Yebra *et al.*, 2005), such scarcely seawater soluble molecules of relatively low molecular weight can be effectively solvated by water after alkaline hydrolysis (Yebra *et al.*, 2005). Therefore, these smaller molecules induce surface erosion not by turning into mechanically weaker reaction products (e.g., TBT-methacrylate into Na-methacrylate), but rather by progressively leaving the seawater-exposed binder phase all the way from the pigment front to the paint surface, thus debilitating the latter (Yebra *et al.*, 2006a, b; see also the section on the modelling of selected paints containing rosin-derivatives later on). In these paints, controlling seawater access to the soluble pigment particles and the soluble binder resins is key to a smooth paint performance. This can be performed via hydrophobic retarders, which also serve as polishing regulators by counterbalancing the effects of seawater sensitive resins.

Hence, the coating-seawater mechanism of self-polishing coatings includes the following rate-influencing steps: hydrolysis and erosion of the active binder, effective diffusion in the leached layer of dissolved binder reaction products, pigment species and other biocides, and external mass transport of relevant components. All the rate-processes are coupled, just as in the TBT-SPC case.

14.4.2 Mathematical models

Modelling tributyltin self-polishing copolymer paints

The first publication describing a mathematical model of a self-polishing coating is that of Kiil *et al.* (2001). TBT-SPC-based model paints containing different amounts of retardant and a constant Cu_2O load of about 40% (in solids volume) were tested at two different rotary speeds and seawater temperatures. In this work, the kinetic and transport parameters of binder and pigment chemistries needed as model inputs are also provided and simulations are verified against experimental rotary data. The remarkable agreement between simulations and experimental data confirmed the working mechanism assumptions presented in Section 14.4.1. In Kiil *et al.* (2002a), the effects of seawater parameters and coating formulation parameters on the coating behaviour are simulated and discussed. As an example of the use of the mathematical model, the effect of seawater pH on the paint polishing and biocide release rate is simulated in Fig. 14.3. It can be seen on the figure that the rate of polishing goes up when the pH is lowered. The reason is that the rate of dissolution of Cu_2O is proportional to the concentration of H^+ . Thus, when the latter increases, the rate of movement of the pigment front is increased. This also leads to a faster rate of move-



14.3 Effect of pH of seawater on the polishing rate and the stable thickness of the pigment-leached layer of a self-polishing antifouling coating. A pH value of 8.2 corresponds to 'normal' seawater. Reproduced from Kiil *et al.* (2002a) with permission from Trans IChemE.

ment of the polymer front, because the movements of the two fronts are coupled. However, the rate of movement of the polymer front is not enhanced as much as the pigment front leading to an increased thickness of the leached layer. The importance of the findings is that the lifetime of a self-polishing antifouling coating, to some extent, is dependent on the pH value of the local seawater, where a given ship is sailing.

Modelling of tin-free paints containing rosin-derivatives

As an adaptation of the TBT-SPC model of Kiil *et al.* (2001), Yebra *et al.* (2006b) developed a model which described the behaviour of simple model paints inspired by commercially relevant tin-free systems. The main seawater reactive resin in the binder of such paints was a synthetic substitute of natural rosin (subject to hydrogenation and distillation) which has been claimed to be more consistent, less sensitive to oxidation (lower number of double bonds) while retaining a suitable seawater solubility (Yebra *et al.*, 2005). This rosin derivative is further reacted to form zinc carboxylate (sometimes called zinc resinate), giving rise to controlled-polishing properties through an alkaline hydrolysis reaction. The zinc derivative entails an increased hardness and faster drying times compared to the hydrogenated rosin.

As a first step, the new model had to be adapted to include the new binder chemistry, determined experimentally in Yebra *et al.* (2005). In such tin-free paint model, the $X_{\max}$ concept introduced for the modeling of TBT-SPCs and verified in Kiil *et al.* (2001) was translated into the amount of rosin-derivative which has been leached away from the binder phase at the paint surface just before it is eroded. The loss of binder resins from the pore walls entailed that the leached layer pore volume fraction was not constant along exposure, as in the TBT-SPC model, but time dependent. Such pore enlargement process is directly linked to changes in the pore wall surface area available for seawater reaction and in the pore tortuosity values (defined as the ratio between the length of the actual tortuous pore diffusion path and the leached layer thickness). From these two parameters, the former influences the net binder reaction rate while the latter modifies the diffusive transport of dissolved species along the leached layer. Additionally, the new model contains a more detailed modelling of the seawater speciation by use of the Extended UNIQUAC ion activity coefficient model. Finally, the Cu(I) oxidation process to Cu(II) within the leached layer was included for the first time, which allowed the assessment of the risk of formation of Cu(II) precipitates.

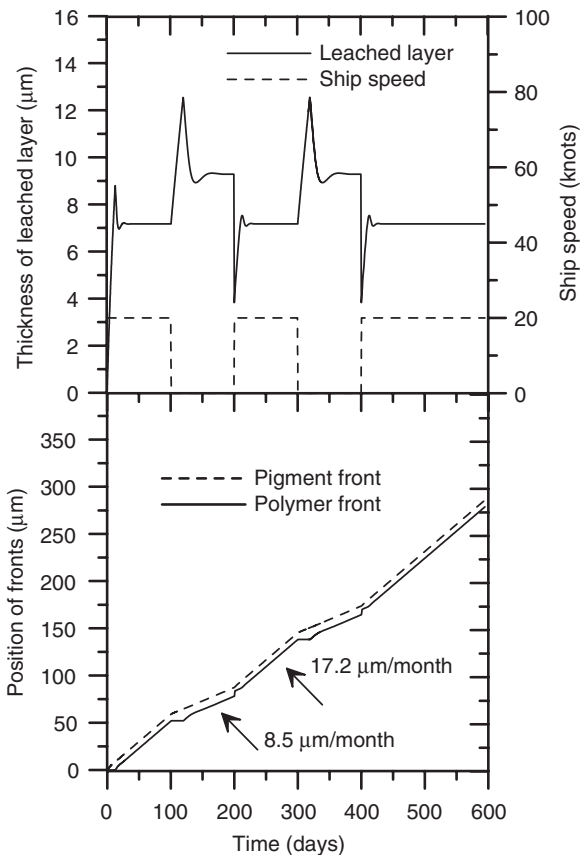
The modelling of experimental data showed that, compared to AF products based on tin-containing acrylic polymers, paints with a large content of rosin derivatives had a faster Cu_2O leaching rate as a result of enhanced water ingress into the paint film and a relatively open leached layer structure. SEMD-EDX results seemed to point at a very fast depletion of the rosin-derivative virtually throughout the leached layer, hence leaving back a porous skeleton consisting of mainly inert paint components. Yebra *et al.* (2006b) hypothesized that it was the physical erosion of such weakened skeleton, and not the zinc-carboxylate depletion kinetics, which determined the polishing rate. Polishing is therefore strongly dependent upon the initial amount of Cu_2O and zinc-carboxylate content of the paint (i.e., water soluble components), which eventually lead to voids at the paint surface.

Other modelling attempts

A recent publication (Howell and Behrends, 2006) contains a qualitative model for the copper leaching from tin-free self-polishing antifouling coating. The exact binder type is not provided, but it is stated that it is a copper-based paint with a proprietary blend of 'binder, plasticizers and resins' containing DCOIT as co-biocide which can be specified for up to 60 months. The model does not consider polishing (very low rotor speeds) and is used to explain in a qualitative manner the experimental observations made (such as an increase in Cu^{2+} release rate when increasing the rotor speed). The model is not used to investigate the effect of formulation variables on polishing and leaching rates due to unknown coating composition.

Dynamic simulations of coating behaviour

The mathematical model underlying the simulations is described in detail in Kiil *et al.* (2001) and used for performing dynamic simulations in Kiil *et al.* (2002b). Here, as an example, the effect of sailing speed changes on the behaviour of a self-polishing antifouling coating is reviewed from Kiil *et al.* (2002b). The sailing speed of large ocean-going ships varies in the interval 0–30 knots (ship in port or at open sea). In Fig. 14.4 the effects of dynamic changes in sailing speed on the rate of movement of the polymer (polishing) and pigment fronts and the thickness of the leached layer are



14.4 Dynamic simulations showing the effects of step changes in sailing (ship) speed on the rate of movement of the pigment and polymer fronts, as well as the thickness of the leached layer. Two points of stable polishing rates are indicated with arrows. From Kiil *et al.* (2002b). With permission of The Federation of Societies for Coatings Technology.

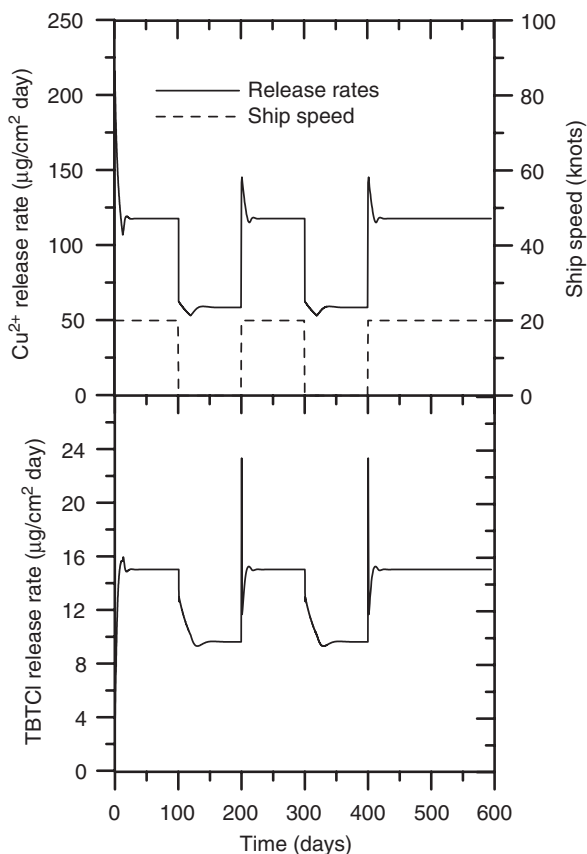
shown. According to the TBT-SPC model, a reduction in sailing speed from 20 to 0 knots initiates a growth of the leached layer. This takes place because the polishing stops completely for 20 days following the speed reduction. At a speed of 20 knots, the value of the surface conversion at which the active polymer is released, $X_{\max}$, is 0.75, whereas at zero speed it is about 0.96 (experimental input provided in Kiil *et al.*, 2001). Thus, the 20 days without polishing corresponds to the time needed for the binder hydrolysis reaction to increase the surface conversion of the active polymer from 0.75 to 0.96. After those 20 days, the polishing is reactivated, though now at a smaller rate, leading to a reduction in the leached layer thickness of about 3 μm . When the speed is increased again from 0 to 20 knots, the leached layer thickness is decreased momentarily from 9 to about 4 μm . The reason for this is that the value of $X_{\max}$ now changes from 0.96 to 0.75. Therefore, a portion (about 60%) of the leached layer is washed away down to the position in the layer at which the conversion of the active polymer is equal to the new value of $X_{\max}$ (i.e., 0.75). From this point and onwards, the leached layer thickness begins to grow again until a stable thickness is reached after about 30 days.

The release rates of Cu^{2+} and TBTCl, during step changes in the sailing speed, are shown in Fig. 14.5. It can be seen that the release rates of biocides drop rapidly when the speed is decreased and become stable after about 80 days. When the speed is increased again to 20 knots, peaks in the release rates are observed. These are due to the aforementioned sudden reduction in the leached layer thickness, which provides a short diffusion path for TBTCl and Cu^{2+} . It should be noticed that the peaks are of a short duration (a few hours or less). It is also evident that even during the periods of a polishing rate of 0 $\mu\text{m}/\text{month}$ there is still a release of TBTCl. This is because the hydrolysis of TBT-polymer takes place throughout the leached layer even when the polymer front is not moving.

In Kiil *et al.* (2002b) dynamic simulations of the effect of pH, temperature, and salinity changes on the polishing and leaching rates are also shown.

Seawater-soluble pigments and their potential use in antifouling coatings

When reading through the open literature, it is clear that a large number of active components are considered for their potential use in self-polishing antifouling paints (see, e.g., review by Rittschof, 2001). These include 'natural' compounds such as terpenoids, steroids, fatty acids, and amino-acids, heterocyclics, and various organic biocides such as copper and zinc pyrithione and Zineb (Yebra *et al.*, 2004). However, the use of 'natural' antifoulants in a functional coating is a technological, financial, temporal and regulatory challenge (Rittschof, 2001). In Kiil *et al.* (2002c) the mathematical model described above was modified to enable a simulation-based



14.5 Dynamic simulations showing the effects of step changes in sailing (ship) speed on the release rates of TBTCI (aq) and Cu^{2+} . Reproduced from Kiil *et al.* (2002b). With permission of The Federation of Societies for Coatings Technology.

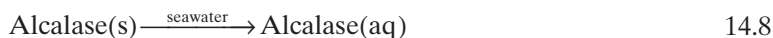
exploration and screening of seawater-soluble pigments for their potential use in a self-polishing antifouling coating. Here, a selected example from that work is discussed (see also Kiil *et al.*, 2006). The reader is referred to the original reference for more details.

‘Pigment’, in the present context, refers to relevant seawater-soluble particulate solids. The reaction that takes place at the dissolving pigment front (see Fig. 14.1) can be written, in general terms, as



The pigment, $P(s)$, can, in principle, be any seawater-soluble particulate solid that is being considered for a potential use in a self-polishing

antifouling paint. Salts, sugars, and proteins (enzymes, peptides, or hormones) are obvious examples. It should be noted that the pigment can also represent a seawater-soluble solid carrier material with any of the aforementioned active ingredients dispersed within the pigment or a coated particle with active material inside. The dissolved pigment species, $S(aq)$, can represent a complex (such as $CuCl_2^-$ in the case of Cu_2O dissolution), an ion (for instance Cu^{2+} if the pigment is $CuCl_2$) or just a physically seawater dissolved species such as sucrose. a and b represent stoichiometric coefficients of a given reaction. Selected examples from the original reference (not all of practical importance) are:



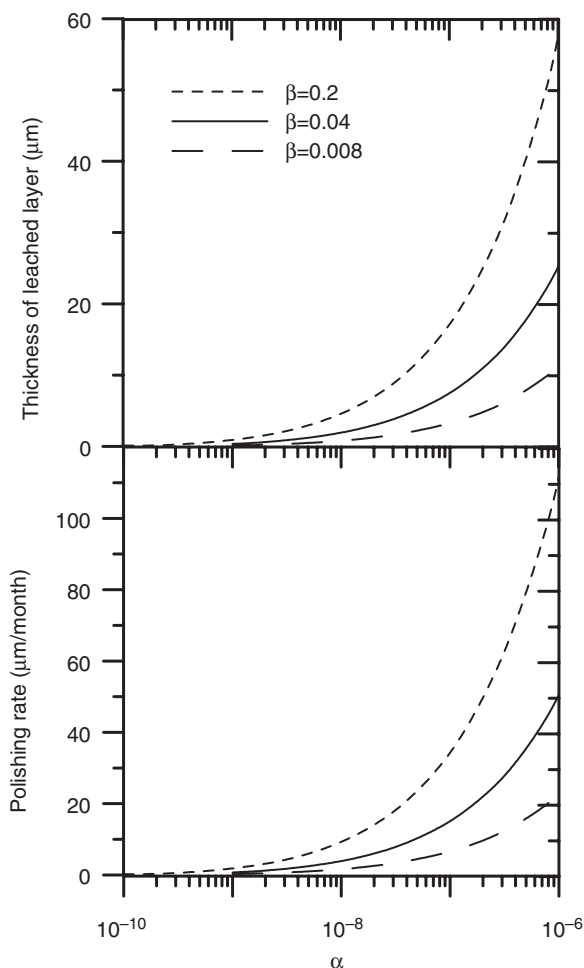
Alcalase is the name of an enzyme that might be used to hydrolyze, e.g., barnacle proteins (see Chapter 23). For the purpose of a general simulation, the model was expressed in such a way that the following two dimensionless parameters could be varied by the user:

$$\alpha = \frac{M_P C_S n}{\rho_P m} \quad 14.9$$

$$\beta = \frac{D_{e,S}}{D_{L,OH^-}} \quad 14.10$$

The parameter α , represents a dimensionless seawater solubility of a given pigment. If the pigment, $P(s)$ of reaction (5), represents a carrier material containing dispersed active ingredients then α must be the dimensionless solubility of this multiphase particulate solid system. β represents a dimensionless effective diffusion coefficient of the dissolved pigment species in the leached layer. Thus, by varying α and β it is possible to see how a given seawater-soluble pigment will influence the polishing and leaching behaviour of a self-polishing antifouling coating.

The effects of the model parameters α and β on the polishing rate and the stable thickness of the leached layer at 30°C are shown in Fig. 14.6. Both parameters have a significant influence on the coating behaviour. Increasing α from 10^{-8} to 10^{-6} , while keeping β constant at 0.04, increases the rate of polishing from 4 to 51 $\mu\text{m}/\text{month}$ and the stable thickness of the leached layer from 2 to 25 μm . Similarly, increasing β from 0.04 to 0.2, while keeping α constant at 10^{-7} , increases the rate of polishing from 15 to 34 $\mu\text{m}/\text{month}$



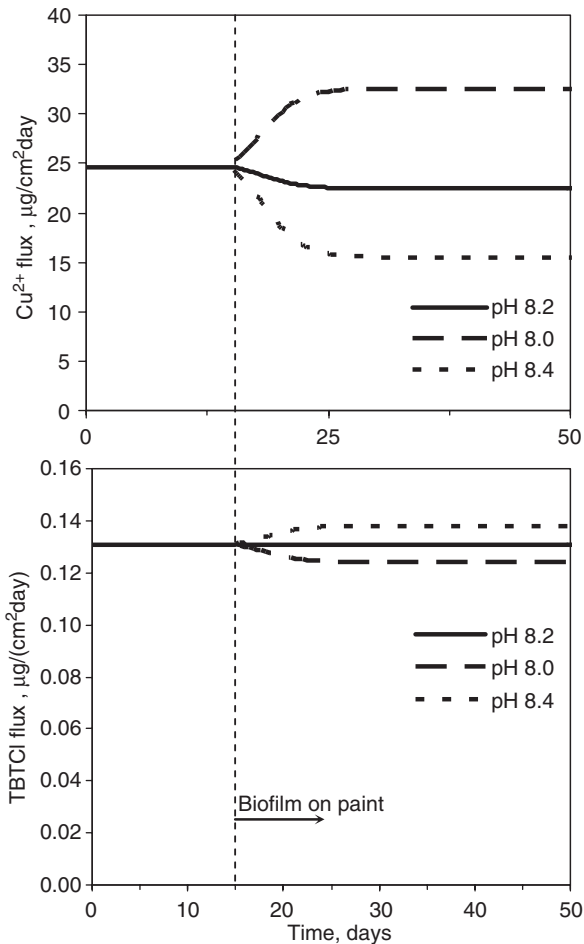
14.6 Simulations showing the effects of the model parameters α and β on the polishing rate (at conditions of a stable leached layer thickness) and the stable thickness of the leached layer. $T = 30^\circ\text{C}$. Reprinted from *Progress in Organic Coatings*, 45, Kiil *et al.*, 'Seawater-soluble pigments and their potential use in self-polishing antifouling paints: simulation-based screening tool', 423–434, 2002, with permission from Elsevier.

and the stable thickness of the leached layer from 8 to 17 μm . At the conditions underlying the simulations of Fig. 14.6, the polishing rate clearly becomes too high at values of α greater than about 10^{-7} ($\beta = 0.04$) or $5 \cdot 10^{-7}$ ($\beta = 0.008$). A commercial self-polishing antifouling coating should typically polish at a rate of 5–15 $\mu\text{m/month}$, depending on the seawater temperature,

pH, salinity and sailing speed. The usefulness of the simulations in Fig. 14.6 is that they can be used for an initial evaluation of a given seawater-soluble pigment. From a calculation of α and β and a reading on the figure, the expected polishing rate and stable leached layer thickness can be estimated. If this exercise suggests a suitable coating behaviour then one may proceed with elaborate rotary experiments. Hence, the mathematical model represents a simulation tool that enables an accelerated screening of new potential seawater-soluble pigments. This is useful information in the design of novel carrier systems or simply for estimating the rate of leaching of a given active ingredient in particulate form.

Effect of marine microbial biofilms on the biocide release rates

The differences between artificial seawater testing (ideal for screening and mechanistic studies) and natural seawater testing should be eventually addressed by AF paint models if a close correlation between simulations and real-life paint performance is desired. A first exploratory attempt was performed by Yebra *et al.* (2006 c,d). In such references, the antifouling (AF) paint model of Kiil *et al.* (2001) and the simplified biofilm growth model of Gujer and Wanner (1990) were coupled to provide a reaction engineering-based insight to the effects of marine microbial slimes on biocide leaching and, to a minor extent, polishing behaviour of AF paints. After pigment/biocide dissolution, dissolved species must diffuse out of the leached layer and through the biofilm layer that will inevitably form on top of the topcoat before reaching the bulk seawater. Along the biofilm, dissolved species will be subject to diffusive limitations as well as to potential adsorption and biodegradation processes. The study by Yebra *et al.* (2006 c,d) examines experimental results reported in the literature to try to explain how marine slimes affect the AF paint processes. The study concludes that the perturbation of the local seawater conditions (e.g., pH), as a consequence of the metabolic activity of the biofilm should not affect the net biocide leaching and binder reaction rates significantly (see e.g., Fig. 14.7). This is related to the thin and poorly active biofilms which presumably grow onto the highly effective modern AF paints (see Chapter 17 for comments on this assumption). According to simulations, the experimental decrease in the biocide leaching rate caused by biofilm growth must be mainly attributed to adsorption of the biocide by the exopolymeric substances secreted by the microorganisms. The effects of biofilms on the leaching of any generic active compound (e.g., natural anti-foulants) are discussed in relation to their potential release mechanisms. The largest influence of biofilms is predicted for those active compounds that are released by a diffusion-controlled mechanism (typically tin-free algaecides).



14.7 Effect of changes in the seawater pH on the Cu²⁺ and TBTCI release rates due to biofilm formation (simulations). The vertical dashed line indicates initiation of biofilm growth, which has been purposely delayed 15 days to better appreciate the effect of the biofilm on the stable performance values. Reprinted from *Progress in Organic Coatings*, 57, Yebra *et al.*, 'Effects of Marine Microbial Biofilms on the Biocide Release Rate from Antifouling Paints: a Model-Based Analysis', 56–66, 2006, with permission from Elsevier.

14.5 Experimental techniques to quantify model input parameters

As it comes from the working mechanisms of chemically active SP paints outlined above, experimental techniques aiming at a model-based characterization of AF paint performance should mainly quantify the

binder reaction, the biocide leaching and the surface polishing processes. We will use these headings to review some approaches used in the literature.

14.5.1 Binder seawater reaction rates

This section will be divided into two subsections, the first one focusing on kinetic studies for the seawater reaction of AF resins. This topic is further divided into two categories depending on whether the seawater-sensitive vehicle to be characterized works through:

- a) hydrolysis of selected pendant groups (see below).
- b) dissolution after seawater reaction (page 352).

Once a kinetic expression for the binder reaction is available, typically in mass loss per unit area and time units, it is necessary to have estimates of the binder surface area available for reaction with seawater within an AF coating. This issue will be tackled on page 354.

Polymeric SP binder with hydrolyzable pendant groups

Even though several current commercial tin-free technologies use resins which are claimed to belong to this family, scientific proof of such a polishing mechanism is only available for TBT-SPCs. In other words, there is no scientific evidence of a controlled hydrolytic cleaving of pendant groups for any of the currently available commercial SP polymers. The reader must be aware that attaining controlled hydrolysis rates for the pendant group is only part of the requirements for a SP resin. First of all, the initial properties of the polymer must fulfil a series of basic *sine qua non* requisites which apply to any generic paint (applicability, recoatability, mechanical properties, weathering resistance, controlled release properties, etc.; see Chapter 13). When this is achieved, it is then necessary to couple the controlled loss of the pendant group to a progressive shift to specific final polymer properties. Only then a suitable polishing rate can take place. It is therefore not surprising that in spite of so many years of research towards novel AF resins, there are only very few well-functioning SP resins in the market. This section will provide sources of information to inspire potential future studies on reaction rates of innovative tin-free hydrolysable polymers.

Kiil *et al.* (2001) used experimental information from different literature sources to build up a kinetic expression for the hydrolysis reaction of poly(tributyltin methacrylate-methyl methacrylate) polymers (TBTM-

MMA) in seawater. The final expression summarizes the relevant parameters to be studied:

$$(-r_{\text{TBTCP}}) = \frac{k_{\text{SL}} \cdot x_{\text{TBTM}} \cdot [\text{OH}^-]^a \cdot [\text{Cl}^-]}{(1 + k_2 \cdot [\text{OH}^-]^b)} \quad \text{for } x_{\text{TBTM}} < 0.28 \quad 14.11$$

$$(-r_{\text{TBTCP}}) = \frac{k_{\text{SH}} \cdot (x_{\text{TBTM}} - \sigma) \cdot [\text{OH}^-]^a \cdot [\text{Cl}^-]}{(1 + k_2 \cdot [\text{OH}^-]^b)} \quad \text{for } 0.28 < x_{\text{TBTM}} < 0.52 \quad 14.12$$

where:

x_{TBTM} : related to the content of hydrolysable groups on the reaction rate (decreasing with reaction time). See, e.g., Takahashi and Ohyagi (1987).

k_{SL} and k_{SH} : kinetic constants with implicit temperature dependence (activation energy). See, e.g., Takahashi and Ohyagi (1987).

$[\text{OH}^-]^{a,b}$: obtained from studying the influence of seawater pH on the reaction rate. See, e.g., Yuanhui *et al.* (1988).

$[\text{Cl}^-]$: obtained from studying the influence of seawater salinity on the reaction rate. See, e.g., Somasekharan and Subramanian (1980).

Finally, an estimation of the reverse reaction kinetics is also necessary (Kiil *et al.*, 2001), even though it will be negligible in most cases when the reaction rate is slow compared to the mass transport rate away from the reacting surface. The numerical values and exact meaning of all these parameters can be found in Kiil *et al.* (2001), and were obtained from TBT⁺ release data from pure TBTM-MMA polymers exposed to seawater. Different techniques, such as, e.g., gas chromatography and atomic force microscopy (AFM), were used to quantify the degradation rates. It is important to stress that experimental kinetic studies must be performed in absence of diffusive limitations or, at least, under well-characterized mass transport conditions. For very slow reactions, the overall reaction rate is usually controlled by the reaction kinetics, and diffusive limitations can be neglected. The reader is forwarded to any book on chemical reaction kinetics for an appropriate design of experiments. Ideally, the potential effects of other compounds which can interfere with the hydrolysis reaction under real-life exposure conditions (e.g., Cu²⁺, Zn²⁺, Ca²⁺, organic biocides, co-binders) should also be assessed.

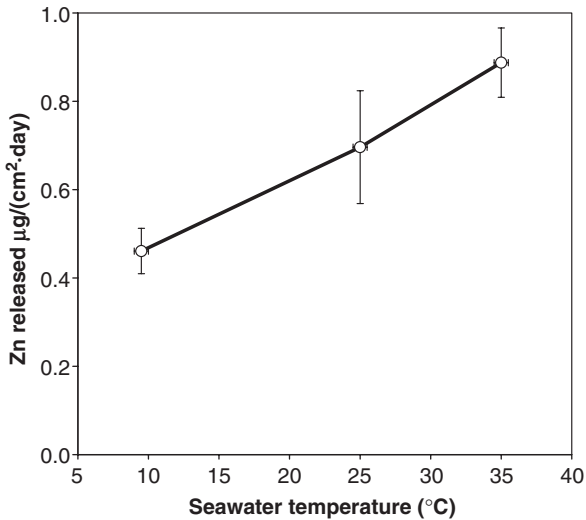
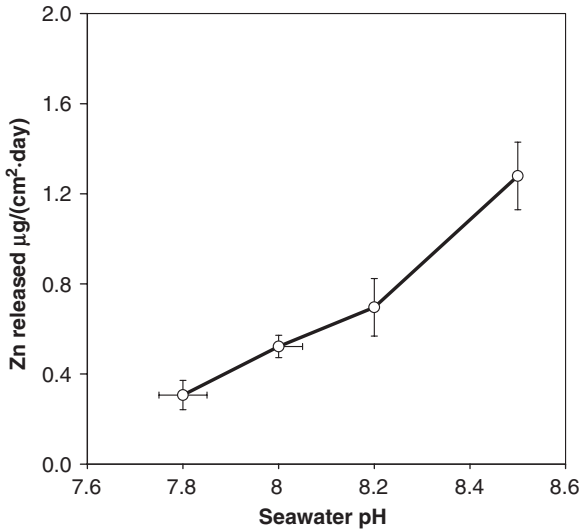
As a reference dealing with tin-free polymers, we can mention the study by Langlois *et al.* (2002) studying the degradation of poly(lactic acid) (PLA) methacrylate, a hydrolyzable macromonomer, and tert-butyl acrylate. These authors investigated the influence of different polymer parameters on the hydrolysis kinetics as well as the seawater temperature. The copolymer degradation was monitored in this case by analyzing the concentration of

dissolved lactic acid, together with pH and size exclusion chromatography measurements.

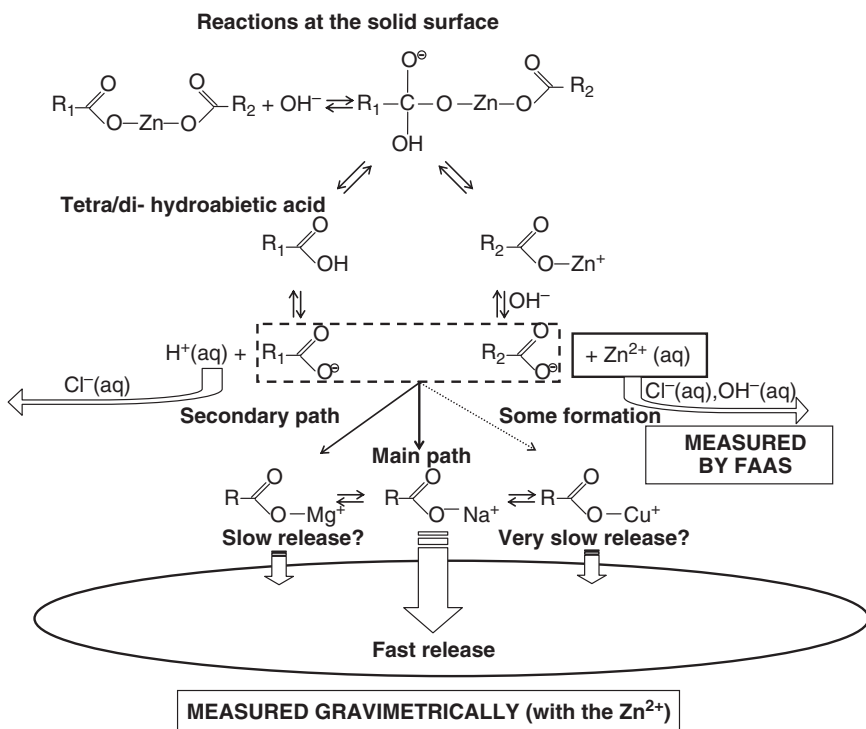
Seawater soluble resins

Rosin derivatives are one of the most widespread AF paint binders, being the main binder of most short specification time AF coatings (i.e., up to 36 months of drydocking intervals) and a few 60+ months SP coatings. Extending the active lifetime from 36 to 60+ months relies on the choice of rosin-derivative (e.g., gum rosin vs. the Zn-carboxylate of tetrahydrogenised abietic acid; Yebra *et al.*, 2005), the choice of co-binders and biocide package and additional formulation improvements (e.g., the addition of mechanically reinforcing microfibres; Yebra *et al.*, 2004). The reasons behind the wide use of this family of resins are their low price, wide availability and a more than acceptable control of the polishing and leaching rates. Contrarily to hydrolysable acrylic SP resins, rosin-derivatives dissolve fairly fast in seawater, leaving behind a weak skeleton of inert binder components which can be chosen to polish at the desired rate through inert paint components. The few research efforts aimed at characterizing the dissolution of this kind of resins were summarized by Yebra *et al.* (2005). From these, perhaps the most comprehensive is that presented in that same paper, in which the dissolution rate of the zinc carboxylate of a purified and hydrogenized derivative of abietic acid was assessed under static/mild dynamic conditions. This resin, used in combination with selected co-binders and mechanically reinforced micro-fibres has been claimed to lead to excellent AF performances to the level of TBT-SPCs (Yebra *et al.*, 2004).

In Yebra *et al.* (2004), very thin glass panels were coated with the relevant physically drying resin so that the exposed surface area was carefully controlled. At least three replicates were used. The films were temperature dried and then exposed to seawater. Subsequently, two experimental procedures were used to characterize the reaction of the seawater sensitive resin: one is based on a gravimetric approach while the other uses flame atomic absorption spectroscopy (FAAS) to determine the total Zn^{2+} released by the resin. The experimental procedures developed were used to investigate the influence of NaCl concentration (12–52 g/l), pH (7.8–8.5) and seawater temperature (10–35 °C) on the rate of reaction of the Zn-carboxylate. Additionally, issues such as the risk of diffusion control in the experiments, the influence of the reverse reaction, the effects of divalent seawater cations on the reaction rate, the nature of the reaction products and the influence of plasticizing co-binders were discussed in the paper. The influence of seawater pH and temperature on the reaction rate as it comes from Yebra *et al.* (2005) is shown in Fig. 14.8, while the proposed reaction mechanism is outlined in Fig. 14.9.



14.8 Steady state Zn^{2+} release rates obtained at 25 °C and different sea water pH values with the AAS-based method (top). The influence of the seawater temperature on the Zn^{2+} release rate is also shown (bottom; pH = 8.2). Reprinted from *Progress in Organic Coatings*, 53, Yebra *et al.*, 'Reaction rate estimation of controlled-release antifouling paint binders: rosin-based systems', 256–275, 2005, with permission from Elsevier.



14.9 Hypothesized ZnR reactions upon immersion according to the experimental information gathered in this work. Reprinted from *Progress in Organic Coatings*, 53, Yebra *et al.*, 'Reaction rate estimation of controlled-release antifouling paint binders: rosin-based systems', 256–275, 2005, with permission from Elsevier.

Morphological characterization of leached layers using BET surface area measurements and mercury porosimetry

Reliable mathematical models for SP coatings rely on an accurate knowledge of the structure of the leached layer. This is because both the binder reaction and the mass transport rates for all dissolved species participating in the paint's working mechanisms depend on the morphology of such a porous layer. In Kiil and Dam-Johansen (2007), BET surface area measurements and mercury porosimetry were used to characterize leached layers formed when seawater-soluble pigments (Cu_2O and ZnO) dissolve during accelerated leaching (1 M HCl) of simple antifouling coatings. Measurements on single-pigment coatings showed that an increasing fraction of Cu_2O or ZnO pigment particles becomes unavailable for dissolution when the concentration of the pigment decreases in the coating and the interparticle distance in the binder matrix becomes larger.

Experimental data for a coating initially containing a mixture of Cu_2O and TiO_2 pigments suggested that a substantial fraction of the smaller and inert TiO_2 particles may be lost from the coating upon dissolution of the larger Cu_2O particles. This inert particle translocation effect is important to take into account when interpreting polishing and leaching data and when developing mathematical models of antifouling coating behaviour because the active binder surface area and porosity of the leached layer are substantially increased. A similar effect was not observed for a coating with a mixture of ZnO and TiO_2 pigments. Both BET and mercury porosimetry-based experimental methods are expected to be useful for future analysis of leaching of seawater-soluble components from commercial antifouling coatings.

14.5.2 Pigment dissolution rates of copper oxide (I) or cuprous oxide

Only one scientific paper dealing with the dissolution rate of Cu_2O in seawater, dating back to 1946 (Ferry and Carritt, 1946) has been found in the open literature. This is surprising since most commercial AF coatings typically contain about 40%_w of this pigment in the dry film. This fact demonstrates that the development of antifouling paints has not relied, at least to a great extent, on chemical reaction studies. In order to determine the seawater reaction rate of Cu_2O , Ferry and Carritt incorporated Cu_2O pigment particles into an insoluble Vinylite binder. The amounts of Cu_2O used were 90%_w and 80%_w in weight (i.e., about 65% and 45% in solids volume percent respectively). Ferry and Carritt used the data obtained from the coating with 90%_w Cu_2O and assumed that the exposed Cu_2O surface area was equal to that of the panel, the portion occupied by the binder being counterbalanced by the irregularities of the pigment surface. That seems a fairly coarse assumption, and it is difficult to assess its effect on the final rate values.

The effect of agitation speed on the dissolution rate was tested in order to investigate how important the diffusion limitation contribution was to the release rate values measured. The results obtained with the coating formulated with 90%_w Cu_2O showed that the higher the agitation speed, the faster the dissolution rate, thus suggesting an influence of external mass transport limitations. Although not stated clearly we assume that the measurements were taken before any leached layer is built up, i.e., the internal diffusion through the Cu_2O -leached paint layer was negligible. The paint formulated with 80%_w Cu_2O showed a constant dissolution rate at relatively fast agitation rates, suggesting kinetic control of the dissolution process.

In order to assess the dependency of the reaction rate on pH, 0.1 M sodium borate buffers between 7.08 and 8.48 were used in 0.48 M NaCl solutions. The results obtained with high agitation rates (kinetic control) show an

almost linear dependency of the H^+ concentration. The buffer at pH 7.88 was used later on to assess the influence of the NaCl concentration. Special care was taken not to modify the ionic strength of the solution to a large extent. A second order dependency with the chloride ions was found.

The measurements at different temperatures were performed in seawater. Subsequently, changes in the temperature affected the pH by perturbing the carbonate system equilibrium with the atmosphere. Although the effect was not very marked, the authors corrected for the pH effect by making use of the linear dependency of the dissolution rate with the H^+ concentration. The results obtained from a paint containing 80%_w Cu_2O led to a good fitting with an Arrhenius-type equation.

The sum of all these experimental inputs led to the following kinetic expression:

$$(-r_{Cu_2O}) = k_1 \cdot [H^+] \cdot [Cl^-]^2 \quad 14.13$$

where $k_1(25^\circ C) = 2.35 \times 10^{-7} \text{ molCu}_2\text{O} \cdot (\text{mol/m}^3)^{-3} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The reverse reaction is assumed to be first order in the concentration of $CuCl_2^-$, thus (Kiil *et al.*, 2001):

$$(-r_{CuCl_2^-}) = k_{-1} [CuCl_2^-] \quad 14.14$$

At equilibrium:

$$(-r_{CuCl_2^-}) = 2 \cdot (-r_{Cu_2O}) \quad 14.15$$

which leads to an expression for k_{-1}

$$k_{-1} = k_1 \frac{2 \cdot K_w}{K_{CuCl_2^-} \cdot L_{CuOH} \cdot \gamma_{\pm}^2} \quad 14.16$$

14.5.3 Pigment dissolution of zinc oxide

Yebra *et al.* (2006e) tried to optimize the kinetic study discussed above to quantify the ZnO dissolution process in seawater. To overcome the uncertainty in the pigment surface area value exposed to seawater, Yebra *et al.* (2006e) chose to use pure ZnO pellets of well-defined geometry. In a first attempt, a few grams of technical grade powdery ZnO (white) were inserted into a 13 mm diameter cylindrical mould together with a drop of distilled water to improve the cohesiveness of the final pellet. A pressure of $8.9 \text{ N} \cdot \text{m}^{-2}$ was applied to the samples by means of a stainless steel cylinder. The pellet was subsequently introduced into an electric oven at $1200^\circ C$ for 24 hours within a porcelain crucible. Four such pellets were placed onto a glass lid and pressed firmly against its bottom surface. Subsequently, highly-hydrophobic, melted paraffin wax was added to the lid in sufficient amounts to cover the pellets. After demolding, the ZnO surfaces which were in

contact with the glass surface were free of any paraffin and ready to be exposed. On a second attempt, the pellets were replaced by monocrystalline $10 \times 10 \times 0.5$ mm ZnO films purchased from MolTech GmbH, Germany. The surfaces were EPI polished (i.e., virtually smooth) and the crystal's flat orientation is (001).

The ZnO samples were subsequently exposed to seawater in a 500 ml jacketed glass reactor. A volume of 300 ml of artificial seawater was added to the reactor, which was closed by means of a rubber lid (paraffin films were used to seal the lid). The lid was fitted with a thermometer, a pH electrode and the capillary of an automatic titration system used to assure a stable pH during the experiments. The temperature was kept constant by flowing water from a thermostatic bath through the reactor jacket. A velocity-adjustable four-blade axial flow impeller was used to stir the solution and avoid mass transport-controlled dissolution rates. At specific time intervals, 5 ml aliquots of the dissolving medium were withdrawn from the reactor and filtered through $0.45 \mu\text{m}$ disposable hydrophilic filters. The samples were stored in paraffin film-sealed polyethylene containers and subsequently placed in a refrigerator. The total Zn content of the samples was finally measured in a Perkin Elmer Sciex ELAN 5000 induced-coupled plasma-mass spectrometer (ICP-MS). The results show dissolution rates slower than those of Cu_2O , about three times faster for the case of sintered pellets compared to the smooth crystals. Pores, surface roughness and impurities are believed to explain such results. Potential uncertainties associated with this experimental procedure are analysed in detail in Yebra *et al.* (2006e).

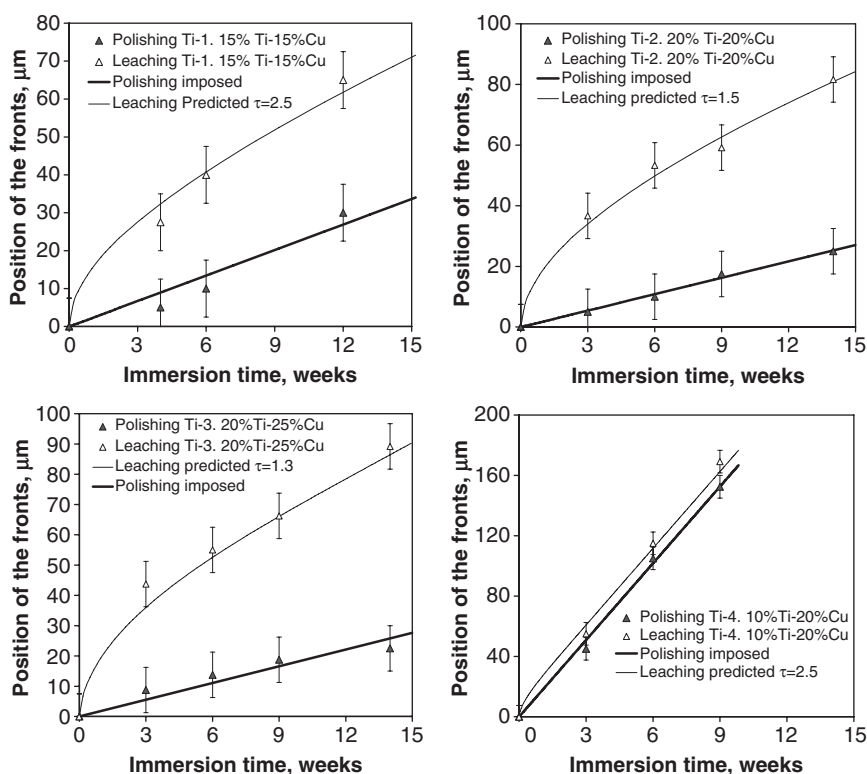
14.5.4 Pigment dissolution rate from AF paints

In Kiil *et al.* (2001), the assumption of kinetically controlled Cu_2O leaching and the subsequent use of Ferry and Carritt's equations was found to fit very well the Cu^{2+} release rates from model TBT-SPC paints. In the TBT-SPC model, the exposed Cu_2O surface area was assumed to equal the pigment volume concentration of this pigment into the formulation. In other words, the Cu_2O particles were assumed to be perfectly surrounded by the very hydrophobic, undegraded TBTM-MMA polymer, which did not allow any water to reach the Cu_2O beyond the "pigment front" (Fig. 14.1). In light of Kiil *et al.* (2001), such assumption seemed fairly realistic.

Unfortunately, such assumptions do not hold for every AF system. Experimental performance testing of model antifouling paints formulated with ZnO and/or Cu_2O (Yebra *et al.*, 2006b,e) demonstrates that the binder/pigment interaction cannot always be disregarded. In those studies, the release rates of those pigments were found to be much faster than predicted from dissolution rate studies with 'pure' pigments. In rosin-derived vehicles, seawater was hypothesized to dissolve pigment particles away from the

'pigment front', potentially leading to saturation of dissolved pigment species in the vicinity of the pigment surface and leading to a diffusion-controlled leaching process. Also, the typical copper leaching curves obtained from rotary ASTM/ISO standardised tests (Haslbeck and Holm, 2005) show a clear diffusion-dependent shape, which cannot be modelled through kinetically controlled Cu_2O leaching equations.

The consequence of the above is that studies based on pure compounds (i.e., pigments and solid organic biocides) may not represent real-life leaching scenarios. In this case, leaching rate studies from full paint formulations should be conducted. Yebra *et al.* (2006b) is an example of such kind of studies. Leaching rate measurements from paint samples exposed to laboratory-scale lab rotors (see Chapter 16) were used to model the Cu_2O leaching rate (see Fig. 14.10). The model prediction relies on the



14.10 Experimental (symbols) and predicted (lines) position of the polishing and leaching front for different model paints containing Cu_2O and TiO_2 . The polishing rate was imposed to a constant value, so that only leaching was modelled. Reproduced from Yebra *et al.* (2006b) with permission from Wiley Interscience.

mathematical solution of a complex set of differential chemical speciation balances including, e.g., H^+ (i.e., pH), Cu(I) and Cu(II) concentration gradients and a pore enlargement process which modifies the effective diffusion coefficients as the binder reaction proceeds.

Experimental Cu_2O leaching data can be obtained via microscopical examination of aged samples. That is because Cu_2O usually possesses a distinctive red colour, so that it is possible to measure the remaining Cu_2O in the film and subsequently calculate the amount of copper released over the test (Yebra *et al.*, 2006b). A different approach is to measure the dissolution products released into the seawater instead. In the ISO/ASTM D4662-99 copper release rate procedures, the paint to be studied is slowly rotated in a known volume of water for one hour, after which the concentration of the relevant dissolution products in the seawater is measured (Chapter 19). Such procedure is repeated after specific exposure times, typically covering ageing times of about 45 days. Between measurements, the coated specimens are kept under static conditions in a holding tank with seawater. Yebra *et al.* (2006b), showed that ASTM/ISO Cu^{2+} release data can be successfully fitted to mathematical expressions using parameters with physical meaning. Even though the reliability of the ASTM/ISO ageing method is questionable, it is hypothesized that the same mathematical expressions would reproduce any other more reliable Cu^{2+} release data. Howell and Behrends (2006) used a rotary set-up in which the seawater was continuously driven through both an ion-exchange column and a solid phase extraction column placed in series to retain the released Cu^{2+} and organic biocides respectively. Quantitative analysis of the biocides trapped in the columns over a certain amount of time allows the characterization of the biocide lixiviation process out of AF paints. The release results were also interpreted by means of simpler diffusion equations and used to explain the paint behaviour. Finally, another technique which has been used to quantify the Cu^{2+} release process is SEM-EDX (see Fay *et al.*, 2005). In this case, the remaining copper in the paint film was measured as a function of exposure time. SEM-EDX is a very powerful technique to monitor the release of other raw materials which cannot be detected with the optical microscope. Fay *et al.* (2005) performed release studies of a set of organic co-biocides from AF paints by means of SEM-EDX.

14.5.5 Surface polishing

With the experimental techniques outlined so far, we should be capable of modelling the dissolution process of soluble particles within the film and the subsequent formation of a leached layer characterized by a certain surface area. The binder exposed at the walls of such pore network will react at a rate estimated through the experimental techniques presented by

Section 14.5.1. However, the remaining question is: to what extent should this binder react to weaken the paint surface to a point in which it can be polished by moving seawater? So far only Kiil *et al.* (2001) and Yebra *et al.* (2006a,b) have addressed this point, with dissimilar conclusions.

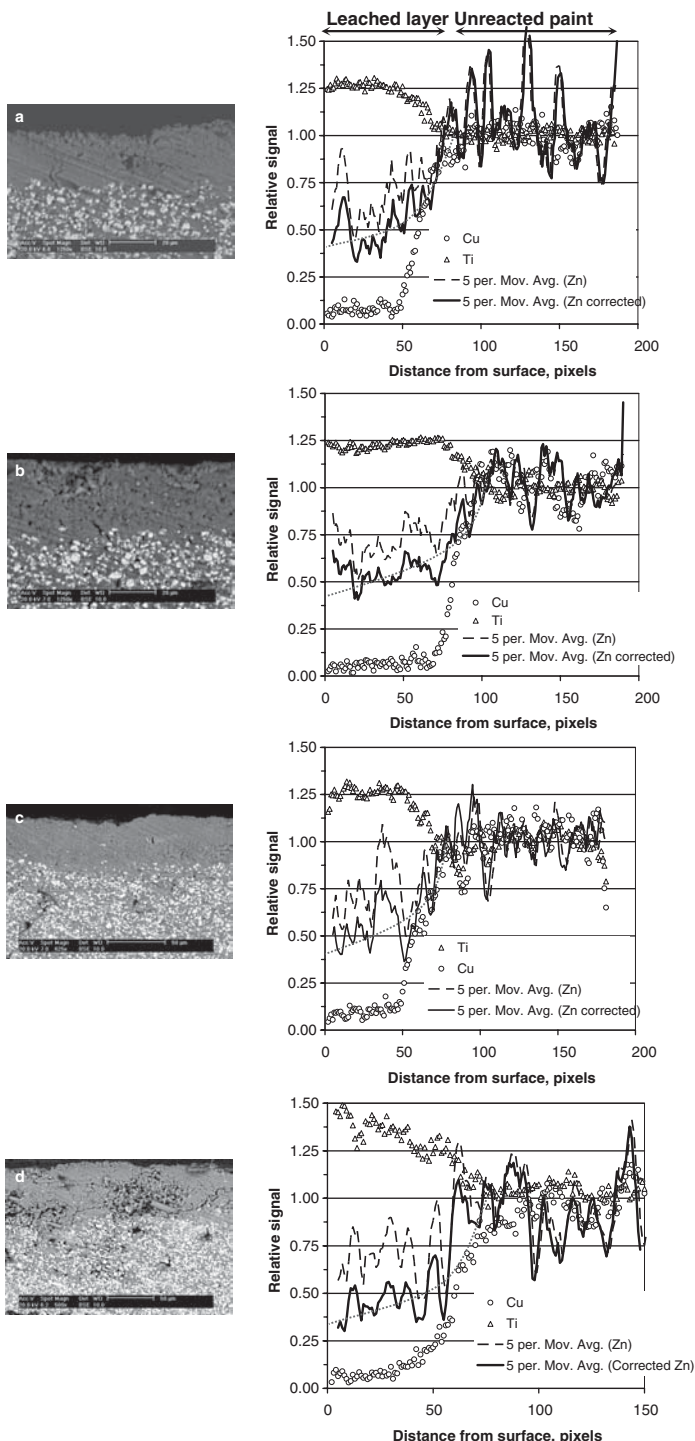
Kiil *et al.* (2001) used SEM-EDX to measure the Cu and Sn profiles along the leached layer of model TBT-SPC paints containing Cu_2O as the only pigment. These paints had been previously subjected to ageing in artificial seawater by means of a laboratory rotary set up described in more detail in Chapter 16 (see also Weinell *et al.*, 2003 and Yebra *et al.*, 2006a). The $X_{\max}$ values measured via SEM-EDX (i.e., Sn relative surface concentration) and those fitted values which led to an excellent modelling of the polishing rate were in close agreement. Kiil *et al.* concluded that $X_{\max}$ values in the range from 0.65–0.75 could predict the polishing of model paints very well and provide a fair estimate of the polishing rate of commercial paints.

Rosin-derived vehicles were found to behave differently. The exposed paints were also analysed by means of SEM-EDX analysis so as to quantify the degree of binder degradation which characterizes the paint surface during the constant polishing regime. According to the experimental evidences gathered, most of the paints presented $X_{\max}$ values within 0.5–0.7 regardless of its composition. Such a conversion value was attained after a short immersion time (less than three weeks; see Fig. 14.11). The fact that a flat and stable binder conversion profile was measured after such a short exposure time suggests that:

- the available seawater sensitive resin dissolves fairly fast throughout the leached layer reaching such maximum conversion value
- part of the soluble binder is unavailable for seawater reaction
- polishing is determined by the rate of erosion of a paint skeleton totally depleted of Cu_2O and partially depleted of the Zn-carboxylate
- the $X_{\max}$ concept cannot be used to model surface polishing from such paints.

14.6 Future trends

Coating producers on the global market of antifouling coatings are facing severe challenges in the attempt to develop efficient and environmentally friendly coatings. New active compounds are being developed and the need for design of efficient and stable release systems for use in various types of coatings is evident. Mapping of controlled release mechanisms and development of mathematical coating models quantifying these systems can help in the design procedure. Much is to be done, considering the complex nature of coating formulations, but the basic foundation has been developed and is available in the open literature. Now it is up to the coating producers to



14.11 SEM pictures (left) and EDX analysis results (right) of TiO₂-containing rosin-derived AF model paint formulations exposed to sea water for a) 3 weeks, b) 6 weeks, c) 9 weeks and d) 14 weeks. The value '0' in the x-axis on the right-hand plots corresponds to the paint surface in the SEM pictures. Reproduced from Yebra *et al.* (2006b) with permission from ACS publications.

implement these advanced tools in the learning and development process of their research laboratories.

14.7 Nomenclature

BET	Brunauer, Emmett, and Teller isotherm for multilayer adsorption, m^2/g
C_s	seawater solubility of pigment, mol/m^3
$D_{e,i}$	effective diffusivity of component i in the seawater-filled leached layer, m^2/s
$D_{L,i}$	molecular seawater diffusivity of component i , m^2/s
i	component or species i
k_{SL} and k_{SH}	Surface rate constant of the hydrolysis reaction in equations [14.11] and [14.12]
k_1	surface rate constant for the Cu_2O seawater dissolution reaction
k_{-1}	surface rate constant for the reverse Cu_2O seawater dissolution reaction
k_2	parameter in rate expression for the tributyltin methacrylate hydrolysis
K_w	equilibrium constant for the water dissociation reaction
K_{CuCl_2}	equilibrium constant for the CuCl_2 complexation reaction
L_{CuOH}	solubility product of CuOH
n	reactant stoichiometric coefficient in equation [14.5]
m	product stoichiometric coefficient in equation [14.5]
M_i	molar mass of species i , kg/mol
P	pigment
$r_{\text{Cu}_2\text{O}}$	rate of dissolution of Cu_2O in seawater
r_{CuCl_2}	rate of formation of CuCl_2 in seawater
r_{TBTCP}	rate of hydrolysis reaction of tributyltin methacrylate monomer units
S	dissolved pigment or pigment product
T	temperature, K
x_{TBTM}	molar fraction of tributyltin methacrylate in the copolymer
X_{max}	value of surface conversion at which the active polymer (TBTCP) is released into seawater
$[i]$	concentration of species ' i ' in seawater
$[\text{OH}^-]^{a,b}$	hydroxyl ion concentration; ' a ' and ' b ' are reaction orders

Greek Letters

- α dimensionless model parameter, defined in equation [14.9]
 β dimensionless model parameter, defined in equation [14.10]

- $\gamma_{\pm}$ mean ion activity coefficient
 σ parameter of equation [14.12]

Superscript

- o diffusivity at 25°C and infinite dilution

14.8 References

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High throughput methods for the design of fouling control coatings

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Abstract: The use of high throughput methods for the synthesis, formulation, and testing of libraries of coatings having differing compositions is discussed. The design of new non-fouling coatings is challenging due to the large diversity in chemical composition and the fact that the effect of composition of the coatings on biofouling is unknown. Combinatorial coating preparation involves the synthesis of a large number of coatings using high throughput methods. These coatings are then screened for key mechanical and surface properties. In order to be able to downselect coatings for field testing, a number of laboratory assays have been developed to challenge these coatings with representative marine organisms such as bacteria, algae, and barnacles. Correlations of the lab assays with field test results indicates that these lab assays are useful predictors of short-term coating performance.

Key words: biofouling, high throughput, combinatorial, coatings, non-fouling coatings.

15.1 Introduction: the need for high throughput methods

The primary method for mitigating biological fouling on underwater structures, such as ship hulls, is to coat those structures with an antifouling coating. While many different types of coatings have been employed in the past, current coatings technology consists primarily of biocides dispersed in what are termed either ablative or self-polishing coating binder systems. In operation, the biocides are slowly leached out of the coatings and the coating binder degrades through processes including hydrolysis, dissolution, and/or erosion. Now that coatings containing tributyl tin (TBT) are being eliminated from use in antifouling coatings due to environmental concerns (Champ, 2000), the most commonly used biocide is cuprous oxide, often supplemented with organic or organometallic ‘booster’ biocides (Jacobson and Willingham, 2000; Omae, 2003). It is expected that future regulations will limit the amount of copper that can be discharged into the environment from antifouling coatings.

Extensive research and development activities are being carried out worldwide to design new antifouling coating systems (Chambers *et al.*, 2006; Rascio, 2000; Yebra *et al.*, 2004). The primary driver for much of this activity is environmental and has two areas of focus: (a) to reduce the amount of volatile organic compounds (VOCs) emitted from the paint during application, and (b) to minimize the impact that the coating has on the environment during its service lifetime. The latter area is receiving the most attention in the research community today.

The term 'antifouling' can be used in two distinctly different ways with respect to coatings technology. In a general sense, antifouling is used to describe any coating system which mitigates fouling by marine organisms, regardless of the mechanism. In a more narrow sense, the term antifouling is used to describe coatings that deter fouling by organisms, usually through chemical means (biocides; see Chapters 12–14). The use of the term in this way emphasizes the contrast between coatings that actually deter fouling and those coatings that do not deter settlement of fouling organisms, but do not allow organisms to form a strong adhesive bond to the coating surface. These latter coatings are often known as fouling-release or easy-release coatings (Brady, 2000; Brady *et al.*, 1987) (see Chapter 22).

In the area of biocidal antifouling coatings, research is being carried out to design coatings containing less toxic biocides, and binder systems that more effectively control the release of biocides into the environment (Thouvenin *et al.*, 2003, 2002; Vallee-Rehel *et al.*, 1999, 1998). In addition, extensive research is being conducted to identify and optimize coatings that are either non-fouling through some physical mechanism or which are effective fouling-release or easy-release coatings (Brady, 1999; Brady *et al.*, 1987; Burnell *et al.*, 2000; Chambers *et al.*, 2006; Genzer and Efimenko, 2006; Hoipkemeier-Wilson *et al.*, 2004; Majumdar *et al.*, 2007).

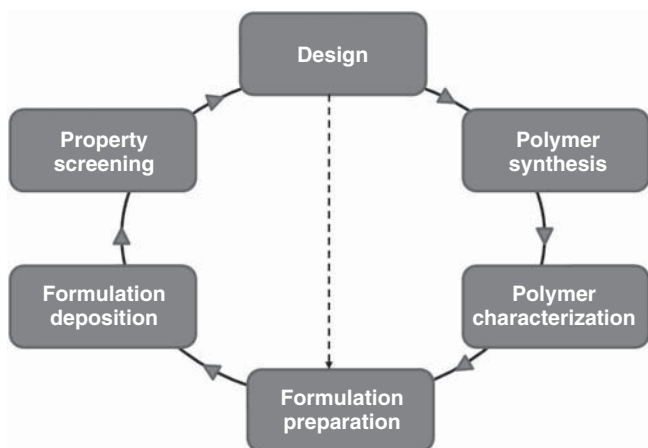
Coatings are highly complex systems which contain a large number of ingredients that are intended to work together to achieve a desired set of application and coating performance properties. A typical coating formulation consists of the binder system, pigments, pigment dispersants, solvents and a whole host of possible additives which can function to adjust the rheological, flow, leveling, adhesion, and durability properties of the coating (see Chapter 27b). Multiple pigments are used to adjust the color to a desired value, multiple solvents are used to control the drying properties of the coating, and multiple additives are used in combination according to their intended (and sometimes non-intended) function. A 'simple' coating formulation may have fifteen ingredients, while it is typical for a commercial formulation to have many more. Polymer binder systems can consist of polymers, oligomers, crosslinkers, and combinations thereof. Variations in polymer molecular weight, composition, functional group concentration, and polymer-crosslinker stoichiometry can all influence the properties of the coating.

The formulator's challenge is to come up with a coating system that meets all of the performance requirements simultaneously. This is usually done through a series of incremental experiments where several experimental batches of paint are prepared in the laboratory, tested for their properties, and the results used to determine the composition of the next set of trial batches. This iterative trial-and-error approach is carried out until a satisfactory solution is found or, more typically, it is time to pick several of the best formulations and conduct field trials. Additional modifications to the formulation are made following field trials. While statistical experimental design methods might be employed in the research stage in order to increase the efficiency of the experimental process, this approach appears to be employed only sparingly.

The conventional approach to experimentation often relies on the skills of an experienced formulator. Knowledge gained is recorded in laboratory notebooks in various formats and may not be accessible to other formulators in an organization. Transferring the knowledge of an experienced formulator to a new scientist is often challenging and it is not uncommon that new formulators often have to begin anew to accumulate their own expertise.

The combinatorial and high throughput approach is a methodology which seeks to overcome these limitations in designing coating formulations. Inspired by combinatorial chemistry – where thousands of compounds are synthesized and screened for activity (Bannwarth and Hinzen, 2006; Fassina and Miertus, 2005; Lowe, 1995) – the combinatorial approach to designing coating formulations involves the preparation of a large number of materials having a systematic variation in composition and screening those materials for their key performance properties. To facilitate combinatorial experiments, high-throughput methods are often employed, which use automated systems to carry out the preparation and characterization of arrays or libraries of materials. An information management system is also employed to assist in experimental design, experiment execution, and for storage of formula composition information and the results of the screening experiments.

Combinatorial and high-throughput methods for polymer synthesis, coating formulation and screening have emerged in the past decade as powerful tools for exploring coating systems. This approach results in a number of advantages. First, by accelerating the rate at which compositions are prepared and screened, it is possible to identify a composition that meets the performance requirements – often called a 'lead' – in a short period of time. In addition, by capturing all of the compositional and screening information in a database, it is possible to develop knowledge about a system at a level that could not be achieved using conventional experimental methods. Finally, using this knowledge, it is also possible to further optimize a system so that a best performing composition or formulation can be identified.

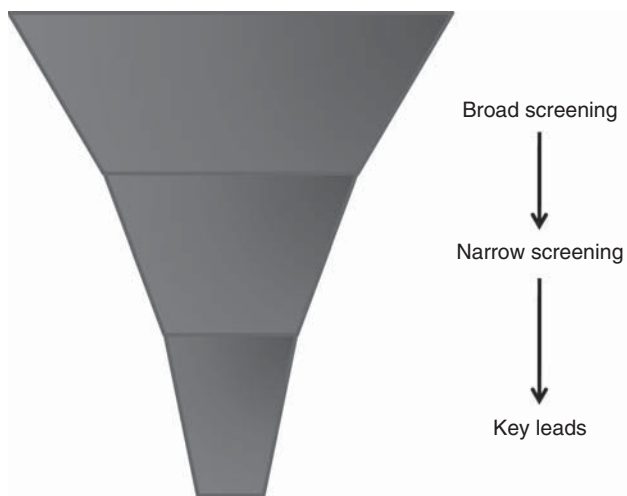


15.1 Combinatorial workflow for designing coating systems.

One useful organizing concept when contemplating a combinatorial approach is that of the workflow. A typical overall workflow for coating development is illustrated in Fig. 15.1. The workflow helps identify and organize the key steps required to conduct successful combinatorial experiments. It is important that each step in the workflow be carried out at approximately the same throughput in order to prevent bottlenecks in the overall process. A series of combinatorial experiments is often carried out where broad screening of a compositional space is conducted first, followed by exploration of a portion of the initial space in more detail until a lead composition or compositional area emerges (Fig. 15.2).

It is also important to understand that combinatorial and high throughput methods do not replace every aspect of research and development. The scientist still has to develop a sound and testable hypothesis before conducting a combinatorial experiment. With the implementation of any high throughput method, it is also important to verify that the experiments conducted using high throughput systems are truly representative of the same material made using conventional methods. This is crucial since any lead composition identified in a high throughput experiment needs to be able to be scaled up to conventional scale and yield the same performance. In addition, there are some test methods which have yet to be developed in a high throughput manner, and, thus, conventional methods must still be used.

In the field of marine coatings, many of the coating systems now being explored are complex and could benefit from a combinatorial and high throughput approach. A few examples from the literature can show how the use of high throughput methods could be of great benefit in the design of new marine coatings. In the area of self-polishing antifouling coatings,



15.2 The 'Combi Funnel'.

Thouvenin *et al.* described an approach to the design of a 'tunable' polymer matrix resin from a copolymer containing a combination of a hydrophilic monomer, hydrophobic monomer, and a hydrolyzable monomer (Thouvenin *et al.*, 2003). Since there are several choices for each monomer type, a range of polymer compositions with each set of monomers, and since the polymer molecular weight can also be varied – all of which are expected to influence the biocide release and polishing rates of the coating – a combinatorial approach could be employed to develop detailed structure-property relationships for coatings as a function of these experimental variables.

Silicone elastomers are a leading candidate for fouling-release coatings and a number of parameters have been identified as critical for obtaining good release properties. Researchers at General Electric used a statistical experimental design approach to explore the effects of a number of variables on their fouling-release performance (Kavanagh *et al.*, 2003; Stein *et al.*, 2003a, 2003b; Truby *et al.*, 2000). The timeframe involved in conducting these experiments could have been shortened considerably if high throughput methods had been used.

15.2 Methods for the preparation of coating libraries

15.2.1 Polymer synthesis

Organic polymers form the basis for most paint and coating systems. Since polymers also have broader uses, methods and equipment for the high throughput synthesis of a number of different types of polymers have been

explored. Since the overall length of time required for carrying out a polymerization reaction is typically fixed, the throughput of polymer synthesis experiments is typically increased by carrying out reactions in parallel. Simple batch parallel polymerization reactions can be carried out in an array of small vials or flasks. Companies such as Chemspeed and Symyx have developed computer-controlled synthesis systems which use liquid handling pipettes to dispense solvents, monomers, and catalysts into individual reactors according to a recipe. The parallel reactors are then heated to initiate the polymerization reaction; stirring can be either through magnetic stir bars or vortexing. By varying the monomer composition, initiator concentration, or other reaction conditions, arrays of polymers having variations in molecular weight, functionality, and/or composition can be synthesized in a single experiment.

The synthesis of libraries of a number of different polymer types has been reported. The Schubert group has described the synthesis of polymer libraries using controlled free-radical polymerization methods such as atom-transfer radical polymerization (ATRP) (Zhang *et al.*, 2003a, 2003b, 2004), nitroxide-mediated polymerization (NMP) (Becer *et al.*, 2006), and reversible addition-fragmentation chain transfer (RAFT) (Fijten *et al.*, 2004, 2005; Guerrero-Sanchez *et al.*, 2006; Paulus *et al.*, 2005). The Webster group has described the parallel synthesis of libraries of acrylic polyols via conventional free radical polymerization using a parallel batch reactor system (Pieper *et al.*, 2007; Webster *et al.*, 2004). Anionic polymerization (Guerrero-Sanchez *et al.*, 2005, 2006; Guerrero-Sanchez and Schubert, 2004), cationic polymerization of 2-oxazolines (Hoogenboom *et al.*, 2004c, 2006), ring-opening polymerization of siloxanes (Ekin and Webster, 2006a), and the ring opening polymerization of ϵ -caprolactone (Ekin and Webster, 2006a, 2006b; Meier *et al.*, 2005) have also been carried out in a combinatorial way. The parallel synthesis of libraries of structurally diverse polyacrylates (Brocchini *et al.*, 1997), polyurethanes (Mant *et al.*, 2006; Tourniaire *et al.*, 2006), and poly β -amino esters (Anderson *et al.*, 2003, 2004, 2005, 2006) have been reported in order to evaluate these as biomaterials. Since the automated synthesizers typically include robotic pipettes to dispense reagents into the reaction vessels, these pipettes can also be used to withdraw samples periodically during the synthesis to determine the kinetics of the polymerization (Hoogenboom *et al.*, 2004a, 2004b, 2004d).

Simple batch processes for polymer synthesis are adequate for most systems and are appropriate when the objective of the experiment is to explore trends in variables such as polymer composition and molecular weight. However, some polymers are sensitive to the process used to prepare them. For example, emulsion polymerization, used to prepare latexes, is highly dependent on the mixing dynamics during the polymerization. Many polymers such as acrylics and latexes are made on an industrial scale by

feeding monomers into the reactor over time. In addition, it is desired to be able to explore the effect of process variables such as temperature, feed rate, stirring speed, etc. The Autoplant and Miniplant synthesis systems by Chemspeed have been designed to be able to conduct more complex polymerization processes. A recent report described the use of this reactor system to prepare polyurethane dispersions – a rather complex multi-step synthesis process (Nasrullah *et al.*, 2007).

15.2.2 Polymer screening

After synthesis, the polymers can be characterized using a number of methods. A rapid gel permeation chromatography system has been developed for polymer molecular weight determination (McConville and Saunders, 2004; Petro *et al.*, 2003). Matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectroscopy has been applied to the characterization of polymer libraries (Meier *et al.*, 2003a, 2003b; Meier and Schubert, 2004, 2005). High-throughput accessories are available for Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy. Gas chromatography and HPLC, using systems equipped with autosamplers, can be used to determine polymer purity and monomer conversion.

15.2.3 Coating formulation preparation

Coating formulations consist of polymers, crosslinkers, solvents, pigments, catalysts, and other additives. To prepare a formulation, these ingredients have to be precisely dosed and mixed. While a number of different approaches to preparing formulations can be employed, most use liquid dispensing robots for the dosing of liquid components of the formulation (Chisholm *et al.*, 2002a, 2002b; Iden *et al.*, 2003; Webster *et al.*, 2004; Wicks and Bach, 2002). High viscosity polymers and solid materials such as catalysts are diluted with solvent for more accurate dispensing. Mixing of the ingredients may be accomplished by shaking, vortexing, magnetic stir bars or mechanical agitators. It is important to understand the process dynamics of any high throughput system: factors such as the dispense accuracy and precision, and mixing capability (Chisholm *et al.*, 2005, 2006, 2003b).

Most systems used to date have been modifications of systems used for combinatorial chemistry. Thus, there may be limitations both in dispensing and mixing of the viscous materials which are typically found in coating formulations. In addition, dispersing solid pigments on a small scale has yet to be addressed. However, new systems are currently being developed by companies such as Symyx, hte AG, Bosch, and Chemspeed to address these issues.

15.2.4 Coating deposition

In order to evaluate key properties, coating formulations are deposited on a substrate and ‘cured’ to form a solid film. The format for deposition may be dictated by the screening experiment to be carried out. Coatings can be deposited in the wells of microtiter plates, in the bottom of an array of vials, and so on. Another method of deposition involves using an automated drawdown system to create coating samples on a substrate (Majumdar *et al.*, 2006; Webster *et al.*, 2004). A silicone template can be used to create temporary ‘wells’ for depositing an array of coatings on a plastic substrate (Cawse *et al.*, 2003). Ink jet printing has also been used to deposit polymers from solution (de Gans *et al.*, 2004; de Gans and Schubert, 2003).

15.3 Screening of coating libraries

15.3.1 Coating film screening: fundamental properties

The measurement of fundamental materials properties of a coating such as glass transition temperature, viscoelastic properties, crosslink density, hardness, toughness, elastic modulus, cure shrinkage, substrate adhesion, and optical clarity enables a basic understanding of the utility of the coating for a given application. While challenging, high-throughput and/or automated measurement methods have been developed to measure fundamental coating properties. With regard to throughput, one of the most impressive tools developed is the parallel dynamic mechanical thermal analysis system (pDMTA) commercialized by Symyx (Kossuth *et al.*, 2004). This system enables the measurement of viscoelastic properties of 96 polymer and/or coating samples at one time. While the system has displayed impressive throughput, data quality and reproducibility does not appear to be as good as with conventional dynamic thermal mechanical analysis systems, a sacrifice often encountered in high throughput methods (NDSU unpublished data).

In addition to the pDMTA, Symyx has developed a semi-automated process for measuring coating adhesion using an automated system based on the pull-off adhesion test (ASTM D4541). The degree of correlation between this semi-automated method and the ASTM standard method was determined by Chisholm and coworkers (Chisholm *et al.*, 2007b). They found a strong correlation between the semi-automated method and the ASTM standard method, but adhesion strength values obtained with the semi-automated method were consistently higher than those obtained with the ASTM method. This difference was explained in terms of the differences in strain rate between the two methods. A high-throughput method based on semi-automation of the tape adhesion test (ASTM D3359) was

developed by Potyrailo *et al.* (Potyrailo *et al.*, 2003). Quantification of the amount of coating removed as a result of the tape pull was enhanced using a fluorescence imaging technique. With the use of a programmable translation stage, automated measurements of hardness and elastic modulus can be obtained from coating arrays using conventional indentation instruments (Schrof *et al.*, 2001). Nanoindentation has also been employed to characterize the hardness of polymer libraries (Kranenburg *et al.*, 2007; Tweedie *et al.*, 2005).

Measurements that involve imaging techniques or the use of electromagnetic radiation are uniquely suited for application in a high throughput process because they are typically fast, quantitative, nondestructive, and amenable to small sample sizes. High throughput optical-based methods have been developed to measure fundamental coating properties such as cure shrinkage (Schrof *et al.*, 2001) and optical clarity (Potyrailo *et al.*, 2002).

The surface properties of a polymer or coating system are of critical importance for many applications, and especially in understanding the properties of fouling-release coatings. Automated surface energy systems have been reported which are based on contact angle measurements of test fluids such as water and methylene iodide (Urquhart *et al.*, 2007; Webster *et al.*, 2004; Wijnans *et al.*, 2004).

15.3.2 Coating film screening: end-use focused properties

The diversity of applications and requirements for protective coatings creates a significant technical challenge for the development combinatorial workflows. Generally, for a given coating application, multiple properties are critical to coating performance. As a result, multiple properties must be screened and optimized. Often, the properties of interest depend on multiple basic material characteristics. For example, coatings are often used to prevent scratching and marring of plastic articles such as head lamps for automobiles or touch pads for home appliances. The ability of a coating to withstand damage when contacted by abrasive particles or sharp objects is most likely a complex function of several fundamental material properties such as elastic modulus, adhesion to the substrate, and toughness. As a result, it is difficult to predict the performance of the coating for a given application using these fundamental material properties. As a consequence, the coatings industry has developed a plethora of application specific testing methods that serve to simulate the actual environment that the coating would see in the application while still being convenient, cost-effective, and faster than actual application testing. In order to apply a combinatorial approach to the effective development of a coating for a specific application, new screening methods are often required that simulate the application specific property

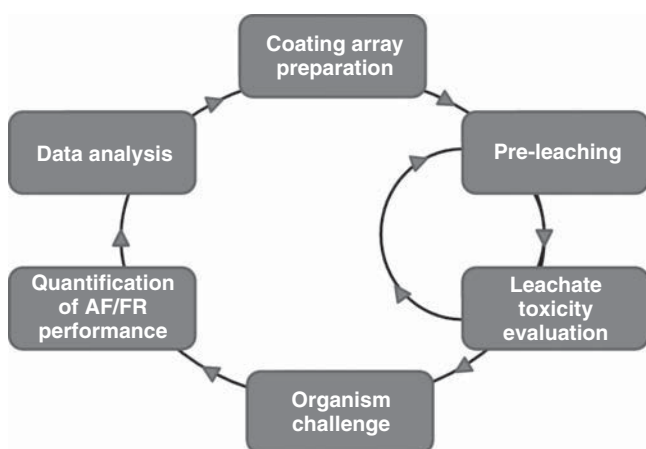
of interest while maintaining the requirements of high throughput and miniaturized samples in an array format.

Chisholm and Potyrailo described a high throughput method for screening abrasion resistance of clear coatings designed for protecting plastic substrates which involved an oscillating sand abrasion process and automated scattered light measurement (Chisholm *et al.*, 2003a; Potyrailo *et al.*, 2002). These researchers also described the development of a high throughput method for determining the ability of the coatings to protect the plastic substrate from degradation by the ultraviolet radiation that is encountered during outdoor exposure (Potyrailo *et al.*, 2005).

As part of a workflow designed to develop novel fouling-release coatings, the semi-automated process described in the previous section of this document for measuring coating adhesion was modified to simulate the release properties of coating surfaces toward barnacles (Chisholm *et al.*, 2007b). He and coworkers recently developed a high throughput electrochemical measurement system for screening the protective capabilities of coatings toward inhibiting substrate corrosion (Chisholm *et al.*, 2007a; He *et al.*, 2008). For many applications, resistance to fluids such as fuels or cleaning fluids is important. Using an imaging technique, Schrof and coworkers (Schruf *et al.*, 2001) developed a high-throughput method for determining coating solvent and stain resistance. Similarly, a high throughput method for determining the scrub resistance of coatings was reported by Vratsanos *et al.* (2001).

15.4 Methods for rapid screening for biofouling

The most common method used to evaluate the performance of antifouling coatings is the static immersion of large raft panels in the ocean (ASTM D3623 and D5618). This method of testing is highly effective and provides detailed information on the durability, weatherability, and overall antifouling performance of a coating towards a complex community of marine fouling organisms. Although static immersion testing in the ocean is effective, it is not well suited to accommodate the large numbers of coatings generated using a high throughput approach. This is due to a number of factors such as the limited capacity of panels that can be analyzed at any one time, the relatively large amount of material required to coat panels, the time required to analyze performance (several weeks to months) and the cost of testing. Current research is focused on developing new non-toxic, nonfouling surfaces and fouling-release coatings. This has led to a need for laboratory test methods to characterize these new research materials. Several laboratory assays have been developed, using a variety of marine fouling organisms, to meet some of these shortcomings (Callow *et al.*, 2007; Ista *et al.*, 2004; Tang and Cooney, 1998). These include assays that utilize relatively small sample sizes (i.e., glass microscope slides) and can be



15.3 High throughput biological workflow for the evaluation of antifouling marine coatings.

evaluated in a few days to a week. However, as with static ocean immersion testing, these methods are also unable to appropriately handle large numbers of coatings at one time. As a result, a high-throughput (HT) biological workflow has been developed to rapidly analyze libraries of coating arrays.

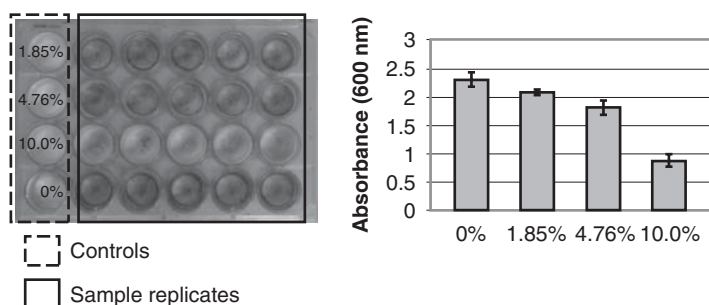
Figure 15.3 shows a general diagram of the HT biological workflow for characterization of non-toxic AF/FR marine coating performance. Coating libraries are robotically prepared on 4" x 8" aluminum panels or cast in the wells of 24-well plates for biological evaluation. Coating arrays are then immersed in a recirculating de-ionized water tank for the appropriate period of time required to sufficiently leach out any toxic impurities (i.e., residual catalyst, monomers, un-reacted biocides, etc.). This is verified by carrying out toxicity evaluations of overnight extracts of the coatings in an artificial sea water (ASW) growth medium. This process is repeated until the majority of the coatings in each library are determined to be non-leachate toxic. Coatings found to be non-leachate toxic are then challenged with one or more marine fouling organisms to rapidly assess their potential as new antifouling (AF) and or fouling-release (FR) marine coating candidates warranting further investigation and development.

15.4.1 Bacterial assays

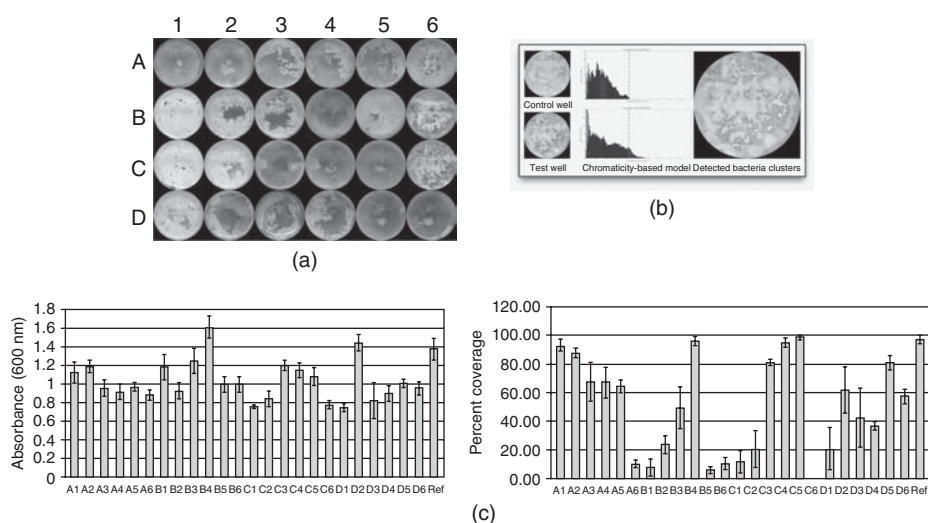
A variety of marine bacterial screening assays have been developed to characterize both the AF and FR performance of coating libraries prepared in 24-well array plates (Stafslien *et al.*, 2007a, 2007b, 2007c, 2006). These assays are the primary 'workhorse' of the HT biological workflow and are typically used to obtain a first approximation of coating performance.

Coatings are challenged with batch cultures of an appropriate marine fouling bacterium. The marine bacterium is suspended in an artificial sea water (ASW) nutrient medium and dispensed into the wells of the coating array plates. The plates are then incubated at the appropriate temperature and duration to facilitate bacterial attachment and biofilm formation. The plates are rinsed three times with de-ionized water, allowed to air dry at ambient conditions, and stained with the biomass indicator dye, crystal violet (CV). The CV stained biofilms retained on the coating surfaces are then extracted with acetic acid, transferred to a 96-well plate and measured for absorbance at 600 nm to determine total biomass retained on the coating surfaces. Figure 15.4 shows an example of this method used to quickly evaluate the AF performance of a series of silicone coatings containing bound ammonium salt groups (Stafslien *et al.*, 2007b). A higher reduction in *Cellulophaga lytica* biofilm retention was observed as the concentration of the bound ammonium salt group was increased in the silicone matrix. The 10.0 weight % coating showed the greatest reduction in biofilm retention (62%) when compared to the 0 weight % control.

Digital images are taken of each CV stained coating array plate, before acetic acid extractions, and percent surface coverage is determined for each coating using an automated imaging program (Stafslien *et al.*, 2007e). The percent surface coverage is used to gauge the degree of bacterial biofilm retraction (i.e., redistribution of the biofilm surface coverage after rinsing and drying) on a coating surface. This measurement gives a quick indication of bacterial biofilm adhesion (Stafslien *et al.*, 2007c). Figure 15.5 shows the analysis of *C. lytica* bacterial biofilm retention and retraction on a series of siloxane-urethane FR coatings. Figure 15.5a shows the relatively large degree of variation in *C. lytica* biofilm retraction obtained on these



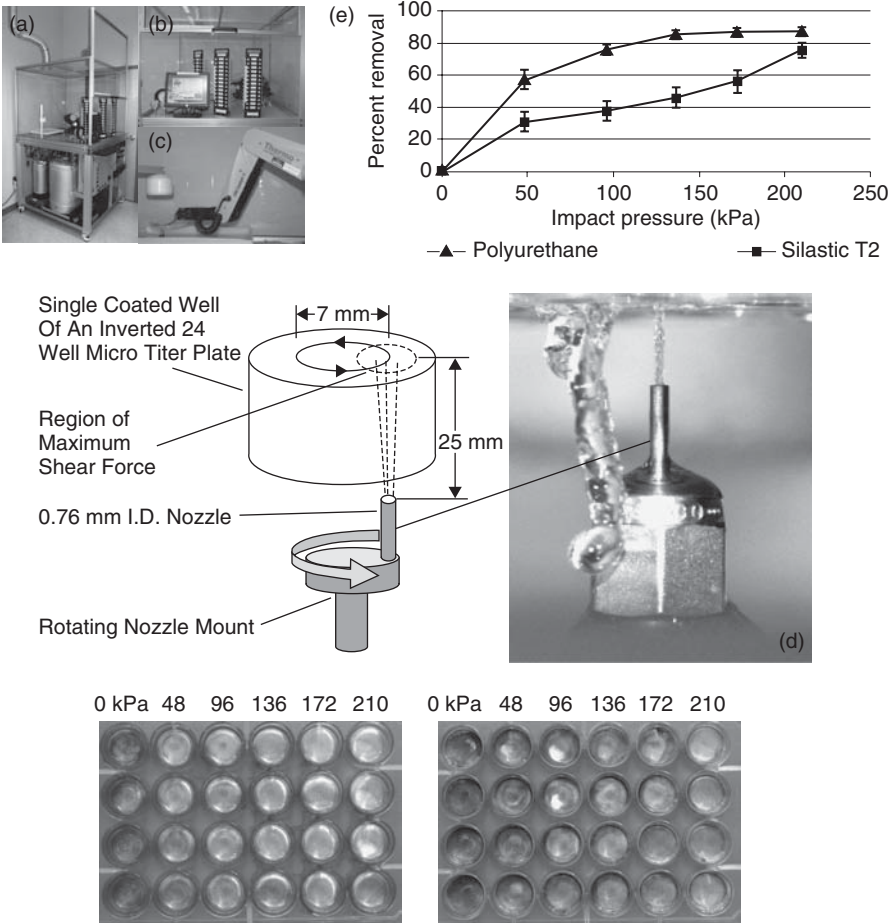
15.4 Image of silicone coatings containing bound ammonium salt groups after CV staining of *C. lytica* biofilms (left). Absorbance values determined from acetic acid extractions of CV (right). Percentages indicate the concentration of the bound ammonium salt groups in the silicone coating.



15.5 (a) Representative image of each coating of the siloxane-urethane coatings library after CV staining of retracted *C. lytica* biofilms. (b) Imaging program used to determine the percent surface coverage of CV stained biofilms. (c) *C. lytica* biofilm retention measurements (left) and percent surface coverage determinations (right) for the siloxane-urethane coatings library.

surfaces. Some CV staining of coatings A6, B6 and C6 was observed, but the automated imaging program was able to adequately detect the retracted regions of *C. lytica* biofilm (Fig. 15.5b). Figure 15.5c shows the biofilm retention and retraction data for each siloxane-urethane coating. The amount of biofilm retained on the coating surfaces was approximately the same for each coating. However, the degree of biofilm retraction was highly dependent on the composition of the coating. Several coatings exhibited 20% or less surface coverage and were identified as promising FR coating candidates.

An automated water jet apparatus was developed to rapidly determine the adhesion strength of bacterial biofilms to coatings deposited in 24-well array plates (Fig. 15.6) (Stafslien *et al.*, 2007a). A robotic arm was mounted on the deck of the apparatus and utilized to transfer coating array plates from plate stacking hotels to the water jet nozzle. The water jet nozzle was designed to be off-center and rotate during operation to facilitate adequate treatment of the coating surfaces. The duration and pressure of the water jet are precisely controlled by a CPU to facilitate accurate and reproducible treatment for each coating within an array plate. Figure 15.6e shows the results of a water jet experiment comparing the adhesion of *Halomonas*



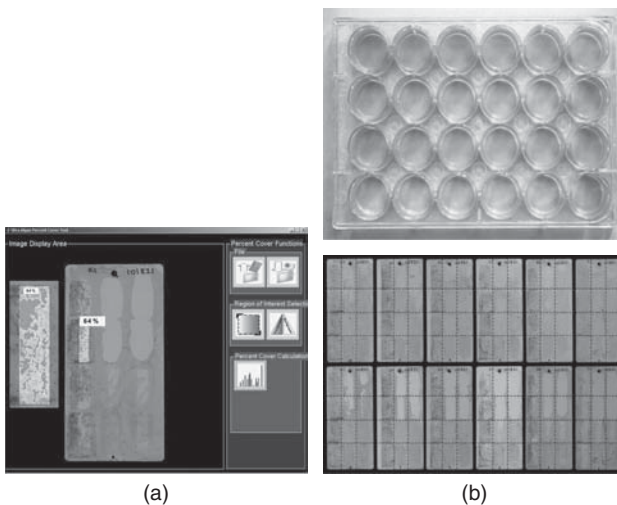
15.6 (a) Automated water jet apparatus. (b) CPU interface and multi-well plate hotels on the deck on the water jet apparatus. (c) Robotic arm used to manipulate multi-well plates. (d) Picture of water jet nozzle (right) and diagram of nozzle configuration used to treat the wells of array plates (left). (e) Water jet treatment of *H. pacifica* biofilms on polyurethane and a silicone elastomer (Dow Corning Silastic® T-2) coating.

pacifica biofilms to a flexible polyurethane (PU) and a silicone elastomer coating. A higher degree of *H. pacifica* biofilm removal was achieved on PU, when compared to the silicone elastomer coating, throughout the range of water jetting pressures evaluated. Maximum biofilm removal was observed at approximately 136 kPa for PU while the highest pressure employed failed to achieve maximum removal for the silicone elastomer.

15.4.2 Algal assays

Marine fouling algae have been routinely employed in traditional laboratory based assays to assess the performance of AF/FR marine coatings and typically include the use of both micro and macro-fouling algae to characterize the coatings broad spectrum performance. These methods have been modified to accommodate the analysis of coatings cast on both array panels and in multi-well plates.

The analysis of coatings cast in multi-well plates is carried out in a similar fashion to that of the marine bacterial assays (Casse *et al.*, 2007b). Algal cultures are resuspended in ASW supplemented with nutrients and dispensed into the wells of the plates. The plates are then transferred to an illuminated growth cabinet for the appropriate period of time and at a temperature required to promote optimal biofilm growth. In the case of the green macroalgae *Ulva linza*, the plates are allowed to sit for 2 hr to promote initial attachment and the nutrient medium is then replaced before being transferred to the growth cabinet. The total biomass on each coating surface is determined by a fluorescence measurement of dimethyl sulfoxide (DMSO) extractions of chlorophyll. The procedure for analyzing coatings prepared on array panels is similar to that of the multi-well plates, but employs an automated imaging program (rather than DMSO extraction of chlorophyll) to quantify the amount of algal biofilm obtained on each coating array patch (Fig. 15.7a) (Casse *et al.*, 2007a). Figure 15.7b provides

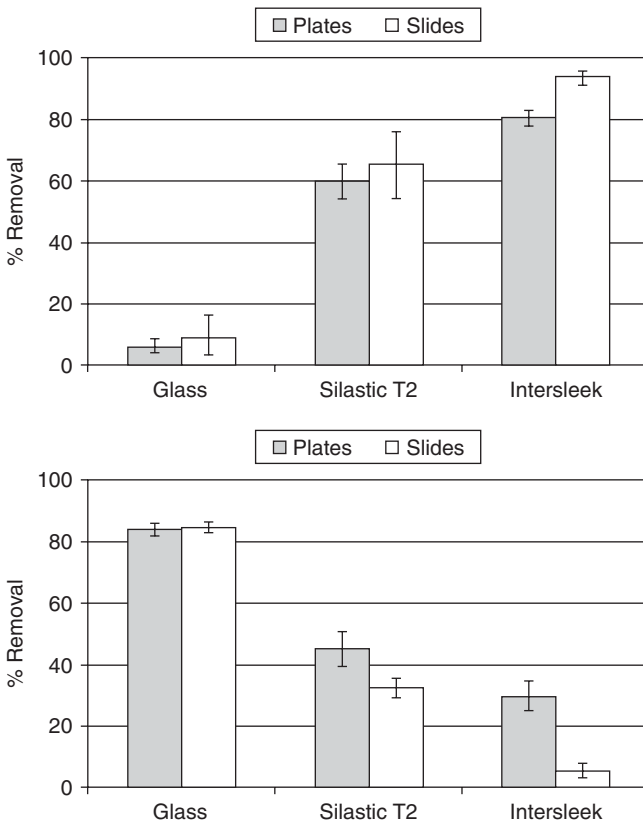


15.7 (a) Automated imaging program used to quantify algal biofilm surface coverage on array panels. (b) Images of *Ulva* biofilms on coatings prepared in multi-well array plates (top) and on array panels (bottom).

an example of *Ulva* biofilm growth obtained on coatings prepared with both array formats.

Coatings cast in multi-well plates are treated with the water jet apparatus, described in the bacterial assays section, to rapidly determine algal adhesion. For array panels, a rastering water jet apparatus utilized to treat coatings prepared on glass microscope slides was reprogrammed to accommodate the 12-patch layout.

Figure 15.8 shows the adhesion of both *Ulva* biofilms and the marine fouling microalgae, *Navicula perminuta* (diatom), to glass and silicone elastomers, Dow Corning Silastic® T-2 (T2) and International Intersleek® (IS), prepared in multi-well plates and on glass microscope slides (Casse *et al.*, 2007b). For both algal species, the multi-well plate adhesion results were in good agreement to the data obtained with the traditional microscope slide-based assay. In general, *Ulva* biofilms were more easily removed from the



15.8 *Ulva* biofilm (top) and *Navicula* (bottom) adhesion to glass and silicone elastomers T2 and IS.

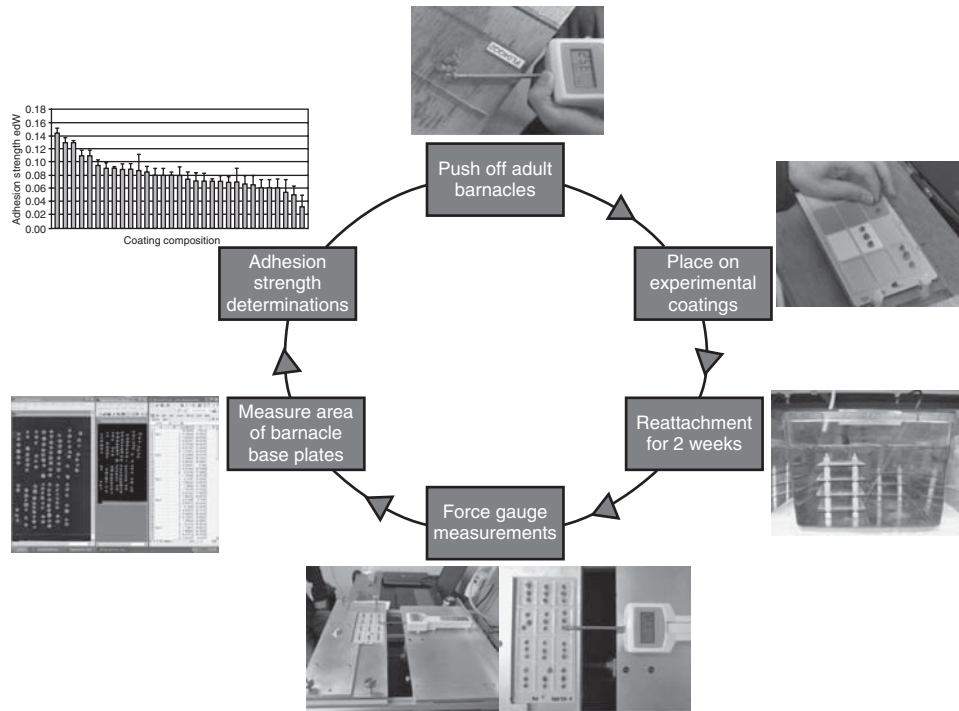
silicone elastomers, but strongly adhered to the uncoated glass. Conversely, *Navicula* was easily removed from glass, but showed a much lower removal from the silicone elastomers. As a result, the identification of coatings exhibiting good removal of both algae may be considered as promising candidates for further development and advanced characterization.

15.4.3 Adult barnacle reattachment assay

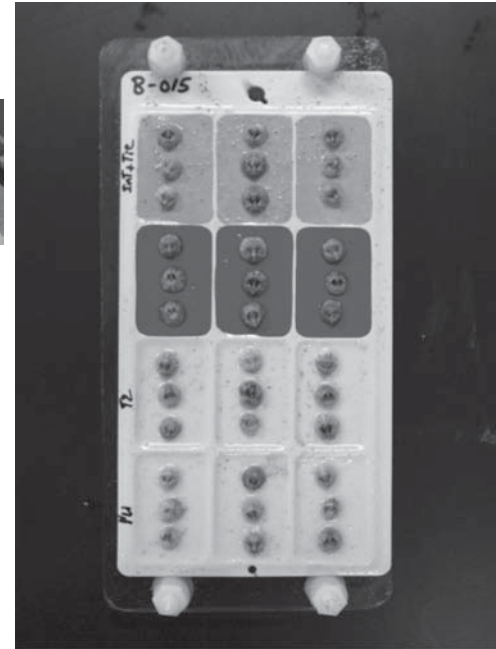
The development of a HT assay employing barnacles was considered to be highly desirable for use as a secondary screen of AF/FR performance. This would allow for more rigorous characterization of promising coating candidates, identified with the bacterial and algal primary screening assays, and provide valuable information regarding the performance of lead candidates with respect to shell fouling. Preliminary efforts to develop a cyprid settlement assay were unsuccessful due to their high sensitivity towards coating formulation components (i.e., solvent, catalyst, monomers etc.) and their preference for settling at the edges of the coatings prepared in array plates and on panels. As a result, an adult barnacle reattachment assay was developed to characterize the FR properties of coatings in an array format.

Figure 15.9a depicts the HT barnacle reattachment workflow (Rittschof *et al.*, 2008). Barnacle cypris larvae are settled on full panels of T2 and reared to a suitable size for testing. The adults are then pushed off the T2 coating and placed on the surfaces of experimental coatings (Fig. 15.9b). After 2–3 hours of reattachment in a humid container, the coating array panels are placed in an aquarium tank and filled with ASW. The array panels then remain in the aquarium tank for an appropriate period of time to promote strong adhesion to the coating surfaces. A hand-held digital force gauge is used to measure the force of removal for each barnacle in shear. The basal plate areas of the adult barnacles removed from the coating surfaces are then measured to enable the measurement of adhesion strength.

The influence of reattachment time on barnacle adhesion strength was investigated and it was shown that it was highly dependent on the surface being evaluated. Silicone elastomers required only 1–2 weeks of reattachment to achieve adhesion strengths comparable to that observed for barnacles settled and reared for 9 weeks in the laboratory. However, PU required approximately 4 weeks of laboratory reattachment to achieve good adhesion. This effect was attributed to the inability of the barnacle to appropriately exclude water during the adhesion process. As a result, the reattachment assay appears to be a useful tool to quickly screen the FR performance of silicone based coatings, but must be used with caution when analyzing other coating systems.



(a)



(b)

15.9 (a) Adult barnacle reattachment assay. (b) Image of adult barnacles reattached to a coating array panel.

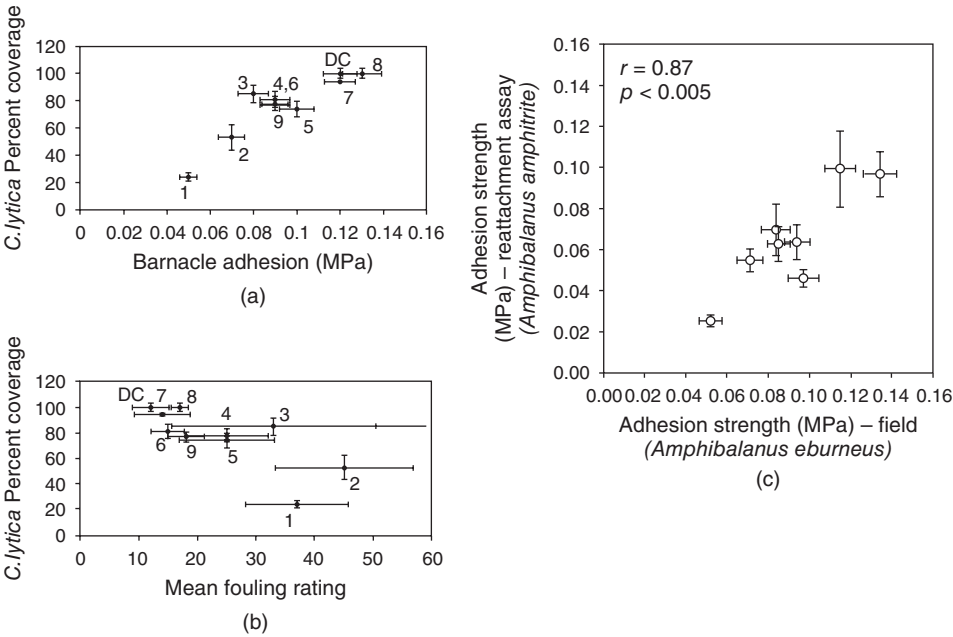
15.4.4 Correlations between laboratory assays and field testing

The development of rapid biological laboratory assays, with the ability to analyze large numbers of coatings in parallel, is a key component to the success of research efforts based on a high throughput approach. In this regard, the HT biological assays must be able to accurately downselect and identify the most promising coating candidates. Ideally, the most effective coatings identified with the HT biological assays would also be the most effective coatings determined using ocean immersion testing. Establishment of correlations between HT laboratory measurements and measurements made using ocean immersion testing would enable a powerful, real-world predictive capability for the HT biological workflow.

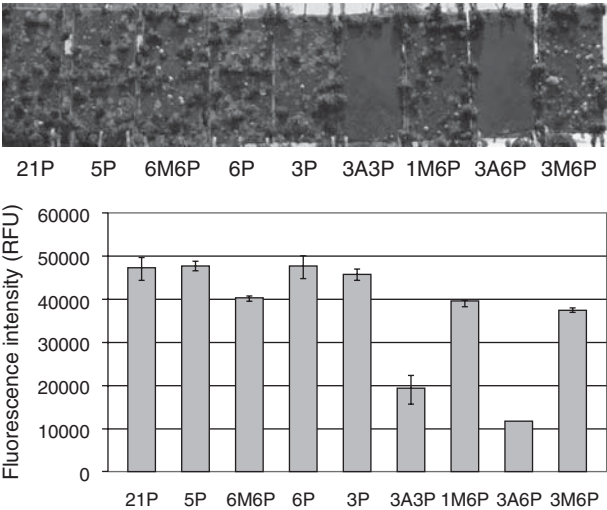
A series of polysiloxane FR coatings and biocide incorporated silicone coatings were prepared and analyzed with both the HT biological assays and ocean immersion testing at the Florida Institute of Technology (FIT) field testing site (Indian River Lagoon, Melbourne FL). A strong correlation was observed for the bacterial biofilm retraction ($r = 0.90$) and the adult barnacle reattachment ($r = 0.87$) assays when compared to barnacle adhesion data obtained on the polysiloxane FR coatings (Fig. 15.10) (Stafslien *et al.*, 2007c). Both HT screening assays correctly identified the best and worst performing coatings observed as a result of ocean immersion testing. A moderate correlation was also observed between the bacterial biofilm retraction assay and the mean fouling rating ($r = 0.77$).

Bacterial biofilm (*Halomonas pacifica*) and algal (*Navicula incerta*) adhesion evaluations using the automated water jet apparatus were also carried out on the polysiloxane FR coatings (Stafslien *et al.*, 2007d). The highest amount of *H. pacifica* biofilm removal was observed on the coating that had a combination of low crosslinker amount and the addition of a silicone oil additive. Conversely, the highest amount of *N. incerta* removal was observed on the coating that had the highest amount of crosslinker and no silicone oil additive. The opposite behavior in adhesion, observed for bacteria and algae on this set of coatings, stresses the importance of using more than one fouling organism to aid in the down-selection process.

Figure 15.11 shows the laboratory and field results obtained on the biocide incorporated silicone coatings (Choi *et al.*, 2007). The two coatings containing the biocide (i.e., Triclosan) grafted by an acrylate functional group (3A3P and 3A6P) showed good resistance to fouling after 29 days of ocean immersion testing. These coatings also showed a substantial reduction in algal biofilm growth (*N. perminuta*) assay when compared to the rest of the coating series. Interestingly, the coatings containing the biocide with a methacrylate functionality (6M6P, 1M6P, 3M6P) showed no antifouling activity in the field or in the laboratory assay.



15.10 Correlations between HT biological assays and ocean immersion testing. a) Bacterial biofilm retraction (percent surface coverage) vs. barnacle adhesion at the FIT field testing site. b) Bacterial biofilm retraction vs. mean fouling rating at FIT. c) Reattached barnacle adhesion vs. barnacle adhesion at FIT.



15.11 Image of biocide incorporated silicone coatings after 29 days of ocean immersion at FIT field testing site (top). 48 hr *N. incerta* biofilm growth on biocide incorporated silicone coatings (bottom).

15.5 Conclusions and future trends

Combinatorial and high throughput methods have the potential to have significant impact in all areas of polymer and coating development, and especially on the design and development of new marine coatings technologies. Automated methods have been developed for the synthesis of polymers and the preparation and testing of coating films. Newer methods are also being introduced to prepare pigmented coating systems and handle higher viscosity coating ingredients. New laboratory methods for screening the interaction of key biological organisms with coating surfaces have been developed which can be used to downselect promising coating candidates for further field testing. The focus of these test methods has been to screen non-leaching biocidal coatings or non-toxic fouling-release coatings.

It is expected that as combinatorial and high throughput methods become more widely adopted, new test methods will be developed for other marine coatings types. For example, there is still extensive development worldwide in the area of ablative or self-polishing biocidal coatings technology that could significantly benefit from high throughput screening methods. As we have seen, there is an extensive compositional range for self-polishing polymer binder systems and, when coupled with the effects of pigmentation and biocides, the number of possible combinations becomes exceedingly large. New test methods, however, will need to be developed in order to screen the key desired properties of these coating systems, for example, biocide release rate or polishing rate of the coating.

The current high throughput screening methods have been developed in order to rapidly downselect coatings compositions from a large number of possible candidates in a short period of time. In other words, researchers can generate numerous concepts for potential marine coating systems, and each of these concepts may reside within a large compositional space. The focus in developing these high throughput methods has been on the rapid determination of the feasibility of these concepts and/or to identify a feasible compositional subset. From this point on, further development would use conventional methods.

The acceleration of long-term marine coatings testing is also an attractive goal; however, there are a number of key issues that have to be carefully considered. If the timescale of testing cannot be reduced, acceleration of throughput will involve conducting the testing of a large number of trial coatings in parallel. Then, issues such as the minimum sample area required, number of replicates required (as well as testing at single or multiple test sites), and so on, have to be considered. The methods used for evaluating these test samples also have to be streamlined as they are currently highly labor intensive. All of this is indeed possible, and hopefully, discussion and use of short-term high throughput methods will stimulate thinking in this direction.

15.6 Acknowledgements

The authors would like to thank the US Navy's Office of Naval Research for support under grants N00014-03-1-0702, N00014-04-1-0597, N00014-05-1-0822, N00014-06-1-0952, N00014-07-1-1099.

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Ageing tests and long-term performance of marine antifouling coatings

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Abstract: Appropriate short-term antifouling (AF) protection in chemically active systems is virtually assured provided that sufficient amounts of active compounds are added to the formulation. Hence, it is no big surprise that many different experimental approaches can claim fouling protection capabilities after a few weeks/months of immersion in natural seawater overperforming blank uncoated panels (e.g., enzyme-based coatings, surface-microtextured coatings; see Chapters 23 to 25). Nevertheless, only very few coatings systems worldwide can demonstrate sustained AF protection for prolonged periods of time (i.e., 60+ months). Since the latter is required by most of the ocean-going trading fleet, sound long-term ageing of experimental coatings is a key step in the AF paint formulation development, also for the non-toxic environmentally friendlier approaches. This chapter is aimed to give a coarse description of ageing methods typically used by industry so that they can become an integral part of the development of cleaner, yet more efficient, AF technologies. Static and dynamic field tests are covered together with an insight into actual ship testing (test areas and full ship applications). Some relevant laboratory setups are also presented, described and discussed.

Key words: field tests, sea stations, static immersion tests, dynamic exposure tests, Couette-type laboratory setups, Turboeroder.

16.1 Introduction

The properties that a successful paint product has to satisfy from a technical point of view can be classified into two main groups: general paint properties such as, e.g. storage stability and sag resistance, and those related with the final purpose of the paint (e.g., antifouling performance). This chapter will focus on the testing of the long-term performance of antifouling (AF) products, while Chapter 13 will tackle the more generalist paint technology issues. Because a large percent of AF paints are applied on ship hulls, we will concentrate on ageing tests simulating exposure conditions similar to those experienced by the hull of ocean-going vessels.

The actual service time for an AF paint is defined by the dry docking interval of the ship, which typically spans from 3 years up to a maximum of

5 years (probably even longer in the near future). Such a long working time combined with the aggressiveness of natural sea water makes AF paints especially difficult products to design (Kiil *et al.*, 2006). There exists a range of tests that can help in the safe development of new AF paint products. Some are relatively fast and require just a few months of testing (see, e.g., Section 16.3). Unfortunately, the information obtained from such methods is rather incomplete, so the real-life potential of a given product must be inferred, more or less directly, from such tests based on previous experience (most often) or mathematical models (see, e.g., Chapter 14). Antifouling paints are very complex, reactive, multicomponent products where the interactions between its commercial-grade ingredients (potentially purchased from more than one supplier and containing impurities) and their effect on the long-term performance of the paint are very difficult to forecast. Furthermore, the same combination of ingredients may end up exposed to an almost infinite diversity of working conditions (seawater parameters, ship speed, fouling pressure) during its service period. All this, combined with the high cost of a potential failure, force paint designers to be cautious and ultimately rely on long-term tests run in natural seawater as the main experimental input for the paint design and optimisation process. The latter is certainly relevant when small changes are introduced into an existing technology, but it is even more important when a new technology or an innovative concept is being developed.

This chapter will describe the most common ageing test procedures available for paint manufacturers to monitor the long-term AF performance of their paint products. The ageing tests will be arranged into two main groups: laboratory tests and field tests. While in the former group, the test conditions are normally well controlled and yield highly reproducible results, those tests run in the natural marine environment will be subject to uncontrolled daily, seasonal and even annual cycles.

16.2 Field tests

This section will deal with ageing tests performed at sea sites. Alternative tests described later on in Section 16.3 may eventually use natural seawater, too, but the setups are placed inland so seawater is typically pumped from the sea, treated and then used in the setup. Testing AF paints in their actual working conditions, i.e. natural seawater, is an essential part of their development. As it comes from the working mechanisms of chemically active AF paints (see Chapter 14), these products experience dramatic changes during their service life (e.g., composition, morphology, mechanical properties). Because it is virtually impossible to predict all the implications of such changes on the overall product performance, long-term ageing tests in conditions as similar as possible as those encountered in service are the

most informative experimental inputs before a paint is launched into the market.

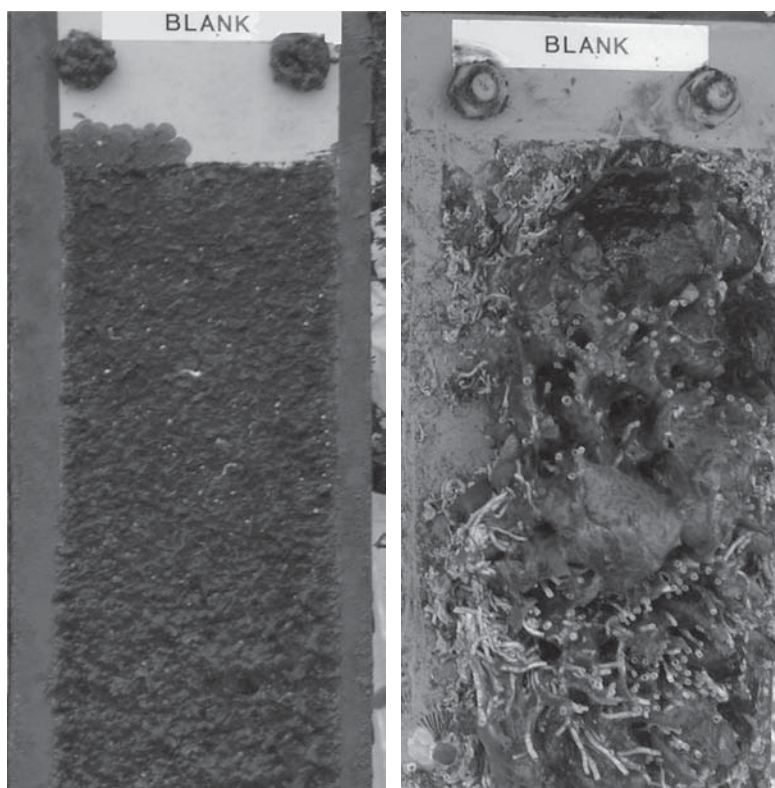
There are two main ways of testing the AF performance of paints in the marine environment: sea testing stations and ships. Ship tests (Section 16.2.4) are most typically used in the last stages of development as a means of confirming the conclusions withdrawn from the preliminary screening and optimisation tests run in the laboratory and in sea testing stations.

16.2.1 Sea station tests

Antifouling paints are routinely tested in one or more sea stations as part of their development and optimisation processes. Tests are normally run for 1 to 5 years depending on their performance and the paint development stage. As a general rule, the formulations tested in screening projects are designed to show as many differences as possible in short periods of time, whereas commercial or close-to-commercial paints need far more testing time in order to actually observe diverging trends. For initial screening processes, tests are usually run with one replicate and at one specific test location (see comments below) with the goal of maximising testing capacity. As a formulation approaches commercialisation, the number of both replicates and test locations increases so as to gain full confidence in the potential new product.

When a paint formulation is tested in natural conditions, many test parameters are not controlled and can only be monitored. That makes these tests different every time, so the conclusions can only be safely taken through comparison to other paints tested simultaneously. For example, the performance of the same paint system after one year of immersion in the same sea station could be significantly different depending on whether the test was started in spring or autumn. Result comparison between tests run at different points in time becomes possible when commercial standards of well-known and predictable behaviour are included in every experimental series. In any case, the differences in performance that are observed between test series run at one specific test site over time are normally much smaller than those experienced when the paint is tested at completely different test sites (e.g., Mediterranean and tropical waters; Fig. 16.1).

Fouling type and intensity depends on factors such as, e.g. water salinity, water temperature, solar radiation, and available nutrients that can vary significantly depending on the test site (Iselin, 1952; Yebra *et al.*, 2004). Fouling is more intense in warm waters than in cold or temperate waters (see Fig. 16.1). During the screening phase of the paint development, it is interesting to see differences already from small changes in the paint composition. The latter are easier to detect in temperate waters than in warm waters, where early fouling may hide subtle differences in the paint



16.1 Fouling pressure can be very different around the world. This figure shows the extent of fouling accumulated on blank panels exposed at two different test sites for 16 weeks. Temperate waters (Mediterranean Sea; sub-tropical: left) versus warm waters (tropical area in the Indic Ocean; right).

performance. On the other hand, warm water makes the test more aggressive and, hence, shorter, so these are ideal conditions to compare commercial paints or experimental paints in the final stages of development.

Typically, information from more than one testing site is used during the development of new paints as a means of assessing the paint performance in different environments. Analogously, within each sea station, two complementary tests are often performed in order to complete the performance assessment of a given product: static tests and dynamic tests. The first one basically consists of the immersion of painted panels into natural seawater which are regularly inspected so as to grade their resistance to fouling attachment (i.e., biocide release rates) and mechanical failures. The water flow on the paint surface is limited to that from coastal currents and tidal flows hence reproducing the exposure conditions experienced by AF paints

during a ship's idle periods. Dynamic testing is more expensive as it involves the use of energy to provoke friction between the paints and seawater. In the dynamic tests, additional parameters to monitor include the decrease of the film thickness during exposure and the position of the biocide leaching front(s) (see Chapter 14).

Both tests can be combined in cyclic tests alternating static and dynamic exposure conditions to better reproduce real ship navigation scenarios. Such tests are especially relevant for those coatings based on self-cleaning principles such as typically fouling release paints (see Chapter 26).

16.2.2 Static tests

These tests basically consist of statically immersing painted panels in natural seawater and then checking their performance after regular periods of time. The results obtained from static tests are especially relevant for those paints designed for the protection of offshore structures and the hulls of ships with a very low activity (i.e., long idle periods) such as pleasure boats or coastal fishing vessels. For experimental biocide-based paints, static tests typically run for no less than one year (note that the absence of significant polishing involves early failure of biocide-based coatings). Fouling release coatings are usually tested for longer periods as it is interesting to monitor their long-term self-cleaning properties.

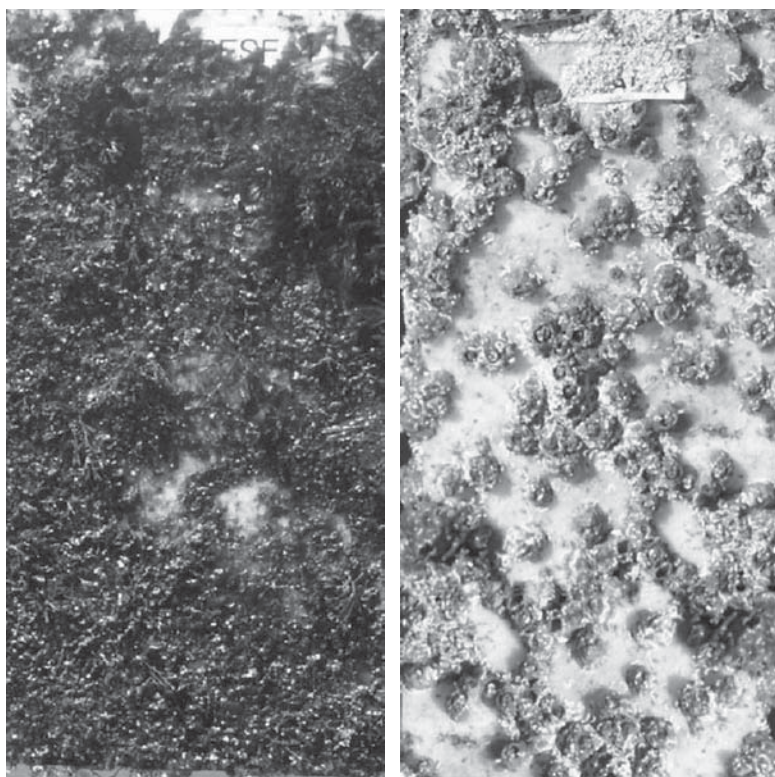
The panel substrate for static tests is selected from different materials such as typically metals (e.g., steel or aluminium) and plastics (e.g., acrylic or polycarbonate). The preferred substrate is normally plastic because of its lower cost and weight. Metals are used when the goal of the test is not only restricted to the AF performance, and properties such as corrosion protection of the full paint system are also under investigation.

Antifouling paint topcoats are typically sprayed in dry film thicknesses (DFT) equal to those recommended in the product's technical data sheet (one or two coats). Previous coats are usually applied only to secure the adhesion between the paint system and the substrate. Typically, paint surfaces around 100 cm² are enough for comparative tests, even though some special tests may require larger surface areas.

Hempel A/S has chosen three different locations for static tests: the Baltic Sea (brackish inland sea with relatively cold waters), the Mediterranean Sea (sub-tropical environment) and in the waters around Singapore (tropical). The algal fouling intensity is very high in the Mediterranean Sea, whereas the animal fouling is particularly aggressive in Singapore. Tests run in the Baltic Sea are useful to test the topcoat's resistance to immersion in low salinity waters (e.g., estuaries) as well as to study the paint reactivity and efficiency at low temperatures. Using such varied locations worldwide provides AF performance results over a wide spectrum of stress conditions.

In this kind of test, results are markedly influenced by the position in which the paint is exposed. Depth and orientation with respect to the sun strongly influence the fouling type and intensity observed during the duration of the test. Hence, panels are always kept in the same position during the whole test in order to avoid irregular performance patterns. Usually, sun-oriented panels will show a higher tendency to develop algal fouling than those receiving smaller amount of solar radiation (Fig. 16.2). Similarly, panels closer to the sea surface will typically show more algae than panels exposed in the deeper positions of the rack.

The time interval between inspections varies from a few weeks up to months depending on the test site and the paint type. Shorter inspection times are used for paints designed for cold waters (i.e., for less fouling pressure) and tested in temperate waters or when the test site is placed in warm waters (aggressive fouling conditions). Contrarily, longer inspection times are employed when the paint to be tested has been designed for warm



16.2 Picture showing blank panels exposed during 23 weeks in the Mediterranean Sea (both panels were immersed in February 2007). The panel to the left was sun-oriented (thick brown slime) while the panel to the right remained in shadow (predominantly animal fouling).

waters and tested in temperate waters or when the test site is placed in cold waters with relatively low risk of fouling.

Inspections basically consist of a visual evaluation of the paint surface regarding fouling attachment and mechanical failures. Additionally, the performance of fouling release paints is further evaluated by checking the 'easiness of cleaning' or, in other words, the adhesion strength of the fouling species settled. By far, the most important parameter to be evaluated is the AF performance of the topcoat and its degradation with time. Such parameter can be evaluated following the standard ASTM D6990-05, even though more detailed and informative performance reports are often used. It is also of interest to report paint failures than can affect the AF performance such as blisters (ISO 4628-2:2003), peelings (ISO 4628-5:2003) or cracks (ISO 4628-4:2003).

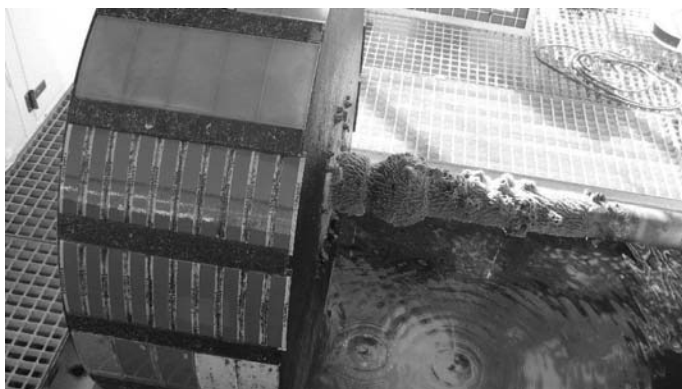
For biocide-based paints, the performance in static tests is mainly related to the release of the biocides. The solubility, AF efficacy and the amount of the biocides used in the paint, as well as the permeability and potential seawater solubility of the other paint components are the main factors to consider when comparing paints exposed to equivalent conditions. If an inert (non-polishing nor leaching) paint spot has been applied onto the AF topcoat, the decrease of paint thickness due to exposure and the position of the leaching front of the different biocides can be measured (Yebra *et al.*, 2006). However, this information is not as relevant for this kind of test as it is for the dynamic tests reviewed in Section 16.2.3.

Fouling release coatings have working principles different to the classical biocide-based paints (see Chapter 26). Their self-cleaning properties are evaluated by grading the pressure needed to remove the fouling attached to the paint surface.

16.2.3 Dynamic tests

Dynamic tests are designed to study the AF performance of paints when exposed to shear stress due to, e.g. moving seawater, hence simulating exposure conditions experienced on ship hulls. Such test conditions are especially relevant for self-polishing (or eroding in general) and fouling release coatings. In the former, the surface polishing effect induced by water friction strongly influences the biocide release rates over time (see also Chapter 14), while in fouling release coatings a certain shear stress is necessary for the 'self-cleaning' effect to occur (see also Chapter 26).

Perhaps, the more common design for dynamic exposure testing is based on a rotating drum immersed into natural seawater (Fig. 16.3). For example, Hempel obtains dynamic test results from a sea station at Vilanova i la Geltrú, 41.13 N, 1.43 E (Mediterranean Sea; Barcelona, Spain). Panels coated with the test paints are placed on the drum's peripheral surface



16.3 Rotor drum exposed to natural seawater. Heavy fouling can be observed on the shaft, even on top of the Zn sacrificial anode despite the high rotational speeds and short static periods (below 1% of the time).

which, through rotation, reaches predefined perimeter velocities up to 22 knots (typical sailing speeds for ships). Contrary to static racks, the low cylinder height and the drum rotation ensures that all positions of the drum are subjected to similar exposure conditions (also checked right after construction of the setup). For other possible setups different from the open-sea rotating drum, please refer to Section 16.3. Normal testing times exceed one year for experimental coatings, while the longest experimental times are spent with commercial and close-to-commercial products. The dry film thickness (DFT) of the paint to be tested is chosen in accordance with its expected polishing rate, the desired testing time and the rotor speed. Typical DFTs are around 200 μm applied in two coats. For both biocide-based and fouling release coatings, the time between inspections depends on the paint properties and the fouling intensity, but as a general rule paints are inspected every few months.

Dynamic tests are especially useful for paints designed to protect ships sailing a large percent of the time with short idle period (e.g., container ships, bulk carriers or LNG carriers) and at speeds similar to those used in the rotary setup. Combined static and dynamic tests are relevant for ships having medium activity (i.e., relatively frequent idle periods) and for fouling release coatings, where fouling is allowed to settle during the static periods so as to evaluate the coating's fouling release capabilities during the dynamic periods.

In addition to all the paint performance variables assessed in the static tests (e.g., AF performance and mechanical properties), dynamic tests provide useful information on the thickness decrease rate of the topcoat and the biocide release rate (through leached layer thickness measurements).

Monitoring DFT loss in eroding (e.g., self-polishing) AF topcoats

Paint polishing is a key parameter for the long-term efficiency of biocide-based paints (see, e.g., Kiil *et al.* 2001). In such paints, the polishing rate influences the diffusion rates of seawater species from the bulk seawater into the paint film and those of paint components (e.g., biocides) out of the paint film into the seawater (see Chapter 14 and Yebra *et al.*, 2004). Hence, polishing is a key parameter for the long-term efficiency of chemically active coatings.

Polishing can be defined as the dynamic loss of thickness experienced by eroding-type AF paints (e.g., self-polishing) when immersed in water. Generally, it is expressed in micrometers per time unit (months) or per distance unit (e.g., 10 000 nautical miles).

There are several parameters that influence the polishing process, the more important being:

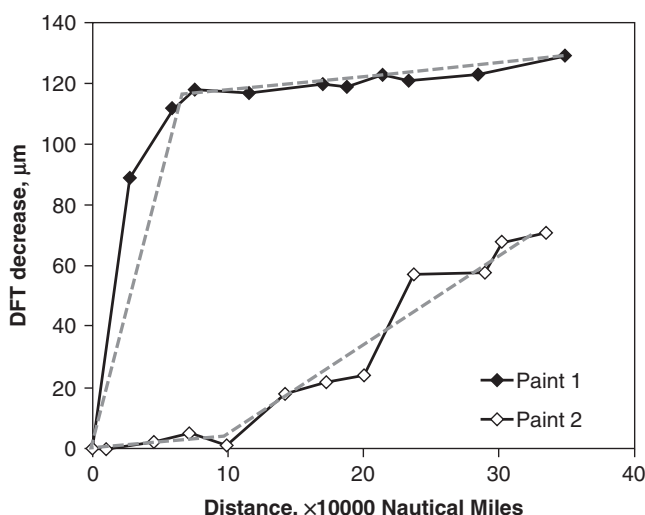
- water temperature, salinity and pH (see also Chapter 14)
- water speed/turbulence
- fouling (see Chapter 17).

The goal of the dynamic tests is to assess the long-term polishing rate and performance of a given AF topcoat, and identify potential optimisation pathways. Some paints polish at a given rate for a few months (e.g., slowly due to the need for a given induction period such as in tributyl copolymer paints; Kiil *et al.*, 2001), which then stabilises into another polishing rate which is maintained more or less stable during the entire testing period (i.e., years; Fig. 16.4).

There are two main methods to monitor the loss of film thickness of the coating during exposure:

- Via non-destructive methods making use of, e.g., the diverging magnetic properties of the metallic substrate and the coating layers.
- Via destructive methods consisting of the analysis of small paint samples of few millimetres withdrawn from the test panel (e.g., through optical microscope analysis of the cross section; see Yebra *et al.*, 2006 and Kiil *et al.*, 2006).

When using the non-destructive method (e.g., eddy current- or magnetic induction-based coating thickness measurement gauges) the test panel must be metallic (e.g., stainless steel or steel respectively) so a complete anticorrosive paint system is applied under the AF paint. When using any of these methods, it is key to measure a sufficient number of spots placed at random, but reproducible, locations along the test panel so as to attain a statistically representative DFT value over time.



16.4 Dry film thickness (DFT)-decrease plots showing a two-step polishing behaviour. Paint 1 shows a high initial polishing while Paint 2 required about 100000 NM (30 weeks at 20 knots) of induction time to start polishing (see Kiil *et al.*, 2001).

When the surface polishing is monitored using a microscopical examination of paint (MEP) procedure exclusively, there is no need for metallic substrates, so plastic panels are normally used in order to avoid potential corrosion problems. Non-destructive methods are usually faster and allow measuring the DFT evolution at many different points of the panel. Nevertheless, they usually do not provide any information on the biocide release process. MEP inspections, on the contrary, are more time consuming but provide a more complete description of the paint performance mechanisms as the relative positions of the biocide leaching front with respect to the paint surface can be determined. Paint surface areas of around 100 cm² are again enough to test polishing for both measurement type methods.

The polishing rate and the evolution of the biocide-depleted layer thickness (i.e. leached layer) are key factors to define the types of vessel and sailing areas where a given paint product has higher chances of success (see also Kiil *et al.*, 2006). Even though such parameters are strongly influenced by the rotor speed and the seawater temperature, the experience of the paint designer together with results from well-known standard paints used as references allows the attainment of conclusions that can be extrapolated to other ship speeds and water conditions. No doubt exhaustive result databases combined with modern statistical tools and computers are rapidly changing the way test information is being handled and analysed. Today, decisions are taken on the basis of a vast amount of historical information

which helps with tackling the large amount of formulation variables inherent to AF coatings.

Fouling release coatings

Fouling release coatings base their performance on keeping the initial paint surface properties during as long an immersion period as possible. Theoretically, should this be achieved, the AF properties of the coating would be maintained indefinitely (experimental fouling release coatings are usually tested for no less than two years). Hence, DFT loss measurements are not relevant for this family of AF topcoats. Except for DFT loss and leached layer thickness measurements, the fouling release performance during dynamic testing is evaluated from the same variables listed in the section dealing with static tests plus the so-called cleanability test. Since DFT loss is not a relevant parameter, there is no specific requirement for the panel substrate provided that adhesion is secured. Nevertheless, since adhesion is a key parameter in these coatings, it is typically recommended to always apply the full paint system, including the special tie-coat that secures the adhesion between the anticorrosive epoxy layers and the silicon topcoats (see, e.g., Hempel's Nexux tie-coat; Grønlund *et al.*, 2005), so as to evaluate the topcoat adhesion properties (see Chapter 13). Similar to self-polishing paints, paint surfaces around 100 cm² are enough to evaluate the effectiveness of a paint system.

The DFT of the topcoat is a relevant design parameter for fouling release systems (see Chapter 26 and Yebra *et al.*, 2004). Hence, it is a parameter to be optimised during the development of novel topcoat formulations.

16.2.4 Ship tests

Testing paints on ship hulls would be the most logical way of checking the performance of AF coatings under development. However, owing to the large time and financial investment involved and the lack of control over the test conditions, these tests are usually restricted to very promising paints or paints that are very close to being launched commercially. The difficulties associated with ship testing include the preparation of a large amount of paints (i.e., arrange pilot productions in the factory), the coordination of the paint application (e.g., ship owner, ship yard, coating advisors) and the regular monitoring of the paint performance via underwater diving inspections.

Ship tests can be carried out on either relatively small hull areas of just several square metres (i.e., a so-called test area), or the full ship (full ship application). The main advantage of a full ship application compared to test areas is that the experimental paint is tested in all the different areas of the

hull. Nevertheless, full ship applications are only employed for those paint formulations which are in the last stage of development just before being launched commercially. The reason for the latter is mainly economic: several thousand litres of paint will be needed to protect the whole underwater area and an eventual paint failure could become very expensive if a dry docking is required to repaint the hull.

Test areas are an intermediate step between laboratory/field tests and full ship applications. Test areas can consist of several paints distributed in patches of a few square metres each or just one big test area. In the former case, the main aim of the test is to compare some promising experimental paints under real ship sailing conditions. In the latter, the experimental paint is compared to the commercial paint applied to the remaining hull surface. The ship(s) selected to carry out these tests must have a sailing pattern according to the test product's characteristics. In this way, the speed and the activity of the vessel must fit with the product profile (fast/slow polishing). The same applies to the sailing route, which is also a key parameter when selecting the hull coating scheme.

The advantages of ship testing for close-to-commercial AF systems are numerous. For the first time, the new paint will have to be produced in large factory batches (robustness check of the formulation with respect to production methods), will be applied under real, uncontrolled atmospheric conditions by professional painters and will be exposed to a wide range of sailing conditions (e.g., seawater parameters and sailing speeds). All these points are relevant and could force the introduction of small changes in the paint formula or even to fully abandoning the commercialisation of the product if a serious problem is detected. Obviously, paint companies do their utmost to design preliminary tests which would detect such failures before getting to the ship testing stage (Chapter 13).

In the worst case, the shift from laboratory production (1 to 2 litre batches) to pilot- or full-scale factory batches of several thousand litres may require a few minor formulation adjustments and is not expected to involve restarting the entire paint design process. The application of the paint in shipyards around the world is the best way to check the robustness of the product with respect to a combination of factors, some of them known beforehand and tested in laboratory conditions (e.g., temperature and humidity), and others that are unexpected or unknown. Properties such as inter-coat adhesion and film appearance are strongly influenced by these factors. Applying the new AF product in real-life conditions will provide useful information that will be used to fine tune the application instructions predefined from the laboratory tests. On the contrary, if there were any failures that could not be explained or were caused by uncontrolled factors that may be repeated in future applications, the paint will need to go through serious reformulation work.

Finally, ship tests provide the best possible input for assessing the AF capabilities of the new paint. During the development of the novel formula-

tion, the AF properties are tested in tightly controlled exposure environments (laboratory tests) and/or in known natural environments (field tests). However, ships operate under a virtually infinite combination of sailing speeds, idle times and seawater conditions impossible to reproduce by any other test. Since protecting ship hulls is, most often, the main use of a new AF paint system, ship tests are necessary in order to relate the performance parameters generated during laboratory/field tests to the actual paint performance on the ship hull. Only if a successful correlation between the final paint performance under real-life exposure conditions and laboratory/sea test results is achieved, such tests become relevant.

The performance follow up during ship tests is made via diving inspections that are normally carried out when the ship is in port. The information collected is similar to that obtained from field tests: a visual evaluation of the AF performance (recorded in pictures and/or video; Fig. 16.5) and assessing the presence of inter-coat adhesion and mechanical failures such as, e.g. blistering, cracking and delamination (see Fig. 16.6). Paint samples are also collected for subsequent analysis in the laboratory looking for estimations of the paint polishing rate and the relative position of the biocide leaching fronts with respect to the paint surface. These inspections are optimally carried out several times during the paint working period but are strongly subjected to the ship's availability. Sometimes, inspections



16.5 Picture of a perfectly clean hull during an underwater inspection showing clear signs of polishing. The darker areas (top left-hand side) correspond to the last micrometers of an antifouling layer containing red inert pigments and which has nearly polished-through. Those areas where the red topcoat has been completely exhausted unveil the presence of a white TiO_2 -containing antifouling undercoat (the leached layer of such paints is completely white). It is interesting to remark that the red topcoat leftovers are found in overlapped areas during paint application (higher initial film thickness).

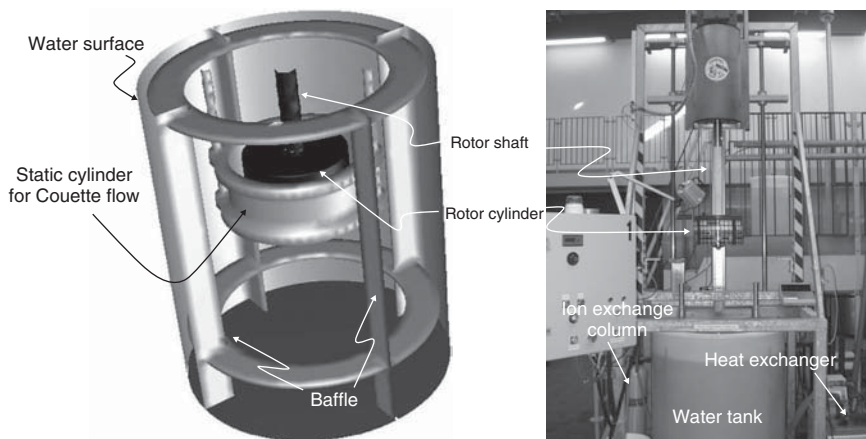


16.6 Antifouling topcoat delamination detected during an underwater inspection.

cannot be performed and part of the information is therefore lost. To overcome this, it is usual to overestimate the number of ship tests to be carried out in order to attain a minimum number of results covering a sufficient spectrum of sailing conditions.

16.3 Laboratory setups

As elaborated again in Chapter 14, the most critical requirement an AF coating must fulfil is to maintain an adequate fouling protection for extended exposure periods. The latter inevitably leads to long (years) seawater exposure periods at both static and dynamic conditions. In order to accelerate paint testing, at least during the screening stages, it would be most useful to be able to correlate AF performance to physical ‘abiotic’ parameters such as polishing and biocide leaching rates. If the latter information is available, artificial seawater tests can be run under well-controlled ‘accelerating conditions’ (e.g., high temperature) investigating such parameters and screening out the most promising systems worth subjecting to slow and costly field tests. Because the correlation between polishing and biocide leaching to real-life performance is never complete, field tests are still the most informative tests for commercial or close-to-commercial coatings. In spite of the latter, laboratory setups are invaluable tools for accelerating, e.g. the development of innovative paint technologies, raw material screening tests, supplier approval for given raw materials and evaluation of factory batches.



16.7 Schematic drawing and picture of a Couette-type rotary set-up. Paints are attached to the outer surface of a rotating cylinder which is immersed in artificial seawater. The design of the tank is aimed at attaining a specific flow pattern at the surface of the paints so as to control the shear stress and simulate real life values.

16.3.1 Couette-type laboratory setups

The rotary set-ups presented in this section (see Fig. 16.7) have been successfully used for the empirical design and optimisation of commercial AF paint systems since the late 90s. In addition, they have been used to validate the mathematical model describing the behaviour of model tributyl tin self-polishing copolymer paints (TBT-SPC) and tin-free, zinc-carboxylate, fibre-based paints (see Chapter 14) and to demonstrate the self-smoothing behaviour of rosin-derived SP products by means of drag measurements (Weinell *et al.*, 2003).

Design

The set-ups (Fig. 16.7) consist of baffled tanks containing 0.4–0.5 m³ artificial sea water (Yebra *et al.*, 2006). The tanks are equipped with two concentric cylinders (inner cylinder 30 cm Ø; gap width of 4 cm; height 17 cm), the innermost in rotation (the rotational speed can be adjusted within a wide range of angular velocities) with the paint samples attached to the outer surface of the rotating cylinder. With this configuration, the flow pattern in the narrow channel between the cylinders approaches Couette flow, which is characterised by a constant shear stress across the channel. The set-up can operate at a wide range of sea water conditions (e.g., sea water temperature, pH and sea water composition) which are tightly controlled.

- Temperature control is achieved thanks to a set of thermostatic valves regulating the flow of cold water through approx. 30 m of a plastic spiral coil fixed to the tank walls.
- For pH control, an automatic titration system has been modified to continuously (e.g., every 10 minutes) check the seawater pH and correct it when necessary by adding a fixed volume of either NaOH or HCl.
- Salinity is monitored through daily conductivity measurements. The seawater level in the tank is adjusted by adding demineralised water on demand via communicating vessels to compensate for evaporation losses. Seawater is typically exchanged every 8 weeks.
- The accumulation in the tank of the Cu^{2+} ions released, which may potentially affect the Cu_2O leaching rate during the experiment, is prevented by continuously forcing the water through a column packed with a strong cationic ion-exchange resin (Na-conditioned Lewatitt TP 207). Turnover time is no more than 30 minutes, with water linear velocity within the column of about 15 m/h.
- An active carbon filter is also used to retain all seawater soluble organic species released as a result of paint immersion, typically binder resins and biocides.

Test procedure

According to Hempel's procedure, paint samples are applied onto a plastic foil by means of a special applicator leaving eight drawdowns of about 500 μm wet film thickness (WFT) and subsequently dried for one day in a ventilated cupboard. The substrate had been previously coated with 20–30 μm of a two-pack AF link coat in order to secure adhesion. Once the paint is sufficiently dry, the foil is cut into 26 mm wide strips which are fixed to the outer surface of PVC discs. Prior to that, a sea water-insoluble acrylic paint is applied on selected parts of the AF paint film in order to use it as reference to monitor the degree of polishing of the exposed AF coating with time. The recoating interval between the link coat and the topcoat, as well as the minimum drying time prior to seawater exposure are fixed so as to assure reproducibility between tests.

Each setup rotates six such discs, each of them having a capacity for 4 panels (i.e. 32 samples). After a predetermined exposure time (standard operation conditions: pH 8.2, 25 °C, 20 knots, Grasshoff water; Yebra *et al.*, 2006), one panel is withdrawn from the setup and each of the eight samples is inspected with an optical microscope. For sample preparation, the specimen is first embedded in paraffin and then sliced by means of a microtome to attain a clean surface for analysis. The cross section of the paint reveals the degree of polishing and the thickness of the pigment depleted layer or

leached layer. Usually, this procedure is repeated at four different times (e.g., every 8 weeks) for each set of eight paints, yielding, e.g. 32 weeks polishing and leaching data. When required, scanning electron microscopy coupled with energy dispersive X-ray sensors (SEM-EDX) can be used to assess composition changes during immersion along the paint's cross section (e.g., biocide lixiviation; Yebra *et al.*, 2006).

In those cases in which the evolution of the leached layer is of little relevance, it is also possible to use a non-destructive method to monitor paint polishing with time. Such a method consists of an optical displacement sensor (ODS) system with a spot size of $\varnothing 1.0$ mm. The measuring location can be reproduced with accuracy higher than ± 5 μ m. At the same time a digital camera gives an image of the sample surface, which can document the surface condition (e.g., colour, cracking). For analysis, the rotor discs are mounted on a horizontal stage which is able to rotate by means of a PC-controlled stepper motor. The PC also controls the movement of the ODS and the camera, both of them oriented perpendicular to the sample surface. At the end of the exposure period, it is again possible to examine the sample optically (or via SEM-EDX) as explained before, hence obtaining one final data point for both the degree of polishing (to verify the ODS measurements) and the leached layer thickness.

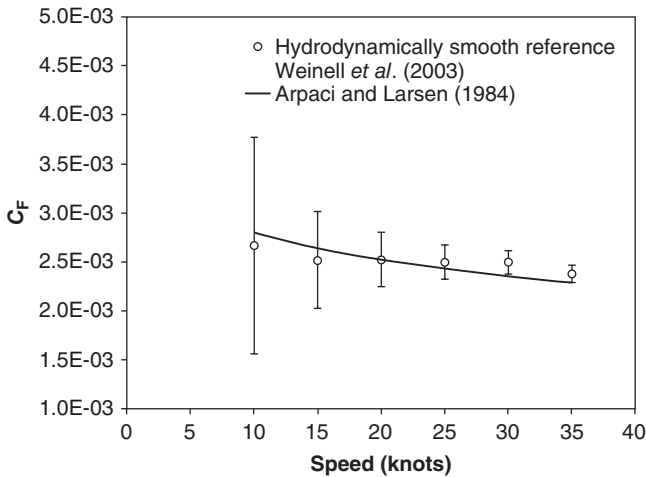
The operation conditions in these setups are close enough to those in natural seawater, with a slightly elevated temperature so as to accelerate the relevant chemical processes. As a comparison, the annual average seawater temperature in Barcelona's coast (see earlier sections) is about 18 °C. When increasing the test temperature and correlating the results to real life, it is important to consider that different product families may behave differently depending on their polishing mechanism. Paint polishing through a chemically controlled mechanism (self-polishing paints) will be markedly affected by temperature changes (Arrhenius-type dependency), while physically eroding paints will be less affected. The latter is usually not a problem when comparing small modifications of the same product.

Set-up validation

Basically the hydrodynamic design criteria for dynamic laboratory setups are:

- to attain a given shear stress at the paint surface
- to assure a homogeneous shear stress distribution along the entire test area.

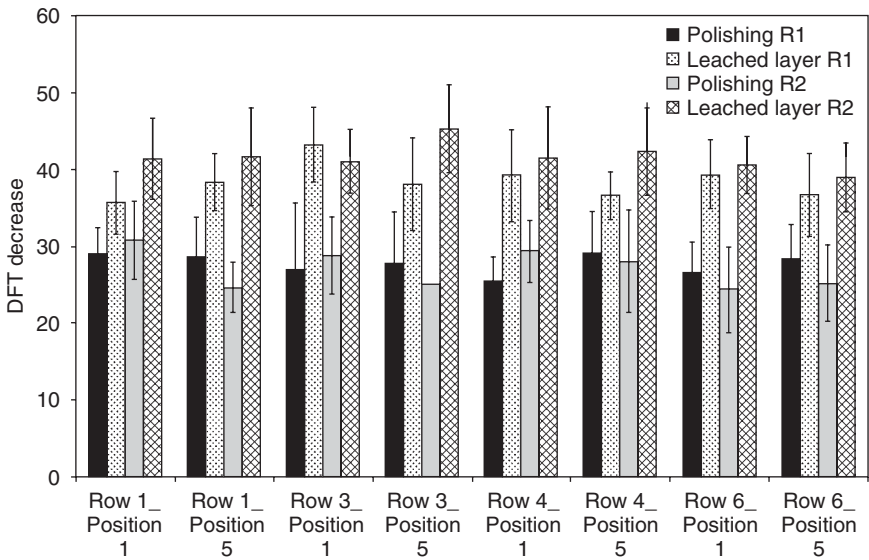
Obviously, the aim would be to approach shear stress values such as those found on ship hulls. For that, rough estimations using, e.g. equations relating



16.8 Friction coefficient values at different rotational velocities calculated from torque measurements on the Couette-type laboratory rotors using a smooth PVC surface. The results show an excellent agreement with the theoretical expression presented in Arpaci and Larsen (1984).

bulk flow and friction coefficient on smooth flat plates, can be used (Schlichting, 1968). Modern computational fluid dynamics (CFD) calculations can be used to provide more accurate estimates for specific ship geometries.

Weinell *et al.* (2003) measured the drag resistance of the rotor set-up at different rotational speeds and compared the results to those obtained theoretically assuming a perfectly smooth surface and Couette flow. The results verify that a controlled shear stress can be attained at the paint surface by means of this set-up (Fig. 16.8) despite non-ideal Couette flow is predicted from CFD simulations (Yebra *et al.*, 2006). Such simulations were also used to demonstrate that all the paints exposed onto the rotating cylinder are subject to the same shear stress independently of the position in the rotor. The results show that there are no important shear stress gradients in the direction parallel to the axis of rotation, which means that all the samples will be subjected to a similar shear independently on their position on the rotor (Yebra *et al.*, 2006). The latter is confirmed in Fig. 16.9, where the short-term thickness loss data for a fast-polishing AF paint tested at different vertical and horizontal positions on the rotating cylinder are shown. No significant differences are observed once the uncertainty associated with the paint inspection process is taken into account.



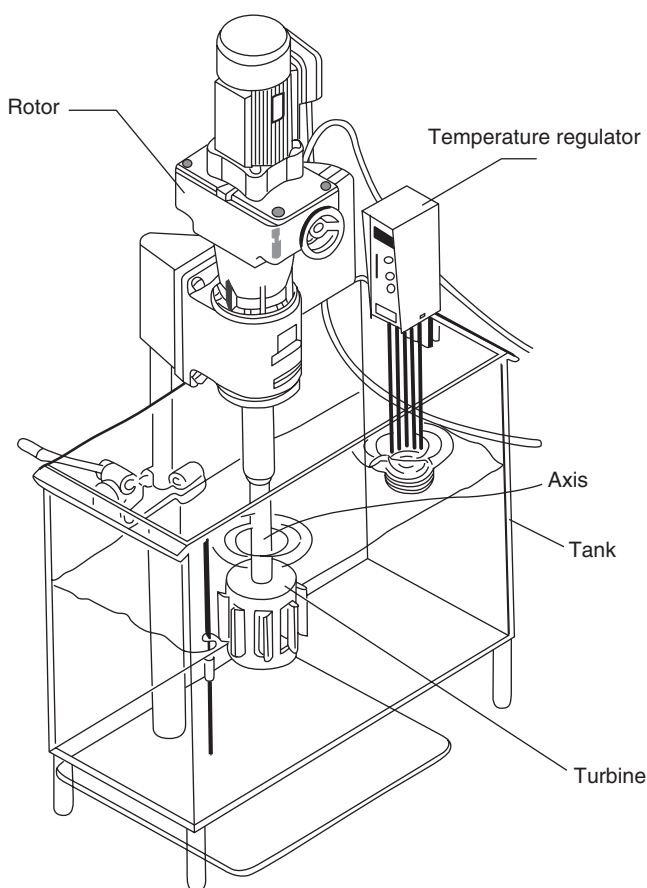
16.9 DFT decrease after 8 weeks of rotor exposure (20 knots, 25 °C, pH 8.2) for a fast polishing abrasive reference paint at different positions in two different laboratory setups, showing no significant differences (95% confidence interval).

16.3.2 'Turbo Eroder®' type

Design

The rotary set-up presented in this section is manufactured under the commercial name Turbo Eroder® (Labomat Essor) (Fig. 16.10)¹. It basically consists of a tank filled with 60 litres of ASTM artificial seawater (D1141-98). A stainless steel cylindrical drum is attached to a motor by a central shaft and then placed within a turbine to obtain the same shear stress distribution along the peripheral surface of the rotating cylinder, with no significant gradients in the vertical direction. The turbine creates a high hydrodynamic flow tangentially to the coating surface and turbulences are produced. Owing to the complex turbine geometry, the velocity profiles at the level of the rotating cylinder applied to the paint surface have not been estimated yet. The drum is rotated at 650 rpm yielding an equivalent surface linear velocity of 3 knots. As a result of the cylinder rotation, heat is generated within the setup, leading to a seawater temperature of 40°C which is maintained throughout the test. The water is neither circulated nor refreshed.

¹ Information provided by Dr. Christine Bressy. See 'Sources of further information'.



16.10 Picture of the Turbo Eroder apparatus (with permission of French Navy).

Test procedure

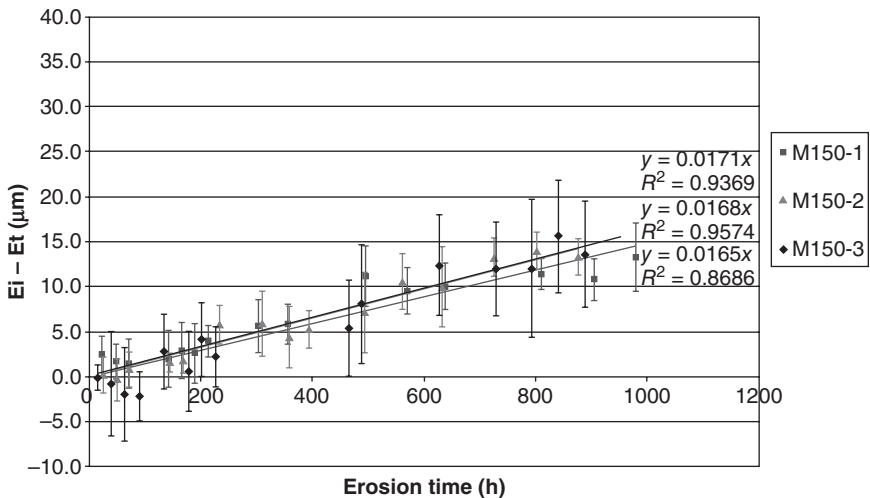
A stainless steel cylindrical drum with a diameter of 45 mm is first coated with 50 μm of an epoxy primer (M121 from Kolorian, France). After 24 hours, the drum is airsprayed with approximately 100 μm dry film thickness (DFT) of both test and reference paints (alternatively, Dr Blade applications on a plastic foil are also possible). Four different paints can be applied on the same drum, which are dried for at least 1 week in the laboratory at room temperature before testing.

Before each thickness measurement (using an Elcometer 355 measurement probe), the rotor is rinsed and dried at room temperature. A template is then adapted on the rotor in order to always measure the coating thickness at the same place of the test area. To protect the coating from mechanical

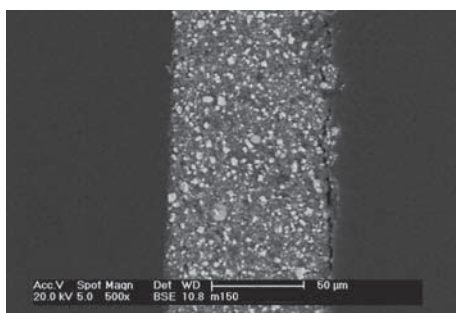
damage during DFT measurements, a foil is placed between the coating and the template. DFT is measured at eight different positions, and ten DFT values are recorded for each of them (aberrant values are not taken into account). Thickness measurements are performed every 50 hours of rotation. Average thickness decrease values are plotted as a function of rotation time. During the inspection, pH, conductivity, and salinity values are collected and adjustment of the water level and the pH is performed when necessary. During the first 24 hours of test, a low speed of 80 r.p.m. is chosen to allow the hydration of the coating. The erosion rate is accounted as the average decrease in film thickness (expressed in $\mu\text{m}/\text{month}$) over the whole test.

Set-up validation

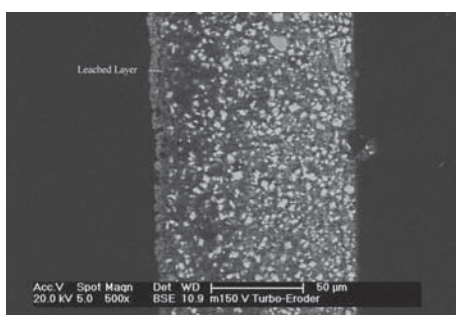
The reproducibility of this technique is shown in Fig. 16.11 where the polishing results of a commercial TBT-based self-polishing reference paint (M150) exposed in three different setups are compared with time. An average thickness loss rate of $12.1 \pm 0.2 \mu\text{m}/\text{month}$ is obtained from three setups compared to about $5 \mu\text{m}/\text{month}$ in 30°C natural seawater. Just as in the Couette-type setup, samples can be withdrawn at different time intervals and analyzed by physicochemical techniques such as scanning electron microscopy (SEM) combined or not with an energy-dispersive X-ray analysis (EDX). These techniques were already reported in the literature for the analysis of AF paints (Fay *et al.*, 2005; Yebra *et al.*, 2006). Combining Turbo Eroder tests with



16.11 Evolution of the thickness loss ($E_i - E_t$, μm) of three different setups of M150 with erosion time in artificial seawater ASTM at 40°C . Test of reproducibility.



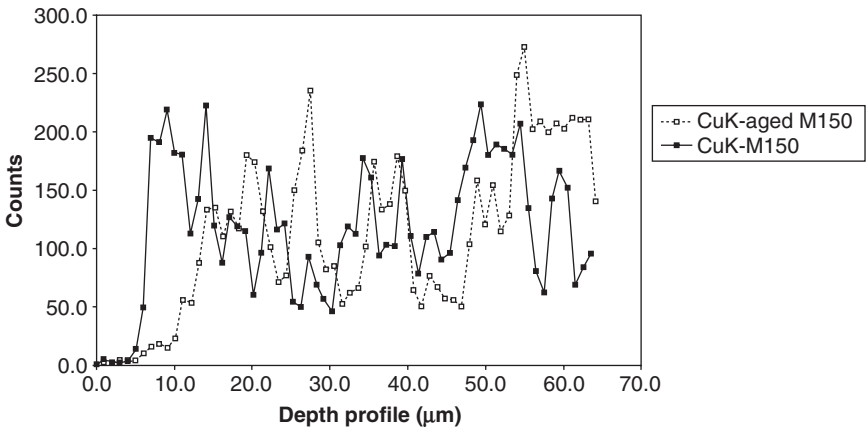
(a)



(b)

16.12 SEM pictures of the cross section of a TBT-based commercial antifouling paint M150 before (a) and after 900 hours of erosion in artificial seawater ASTM at 40 °C in a Turbo Eroder® setup (b). Magnification is 500×.

SEM and EDX analysis is a powerful technique to differentiate between self-polishing paints and controlled-release ones depending on whether the dissolution of fillers or pigments occurs near the coating surface (thin leached layers) or inside the film (thick and undefined leached layers) respectively. Figure 16.12 shows SEM micrographs of the commercial TBT-based self-polishing reference paint (M150) before and after the erosion test. A leached layer of 5–10 μm thickness range could be easily observed. EDX spectra of both the leached layer and the bulk of the coating reveal the presence of copper, zinc, titanium and tin atoms with an important decrease of copper content within the leached layer (Figure 16.13). To differentiate between a self-polishing and a controlled-release behaviour, X-ray profiles are recorded over 60 μm-deep analysis area. Figure 16.13 shows average data obtained from three copper content profiles performed on both aged and non-aged M150. These data reveal a significant release of copper-based compounds near the surface without gradient concentration from the copper leaching front to the substrate. An average leached layer thickness can be estimated by EDX analysis profiles close to 7–8 μm.



16.13 Average data obtained from three copper content profiles performed on both aged and non-aged M150.

Table 16.1 Advantages and drawbacks of the Turbo Eroder® in comparison with traditional, in-situ dynamic drum apparatus

Advantages	Drawbacks
Both artificial and natural seawater can be used	Only four different paints can be tested simultaneously in each setup
Tight control of the seawater composition (pH, salinity, conductivity)	The geometry of the turbine blades is important to prevent deviations in thickness loss results
Temperature range from 25 °C up to 40 °C	Minimal temperature of 40 °C without additional temperature controller
No development of fouling on the coating surface which could diminish the reliability of the thickness loss measurements	Test temperature away from natural seawater conditions
Short-term polishing results	Equivalence to polishing in natural seawater not established yet

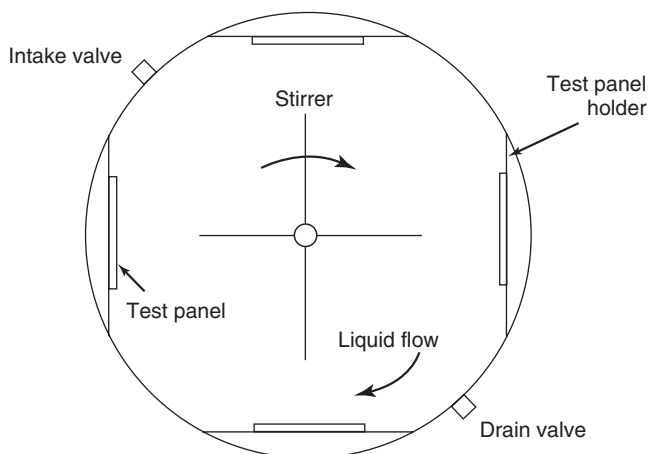
Summing up, the Turbo Eroder® provides reproducible experimental data on the DFT loss and biocide release rate from a paint film. This test can be used to compare the erosion rate of AF paints to a self-polishing reference paint. The value is not necessarily indicative of actual service performance, but rather a means of comparing products or different batches of the same product line. Compared to other ageing methods (ASTM 4938-89; Rabenhorst 2005), the advantages and drawbacks of the Turbo Eroder® apparatus are listed in Table 16.1. Temperature values above room temperature could be used here to accelerate the water sorption, solubility and

permeability of organic coatings (Blahnick, 1983; Li *et al.*, 1998) leading to higher dissolution rates of hydrolyzed and ionised species (e.g., self-polishing binders, seawater soluble pigments and biocides) out of the coating. Moreover, chemical reaction and diffusion phenomena are key mechanisms in the performance of chemically active AF paints, and these latter can be markedly affected by seawater conditions (Yebra *et al.*, 2006).

16.3.3 Other setups

Florida Institute of Technology (FIT) dynamic ageing system

A 1.6 meter diameter cylindrical tank containing either artificial or natural seawater has been recently patented for AF dynamic and combined static/dynamic ageing tests (Swain and Touzot, 2006). Currently, seawater is supplied from the Indian River Lagoon in Melbourne, Fla., and completely renewed every 3 hours (FIT's web page; last accessed November 2007). Substantially rigid rectangular panels ($0.254\text{ m} \times 0.305\text{ m}$) are inserted into the tank and positioned so that top and bottom connect to points on the inner circumference of the tank (Fig. 16.14; Swain and Touzot, 2006). With this design, the test panels are not required to have a circular shape conforming to the diameter of the tank. Fouling control coatings to be tested are applied or attached to the test panel holder so that they are at or beneath the surface of the liquid contained in the circular tank. A motor (1.5 hp dc 30:1 reduction) driven stirrer has one or more paddles ($0.5\text{ m} \times 1\text{ m}$ high PVC plates) which emanate from a centre point of rotation causing the seawater to rotate and impart a rotational velocity to the liquid in the



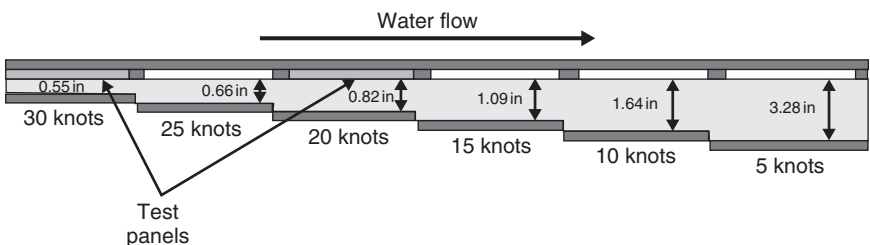
16.14 Top view of a test tank (adapted from US2006016250).

circular tank. Compared to other dynamic ageing methods, which require large motors and to increase the velocity of the liquid from zero to the desired velocity on a continuous basis, the energy required from this setup is only that required to overcome losses as the liquid rotates. Salinity, pH, temperature, and velocity of the test liquid are some of the parameters that can be monitored during the test duration. Instrumentation for such monitoring can be mounted to the side of the tank or to the framework for mounting the stirrer and drive assembly to the tank.

When natural seawater is used, this setup also allows alternating static and dynamic cycles of approximately 30 days each for a total length of time or until some degree of fouling is reached. The seawater can flow through the tank during the static period allowing the test panels to become fouled. When the conditions for terminating the static interval have been satisfied, the dynamic cycle can run for a desired length of time or may be stopped periodically to test the effectiveness of the paints tested.

ASTM D4938-high speed water channel

The high velocity water tunnel consists of a large pump which forces water through a four-sided rectangular section with diminishing width to generate water flows of up to 18 m/s (Fig. 16.15). For example, the Naval Research Lab Key West facility uses a 3.6 m³/s pump and a 14 cm high rectangular cross section with widths of 8.33, 4.17, 2.77, 2.08, 1.68, and 1.40 cm which generate water velocities of 5, 10, 15, 20, 25, and 30 knots respectively. Coating thickness measurements can be taken at certain time intervals. As weak points, the system is expensive to build and requires a large horsepower pump to achieve the high velocity of water flows due to inefficiencies caused by large pressure losses in the system. The system is not suited to test a large number of samples as it can only accommodate one test panel at each velocity. The flow characteristics are poorly defined. Finally, the narrow width (1.4 cm) required to generate high water velocities precludes the testing of panels with large macrofouling communities.



16.15 Diagram of a high-speed seawater flow channel (top view).

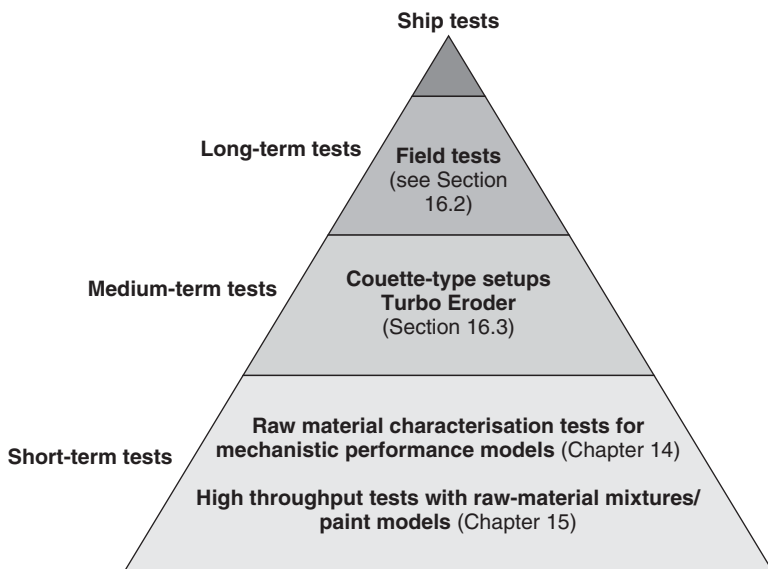
16.4 Future trends and conclusions

As already mentioned several times throughout this chapter, the main difficulty in formulating new AF coatings (both chemically active and fouling release) is to maintain a sufficient level of protection against colonisation by fouling organisms for extended periods of time irrespectively of the exposure conditions. Unless the paint product is specified for short activity periods at very specific marine locations (e.g., pleasure boats), reliable paint testing must necessarily include long (i.e., years) ageing periods in as realistic and varied exposure conditions as possible. This is most realistically achieved through ship testing which is an economically risky and extremely time and resource-consuming practice. Hence, paint companies need to base their paint design and optimisation processes on alternative ageing tests which yield reliable estimations of the actual real-life paint performance in as little time as possible.

Without any doubt, the most informative paint ageing methods are those carried out in natural seawater at sea stations for long (i.e., years) periods of time. This chapter has described examples of such tests simulating both static and dynamic exposure conditions (and the combination of both). Though fairly cheap compared to ship testing, reliable dynamic field tests are still rather slow (not shorter than one year) and expensive, so any means of accelerating the screening so as to select the most promising products before natural seawater long-term testing would be most useful (see, e.g., Chapters 14 and 26). Both with accelerated and long-term testing procedures, it is key to extract as much information as possible during and after the test. Actually, the efficiency of the design and optimisation process is enhanced markedly when we can use test results to build paint performance models assisting in the selection of new promising formulations for the next stage of development. These models may be:

- mechanistic (see, e.g., Chapter 14).
- statistic (use of design of experiment and multivariate data analysis tools).

An ideal work flow for paint design is illustrated in Fig. 16.16. The width of the pyramid stands for the number of variables/formulations tested. Optimally, short tests should be used to screen out a large number of formulation variables, while long-term field tests are used for final fine-tuning of close to commercial formulations. Obviously, meaningful short-term tests to characterise the long-term reactivity and performance of a multicomponent paint system exposed to changing environments are no easy task. Unlike fouling release coatings (see, e.g., Chapter 26), short term performance tests with broad-spectrum biocide-based paints are usually not very useful for paint design, as it is the sustained release of the active compounds over the years which determine the success of the product. Hence, we



16.16 Work flow for an efficient antifouling paint design and optimisation. Short-term lab tests are used so as to evaluate the potential of a given formulation/raw material and suggest preliminary formulations for medium-term (i.e. months) polishing and biocide leaching testing. Those formulations yielding promising polishing biocide leaching are then subject to long-term optimisation tests in the field. The best products will be then ready for marketing provided that a sufficient number of successful results on actual ships is gathered.

believe that the development of novel fouling control coatings during the coming many years will strongly rely on medium and long-term optimisation approaches similar to the ageing tests described in this chapter potentially assisted by statistic and mechanistic models.

16.5 Sources of further information and advice

The information about the Turbo Eroder[®] was facilitated by Dr. Christine Bressy. Dr. Bressy is a researcher at the Institut des Sciences de l'Ingénieur de Toulon et du Var. Paint samples to be tested in the Turbo Eroder can be sent to the postal address shown below:

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I.S.I.T.V.

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Dr. Bressy's group is planning to set up a test-package including erosion tests, raft immersion test and exposure tests for AF and anticorrosive paints, and create a test network with several laboratories.

Geoffrey Swain and Arthur Touzot are responsible for the design of the FIT's dynamic ageing system (US2006016250)

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16.6 Acknowledgements

In addition to the researchers who have provided information for this chapter, the authors wish to thank Elisabeth Haslbeck for her kind help suggesting sources of information for the existing ageing methods. Claus Weinell is also acknowledged for his help in the description of the Couette-type setups. Finally, Pere Català and Santiago Arias provided their extensive experience within field tests, which greatly improved the manuscript.

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Testing the impact of biofilms on the performance of marine antifouling coatings

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Abstract: This chapter covers the role of marine microfouling and biofilms in release rate testing and looks at how the results of dynamic release rate testing in natural seawater can inform on the acknowledged discrepancies between current release rate test methods. The effects of biocidal AF coatings on biofilms are considered, along with the consequences of shear stress on biofilm formation and how this may impact on test method design. The effects of biofilms on the performance of biocidal antifouling coatings is then considered with regard to novel test methodologies. Current test methodologies are then reviewed in light of the importance of biofilm formation, and suggestions are proposed for ensuring future test methodologies can account for the effects of biofilms.

Key words: biofilm, release rate, test methodology, biocide, hydrodynamics, micro-fouling.

17.1 Introduction

There is an acknowledged need for rigorous, standardised, global testing of novel antifouling (AF) coatings for regulatory standards. Such testing would not only enable coatings manufacturers to harmonise their global business, but it would also ensure effective protection of the marine environment. It is of paramount importance for both regulators and coatings manufacturers that a fast, reliable, repeatable and reproducible method for measuring biocide release rates is developed.

There are acknowledged problems with the ASTM and ISO release rate methodologies (Valkirs *et al.* 2003; Haslbeck *et al.* 2005a; 2005b; Finnie 2006). The methodology that appears to measure ‘reality’, the US Navy dome method (Valkirs *et al.* 2003), has only been tested in one geographic location (San Diego Bay) and is prohibitively expensive to use. This chapter looks at how the results of dynamic release rate testing in natural seawater can inform on the acknowledged discrepancies between current release rate test methods.

17.2 Marine microfouling

Marine fouling consists of macrofouling (e.g., mussels, barnacles) and microfouling in the form of algal or bacterial biofilms. Biofilm formation begins with the adhesion of a small number of bacterial cells to a surface. These isolated cells give rise to small colonies which increase in size and coalesce to form a bacterial film (Yebra *et al.* 2006a). The attached bacterial cells synthesise new exopolysaccharide (EPS) material in order to cement their adhesion both to the surface and to other bacterial cells in the developing biofilm (Costerton 1999).

Diatoms, which form another component of marine biofilms and act as a settlement mediator for larger fouling (Cassé *et al.* 2006), secrete large amounts of EPS from a slit (raphe) or apical pore in the frustule (Hoagland *et al.* 1993). Diatom fouling is dominated by a restricted number of genera and previous studies have shown that the raphid diatoms *Navicula* and *Amphora* are the most common on conventional AF coatings, whilst *Acanthos* is very common on copper free, TBT based coatings (Robinson *et al.* 1985; Callow 1986).

In the 1970s and 1980s, many attempts were made to develop mathematical models to describe processes in biofilms. To simplify the equations used in these models, an assumption was made that biofilms were homogenous structures, allowing the calculation of diffusion gradients (Wimpenny *et al.* 2000). With the development of new tools for in situ examination (e.g., confocal laser scanning microscopy (CLSM), and differential interference contrast microscopy (DICM)), the structural heterogeneity of natural biofilms has been demonstrated in more detail (Wimpenny *et al.* 2000). It has been shown that biofilms are not homogenous planar layers, but aggregations of cells with channels running between them where convective flow is possible to supply oxygen and nutrients inside the biofilm (Stoodley *et al.* 1994). This has obvious importance in the case of biofilms growing on AF coatings, as not only will these channels transport nutrients from the surrounding water into the biofilm, but they will also transport biocides from the surface of the AF coating through the biofilm to the surrounding water.

When investigating biocidal AF coatings, the most important factors in biofilm development are:

- Biocide release rate and its effect on biofilm development, species composition and transport of biocides through the biofilm.
- Differences in shear stress at the surface of the coating, important not only for biofilm formation but also for biocide release rate.

Characterising the effects that these factors have on bacterial and algal diversity is becoming more important as AF technology becomes more concerned with preventing fouling at smaller and smaller scales through the

manipulation of micro-textured surfaces (Berntsson *et al.* 2000; Petronis *et al.* 2000; Callow *et al.* 2002; Jelvestam *et al.* 2003; Scardino *et al.* 2003; Hoipkemeier-Wilson *et al.* 2004; Carman *et al.* 2006; Scardino *et al.* 2006; Schumacher *et al.* 2007; see also Scardino Chapter 25 in this volume). There is a growing body of work looking at how biofilms affect a coating's performance in terms of hydrodynamic drag (WHOI 1952; Watanabe *et al.* 1969; Loeb *et al.* 1984; Mihm *et al.* 1988; Candries *et al.* 2003; Valkirs *et al.* 2003; Holm *et al.* 2004; Schultz 2004) and biocide release rate (WHOI 1952; Mihm *et al.* 1988; Valkirs *et al.* 2003; Yebra *et al.* 2006b; Howell 2007), although there has been very little work done in the last 20 years investigating how species diversity in biofilms is affected by biocide concentrations from AF coatings (WHOI 1952; Dempsey 1981; Tang *et al.* 1998; Boivin *et al.* 2005; 2006; Cassé *et al.* 2006; Howell 2007), or by changes in shear stress (Beyenal *et al.* 2002; Rickard *et al.* 2004; Tsai 2005; Cassé *et al.* 2006; Howell 2007). Species diversity could be important as new antifouling technologies based on micro-textured surfaces will need to address the community composition of biofilms in order to be fully successful.

Most algal or bacterial biofilms in their natural environments are likely to consist of a variety of species that influence each other in synergistic and antagonistic ways (Burmolle *et al.* 2006). Having a system that is stressed in some way (e.g., by copper or shear stress) will affect this natural species diversity (e.g., copper-tolerant species becoming dominant (Boivin *et al.* 2005)).

Some marine bacteria produce antibacterial substances to give them a competitive advantage, such as *Pseudoalteromonas tunicata* (Holmstrom *et al.* 1998). Rao *et al.* (2005) showed that in a mixed-species biofilm, *P. tunicata* removed the competing strain unless its competitor was relatively insensitive to the antibacterial protein AlpP (*Pseudomonas gracilis*), or produced strong inhibitory action against *P. tunicata* (*Roseobacter gal-laeciensis*). Their data suggested that the dominance of *P. tunicata* could be attributed to their ability to rapidly form microcolonies and produce extracellular antibacterial compounds. Burmolle *et al.* (2006) found that in biofilms subjected to invasion by *P. tunicata*, mixed-species biofilms resisted invasion to a greater extent than single species biofilms, suggesting that synergistic effects promote biofilm biomass and resistance of the biofilm to antimicrobial agents and bacterial invasion in multispecies biofilms.

17.3 Effects of biocidal antifouling coatings on biofilms

When looking at bacterial biofilms, it is important that one takes into consideration the fact that cultural methods for identifying bacteria are always restrictive, because not all the bacteria can grow under chosen cultural

conditions in the laboratory. The most important findings are therefore not the types of bacteria that are found but the differences in community composition between different conditions (Cassé *et al.* 2006). If a coating is causing selective colonisation by particular bacteria, it is possible that thick biofilms could develop which would affect biocide release and hydrodynamic performance. If it was possible to know the community composition of any biofilm and how it was being affected, it should be possible to ensure that any biocides within a coating will target all bacterial species and give better antifouling performance.

17.3.1 Heavy metals

There has been little work done specifically investigating how biofilms affect biocide release rate from AF coatings. However, there is a body of work looking at the effects of heavy metals on biofilms, investigating tolerant species, the ability to bind heavy metals in the EPS structure and the differences in species composition brought about by copper stress. Once copper tolerant species have been identified, changes in community structure can then be related to release rates.

On a copper-based AF coating, Howell (2007) showed there were different bacterial and diatom communities present at static, 1 knot and 4 knots, and on the surface of the coating being tested as opposed to the container wall within which the coating was immersed. The container wall in the static condition, which had a thick algal biofilm, showed a move from a community made up of gram-positive and gram-negative bacteria, to one dominated by gram-negative γ -Proteobacteria, particularly the Cu^{2+} resistant *Pseudoalteromonas elyakovii* and *Pseudoalteromonas tunicata*.

In contrast, the container wall in the rotating condition only had α -Proteobacteria when the cylinder was rotating at 1 knot, and became more diverse after the speed was increased to 4 knots. This change in community structure could either concern oxygen levels within the container increasing with increasing speed or an observed spike in release rates when the speed was increased. The spike in release rates may have disturbed the bacterial community on the container walls sufficiently for recolonisation to take place.

On the coating in the static condition, gram-negative bacteria dominated the community at both 1 and 4 knots. In the γ -Proteobacteria, there was a move away from *Alteromonas* sp., *Idiomarina* sp. and *Colwellia* sp. towards *Marinobacter* sp., which have been shown to have Cu^{2+} binding capabilities (Bhaskar *et al.* 2006). In the gram-positive bacteria there was a move from *Corynebacterium* sp. to *Micrococcus* sp. which has also been shown to have Cu^{2+} binding capabilities (Lo *et al.* 2003). In general the community changed towards a more copper-tolerant species.

Table 17.1 Viable bacteria in biofilms found on test surfaces after 57 and 62 days immersion (Howell 2007)

Immersion	Container wall				Cylinder surface			
	Static (Day 57)	Static (Day 62)	Rotating (Day 57, 1 knot)	Rotating (Day 62, 4 knots)	Static (Day 57)	Static (Day 69)	Rotating (Day 57, 1 knot)	Rotating (Day 69, 4 knots)
Total	3	4	2	16	7	11	6	5
Gram-negative								
α-Proteobacteria			2		3	2		
β-Proteobacteria							1	
γ-Proteobacteria	1	3		3	3	4	1	4
Flavobacteria				5			2	3
Gram-positive								
Actinobacteria	1			5				1
Firimicutes	1	1		3	1	2	1	1

There was a clear decrease in bacterial species diversity on the coating in the rotating condition after the speed increase, with γ-Proteobacteria becoming the dominant group, in particular *Pseudoalteromonas* sp., *Glaciecola* sp., and *Idiomarina* sp.. Although the speed decreased the diversity, it did not significantly decrease the number of species, implying that γ-Proteobacteria have a competitive advantage in conditions with higher shear stress. Unlike the findings of Rickard *et al.* (2004), who found that in freshwater biofilms, shear rate did not select for a single cluster of species, genera or taxonomic group, Howell (2007; see Table 17.1) found that the combinatory influence of an increase in shear rate plus a very high pulse of Cu²⁺ when the speed increased selected for a particular taxonomic group that has both high Cu²⁺ tolerance, and the ability to withstand high shear stress.

Cassé *et al.* (2006) found marked differences in the community composition and density of diatoms and bacteria in the microfouling communities that developed on commercial AF surfaces, and that these communities were impacted by differing hydrodynamic conditions. They tested a TBT SPC, a copper SPC, a copper ablative and a fouling release coating at static conditions and a dynamic velocity of 8–10 knots. They found that after static immersion for 60 days, all four coatings had >80% fouling cover, dominated by slime films. The only bacterial genera found on all coatings were *Micrococcus* and *Pseudomonas*, although *Pseudomonas* disappeared from biocidal coatings after dynamic immersion. Only pinnate diatoms were observed, represented by two classes, *Raphinidae* and *Araphinidae*. Within these classes *Amphora*, *Navicula* and *Syendra* were found on all surfaces.

There was an eight-fold reduction in diatoms for all coatings after dynamic immersion, although *Amphora* and *Navicula* remained present on both SPC coatings.

Boivin *et al.* (2005) characterised the effects of copper and temperature on bacterial communities in photosynthetic biofilms. They found that after 3 days exposure to 3 μM copper solution, the copper concentration in the biofilm significantly increased. Community level physiological profiling was used to investigate the relationship between exposed and reference bacterial communities. They found that successional changes in semi-natural biofilms took place because of the presence of copper. Changes in bacterial genetic and metabolic profiles in copper exposed biofilms were unequivocal: community characteristics developed distinctly from reference systems and such changes were marked in a short period of time.

It is not always the case that Cu^{2+} increases secretion of EPS. Richau *et al.* (1997) found that cells defective in EPS synthesis appeared during cultivation of *Sphingomonas paucimobilis* with inhibitory concentrations of Cu^{2+} . The percentage of less mucoid colonial variants dramatically increased with the increase in Cu^{2+} , reaching 85% of total viable cells at the maximal concentration for growth. They found that the level of increased Cu^{2+} tolerance of the mutated variants, assessed by the inhibitory effect of Cu^{2+} on growth, correlated with the degree of loss of the ability to secrete high molecular mass EPS.

Tang *et al.* (1998) investigated the development of biofilms of *Pseudomonas aeruginosa* on materials coated with a biocide free AF coating (VC 18, International Paints), and on two different conditions where materials were coated with the same coating containing either Cu_2O or TBTF at concentrations given in commercial paints. They found that Cu_2O decreased viable counts initially, but after 6 days, numbers were equivalent to uncoated surfaces. These results may not be due to copper tolerant species however, as Cu^{2+} release rates would have dropped to negligible levels in a short time once the surficial copper oxide particles dissolved. The remaining particles would be bound up in the hard matrix of the coating and be inert, leading to a reduction in Cu^{2+} release rate and subsequent bacterial recolonisation.

17.3.2 Shear stress

Shear stress is an integral part of any test design as it has been shown that changes in rotary speed in artificial water without any biofilm formation can cause changes in the shear stress which can create marked differences in biocide release rate (Howell *et al.* 2006a; Howell 2007). Shear stress can also effect biofilm formation and therefore create different issues that need to be taken into account when designing test systems in natural water.

Although shear stress is not strictly an effect of AF coatings per se, the consequences of increased or decreased shear must be taken into account when investigating the interactions between AF coatings and biofilms, as shear stress is an integral part of the physico-chemical system under investigation. Most biofilms can be described as biopolymers and are viscoelastic (Stoodley *et al.* 1999), meaning they will immediately deform when subjected to an applied force and return to their original form when the force is removed. In addition to this elastic response, an applied stress will also cause a viscoelastic polymer to plastically deform (or flow) in a time-dependent manner. This deformation increases over time (creep), and when the force is removed there is an immediate elastic recovery and a much slower creep recovery (Stoodley *et al.* 1999). The shape of the biofilm will vary not only through the growth cycle of the biofilm but also with variations in fluid shear stress. Changes in biofilm shape will affect its porosity and density and therefore the transfer of solutes into and through the biofilm (Stoodley *et al.* 1994; 1999; 2002).

Beyenal *et al.* (2002) hypothesised that depending on the flow velocity at which they are formed, biofilms arrange their internal architecture to control: (1) nutrient transport rate; and (2) the mechanical pliability to resist the shear stress of the water flowing past them. It appears that biofilms attempt to satisfy the second goal first at the expense of the nutrient transfer to deeper layers.

The strength increase is associated with an increase in biofilm density, which slows down the internal mass transfer rate. Biofilms grown at low flow velocities (0.15 knots) exhibit low density and high effective diffusivity, whereas biofilms grown at higher flow velocities (0.54 knots) have high density and low effective diffusion. External shear force will not only change the shape of a biofilm, but it can also influence biofilm detachment (Horn *et al.* 2003), which in turn influences biofilm formation and the microbial ecology within it. Tsai (2005) investigated the impact of flow velocity on the dynamic behaviour of biofilm bacteria. He found that the maximum biofilm biomass did not change when flow velocity was increased from 0.38 to 0.77 knots, but was significantly affected when flow velocity was further increased to 1.16 knots. The concentration of bacteria within the biofilm was substantially reduced by the higher shear stress.

Evidence is emerging that multispecies communities that develop under high shear stress are less diverse than those that have developed at lower shear stress (Liu *et al.* 2002; Soini *et al.* 2002; Rickard *et al.* 2003; 2004; Cassé *et al.* 2006; Howell 2007). Rickard *et al.* (2004) investigated the effects of different hydrodynamic shear forces on the formation and diversity of freshwater biofilm communities. They showed that the biofilm that developed at the lowest shear rate showed the highest diversity, and conversely, at the highest shear rate the least diverse biofilm developed. They also

showed an inverse relationship between shear rates and the quantities of distinct colony morphotypes isolated. Each shear rate did not select for a single cluster of species, genera, or taxonomic group. Rather, it selected for groups of genetically unrelated strains to generate taxonomically diverse multispecies biofilms. The proportion of biofilm bacteria that were able to autoaggregate was also inversely related to the shear rate. In the biofilm that developed at the lowest shear stress, 24% autoaggregated as opposed to the highest shear stress where 83% autoaggregated. They concluded that fluid flow velocity moderates biofilm diversity, and that the ability to autoaggregate is an important determinant of biofilm formation at high shear stress.

17.4 Effects of biofilms on the performance of biocidal antifouling coatings

17.4.1 Hydrodynamic coating performance

The total drag on a ship is the sum of all the aerodynamic and hydrodynamic forces, in the direction of the external fluid flow, acting to oppose forward motion. There are two types of drag: viscous drag, which predominates at low speeds and is largely determined by the frictional resistance caused by the surface roughness of a hull; and wavemaking drag, which predominates at high speed and is determined by the length of the ship and the beam to draft ratio (Howell *et al.* 2006b). In smooth new ships, viscous drag accounts for 80 to 85% of the total resistance in slow speed vessels and as much as 50% in high speed vessels (Todd 1980).

Viscous drag occurs in the layer of fluid in the immediate vicinity of a bounding surface, known as the boundary layer. At high Reynolds numbers (the Reynolds number is the ratio of inertial forces to viscous forces), it is desirable to have a laminar boundary layer as this imparts less friction to the bounding surface. As a fluid flows along a surface, it inevitably becomes turbulent, imparting more drag, although a laminar sublayer remains immediately adjacent to the surface below this turbulent boundary layer. Extra turbulence is caused by the projection of roughness elements through this laminar sublayer. Schultz (2003) showed an increase in the boundary layer thickness and the integral length scales occurred in an unsanded, painted surface compared to a smooth wall. If the height of the roughness elements is small relative to the thickness of the sublayer, then the surface will behave as if it is hydraulically smooth, and the transition to rough behaviour will be due to the decrease of thickness of the laminar sublayer with increasing Reynolds number. Extra turbulence is caused by the projection of roughness elements through this laminar sublayer (Howell *et al.* 2006b). A biofilm growing on an AF coated surface will project into the laminar sublayer and

cause extra turbulence, thus increasing the hydrodynamic drag of that surface.

On a ship, there are three levels of roughness: (1) structural roughness caused by the construction process (e.g., weld spots); (2) roughness caused by the coating application process (i.e., a coating will be rougher when applied with a roller than a spray method); and (3) micro-roughness that is a function of the components and structure of the coating system, which can often get hidden in the laminar sublayer (Howell 2007). When modelling the relationship between 'macro-roughness' (cutoff 50 mm), 'micro-roughness' (cutoff 10 mm) and drag resistance, Weinell *et al.* (2003) found that in an ideal painted surface the coefficient attributable to micro-roughness was an order of magnitude higher than that attributable to macro-roughness. The coefficient attributable to macro-roughness had a negative sign at higher velocities meaning that it had a decreasing effect on drag resistance as velocity increased. An increase of 5 μm in micro-roughness corresponded to a ~4% increase in drag resistance. This is not to deny the importance of large-scale irregularities in drag. Weinell *et al.* (2003) also reported that an increase of 3% coverage in simulated weld seam doubled the drag. However, once structural roughness has been eliminated as much as possible, a 4% difference in drag between coatings is a significant saving in a highly competitive market.

The effect of biofilms on hydrodynamic efficiency was first reported in 1952 during towing tank experiments performed by the US Navy, where the drag of AF coated plates was tested after varying immersion times (WHOI 1952). After 1 day, the increase in resistance was very small, but after 10 days, the resistance had increased by 10%, attributed to a biofilm. It was also observed that a significant part of the biofilm released during testing, and that after 30 days the biofilm consisted of an upper layer which sloughs off and a harder layer underneath which does not release. Watanabe *et al.* (1969) measured the impact of a biofilm on resistance, using a rotor study involving cylinders and disks, and a towing tank experiment with a 9 m long ship model. They not only predicted a 9–10% increase in total resistance but also reported that when the speed exceeded 8 m s^{-1} , 90% of the biofilm detached. Loeb *et al.* (1984) reported a drag increase of 5–8% at 40 knots using a rotary disk and Holm *et al.* (2004) reported drag penalties due to microfouling from 9% to 29%.

17.4.2 Biocide release rate

Owing to the difficulties in directly measuring biocide release rate in a dynamic testing system, and the irregularities in reported results that this has caused (Valkirs *et al.* 2003; Haslbeck *et al.* 2005a; Finnie 2006; Howell *et al.* 2006a; Howell 2007), there are currently very few practical studies

investigating the effects that biofilm formation can have on real-time biocide release rate (Howell 2007). Yebra *et al.* (2006b) completed a model based analysis of the effects of biofilms on biocide release rates, but at this moment in time, as Yebra *et al.* (2006a) note, many questions such as the AF effect of biocide saturated biofilms and the rate of renewal of biofilms, remain unanswered.

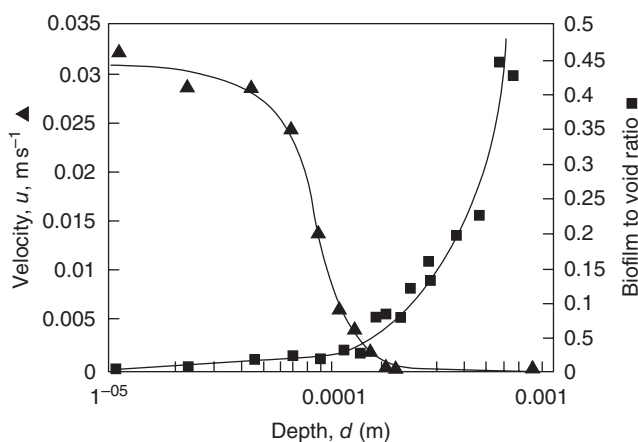
Both bacterial and algal biofilms have a recognised ability to bind metals within the EPS structure of the biofilm itself (WHOI 1952). In this way the biofilms could behave as biocide capacitors and build up high levels of biocides within them. In doing so, the release of biocides to the wider environment could be reduced, thus creating a better environmental profile whilst in port. The performance of the coating to target organisms could also be improved as the biofilm may have built up high concentrations of biocides.

Once the EPS is saturated with biocides, the structure of the biofilm could also affect release rates. It has been shown (Howell *et al.* 2006a) that the width of the leach layer, and therefore the effective path of diffusion from the pigment front to the polymer front, has an effect on release rates. The presence of the EPS structure would increase the length of this path of diffusion, modify the concentration gradients along the diffusion zone, and consequently potentially reduce the release rates. This effect may not be as pronounced in turbulent conditions as turbulent diffusion at the aqueous edge of the biofilm will increase the mass transport in the biofilm (Howell *et al.* 2006a).

Transport through a biofilm has been demonstrated by Stoodley *et al.* (1994), who investigated liquid flow in a model biofilm of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Klebsiella pneumonia* in a conduit reactor with an average flow velocity of 0.07 m s^{-1} (0.13 knots). They measured velocity and biofilm to void ratio through the biofilm and found that the velocity decreased from the bulk fluid to the substratum, as shown in Fig. 17.1. They also found that the channels are approximately $20 \text{ }\mu\text{m}$ in diameter, larger than the pores in a commercial SPC AF coating which are about $5 \text{ }\mu\text{m}$ (Howell *et al.* 2006a). Although this work was originally done to investigate the transport of nutrients into a biofilm, it can just as well describe the transport of biocides out of a biofilm.

Mihm *et al.* (1988) cite two experimental studies dealing with the effects of biofilms on TBT release rate. The inoculation of marine bacteria and posterior settlement on a TBT-SPC coated panel led to a decrease in release rate of about 70%, although when an algal biofilm was investigated, a slight acceleration of the TBT release rate was observed. An important increase in the release rate from all four specimens was observed after biofilm removal.

Valkirs *et al.* (2003) attempted an investigation of the effect of an accumulated biofilm on Cu^{2+} release rate. They tested seven self-polishing,



17.1 Velocity profile measured in a biofilm channel as related to the biofilm to void ratio (after Stoodley *et al.* 1994).

TBT-free AF coatings, applied onto panels and exposed in natural seawater. Biofilms were gently removed with a soft plastic brush and any change in release rate was observed. In four of the coatings, an increase was observed, and in six of the replicates, this increase was 2–3 fold. Although it is difficult to determine what proportion of the release rate is attributable to biofilm removal and what is attributable to disturbance of the coating surface, they concluded that the presence of established biofilms in the static condition leads to significantly lower Cu^{2+} release rates compared to the fouling-free case. The experimental flaw in this study is highlighted by Yebra *et al.* (2006a) who point out that both the study by Valkirs *et al.* (2003) and WHOI (1952) base their approach not on a drop in release rate due to biofilm formation, but an increase after ‘careful’ biofilm removal.

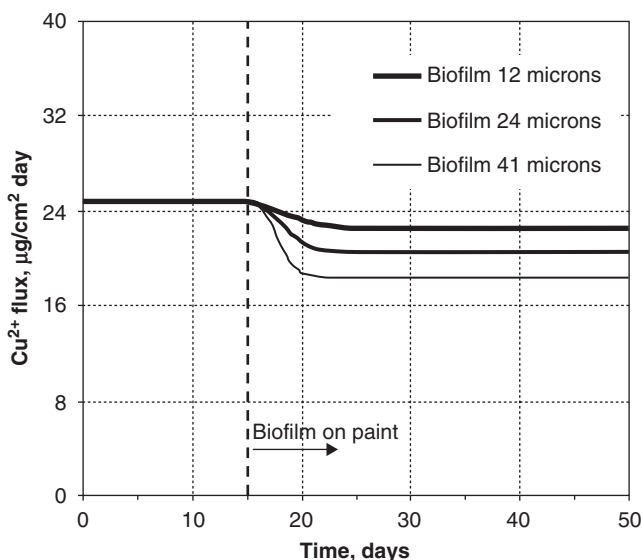
It is possible that exudates from the biofilm could reduce the pH at the coating surface (Liermann *et al.* 2000) thus increasing Cu_2O dissolution and therefore release rates. It has been shown (Howell, 2007) that biofilms could potentially increase biocide release rate in copper-based coatings. This has also been observed on TBT AF coatings (Mihm *et al.* 1998) where algal biofilms increased release rates rather than decrease them. Changes in biofilm shape will affect the porosity and density of that biofilm and this in turn will affect the transfer of solutes into and through the biofilm (Stoodley *et al.* 1994; 1999; 2002) and the leach layer, thus affecting release rates. Beyenal *et al.* (2002) hypothesised that biofilms arrange their internal architecture to primarily control mechanical pliability to resist shear stress and that this strength increase is associated with an increase in biofilm density, which slows down the internal mass transfer rate.

17.5 Multi-parameter studies on antifouling coatings

Owing to the difficulties in directly measuring biocide release rate in a test system running at low speeds (1–4 knots) and the irregularities in reported results that this has caused (Valkirs *et al.* 2003; Haslbeck *et al.* 2005a; Finnie 2006; Howell *et al.* 2006a), there is currently only one practical study investigating the effects that biofilm formation can have on real time biocide release rate in a dynamic system (Howell 2007). Yebra *et al.* (2006b) have completed a model-based analysis of the effects of biofilms on biocide release rates, and Cassé *et al.* (2006) investigated bacterial and algal species succession during static and dynamic immersion testing of AF coatings but did not report release rates.

Yebra *et al.* (2006b) reported a model-based analysis of release rates with biofilms based on various assumptions (see Fig. 17.2):

1. Only heterotrophic bacterial species were considered. Algal species were disregarded for the purpose of simplification.
2. As bacterial species only were considered, the biofilms were considered to be thin, and therefore anaerobic processes were not considered.
3. Biofilm porosity and effective diffusion coefficients were assumed constant throughout the biofilm for the purposes of simplification.



17.2 Simulated Cu^{2+} release rate decrease as a function of the diffusion resistance (i.e., biofilm thickness) exerted by the EPS matrix, after Yebra *et al.* (2006b).

4. Nutrient concentration gradients were not considered, only the diffusive transport of the AF biocide.
5. No effect of EPS, seawater heavy metals, acidic metabolism products and H_2O_2 on pH were considered, meaning that hydrolysis of the coating was as it would be in seawater and release rates would not be affected.
6. The biofilm was considered a homogenous sheet of bacteria covered with EPS, covering the surface with increasing thickness over time.
7. Biofilm growth occurs once the AF coating has reached a stable release rate.

Transport through the biofilm was based on the premise that the heterogeneous structure of many biofilms permits convective transport within voids and water channels permeating through the biofilm. The dissolved biocides will move through the biofilm pores by convective currents caused by spreading biomass (advection) and diffusion only.

Under the assumption of only thin biofilms being formed and considering just diffusive-resistance effects, they concluded that microbial biofilms do not appear to exert a significant effect on the net biocide release rate (i.e., the amount of biocide dissolved from the pigment front) and paint polishing processes; however, a decrease in the measured release rate in the bulk phase will be observed as biocides are bound in the EPS.

Howell (2007) carried out the first practical study that investigated the bacterial composition of biofilms growing on a commercially available tin-free, self-polishing, hydrolysing, Cu_2O containing, AF coating under dynamic conditions and the effects that these biofilms had on dynamic biocide release in this novel biocide release rate method.

This study showed that biofilms can have an effect on biocide release rate by both changing the conditions at the coating surface, and storing biocide in EPS. It appears that the biofilm increased release rate initially, due to a possible change in pH at the coating surface affecting hydrolysis. Biofilms also have the ability to act as biocide capacitors, and to release high levels of biocide after an increase in shear stress.

Spikes were observed in this study, but were of a much greater magnitude than those observed in previous studies (Howell *et al.* 2006a, b) and were apparent on both the condition that had a speed increase, and that which did not. This was due to the experimental methodology, which required removal of the cylinders before a speed increase to sample the coating and the biofilm. When the cylinders were restarted, even the small increase in shear stress from 0 to 1 knot caused the outer layer of the biofilm to detach. At the time of year that this test was run (May), it is difficult to devise a methodology using natural seawater as there will always be the added complication of a biofilm. Consequently, the magnitude of these spikes will be difficult to assess for individual coatings, as different coatings in different

environments will be colonised by different bacterial and algal species. One can say, however, that the burst effect demonstrated here is of a greater magnitude in natural waters and is important in assessing the environmental impact of AF coatings in natural, tidal waters.

These results also strongly suggest that a peak in total copper, of a much higher magnitude than expected, will occur after a change in rotary speed on an AF coating covered with a biofilm. This is due to potential remobilisation from the biofilm and high levels of organically bound copper from the EPS that has sloughed off. This has implications on the burst effect of biocides from AF coatings in harbours when vessels go underway, and when AF coatings are being spray-cleaned to remove slime fouling.

Although it is impossible to make quantitative comparisons with the model results of Yebra *et al.* (2006b) regarding release rates into the bulk phase. It is possible, however, to make qualitative comparisons with the assumptions made by Yebra *et al.* (2006b). Contrary to what they have assumed:

1. Algal biofilms do need to be considered.
2. Release rates were affected more significantly by the biofilm formation and subsequent detachment, than they were by the increases in hydrodynamic shear within the speed range studied.
3. Algal biofilms increase release rate and variation rather than decrease them.
4. The biofilm is not a homogenous layer, but deforms under shear stress and it is likely that it also becomes thinner under higher shear when some of the biofilm detaches.

This work has shown that biofilms can have an effect on biocide release rate by changing the conditions at the coating surface, and storing biocide in EPS. It appears that the biofilm increased release rate initially, due to a possible change in pH at the coating surface affecting Cu_2O dissolution. Biofilms also have the ability to act as biocide capacitors, and to release high concentrations after an increase in shear stress. The majority of this biocide may be chelated and therefore potentially not bioavailable. The burst effect demonstrated by Howell (2007) is of a greater magnitude in natural water than in artificial seawater (Howell *et al.* 2006a) and is important in assessing the copper loading from AF coatings in harbours.

17.6 Current test methodologies

Biofilms are more likely to occur on an AF surface under conditions that are not static, and Howell (2007) showed that in some cases, a biofilm may only develop after a speed decrease, due to a reduction in release rates making the coating more vulnerable. Both of these observations have implications for assessing the behaviour of hydrolysing AF coatings in reality:

- A vessel will be most vulnerable to biofilm formation in the period after it has docked, and release rates drop.
- Biofilms would be more likely to grow on vessels moored in regions where there are larger currents.
- In more static waters there will be less dilution of biocide creating a less favourable environment.
- Once these biofilms develop, rather than inhibiting biocide release, they could potentially increase biocide release, as the decrease in pH levels within the biofilm (Liermann *et al.* 2000) could lead to faster Cu_2O dissolution.

In order to fully evaluate the implications of biofilms growing on AF coatings one must look at the findings of current research when compared to published release rate data using different methodologies. In his review of current test methods, Finnie (2006) reported that when using the ASTM/ISO method, a given laboratory's estimate could be from 4–54% off from the true concentration of the standard solution. When compared to release rates for the dome method, the ratio of ASTM:dome is 10.4, showing that the ASTM method significantly overestimates the 'true' environmental input of copper from an AF coating (Finnie 2006). Finnie (2006) also showed that the CEPE calculation method provides higher release rate values than those determined directly from a ship's hull, and when compared to the dome method, it overestimates by a factor of approximately 10–12.

ASTM results for SPC coatings are much closer to the peak results shown by Howell (2006a; 2007) than the stable release rates themselves. This is due to a major flaw in the ASTM methodology, which is that the coatings are not under constant dynamic immersion. It has been shown (Howell *et al.* 2006a; 2007) that a rotary speed step change will cause burst effects that can be up to an order of magnitude higher than the stable release rates, and that these can take up to 6 hours to develop. What the ASTM method is measuring is these burst effects, as the cylinders are rotated for one hour only, and then the value is extrapolated to a daily release rate. As these peaks are inherently unstable and show variation between replicates, there will be even more variation when comparing between laboratories, as has been shown (Haslbeck *et al.* 2005b).

Yebra *et al.* (2006a) hypothesised that one of the potential reasons for the divergence between different release rate testing methods, is the inevitable formation of marine biofilms on AF coatings in natural environments reducing release rates. Howell (2007) showed that this is certainly not always the case, and it should not be automatically assumed that biofilms will reduce release rates. The differences between the results shown by Howell (2007) and those of the model based analysis of Yebra *et al.* (2006b)

highlight the importance of reliable, practical, real-world testing. It is likely that there is a balance between increased dissolution of Cu_2O at the coating surface, caused by decreased pH in the biofilm, and decreased mass transport of biocide from the pigment front, due to the low effective diffusion in the biofilm. Ultimately, it appears that the increased dissolution is more important than the low effective diffusion.

However, the question still remains – which method should be used for risk assessment? If one is building an environmental risk model, or assessing environmental emissions, it is important to realise that factors affecting biocide release rates from hydrolysing AF coating systems are much more complex than a simple measurement of biocide release rates in artificial seawater. These coatings are designed to interact with the surrounding hydrodynamics and seawater chemistry, and therefore could behave very differently in a harbour with fast tidal flow than they would in a harbour with minimal tidal flow. Although many harbours are semi-enclosed and have minimal water flow, there are also those that are built in estuaries that will experience considerable daily water movement. For example, all river berths on the River Tyne are tidally influenced, and tidal streams in the river vary, reaching a maximum of 3 knots (Port of Tyne 2007). One of the largest docks in Europe, in Southampton Water, is tidally dominated, with a maximum tidal flow of 1.2 knots (Shi 2000).

It is not unrealistic to expect the differences seen between static testing or the dome method when compared to the dynamic testing, to occur when a vessel is in harbour. This could not only be due to the movement of that vessel through the water upon arrival and departure, but also to tidal flow. Therefore, the issue becomes less about which method is the correct one to choose, and more about calculating worst case scenarios with the information we now have regarding how hydrolysing AF coatings react to small changes in hydrodynamics, and also how copper release rates can be influenced by biofilm formation.

The current environmental risk models such as REMA (Mackay *et al.* 1983), MAM-PEC (CEPE 1999), and USES (Luttik *et al.* 1993) do not give reliable estimates as they use the CEPE method as an input for leaching calculations. This method is flawed, as one of its fundamental components is that release rates do not change for the life of the coating, over-simplifying release rate behaviour in harbours. The Emission Scenario Document on Antifouling Products (OECD 2005) states that the ‘data calculated by this method for the release of copper from an organotin copolymer paint shows good agreement with copper release rates measured via the ISO method, although that agreement was less good for copper release from rosin based paints’. Many of the tin-free coatings on the market today are rosin based. Thouvenin *et al.* (2002) stated that ‘the models developed in the standard ISO 15181 to estimate the surrounding cumulative release

seem to be inadequate, due to lack of specificity of the parameters used in the calculation, and assumptions not validated'.

The hydrodynamics of the dome system have never been quantified and it is assumed that it gives a measure of static flow. It has never been proven that the hydrodynamic conditions inside the dome accurately reflect environmental conditions. The dome method therefore gives results that are potentially close to reality, but only for a given environmental scenario, i.e., static flow in San Diego Bay.

17.7 Future trends

The problem of copper loading in docks and marinas is one that is becoming very important. In several studies in the US and Europe looking at copper concentrations in marinas, the areas with the highest copper concentrations tended to be associated with the highest vessel density and lowest water circulation, as expected (Hall *et al.* 1992; 1999; Matthiessen *et al.* 1999; Schiff *et al.* 2007; Srinivisan *et al.* 2007). As an example of how severe the problem is becoming, approximately 75% of the volume in the marinas of the San Diego region exceeded water quality thresholds for dissolved copper (Schiff *et al.* 2007). As more information becomes available, showing how AF coatings are a major source of copper loading in these coastal environments, environmental protection agencies are becoming increasingly keen to set regulatory limits on these coatings.

It is a relatively simple matter to set the limit of copper release into a coastal zone or harbour, taking into account water exchange, toxicity levels and dilution factors. It is much harder to relate such limits to the sources of copper, such as AF leachate, and attempt to prohibit coatings using standard test methods only. Current research has shown that the two methodologies that have received the most scrutiny, the ASTM/ISO method and the US Navy dome method, are essentially measuring different things and cannot be compared.

It has been shown that small changes in hydrodynamics as well as biofilm growth can result in large changes in release rates. Appropriate testing methods that will be used for the source of data for risk assessment models should include both static and dynamic testing and a measure of how coatings are affected by biofilm growth. The risk assessment models themselves should couple tidal flow, biofilm formation and vessel movement with release rates to reflect the true behaviour of a coating under natural conditions.

17.8 References

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Part III

Chemically active marine
antifouling technologies

Tin-free self-polishing marine antifouling coatings

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Abstract: Many tin-free binders have been developed to mimic the TBT-SPC binders in sea water soon after the recognition of the detrimental side effects of tributyltin (TBT)-based compounds on non-target species in the early 80s. Throughout the different sections of the chapter, the requirements for self-polishing antifouling coatings are displayed in order to try to reproduce the control of the polishing rate and biocide release rate of TBT-based coatings. The influence of parameters including the hydrophobic/hydrophilic character of the binder, its molecular weight, its microstructure and the paint formulation is discussed. Research works from both industry and university laboratories are considered.

Key words: antifouling paints, self-polishing binders, controlled polishing rate, biocide release, hydrolysis and ion exchange reactions.

18.1 Introduction

Chemically active antifouling technologies can be subdivided into three main categories: contact leaching coatings, controlled depletion paints (CDP) and self-polishing copolymers (SPC). Hybrid systems which combine properties of controlled depletion system and self-polishing polymers are also available. All these technologies aim at the same objective, the controlled release of bioactive molecules, but act via various chemical mechanisms, many of which remain partially understood. The most successful antifouling paints in terms of long-term efficiency in service life are tin-based SPC paints. In such paint, the biocidal compound is chemically bonded to the binder, gradually hydrolysed or dissolved in water to release the antifouling agent.

Soon after the recognition of the detrimental side effects of tributyltin-based compounds on non-target species in the early 80s, tin-free alternatives were developed and dominate the market today. Chemists from industry and university research laboratories are currently busy trying to

improve these so-called tin-free, self-polishing copolymers to match the effectiveness of TBT-containing antifouling paint.

Many alternatives have been developed to mimic the TBT-SPC binders in sea water and need to be described and compared. Throughout the different sections of the chapter, the requirements for self-polishing coatings are displayed. The influence of both the binder design and the formulation is discussed. Then a section on future trends in the development of tin-free substitutes is presented. The chapter ends with conclusions and a look ahead on the related costs and benefits for SPC to prevent or control the marine biofouling on surfaces exposed to the marine environment.

18.2 Requirements for self-polishing coatings

Although the terms ‘self-polishing polymers – coatings’ are largely used, they are unclear and confuse people because they involve a lot of phenomena that are very intricate to investigate and the properties are difficult to obtain (Von Burkensroda *et al.*, 2002).

The SP paints are characterised by three common properties, which constitute the basis of efficiency determination:

- A controlled biocide release which allows a constant rate of leaching over time.
- A polishing rate which increases linearly and not exponentially with the sailing speed. In stationary conditions, the remaining polishing allows antifouling protection.
- A stable leached layer thickness (10–20 μm for TBT SP coatings), which is established between two distinct moving fronts, the dissolving pigment or leaching front and the eroding polymer front.

Whilst the polishing of these products and the consequent constant biocide release rate were the main characteristics, it was also noted that any initial roughness due to application was smoothed out during sailing (Howell and Behrends, 2006). The name ‘self-polishing’ for these products was therefore applied by the marine coatings industry to indicate smoothing properties, although whilst the paint itself became smoother, the hull, overall, often became rougher due to surface damage (Anderson *et al.*, 2003; Yebra *et al.*, 2006b). Furthermore, the analysis of tin-free SPC coatings has shown that roughness and thermodynamics are not sufficient to specifically characterise the surfaces and that it is necessary to consider various parameters such as texture (Candries *et al.*, 2003).

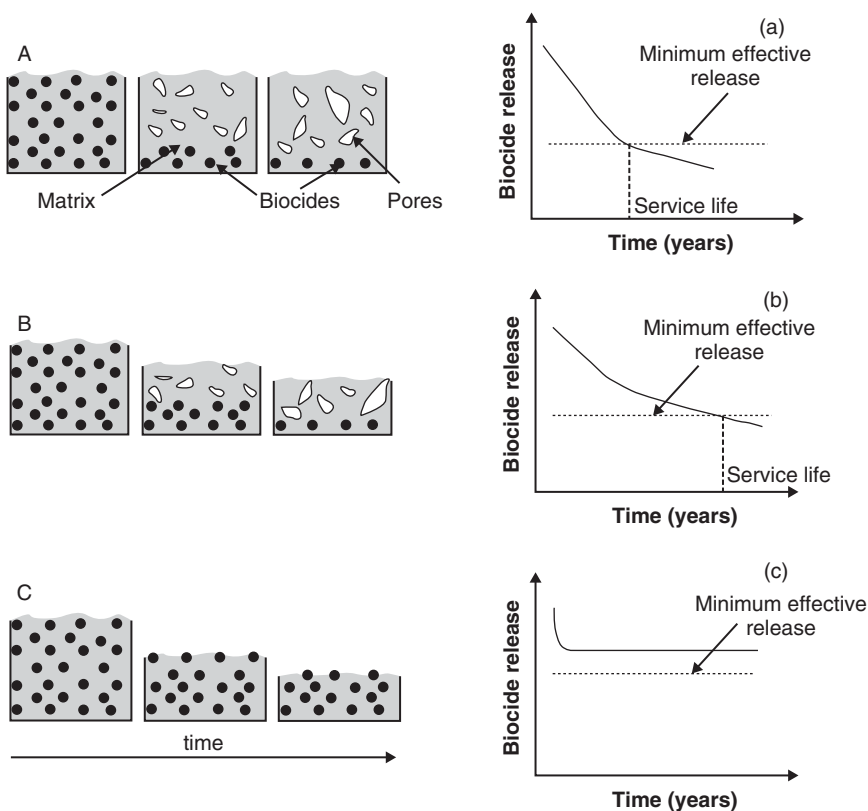
Additionally, the paint system must also fulfil a series of practical requirements (e.g., applicability, mechanical properties, adhesion; Chapter 13), which are also key to long-term efficiency in real life.

The definition of SP paints has continuously evolved according to the development of analytical tools making possible the investigation of the paint film behaviour during its immersion. That is the reason why this section will be structured as follows: a brief description of paint properties followed by a presentation of available analytical tools.

18.2.1 Biocide release and antifouling activity

Many mechanisms are involved in the release of entrapped molecules: erosion, simple diffusion, diffusion coupled with erosion, swelling, specific interactions between molecules inside the polymer matrix (Langer, 1995; Ritger and Peppas, 1987). The emerging coating systems control the release rate through a combination of binder hydrolysis, particle dissolution and surface erosion in sea water (Valkirs *et al.*, 2003). Their performance is, hence, dependent on heterogeneous liquid-solid reactions related to both the pigment and binder phases (Yebra *et al.*, 2006d). The solubilisation and subsequent diffusion process of biocidal molecules is controlled by the accessibility of water in the polymer matrix, which can contain hydrophobic and/or hydrophilic functions and by the structure of the pore network (Thomas *et al.*, 1999; Yebra *et al.*, 2006b). It has been shown that chemical reactions and diffusion phenomena are key mechanisms in the performance of biocide-based paints and that these can be affected by the exposure conditions (Kiil *et al.*, 2002b; Trentin *et al.*, 2001). The antifouling activity depends on both the biocides themselves and the technology used to control their release (Almeida *et al.*, 2007). Nowadays, coatings can be classified into three categories (see Fig. 18.1) (Thouvenin *et al.*, 2002b; Faÿ *et al.*, 2005; Howell and Behrends, 2006):

1. **Contact leaching coatings** The thickness of these paints remains constant, which means there is essentially no dissolution or decrease of the paint film thickness over time. As more and more biocide is leached out, a thick depleted layer develops at the surface. As this layer increases in depth, the diffusion of biocides from the bulk of the film slows down, meaning few products of this type have lifetimes in excess of 24 months.
2. **Controlled depletion coatings** Rosin and chemical derivatives of rosin allow sea water to penetrate the paint film and to lead to the release of biocides by a diffusion process. This results in an 'exponential' leaching rate, with an excessive release in the early days of immersion, which falls quite rapidly, eventually to a level which becomes too low to prevent fouling.
3. **Self-polishing coatings** The initial leaching rate of the SP coatings is much lower than that of the rosin-based system, and then a 'steady state' constant rate is achieved as a steady polishing period is established. This



18.1 Release kinetics of three categories of antifouling coatings. (a) Contact leaching coatings; (b) Controlled depletion coatings; (c) Self-polishing coatings.

steady state continues for as long as antifouling paint film remains. The leaching rate of copper and booster biocides in the self-polishing copolymer are controlled by the degree of polymerisation (molecular weight) and the hydrophilicity of the copolymer, which depends on both the ratio of hydrophilic or sea water hydrolysable groups and the chemical nature of the macromolecular chains (Omae, 2003b).

The biocide release rate is usually estimated by analysing the surrounding water composition. Many protocols have been developed and standards (ISO 15181 for example) are available (Finnie, 2006). These methods are based on successive phases of dynamic or static ageing and the use of models to determine the cumulative leaching from instantaneous analysis. The accurate control of the test conditions and the models used are still under debate. The released molecules (copper derivatives or organic molecules)

are quantified by classical techniques like inductively coupled plasma spectrometry liquid chromatography (HPLC-UV).

This approach is relevant to determine the environmental impact of biocide leaching but it is insufficient to evaluate the antifouling activity of paint films during their service life. More precisely, antifouling action implies molecules, which are available for sea water dissolution and subsequent diffusion towards the surface. However, blended molecules are often entrapped and can not diffuse and become inactive. Because of this, the optimisation of coating formulations by paint companies relies on more advanced analysis of the biocide leaching process including the localisation of the molecule in the paint film thickness, i.e. characterising the so-called leached layer.

18.2.2 Self-polishing and degradation

The term 'self-polishing' has been associated with a progressive thickness depletion confined at the extreme surface of the paint, which leads to a continuously renewed bioactive surface (Omae, 2003a). The self-polishing effect is the result of many degradation processes which need to be controlled to obtain a constant biocide release rate. In order to present these phenomena, we propose to distinguish the data relevant to the binder (mixture of polymers) and those focussed on the paint film (complete formulation). The TBT SP paints were based on an acrylic polymer (often methyl methacrylate) with TBT groups bonded onto the polymer backbone by an ester linkage. The main working mechanisms of these paints were modelled by Rascio *et al.* (1988) and Kiil *et al.* (2001, 2002a). After immersion, the soluble molecules (Cu_2O , for example) in contact with sea water begin to dissolve. This dissolution leads to the creation of pores that are progressively filled by sea water. The carboxyl-TBT groups at proximity can react in the same way as the extreme surface of the film. The controlled hydrolysis enables the release of the organotin biocide from the film and thus provides the antifouling action. Once a sufficient number of TBT moieties have been released, the partially reacted brittle polymer backbone can be easily eroded by the moving sea water and a continuously renewed bioactive surface is exposed (Omae, 2003a).

Degradation of the binder

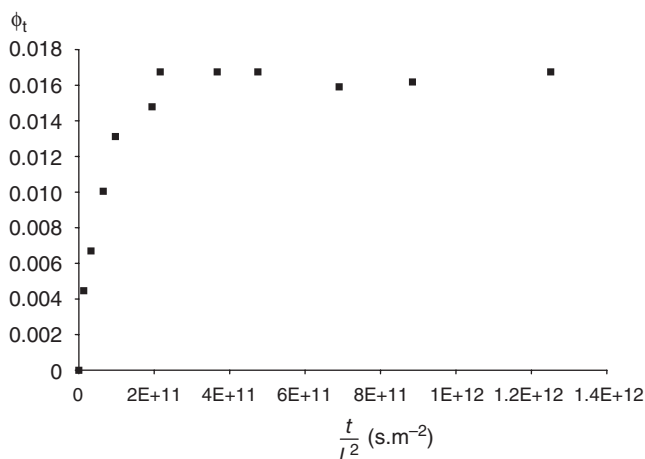
The self-polishing effects can be achieved by a controlled degradation of the binder used in the formulation. To control this process, it is convenient to involve hydrolysis, which is an equilibrated reaction from the thermodynamic point of view. Nevertheless, hydrolysis of macromolecular chains implies many other phenomena such as, e.g. hydration and molecular

diffusion. The relative kinetics of these reactions leads to the distinction of two types of polymer matrices: surface-degrading resins and bulk-degrading resins (Tamada and Langer, 1993; Göpferich, 1997). If the diffusion of water into the polymer is faster than the degradation of polymer bonds, the polymer will undergo bulk degradation because degradation is not confined to the polymer surface. If, however, the degradation of the polymer bonds is faster than the diffusion of water, it will be consumed by hydrolysis of bonds on the polymer surface and thus will be prevented from diffusion into the bulk. Degradation processes are then strictly confined to the matrix surface.

Hydration kinetics

The matrix hydration is the limiting step of degradation and needs to be controlled. Research works published so far on polymer hydration approach this phenomenon in both qualitative and quantitative ways. The main properties studied are the absorbed water amount (by gravimetric and coulometric methods), the various classes of water diffusion kinetics (Fickian or non-Fickian behaviours), the forms of absorbed water (bound, free and clusters), the water localisation (identification of hydrated functions in the polymer matrix), the effects of the chemical structure of the binder on the hydration and the effects of hydration on structural and dynamic properties of macromolecular chains (Muller and Schmitt, 1997). Owing to their complex composition, the study of hydration is often limited to the binder and is not extended to the coating. It is clearly a major approximation because the formulation and more precisely the pigments play a key role in the hydration of paint film (Thouvenin *et al.*, 2002a; Hulden and Hansen, 1985).

Non-destructive methods are particularly powerful tools to determine hydration kinetics. For example, the spectroscopic technique ATR-FTIR combined with a mathematical model (second Fick's law) enables the calculation of kinetic constants such as diffusion coefficients (Thouvenin *et al.*, 2002c). The uptake and diffusion rates of water into binders can be also quantified by electrochemical impedance spectrometry (EIS) measurements. The introduction of water in a polymer film or a coating introduces a change in the dielectric properties of the material, i.e. an increase of the polarisability is observed, which is reflected by an increase in the permittivity of the polymer. This increase in permittivity can be determined by deriving the capacitance from impedance measurements (Kittel *et al.*, 2001). By following the variations of the volume fraction of water ϕ in the coating as a function of time, it is possible to define the water diffusion behaviour (Fickian or non-Fickian) of supported films immersed in artificial sea water and to observe water accumulation within the coating which leads to its swelling. Kinetic

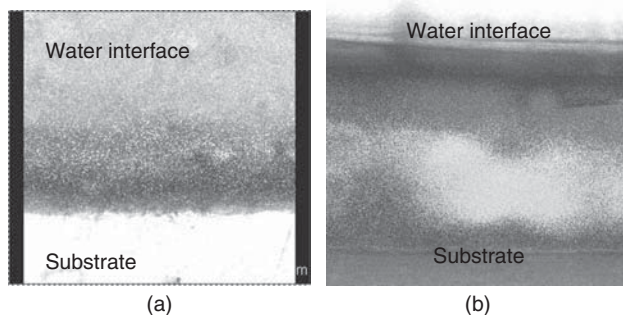


18.2 Evolution of the volume fraction of water ϕ_t in coatings with $\frac{t}{l^2}$ (s.m^{-2}) in artificial sea water at 23 °C. l is the coating thickness. TBT-SPC binder (CUTINOX® 1120). (Adapted from Bressy *et al.*, 2009.)

constants such as apparent diffusion coefficient, solubility and permeability of water can be estimated using the second Fick's law. Figure 18.2 illustrates the water sorption curves of a TBT SP binder.

Furthermore, the ATR-FTIR spectroscopy enables the structural analysis of the water dissolved in the polymers. Fluorescence spectroscopy, a second non-destructive method, was used to evaluate the impact of hydration on macromolecular cohesion (Thouvenin *et al.*, 2002c). The results obtained for rosin-based binders (blends of an acrylic polymer and rosin) indicate that the chain fluctuations are highly enhanced as water diffuses. The increased motions of the acrylic polymer chains, along with the large swelling observed, clearly indicate that both the structure and dynamics of the copolymer are drastically altered as a consequence of hydration. For the TBT-SPC coating, no variations were observed, meaning that water cannot penetrate deeply in the matrix, at least during the experimental time.

Microscopy also leads to relevant experimental data and more precisely gives the opportunity to visualise hydration. A recent study based on confocal laser scanning microscopy (CLSM) investigates the hydration of biodegradable binders and compares their hydration properties to a TBT-SPC polymer (Faÿ *et al.*, 2007d). Figure 18.3 displays the cross-section micrographs of rosin-based and self-polishing binders after various immersion times. In the case of rosin-based binder, the hydration could be considered



18.3 CLSM micrographs of the cross-section of a rosin-based paint (a) and TBT-SPC coating (b) after two months of immersion in fluorescein. Paint and water were simultaneously observed in white (λ comprises from 650 to 750 nm) and black (λ comprises from 500 to 550 nm), respectively. (Adapted from Faÿ *et al.*, 2007d.)

homogeneous. For SPC, the micrograph reveal a hydration front even after immersion. Water cannot diffuse largely in the entire thickness of paint. This binder undergoes a surface hydration.

Most studies on the sorption and permeability of water through organic coatings and films are based on the assumption of their structural homogeneity, and it is assumed that water is transported by the mechanism of activated diffusion. Some deviations from the ideal state can be encountered due to inhomogeneities caused by micro-cracks, the presence of fillers, and the interaction of water with the coating components.

Degradation kinetics

The intrusion of water triggers chemical polymer degradation, changing the microstructure of the film through the formation of pores, leading to the ablation of the surface and the release of bioactive molecules. These processes can be related to hydrolysis, ionisation, protonation of the binder via a pendant group or by a backbone cleavage (Heller, 1984). Hydrolysis is the main mechanism involved in the degradation of antifouling binders. This reaction can be conducted through pendant ester groups in the case of acrylic polymers and copolymers or within the polymer backbone as in the cases of polylactides, polyamides, polyhydroxyalkanoates and polyanhydrides (Omae, 2003b; Yebra *et al.*, 2004; Faÿ *et al.* 2006, 2007a, 2007b, 2007c). For (meth)acrylic copolymers bearing hydrolysable side ester groups in sea water, the reaction mechanism yields the conversion to sodium salt of some

of its monomer units. As a result of this chemical weakening, the paint surface can be removed by moving sea water. The type of bonds (ester, anhydride, amide) determines the rate of hydrolysis but rankings must be viewed with circumspection. Reactivities are dependent on the mode of catalysis or on the chemical neighbourhood of the affected functional group through steric and electronic effects. It is therefore difficult to rank the reactivity of ester functions from the corresponding low molecular weight compounds containing the same functional group. In addition, the chemical structure of the pendant group affects: i) the hydrophobic/hydrophilic balance of the matrix, ii) the change of the glass transition temperature during hydrolysis and iii) the water absorption and possible swelling of the polymer (Vallée-Réhel *et al.*, 1998). Furthermore, the distribution of the different monomer units in the copolymers, the possible differentiation between the characteristics of the surface and the bulk of the material, and the interactions and associations between pendant groups must also be taken into account.

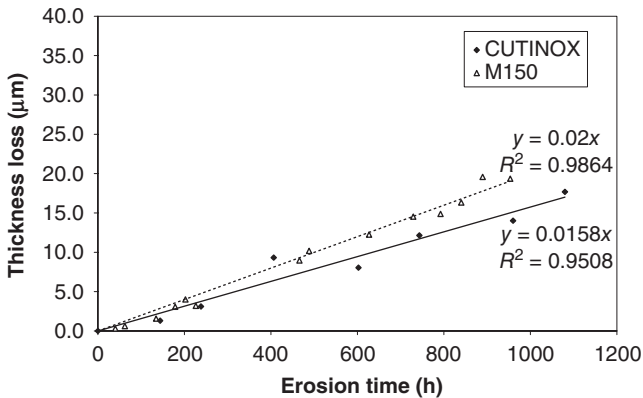
Among the SP copolymers, a second mechanism can be involved in the degradation: an ion exchange. This reaction is identified as the degradation mechanism of (meth)acrylic binders blocked by nitrogen compounds (Hugues *et al.*, 2003; Bressy *et al.*, 2008b), copper- (Yebra *et al.*, 2004) and zinc-acrylates (Yonehara *et al.*, 2001).

Erosion of the paint film

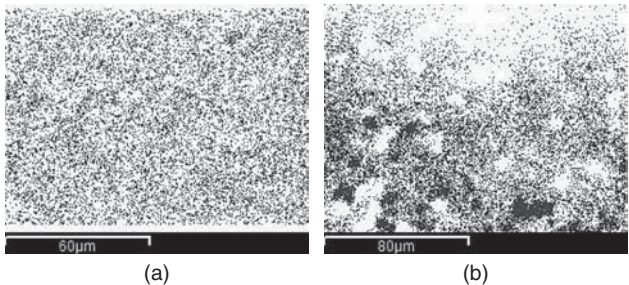
Erosion of the paint film is caused by binder degradation, dissolution of soluble molecules entrapped in the matrix, and progressive loss of mechanical strength. The main method used to evaluate erosion rate is the determination of the film thickness decrease with time. Nevertheless, the variation of paint thickness is the superposition of many phenomena with opposite effects such as degradation and swelling. The test conditions have to be optimised in order to observe clearly the erosion. Rotary setups are generally used to generate performance data for antifouling paints (see Chapter 16). This dynamic test is also a reliable tool to classify erodable binders based on the evolution of the thickness loss of the coating versus erosion time. Figure 18.4 illustrates clearly that the controlled polishing rate of SP paints is mainly affected by the inherent property of the binder.

18.2.3 Observation of the leached layer

Microscopy can constitute a convenient approach which enables observation of the distribution of active molecules in the film. In the following



18.4 Evolution of the thickness loss ($E_i - E_t$, μm) of a commercial TBT-based self-polishing paint (M150) and a TBT-SPC binder (CUTINOX® 1120) with erosion time in artificial sea water ASTM at 40°C. Turbo-eroder Test (Cambon, 1994).



18.5 Distribution of organic biocides in cross-section of (a) rosin-based and (b) TBT-SPC paints. The biocide molecules are represented by black pixels. (Adapted from Faÿ *et al.*, 2005.)

examples, scanning electron microscopy and energy-dispersive X-ray analysis (EDX) were used to record the evolution of biocide distribution during paint immersion (see Fig. 18.5).

As shown on the previous figure, in the case of rosin-based paint, no pigment front (for organic biocides) could be observed. The dissolution and diffusion of the biocide took place in all the film thickness. For the TBT-SPC paint, a pigment front was clearly observed. In the inner part, the biocide molecules were not in contact with water and did not diffuse. Two moving fronts coexist: the dissolving pigment front and the eroding polymer front.

Table 18.1 Values of Young's modulus (E), hardness (H) and maximal penetration ($H_{\max}$) for paints

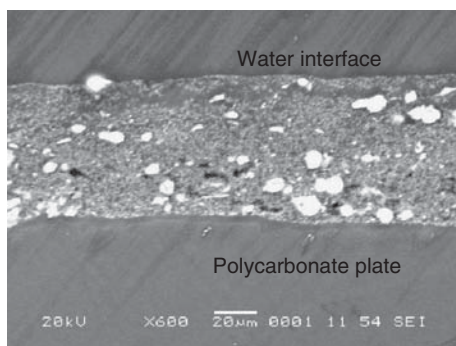
Paint	Time (weeks)		E (GPa)	H (GPa)	$H_{\max}$ (μm)
Rosin-based	0		3.50 (0.21)	0.106 (0.019)	11.50
	36		3.42 (0.05)	0.086 (0.018)	12.81
TBT-SPC	0		2.78 (0.92)	0.075 (0.006)	13.44
	23	Inner zone	3.22 (0.45)	0.081 (0.013)	13.55
		Outer zone	1.54 (0.42)	0.022 (0.006)	24.59

The standard deviation is in brackets.

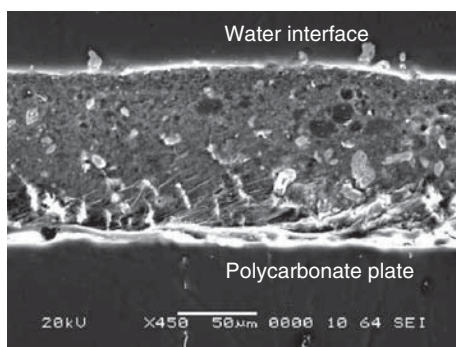
The resulting leached layer can be revealed by many techniques including microscopy and nanoindentation.

Nanoindentation has been established as an important tool for measuring mechanical properties of both bulk solids and thin films at the submicron scale. In nanoindentation, the depth of penetration combined with the known geometry of the indenter provides an indirect measure of mechanical parameters such as Young's modulus (E) and hardness (H). It enables evaluation of the mechanical properties of films and their potential heterogeneities. In the case of SP coatings, two zones are clearly distinguished: i) an intact zone where the mechanical characteristics are constant and ii) a degraded zone where a loss of mechanical resistance is observed (Faÿ *et al.*, 2007d).

After immersion, the rosin-based paint (bulk erosion) indicated homogeneous mechanical properties throughout the entire film thickness (see Table 18.1). Values for TBT-SPC were clearly different. The inner zone, considered intact, was hard: initial mechanical properties were not modified. In the opposite zone, the outer hydrated part has lost its mechanical properties. These changes of E and H values may be explained on the basis of the hydration-degradation process: absorption, swelling, release of components (biocides, fillers) and hydrolysis. These results were confirmed by microscopy (see Fig. 18.6). After immersion, the paints have clearly different aspects. The rosin-based coating is relatively homogeneous. In contrast, the micrograph of the TBT-SPC paint reveals two zones separated by a front. Near the water interface, the region seems spongy and porous, corresponding to the hydrated zone: the polymer is hydrolysed. The inner part keeps being compact and dense (Faÿ *et al.*, 2005).



(a)



(b)

18.6 SEM micrographs of cross-sections of rosin-based (a) and TBT-SPC (b) paints after six months of immersion. (Adapted from Faÿ *et al.*, 2005.)

18.3 Chemical structures of self-polishing binders

It has often been claimed that the only way to reproduce the performance of tin-based paints is to mimic their chemistry as closely as possible. Actually, tin-free paints do not have any efficient biocide linked to the self-polishing binder, which was the key to success of tin-based paints. In tin-free systems, contrary to tin-based paints, polishing and biocide leaching are not directly related but are interrelated processes. Tables 18.2 and 18.3 summarise the chemical structures of binders used in commercial (well-performing) chemically active paints and in experimental ones. In each section, the binders are differentiated by the reaction mechanism involved in sea water. This classification is still a very debatable issue due to the evident lack of scientific data reported on the working mechanism of the corresponding anti-fouling paints.

Table 18.2 Classification of commercial binders for tin-free chemically active paints

Binder classification	Chemical structure	References
Trialkylsilyl-based (meth)acrylate	Polymer-COOSiR ₃ Random copolymers	Russel <i>et al.</i> , 1984; Gitlitz and Leiner, 1986; Masuoka and Hondou, 1989; Durand <i>et al.</i> , 1992; Camail <i>et al.</i> , 1992; Durand, 1993; Durand <i>et al.</i> , 1994; Gillard <i>et al.</i> , 2001a; Gillard and Vos, 2003a; Abrams <i>et al.</i> , 2006; Tsuboi <i>et al.</i> , 2000; Itoh <i>et al.</i> , 1997; Nakamura <i>et al.</i> , 1997; Honda <i>et al.</i> , 1995; Matsubara <i>et al.</i> , 1996; Vos <i>et al.</i> , 2001; Gillard <i>et al.</i> , 2001b; Oya <i>et al.</i> , 2005; Jackson <i>et al.</i> , 2003; Gillard and Vos, 2003b; Guittard <i>et al.</i> , 2003.
Cu-based acrylate	Polymer-C(O)OCuO(O)CR	Omae, 2003b; Yebra <i>et al.</i> , 2004; Shilton, 1997; Yamamori <i>et al.</i> , 1986; 1987; 1989; 1992; Matsuda <i>et al.</i> , 1997; Yamamori <i>et al.</i> , 2001; Osamu and Shigeo, 1997; Green <i>et al.</i> , 1991; Fox and Finnie, 2000
Zn-based acrylate	Polymer-COO-Zn-X	Yonehara <i>et al.</i> , 2001; Yamamori <i>et al.</i> , 1989; Osamu and Shigeo, 1997; Green <i>et al.</i> , 1991; Fox and Finnie, 2000; Kuo, 1995; Kuo and Chuang, 1996.
Nanocapsule acrylate particles	Core-shell morphology Core: Polymer-COOH Shell: Polymer-COOR	Omoto <i>et al.</i> , 2003

18.3.1 Binders for commercial chemically active paints

Below, the most important self-polishing copolymers employed in top-tier commercial AF paints will be described in detail. The reason is that these binders show the best control of the polishing and biocide leaching processes, so they are likely candidates to be used in future low-biocide formulations or to control the release of novel more environmentally friendly active agents. Nevertheless, it is still relevant to point out that the market share of the above resins is very limited compared to that of rosin derivatives, which clearly dominate the market (see Yebra *et al.*, 2005).

Table 18.3 Classification of experimental binders for tin-free chemically active paints

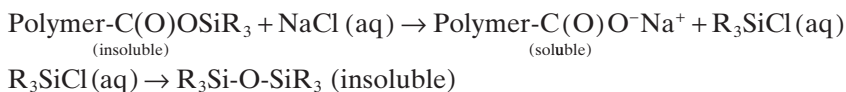
Binder classification	General chemical structure	References
Trialkylsilyl-based (meth)acrylate	Polymer-COOSiR ₃ Block copolymers Core-shell morphology	Arimura <i>et al.</i> , 2002; Bressy <i>et al.</i> , 2004; Nguyen <i>et al.</i> , 2005; Nguyen, 2006; Bressy <i>et al.</i> , 2008a. Omoto <i>et al.</i> , 2003.
Titanate-based acrylic	Polymer-C(O)OTi(OR) ₃	Humbert-Vandenabeele, 1996; Camail <i>et al.</i> , 1998a; Camail <i>et al.</i> , 1998b; Humbert <i>et al.</i> , 2000; Cerf and Senff, 2002.
Trialkylsilyl-based sulfonate	Polymer-S(O)OSiR ₃	Tuneta <i>et al.</i> , 2001
Polyesters	Graft copolymers with oligoester branches or oligolactic branches	Fukuda and Yokoi, 1995; 1999.
	Copolymers of ϵ -caprolactone	Vallée-Réhel <i>et al.</i> , 1999; Miyamoto <i>et al.</i> , 2001; Langlois <i>et al.</i> , 2002; Paul <i>et al.</i> , 2003; Faÿ <i>et al.</i> , 2006.
	Poly(ester-anhydride)s	Faÿ <i>et al.</i> , 2007a, b, c.
Cu-based acrylate	Polymer-C(O)OCuO(O)C-Polymer	Samui <i>et al.</i> , 2006.
Sulphonic or carboxylic acid functions blocked by nitrogen compounds	Polymer-SO ₃ ⁻ NR ₄ ⁺	Murfin, 1977; McLearn <i>et al.</i> , 1991; 1994.
	Polymer-SO ₃ ⁻ HNR ₃ ⁺	Hunter <i>et al.</i> , 1995; Tsuda, 1989; Finnie <i>et al.</i> , 1999
	Polymer-CO ₂ ⁻ HNR ₃ ⁺	Hugues <i>et al.</i> , 2003; Cambon, 1996; Finnie and Lenney, 1996; Arias Codolar <i>et al.</i> , 2000.

Rosin compounds are key ingredients in most 60 months AF formulations and in all hybrid and CDP formulations. The reason is that these abundant and cheap compounds feature a low water solubility which, combined with adequate co-binders and other paint ingredients can provide very cost-effective AF protection actually very difficult to match even by sophisticated polymeric systems. However, their intrinsic limitations hinder further optimisation so the scientific research on this type of resins is very limited. Additionally, the growing concern about fuel costs and the emissions of green house gases is progressively making advanced self-polishing polymers

more economically attractive in spite of their higher price. Hence, their importance in the near future is expected to increase significantly.

Polymer backbone with hydrolysable ester linkages

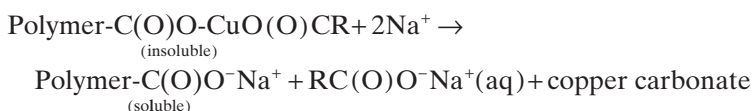
The binder technology, which has been pursued with a certain success as tin-free self-polishing binders, is based on silyl acrylates. The use of triorganosilyl groups as hydrolysable groups was first disclosed in 1984 (Russel *et al.*, 1984). The hydrolysis of the ester bond of a trialkylsilyl (meth)acrylate was reported to yield to a water-soluble binder and a non-toxic R_3SiCl side product which tends to form siloxanes by hydrolysis (Yebra *et al.*, 2004; Pelaprat *et al.*, 1996):



Several chemical reactions have been reviewed for the synthesis of trialkylsilyl (meth)acrylate monomers (Tonomura *et al.*, 2002; Paul and Rondini, 2003; Plehiers *et al.*, 2003; Mancardi *et al.*, 2007). Although a complete list of alkyl groups linked to the silicon atom could be found in patents, tri-*n*-butylsilyl and tri-isopropyl groups are often mentioned. The drawbacks related to the trialkylsilyl-based antifouling paints were poor mechanical and adhesion properties (cracking and peeling), no erosion rate and low storage stability (Vos *et al.*, 2001). Further tentative improvements have been disclosed in patents. Random (meth)acrylic co- and ter-polymers were synthesised from trialkylsilylated monomers and hydrophobic or hydrophilic acrylate or methacrylate comonomer units having alkyl, alkoxy, tertiary amino, quaternary ammonium salts, nitrogen-containing heterocycle, and hydroxyl groups (Oya *et al.*, 2005). Copolymers of trialkylsilyl monomers and ether comonomers (Honda *et al.*, 1995), copolymers with co-monomers containing a hemiacetal ester group (Yoshiro *et al.*, 1996), copolymers with N-vinylpyrrolidone co-monomers (Gillard *et al.*, 2001b; Vos *et al.*, 2001), and terpolymers with fluorinated methacrylate and acrylic monomer units (Guittard *et al.*, 2003) are proposed as self-polishing binders. Blends of trialkylsilyl copolymers with compounds selected from rosin (Itoh *et al.*, 1997; Tsuboi *et al.*, 2000) or with chlorinated paraffin (Nakamura *et al.*, 1997) have also been reported to improve the ability to erode at static conditions, the recoatability and the resistance to cracking. Improved mechanical properties have been claimed by incorporating incompatible, phase-separating (meth)acrylate polymers having a low glass transition temperature (Jackson *et al.*, 2003). In addition, polymers and copolymers of polyorganosilylated carboxylate monomers containing siloxane units have been reported to give films which undergo neither cracking or peeling and show moderate hydrolysability to dissolve into sea water at an adequate rate to exhibit long-term antifouling properties (Plehiers and Gillard, 2004).

Polymer backbone with ionic bonds: systems based on copper-acrylates

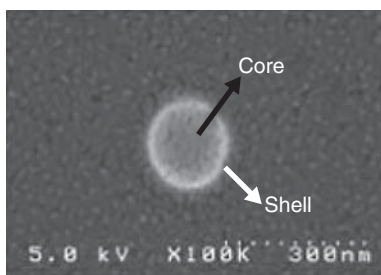
The first self-polishing copolymer technology disclosed in 1986 was based on an acrylic matrix bearing copper salts of an organic moiety (Yamamori *et al.*, 1986). These early copper acrylate-based systems have been reported to be active for up to three years, although performance data were only found for up to 42 months (Shilton, 1997; Yebra *et al.*, 2004). As mentioned in the literature, the self-polishing characteristics of copper acrylate copolymers result from a disrupting of the ester function. Nevertheless a disagreement remains about the sea water reaction of this copolymer (Yebra *et al.*, 2004). There is a lack of experimental evidence in the literature about copper acrylate copolymers and their self-polishing properties. The chemical reaction assumed today is the release of both a copper carbonate and an organic acid which is present in the sodium salt form if such groups are exposed to salt water:



However, these latter provide insufficient biocidal properties at their released concentration (Shilton, 1997). Therefore, antifouling efficacy of the corresponding paints comes from additional biocides. As an example, non-scientifically supported data are provided showing the linear behaviour of the polishing rate of Cu-acrylate paints with changing sailing speed (Omae, 2003b; Yebra *et al.*, 2004).

Polymer backbone with ionic bonds: systems based on zinc-acrylates

The use of zinc-acrylate copolymers as potential tin-free binders was first disclosed in 1989 (Yamamori *et al.*, 1989). It is assumed today that the leaching is based on an ion exchange reaction between zinc in the polymers and sodium in sea water. Nevertheless, an ion exchange reaction is a reversible chemical reaction during which ions of the same charge may be interchanged. In this binder technology, the polishing of Zn-acrylate films depends on both the zinc acrylate content (influencing the binder reaction) and the nature of the co-monomers which could influence both the water uptake and the mechanical properties (Yonehara *et al.*, 2001). Fouling prevention mainly relies on the release of other biocidal ingredients contained in the paint formulation since the dissolved zinc does not provide sufficient biocidal efficiency. In order to assure the long-lasting antifouling performance, it is essential to keep the sufficient release of the biocides as long as possible and therefore to control the erosion rates. According to this study, the release rate of copper in static conditions is 20 µg/cm² per day, after very few days of immersion.

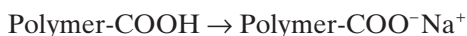


18.7 Nanocapsule acrylate particles obtained by field emission scanning electron microscopy (FE-SEM) after dilution in n-hexane. (Courtesy of Hempel A/S.)

Polymer backbone with ionic bonds: systems based on nanocapsule acrylate particles

The nanocapsule technology described hereafter is used in HEMPEL's GLOBIC NCT series, which offers ship hull protection for more than 60 months (see also Section 18.4.2). NCT is based on encapsulating the active polymer in a shell of hydrophobic acrylic polymer (see Fig. 18.7).

The core consists of a highly reactive polymer which, upon sea water exposure, yields the formation of sodium salt moieties on the polymer backbone triggering the polishing process (see Fig. 18.8):



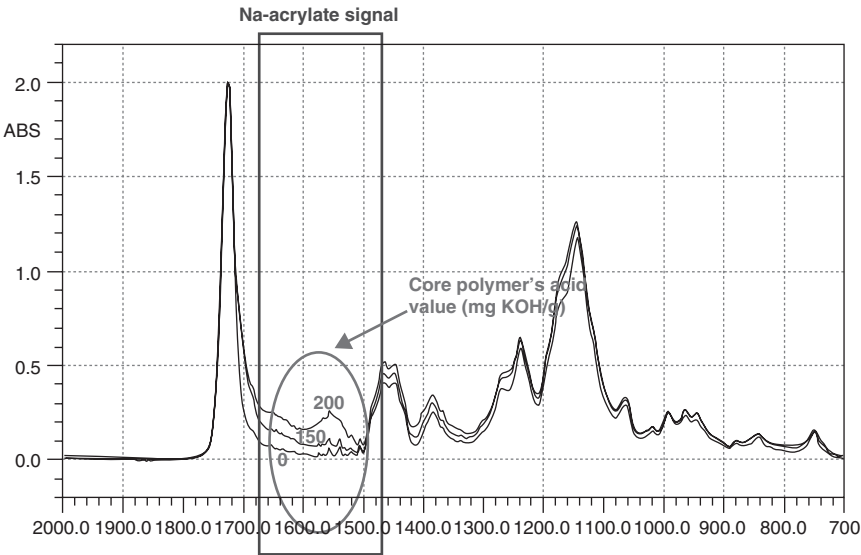
The hydrophobic shell assures thin leached layers which minimise the diffusion resistance that would otherwise prevent the active ingredients reaching the surface in effective concentrations (see Fig. 18.9).

In addition to a controlled copper release rate, the NCT can demonstrate a tight control over the release of the entire biocide package. Figure 18.10 shows a drop in the copper signal a few micrometers from the paint surface. The drop in the sulphur signal, associated with the co-biocide, is located even closer to the paint surface, which shows that the biocide is in balance with the polishing process. Thus sustained release of the active ingredient is assured over the entire paint lifetime.

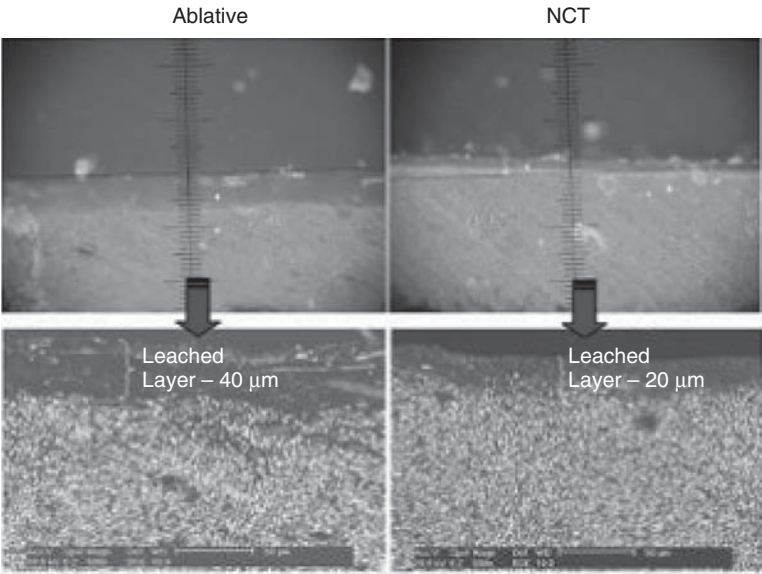
18.3.2 Experimental binders for solvent-based paints

Polymer backbone with hydrolysable ester linkages: systems based on silyl acrylates

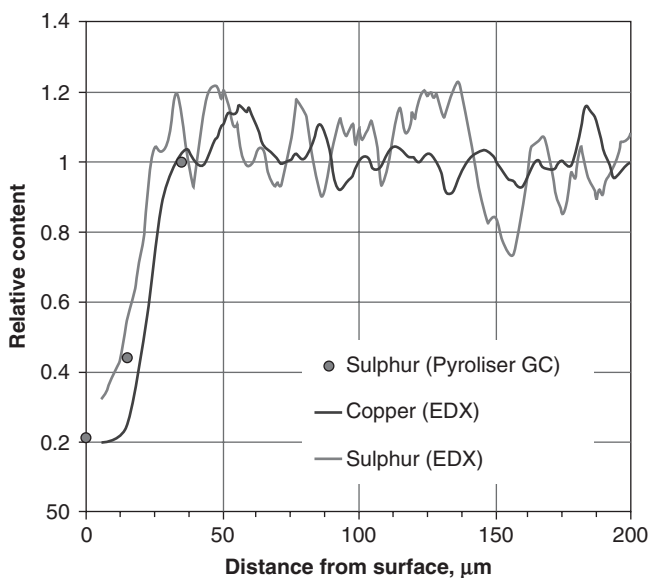
Recently, copolymers with controlled architectures such as di-, tri-block and star-type copolymers containing an organosilyl ester group have been investigated to enable good film formation properties, less cracking tendency and



18.8 Attenuated total reflectance Fourier transform infrared analysis of NCT films exposed to artificial sea water at 50 °C for 10 days. The spectra show a visible formation of Na-acrylate groups, the extent of which depends on the core polymer's acidity. (Data courtesy of Hempel A/S.)



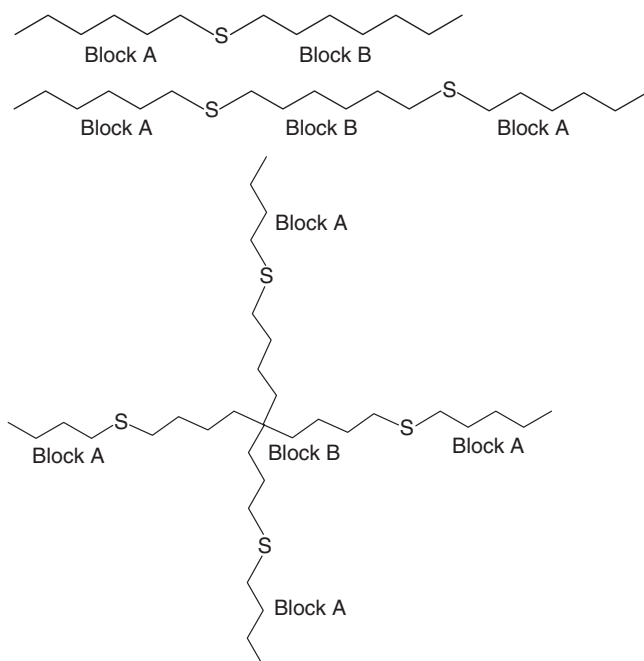
18.9 A comparison of leached layers using paint models formulated with large amounts of cuprous oxide and tested in laboratory rotors (optical microscope, top; scanning electron microscope, bottom). The NCT binder demonstrated excellent controlled biocide release properties. (Courtesy of Hempel A/S.)



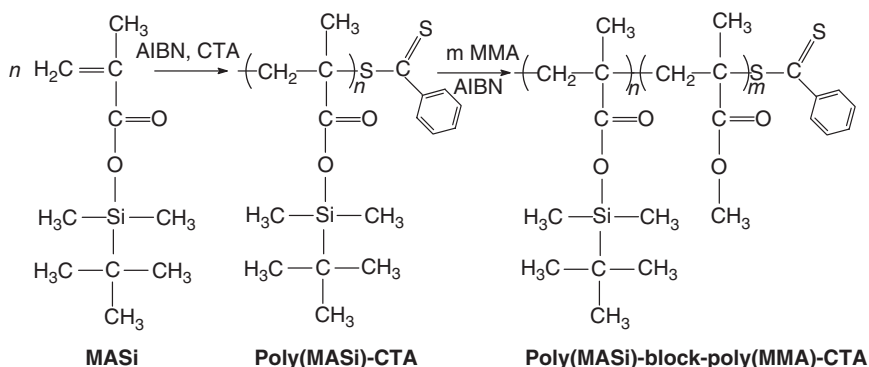
18.10 Chemical analysis of an exposed paint sample using energy dispersive X-ray analysis (EDX) and pyrolyser-gas chromatography techniques. The co-biocide-depleted layer is even thinner than the cuprous oxide one. (Courtesy of Hempel A/S.)

a controlled erosion rate. Copolymers with poly(oxyalkylene)s blocks have been synthesised via a conventional radical polymerisation process with thio-compounds as chain transfer agents (Arimura *et al.*, 2002). In that case, the poly(oxyalkylene) block is linked to the silyl ester block through a thioether bond $-S-$ (see Fig. 18.11).

The synthesis of experimental diblock methacrylic-based copolymers containing silyl ester hydrolysable groups was also investigated using the reversible addition-fragmentation chain transfer polymerisation (RAFT) (Bressy *et al.*, 2004; Nguyen *et al.*, 2005; Bressy *et al.*, 2008a). The RAFT process has been selected as an interesting radical technique for preparing well-defined polymers or copolymers with controlled and awaited molecular weights and low polydispersity indexes (DPI). Figure 18.12 illustrates the chemical reaction which occurs in the synthesis of these copolymers. The poly(silylated methacrylic) block is directly linked to the poly(methyl methacrylate) block using dithioester compounds as chain transfer agents (CTA). The authors demonstrated that diblock copolymers poly(methyl methacrylate-*b-tert*-butyldimethylsilyl methacrylate) showed a better control of the erosion with a constant polishing rate over a long-time service in water at pH = 8.2. Experiments showed that the erosion rate could be modulated by varying the molar proportion of hydrolysable side groups



18.11 Type of polymer microstructures as potential binders for self-polishing antifouling paints.



18.12 General scheme for the synthesis of diblock copolymers using dithioester compounds as chain transfer agent (CTA)- RAFT process.

onto the copolymer and the weight amount of copolymers mixed with PMMA in aromatic solvents (Nguyen *et al.*, 2006) (see also Section 18.4.2).

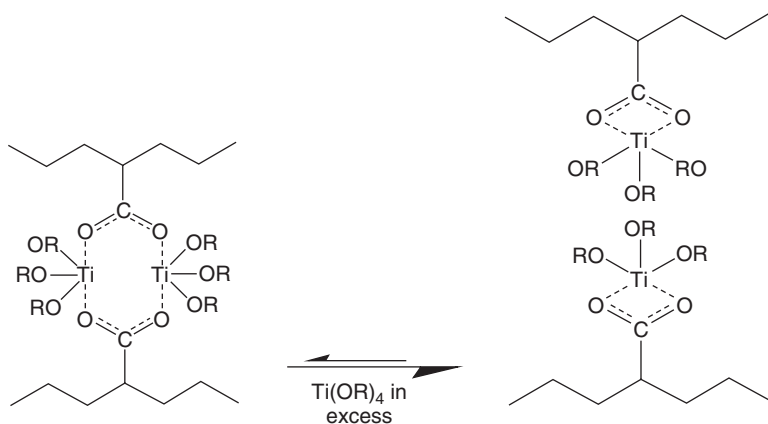
In addition, binders with controlled morphology have been investigated to enable good film formation properties, less cracking tendency, excellent adherence and a controlled erosion rate. Resins with a core-shell structure

comprising silyl esters of acrylic polymers in the core and/or in the shell component of the particles have first been patented in 2003 (Omoto *et al.*, 2003). These non-aqueous dispersions (NAD) based paint compositions are claimed to be constituted of low levels of silyl ester groups (less than 3% in weight of the total acid groups) with a total acid value of the NAD resin after hydrolysis comprising between 15 mg KOH/g and 400 mg KOH/g to obtain a high durability of the paint coat in sea water.

Polymer backbone with hydrolysable ester linkages: systems based on titanate acrylics

Experimental polymers containing titanate hydrolysable side groups are described in both patented and scientific literature (Humbert-Vandenabeele, 1996; Camail *et al.*, 1998a; 1998b; Humbert *et al.*, 2000; Cerf and Senff, 2002). Initial methacrylic monomers and polymers bearing carboxylic acid group have been considered to react with tetraalkoxytitanane compounds via an esterification reaction leading to the structures in Fig. 18.13.

Several trialkoxytitanate methacrylic monomers have been synthesised varying the alkyl groups linked to the titanium atom. Copolymers with methyl methacrylate have been prepared and an excess of the molar ratio $\text{Ti}(\text{OR})_4/\text{-COOH}$ of 4/1 was reported to avoid gel formation and to enhance the solubility of the resulting binders in organic solvents such as aromatic solvents. Paints derived from these polymers show a linear thickness loss versus erosion time. The erosion rate assessed with a Turbo-eroder apparatus depends on the carboxylic acid group content of the poly(methacrylic) copolymers (Humbert-Vandenabeele, 1996).



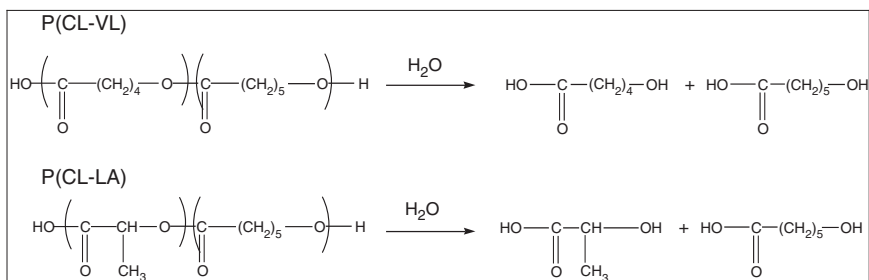
18.13 Chemical structures of carboxylic acid-based polymers in presence of tetraalkoxytitanane in excess.

*Polymer backbone with hydrolysable ester linkages:
systems based on polyesters*

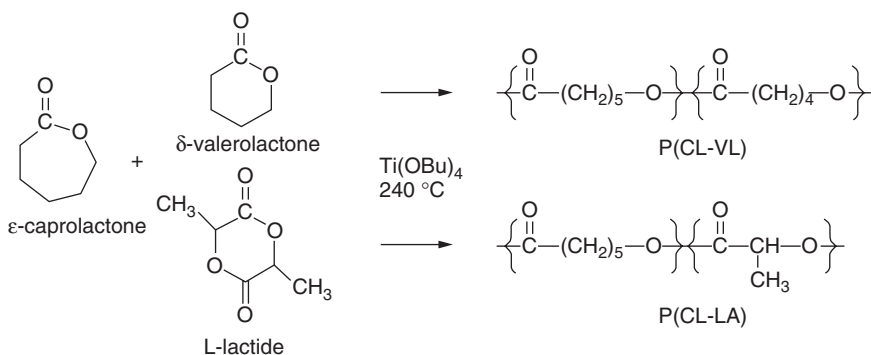
As environmental concerns increase, biodegradable polymers are watched with interest as self-polishing binders for chemically active paints. Among the family of biodegradable polymers, aliphatic polyesters such as PCL and ϵ -caprolactone (CL) copolymers are particularly described as potential binders which undergo a cleavage of the polymer backbone in water (see Fig. 18.14).

Graft copolymers have been synthesised from a cyclic lactone in a ring-opening process using a catalyst such as organic tin compounds (Fukuda and Yokoi, 1995; 1999; Vallée-Réhel *et al.*, 1999; Miyamoto *et al.*, 2001). Further, (meth)acrylic copolymers bearing hydrolysable poly(hydroxyacid) side groups have been investigated. The corresponding hydrolysable monomers were synthesised by reacting (meth)acrylic acid (Vallée-Réhel *et al.*, 1999; Langlois *et al.*, 2002), 2-hydroxyethyl methacrylate and methacrylic anhydride (Paul *et al.*, 2003) with oligo(lactic acid)s. In such copolymers, tin-based catalysts are used. Copolymers obtained by ring-opening polymerisation of ϵ -caprolactone and *L*-lactide or δ -valerolactone using tetrabutoxy-titane $\text{Ti}(\text{OBu})_4$ as initiator have been investigated as potential environmentally friendly self-polishing binders (see Fig. 18.15) (Faÿ *et al.*, 2006; 2007c). The authors stated that the hydrolytical properties of such caprolactone-based copolymers were affected by both the incorporation and the composition of comonomers. Furthermore, the biodegradable resins need to be soluble in common aromatic solvents, compatible with fillers, and show a controlled degradation and molecule release.

Biodegradable block copolymers composed of poly(ϵ -caprolactone) (PCL) and poly(sebacic acid) (PSA) have been studied to enhance these awaited properties (Faÿ *et al.*, 2007a; 2007b). The prevention of biofouling settlement and growth was obtained on a natural site. Nevertheless, the corresponding coatings were completely eroded after 10 weeks of



18.14 Backbone cleavage of biodegradable polymers in water.



18.15 Synthesis of copolymers poly(ϵ -caprolactone- δ -valerolactone) and poly(ϵ -caprolactone *L*-lactide). (Adapted from Faÿ *et al.*, 2006.)

immersion, which is too fast. Future work will include the development of binders with suitable biodegradation and erosion to maintain efficiency for many months.

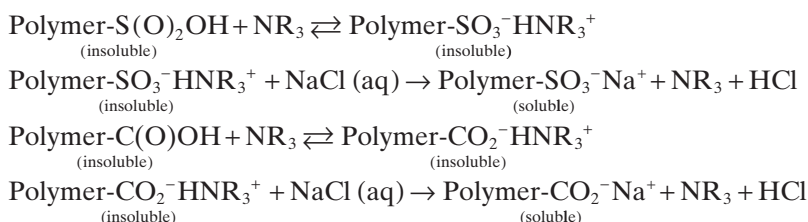
Polymer backbone with ionic bonds: systems based on copper-carboxylate bonds

Recently, copper salt of polyester amide have been explored as self-polishing binders (Samui *et al.*, 2006) This technology is based on metallic soaps which are defined as water insoluble compounds containing heavy metals combined with monobasic carboxylic acids of 7–22 carbon atoms. In order to enhance the film properties of copper soaps, the authors synthesised organo-copper polyester-amides with a copper content around 4% wt. To impart mechanical defects, chlorinated rubber resin was blended with these polymers. Controlled copper leaching rates in sea water have been found. The self-polishing behaviour was revealed by a surface smoothness which remains throughout the exposure in sea water.

Polymer backbone with ionic bonds: (meth)acrylic binders blocked by nitrogen compounds

Another interesting (tin-free) paint technology is self-polishing antifouling paint based on binder polymer having pendant acid functional groups, e.g. sulphonic acid (Murfin, 1977; Tsuda, 1989; McLearn *et al.*, 1991; 1994; Hunter *et al.*, 1995, Finnie and Lenney, 1996), and carboxylic acid groups (Finnie and Lenney, 1996; Cambon, 1996; Arias Codolar *et al.*, 2000) blocked by nitrogen compounds. In this classification, the binders are characterised in that the sea water-erodible polymer contains acid groups in ammonium

salt form. Thus, the binders can be defined as ionic exchange resins as the ammonium salt could be exchanged by Na^+ prior to polishing of the paint. The amine salt gradually dissociates on prolonged immersion and the amine is gradually released into the sea water. The remaining acid-functional polymer converted to Na^+ salt becomes sea water-soluble and is gradually swept from the substrates.



Hugues *et al.* (2003) and Bressy *et al.* (2008b) have discussed the chemistry of these SP binders bearing pendant acid functional groups blocked by biocidal tertiary alkylamines. Infrared spectrometric investigations reveal that a proton transfer reaction occurs between an acrylic resin and the base reagent resulting in the formation of an ionic complex. The authors show that the formation of this ionic complex depends strictly on the difference of pKa values between the acid and amine moieties in such a macromolecular system. Therefore, the proton transfer reaction involves an equilibrium process which was estimated to be independent of the alkyl chain length of the tertiary dimethylalkylamine base reagent. Because of a limiting value of the degree of complexation, tertiary amines have different bonding states within the polymer film, resulting in its plasticisation.

Alternative amines which can be used as blocking groups are biocidal aliphatic amines containing one or two organic groups of at least eight carbon atoms, aralkylamines or a primary amine derived from rosin which is sold commercially as 'Rosin Amine D' (Hunter *et al.*, 1995; Finnie and Lenney, 1996). Tri-amino compounds have been used as blocking groups for poly(meth)acrylic copolymers (Cambon, 1996). The resulting antifouling paint has shown good antifouling efficacy (up to two years) with controlled erosion properties assessed with an erosion test patented by the same inventor (Cambon, 1994). Linear thickness loss has been obtained and compared with TBT-based paints.

Acid star-type copolymers were patented as potential self-polishing binders when they are combined with nitrogen compound (Finnie and Lenney, 1996). The binder polymer has at least three limbs radiating from a central core, the acid functionality being present in the said limbs of the polymer. It has been found that self-polishing antifouling paints comprising polymers of the type specified herein seem to possess certain deficiencies under conditions corresponding to long-term exposure to weathering. These

properties can be improved by the incorporation of fibres (Arias Codolar *et al.*, 2000).

18.3.3 Experimental binders for water-based paints

Environmental antifouling paints are actually developed to mimic the behaviour of tin-based paints through the synthesis of core-shell copolymer particles in an aqueous media. The mini-emulsion polymerisation technique has been chosen to get nanoparticles, usually under 100 nm radius, and to limit the use of surfactant in comparison with the emulsion process (Lovell and El-Aasser, 1997). Results tend to show a narrow particle size distribution and a long shelf stability of the latexes. Kinetic measurements show that each polymerising droplet behaves as an isolated batch polymerisation reactor, thus introducing the nanoreactor idea (Landfester, 2001). When the recipe of the optimised miniemulsion was established, a series of (meth) acrylic core-shell latexes with several compositions were synthesised. The methacrylic acid (MAA) monomer unit was chosen for its hydrophilic character and enables the formation of the well-known carboxylate sodium salt moieties, which contribute to the erosion mechanism. Core-shell morphology is pointed out by an increase of the particle diameter without an evolution of the particle size distribution of the latex (Ni *et al.*, 2005) (no formation of a secondary population of particles) and later on by staining method using a transmission electronic microscope. The ratio between the core and the shell and also their compositions were varied to control film formation and polishing properties of these waterborne coatings (Kermabon *et al.*, 2007a; 2007b). The interest of such systems is that biocides could be introduced in polymer nanoparticles as already reported in the literature (Zhang *et al.*, 2007). Potential problems associated with the use of water based systems in continuously immersed marine structures are discussed in Chapter 13.

18.4 Key parameters for binder design

18.4.1 Molar composition effect

The molar composition of binders affects the hydrophilic/hydrophobic balance, which may lead to severe changes of the coating properties.

Effect on surface energy

Over the past two decades, marine bacterial adhesion to surfaces with different surface free energies has been investigated with the frequent conclusion that bacterial adhesion is less to low energy surfaces and easier to clean

because of weaker binding at the interface. However, there are also a number of contrary findings, i.e. that hydrophilic surfaces (or high energy surfaces) have a lower biofouling tendency than hydrophobic surfaces (Baier, 1980). It is suggested that hydrophobic interactions play an important role in the initial adhesion process in some organisms (Zhao *et al.*, 2005). This parameter is far less studied for SP paints than fouling release coatings but it must be kept in mind and will probably be investigated in the near future.

Effect on film hydration

The effect of hydrophobic/hydrophilic balance on water sorption and erosion of coatings was investigated with a series of methacrylic acid-based polymers blocked by nitrogen compounds (Bressy *et al.*, 2009). Several 'ion-exchange' binders were prepared by mixing a poly(methacrylic) resin with different dimethylalkylamines in organic solvents. Table 18.4 shows the decrease of the water sorption of coatings with increasing the hydrophobic character of the blocking groups. Lower values of the apparent coefficient of water diffusion D_{app} at the initial stage and of the water content at saturation S are obtained with increasing both the alkyl chain length and the amount of amines. Therefore, the use of hydrophobic amines results in significantly improving the binder protection against water penetration. As the permeation coefficient P is a product of the solubility and the diffusion coefficient, a decrease in P can occur with increasing the alkyl chain length and the amount of amines. Thus, the corresponding coatings show a higher resistance to erosion in artificial sea water under static conditions.

Table 18.4 Values of the water solubility S , the apparent water diffusion D and permeation P coefficients of different acrylic coatings immersed in artificial sea water at 23 °C. Data from EIS measurements. FE1 and FE1CXN/1 or 2 = binders consisting of initial acrylic resin and acrylic resin blocked by dimethyloctylamine (C8N), dimethyldodecylamine (C12N) and dimethylhexadecylamine (C16N), respectively. The molar ratio of amine to acid functions varies from 1 to 2. (Adapted from Bressy *et al.*, 2009)

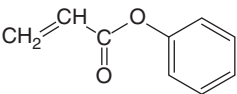
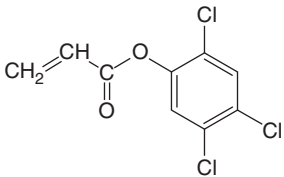
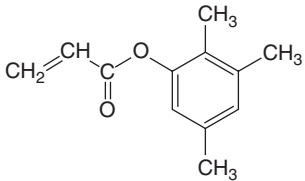
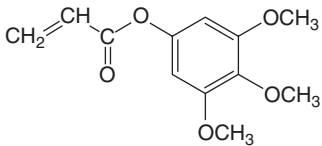
Type of coatings	S (kg.m ⁻³)	D_{app} (m ² .s ⁻¹)	P (kg.m ⁻¹ s ⁻¹)
TBT-based binder	17	12.10^{-13}	2.10^{-11}
FE1	1807	119.10^{-13}	2150.10^{-11}
FE1C8N/1	1326	68.10^{-13}	901.10^{-11}
FE1C8N/2	1320	56.10^{-13}	739.10^{-11}
FE1C12N/1	960	16.10^{-13}	151.10^{-11}
FE1C12N/2	927	5.10^{-13}	45.10^{-11}
FE1C16N/1	551	4.10^{-13}	21.10^{-11}

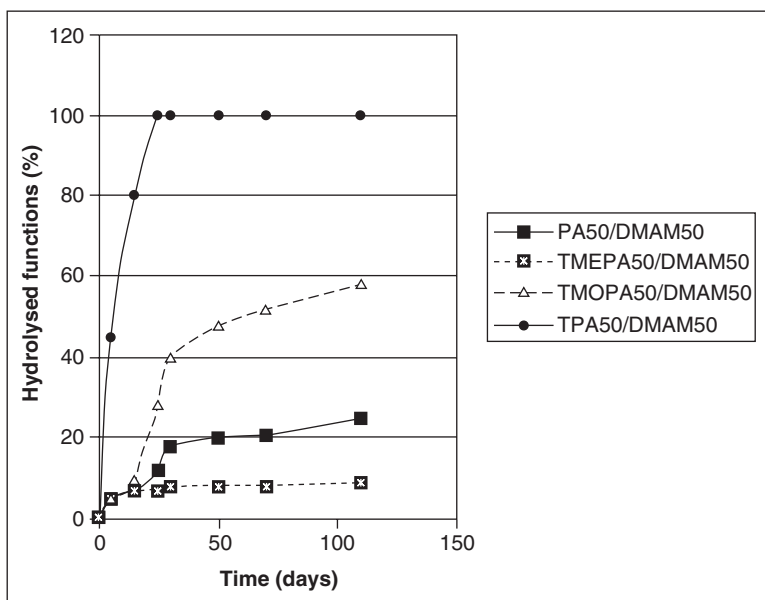
Effect on binder degradation

The hydrolysis of acrylic copolymer lateral ester groups was studied in order to identify the parameters that control this reaction and to better understand the relationship between chemical structure and polishing properties (Vallée-Réhel *et al.*, 1998). Several monomer units were combined within random copolymer chains composed of:

- hydrolysable groups: derivatives of phenyl acrylate (phenyl, trichlorophenyl, trimethylphenyl and trimethoxyphenyl)
- hydrophilic groups: dimethylaminoethyl methacrylate which is protoned at pH = 8
- hydrophobic groups: *t*-butyl methacrylate which enables the improvement of the mechanical properties of the film and to modulate the hydrophilic/hydrophobic balance (see Table 18.5).

Table 18.5 Chemical structure of phenyl acrylate derivatives

Hydrolyzable monomer	Chemical structure
Phenylacrylate	
Trichlorophenylacrylate	
Trimethylphenylacrylate	
Trimethoxyphenylacrylate	



18.16 Hydrolysis of copolymers based on trimethylphenylacrylate, phenylacrylate, trimethoxyphenylacrylate and trichlorophenylacrylate. (Adapted from Vallée-Réhel *et al.*, 1998.)

The different copolymers underwent a regular degradation by hydrolysis which led to a decrease of their glass transition temperature (T_g). The degradation of the copolymer based on trimethyl phenyl acrylate was the smallest indicating that the donor inductive effect combined with the hydrophobicity and the bulky character of the methyl groups prevent hydrolysis (see Fig. 18.16). In the case of the methoxy substituents, the easier hydrolysis can be explained by considering an attractive effect of the methoxy groups. Additionally, the presence of chloro functions on the phenyl group weakens the ester linkage through an electron-withdrawing inductive effect.

The ratio of hydrophilic comonomer and the hydrophobicity of phenyl acrylate derivatives were varied to evaluate their impacts on the degradation. It was concluded by comparing methacrylate and acrylate series of phenyl derivatives that the methacrylate compounds did not undergo any degradation because of the methyl group which has a marked hydrophobic effect and reduces water penetration in the film. On the contrary, the increase of hydrophilic comonomer favoured the hydrolysis.

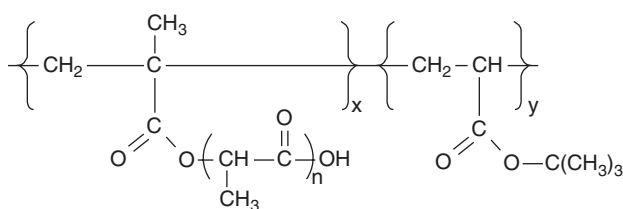
The hydrolysis of biodegradable copolymers based on poly(ϵ -caprolactone) (PCL) were also investigated. Poly(ϵ -caprolactone) (PCL) is a semi-crystalline linear aliphatic polyester, generally described as a biodegradable

polymer because of the susceptibility of its aliphatic ester linkage to hydrolysis. Applications of PCL are limited because degradation kinetics is considerably slow due to its hydrophobic character and high crystallinity. In antifouling paints, crystallinity reduces solubility in common solvent and shortens dramatically the storage time of corresponding coatings. Copolymerisation of ϵ -caprolactone with different lactones is a simple way to tailor the PCL properties. It has been demonstrated that the degradation rate of copolymers of ϵ -caprolactone and L-lactide can be adjusted by the molar compositions of the copolyesters (Ye *et al.*, 1997). The incorporation of comonomer such as L-lactide or δ -valerolactone led to a faster degradation than that of PCL homopolymer. Therefore, the release of biocides could be correlated with the degradation of copolymer (Faÿ *et al.*, 2006; 2007c). In another study, graft copolymers containing poly(lactic acid) side chains were investigated (Langlois *et al.*, 2002). These copolymers are very interesting because of the versatility of controlled structures that can be obtained. They make possible the study of the effect of hydrophobic/hydrophilic balance on degradation. It appeared that the degradation rate of these binders bearing hydrolysable segments derived from α -hydroxyacids (such as lactic acid) may be modulated by changes in chemical composition. Specifically, increasing the proportion of lactic acid units leads to faster degradation. The chemical structure of the copolymers is detailed in Fig. 18.17.

The synthesis pathway enables the control of the ratio of incorporated lactic units and the nature of the end-group of the side chains (hydrogen or methyl groups). Table 18.6 summarises the characteristics of the polymers.

Four copolymers having different *t*-butyl acrylate/macromonomer molar ratio were submitted to ageing. The degradation of copolymers containing less *t*-butyl acrylate ratio is faster (see Fig. 18.18). This result indicates that the presence of hydrophobic units such as *t*-butyl acrylate decreases the hydrolysis dramatically. With these structures, it is possible to design copolymers to obtain the desired degradation rate.

Figure 18.19 illustrates the impact of the end group of the side chain on hydrolysis. By controlling this parameter, it is also possible to modulate the

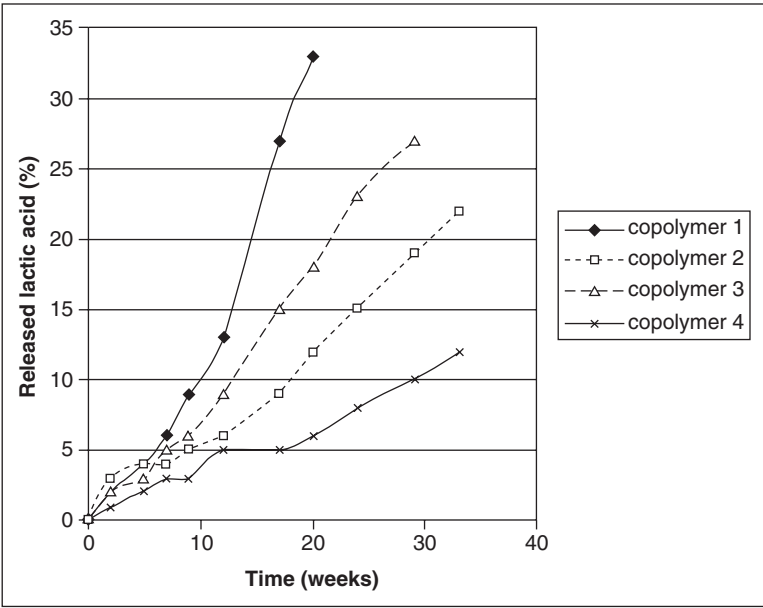


18.17 Graft copolymer with hydrolysable side chains. (Adapted from Langlois *et al.*, 2002.)

Table 18.6 Characteristics of graft copolymers with oligoester branches

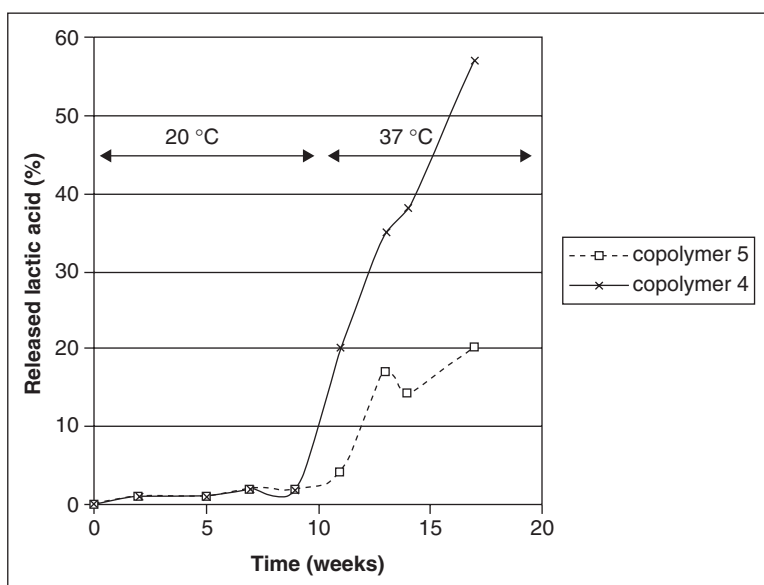
No.	Molar ratio ^a		Average length of oligoester branches (n)	End group of oligoester branches
	Initial comonomer feed	Copolymer		
1	10/1	13/1	27	H
2	20/1	24/1	27	H
3	10/1	13/1	14	H
4	20/1	24/1	14	H
5	10/1	19/1	12	CH ₃

^at-butylacrylate/macromonomer PLA molar ratio.



18.18 Effect of the molar composition of the copolymer on the hydrolysis. (Adapted from Langlois *et al.*, 2002.)

hydrophilicity of the binder and to control its degradation. In this case, the hydrophobicity of methoxy groups decreased the penetration of water into the copolymer. Moreover the difference in the kinetics of degradation can be explained by the presence of carboxylic acid functions on the copolymer. These acid groups increase the local acidity and can catalyse the hydrolysis process. Such a proximity or concentration effect is known in polymer chemistry.

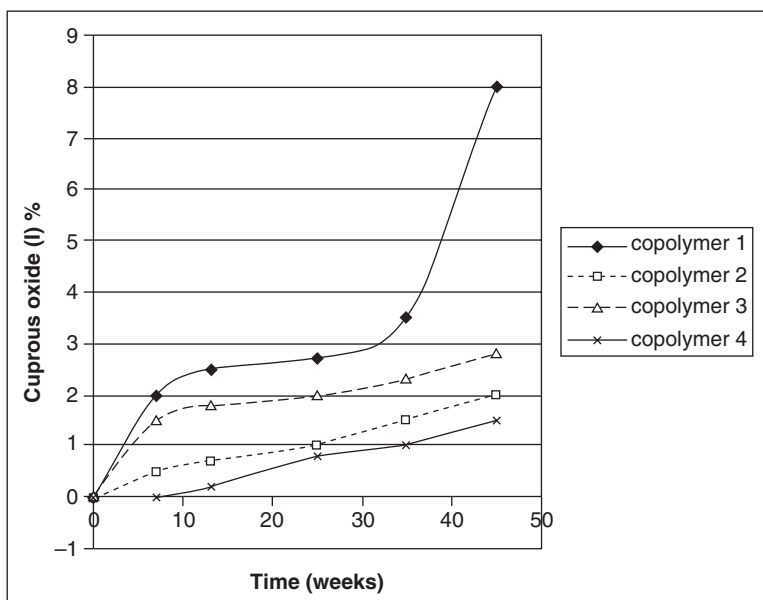


18.19 Effect of the nature of side chain end group on the hydrolysis. (Adapted from Langlois *et al.*, 2002.)

Effect on biocides release rate

The controlled structure of graft copolymers containing poly(lactic acid) side chains makes possible the study of the effect of hydrophobic/hydrophilic balance on polishing and biocide release (Langlois *et al.*, 2002). The rate of copper release increases with the number of the hydrolysable monomer units of oligolactic branches. Thus, the rate of biocide release may be correlated to the amount of lactic acid units (see Fig. 18.20).

One typical example of the effect of the molar composition on the controlled release of biocides is reported with the use of zinc acrylate copolymers as binder resin. The authors demonstrated that the leaching out of the polymers depends on the zinc acrylate content and the hydrophilicity of comonomers. Nevertheless, with respect to the physical property, the paints tended to be less flexible as zinc acrylate content increased. It was concluded that controlling the release by varying the hydrophilicity was more advantageous than by zinc acrylate content. The zinc acrylate content produces a significant effect on the copper release rate. Almost no effect was observed by the zinc acrylate/zinc methacrylate ratio. The type of alkyl groups in comonomers produces considerable effect on erosion rate and copper release rate. The most powerful effect was produced by MEA (methoxy ethyl acrylate) content (Yonehara *et al.*, 2001).



18.20 Effect of the *t*-butylacrylate molar ratio on the release of cuprous oxide. (Adapted from Langlois *et al.*, 2002.)

Effect on polishing rate

The polishing rate of coatings is also affected by the molar composition of the binder. Few scientific data are available for commercial self-polishing binders. Most of the information is disclosed for experimental binders. Several co- and ter-polymers containing methyl methacrylate, trialkylsilyl methacrylate and methacrylic acid monomer units have been synthesised as long-term controlled biocides delivery systems (Durand, 1993; Durand *et al.*, 1994). Table 18.7 shows the effect of the silicon atom substituents on polishing rate in artificial ASTM D1141-90 sea water. The type of alkyl groups in silylated comonomers affects subsequently the polishing rate, and a great effect was observed with phenyl groups as silicon atom substituents. Lower polishing rates could be obtained by replacing phenyl groups with more hindered groups such as tert-butyl groups. Both the polarity and the steric hindrance of the side groups linked to the silicon atom affect the polishing rate of the corresponding coatings. Furthermore, the polishing rate increases with increasing the molar proportion of hydrolysable monomer units within the macromolecular chains. Investigations done with TBuMe₂SiMA-based copolymers showed that depletion in coating thickness can be obtained at a rate substantially the same as that of a commercial self-polishing copolymer by the adjunction of a hydrophilic comonomer such as methacrylic acid (Durand *et al.*, 1992).

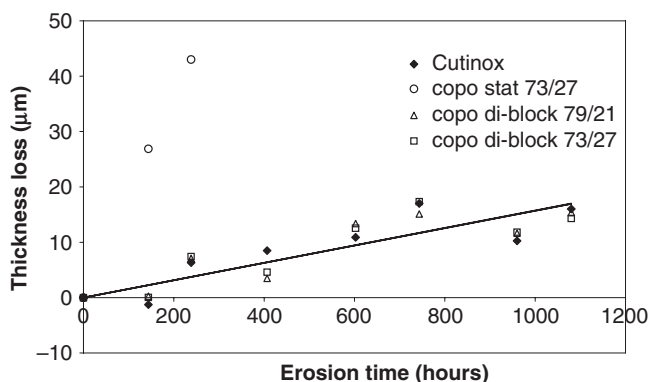
Table 18.7 Features of trialkylsilylated polymers and the tributyltin-based poly(meth)acrylate TBT-MA reference: monomer composition, weight-average molecular weight of polymers and polishing rate of the corresponding coatings

Exp. Nr	Type of hydrolysable monomer units	Hydrolysable monomer composition (% mol.)	Mw (g.mol ⁻¹)	Polishing rate (µm/day)
0	TBT-MA	32	80 000	1.0
1	TBuMe ₂ Si-MA	35	34 000	0
2	φ ₂ MeSi-MA	35	47 000	1.5
3	Me ₂ φSi-MA	35	56 000	Instantaneous
4	TBuMe ₂ Si-MA/ MAA	30/5	127 000	1.1

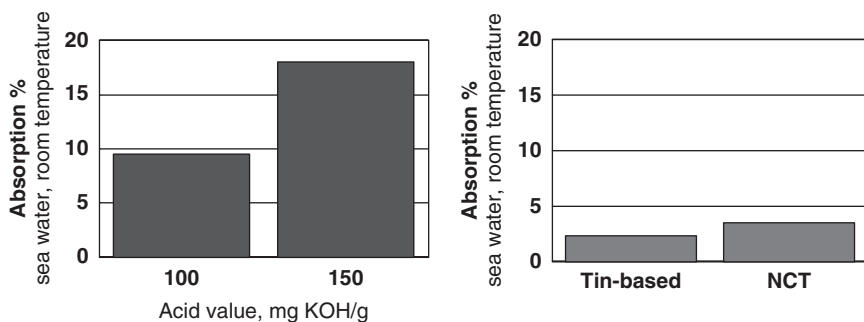
In a more recent patent, Abrams *et al.* (2006) reported the effect of the molar composition of hydrolysable silylated monomer units in copolymers on both the mechanical and polishing properties of the resulting coating. The authors mentioned that poor film formation properties are obtained for methacrylic polymers containing more than 20 mole% of tribenzylsilyl hydrolysable pendant groups. The authors revealed that the polishing rate of copolymers containing tribenzylsilylmethacrylate monomer units (TBzSMA) was quite linear and depended on the silyl ester content.

18.4.2 Copolymer microstructure and morphology effect

The effect of the microstructure of polishing binders on the polishing rate has been recently established. Well-defined random and diblock copolymers bearing silylated ester groups as hydrolysable pendant groups were synthesised by the RAFT process (Bressy *et al.*, 2008a). Data from erosion tests clearly show that a diblock copolymer poly(methyl methacrylate-*b*-*tert*-butyldimethylsilyl methacrylate) enable better control of the erosion over long-term service than the corresponding random copolymer (see Fig. 18.21). This latter was totally removed from the rotor after 250 hours of erosion. Moreover, the polishing properties of these diblock copolymers containing 21 to 27 mole% of hydrolysable groups are shown to be similar to the TBT-based binder with a linear thickness decrease over time. The authors mentioned that these results could be explained by a specific surface morphology due to a local chain organisation of the two incompatible blocks composed of PMMA and poly(*tert*-butyldimethylsilyl methacrylate). A phase separation in the bulk state of the hydrophobic PMMA block and the hydrolysable silylated block could modulate the erosion as the two phases are attached by one end. With low content of the silylated block (less than 50 mole%), the morphology could be close to hydrolysable domains dispersed in a hydrophobic matrix phase.

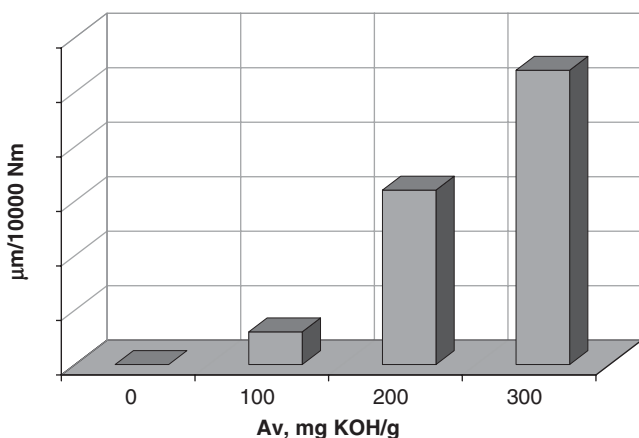


18.21 Plot of film thickness loss (μm) versus erosion time from rotor test (called Turbo-eroder) of tert-butyldimethylsilyl methacrylate-MMA statistical and diblock copolymers and of CUTINOX® 1120 as TBT-based reference. Films were cast on the same rotor and tested in artificial ASTM sea water (Bressy *et al.*, 2008a).



18.22 In the plot to the left, we show that core polymers are fairly hydrophilic, the water uptake being dependent upon the amount of acid monomers in the polymer (7 days immersion in sea water at room temperature). The encapsulation of such polymers by hydrophobic acrylates (right) strongly reduces the water uptake of the NCT binder (comparable to that of the best tin-based co-polymers). (Courtesy of Hempel A/S.)

In the commercial nanocapsules technology, the core-shell morphology of the binder is feasible for AF purposes. In the liquid and the dry film, the shell protects the core from reaction with other paint components. Moreover, in the dry film, the encapsulation of the hydrophilic polymers by hydrophobic acrylates strongly reduces the water uptake (comparable to that of the best tin-based co-polymers) (see Fig. 18.22) thus regulating the polishing of the paint (see Fig. 18.23).



18.23 Polishing rate trends obtained from NCT-based paints after 240548 nautical miles in natural sea water at 22 knots. In accordance with the polishing mechanism being postulated, the polishing of the paint is increased with increasing amounts of acid groups. (Courtesy of Hempel A/S.)

By varying the shell polymer composition, it is also possible to control water access to the reactive polymer and therefore fine-tune the release rate of active compounds (see Fig. 18.24).

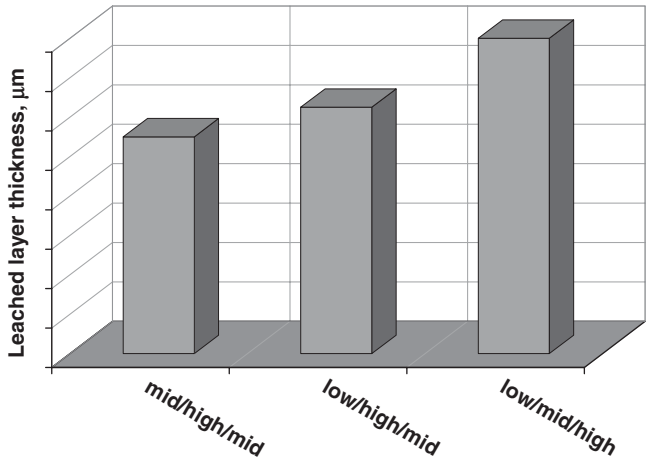
18.4.3 Macromolecular chain length effect

Few scientific results are reported in the literature on the effect of the macromolecular chain length on both the hydration and the erosion of self-polishing binders. Durand (1993) has shown the effect of the macromolecular chain length on the erosion rate of paints containing silyl acrylate binders. Figure 18.25 illustrates the decrease of the erosion rate of antifouling paints with increasing the molecular weight of binders for given pigment volume concentration and solid content.

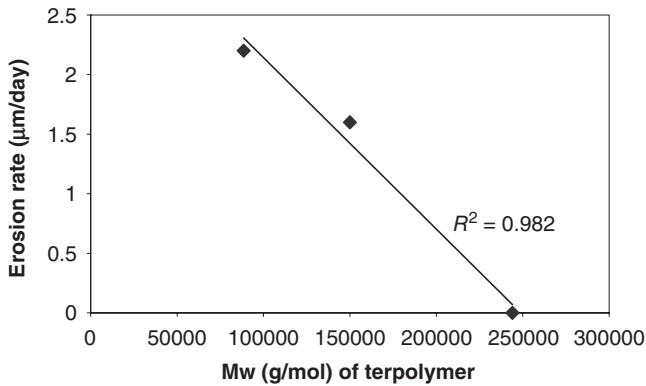
In the NCT technology, the molecular weight of the hydrophobic shell polymer has been found to have a visible effect on both the polishing rate and the leached layer thickness, probably due to changes in the water uptake kinetics (Yebra, 2008).

18.4.4 Formulation effect

Paints are complex materials with regards to the large number of compounds introduced in their formulation. The substitution of one major paint component may cause a dramatic imbalance in the performance which often takes years of research and development (Yebra *et al.*, 2006b; Kiil



18.24 Leached layer thickness trends for NCT-based paints after 6 months exposure to artificial sea water at 20 knots and 25 °C. Varying the shell monomer composition (Monomer 1/ Monomer 2/ Monomer 3. Low < 10%, Mid 10–40%, High > 40%) has a strong influence on the water uptake and, subsequently, on the biocide leaching process. (Courtesy of Hempel A/S.)



18.25 Effect of weight average molecular weights Mw on erosion rate of paints containing 35.9% wt. of zinc pyrithione, 15% wt. of titanium dioxide and TBuMe₂SiMA-based terpolymers with a molar proportion of MMA/TBuMe₂SiMA/MAA monomer units of 68/27/5. Erosion rate value of 1.0 μm/day for the TBT-MA reference paint (32% of hydrolysable monomer unit, Mw = 80 000 g/mol.).

et al., 2007; Chapters 13 and 14). The optimisation of the protective activity of paints requires the understanding of the precise function of each component, the definition of the mechanisms involved in polishing and antifouling phenomena, the resulting properties and the influential factors.

The adjustment of desired release and polishing kinetics are mainly affected by the hydrophobic or hydrophilic properties of the paint components including SP binder, co-binders, plasticisers, fillers, and biocidal pigments and molecules. Many studies propose to develop mathematical models with the main objectives to control the erosion rate and the biocides release rate (Yebra *et al.*, 2005; 2006b; 2006c). Nevertheless, the effects of both factors and their influence on the resulting antifouling efficiency seem to be closely related to the initial chemical structure of the binder as stated by Thouvenin *et al.* (2002a) through conducting a factorial experiment design on a commercial and a polyester-based binder. For polyester-based binders both the pigments and the biocides affect the antifouling efficiency by modifying the accessibility of the ester functions toward water and/or by decreasing the diffusion of the products resulting from the degradation. A strong interaction between the biocide and polymers and/or other non-diffusing substances in the coatings is needed in order to prevent rapid diffusion of the active molecules through the paint film (Handa *et al.*, 2006). For example, the antifouling molecule Medetomidine has been proven to interact with antifouling binder and therefore to affect its properties and efficiency (Shtykova *et al.*, 2004).

Like NMR and FTIR spectroscopies, SEM is a powerful tool to investigate such interactions by imaging the active molecule distribution.

Rosin-derivatives could be used in chemically active paints to adjust the intrinsic hydrophobicity of acrylic-based AF systems (Yebra *et al.*, 2004). In addition, hydrophobic polymers could be added as a retardant polymer resulting in a decrease of the coating erosion rate (Yebra *et al.*, 2006a). The same authors revealed how the use of a highly hydrophobic self-polishing binder resin is capable of correcting the copper release rate of rosin-based systems to match that of TBT-SPCs (Yebra *et al.*, 2005; Kiil *et al.*, 2007). Furthermore, the use of polymer blends is not only restricted to the adjustment of desired release kinetics and water permeability but can also offer major advantages including improved film formation, mechanical properties and storage stability (Kiil *et al.*, 2002a).

The presence of pigments in a coating makes the situation much more complex for permeability investigations. Besides the shape and the degree of dispersion of the pigment, the interactions between binder, pigment and water have to be considered (Hulden and Halsen, 1985). It is known that copper oxide enables regulation of erosion and favours the mechanical cohesion of the coating. The cohesion is also dramatically affected by the pigment volume concentration (PVC) which is another key parameter for describing paint (Chapter 13). The higher the PVC, the lower is the concentration of polymeric binder within the paint and the higher is the portion of pigment and filler particles. Above the critical pigment volume concentration (CPVC), the solid particles can no longer exist as separated phases

and the coating becomes porous. If the coating contains pores, the transport of water can occur by capillary flow and enable higher water permeability. SEM constitutes an excellent technique to confirm that the critical pigment volume concentration (CPCV) is not reached, that there is enough paint vehicle to fill the interstices between the particles.

The type of pigments and fillers has also been reported to contribute to the polishing rate of the coating (Yebra *et al.*, 2006a). The tendencies that are observed with the addition of insoluble pigments (i.e., TiO_2 and Fe_2O_3) indicate that the higher the insoluble pigment content, the lower the polishing rate. For soluble pigments such as Cu_2O and ZnO , the polishing rate is affected by both their volume ratio and their respective size (i.e., surface area) (Kiil *et al.*, 2002b).

Finally, it has to be pointed out that the addition of microfibrils improves the mechanical properties of binders under conditions corresponding to the long-term exposure to weathering. Patented data were reported for self-polishing antifouling paints comprising metal acrylates, silylated acrylates, nitrogen-blocked acrylics, and NAD binders (Yebra *et al.*, 2004; Arias Codolar *et al.*, 2000). Nevertheless, the Hempel microfibre technology actually has no significant effect on polishing rate (Yebra, 2008).

18.5 Future trends

The question: 'Where will chemically active antifoulings be in ten years time?' is very difficult to answer. Solomon (2007), Worldwide Marine Antifouling and Foul Release Business Manager from International Paint Ltd provides us his feeling:

Ten years after the first commercial and patented copper acrylate polymer-based product Intersmooth® 460 SPC that gave the same performance benefits as TBT systems was introduced by International Paint Ltd, we have got to consider where this technology-led market will go next. A single word to sum-up the external drivers for the development of antifouling paints is 'regulation'. In summary, the drivers that will control the development of antifoulings over the coming years will be to:

- Ensure that all raw materials are as safe as possible for human use.
- Reduce VOC emissions as far as practicable with high solids content paint or water-based and curing systems but still based on acrylics or rosin/rosin derivatives.
- Develop products containing environmentally benign substances.

Typical ways of reducing the solvent requirement of acrylic polymers is to either modify the molecular weight of the polymer chain or to change the monomers. Creating polymers with lower solution viscosities means that the resultant formulated paints can be higher in solids content and lower in VOC emissions. However, polymer modifications of this type can only reduce the

solvent requirements to a point before the other properties of the paint start to become affected (such as mechanical strength which is needed for antifouling paints to resist the stress and strain caused by the intermittent wetting and drying when on an immersed underwater hull). There is a balance of most properties of an acrylic polymer with molecular weight; those include viscosity, mechanical strength and glass transition temperature T_g .

The choice of active ingredients is currently limited and the future anticipated choice is much more so. Any active ingredient must be shown to be non-bioaccumulative and non-persistent in the environment. As such, future antifouling products will be based on a limited set of active ingredients which are released into the environment in a controlled way in order to minimise their impact.

In the ongoing debate concerning global warming, Johansen (2007) from Jotun believes that emission to air will become even more important in the shipping industry. Clean and smooth vessel hulls are needed to meet this demand and Jotun believes that the best way to reach fouling-free hulls is SPC coatings with binders becoming dissoluble via hydrolysis of an ester bond. It is an important ship-building nation and many of the yards are situated in China's numerous freshwater rivers. The vessels built at these yards spend the outfitting period in freshwater, which will increase the diffusion of water molecules into the coatings. Too high water uptakes will risk the lifetime and performance of the coatings. It is also important that the solubility of the binder resin in freshwater does not exceed that in sea water, as this will change the properties of the paint film. Jotun's scientists have found binder resins/antifouling paints based on covalently bonded leaving groups, such as the trialkylsilyl esters. This technology minimises water penetration into the bulk of the film to confine the reaction with sea water to near the coating surface. In addition, the covalent bond of trialkylsilyl esters has proven to be rather inert to freshwater exposure.

Scientists from Hempel postulate that the coming years will experience an increased interest in the quantification of the efficiency of the different AF products, closely related to fuel savings, release rates of harmful exhaust gases and the translocation of invasive species (Yebra *et al.*, 2007). Hence, 'artificial' discussions such as the exact definition of the self-polishing mechanism will be finally left behind in favour of those technologies offering the best AF protection with the lowest environmental impact (safe active compounds and lower VOCs) independently of their chemistry. The NCT concept described earlier in this chapter is innovatively based on 'sharing' the binder's required properties (good polishing and biocide release rates) between two different acrylate polymers (core and shell), each of them specifically designed to carry out their mission in the simplest and most flexible way possible. Unlike in 'one-polymer' acrylates, adjusting the reactivity of the binder (i.e., paint polishing) does not significantly affect the

paint's biocide release mechanisms. This flexibility is believed to be useful when adapting the formulation to the use of non-toxic or, at least, environmentally friendly active agents. Improved polishing behaviour by acid-containing polymers in freshwater is another key advantage of NCT. Contrary to 'self-polishing' acrylates and even tin-based paints, NCT acid groups are very reactive even at neutral pH values and low sodium ion concentrations. Thus, they can also self-polish in fresh water environments. At the same time, contrary to 'ionic-type' self-polishing acrylates, the very hydrophobic shell polymer avoids accelerated osmotic water ingress and film damage.

18.6 Conclusions: advantages of self-polishing coatings

The antifouling paints are usually based on biocides and prevent undesired marine organisms because of their toxic properties. The ban on harmful substances in antifouling paints requires the development of new antifouling strategies with acceptable standards for use conditions and negligible effects during occupational use, for consumers and for the aquatic environment. Alternatives to conventionally toxic paints have to compete against highly efficient, long-lasting and well-priced paints and their applicability has to be proven in an appropriate way.

Most self-polishing paints contain acrylic or methacrylic copolymers which are hydrolysed in sea water. This hydrolysis/polishing process results in a smooth surface on the copolymer and an ability of controlling/regulating biocide leaching rates. Alternative tin-free antifouling paints used the same type of self-polishing copolymers which are used in the case of organotin antifouling paints by bonding copper, silicon, zinc or titanium, oligomer groups with its carboxylate side chain instead of the TBT groups. The leaching rate of copper and organic biocides in the self-polishing copolymer is controlled by the degree of polymerisation (molecular weight) and the hydrophilic character of the copolymer (depending on the ratio of carboxylic group in the molecule and kinds of copolymers).

Present modern methods of biofouling control are effective alternatives to the TBT antifouling coatings, but not yet their equal. In Table 18.8, the cost of alternatives can be seen (Chambers *et al.*, 2007).

The use of silicone rubber finishes incorporating silicone or fluoropolymer oils tested as antifouling methods allows the temporary fixing of algae and barnacles with an easy removal by water jets or simply by the friction of sea water in the case of fast ships (Williams *et al.*, 2002). However, the spreading of this technology to the majority of the world fleet is mainly restricted by their poor efficiency at low speeds or in ships with frequent and/or long idle periods. The tin-free self-polishing binders exhibit many advantages:

Table 18.8 Commercial antifouling alternatives to TBT-SPC paints: mechanisms of action, lifetime, cost and drawbacks. (Adapted from Chambers *et al.*, 2007)

Antifouling system	Leaching rate	Lifetime	Cost (dollar)/m ²	Problems
(TBT) self-polishing copolymer paints	Chemical reaction through hydrolysis. Reaction zone of ablation 5µm deep.	4–5 years	\$680884	Banned 2008
(Tin-free) self-polishing copolymers	Chemical reaction through hydrolysis of copper, zinc, and silyl acrylate	5 years	\$691355	Lifetime shorter than TBT-based paint systems
Foul release	Low energy surface, leaching of silicone oils	2–5 years	\$1357786	Prone to abrasion damage Increase the ability to translocate alien species

- Compatibility with the existing antifouling systems: few issues are observed with film adhesion, no primer coatings are needed. Furthermore these binders are compatible with the existing painting method.
- Durability: these binders are perceived to keep their mechanical properties and can easily be repaired if necessary.
- Wastes: the self-polishing coatings do not lead to the formation of a residual degraded layer very difficult to remove. The cleaning before re-painting is easy and the corresponding wastes are produced in important quantities. Furthermore, in the case of biodegradable paints, the wastes are not toxic or polluting.

Although the current alternatives to TBT-SPC paints are not efficient enough, the research on these systems keeps being fascinating because of the complexity of their activity. More profound investigations have to be carried out in order to better understand the hydration, the degradation, the polishing mechanism and the biocide release. It is a real challenge to the scientists to control such intricate systems and to propose coatings which respect the marine environment and its biodiversity.

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The use of copper as a biocide in marine antifouling paints

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Abstract: This chapter provides an overview of the current information concerning the concentration and environmental effects of copper in the coastal marine environment. Copper inputs from antifouling paints into the local marine environment are evaluated and how these inputs contribute to the overall copper concentration and whether regulators should be concerned. The importance of copper speciation and how this influences the bioavailability and toxicity of copper to marine organisms is discussed. Overall, the risk of copper in the marine environment from an antifouling paint perspective is critically evaluated, looking at the current methods of measurement and prediction and whether these provide adequate protection for marine life.

Key words: copper, risk assessment, bioavailability, biotic ligand model, antifouling biocide.

19.1 Introduction

There has been much recent attention by regulators and scientists on the environmental effects of copper, which has been due partly to its widespread and increasing usage in marine antifouling paints following controls on tributyltin (TBT, IMO, 2001). It is certain that many Western countries are beginning to re-evaluate their risk assessments in order to provide adequate protection for the environment against the potentially harmful effects of copper. A few European countries have recently restricted or banned the use of copper-based antifouling paints on small recreational vessels (e.g., Sweden and The Netherlands). These controls are restricted to enclosed freshwater systems with limited water exchange where water-borne copper concentrations were found to exceed water quality thresholds. Other European nations such as Finland have recently begun to question the environmental impact of copper from antifouling paints and could follow suit in banning copper biocides from recreational vessels. However, there are conflicting views on the bioavailability and toxicity of copper in the natural environment, and laboratory studies may overestimate environmental risk.

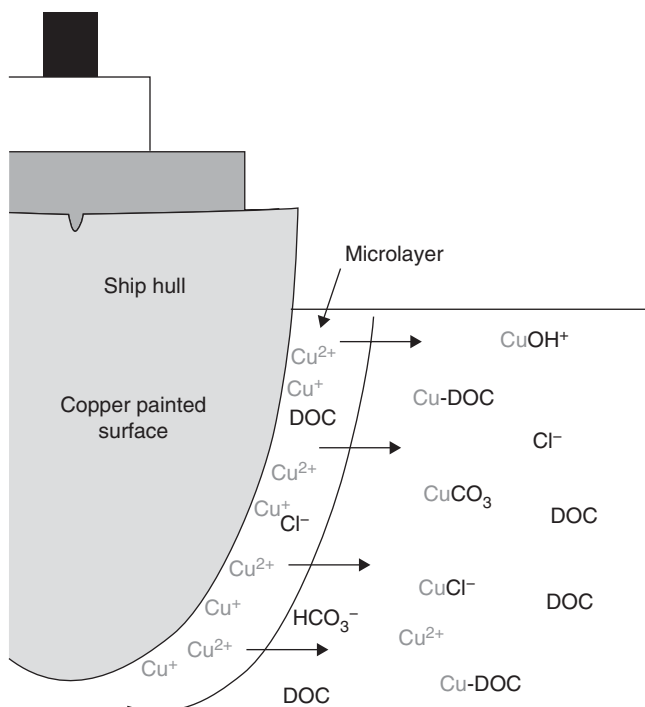
The following chapter provides an overview of the current information relating the concentrations of waterborne copper in the marine environment and the potential effects that these concentrations can have on animal and plant species. The inputs of copper from antifouling paints will be a main focus, examining how these contribute to total seawater copper concentrations and whether or not regulators should be concerned. The importance of copper speciation influencing copper bioavailability and toxicity will be re-evaluated with respect to the important environmental parameters such as dissolved organic carbon. Overall, the risk of copper in the marine environment will be critically assessed, looking at the current methods of measurement and prediction, and whether these provide adequate protection for marine life.

19.2 Copper as a biocide in antifouling paint coatings

Marine biofouling is the undesirable accumulation of biological matter on the surfaces of submerged objects such as ship hulls and pier pylons. There are over 4000 marine species that have been identified as biofouling organisms, all of which are sessile forms (Anderson and Hunter, 2000). Marine antifouling paints can be described as ‘highly specialised coatings that protect ship hulls from biofouling by releasing active compounds in a controlled manner’ (Yebra *et al.*, 2006, see Fig. 19.1). These active compounds prevent colonisation by the biofouling organisms.

There are strong economic arguments for developing effective antifouling coatings (see Chapter 9). The main problems of biofouling on marine vessels are associated with the increased friction created as a result of the presence of the organism(s) on the vessel surface. This can lead to reductions in manoeuvrability, increased weight and reduced speed, all of which can result in increased fuel consumption and cost. The increased fuel consumption as a result of biofouling has been estimated to be as much as 40% (Yebra *et al.*, 2004). This provides a large incentive to vessel owners and coating companies to develop strategies to prevent the attachment of organisms to vessel surfaces. In addition to the more obvious frictional effects, high levels of biofouling activity can lead to increased frequency of dry-docking operations and overall reduction of the integrity of the ship hulls – both of these factors would have significant financial impacts to the vessel owners.

There are also environmental drivers for developing effective antifoulings, e.g. fuel savings mitigate CO₂ emissions. Biofouling communities also have the potential to transport invasive non-native species across geographical niches; such activities can have disastrous effects on native populations and communities (Conlan, 1994). Therefore the need to protect submerged surfaces from biofouling organisms is both of economic and environmental importance.



19.1 The theoretical release of copper from an antifouling paint. Copper leaches from the boat into the seawater as unstable ion Cu^+ , which quickly oxidises to form the more stable Cu^{2+} . Complexation reactions between Cu^{2+} and organic and inorganic ligands occur within a few micrometres of the boat surface. Consequently, high toxicity of copper on the boat surface prevents biofouling and there is a significant reduction in copper toxicity through ligand interactions within the a few micrometres of the boat surface, reducing its effects on non-target organisms.

Conversely, the coatings themselves may cause environmental damage. TBT has been widely used as the main biocide in antifouling paint coatings on marine vessels over the last 40 years and until recently, covered approximately 70% of the world's shipping fleet. Although TBT was an effective biocide, it was also found to be highly toxic to a wide range of aquatic species at very low concentrations (Thain, 1986; Alzieu, 1991). For example, low ng/L TBT concentrations were found to cause 'imposex' in dog whelks (*Nucella* sp.), a condition that causes females to produce male genitalia, a phenomenon that has been very well documented in the scientific literature (Thain, 1986; Thain and Waldock, 1986; Bryan *et al.*, 1989; Smith *et al.*, 2006). The legacy of TBT and the occurrence of imposex are still prevalent

to the present day despite the restrictions on TBT usage in antifouling paints.

The detrimental effects of TBT on aquatic life and the potential effect on humans due to the persistent bioaccumulation of the biocide led to its eventual ban by many countries on all small vessels, e.g. the UK restricted use to vessels above 25 m in length. TBT-based paints were effectively withdrawn from the assortment of the major antifouling paint companies in 2003, and a complete ban on the use of TBT as a biocide on all vessels will be imposed by 2008 (IMO, 2001). As a consequence, copper has been increasingly used as the main biocide ingredient in antifouling paint coatings. However, the use of copper as a biocide is not new; the first recorded use of copper as an antifouling agent was in the British patent of William Beale in 1625, although it was not until the 19th century that the dissolution of copper was found to be responsible for preventing biofouling (Yebra *et al.*, 2004).

In the present day, most chemically active paint systems rely on the use of seawater soluble copper oxide (Cu_2O) pigment in combination with one or various organic booster biocides for the prevention of biofouling. Other copper pigments which have been used include copper (I) thiocyanate, cuprous bromide, cuprous iodine and cuprous cyanide. Overall, Cu_2O is preferred over these other pigments due to a combination of cost, solubility and toxicity. However cuprous thiocyanate is often used as an alternative to Cu_2O when lighter colours of hull coatings are required. Cuprous thiocyanate and Cu_2O have led to similar seawater concentrations of CuCl_2 at pH 8.4 (Yebra *et al.*, 2004). In our laboratory, the toxicity of copper to the development of the early life-stages of the mussel, *Mytilus edulis* was found to be identical whether Cu_2O or copper sulphate was used as the contaminant source.

The amount of copper used within any antifouling paint varies widely from 20% to 76% of the total, although some environmentally conscious paint manufacturers have recently been trying to reduce this proportion, without compromising the effectiveness of the paint.

Antifouling paints are largely formulated in three ways: insoluble matrix, soluble matrix, and self-polishing (see Chapter 12). The insoluble and soluble matrix paints contain biocides (i.e., copper) entrained within the paint. The insoluble paint matrix only releases the biocide into the water by contact leaching, whereas the soluble matrix and self-polishing formulations provide a more controlled dissolution of the biocide (Thomas *et al.*, 1999). Both the insoluble and soluble matrix paints are characterised by high initial leaching rates followed by exponentially decreasing rates over a relatively short time (~2 years).

Self-polishing co-polymer paints are essentially only used with TBT as the main biocide ingredient. In this case the TBT is bonded to the polymer

with an unstable release layer that gradually erodes due to hydrolysis. As a result, self-polishing co-polymer paints are characterised by a short initially high leaching rate followed by a relatively long (~5 years) constant leaching rate (Thomas *et al.*, 1999). However, since the ban on TBT, paint manufacturers have been trying to produce a more controlled delivery system for TBT-free biocides. Other formulations for the inert matrix and delivery have been developed, such as ablative and epoxy-based formulations. The passive flux of the biocide such as copper from each of these formulations differs as a result of the matrix delivery system. For example, epoxy-based coatings use a honeycomb matrix that enables the impregnated cuprous oxide to leach through micro-channels in the coating (Schiff *et al.*, 2004). These have provided a more controlled delivery system for the biocide.

The rate of copper biocide release has been investigated for a number of years. Release rate has been shown to be dependent on a variety of physico-chemical factors including water flow, temperature, pH, salinity, as well as biofilms (Yebra *et al.*, 2004; 2006; Thomas *et al.*, 1999). Regulatory assessment of paint leaching rates uses the standard American Society of Testing Materials (ASTM) method that determines biocide release in a container of artificial seawater. However, the method yields biocide release rates significantly higher than those obtained through testing with natural seawater (Valkirs *et al.*, 2003; Thomas *et al.*, 1999). ASTM laboratory tests predicted values of 22–65 $\mu\text{g Cu/cm}^2/\text{day}$, whereas *in situ* measurements made directly on the antifouling paint surface of vessels provided leaching rates of approximately 8.2 $\mu\text{g Cu/cm}^2/\text{day}$ on recreational craft and 3.8 $\mu\text{g Cu/cm}^2/\text{day}$ on naval vessels (Valkirs *et al.*, 2003). The authors suggested that the presence of established biofilms was the reason for the large difference in laboratory and field measurements. Despite knowing that microbial biofilms in natural seawater can alter leaching rates, there is a paucity of understanding of the build-up of microbial biofilms on boat surfaces. The high leaching rates found in the pleasure craft compared to the naval vessels was thought to be due to the increased cleaning activity of the former, compounded by differences in paint formulation (Valkirs *et al.*, 2003).

Current regulations for the measurement of leaching rates of biocides from antifouling paints are still based on controlled laboratory tests (ASTM, 2000). The significant differences between leaching rates based on controlled laboratory testing and those carried out in the natural environment have led to an overestimation of biocide leaching rates. A more realistic estimate of leaching rates in the natural marine environment could be achieved with better understanding of the build-up of microbial biofilms and the effects of boundary layers on the antifouling paint mechanisms in natural seawater.

19.3 Comparative copper inputs from antifouling paints

Although it is often difficult to separate anthropogenic and natural inputs of copper into the environment, it has been estimated that the input of copper into seawater from antifouling paints equates to approximately 3000 tonnes per year. This is a small amount compared to the natural weathering of copper from land estimated at 250 000 tonnes per year (Pidgeon, 1993). However, it is the localised scale of input of copper from boat surfaces that can potentially lead to elevated concentrations in enclosed water bodies such as marinas and harbours. In coastal waters with high levels of boating activity, the contribution of copper from antifouling paint is believed to be a small proportion of the copper input, with urban-runoff considered as the main contributor (Thackray, 1992). The inputs of copper from antifouling paints have been quantified in a number of studies. For example, copper input from marine antifouling paints was estimated to contribute about 2% of the total copper load in the New York/New Jersey harbour area (HydroQual Incorporated, 1995). Slightly higher contributions of copper from antifouling paints were reported in San Diego Bay (Valkirs *et al.*, 1994). In the UK, copper from antifouling paints was reported to contribute about 10% of the overall anthropogenic inputs (IMO, 1997). Although the proportions differ to a certain extent, they are in agreement that natural sources are by far the main contributors.

The inputs of copper from antifouling paints can come from a number of activities, these include passive leaching from the boat surface, elevated inputs as a result of cleaning and inputs from paint chippings and flakes often resulting from boat maintenance and cleaning activities. Schiff *et al.* (2004) compared the inputs of copper from recreational vessels from both hull cleaning activity and passive leaching. They found that about 95% of copper inputs from recreational vessels were from passive leaching, whilst cleaning activities contributed the remaining 5%. Hull cleaning activities were reported to contribute between 3.8 and 8.6 μg dissolved Cu/cm²/event (Schiff *et al.*, 2004). Recreational vessels have been reported to leach copper in excess of 2 kg of Cu per annum per vessel (approx 28ft, Boxall *et al.*, 2000).

Cleaning activities for small vessels, often on an annual cycle, require the boat to be removed from the water and placed on a dry dock area to gain access to the hull. Such cleaning activities include pressure hosing and or complete stripping including shot blasting, sanding and stripping. It is well known that these cleaning activities can lead to large quantities of paint chippings and flakes. Although there are current safeguards for the collection and safe disposal of discarded chippings and flakes in many Western countries, evidence suggests that much of this material is washed into the water (Boxall *et al.*, 2000; Thomas *et al.*, 2000; 2003).

Recent work involving the chemical characterisation of paint chippings collected from a recreational boat yard in the UK revealed copper as the most abundant metallic compound (Turner *et al.*, 2008). Contaminant hotspots with elevated copper concentrations were found in the underlying sediments of the marina, these hotspots were attributed to the presence of paint chippings. Previous research has also found elevated concentrations of copper close to slipways and boat hoists where cleaning activities take place, suggesting that cleaning activities may be responsible for these elevated copper concentrations (Thomas *et al.*, 2000; 2003).

19.4 Copper concentrations in the marine environment

Typically, background copper concentrations within estuarine and coastal seawater range from 0.5 to 3 µg/L, although concentrations can become elevated in areas of high run-off close to locations of historical mining activity (e.g., Restronguet Creek, Cornwall, UK, (Bryan and Gibbs, 1983)). Dissolved copper has a tendency to adsorb on to colloids and suspended particulates, which then settle and accumulate in the sediment. Through this mechanism copper concentrations in the sediment can often be two to three orders of magnitude higher than in the water column. In the UK, sediment copper concentrations can range between 5 and 2400 mg/kg (HSE, 1999a), with highest sediment concentrations often as a result of runoff from historical mining (Bryan and Langston, 1992).

In aerobic sediments, copper is mainly bound to metal oxides and high molecular weight organic matter. Copper adsorbed to the metal oxides and organic matter can be released into the sediment pore waters during oxidation reactions (Skrabal *et al.*, 1997). Movement of dissolved copper from the pore water to the water column have been identified (Skrabal *et al.*, 1997). However the speciation of copper in the pore waters has been found to greatly affect these fluxes, with the free ion and inorganic species more available than organically bound copper. The pore waters of coastal and estuarine sediments are typically loaded with organic matter resulting in a reduction in exchangeable copper. A recent study found over 15% of copper existing in an exchangeable form, ready to contribute to the copper concentration of the water column (Choi *et al.*, 2006), although a previous study claimed that only 5% of the total sediment copper concentration was easily exchangeable (Roper, 1990). In anaerobic sediments, the formation of copper sulphides reduces copper bioavailability. However, for both aerobic and anaerobic conditions, sediment disturbance events, such as dredging and storms can significantly increase the copper input into the water column from the underlying sediment.

There is potential for copper concentrations in the marine environment to build up as a higher proportion of vessels change to copper-based formulations. This is particularly important in enclosed water bodies such as harbours and marinas where the high density of berths, combined with limited water exchange, can lead to elevated concentrations resulting in adverse environmental effects. For example, dense berthing of marinas in the San Diego Bay region of California, with over 17000 recreational vessels, was suggested to be responsible for the elevated copper concentrations within the Bay (Schiff *et al.*, 2007). However, despite the projected problem with respect to elevated copper contamination within these areas, there has been relatively little monitoring work carried out. Work that has been carried out in the USA has found harbours containing elevated copper concentrations exceeding water quality thresholds (Zirino *et al.*, 1998; Noblet *et al.*, 2002; Schiff *et al.*, 2007).

In the San Diego Bay region, dissolved copper concentrations in the surface waters ranged from 1.1 to 21.0 µg/L and averaged 8.5 µg/L (Schiff *et al.*, 2007). The Environmental Quality Standard (EQS) of 5 µg/L Cu was exceeded in 86% of the marina surface water areas. However, biological investigations using the sensitive larvae of the mussel, *Mytilus galloprovincialis* revealed only 21% of the marina surface water area was deemed toxic. This value was significantly less than that suggested from the chemical measurements of dissolved copper within the same area. The proposed reason for this was the presence of organic ligand complexes within the San Diego Bay area reducing the bioavailable proportion of the dissolved copper. This highlights the importance of measuring copper speciation in order to understand the relationship with copper toxicity.

Most monitoring studies that report on copper concentrations in the marine environment measure total dissolved copper concentration. Although information on the total dissolved copper concentration in the environment is useful to a certain degree, it does not provide any indication of the copper concentration that is bioavailable. As will be described in detail in the following section, the complexation and speciation of copper is fundamental to its bioavailability and toxicity, with the free ion considered as the most toxic form. A recent study carried out by Jones and Bolam (2007) measured the concentration of copper in seawater samples collected from a series of marinas, harbours and ports around the UK. Copper was measured as total dissolved copper and labile (free ion and inorganically complexed) copper, with the difference between the two calculated as the organically complexed copper.

Total dissolved copper ranged from 0.30 to 6.68 µg/L, although only one value out of 306 measured was above the EQS of 5 µg/L. This higher value was found in an enclosed marina with little to no water exchange with the adjoining estuary. Total dissolved copper tended to be higher in enclosed

marinas compared to the estuarine ports where sufficient water flux was likely to have contributed to increased dilution. Labile copper concentrations ranged from 0.02 to 2.69 $\mu\text{g/L}$, with labile copper making up 10 to 30% of the total dissolved copper concentration. Or inversely, the proportion of copper bound to organic ligands was between 70 and 90% (Jones and Bolam, 2007). Even at elevated concentrations of total dissolved copper the proportion of labile copper was consistent. It was suggested that this was because of the buffering capacity of the natural environment, mainly due to the presence of dissolved organic carbon.

A recent monitoring study within a Finnish marina measured the concentrations of total dissolved and labile copper in surface waters at seasonal intervals (Brooks, 2006). Total dissolved copper concentrations ranged between 0.62 and 3.89 $\mu\text{g/L}$, with the highest concentrations found in the summer months, which was likely due to the increased boating activity in the marina at this time of the year. With the exception of a single elevated labile copper concentration of 2.07 $\mu\text{g/L}$, labile copper concentrations were found to stay within a narrow range below 1 $\mu\text{g/L}$. The dissolved organic carbon (DOC) content within these waters was found to be particularly high, ranging from 4.90 to 10.60 mg/L , and was thought to account for the constant labile copper concentration within and around the marina. The labile copper concentration was similar to that found in the UK survey (Jones and Bolam, 2007). Overall, the relative contribution of the marina with respect to copper inputs into the local area was considered to be low (Brooks, 2006).

19.5 Copper speciation and toxicity

It is important to realise that copper is an essential metal ion required by all organisms for cellular processes. Most organisms have developed uptake mechanisms to enable them to sequester copper in nutrient limiting environments (e.g., production of the metal-binding protein metallothionein). Conversely, these mechanisms can also be used to expel cellular copper and maintain it at cell tolerance limits. It is only when these metabolic systems are exceeded that copper becomes toxic (US EPA, 1985).

Copper speciation and toxicity to aquatic organisms both in fresh and seawater media has been well documented in the scientific literature over the last 30–40 years. There is general agreement that the free copper ion (Cu^{2+}) is the most bioavailable and thus the most toxic form of dissolved copper to aquatic life. This free ion is capable of crossing biological membranes where it can enter cells to elicit a toxic effect. Correlations between the concentration of the free copper ion and toxic effects have been highlighted (Zamunda and Sunda, 1982; Sanders *et al.*, 1983; Lorenzo *et al.*, 2006).

In water, free copper ions have a strong tendency to form complexes with both inorganic and organic ligands. This often results in the free copper ion concentration only making up a small percentage of the overall dissolved copper concentration, although this is dependent on the concentration of soluble organic matter in the system. The complexation of copper with inorganic and organic ligands is believed to reduce the bioavailability of the metal. Although copper bound to inorganic ligands is believed to be bioavailable to a certain extent (MacRae *et al.*, 1998), copper bound to organic ligands is considered to be largely non-bioavailable and thus non-toxic (Arnold *et al.*, 2005). However, the wide variety of inorganic and organic ligands with different copper binding characteristics have raised questions as to whether copper bound to certain organic ligands is bioavailable (MacRae *et al.*, 1999).

There are clear differences between the speciation and toxicity of copper in fresh and seawater, mainly due to the absence/presence of ions such as chloride, and the influence of organic matter. For the purpose of this review, particularly dealing with the impacts of copper from an antifouling perspective, copper speciation and toxicity dynamics within a seawater matrix is the main focus.

A wide range of physical and chemical characteristics have been found to influence the speciation and hence the toxicity of copper in marine systems. These include, pH, alkalinity, ion concentrations, as well as concentrations of dissolved and particulate organic carbon. Of these variables DOC has been found to influence copper toxicity to the greatest extent. The presence of biofilms, as a source of DOC, on the vessel surface is likely to influence copper speciation prior to entering the water column. This would ensure the free copper ion is immediately complexed as it leaves the vessel surface, although the extent of this interaction is uncertain.

Increased DOC content in the seawater media of controlled laboratory tests, using measured concentrations of copper, have been found to have protective effects in fish (Playle *et al.*, 1993a, b), echinoderms (Lorenzo *et al.*, 2006), bivalves (Brooks *et al.*, 2007), macroalgae (Brooks *et al.*, 2008), and unicellular organisms (Florence and Stauber, 1986). In all these cases the reduction in copper toxicity with the addition of DOC was due to an increase in the organically bound copper and the concomitant reduction in the free ion concentration.

Marine organic matter is composed of a myriad of different compounds, covering a wide range of molecular weights, chemical structures and functions, all derived from a variety of sources both natural and anthropogenic. Many laboratory tests have been carried out using artificial organic ligands, such as ethylene diamine-tetra-acetic acid (EDTA), nitrilo tri-acetic acid (NTA) and diethylenetriamine penta-acetic acid (DTPA). All were found to show detoxifying mechanisms with respect to copper toxicity (Muramoto, 1982).

Several groups of organisms have developed active defence mechanisms against metal toxicity by using the complexation capacity of organic matter. Defence mechanisms include the absorption of ligands to the external surface of the organism thereby preventing metal interaction with the cell surface, and the active release of organic ligands, produced by the organism, into the water. These defence mechanisms have been found in bacteria (Geesey *et al.*, 1992), cyanobacteria (Moffet and Brand, 1996), diatoms (Fisher and Fabris, 1982), flagellates (Nagata and Kirchman, 1992) and macroalgae (Sueur *et al.*, 1982). In most cases the defence system was thought to be induced by the presence of the metal in the external media (Karmen *et al.*, 1999).

Conversely, a recent laboratory study has shown DOC to be directly toxic to two gammarid amphipods (Timofeyev *et al.*, 2006). Exposure to elevated concentrations of DOC was reported to be responsible for increases in lipid peroxidation, hydrogen peroxide concentration, catalase, peroxidase and glutathione S-transferase activities. However, in our laboratory, environmentally relevant DOC concentrations (up to 5 mg/L DOC as humic acid) were found to have no adverse effects on the development of the oyster larvae or growth of germlings of a macroalgal species (Brooks *et al.*, 2007; 2008).

There is reasonable consensus that copper toxicity is associated with the free copper ion that acts on target sites to elicit a toxic effect in aquatic species. However, other evidence suggests that copper weakly bound to ligands, both organic (i.e., amino, humic, fulvic acids) and inorganic (i.e., Cl^- , SO_4^{2-} , CO_3^{2-}), can contribute to the response when the metal-ligand binding affinity is weaker than that of the biological ligand (MacRae *et al.*, 1999; Playle *et al.*, 1993a, b). The biological ligand refers to the main target site for copper toxicity such as the gills and intestine epithelium. Since the complexation of copper with ligands is reversible, weakly bound copper-ligand complexes can dissociate and become bound to the biological ligand, resulting in toxicity. Information on the various copper-ligand complexation capacities in seawater is essential for a better understanding of copper bioavailability and toxicity.

The complexation capacity of organic, inorganic and biological ligands has been quantified, with examples shown in Table 19.1. The strength of each copper-ligand complex is described as the stability constant (K), with the value of K determined by the equation $K = [ML] / [M] [L]$, where M is the metal and L is the ligand. A ligand with a high K value is more likely to bind and stay bound to the metal (Stumm and Morgan, 1996). Ligands with a stability constant between 8 and 13 were found to have the largest impact on the abundance of the free copper ion (Karmen *et al.*, 1999). Alternatively, a low K value in the presence of a biological ligand would more likely dissociate from the weakly bound environmental ligand and

Table 19.1 A selection of stability constants for copper-ligand interactions. A Log K value below that of the biological ligand would suggest bioavailability

Ligand type	Specific ligand	Stability constant (Log K)	Source
Biological	Rainbow Trout gill	6.4 to 7.5	MacRae <i>et al.</i> , 1999
	Brown Trout gill	7.25	MacRae <i>et al.</i> , 1999
	Daphnia	8.02	Karel <i>et al.</i> , 2001
	Fathead minnow gill	7.4	Playle <i>et al.</i> , 1993b
Inorganic	Cu(OH)	7.66	van den Berg, 1984
	Cu(CO ₃) ₂ ²⁻	8.92	Smith and Martell, 1976
	CuCO ₃	5.75	Smith and Martell, 1976
	Cu(OH) ₂	12.66	van den Berg, 1982
Organic	Nitrilotriacetic (NTA)	10.3	MacRae <i>et al.</i> , 1999
	2,6-pyridine-dicarboxylic	8.6	MacRae <i>et al.</i> , 1999
	Ethylenediamine (EDA)	6.9	MacRae <i>et al.</i> , 1999
	Citric acid	6.4	MacRae <i>et al.</i> , 1999
	Malonic	5.6	MacRae <i>et al.</i> , 1999
	Tartaric	4.2	MacRae <i>et al.</i> , 1999
	Fulvic acid	10	MacRae <i>et al.</i> , 1999
	EDTA	18	MacRae <i>et al.</i> , 1999
	Natural Irish Sea organics	10–10.4	van den Berg, 1984
Exudates of	Cyanobacteria	9.2–9.5	Gouvêa <i>et al.</i> , 2005
	<i>Synurophycea</i>	8.42	Lombardi and Vieira, 1998
	<i>Synechococcus</i>	12.3–13.8	Moffett and Brand, 1996
			Croot <i>et al.</i> , 2000
	Diatoms	8.6–8.8	Gouvêa <i>et al.</i> , 2005
	<i>Thalassiosira weissflogii</i>	10.6	Croot <i>et al.</i> , 2000
	<i>Skeletonema costatum</i>	11.6–12.3	Croot <i>et al.</i> , 2000
	Coccolithophore		
	<i>Hymenomonas carterae</i>	10.8	Croot <i>et al.</i> , 2000
	Dinoflagellates		
	<i>Amphidinium carterae</i>	11.8–12.2	Croot <i>et al.</i> , 2000

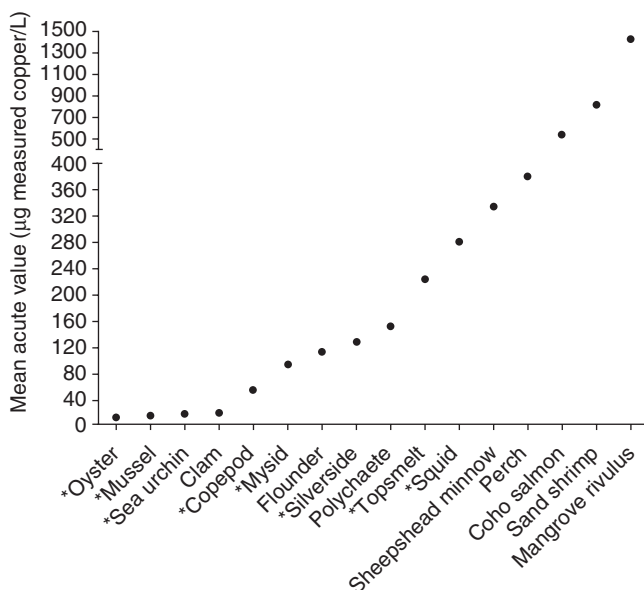
bind to the biological ligand. Low binding affinity copper ligands have been found to contribute to the observed toxicity to early life stages of rainbow trout (MacRae *et al.*, 1999). While this observation was seen in freshwater, similar mechanisms would be expected in seawater, although increased ion competition at the biological ligand may reduced copper binding and toxicity. Overall, copper toxicity is unlikely to be just a measure of the free-ion concentration; instead the binding characteristics of the individual ligands should also be taken into account.

The marine diatom, *Skeletonema costatum* has been found to produce two types of organic ligand with different binding affinities to the copper ion (Lorenzo *et al.*, 2006). These include an organic ligand exuded by the phytoplankton and a refractory humic-like material produced as a by-product of the bacterial degradation of phytogetic material. The humic-like material was found to have significantly higher copper-ligand stability constant than that of the exuded organic ligand. Therefore, copper bound to the exuded organic ligand was potentially more bioavailable than that bound to the humic-like material. Other studies have shown phytoplankton blooms to be responsible for increasing the concentration of organically bound copper (Jones and Thomas, 1988).

The strength of the organic copper-ligand complex over time has particular significance for ecotoxicity testing. Previous authors have reported that a 24 h equilibration time was essential to allow copper complexation reactions to take place and enable copper to be distributed in its different chemical forms (Ma *et al.*, 1999). However, Lorenzo *et al.* (2006) found that the complexation equilibrium in chemically defined seawater was reached after only approximately 12 h. In contrast, Hollis *et al.* (1996) found that the copper-DOC binding affinity did not get any stronger over time. Metals just added into the test mixture minutes before the start of the test produced similar toxicity results as those that were aged for 2 and 3 weeks before testing. It is therefore unclear how long it takes for copper complexation reactions to reach equilibrium in seawater, since detailed speciation studies of organic substances in seawater media are lacking.

Although the mechanisms of copper toxicity are not fully understood, it is known that particularly for freshwater organisms the main site of copper uptake is the gills, where copper targets osmoregulatory enzymes involved in the maintenance of the intracellular ion concentrations essential for life, such as gill Na^+ , K^+ -ATPase, carbonic anhydrase and ion channels (Brooks and Lloyd-Mills, 2003; Bianchini *et al.*, 2004; Grosell *et al.*, 2002). This results in osmoregulatory disruption leading to cellular and organism death. However, in marine organisms, osmoregulation is less important, since intracellular ion concentrations are often similar to that of the external environment. Therefore, in marine species the oxidation of sulfhydryl groups of other intracellular proteins essential for cell survival is more likely targeted, resulting in cell death.

Over the last 40–50 years laboratory tests with copper have mainly provided results of nominal copper concentrations or at best the total dissolved copper concentration measurements. The most recent studies have acknowledged the importance of copper speciation and its direct effects on toxicity, and have consequently reported measured copper speciation values to support toxicity measurements. Tests using nominal copper concentrations



19.2 Mean acute copper toxicity values calculated as the geometric mean of the LC/EC₅₀ values for each group of marine organism. Calculations made with 87 LC/EC₅₀ data points from tests using measured copper concentrations only. Data obtained from the Environmental Protection Agency (EPA) website: <http://www.epa.gov/waterscience/criteria/copper/2003/index.htm>. * indicates embryo/larvae of the species were tested for copper toxicity.

have lost much of their scientific credibility due to the importance of understanding copper speciation.

A summary of copper toxicity to a range of marine species can be seen in Fig. 19.2. Toxicity data is expressed as mean acute toxicity and was taken from tests where only measured concentrations of copper were specified. The mean acute value was calculated from the LC₅₀/EC₅₀ concentrations for each of the species. The three most sensitive species are the embryo/larvae of the oyster, mussel and sea urchins, with mean acute values of 10.96, 13.85 and 17.11 µg/L copper. The embryo/larvae are in general the most sensitive life-stage of all organisms. The oyster, mussel and sea urchin embryo tests, which measure larval abnormalities during contaminant exposure, are regularly used in water quality assessment due to their high level of sensitivity to environmental contaminants such as copper.

Overall, of the groups shown in Fig. 19.2, bivalves were the most sensitive to copper exposure, whilst fish were the least sensitive, e.g., *Mangrove rivulus* showed mean acute values of 1.42 mg/L copper. Differences in mean acute values between bivalves and fish species were up to a 100-fold. In an

assessment of acute copper toxicity in marine fish, 96 h LC₅₀ concentrations were reported to range from 11.9 to 1690 µg/L, with 77% of the LC₅₀ concentrations less than or equal to 460 µg/L (van Sprang, 2004).

Crustaceans showed a large range in toxicity from approximately 50 µg/L in certain copepod species to over 800 µg/L in the sand shrimp (Fig. 19.2). This large variation was supported by assessments of copper toxicity with 96 h LC₅₀ concentrations ranging from 39 to 2100 µg/L (van Sprang, 2004).

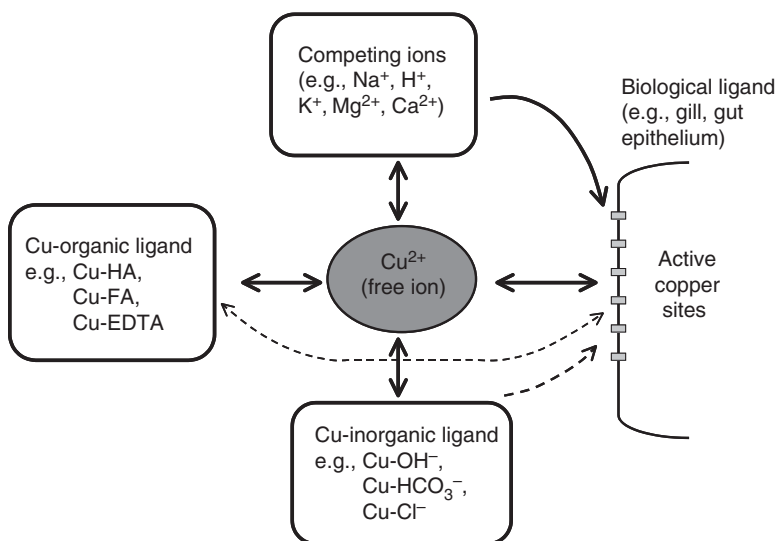
Significantly better relationships between toxic effects and the concentration of copper free ion have been found than when using total dissolved copper data (Zamunda and Sunda, 1982; Sanders *et al.*, 1983). Work recently carried out at the Cefas laboratory has found a good correlation between labile copper concentration (Cu²⁺ and inorganic Cu) and the toxic effects to oyster embryo larvae (Brooks *et al.*, 2007) and *Fucus* germlings (Brooks *et al.*, 2008).

Cyanobacteria are the most sensitive group of organisms to copper toxicity, with growth effects in the genus *Synechoccus* detected at a free copper concentration of approximately 0.63 ng/L (Moffet and Brand, 1996). However, most phytoplankton species have been reported to show adverse effects to copper exposure at higher concentrations of around 0.2 µg/L. The particular sensitivity of cyanobacteria has raised conflicting views by both scientists and regulators as to whether or not it should be included in water quality risk assessments, since toxicity values are lower than the natural background concentration of copper in certain water bodies.

19.6 Copper toxicity models in the marine and estuarine environment

Although there have been a wide range of mathematical models for the prediction of metal toxicity in aquatic systems, the Biotic Ligand Model (BLM) has shown the best potential in predicting waterborne copper toxicity. The BLM is an adaptation of previous predictive models and incorporates the gill surface interaction model (GSIM, Pagenkopf, 1983), a chemical equilibrium model (CHESS, Santore and Driscoll, 1995), and a metal-dissolved organic matter interaction model (WHAM v.5, Tipping, 1994). For a detailed description of the BLM model and associated equations readers are directed to Di Toro *et al.* (2001).

The BLM predicts the proportion of bioavailable copper in the water taking into account the factors that can reduce or inhibit copper bioavailability. These include DOC concentration, competing ions (e.g., Ca²⁺, Na⁺ and H⁺), water chemistry (pH, alkalinity, hardness), and the concentration of particulate matter. The proportion of copper in the water is then combined with estimates of metal accumulation rates of biological ligands (Arnold *et al.*, 2005), since it is assumed that the amount of metal bound to



19.3 Adapted from Arnold *et al.* (2005). Copper speciation and toxicity in aquatic systems, and the fundamental basis of the Biotic Ligand Model (BLM). The free copper ion (Cu^{2+}) is the most bioavailable species interacting with the target sites of the biological ligand. Cu^{2+} has a tendency to form complexes with both inorganic and organic ligands, which significantly reduces its toxicity. The binding strength of the Cu-ligand complex in relation to that of Cu-biological ligand, influences its toxicity. Interactions with ions present in the water can reduce the overall toxicity of copper by increasing competition at the active sites on the biological ligand.

the biological ligand leads to acute toxicity. The principles of the BLM can be seen in Fig. 19.3, adapted from Arnold *et al.* (2005).

The freshwater BLM has been found to predict known copper toxicity values to within a factor of ± 2 (Di Toro *et al.*, 2001; Santore *et al.*, 2001; US EPA, 2003). The success of the BLM has resulted in its consideration by the US EPA for the direct calculation of copper criteria in US freshwaters (US EPA, 2003). It is designed to be protective of 95% of aquatic life.

The BLM has been applied to several freshwater organisms such as Cladocerans (*Daphnia*) and fish (Di Toro *et al.*, 2001; Zhou *et al.*, 2005), amphipods (Borgmann *et al.*, 2005) and algae (De Schampelaere *et al.*, 2005). In most of these cases the models have been extrapolated from the fish BLM, which has caused scientists to question their reliability for toxicity assessments. Despite the initial success of the BLM in predicting acute copper toxicity in freshwaters, the model is still developing and several limitations to the model have been highlighted (Niyogi and Wood, 2004).

One of the main difficulties of the BLM is how to deal with the large variety of natural and anthropogenic DOC, in terms of stability constant and concentration. As discussed in the previous section, organic ligands can significantly reduce the toxicity of waterborne copper by complexing the free copper ion. Alternatively, copper weakly bound to organic ligands can potentially disassociate and bind to biological ligands resulting in toxicity. Therefore, information on the type and concentration of each organic ligand in a natural system would benefit the model for determining the proportion of potentially bioavailable copper and predicting toxicity.

In addition, the biological ligand has been reported to contain more than one copper binding affinity. This has led to the development of multiple ligand models for the prediction of copper toxicity. However, there is no evidence that multiple ligand models do any better at predicting toxicity than single site models and vice versa. To tackle this problem, adjustment factors for the different protective properties of organic ligands have been applied. These are relatively new and more research is required before they can be applied effectively (Niyogi and Wood, 2004).

A major consideration that is often overlooked with the BLM is the dynamic nature of the ligand-metal binding properties and how these are affected by a number of key factors. Such factors include pre-exposure to waterborne copper, dietary copper levels, dietary ions, and age of organism (Niyogi and Wood, 2004). In addition, temperature effects on biological membrane permeability have also been identified; with a 2 to 5 fold decrease in membrane permeability following a temperature reduction of 17 °C (Hassler *et al.*, 2004). The BLM takes no account of these factors that can potentially lead to significant differences in metal bioavailability and bioaccumulation. Site-specific assessments of water quality criteria incorporating all of these factors into the BLM would prove useful.

Although the BLM was first developed to predict copper toxicity in freshwater, the success of the freshwater BLM has led to the current development of a marine BLM to predict copper toxicity in estuarine and seawater systems (Arnold *et al.*, 2005). Arnold *et al.* (2005) used the marine mussel *Mytilus* sp. to test the suitability of a marine BLM to predict copper toxicity in estuarine and coastal waters. They used measured chemistry data over a large range of seawater quality characteristics. A strong correlation was found between the measured and the BLM predicted EC_{50} s, with the BLM predicting within a factor of ± 2 of the measured EC_{50} s in 41 out of 44 cases. However, the authors did report that the model predicted lower EC_{50} s when the measured EC_{50} values were less than 10 $\mu\text{g/L}$ copper. This was suggested to be due to limitations of the metal-DOC interaction model, a limitation similar to that described for the freshwater model above.

When developing the seawater model, the increased protection due to competition by cations (e.g. Na^+ , K^+ , Ca^{2+}) at the biological ligand must be

considered. This can potentially decrease copper binding to the biological ligand and therefore reduce toxicity. A further complication of the model is that marine fish drink water to replace losses from osmosis across the gills; therefore, the gut epithelium may be a more important target for copper interactions in brackish and marine fish than in freshwater fish (Bianchini *et al.*, 2004). Also, digestive processes within the gut can potentially enable copper-ligand complexes to be altered in such a way as to make them bioavailable, a process that may be more significant in marine species (Lewis, 1995).

Information on the routes of copper accumulation and mechanisms of copper toxicity in brackish and marine organisms is scarce. However, the mechanisms of copper toxicity to marine organisms are likely to be different than that seen in freshwater organisms. In freshwater, the principle target of copper toxicity is the ion-regulatory enzyme Na^+ , K^+ -ATPase (Grosell *et al.*, 2002), which is important in maintaining intracellular ion concentrations within cell limits in freshwater species.

The exposure of organisms to elevated copper concentrations, combined with increased osmoregulatory stress in low salinities was found to increase species vulnerability to trace metal toxicity (McLusky *et al.*, 1986). However, in seawater the Na^+ , K^+ -ATPase enzyme is less important since many species do not regulate their intracellular ion concentrations but often conform to that of the external media (Brooks, 2003). Consequently the mechanisms of copper toxicity in marine organisms are still uncertain. Better understanding of the mechanisms of acute toxicity in these organisms is important to further develop the marine BLM.

A future development of the BLM would be to incorporate a series of metals into the model, since as with all environmental contaminants they do not occur in isolation, but rather as mixtures of chemicals. Metal-metal interactions are likely to influence copper accumulation at the biological interface, thereby altering the toxicity of copper. Incorporating additional metals into the model will enable it to provide a more realistic environmental evaluation of metal toxicity and risk (Playle, 2004). A recent literature review on the effects of metal mixtures on aquatic organisms revealed that 43% of the publications reviewed showed less than additive interaction, 27% no interaction (strictly additive) and 30% more than additive interactions (Norwood *et al.*, 2003). This therefore shows that metal-metal interactions have a role to play in predicting copper toxicity.

Although the technology and validation of the marine BLM is limited at present, increased research in this area will improve future predictions and in time enable the model to be validated for a wider range of marine as well as estuarine species. The benefits of an accurate predictive model in marine and estuarine systems, particularly for regulatory risk assessment are enormous, reducing the cost of environmental monitoring programs and

potentially increasing the level of environmental protection. However, there is still a long way to go before valid models are available for toxicity prediction for all marine organisms, particularly since there are uncertainties about copper uptake pathways and target sites in marine species.

19.7 Risk assessment of copper

A risk quotient (RQ) approach has often been applied to determine the environmental risk of copper. The RQ is determined by the simple equation shown below (equation 19.1). In general, if the exposure level or the predicted environmental concentration (PEC) is greater than the sensitivity of the species referred to as the predicted no effect concentration (PNEC), the RQ will be > 1 indicating the likelihood for environmental harm. In this situation, further investigations would be required to fully assess the potential for harm within a given environment.

$$\text{Risk quotient} = \frac{\text{Predicted environmental concentration (PEC)}}{\text{Predicted no effect concentration}} \quad 19.1$$

(if > 1 = harm)

A crucial consideration when calculating the potential environmental risk of a contaminant, such as copper, is to ensure that the calculations are based on reliable and valid data and reflect what is happening in the real environment. In the past, PECs have been inferred from measured total copper concentrations or simple models, whereas PNECs have been taken from laboratory tests using nominal copper concentrations in clean filtered seawater with much less binding potential than would be found typically within the natural environment. As mentioned above, copper speciation and toxicity are highly influenced by the seawater parameters, parameters that would be significantly altered by laboratory manipulations of testing solutions. Since the current understanding of metal toxicity supports the conclusion that only bioavailable metals can elicit a toxic response, it is important that only effect levels based on actual (measured) concentrations of the appropriate metal species should be considered reliable.

Due to the difficulty in measuring the free ion concentration, the environmental concentrations and/or concentrations used in toxicity tests are often based on total or dissolved concentrations (Allen and Hansen, 1996). This has led to the majority of risk assessments based on total or dissolved copper concentration rather than the free copper ion or labile copper concentration. Generally between 70 and 99% of the toxic free copper ions are bound to naturally occurring organic ligands leaving a small proportion bioavailable to marine organisms. Therefore, the current risk assessments based on total dissolved copper concentrations provide a very conservative estimate of risk. A recent study measuring copper speciation in UK

harbours and marinas concluded that using total dissolved copper as an indicator of impact overestimated the risk of copper toxicity by a factor of four (Jones and Bolam, 2007).

The RQ approach has been used to predict the ecological risk of copper in European marine environments (Hall and Anderson, 1999). The PEC data, referred to in this case as the observed environmental concentration (OEC), was based on measured data collected from eight separate studies in marinas, harbours, estuaries and the open waters of the Mediterranean Sea, Baltic Sea and the North Sea between 1986 and 1997. The overall geometric means were 1.53, 1.49 and 0.68 $\mu\text{g/L}$ total dissolved copper for marinas/harbours, estuaries and coastal/open seas respectively. The PNECs were derived from acute tests with 65 seawater species from algae to fish. The PNEC for all species with a 95% protection level was 5.6 $\mu\text{g/L}$ total dissolved copper. The authors reported RQs of > 1 in 3 out of 101 sites and concluded that the ecological risk of copper in European marine environments was generally low (Hall and Anderson, 1999).

Owing to the lack of marine environmental data for the concentration of the free copper ion, many risk assessments have used modelling tools to determine the PEC. Models such as REMA (regulatory environmental modelling of antifoulants) and MAMPEC (marine antifoulant model to predict environmental concentrations) have evolved to provide PECs of antifouling products, such as copper in marine scenarios. The REMA model has been validated for several UK estuaries (HSE, 1999b). However, the model is limited to typical estuaries in the UK and cannot easily be adapted to other exposure scenarios. The MAMPEC model is an improvement on the REMA model and can be applied to a wider range of marine scenarios including open sea, shipping lane, estuary, commercial harbour, and yachting marina. The model takes into account many of the parameters specific to antifouling products such as leaching rate, vessel and compound related factors and processes, physicochemical factors and hydrodynamic processes of typical marine environments. Although validation of this model to known environmental copper concentrations is limited, those studies that have taken place have found a reasonable agreement between measured and predicted concentrations of antifouling biocides (van Hattum *et al.*, 2002).

Copper models in the marine environment are extremely complex and simplification of the models is often employed for practicality. Development of these models, such as the marine BLM and MAMPEC, will offer significant advances towards providing accurate predictions of copper in risk assessments. Models do provide a fast and cost-effective method for predicting risk in the marine environment; and further development of these models to include site-specific risk assessments will only help to improve protection.

If risk assessments of copper are to be effective for management purposes improvements to the assessments should include: 1) better determination of the environmental concentration of copper species from measured concentrations; 2) improvement of predictive models based on measured environmental copper species; 3) the use of chronic copper toxicity end-points, or at least the use of the most sensitive life stages of the most sensitive species for the assessment of risk; 4) site-specific risk assessments, due to the variability in copper toxicity to environmental parameters.

The way in which these tools are used varies from country to country and what is deemed to be acceptable in one regulatory regime is not in others. For example, in the UK, most ports, harbours and marinas are regarded as mixing zones and the level of environmental protection is set to protect the most sensitive species outside of this zone. Some countries set standards based on 95 percentiles of species no matter what the situation, and other approaches provide safety factors for untested species (Hall and Anderson, 1999), so it is unlikely that regulators would come to a consensus view even when given the same package of information.

Notwithstanding the weaknesses in current risk assessments, it is pertinent to compare the likely damage caused by copper with alternative anti-fouling biocides. Thomas *et al.* (2001) compared the likely impact of so called 'booster biocides' with that of TBT. A site-specific case at Southampton water showed that the impact of TBT at the height of its use was around 500 times more damaging than the replacement biocides of irgarol and diuron. Updating this information with results of copper, two values for copper may be derived. The first of these is a highly precautionary quotient based on comparison of worst-case concentrations of total dissolved copper measured by Jones and Bolam (2007, Table 19.2) against the UK EQS (equation 19.2). The second is a more realistic worst-case quotient based on the labile species present (equation 19.3).

$$RQ1 = \frac{\text{measured total dissolved copper } (\mu\text{g/L})}{\text{UK EQS } (\mu\text{g/L})} \quad \frac{6.68}{5} = 1.34 \quad 19.2$$

$$RQ2 = \frac{\text{measured labile copper } (\mu\text{g/L})}{\text{UK EQS } (\mu\text{g/L})} \quad \frac{2.69}{5} = 0.54 \quad 19.3$$

The result suggests that based on the measured speciation data, even in the worst-case marina site, the likelihood for environmental harm would be small. In addition, the EQS of 5 $\mu\text{g/L}$ was derived from laboratory tests using nominal total dissolved copper concentrations, and therefore provides a conservative estimate.

Substituting values for a site outside a marina, in the Hamble estuary, the comparative figures are 0.72 and 0.08 for quotients based on total dissolved and labile copper measurements respectively (Jones *et al.*, 2005). The latter

Table 19.2 Data for copper concentrations in UK estuaries from Jones and Bolam (2007)

	Total dissolved copper ($\mu\text{g/L}$)	Labile copper ($\mu\text{g/L}$)
Number of samples	108	108
Mean	1.68	0.38
95% ile	4.18	0.71
Maximum value	6.68	2.69

Table 19.3 Comparative risk quotients for selected biocides from similar locations but at different times (updating Thomas *et al.*, 2001)

Location	Risk quotient (algae) ^a		Risk quotient (fish and crustacean)		Risk quotient (based on UK EQS)		
	Irgarol 1051 ^b (1998)	Diuron ^c (1998)	Irgarol 1051 (1998)	Diuron (1998)	TBT (1998)	TBT (1987)	Copper (2002/3)
Ocean Village marina	0.5	0.2	0.01	0.1	14	48 ^d	0.54
Hamble Mouth	0.5	0.1	0.01	< 0.1	3	180	0.08

(a – measured environmental concentration (MEC)/no effect concentration (NEC); b – algal toxicity data from Hall *et al.* (1999); c – diuron toxicity from Verschueren (1996); d – 1989 value).

value is placed in context with those measured by Thomas *et al.* (2001), for a variety of biocides at the same location (Table 19.3). The comparative data suggests that copper is around three orders of magnitude less damaging than TBT at the open estuary location.

19.8 Conclusions

Despite the wealth of information on the use, fate and effects of copper in antifouling paints, there is still an ongoing debate about the potential harm to non-target organisms in estuarine and marine environments. This review provides an update on some of the facets of uncertainty and the current state of risk assessments.

A summary of the use of copper in different paint formulations is provided, which offers some insights into the source of anthropogenically derived copper compared with natural inputs. Information is provided on

the current concentrations of copper measured in the water column and the likely speciation of copper as ionic forms and those bound to inorganic cations and organic ligands in the natural environment.

We report recent findings on the toxicity of copper and provide data that the most toxic forms of copper are only present in natural systems as a relatively small component of the total dissolved copper loading to seawater.

Since full speciation of copper in seawater is a complicated and time-consuming task, many assessments of risk are based on models of both the likely toxicity of copper and the likely environmental fate. We provide some information on biological effects models, for example the biotic ligand model, that have good track records in estimating the likely effects of copper in animal systems.

Finally, the risk assessment of copper is re-evaluated. We contrast the conclusions that may be drawn from comparing: a) laboratory-based studies on copper in artificial media, supported by measured concentrations of total dissolved copper and; b) a more realistic measure of toxicity determined experimentally in the presence of organic ligands in the laboratory, supported by measured concentrations of labile copper in the environment. We compare the relative risk of copper with other biocides such as TBT and conclude that the potential for harm from copper is low and unlikely to cause harm even in estuarine locations in the UK frequented by large numbers of pleasure craft.

Overall, copper toxicity from an antifouling source only becomes a problem in the marine environment in isolated water bodies, such as enclosed marinas and harbours that experience little water exchange with high levels of boating activity. As previously mentioned, a comprehensive UK monitoring programme found copper concentrations within safe limits, suggesting that copper is not a major environmental concern (Jones and Bolam, 2007). This is not to say that it may not become a concern in the future, due to increased boating activity and contaminant build up. For example, in the US, elevated concentrations above those considered as harmful to marine life have been frequently found in certain coastal water bodies, which have been thought due to the high levels of boating activity in the area (Schiff *et al.*, 2007). Restrictions in vessel mooring densities in these areas may be the best course of action by regulators to reduce harmful effects in isolated cases.

Despite the low environmental risk of copper from antifouling paints in the marine environment, the advancement of new improved and environmentally friendly antifouling products should be encouraged. This would potentially reduce copper usage and relieve the pressure of copper inputs in high risk water bodies. However, at present, copper is arguably the best biocide available, and its benefits as an antifouling biocide currently outweigh its environmental risk. It is unsure how the continual use of copper

as a biocide will impact the environment in the future. At present, the inputs of copper from antifouling are relatively small compared to the large quantities in natural inputs from land erosion and weathering. And so far the natural environment appears from monitoring studies to be buffering the additional copper to below harmful levels for aquatic life.

19.9 Sources of further information and advice

Further information on copper in the marine environment is provided below; all information and links were valid at the time of publication.

Copper as an antifouling biocide at <http://www.antifoulingpaint.com/>

Copper toxicity database at http://www.pesticideinfo.org/Search_Chemicals.jsp

Copper toxicity models in the marine and estuarine environment

The biotic ligand model at http://www.hydroqual.com/wr_blm.html

MAMPEC at <http://www.antifoulingpaint.com/downloads/mampec.asp>

At the time of writing this chapter a European risk assessment of copper in the marine environment was being compiled:

EU Risk Assessment – [Copper, Copper II Sulphate pentahydrate, Copper(I)oxide, Copper(II)oxide, Dicopper chloride trihydroxide] CAS [7440-50-8, 7758-98-7, 1317-3-1, 1317-38-0, 1332-65-6].

19.10 References

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The use of broad-spectrum organic biocides in marine antifouling paints

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Abstract: This chapter provides a statement on our current knowledge of the occurrence, fate and risks associated with organic antifouling paint biocides. As a group of compounds they are chemically diverse and have been used at different rates in various parts of the world. In Europe all antifouling paint biocides are currently being reviewed under The European Union's (EU) Biocidal Products Directive (BPD) (98/8/EC), however much of these data are confidential. Published peer-reviewed data suggest that the extensive use of Irgarol 1051 and diuron in areas with low water exchange poses an environmental risk to certain aquatic organisms due to increased concentrations resulting from high aqueous persistence. Many of the other biocides have not had the levels of market penetration shared by Irgarol and diuron and therefore few reliable data are available on their occurrence. For these biocides, sound environmental risk assessment, as performed by the BPD, is essential in order to protect the aquatic environment. The data available suggest that there are biocides which have a low environmental persistence (e.g., zinc pyrithione, DCOIT and dichlofluanid) and may therefore not accumulate to the levels observed by diuron and Irgarol 1051. Modelling of relevant exposure scenarios will provide valuable data in predicting environmental concentrations (PEC) where occurrence data are not available. Exposure is only part of establishing environmental risk. If exposure concentrations are greater than those where no effects are observed then there is a potential risk to the aquatic environment. Current data suggest that the concentrations of Irgarol 1051 and diuron exceed the predicted no effect concentrations. What is important in assessing the risks of other biocides, which currently do not occur at concentrations > PNEC, is that the PEC following increased use and release does not exceed the PNEC. Regulatory tests and environmental risk assessments are based upon 'normal' use scenarios where the biocide enters the water from a painted surface. Other exposure scenarios such as those related to the careless and inappropriate disposal of paint particles following hosing and scrubbing activities should also be considered to fully protect the aquatic environment.

Key words: antifouling paint biocides, Irgarol 1051, diuron, DCOIT, zinc pyrithione, dichlofluanid, fate and effects, environmental risk.

20.1 Introduction

Tributyltin (TBT)-containing antifouling paints dominated the market from the early 1960s to 1990s. TBT, especially in self-polishing formulations, was a very efficient biocide; however, well-documented adverse environmental effects led to, first restrictions in its use on boats > 25 m in length, and then in 2003, this was extended by the International Maritime Organisation (IMO) to all ships (Anon, 1999b). From 1 January 2008 ships painted with TBT-containing antifouling coatings will not be allowed to enter European ports, whilst from September 2008 they will not be allowed to enter the ports of countries which are signature to the IMO Antifouling Systems (AFS) Convention. In response to this, alternative antifouling biocides were developed initially for the 'small boat' market (< 25 m in length) alongside copper oxide, but also used to enhance the efficiency of TBT-based antifouling paints.

Examples of the compounds that have been and are currently used as antifouling biocides include:

- 2-methylthio-4-tertiary-butylamino-6-cyclopropylamino-s-triazine (Irgarol 1051)
- 1-(3,4-dichlorophenyl)-3,3-dimethylurea (diuron)
- 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT)
- *N*-dichlorofluoromethylthio-*N'*,*N'*-dimethyl-*N*-phenylsulphamide (dichlofluamid)
- *N*-dichlorofluoromethylthio-*N'*,*N'*-dimethyl-*N*-*p*-tolylsulfamide (tolylfluamid)
- 2,4,5,6-tetrachloro iso phthalo nitrile (chlorothalonil)
- Bis(1hydroxy-2(1H)-pyridethionato-O,S)-T-4 zinc (zinc pyriithione)
- Bis(1hydroxy-2(1H)-pyridethionato-O,S)-T-4 copper (copper pyriithione)
- 2-(thiocyanomethyl thio)benzthiazole (TCMTB)
- 2,3,5,6-tetrachloro-4-(methyl sulphonyl) pyridine (TCMS pyridine)
- pyridine-triphenylborane (TPBP)
- cuprous thiocyanate
- arsenic trioxide
- zineb
- folpet
- thiram
- oxytetracycline hydrochloride
- ziram
- maneb.

It is estimated that worldwide, around eighteen compounds are used as biocidal antifouling additives in professional and amateur antifouling products. As with TBT, restrictions have been placed on a number of

Table 20.1 Approved uses of booster biocides post HSE review (September 2000)

Active substance	Amateur use on vessels (< 25 m in length)	Professional use on vessels (> 25 m in length)
Chlorothalonil	Revoked	Max. conc. 5% w/w
Dichlofluanid	Max. conc. 10% w/w	Max. conc. 10% w/w
Diuron	Revoked	Revoked
Irgarol 1051	Revoked	Max. conc. 10% w/w
DCOIT	Revoked	Max. conc. 10% w/w
TCMTB	Revoked	Not supported
Zinc pyrithione	Max. conc. 4% w/w	Max. conc. 4% w/w
Zineb	Max. conc. 20% w/w	Max. conc. 20% w/w

broad-spectrum organic biocides with the use of selected compounds being prohibited in certain European countries (Table 20.1). For example restrictions have been placed on the use of Irgarol 1051 and/or diuron in Sweden, Denmark, the United Kingdom and the Netherlands. The European Union (EU) Biocidal Products Directive (BPD) (98/8/EC) is now active and a review of all antifouling biocides submitted for approval has begun (Chapter 10). A decision on the acceptability of these biocides is not expected before late 2008. Assessment of antifouling biocides involves the provision of a large amount of environmental data that often remains confidential. It is a shame that these data are not made public so that they can contribute to the pool of data available in the public domain. Therefore this chapter presents only those data available in the public domain.

The fate and behaviour of any compound released into the environment is subject to a number of complex processes such as transport, transformation, degradation, cross media partitioning, and bioaccumulation. As this chapter will show, antifouling paint biocides are a very diverse group of compounds and vary considerably in their chemical and physical characteristics (Table 20.2). Consequently, the processes that control their fate and behaviour differ, which invariably leads to differences in environmental persistence and likely harm to non-target species.

Reviews on the fate and effects of specific antifouling paint biocides have been performed and offer an overview of the type of environmental data available for antifouling paint biocides. Reviews of antifouling biocides (Thomas, 2001; Konstantinou and Albanis, 2004) Irgarol 1051 (Hall *et al.*, 1999), chlorothalonil (Caux *et al.*, 1996), DCOIT and ZPT (Grunnet and Dahllof, 2005) have been performed and data from these papers are used to complement this chapter. In order to provide a point of reference for the data currently available, this chapter provides data on the release, occurrence, transport, transformation, degradation, cross media transfer, volatili-

Table 20.2 Physico-chemical properties of common antifouling paint booster biocides

Biocide	Log K_{ow}	K_{oc} (soil)	K_{oc} (marine sed.)	K_d	Solubility (mg L ⁻¹)	Calculated* Log K_{oc}
Chlorothalonil	4.0	1300–14000			0.9	3.8
Dichlofluanid	3.7				1.3	3.5
Diuron	2.8	251–1010		8.9 ± 13.4	35	2.6
Irgarol 1051	3.95	820	990	3.49	7	3.7
DCOIT	2.8	7865	15441		14	2.6
TCMTB	3.3	282–2270		27	10.4	3.1
Zinc pyrithione	0.9	10633	10633		8	0.7
Zineb	0.8	1230			0.07–10	0.6

* Log $K_{oc} = \log K_{ow} - 0.21$ (Karickhoff, 1981).

sation, and bioaccumulation of organic antifouling paint booster biocides currently available in the public domain.

20.1.1 Leaching rate

One important factor in assessing the environmental risk posed by antifouling paint biocidal additives is the rate at which they are released from the paint surface. This is often referred to as the paint's leaching rate and many regulatory bodies use leach rate data generated using laboratory based protocols for risk assessment purposes. Typically leaching rates are used to calculate predicted environmental concentrations (PEC) that are the basis of risk assessments performed during the registration or evaluation of biocide (e.g., EU BPD 98/8/EC).

Leaching rate is highly dependent on the type of compound, type and age of the paint matrix, the speed of the ship, as well as environmental factors such as salinity and temperature. Examples of reported biocide leaching rates are presented in Table 20.3. Two standardised protocols are currently employed to generate release rate data. The US EPA has adopted the American Society of Testing Materials (ASTM) method (D5108-80), whilst the UK HSE and many other European regulatory bodies have adopted the International Standards Organisation (ISO) method (ISO/DIS 15181-1,2) for assessing leach rates under standard conditions. Both test systems consist of a polycarbonate cylinder painted with the candidate paint. The cylinder is rotated in a baffled beaker containing synthetic sea water at 60 rpm and concentrations of biocide are measured periodically to calculate the release in terms of micrograms of active ingredient released from each

Table 20.3 Comparison of biocide release rates from antifouling coatings

Biocide	Alternative trade name	Release rate ($\mu\text{g cm}^{-2} \text{ day}^{-1}$)	
		ISO test system	Flume system
Cuprous oxide		25–40 ^a	18.6 $\pm$ 6.5
TBT		1.5–4.0 ^a	1.6
Irgarol 1051		5.0	2.6 ^b
Diuron		3.3	0.8
Dichlofluanid	Preventol A4, Euparen	0.6	1.7
Zinc pyrithione	Zinc Omadine	3.3	— ^c
DCOIT	Sea-Nine 211, DCOI	2.9	3.0
TCMTB	Busan	— ^c	0.9
TCMS pyridine	Densil S	0.6	3.8

Table reproduced from Ref. Thomas *et al.* (1999). ^aObtained from Thomas *et al.* (1999) for comparison. ^bMean of two data points. ^cNo data available.

square centimetre of painted surface per day ($\mu\text{g cm}^{-2} \text{ day}^{-1}$). The method is designed to allow close control of pH, temperature and salinity and provide a comparable laboratory measurement for different formulations. Both these methods have come under open criticism since they are considered overestimate leach rates when compared to real-life conditions.

If laboratory-based release rate measurements are to be used in environmental risk assessments then they need to be representative of actual environmental conditions. A study commissioned by the UK HSE showed that the ISO protocol could be used to obtain a release rate for a number of booster biocides (Table 20.3) (Thomas *et al.*, 1999) When these data were compared with those determined using a flume system, designed to simulate environmental conditions (Thomas *et al.*, 1999), the release rates determined using the flume were consistently lower than those obtained using the ISO method for all biocides, other than dichlofluanid and TCMS pyridine. It was observed that changes in salinity, temperature, vessel speed, pH and the concentration of suspended particulate matter had very little or no effect on the release rate of all biocides tested. The effects of longer term changes (3 months) in temperature and salinity on Irgarol 1051 release was more pronounced, with an increased Irgarol 1051 release of 0.4 to 2.0 $\mu\text{g cm}^{-2} \text{ day}^{-1}$ when the temperature was increased (15–25°C).

20.2 Environmental occurrence of antifouling biocides

The environmental occurrence of antifouling biocides is very much dependent on the rate at which the active ingredient is released and its persistence and fate in the aquatic environment. For relatively persistent biocides, such

as Irgarol 1051 and diuron, seasonal variations in their concentrations are observed, with higher concentrations being measured in areas with a high boat density at the beginning of the pleasure-boat season which coincides with the scrubbing, repainting and subsequent introduction of newly painted vessels (Thomas *et al.*, 2002). With a decrease in release rates as the boating season progresses lower concentrations are observed. No or limited data are available for certain biocides such as TCMS-pyridine, chlorothalonil, zineb and TPBP, with what data there is showing levels below detection limits.

20.2.1 Irgarol 1051

The *s*-triazine herbicide Irgarol 1051 was the first booster biocide to gain prominence as an environmental contaminant. The presence of Irgarol 1051 was reported in 1993 in the surface waters of marinas on the Côte d'Azur, France by Readman *et al.* (1993) at concentrations of up to 1700 ng L⁻¹. Since 1993, the occurrence of Irgarol 1051 has been reported widely (Table 20.4).

In the UK, Irgarol 1051 has been found in both coastal and in-land areas with high boating activity. For example, the south east coast, the Humber estuary, Plymouth Sound, Southampton Water, the Crouch estuary and the Norfolk Broads. Similar concentrations have been reported in other areas of Europe including the Mediterranean; Lake Geneva, Switzerland; west coast of Sweden; Stockholm archipelago, Sweden; Oslofjord, Norway, Western Scheldt, The Netherlands; Sas van Gent and Schaar van Ouden, The Netherlands; and the Baltic and North Sea marinas of Germany. Outside Europe the occurrence of Irgarol 1051 has been reported in sea grasses from Queensland, Australia and in surface waters at marinas and ports in Japan, Canada, the US and Bermuda. The occurrence of the Irgarol 1051 metabolite 2-methylthio-4-tert-butylamino-6-amino-*s*-triazine (GS26575 or M1) has also been reported in Irgarol 1051 contaminated surface waters and sediments (Thomas *et al.*, 2000, Thomas *et al.*, 2002). Environmental degradation of Irgarol 1051 appears not to be the only source of GS26575 since it has also been reported to occur in Irgarol 1051 paint formulations (Thomas *et al.*, 2003). Typically concentrations of between < 1 and 230 ng L⁻¹ have been reported (Thomas *et al.*, 2002). Regulation of the use of Irgarol 1051 in the UK has successfully led to reduced concentrations of Irgarol 1051 being detected (Cresswell *et al.*, 2006).

20.2.2 Diuron

Diuron, a phenylurea herbicide, has been in use since the 1950s. Predominantly this use has been associated with weed control in non-agricultural

Table 20.4 Summary of biocide environmental occurrence data

Biocide	Sample type	Concentration range	Geographical region	Comment	References
Irgarol 1051	Marina, port and estuary surface waters, inland water ways and lakes, sediments and macrophytes	Marinas- <1–670 Coastal- <1–92 Lakes- Inland waterways- Sediments-	Europe (Norway, Sweden, Denmark, The Netherlands, UK, Spain, Italy, France, Greece), Australia, North America (USA, Canada, Bermuda), Caribbean, Asia (Japan, Singapore, Thailand, Vietnam)	Found ubiquitously in areas where Irgarol 1051 containing paint formulations are used	(Biselli <i>et al.</i> , 2000, Bowman <i>et al.</i> , 2003, Carrasco <i>et al.</i> , 2003, Cresswell <i>et al.</i> , 2006, Gatidou <i>et al.</i> , 2005, Gatidou <i>et al.</i> , 2007, Lambert <i>et al.</i> , 2006, Lambropoulou <i>et al.</i> , 2002, Lamoree <i>et al.</i> , 2002, Scarlett <i>et al.</i> , 1999, Tolosa <i>et al.</i> , 1996, Albanis <i>et al.</i> , 2002, Di Landa <i>et al.</i> , 2006, Sakkas <i>et al.</i> , 2002, Thomas, 1998, Thomas <i>et al.</i> , 2000, Thomas <i>et al.</i> , 2001, Thomas <i>et al.</i> , 2002, Voulvoulis, 2006)
Diuron	Marina, port and estuary surface waters, inland water ways and sediments	Marinas- <1–6742 Coastal- <1–465 Inland waterways- Sediments-	Europe (Sweden, Denmark, The Netherlands, UK, Spain, Italy, France, Greece), Asia (Japan, Vietnam, Thailand)	Also contributions from non-agricultural applications	(Gatidou <i>et al.</i> , 2005, Lambert <i>et al.</i> , 2006, Lamoree <i>et al.</i> , 2002, Thomas, 1998, Thomas <i>et al.</i> , 2000, Thomas <i>et al.</i> , 2001, Thomas <i>et al.</i> , 2002, Harino <i>et al.</i> , 2005, Harino <i>et al.</i> , 2007)

DCOIT	Marinas, ports and coastal waters	Marinas- <1–3700 Ports- <1–1	Europe (Sweden, UK, Spain, Greece), Asia (Japan, Vietnam, Thailand)	Only licensed for use on small vessels	(Thomas <i>et al.</i> , 2001, Sakkas <i>et al.</i> , 2002, Harino <i>et al.</i> , 2005, Harino <i>et al.</i> , 2007)
Dichlofluanid	Marinas, ports, coastal waters and sediments	Marinas <1–760 Ports <1–88 Coastal <1–7 Sediments <0.4–4	Europe (Sweden, UK, Italy, Spain, Greece)		(Hamwijk <i>et al.</i> , 2005, Sakkas <i>et al.</i> , 2002, Voulvoulis, 2006)
Chlorothalonil	Marinas, ports, coastal waters and sediments	Marinas- <1–63 Ports Coastal sediments	Europe (Sweden, UK, France, Spain, Greece), Asia (Japan)		
Zinc pyriithione	Marinas, ports, coastal waters and sediments	Generally below detection limits although 1 report of 100 nm	Europe (UK), and Asia (Japan)	Very few surveys performed	(Thomas, 1999, Thomas <i>et al.</i> , 2001)
Copper pyriithione	Marinas, ports, coastal waters and sediments	<2–420 $\mu\text{g kg}^{-1}$ dry weight	Japan	Very few surveys performed	(Harino <i>et al.</i> , 2006)
TCMTB	Marinas and ports	<LoD	UK	Very few surveys performed and biocide not widely used	
TCMS-pyridine	Marinas and ports	<LoD	UK		

applications. Several studies have investigated the impact of non-antifouling use on diuron release into the aquatic environment (Albanis *et al.*, 1994); however, the assessment of inputs from antifouling applications has been restricted to a few studies (Lamoree *et al.*, 2002, Gatidou *et al.*, 2005, Lambert *et al.*, 2006, Gatidou *et al.*, 2007, Thomas *et al.*, 2000, 2001, 2002). For example, Dahl and Blanck (1996) reported diuron concentrations in Swedish marinas of between 10 and 100 ng L⁻¹. A study of marinas on the Spanish Mediterranean coast reported similar concentrations (10–100 ng L⁻¹), whilst a study in the UK reported much higher concentrations in marinas (4–6742 ng L⁻¹) (Thomas *et al.*, 1999, 2001, 2002) probably reflecting the level of diuron use in the UK at the time. This study also reported diuron concentrations of between 5 and 226 ng L⁻¹ in water samples collected from estuaries, of between 1 and 45 ng L⁻¹ in coastal waters and < 8 ng L⁻¹ offshore. In freshwater boating areas diuron has been shown to occur at concentrations less than 170 ng L⁻¹ (Lambert *et al.*, 2006) suggesting diuron containing paints are not so extensively used on inland waterways. The principal metabolites of diuron, 1-(3-chlorophenyl)-3,1-dimethylurea (CPDU), 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) and 1-(3,4-dichlorophenyl) urea (DCPU) have also all been detected at measurable concentrations in surface water samples collected from the UK (Thomas *et al.*, 2002), albeit at concentrations much lower than diuron itself.

20.2.3 DCOIT

Only a few studies have reported the environmental occurrence of DCOIT. Short-lived concentrations of DCOIT have been reported in samples collected at marinas in Spain, Denmark and Greece (Lambropoulou *et al.*, 2002, Steen *et al.*, 2004). Studies in the UK and Norway have reported concentrations below detection limits, although it is not extensively used as a biocide on recreational vessels.

20.2.4 TCMTB

Environmental data are available from the study of point discharges relating to the use of TCMTB as a fungicidal agent in seed coatings and timber treatments (Walsh, 1997). Few studies have assessed its occurrence due to antifouling use. In the UK a study in the late 1990s reported that no TCMTB was found in UK marinas (Thomas, 1998), whilst the same was also true for Mediterranean marinas (Martinez *et al.*, 2001).

20.2.5 Dichlofluanid

Dichlofluanid has been reported to occur in marina surface waters from Greece and Spain as well as UK sediments (Voulvoulis *et al.*, 2000, Sakkas

et al., 2002, Readman, 2006). Other extensive studies in areas of intensive boating activity in the UK have reported concentrations below detection limits for both surface waters and sediments. Interestingly the occurrence of dichlofluanid in Greek (and other) surface waters and sediments has been challenged by a study which showed that the dichlofluanid metabolite N-dimethyl-N-phenyl-sulphamide (DMSA) occurs in surface waters and sediments following rapid degradation and that previous reports of dichlofluanid may be the result of 'false positives' arising from the use of non-specific detectors or inappropriate confirmation ions when using GC-MS (Hamwijk *et al.*, 2005, Schouten *et al.*, 2005).

20.2.6 Copper/zinc pyrithione

Very few monitoring studies have been performed for ZnPT and CuPT (Thomas, 1999, Thomas *et al.*, 2001, Dahllöf *et al.*, 2005, Harino *et al.*, 2007) since the analysis until recently was difficult to perform due to problems with photodegradation during sample work-up and transchelation which resulted in problems with limits of detection and reproducibility. The occurrence of ZnPT has only been reported once in the open aquatic environment at a marina on the Mersey (UK) at a concentration of around 100 nM, however it was uncertain as to whether shampoos or antifouling paints were the source (Mackie *et al.*, 2004). The occurrence of CuPT has been reported in sediment collected from a Japanese Port (9–22 $\mu\text{g kg}^{-1}$) (Harino *et al.*, 2007) with the CuPT possibly present in the form of antifouling paint particles. An extensive survey in the UK failed to detect zinc pyrithione above detection limits in surface waters (Thomas *et al.*, 2001).

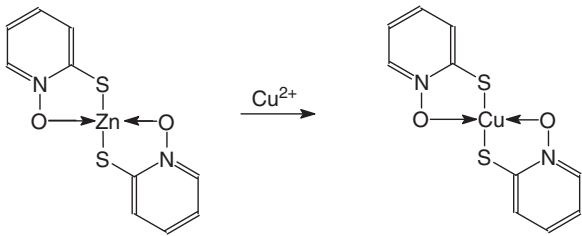
20.3 Environmental fate of antifouling biocides

20.3.1 Chemical transformation

The only biocide that is known to have the potential for chemical transformation is zinc pyrithione (ZnPT). ZnPT is the zinc chelate of 2-pyridinethiol-1-oxide (Fig. 20.1). It has been reported that ZnPT can transchelate with other cationic metals (e.g., Cu^{2+}). Copper pyrithione (CuPT), with the lowest dissociation constant, would be likely to be the most abundant (Turley *et al.*, 2000).

20.3.2 Degradation

A summary of degradation half-lives ($t_{1/2}$), mode of degradation and principal degradation products for the most intensively studied biocides is presented in Table 20.5. The persistence of a biocide is very much dependent



20.1 Transchelation of zinc pyrithione to copper pyrithione.

Table 20.5 Degradation data for antifouling paint booster biocides

Biocide	Half-life in natural seawater	Principal mode of degradation	Principal degradation products
Chlorothalonil	1.8 days	Biotic degradation	4-hydroxy-2,5,6-trichlorisophthalonitrile, 5-cyano-4,6,7-trichloro-2H-1,2-benzisothiazol-3-one
Dichlofluanid	18 h	–	DMSA
Diuron	–	Biotic degradation	3-(3,4-dichlorophenyl)-1-methylurea and dichlorophenyl urea
Irgarol 1051	100 days	–	GS26575 CGA234575 CGA234576
DCOIT	< 24 h	Biotic degradation	N-(octyl)carbamic acid
TCMTB	740 h	Hydrolysis	MBT, MTBT
TPBP	1–34 days	Photolysis/ hydrolysis	Pyridine, phenol and benzene
Zinc pyrithione	< 24 h	Photolysis	2-pyridine sulphonic acid
Zineb	96 h	Hydrolysis	ETU, DIDT, EDI

on its principal mechanism of degradation and in which compartment it resides. For example diuron is very persistent in surface waters but less so in anaerobic sediments (Thomas *et al.*, 2002, 2003). Both zinc and copper pyrithione undergo rapid photolysis in the presence of light but have the potential to persist under reduced light conditions in sediments or deep in the water column. How a biocide enters the environment is also important. Typically, biocide degradation is based upon its presence as a biocide released from a painted surface and rates determined for its degradation in various compartments. Not all biocidal material enters the environment in this form. It has been shown that scrubbing and paint removal activities can result in significant localised short-term pulsed inputs of biocidal com-

pounds into the environment. Much of the biocidal load in these unregulated discharges often resulting from amateur use is associated with paint particles. Biocides incorporated into the matrix of paint particles can persist much longer than those which are released directly from the painted surface (Thomas *et al.*, 2003; Table 20.6).

Irgarol 1051

Irgarol 1051 is not considered to be easily degraded in natural seawater and has a reported half-life of between 100 and 350 days (Scarlett *et al.*, 1999, Hall *et al.*, 1999, Thomas *et al.*, 2002). Biodegradation (Liu *et al.*, 1997), photodegradation (Okamura *et al.*, 1999) and chemical hydrolysis (Liu *et al.*, 1999a,b) of Irgarol 1051 all result in *n*-dealkylation to yield 2-methylthio-4-tert-butylamino-6-amino-s-triazine (M1/GS26575) as the principal degradation product (Fig. 20.2) along with M2 (3-[4-tertbutylamino-6-methylthiol-s-triazin-2-ylamino]-propionaldehyde) (the N-propionaldehyde derivative of M1) (Lam *et al.*, 2005) and M3, *N'*-di-tert-butyl-6-methylthiol-s-triazine-2,4-diamine. Concentrations of Irgarol 1051, M1 and M3 have been determined in the coastal waters of Hong Kong, and found to be 0.1–1.6 $\mu\text{g L}^{-1}$, 36.8–259.0 $\mu\text{g L}^{-1}$ and 0.03–0.39 $\mu\text{g L}^{-1}$ respectively (Lam *et al.*, 2006). GS26575 is a relatively stable degradation product with a reported half-life of between 80 and >200 days (Okamura, 2002; Thomas *et al.*, 2002). As discussed above GS26575 has also been reported to occur in Irgarol 1051 antifouling paints as well as in marinas (e.g., in Japan, Spain, and UK; Thomas *et al.*, 2000, 2003, Okamura *et al.*, 2000a,b, Ferrer *et al.*, 1997). In sediment Irgarol 1051 appears to be more persistent with very little degradation while GS26575 has a sediment half-life of around 260 days (Thomas *et al.*, 2002).

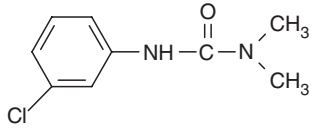
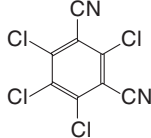
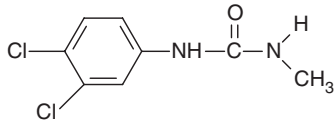
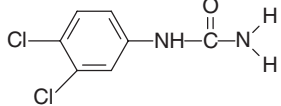
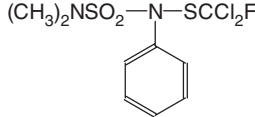
Diuron

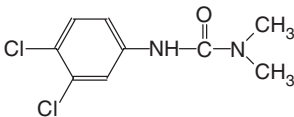
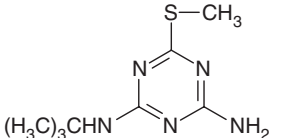
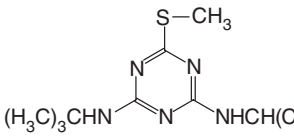
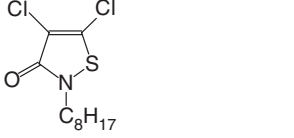
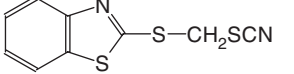
Diuron is also considered to be persistent in seawater (Callow and Willingham, 1996, Thomas *et al.*, 2002). Diuron is less persistent in marine sediments with a half-life of 14 days; however, when incorporated in paint particles this increases significantly (Thomas *et al.*, 2003). Aerobic degradation of diuron is reported to result in the formation of 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) and 1-(3,4-dichlorophenyl)urea (DCPU), whilst anaerobic degradation in sediments results in the formation of 1-(3-chlorophenyl)-3,1-dimethylurea (CPDU) (Ellis and Camper, 1982) (Fig. 20.3).

DCOIT

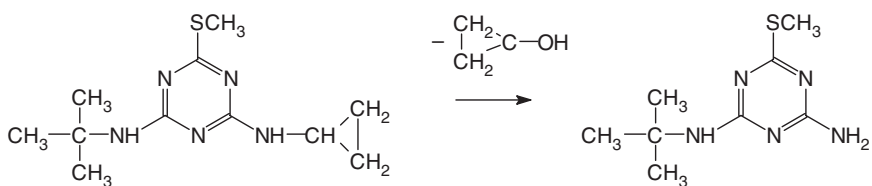
DCOIT degrades rapidly in natural seawater and sediment (Callow and Willingham, 1996, Jacobson and Willingham, 2000, Thomas *et al.*, 2002,

Table 20.6 Pseudo-first order anaerobic degradation rate constants (k_h) and half-lives ($t_{1/2}$) for antifouling booster biocides and degradation products in marine sediments and associated with paint particles in marine sediments^a

Compound	Chemical structure	Free biocide			Paint particle bound		
		k_h (h ⁻¹)	$t_{1/2}$ (days)	RSD (%)	k_h (h ⁻¹)	$t_{1/2}$ (days)	RSD (%)
CPDU		0.02	35	50.1	–	–	–
Chlorothalonil		–	< 0.5 ^b	–	–	–	–
DCPMU		0.68	1.0	11.7	–	–	–
DCPU		0.23	3.0	17.1	–	–	–
Dichlofluanid		–	< 0.5 ^b	–	0.48	1.4	9.1

Diuron		0.05	14	1.7	–	No degradation	–
GS26575		0.003	226	70.0	–	–	–
Irgarol 1051		–	No degradation	–	–	No degradation	–
DCOIT		–	< 0.5 (< 0.04) ^c	–	0.07	9.9	29.3
TCMTB		0.46	1.5	24.0	–	–	–

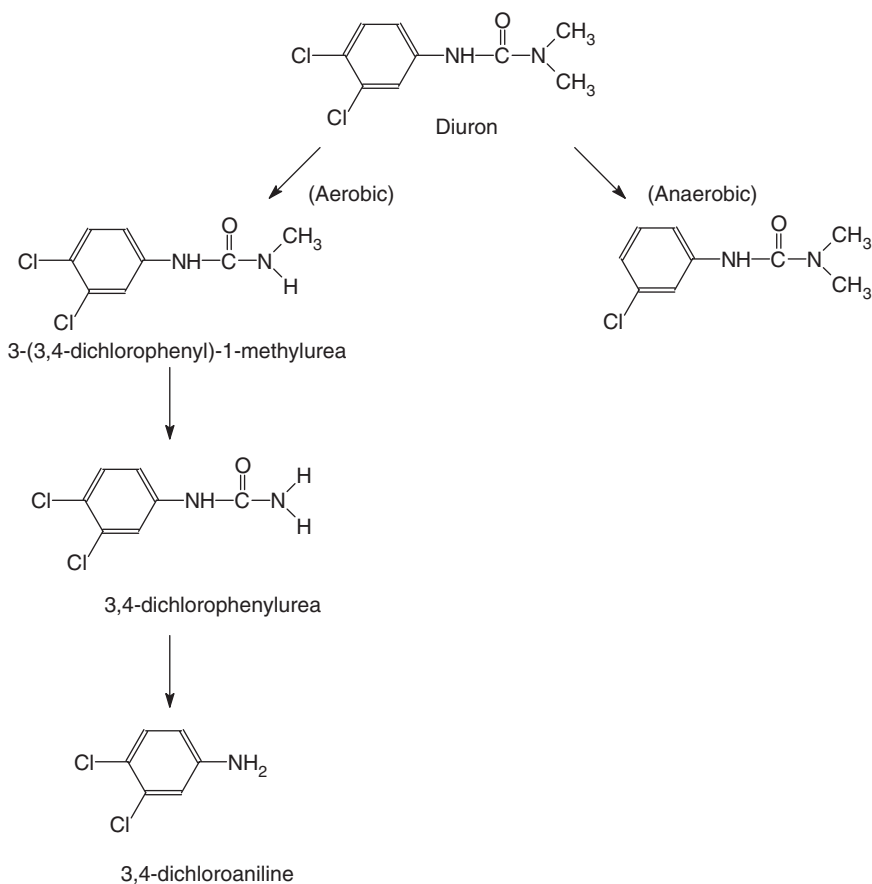
^a Number of each degradation experiment. ^b 100% degradation observed following 24 h. ^c Willingham and Jacobson (1996).
RSD = relative standard deviation.



Irgarol 1051 (MWt. 253)

GS 26575/M1 (MWt. 213)

20.2 Degradation of Irgarol 1051.



20.3 Proposed degradation pathway for diuron under aerobic and anaerobic conditions (Ellis and Camper, 1982).

2003). Degradation half-lives have been determined for DCOIT in a number of environmental matrices. Biological degradation is considered to be over 200 times faster than hydrolysis or photolysis (Willingham and Jacobson, 1996, Jacobson and Willingham, 2000). Anaerobic sedimentary degradation of SeaNine containing paint particles produced a half-life of 10 days compared to less than half a day when present in its free form (Thomas *et al.*, 2003). Biodegradation involves cleavage of the isothiazolone ring and subsequent oxidation of alkyl metabolites. The primary degradation products are N-octyl oxamic acid, 4,5-dichloro-thiazole and N-octyl carbamic acid (Fig. 20.4).

TCMTB

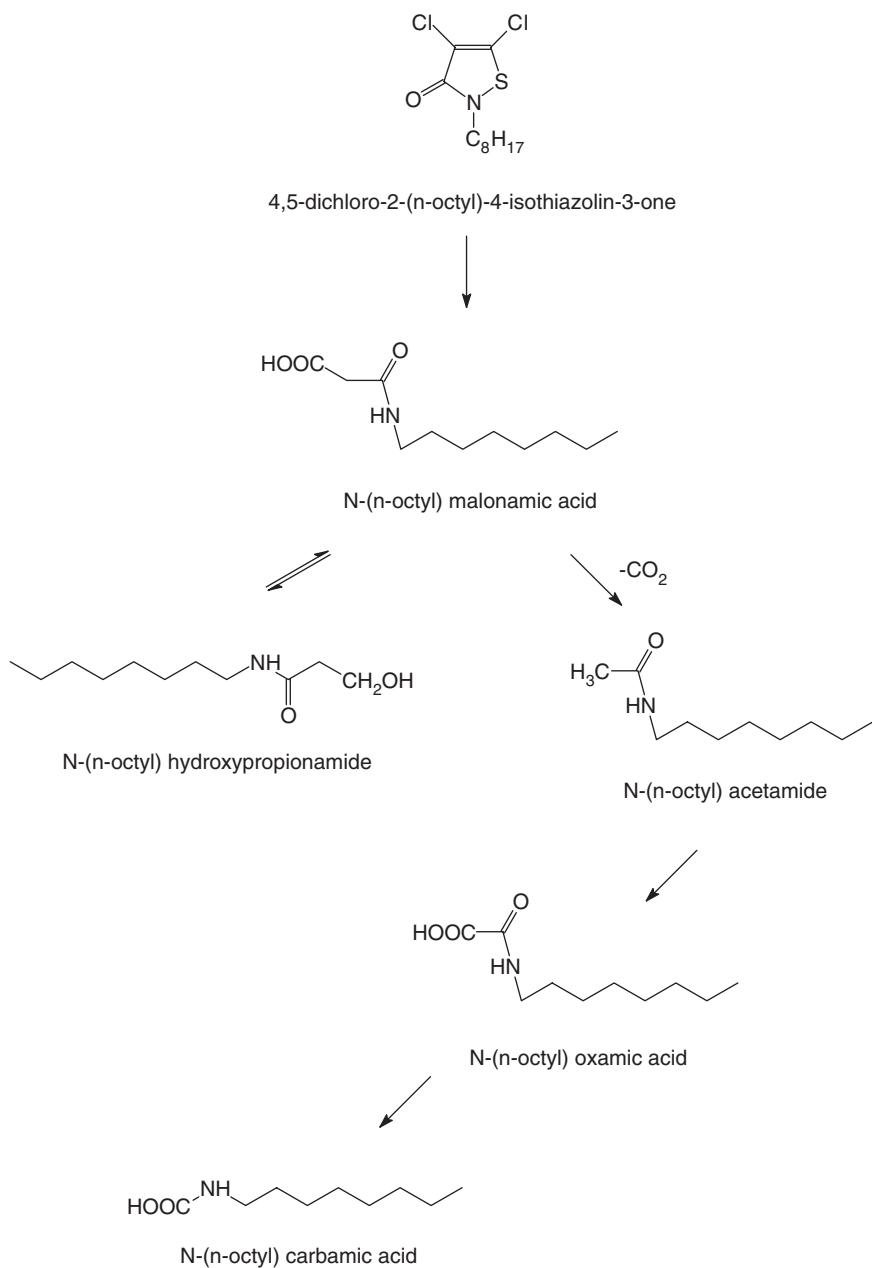
TCMTB is considered unlikely to persist in the environment (Brownlee *et al.*, 1992). Laboratory experiments have shown that TCMTB rapidly degrades to 2-mercaptobenzothiazole (MBT) and 2-(methylthio)benzothiazole (MTBT). This probably occurs through hydrolysis followed by biological methylation (Fig. 20.5). The half-life of TCMTB in natural seawater is between 30 and 40 days (Thomas *et al.*, 2002). TCMTB is also degraded rapidly (< 0.5 h) by photolysis (Brownlee *et al.*, 1992).

Zinc pyrithione and copper pyrithione

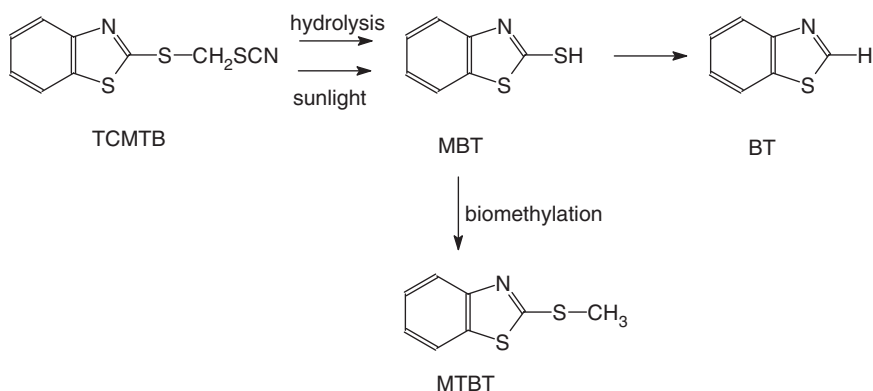
Zinc pyrithione (ZnPT) is rapidly degraded in natural seawater with a reported half-life of < 24 h (Turley *et al.*, 2000). ZnPT photolysis is extremely rapid ($t_{1/2}$ < 1 h), whilst, by contrast, hydrolysis half-lives range from 96–120 days. Photolysis rates can be greatly reduced at depth where light levels are lower. For example, the half-life of ZnPT has been reported to increase to 200 h at depths of over 25 m. Both aerobic and anaerobic biotic degradation also appear to be very rapid ($t_{1/2}$ < 2 h). The primary degradation product of ZnPT has been reported to be 2-pyridine sulfonic acid with Zn^{2+} released into the aqueous phase. Copper pyrithione (CuPT) is also rapidly degraded in natural seawater with a reported half-life of 0.5 h (Turley *et al.*, 2000).

Dichlofluanid

Dichlofluanid rapidly undergoes hydrolysis in water to form N'-dimethyl-N-phenyl-sulphamide (DMSA) (Hamwijk *et al.*, 2005) with a reported half-life in seawater of less than 20 h (Callow, 1995, Thomas *et al.*, 2002). Degradation in sediments is equally rapid with a half-life of < 10 h in marine sediments. Even when incorporated in paint particles the anaerobic



20.4 Proposed metabolic pathway for DCOIT under aerobic conditions (Anon, 1999b).



20.5 Proposed degradation pathway for TCMTB (Brownlee *et al.*, 1992).

degradation of dichlofluanid is relatively rapid with a half-life of 1.4 days (Thomas *et al.*, 2003).

Chlorothalonil

Degradation half-lives of between 4 and 150 h have been reported for chlorothalonil in natural fresh water (Davies, 1987) and between 1.8 and 8 days in natural estuarine water and sediment water test systems (Walker *et al.*, 1988). A bioassay system to measure the degradation of antifouling biocides showed much slower degradation in natural seawater at 25 °C, reporting a half-life of ~8 weeks (Callow and Willingham, 1996).

Zineb and mancozeb

Zineb is known to rapidly degrade, through hydrolysis, to for 5,6-dihydro-3H-imidazo (2,1-c)-1,2,4-dithiazole-3-thione (DIDT), ethylene diisothiocyanate (EDI), and ethylenethiourea (ETU) (Hunter and Evans, 1991, Silver Platter Information, 1997). A half-life of 96 h has been reported at pH 10 and a temperature of 20 °C. Mancozeb is considered to have a similar degradation rate to that of Zineb.

20.3.3 Sediment/water partitioning

The partitioning of contaminants between aquatic compartments has implications for other fate mechanisms discussed within this review. The ability of a compound to partition between compartments is determined by its

adsorption coefficient (K_d) and partition coefficient related to organic carbon (K_{OC}).

$$K_d = \frac{C_{sed}}{C_{water}}$$

where K_d = sorption constant (kg L^{-1})

C_{sed} = equilibrium concentration of solute on sediment (mg kg^{-1})

C_{water} = equilibrium concentration of solute in water (mg L^{-1})

$$K_{OC} = \frac{K_d}{f_{OC}}$$

K_d and K_{OC} have units of kg L^{-1} and f_{OC} is the dimensionless weight fraction of organic carbon in the sediment.

It is considered that organic contaminants with a $K_d < 10^5$ exist mainly in the dissolved phase (Duinker, 1986). Another factor, specific to antifouling paint biocides, is that the biocides may become incorporated into sediments as paint particles from boats and ships that have been hosed down prior to re-painting. In such cases, the K_d and K_{OC} have very little influence on the presence of booster biocides in sediments.

Irgarol 1051

K_d of between 142 and 3100 kg L^{-1} and K_{OC} of between 251 and 63095 kg L^{-1} have been reported for Irgarol 1051 (Tolosa *et al.*, 1996, Biselli *et al.*, 2000, Thomas *et al.*, 2002), which suggest that Irgarol 1051 will be mainly associated with the dissolved phase. Calculations using the Mackay fugacity model estimate that 4.4% of the Irgarol 1051 present in marina waters will partition to sediments (Rogers *et al.*, 1996). Irgarol 1051 has been reported in sediments collected from marinas in the UK (<10 – 132 ng g^{-1} , 1993; <1 – 30 ng g^{-1}) ((Gough *et al.*, 1994); (Thomas *et al.*, 1999, 2001, 2002), Switzerland (2.5 – 8 ng g^{-1}) (Tóth *et al.*, 1996), Sweden ($< 10 \text{ ng g}^{-1}$) (Hall *et al.*, 1999) and Germany (<1 – 220 ng g^{-1}) (Biselli *et al.*, 2000). Few studies have also reported the determination of the Irgarol 1051 metabolite GS26575 in sediments. Thomas *et al.* (2000) reported GS26575 concentrations of <0.4 – 5 ng g^{-1} in UK marinas, whilst Hall *et al.* (1999) reported concentrations $< 10 \text{ ng g}^{-1}$.

Diuron

Diuron is relatively soluble in water (35 mg L^{-1}) with a $\log K_{OW}$ of 2.8. Diuron is reported to have a K_d of $8.9 \pm 13.4 \text{ kg L}^{-1}$ in freshwaters (Table

20.2) (House *et al.*, 1997), $4 \pm 1 \text{ kg L}^{-1}$ in saline waters and a K_{OC} of around 250 kg L^{-1} (Thomas *et al.*, 2002), which suggests that diuron will be predominantly found in the dissolved phase and only weakly sorbed to sediments. Diuron has been detected at low concentrations in sediments in the UK, Netherlands and Spain (Thomas *et al.*, 2000, Lamoree *et al.*, 2002) while Thomas *et al.* (2000) reported high levels (1420 ng g^{-1}) of diuron in an enclosed UK marina; however, this was thought to be due to the presence of paint particles within the sample.

DCOIT

DCOIT also has a reported $\log K_{OW}$ of 2.8 and a solubility of 14 mg L^{-1} . A K_d of between 250 and 625 kg L^{-1} has been reported for DCOIT in aquatic sediments (Table 20.2), however a K_{OC} of around $1.6 \times 10^4 \text{ kg L}^{-1}$ suggests DCOIT will bind strongly and essentially irreversibly to sediment (Willingham and Jacobson, 1996, Thomas *et al.*, 2002).

TCMTB

A calculated $\log K_{OC}$ of 2.74 kg L^{-1} and a K_d of 27 kg L^{-1} for a sediment containing 5% organic carbon (Brownlee *et al.*, 1992) correspond well with the experimental $\log K_{OC}$ of 2.7 kg L^{-1} reported by Thomas *et al.*, (2002).

Dichlofluanid

Few data are available on the partitioning of dichlofluanid to sediments, possibly due to its rapid degradation in test systems. Dichlofluanid has been reported to occur in marine sediments (Voulvoulis *et al.*, 2000) however, as with occurrence data in water, these data have been challenged.

Zinc pyrithione

ZnPT is considered to bind strongly to sediments; however, due to its short half-life, the influence of partition on the fate of ZPT is considered insignificant (Turley *et al.*, 2000).

Chlorothalonil

The adsorption of chlorothalonil to sediments is considered to be an important route of removal from the dissolved phase (Davies, 1987, Walker *et al.*, 1988). Thomas *et al.* (2002) reported a K_d of 37 kg L^{-1} and a $\log K_{OW}$ of 3.3

for chlorothalonil. Once bound to the sediment chlorothalonil is thought to be biodegraded.

Zineb

It is reported that Zineb rapidly absorbs to sediments leading to faster degradation rates (Silver Platter Information, 1997).

Volatilisation

The ability of a compound to partition across the air–water interface is determined by its air–water partition coefficient, referred to as Henry's law constant. Henry's law constant was only available for chlorothalonil. Estimated constants for other biocides suggest that for all the biocides listed in Table 20.7, volatilisation is not an important fate mechanism.

Bioaccumulation

There have been very few reports of studies relating to the biological uptake and bioaccumulation of booster biocides. Biological uptake of Irgarol 1051 has been shown to occur in fresh water macrophytes (Tóth *et al.*, 1996) with bioconcentration factors (BCFs) of up to 30000x, and

Table 20.7 Measured and estimated Henry's law constant for selected booster biocides

Biocide	Henry's law constant (atm m ³ mol ⁻¹)	Vapour pressure (atm @ 25°C)	Solubility (mol m ⁻³)	Ref.
Chlorothalonil	2×10^{-7}	–	–	(Caux <i>et al.</i> , 1996)
Dichlofluanid	$^{\dagger}9.5 \times 10^{-8}$	3.7×10^{-10}	3.9×10^{-3}	(Silver Platter Information, 1997)
Diuron	$^{\dagger}2 \times 10^{-6}$	3.6×10^{-7}	0.18	(Silver Platter Information, 1997)
Irgarol 1051	$^{\dagger}2.5 \times 10^{-8}$	8.7×10^{-10}	0.035	(Hall Jr LW <i>et al.</i> , 1999)
DCOIT	$^{\dagger}5.1 \times 10^{-7}$	9.7×10^{-9}	0.019	(Anon, 1999b)
TCMTB	$^{\dagger}2.7 \times 10^{-7}$	1.17×10^{-8}	0.044	(Silver Platter Information, 1997)
Thiram	$^{\dagger}3.1 \times 10^{-7}$	2.3×10^{-8}	0.075	(Silver Platter Information, 1997)
Zinc pyrithione	Non-volatile	–	–	

[†]estimated from Henry's law constant (H) = P/S ; where P = vapour pressure (atm) and S = solubility in water (mol m⁻³).

within marine macrophytes (Scarlett *et al.*, 1997, 1999). Dyer *et al.* (2006) showed Irgarol 1051 BCFs of up to 150000 ml g⁻¹ in the green algae *Tetraselmis suecica*. Predicted BCFs of 75 and 22 calculated for diuron would suggest that the compound would not appreciably bioaccumulate in aquatic organisms (Kenaga, 1980). Bioaccumulation of DCOIT in fish is considered to be very low (< 1% parent compound) (Willingham and Jacobson, 1996). Metabolism of DCOIT is considered to be rapid, with the metabolites being associated with proteins. Both zineb and thiram are reported not to bioaccumulate (Silver Platter Information, 1997). No pertinent data were available for any of the other biocides.

Transport

There are three main processes that can transport a chemical within the aquatic environment; bulk transport with dispersion, sedimentation and diffusion. Bulk transport with dispersion refers to the dispersion and dilution of a compound in a body of water. Sedimentation refers to the transport of a compound whilst it is associated with bottom and suspended sediments, whilst diffusion refers to the movement of molecules from areas of high concentration to areas of low concentration. Very little information on these three processes is available for antifouling paint booster biocides. From the occurrence data described above it appears that these compounds mainly occur in areas of high boating activity and/or limited water exchange and that as they are transported to off-shore areas dilution plays an important role since the concentrations of booster biocides in off-shore waters are low (Thomas *et al.*, 1999).

20.4 Risk assessment

The thorough risk assessment of the biocides used in Europe is currently underway through the EU's Biocidal Products Directive. The Biocidal Products Directive aims to harmonise the European market for biocidal products and their active substances. At the same time it aims to provide a high level of protection for humans, animals and the environment. In terms of environmental protection there is a comprehensive assessment of the environmental properties and ecotoxicology of each biocide using data provided by the applicant. Typically this is a biocide manufacturer.

Much simpler 'preliminary' risk assessments have been performed using available literature data to determine predicted environmental concentrations (PEC) and predicted 'no-effect concentrations' (PNEC). These are then used to determine a risk quotient that is used to characterise the potential risk presented to the aquatic environment.

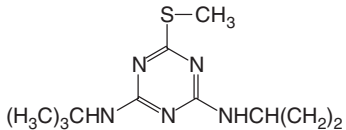
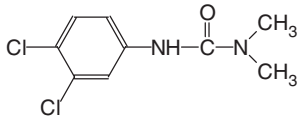
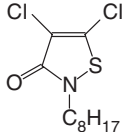
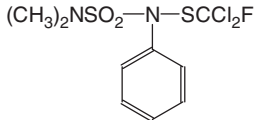
$$\text{Risk quotient} = \frac{\text{Predicted Environmental Concentration (PEC)}}{\text{Predicted no-effect concentration (PNEC)}}$$

For antifouling biocides there are a number of models that use the environmental fate and leach rate data available to estimate PEC under various scenario conditions (e.g., marinas, harbours and coastal areas). MAMPEC (Marine Antifoulant Model to Predict Environmental Concentrations) is one of the most commonly used models and available as a download from <http://www.antifoulingpaint.com/downloads/mampec.asp>.

PNEC are typically generated from the lowest acute or chronic toxicity value generated from a bioassay. It is normal for uncertainty factors to be applied in accordance with either OECD or EU guidelines. For example, for data generated using an acute test an uncertainty of 100 is typically used. A number of published studies have compared the environmental risks posed by antifouling biocides (Bellas, 2006, Ranke and Jastorff, 2000, Turley *et al.*, 2000, Nehring, 2001, Thomas *et al.*, 2001, Ranke and Jastorff, 2002, Boxall, 2004, Lambert *et al.*, 2006) whilst in various European countries regulatory assessments have led to the restriction in the use of various biocides (e.g., UK, Table 20.8).

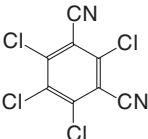
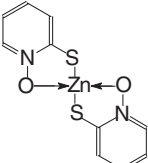
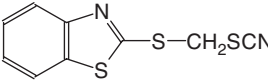
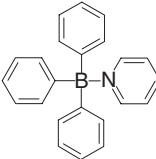
The simple risk assessment of Irgarol 1051 has shown that in areas of high boating intensity and low water exchange Irgarol 1051 can pose a risk to aquatic organisms, in particular algae and seagrasses (Ranke and Jastorff, 2002, Thomas *et al.*, 2001, Boxall, 2004). Scarlett *et al.* (1999) showed that *Zostera marina* beds that occur close to marinas, or other areas where boat density is high, are likely to experience reduced photosynthetic efficiency of photosystem II. A broader assessment based on measured environmental concentrations (MECs) supported these findings and showed that the concentrations of Irgarol 1051 found in marinas, and in particular enclosed marinas, posed a potential risk to marine algae (Thomas *et al.*, 2001). This study also showed that diuron can also pose an acute risk in enclosed marinas. In freshwater environments both Irgarol 1051 and diuron appear to pose a risk to macrophytes, in particular *Najas nodiflorum* and *Chara vulgaris* (Lambert *et al.*, 2006). A recently published comparative risk assessment suggests that in addition to Irgarol 1051, its metabolite M1 and diuron, DCOIT also poses an acute environmental risk; however, this conclusion is based upon the very limited MEC data available. Limited public domain data for establishing environmental risk are a weakness in many of the preliminary risk assessments performed to date. Over the past decade the amount of effects data has improved considerably but the occurrence and behaviour of certain biocides is still uncertain (i.e., DCOIT, ZnPT, TPBP and dichlofluanid). Good modelling tools are essential for effective environmental risk assessment since the market share and subsequently the amount of each biocide can frequently change. Following the outcome of

Table 20.8 Summary of the environmental fate of common antifouling paint biocides showing chemical structure, aqueous half-life, principal degradation products and main environmental compartment where the compound occurs

Compound	Chemical structure	Half-life in seawater	Principal degradation product(s)	Main compartment found
Irgarol 1051		> 100 days	GS26575 CGA234575 CGA234576	Water
Diuron		Persistent	3-(3,4-dichlorophenyl)-1-methylurea and dichlorophenyl urea	Water
DCOIT		< 24 h	N-(octyl)carbamic acid	Sediment
Dichlofluanid		18 h	DMSA	Sediment

(continued)

Table 20.8 Continued

Compound	Chemical structure	Half-life in seawater	Principal degradation product(s)	Main compartment found
Chlorothalonil		43 h	4-hydroxy-2,5,6-trichlorisophthalonitrile, 5-cyano-4,6,7-trichloro-2H-1,2-benzisothiazol-3-one	Sediment
Zinc pyriithione		< 24 h	2-pyridine sulphonic acid	Water
TCMTB		–	MBT, MTBT	
TPBP		1–34 days	Pyridine, phenol and benzene	Water

the BPD it is expected that there will be a much clearer picture as to which biocides pose a potential environmental risk and we shall know the identity of those that will remain approved for use within Europe at least.

20.5 Conclusions

The wide range of environmental properties exhibited by antifouling paint biocides indicates that the fate and behaviour of each one will differ considerably. Data are available for the biocides most commonly used in Europe, North America and Asia. Good data are available on the occurrence of many biocides that are commonly used, although this is not the case for ZnPT and CuPT which are difficult to analyse. The occurrence of certain biocides has been disputed (e.g., dichlofluanid), which further clouds the picture. From the degradation data it appears that chlorothalonil, dichlofluanid, DCOIT, zinc pyrithione, and Zineb rapidly degrade ($t_{1/2} < 4$ days), whilst Irgarol 1051 and diuron seem more persistent. A better understanding of biocide fate has led to improved modelling techniques (i.e., MAMPEC) that are invaluable in determining good PEC data that are so important for risk assessment. Comparative preliminary risk assessments have thus far identified Irgarol 1051 and diuron as potentially posing an environmental risk and there are restrictions in certain European countries on their use. The outcome of the BPD assessment, expected in late 2008, should further clarify this position.

Some caution should however be taken since antifouling paint biocide risk assessments are based on the fate of the substance released from a painted hull and typically use data from standard regulatory tests. The risk associated with release in the form of paint particles nor the effect of relevant factors such as depth are considered and this is a major gap in biocide risk assessments.

20.6 List of abbreviations

AFS	Antifouling systems
ASTM	American Society of Testing Materials
BPD	Biocidal Products Directive
CuPT	Copper pyrithione
DCOIT	4,5-dichloro-2-n-octyl-4-isothiazolin-3-one
DMSA	N'-dimethyl-N-phenyl-sulphamide
HSE	Health and Safety Executive (UK)
IMO	International Maritime Organisation
ISO	International Standards Organisation
PEC	Predicted environmental concentration
PNEC	Predicted no-effect concentration
TBT	Tributyltin

TCMS pyridine	2,3,5,6-tetrachloro-4-(methyl sulphonyl)pyridine
TCMTB	2-(thiocyanomethyl thio)benzthiazole
TPBP	pyridine-triphenylborane
ZnPT	zinc pyrithione

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Organic alternatives to copper in the control of marine biofouling

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Abstract: Since the ban of tributyltin, the use of large amounts of copper in antifouling formulations has been the main reason for a low occurrence of animal fouling on ship hulls and, consequently, important fuel savings (Schultz, 2007). However, doubts about its environmental profile (see Chapter 19) have encouraged considerable efforts aimed at identifying antifouling alternatives to copper. This chapter includes information about three of the new antifouling candidates that are being identified worldwide. First, the use of sodium benzoate and ferric benzoate, which have demonstrated antifouling effects both in aqueous solutions and as part of soluble matrix paints, is presented. Secondly, medetomidine, a non-toxic pharmacological substance showing activity at very low amounts and interacting strongly with paint surfaces, is presented. Finally, we discuss the use of quebracho tannin and aluminium tannate, and their potential activity with fouling organisms. The use of unconventional organic chemicals provides an alternative solution in response to the demands for an effective antifouling strategy to replace copper.

Key words: sodium benzoate, ferric benzoate, medetomidine, quebracho tannin, aluminium tannate.

21.1 The use of benzoates

Current antifouling coatings are based on toxic compounds that can be harmful to marine ecosystems. Environmental concerns about the use of toxic antifoulants have led to increased interest in non-toxic or at least benign alternatives. For this reason, a study was carried out on the effects of sodium benzoate, a synthetic compound commonly used as food preservative, on the cosmopolitan barnacle *Balanus amphitrite* (Cirripedia, Balanidae). Sodium benzoate is an antimicrobial product and it is hypothesized that it could affect the first step of the sequence and consequently interrupt biofouling development. It is well known that biofilms can play an important role in mediating settlement and metamorphosis of larvae. Mitchell *et al.* (1975), Chet *et al.* (1975) and Chet and Mitchell (1976)

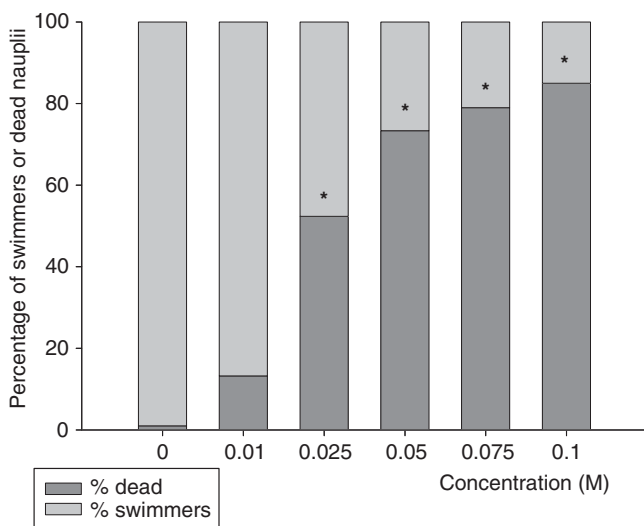
reported the use of non-toxic organic repellents, such as benzoic acid, tannic acid and acrylamide to inhibit the marine microbial film formation. Additionally, the sodium benzoate incorporated to a silicone coating was found to exhibit enhanced antibacterial performance in freshwater (Haque *et al.*, 2005; Al-Juhni and Newby, 2006).

It is recognized that compounds with high solubility represent a disadvantage for a marine paint. The successful employment of soluble compounds (benzoic acid, sodium benzoate, etc.) in anticorrosive and antifouling paints is limited by their lixiviation. However, it is possible to prepare insoluble metallic benzoates with certain cations (iron, zinc, aluminium, etc.) which are widely used in paint technology. Ferric benzoate was used, since it is hydrolyzed and consequently it produces a pH decrease.

The use and characterization of benzoates such as sodium benzoate, benzoic acid, calcium benzoate, zinc basic benzoate, aluminium basic benzoate and ferric benzoate as corrosion inhibitors and anticorrosive pigments for protective paints is well documented (Blustein and Zinola, 2004; Blustein *et al.*, 2006, 2007a,b).

The incorporation of benzoate salts in aqueous media produces hydrolysis of the compound, i.e. equilibrium between benzoate anion and benzoic acid. The mechanisms of action of these products on organisms are different: benzoic acid inhibits the enzymes that control acetic acid metabolism and oxidative phosphorylation in bacteria and yeast, it takes part in different points of citric acid cycle and acts on cellular wall (Lueck, 1980); sodium benzoate generates disruption of cell internal pH and, consequently, interferences in a wide variety of metabolic functions (Zhao *et al.*, 1998; Denyer and Maillard, 2002). In contrast, there is no information available about the mode of action of ferric benzoate on cells. However, it is well known that this compound is easy to synthesize in the lab, non-polluting and biodegradable.

It was demonstrated that sodium benzoate solutions have narcotic effects on nauplii (Fig. 21.1), cyprids and adults of *Balanus amphitrite*. Larvae exposed to different concentrations of the solution could recover when they were placed in benzoate-free seawater and that confirms the temporary effect. This behaviour is not in accordance with earlier experiments (Eighmy *et al.*, 1992) that indicated narcotic effects on cyprids when larvae closely approach to dissolving benzoic acid crystals, but not benzoate in solution. Cirral activity of barnacle adults was strongly reduced in the function of benzoate concentration in seawater (Pérez *et al.*, 2001). Cirral beating reflects physiological and behavioural conditions of the barnacles (Willemssen and Ferrari, 1993). It is hypothesized that this response could affect not only its ability for filtering food but also its reproductive activity (e.g., development of ovigerous mass, larval viability, etc.). The narcotic effect of benzoate anion on barnacle larvae and adults was also confirmed in experiments using calcium benzoate.



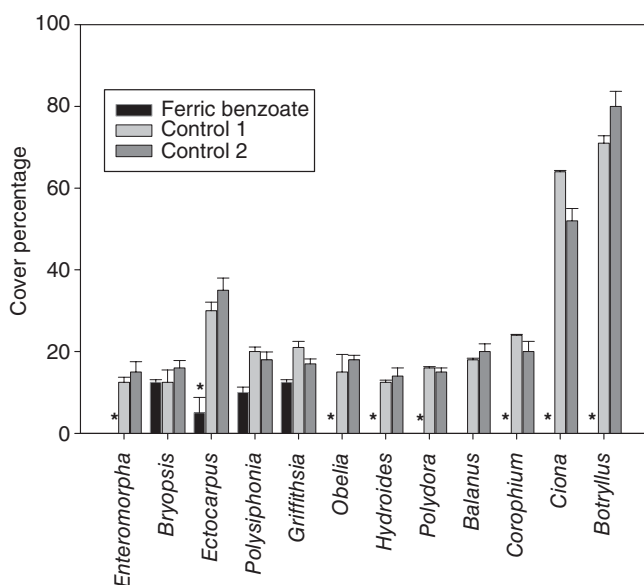
21.1 Effect of sodium benzoate on nauplii survival of *B. amphitrite* for 60 min exposure. (*) indicates significant differences between treatment and controls, $p < 0.05$.

On the other hand, it was noticed that sensitivity of *Artemia salina* larvae to benzoate varied with the concentration of the solutions and responses were similar to that of *B. amphitrite* nauplii. However, the effect of benzoate on *A. salina* was lethal because none of the larvae could recover when they were transferred to fresh seawater.

Although the screening of non-conventional compounds using a laboratory-based bioassay is a useful tool in the search for novel antifouling compounds, the ecological significance of laboratory bioassays appears to be very limited and must be confirmed with the results of field experiments.

In the evaluation of the results, we commented chiefly on two aspects: the biocide range and the degradability. In relation to the first aspect, benzoates showed efficacy as settlement inhibitor for common fouling organisms at Mar del Plata harbour (Argentina) (del Amo *et al.*, 2008). Benzoates are chemically stable in seawater conditions, thus restricting their toxicity spatially and temporally for both human beings and environment. Benzoic acid and sodium benzoate are classified in the United States as Generally Recognized as Safe (GRAS) and their use in food is permitted up to the maximum level of 0.1% (Sagoo *et al.*, 2002; Al-Juhni and Newby, 2006).

The difficulty in making satisfactory antifouling paints has been in devising formulations which release the biocide at a rate sufficient to provide an inhibitive concentration at the surface but still slow enough to prevent the rapid and complete exhaustion of the toxic reserve. In fact, sodium benzoate



21.2 Coverage percentage for macrofouling organisms on ferric benzoate painted panels and controls, six months exposure. *Control 1*: acrylic panels; *control 2*: paint without ferric benzoate. Bars = mean $\pm$ SE. (*) indicates significant differences from controls, $p < 0.05$.

paint showed good antifouling performance but the biocide was quickly leached out. The incorporation of ferric benzoate into the soluble matrix paint represented an advantage because it allowed a controlled pigment release with satisfactory antifouling action during six months exposure. Antisettlement activity was principally observed on algae, calcareous species (barnacles and tube-worms) and ascidians (Fig. 21.2).

The paints significantly limited the strength of the joint between fouling and panel, making the bond so weak that it could be broken by the weight of the fouling or by the motion of the water. These results were similar to those expected for another type of paint like 'fouling-release coatings' (Brady, 2000; Hare, 2000). However, the modes of action of both paints are different.

Surfaces of benzoate paints are not slippery and the antifouling mechanism is due to the effect of low pH produced by the hydrolysis of ferric benzoate combined with an increase in soluble benzoate (and/or benzoic acid) concentration. At the paint/seawater interface the estimated pH value was about 4 (Pérez *et al.*, 2001); it is suggested that this pH would affect mechanisms of cementation to substrate of some fouling organisms, particularly those that attach themselves by proteinaceous cements.

Two effects are possible when a barnacle larva approaches and tries to attach itself to any surface coated with benzoate paint: (1) the larva may swim away as though repelled or (2) it may become temporarily quiet through narcotic effect. In both cases, larvae can complete their life cycles on natural or uncoated substrates and this represents an advantage from an ecological point of view.

In general, the screening of non-toxic antifoulant represents a promising approach to the search for new antifouling agents. In combination with new paint technologies that facilitate easy release of biofoulers (Chapter 26) or that self-polish (Chapter 18), it is now possible to incorporate non-polluting compounds and take a multistrategy approach toward solving the problem of biofouling.

The information obtained from these biological assays (see also Chapter 12), which require no expensive equipment, is easy to perform and to reproduce. However, further research must be carried out in two major lines: (1) narcotizing action on larvae of other marine fouling organisms than barnacles and (2) long-term assays for antifouling paints in experimental rafts.

21.2 The use of medetomidine

Marine biofouling is a well-known problem and barnacles are one of the main foulers and also the one that probably causes most problems. As soon as they have the possibility to attach to a ship hull, or any other surface within the marine environment, they will glue themselves to the surface and create a calciferous bottom plate, building up walls around their soft body. Apart from protection, the shells also create surface turbulence. For a ship of any size that will increase the fuel costs dramatically, lower the speed, increase costs regarding maintenance and thereby reduce efficiency, both in financial as well as safety terms.

Among Crustacea, the barnacles (order Cirripedia) are different in many aspects: the body plan is not equipped with an abdomen (Mouchel-Viehl *et al.*, 1998) and they are sessile. They have a unique larval form, the cyprid larva, which is characteristic for the order and has only one purpose: to find a surface suitable for settlement. Therefore, for antifouling purposes, the cyprid larva is of utmost importance. The goal of any antifoulant is to prevent attachment which precedes settlement. One may do so by simply creating a very toxic surface that will kill the larva, or use the larva's own behavioural cues. It is possible, with specific molecules, to change their behaviour into another and thereby, without killing, avoid attachment and settlement.

Surface attachment precedes settlement and it is essential for metamorphosis, which is the process that leads from a free swimming larva to a

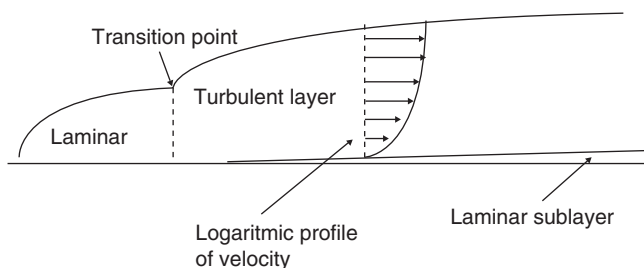
sessile juvenile. Attachment may occur passively, resulting from physical parameters such as buoyancy or gravity, or as a random process due to surface vortices. Cyprid larva is equipped with a pair of antennae and six pairs of thoracic legs which are used for attachment. Pheromones will guide the larva to areas which have already been occupied by conspecific individuals (Dreanno *et al.*, 2007), which is followed by an extensive exploration of the surface (Lagersson and Hoeg, 2002) and finally, cement secretion (Ödling *et al.*, 2006) and settlement, which is irreversible and permanent.

A key to prevent fouling, without killing, is to understand how the neurobiology governs the pre-attachment and pre-settlement behaviour. For that purpose, different pharmacological tools have been used to mimic or block neurotransmission and modulate the physiology of the barnacle larva. Since many of the physiological processes are communicated with neurotransmitters such as dopamine, serotonin or histamine, well-established pharmacological tools, may be used effectively for antifouling purposes. The underlying mechanisms are the same; neurotransmission releasing a neurotransmitter that activates its specific receptor on the target cell, inducing a cellular response. Those mechanisms are not different if studying humans or barnacles. Therefore, there are substances available that can be explored for antifouling purposes, often developed as pharmaceuticals and as such, specific, low toxicity and possible to synthesize at an industrial scale.

Early studies used antagonists, receptor blockers (Yamamoto *et al.*, 1996, 1998) as a way of blocking either attachment or settlement. One possibility is to use antagonists to inhibit cement secretion, which would block cement secretion and thereby, prevent settlement. It was discovered that cement secretion is controlled by dopamine (Okano *et al.*, 1996; Ödling *et al.*, 2006) and consequently, dopamine receptors controlling exocytosis and release of cement proteins (Ödling *et al.*, 2008). However, rather high concentrations of antagonists must be used to fully block cement secretion and settlement.

Metamorphosis is another target for new antifoulants. Several serotonergic and dopaminergic substances, both agonists and antagonists, have been investigated (Yamamoto *et al.*, 1996; Zega *et al.*, 2007) but with limited results. One of the most promising substances of natural origin, barretin, seems to have serotonergic characteristics (Hedner *et al.*, 2006).

The most promising way to inhibit fouling is probably preventing attachment; by keeping the larva away from the surface. A cyprid larva is dependent on its own mobility to reach a surface rather than relying on passive mechanisms. The larva has to move to be able to reach the laminar sublayer (Fig. 21.3). For that, it needs to rely on self propulsion and overcome the strong surface–water interaction forces. Only a small disturbance in the

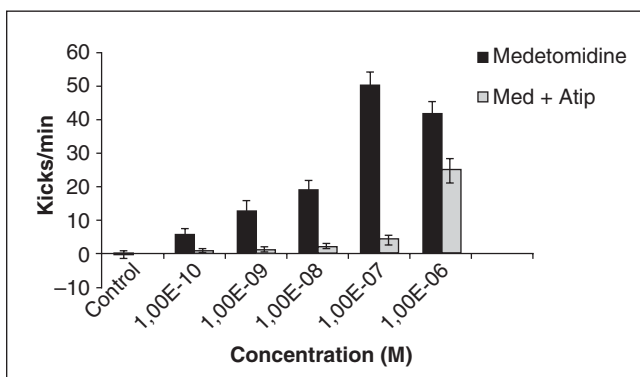


21.3 Leakage from a paint layer is a function of flow velocity and concentration of the leaking substance. The theory is that leaching rate is determined by the rate of hydrolysis and dissolution from the polymer matrix and then viscous and turbulent shear stress accelerate the diffusion of the molecule through the laminar sublayer and boundary layer into the bulk. It is also the laminar layer that is of interest because cyprid larvae attach to the surface. The ideal is that the biocide is accumulated within the laminar layer and does not diffuse into the bulk. A cyprid closing in on a medetomidine covered surface will be affected and avoid attachment. (Redrawn from Stanislaw R. Massel 'Fluid mechanics for marine ecologists', Springer-Verlag, Berlin 1999.)

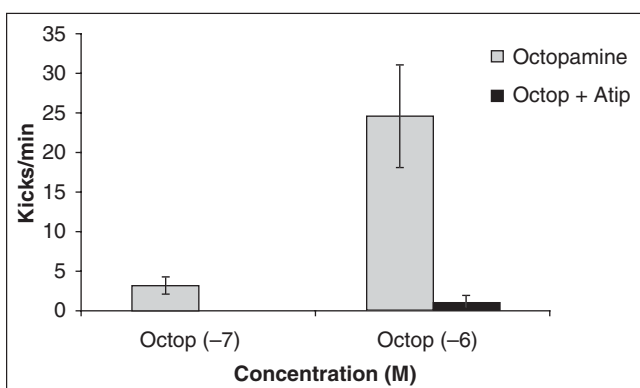
self-propulsion mechanisms will dramatically limit the possibility for the larva to reach the surface (Zilman and Benayahu, 2008).

Medetomidine is a pharmacological substance used within veterinary medicine for more than twenty years. It is known for its high efficacy in activating vertebrate α_2 -adrenoceptors (Lundström *et al.*, 1993). However, invertebrates are not equipped with adrenergic receptors, but instead use octopamine or tyramine receptors for corresponding biological responses (Roeder, 2005). The octopamine receptors are of three different subtypes (Evans and Maqueira, 2005) and can be stimulated by agonists. One of the octopamine receptor subtypes is similar to adrenergic α -adrenoceptors and the gene sequence is known for both *Balanus amphitrite* as well as *Balanus improvisus* (Isoai *et al.*, 1996; Lind, 2008). Among insects, this ortholog subtype is known to be activated and blocked by α_2 -adrenoceptor activating molecules (Minhas *et al.*, 1987). We believe that medetomidine acts through activating the octopamine receptor to induce change in motility (Figs 21.4 and 21.5). The increased motility will cause dramatic change in the larval chances to reach and attach a surface. Medetomidine will induce thoracic leg movement that cannot be controlled by endogenous behaviour and, therefore, the larva loses its ability of self-propulsion towards the surface. The mobility becomes haphazard and behaviourally controlled attachment becomes impossible.

A special characteristic of the medetomidine molecule is its surface affinity (Dahlström *et al.*, 2004). Whenever medetomidine is added into a solu-



21.4 Dose-response curve for medetomidine for the induction of swimming movements on cyprids of *B. improvisus*. The effect starts to show at 0.1 nM and reaches a maximum at 0.1 μ M (black bars). An antagonizing effect was seen with 1 μ M atipamezole (grey bars), a substance that is a competitive antagonist against medetomidine. $n = 35\text{--}60$. * = $p < 0.05$.



21.5 Also the endogenous neurotransmitter octopamine (0.1 and 1 μ M) induced increased swimming motility. As with medetomidine, the effect was effectively blocked by 1 μ M atipamezole. $n = 32\text{--}60$; * = $p < 0.05$.

tion, it will concentrate itself on the surfaces. The same phenomenon is hypothesized when medetomidine is incorporated into a paint matrix. Its low leakage rate and high efficacy even in very low amounts, point at a surface enrichment at the paint–water interface upon exposure. Taking into account such surface affinity, it is also possible to use medetomidine’s imidazole group for complexation reactions with the surface of metal oxides,

such as ZnO or CuO. By doing so, the medetomidine release process can be controlled and leakage rate can be kept low (Fant *et al.*, 2006).

One of the major advantages regarding medetomidine is its high intrinsic efficacy. Only very low amounts of the compound needs to be incorporated into a paint to have total barnacle free surfaces. After one year of field trials, only 0.025% w/w was needed to totally inhibit barnacle settlement. That means as well, that medetomidine leakage rate is low, estimated to be within low ng/cm²/day range (Dahlström, 2004; Bogert, 2004) and it will not affect the paint formulation *per se*. It can be incorporated in present formulations and, so far, we have not seen medetomidine interfere with other biocides. This is important since medetomidine will need a co-biocide to prevent algae fouling.

As a new antifoulant, ecotoxicological studies are needed to ensure its sustainability and environmentally benign profile. Medetomidine has shown very low predicted environmental concentrations (PEC) in prediction models (e.g., MAMPEC). To evaluate effects on non-target organisms and for risk assessment in the marine environment, a vast number of different bioassays on a number of marine organisms have been performed. It has been concluded that it has no bioaccumulative tendencies, it has no endocrine effects, and its lethality is low for all tested organisms. In the risk ratio, expressed as the PEC/PNEC (predicted no effect concentration), the values come out well below unity for all predicted environmental scenarios.

Copper and cuprous oxide is a limited natural resource and when used as antifoulant, impossible to recycle. Due to its toxicity, it is also questioned within marine areas with high maritime activity, such as the Baltic Sea, Chesapeake Bay or San Diego Bay. Also, the cost for cuprous oxide has increased dramatically during the last years and to predict a future price is indeed difficult. Medetomidine is a promising candidate to reduce or omit cuprous oxide within marine antifouling paints. It has an efficacy profile against hard foulers and especially barnacles, it has a benign environmental profile, and it can be produced industrially and incorporated into existing paints, shortcutting the need for long term paint formulation and evaluation. Furthermore, the leakage rate is low and can be controlled and it will be able to combine medetomidine with different co-biocides, needed to control algae fouling.

By using an in-depth knowledge about a fouling organism, it has been possible to develop a new antifouling substance. It indicates that a rational approach based on sound science will in the future be the platform for development of other antifouling substances. For the future developmental work, industry, regulatory bodies representing society, as well as science, will benefit from an approach not relying only on substance toxicity. It is indeed possible to use other endpoints than death in preventing marine biofouling.

21.3 The use of condensed tannins

The use of antifouling paints is the only truly effective method for the protection of underwater structures from the development of fouling organisms. However, they are based on toxic compounds (usually copper) that are dangerous to natural environment. A promising alternative to these compounds is the use of natural products or synthetic ones (that occur in natural organisms) that are non-toxic and present good antifouling properties.

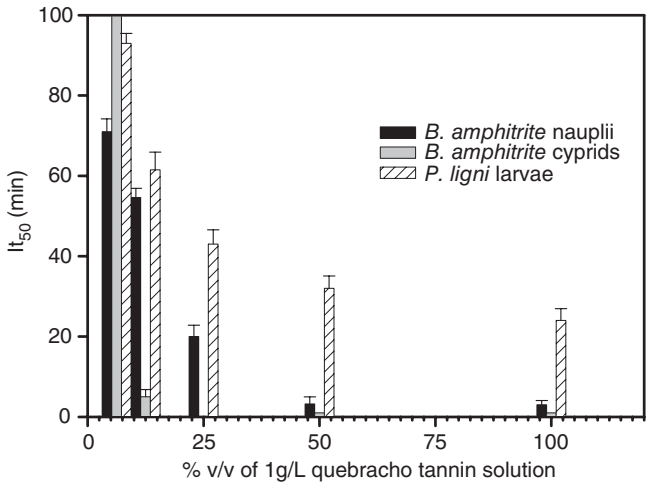
Tannins are natural compounds usually defined as water-soluble polyphenolic substances that have high molecular mass (> 500). They are common in brown algae and higher plants and possess the ability to precipitate proteins. They have also been described to have anticarcinogenic properties and offer cellular protection from oxidative damage. In addition, tannins showed anticorrosive properties and could be used as pre-treatment primers for use of rusted steel without requiring complete removal of the corrosion product (Knowles and White, 1958; Matamala *et al.*, 2000).

Phlorotannins are a type of tannins enclosed in subcellular structures of brown algae (Ragan and Glombitza, 1986). They are polymerized phloroglucinol (1,3,5-trihydroxybenzene) and are extremely water soluble; phlorotannins are released continuously from the algae under normal conditions but at an increased rate under stress. Some antifouling properties have been described: phlorotannins exuded from *Ralfsia verrucosa* into the surrounding environment reduced the survival of barnacle, mussel larvae and algal species and also showed inhibitory effects with microorganisms (Lau and Qian, 1997). In addition, molecules structurally related to phlorotannins, such as phloroglucinol and tannic acid, have also been suggested to be detrimental to both invertebrates and microorganisms (Sieburth and Conover, 1965; Ryland, 1974; Targett and Stochaj, 1994; Lau and Qian, 1997).

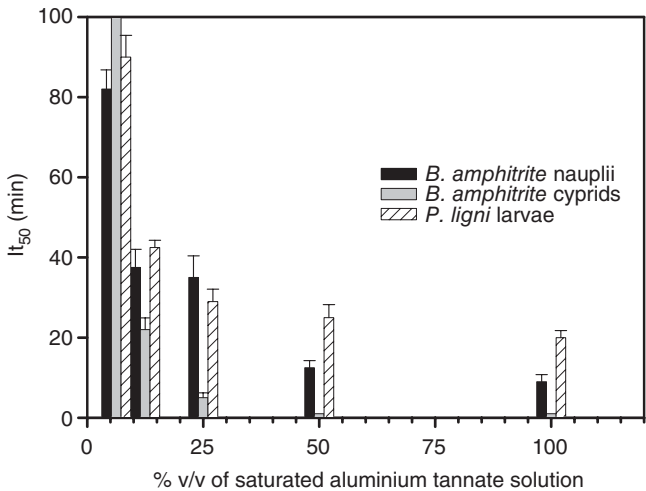
It is well known that condensed tannins have inhibitory properties; they are used as antifungal agents against yeast and bacteria (Zucker, 1983; Scalbert, 1991; Digrak *et al.*, 1999). However, few data are available on the activity of condensed tannins on fouling macroorganisms (Ayoub, 1982). In an earlier study (Pérez *et al.*, 2006), it could be established that quebracho tannin, condensed tannin from *Schinopsis* sp., combined with a low copper content have an antifouling performance as good as a conventional paint.

Current antifouling technology is focused to the use of environmentally friendly compounds. One of the alternative technologies to heavy metal-based antifouling paints is the development of coatings whose active ingredients are compounds that occur in nature. However, the excessive use of natural products can promote environmental damage. Fortunately, several natural compounds like tannins or mimetic ones can be synthesized in the lab by means of green chemical procedures (Feldman *et al.*, 2000; Halkes *et al.*, 2002; Saito *et al.*, 2003).

Some experiments revealed that quebracho tannin (*Schinopsis* sp.) and aluminium tannate, a less soluble compound obtained from quebracho tannin, inhibited the activity of nauplii II and cyprids of *Balanus amphitrite* (Cirripedia, Balanidae) and 15–16 setigerous larvae of *Polydora ligni* (Polychaeta, Spionidae) in a broad range of concentrations (Figs 21.6 and 21.7). In addi-



21.6 Fifty inactivity time (It_{50}) for *B. amphitrite* and *P. ligni* larvae in dilutions from 1 g/L quebracho tannin solution. Error bars indicate SE.

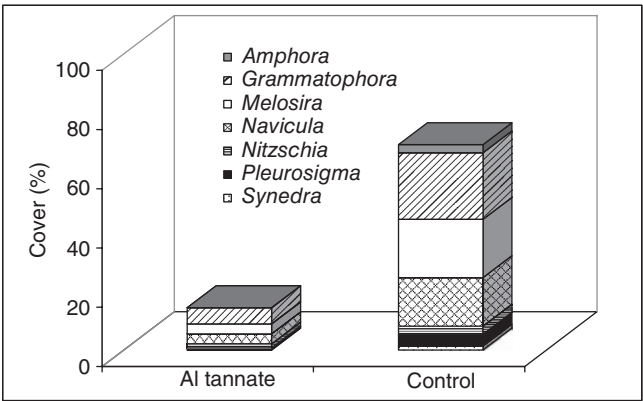


21.7 Fifty inactivity time (It_{50}) for *B. amphitrite* and *P. ligni* larvae in dilutions from saturated aluminium tannate solution. Error bars indicate SE.

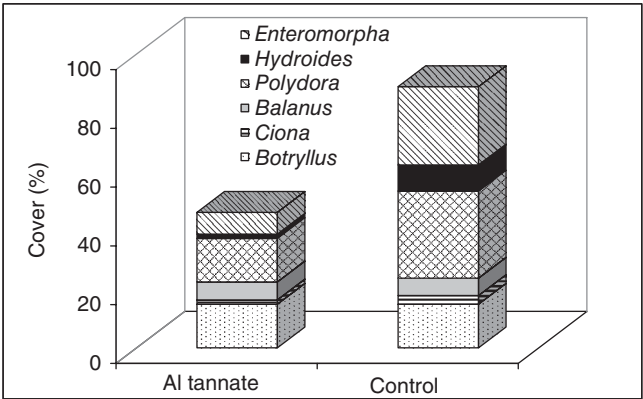
tion, aluminium tannate solutions showed low pH values and this suggests that larvae were affected not only by anion tannate but also by pH decrease (Pérez *et al.*, 2001). The loss of activity was not an indication of the death of organisms, because when larvae were transferred to fresh, non-toxic artificial seawater after exposure which made them inactive, they were able to recover immediately and follow their development.

Aluminium tannate gels exposed in Mar del Plata harbour (Argentina) for 28 days showed a clear antifouling effect: fouling cover was significantly lower for both micro- and macrofouling species than those on the control gels (Figs 21.8 and 21.9) (Pérez *et al.*, 2007).

The power of laboratory-based bioassays is the rapid screening of potential compounds for antifouling toxicity and effectiveness (Rittschof *et al.*,



21.8 Cover percentage for microfouling organisms.



21.9 Cover percentage for macrofouling organisms.

1992). However, there are some aspects to compare between lab and field trials, for instance organism response in the static conditions of water vs. flow conditions (Hay *et al.*, 1998) and the use of one or two species for evaluating compound activity in the lab vs. all fouling organisms in the sea (Rittschof, 2001).

As a result of the need for environmentally safe antifouling systems, in the last twenty years a wide variety of natural compounds have been isolated and identified as settlement inhibitors. However, the possibility of carrying out lab-synthesis of antifouling compounds with molecular structure similar to natural ones applying the postulates of green chemical procedures (i.e., final and intermediate products should be non-toxic) allows a new perspective for antifouling technology. Undoubtedly, the identification of an active compound and its properties is just one of the steps before they can be incorporated in an antifouling formulation. A good antifouling coating will probably need to contain a number of different compounds in order to ensure that all biofouling organisms are repelled from a surface. The use of quebracho tannin is an environmentally benign option for antifouling technology, and is a cheap natural product. It is recognized that compounds with high solubility represent a disadvantage for a marine paint. However, tannin precipitation as aluminium tannate solved this problem because this compound is less soluble and is as effective as quebracho tannin. It is suggested that tannins, their derived and mimetic-tannin compounds are potentially very important pigments for antifouling coatings technology.

21.4 Conclusions

It is clear that investigations about novel candidates to replace copper are in their infancy. The present chapter examines some of the available data on new organic alternatives to copper. Synthetic compounds (benzoates and medetomidine) and natural-origin products (quebracho tannin) showed antifouling activity against fouling organisms in lab and field trials; they are chemical compounds which are commercially available at low cost and are effective in small amounts.

The next step regarding medetomidine is registration according to the regulatory demands within the European Union as well as EPA. It is known from scientific studies that it has a benign profile regarding non-target organisms, fate in the environment and it does not bioaccumulate. Together with the fact that leakage is low and release is possible to control, it will minimize concentrations in the marine environment and consequently undesirable effects. In the case of benzoates and condensed tannins, further research is needed to investigate responses of non-target organisms before commercialization; also information on degradation, bioavailability

and bioaccumulation is required to determine environmental risks associated with their use and to predict realistic concentrations in natural environments.

Recently, several alternative antifouling agents have reached the market. The so-called organic booster biocides can be classified into metallic and non-metallic compounds. The first group includes maneb, zinc pyrithione, zineb and ziram, and the second one includes Borocide™ P, chlorothalonil, dichlofluanid, diuron, Ecomea™, Irgarol, Kathon, Sea-Nine™, TCMS pyridine, TCMTB, thiram and tolylfluanid (Voulvoulis *et al.*, 1999, 2002; Thomas *et al.*, 2001, 2003; Omae 2003; Konstantinou and Albanis, 2004; Yebra *et al.*, 2004; Chambers *et al.*, 2006). Some of these compounds are incorporated into ablative or self-polishing coatings (e.g., Alumacoat SR, Mission Bay, E Paint, Interlux Pacifica, SeaAlu Jotun, Pettit Vivid Free). There is, however, a growing concern about the environmental fate and potential risks of these booster biocides and their use has been banned or restricted in many countries.

Another implication of a future abandonment of copper, hardly ever discussed, is the role of Cu₂O particles as polishing and co-biocide leaching modulator (Kiil *et al.*, 2002) (Chapter 14). If Cu₂O was to be removed from current paint formulations, an alternative pigment/filler should be added in large amounts (probably over 15% in volume of the dry film) in order to attain reasonable pigment volume concentrations (see Chapter 13). These pigments/fillers should not retard the paint polishing process, and must allow the use of fairly hydrophobic, controlled-release binders (see Chapter 8), otherwise the fast water ingress into the film would jeopardize the integrity and the controlled release properties of the paint. To date, such metal-free soluble pigment replacement is not available. Innovative ways to solve this inconvenience are to obtain a less soluble compound by precipitation and/or to use microencapsulation techniques, as this allows a controlled release, permitting the work-life of the protective coating to be increased.

However, there are pigments that have a comparable solubility to cuprous oxide that could be incorporated into a soluble matrix system with successful performance, e.g. ferric benzoate.

21.5 References

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Natural marine products with antifouling activities

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Abstract: Marine organisms have usually been viewed as sources of compounds with interesting and useful biological activities, in particular with the ability to inhibit attachment, settlement, or growth of various marine organisms, i.e. with antifouling (AF) activity. Over hundreds of millions of years, marine organisms evolved in an environment where strong competition for space and epibiosis pressure selected for the presence of AF mechanisms, of which one that seems to be particularly widespread is the use of natural marine products with AF activity. In this chapter, we pursue the objective of reviewing, although not exhaustively, the recent (last ten years) research on marine natural AF products. We preferred rather to focus on interesting cases that would then allow the reader to understand the main trends that emerge from this field, and indicate future and promising directions of research. Among all marine organisms, sponges seem to constitute the most prolific source of novel antifoulants, most likely due to their association with single-cell symbionts. Marine macroalgae are also prominent producers of antifoulants, and within this group, red seaweeds seem particularly active. Although the tropical marine organisms are usually seen as better defended than their extra-tropical counterparts, current data does not support a latitudinal pattern in AF defences. However, the production of AF compounds has been demonstrated to vary spatially (i.e., geographically) and temporally (i.e., seasonally), in the limited number of cases that have been investigated. Similarly, only a few studies have assessed the activity spectrum of natural antifoulants in field experiments. The means to produce natural marine products at a large scale appears possible in a number of cases, either by mass cultivation or by laboratory synthesis. Nevertheless, there are significant regulatory hurdles that must be overcome before any new antifoulant reaches the market.

Key words: natural products, extracts, bioactivity, invertebrate, algae, microorganisms, chemical communication, epibiosis.

22.1 Introduction

As replacements for tin-based coatings, most marketed formulations include high concentrations of heavy metals, e.g. copper and zinc, in combination

with booster biocides such as Irgarol, Diuron and SeaNine 211 (Chesworth *et al.*, 2004; Konstantinou and Albanis, 2003; Chapter 12). As concerns have been expressed about the environmental loading and effects of these alternatives (Omae 2003), non-toxic or environmentally benign alternatives to control surface colonisation are desirable. One suggested route is to exploit natural defences utilised by marine organisms against epibionts, specifically, natural marine product (NMP) antifoulants (Clare, 1996; Clare, 1998; Rittschof, 2000). Many sessile marine organisms do not present, *a priori*, any physical or mechanical means of defence against possible colonisers, or predators, but nevertheless resist overgrowth by epibionts that colonise adjacent living and artificial surfaces. This ability is associated primarily with products of secondary metabolism, which are well known for their role in the chemical defence of marine organisms (Amsler, 2008). One particularly exciting area that is being pursued is the ability of the basibiont to 'attract' epibionts (e.g., bacteria) that will deter further colonisation of the surface by chemical means. The aim of all these studies is to identify allelochemicals, notably allomones, as lead compounds that have commercial potential as antifoulants.

Beyond biological activities, current studies are focusing on ecological roles of molecules (Amsler, 2008), their localisation in organisms and their efficacy at physiological concentrations (Sudatti *et al.*, 2008). This interest stems from the knowledge that certain organisms present antifouling (AF) activity at their surface due to secretion of molecules, whereas other organisms contain molecules with AF activity within their bodies, where they would not necessarily be implied in such a process of biological defence (Steinberg and de Nys, 2002a). However, fouling organisms are not necessarily restricted to the surface of basibionts, and some are known to penetrate the bodies of their host organisms, such as macroalgae, where they would experience within-body concentrations of natural products (Pereira and Da Gama, 2008).

The characterisation of NMPs has enjoyed considerable success (Blunt *et al.*, 2003 to 2007), but relatively few have been identified with AF action. The challenge is to characterise a natural product with low toxicity, stable in a paint formulation, biodegradable when released, with broad-spectrum activity and which can be obtained in commercially relevant quantities either by chemical synthesis, recombinant technologies and culture (aquaculture, *in vitro* and fermentation technology; See also Rittschof Chapter 28). The literature of AF tests suggests that sponges, corals and macroalgae and/or their associated microflora have the most potential for exploitation.

Solvents of different polarity can be used to isolate a wide range of molecules from the same organism. In this chapter, we have summarised the recent results of screening extracts of invertebrates for AF properties (see Tables 22.1–22.7). These extensive results will not be commented upon, but

we chose to present the information as it would be useful for natural product chemists who are looking for specimens to investigate. A set of AF tests using targeted biofouling species (barnacles, bryozoans, tubeworms, mussels and a wide variety of microorganisms; see Chapter 12 on laboratory bioassays) are carried out using various concentrations. Field tests are more seldom performed, although they are always more ecologically relevant than laboratory tests (Da Gama *et al.*, 2008a).

To date, purification of active products has yielded *ca.* 200 molecules with some degree of AF activity against a wide range of marine fouling organisms, assayed mainly through laboratory tests. Clare (1996, 1998), Fusetani (2004), Rittschof (2000), Railkin (2004), and Chambers *et al.* (2006) have reviewed the literature on natural marine products with AF activity, while Lane and Kubanek (2008) focused specifically on antifoulants from photosynthetic organisms, such as seaweeds, and Krug (2006) reviewed the literature on antifoulants from marine invertebrates.

Development of natural AF research projects is supported worldwide but the challenge of finding a natural product which fulfils the required criteria of low toxicity, broad-spectrum activity and ease of production has yet to be realised and is the main reason why they have not been so far successfully commercialised. There are various options available for a sustainable production of NMPs: chemical synthesis, controlled harvesting, aquaculture, *in vitro* production, microbial fermentation and transgenic or enzymatic production. Despite the fact that all these technologies are available, the only NMPs that have been scaled up are for pharmaceutical applications.

In this chapter, marine AF natural product research of the last ten years has been reviewed, but not exhaustively. Rather, we preferred to focus on interesting cases that would then allow the reader to understand the main trends that emerge from this field, and indicate future and promising directions of research.

22.2 Microorganisms

Unlike inanimate surfaces, which are colonised in a predictable manner by a diverse assemblage of marine microbes (Wahl, 1989), submerged biotic surfaces frequently harbour species-specific microbial communities that can be highly variable and distinct from those found in the surrounding environment (Engel *et al.*, 2002). Interactions between epiphytic bacteria and their host play a central role in marine ecology. Understanding the types of microbial behaviours that may be chemically mediated, such as biofilm formation, is of high importance for the development of new AF formulations (Amsler *et al.*, 2000). The impact of the formation of biofilms on the subsequent colonisation by other organisms is not well understood.

22.2.1 Microbe–microbe interactions

Numerous bacteria employ cell-cell communication systems that rely on small signal molecules to express certain phenotypic traits in a density-dependent manner. This regulatory principle is referred to as quorum-sensing (QS) (Fuqua *et al.*, 2004). Among Gram-negative bacteria the most intensively investigated signalling molecules are N-acyl-homoserine lactones (AHLs). At low population densities cells produce a basal level of AHL via the activity of the AHL synthase. As the cell density increases, AHLs accumulate in the growth medium. On reaching a critical threshold concentration, the AHL molecule binds to its cognate receptor, which in turn activates or inhibits expression of target genes (Fuqua and Greenberg, 2002). Using QS, bacterial populations can coordinate important biological functions including aggregation, biosynthesis of antibiotics or biofilm maintenance and differentiation (Romero *et al.*, 2008). The disruption of signalling could be a good approach for the development of new AF formulations. Inhibition of the QS (mechanism known as quorum quenching (QQ)) is a strategy adopted by many organisms to inhibit the growth of a potential competitor. de Nys *et al.* (2001), who investigated the natural products chemistry of *Vibrio angustum* S14, isolated a new compound ([1-(2'-methylpropoxy)-2-hydroxy-2-methylpropoxy]butane) from the stationary phase supernatant which was able to up-regulate the AHL reporter system in *Agrobacterium tumefaciens* and bioluminescence in *Vibrio harveyi*.

Leadbetter and Greenberg (2000) addressed the question of whether AHLs can be degraded biologically and demonstrated that *Variovorax paradoxus* was able to degrade acyl-HSLs and utilise the products as its sole energy and nitrogen sources. Interestingly, some bacteria are able to block the AHL-based QS through an enzymatic inactivation. Thus, two main types of enzymes have been linked with inactivation of the signal molecules: the lactonases and the acylases (Dong *et al.*, 2007). Lactonases have been detected in several strains of *Bacillus*, *Arthrobacter* and *Klebsiella* (Dong and Zhang, 2005). AHL-cyclase activities have been highlighted in various strains of *Pseudomonas*, *Ralstonia*, *Streptomyces*, *Rhodococcus*, *Variovorax* and *Anabaena* (Romero *et al.*, 2008). The main advantages for the use of such enzymes in marine paints are that they are active at temperatures that are relevant to maritime operations (15–25 degrees). Difficulties in the use of enzymes as antifoulants are further discussed in Chapter 23 'Enzyme-based solutions for marine antifouling coatings'.

Boyd *et al.* (1999) and Hentschel *et al.* (2001) screened bacterial strains isolated respectively from macroalgal and sponge surfaces for their antibiotic properties against a range of fouling bacteria. They found that 30%

of the surface-associated isolates showed antibiotic properties. A general pattern was observed in that Gram-positive bacteria inhibited Gram-positive strains while Gram-negative bacteria inhibited Gram-negative isolates.

22.2.2 Microbe–algae interactions

Many reports have revealed the existence of bacteria capable of killing phytoplankton. These compounds used by algicidal bacteria that require prey contact to kill (e.g. *Cytophaga* and *Saprospira*) remain largely uncharacterised, although they may be similar to those from bacteria that kill through dissolved algicides (e.g. *Alteromonas* and *Pseudomonas*) (Mayali and Azam, 2004). Active compounds are released from the surface only after contact with the prey. An antimicroalgal substance, 2-[methyl-(3-phenylpropionyl)amino]-benzoic acid, isolated from a culture of the marine *Streptomyces* sp. strain B7747, showed activity against *Chlorella vulgaris*, *C. sorokiniana* and *Scenedesmus subspicatus* (Minimum Inhibitory Concentration (MICs) $\geq 20 \mu\text{g ml}^{-1}$) (Biabani *et al.*, 1997). The activity is lower than the commercially available antibiotics but could be potentially improved by slight modification of the chemical structure during synthesis. *Pseudoalteromonas piscicida* inhibit the growth of microalgae due to the production of an unknown brominated antibiotic (Bowman, 2007) that can lyse algal cells in a matter of hours.

Pseudomonas aeruginosa biofilms have been shown to have anti-microalgal properties towards phytoplanktonic species such as *Chatonella*, *Gymnodinium*, *Heterosigma* and *Skeletonema* (Lee *et al.*, 2000). Ecto-enzymes, particularly ectoproteases, are believed to be the algicidal agents (Lee *et al.*, 2000). An extracellular serine protease, which is regulated by AHLs, is responsible for the algicidal activity of *Pseudoalteromonas* sp. strain A28.

Attachment of *Ulva* zoospores is also affected by bacterial biofilms (Joint *et al.*, 2000). Monospecies biofilms of specific strains of bacteria displayed an age-dependent stimulation of spore settlement. Using *Vibrio anguillarum* as a model system, a number of lines of evidence suggested that the stimulation of spore settlement by this strain is a response to certain AHLs involved in bacterial QS (Joint *et al.*, 2002). *Ulva* spores, however, responded differently to monospecific biofilms of different bacterial strains (Patel *et al.*, 2003). The effect of bacterial biofilms could not be assigned to species or even genus level. Moreover, the analysis was complicated by strain-specific influences of biofilm age. Patel *et al.* (2003) concluded that one or more mechanisms of action or types of cue might be involved. Bacterial QS signal may elicit specific responses in eukaryotes relating to the nature of the microbial interaction (Wheeler *et al.*, 2006). Elucidating such

phenomena could lead to new ways of inhibiting the settlement of *Ulva* and perhaps other organisms.

22.2.3 Microbe–invertebrate interactions

Production of active compounds

In an extensive screening study of 163 marine bacterial strains, Holmström *et al.* (1996) reported significant settlement inhibitory activities against barnacle (cypris of *Balanus amphitrite*), macroalgae (*Ulva lactuca*) and diatom (*Amphora* sp.). It is suggested that most *Pseudoalteromonas* species are efficient producers of AF agents that may aid the host against colonisation of its surface by other epibionts. To determine the extent to which AF activity and the production of bioactive compounds are distributed amongst the members of the genus *Pseudoalteromonas*, Holmström *et al.* (2002) tested 10 different species, in a broad range of bioassays. *P. tunicata* exhibited the broadest range of inhibitory activity (on settlement of *Hydroides elegans* and *B. amphitrite*, and spores of *U. lactuca* and *Polysiphonia* sp., growth of bacteria and fungi). *P. tunicata* produces at least four target-specific AF compounds (James *et al.*, 1996; Holmström *et al.*, 1998; Boyd *et al.*, 1999). A positive correlation between the AF activity of the dark form of *P. tunicata* and the expression of pigmentation was demonstrated (Egan *et al.*, 2002). Using transposon mutagenesis, the loss of AF activity was correlated with the loss of the yellow pigment. The identification of a gene involved in regulating the expression of AF phenotypes would be a major contribution to understanding the interactions between bacteria and other surface-colonising organisms in the marine environment.

Two strains (UL12 and UL13) of *Pseudoaltermonas ulvae* (collected from *U. lactuca* surface) inhibited the germination of *U. lactuca* and *Polysiphonia* sp. spores and settlement of *B. amphitrite* cyprids (Egan *et al.*, 2000, 2001).

Seawater conditioned by *Ulva reticulata* inhibited larval settlement of *B. neritina* (Walters *et al.*, 1996) and *H. elegans* (Harder and Qian, 2000). The putative AF compound (isolated from *Vibrio* sp. collected from *U. reticulata*) had a molecular weight of > 100 kDa and inhibited larval settlement of *H. elegans* induced by 3,7-Dihydro-1-methyl-3-(2-methylpropyl)-1H-purine-2,6-dione (IBMX) (Dobretsov and Qian, 2002). This is evidence for an unknown pathway that can either inhibit or enhance the metamorphosis of larvae. Overall it would appear that the AF mechanism of *U. reticulata* is dependent not only on materials from the alga itself but also on the epibiotic bacteria on the algal surface.

Lee and Qian (2003) analysed the effects of monospecific biofilms (from bacteria isolated from the surface of the sponge *Mycale adherens*) on settlement of the polychaete *Hydroides elegans*: 75% were non-inductive

and 40% were inhibitory. The authors hypothesised that *M. adherens* has the ability to nurture a specific surface-associated bacterial community that controls macrofouling on its surface.

Olguin-Urbe *et al.* (1997) isolated 6-bromoindole-3-carbaldehyde from *Acinetobacter* sp., a bacterium associated with the ascidian *Stomozoa murrayi* that exhibited *in vitro* settlement inhibition of barnacle larvae and moderate antibacterial properties.

Importance of biofilm in the inhibition of invertebrate settlement

Wieczorek and Todd (1997) studied the effect of biofilm age on settlement of the bryozoan *Bugula flabellata* and the ascidian *Ciona intestinalis*. They concluded that while larvae of *B. flabellata* were inhibited by biofilm, irrespective of film age, settlement of *C. intestinalis* larvae was facilitated on biofilmed substrata, and the numbers of settled larvae generally increased with biofilm age.

Lau *et al.* (2003) isolated *Micrococcus* sp., *Rhodovulum* sp. and *Vibrio* sp. from natural biofilms. When *B. amphitrite* cyprids were presented with a 'choice' of filmed surfaces prepared from these isolates and unfilmed surfaces, the latter were favoured. Inhibitory activity required larval contact with the biofilm rather than the perception of diffusible bacterial products. *Vibrio* was the most inhibitory of the strains assayed. A correlation between increasing *Vibrio* cell density and inhibition of cyprid settlement was highlighted, suggesting that the response was not merely modulated by free-space availability. The general inhibitory effect of bacteria on barnacle settlement extends to isolates from barnacle shell plates. Khandeparker *et al.* (2003) isolated *Bacillus pumilus* and *Citrobacter freundii* from the shell surface of *B. amphitrite* and found biofilms of both strains to be inhibitory to cyprid settlement.

The inhibition of barnacle cyprid settlement by bacterial biofilms in the laboratory has been confirmed in the field. Olivier *et al.* (2000) determined that *B. amphitrite* cyprids are inhibited from settling on surfaces that bear bacteria and that the degree of inhibition increased with biofilm age. Lau and Qian (2000) concluded that the general inhibitory effect of bacteria suggests that they are a rich source of potential natural inhibitors of barnacle settlement.

22.2.4 Testing of bacteria and products in prototype coatings

Armstrong *et al.* (2000) combined active compounds from the cells and culture supernatants of two bacterial strains (F55 and NudMB50-11), isolated from the surface of *Fucus serratus* and *Archidoris pseudoargus*, with acrylic-based paint resin and assayed for AF activities. This was the

first report of the formulation of a marine paint that incorporated marine microbial natural products and represented a significant advance towards the production of new environmentally-friendly AF technologies that utilise a sustainable supply of natural biodegradable compounds.

Perry *et al.* (2001) demonstrated that metabolites from *Pseudomonas marina*, when combined with a polyurethane coating, led to a total inhibition of settlement of *B. amphitrite* and stopped the complete development of *B. neritina* larvae. Cellular components or extracellular metabolites of *P. marina* were suggested as the active agents. The maintenance of activity of the bacterial metabolites within a polyurethane coating suggested that it might be possible to create an effective antifouling coating using these chemicals.

Yan *et al.* (2002) studied two marine bacterial strains (EI-34-6 and II-111-5) isolated from the surface of *Palmaria palmata*. The culture supernatant exhibited a different spectrum of antibiotic activity when the strains were grown using an agar roller bottle method compared to shaking flask cultures or non-agar bottle cultures. These results suggested that biofilm formation is an important factor in the production of antimicrobial compounds by these two strains and that there is a potential to increase production of useful secondary metabolites such as antibiotics from marine epibiotic bacteria; the controlling factors are likely to be freely diffusible from the biofilm. Yan *et al.* (2003) reported biofilm-specific, cross-species induction of antimicrobial compounds in *Bacilli*. They designed an air-membrane surface (AMS) bioreactor to allow bacteria to grow attached to a surface as a biofilm in contact with air. Bacteria grown in that way were able to induce planktonically grown cells to behave as if they were in a biofilm by regulating the expression of pigments and antimicrobial compounds.

Biojelly® is a polymer that is formed on a cellulose acetate membrane immersed in seawater (Hayase *et al.*, 2003), which inhibits attachment of marine organisms such as algae and barnacles. Hayase *et al.* (2003) successfully isolated several marine bacteria from Biojelly®-attached microorganisms. One of these isolates, a strain of *Alteromonas* SHY1-1, produced water-insoluble polymeric materials in natural seawater supplemented with yeast extracts and glucose. Biojelly® and the polymer film produced by *Alteromonas* sp. SHY1-1 were characterised by Fourier transformed infrared (FT-IR) spectroscopy and thin-layer chromatography (TLC) as a mucopolysaccharide consisting of amino sugars. In addition, both polymer films had similar antifouling activity against barnacle (*B. amphitrite*) cypris larvae.

22.3 Macroalgae

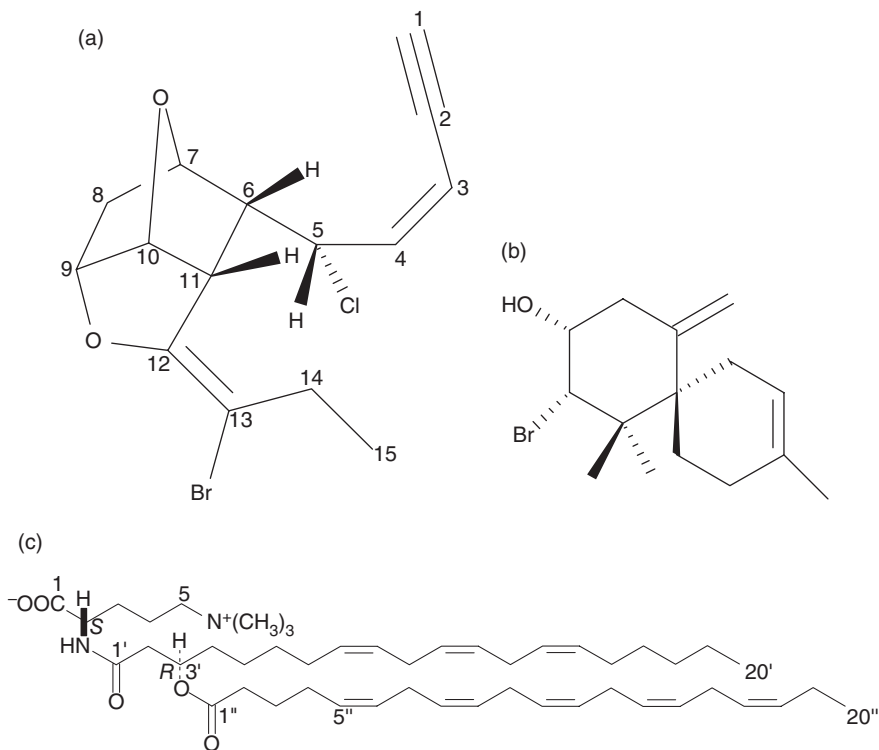
Marine macroalgae have developed chemical cues to deter settlement of organisms involved in biofouling (Walters *et al.*, 1996; Steinberg *et al.*, 2002a,b). The localisation and surface quantification of these antifouling compounds

has been studied to understand their ecological roles and the chemical defence strategies of seaweeds (de Nys *et al.*, 1998; Dworjanyn *et al.*, 1999; Salgado *et al.*, 2008; Sudatti *et al.*, 2008). In order to determine biological activities of algae, a large range of extracts was tested against strains of bacteria and fungi, algal spores and marine invertebrate larvae (Steinberg *et al.*, 1997; Hellio *et al.*, 2000a, 2000b, 2001a, 2001b, 2002, 2004b; Da Gama *et al.*, 2002, 2003, 2008a, b; Nylund and Pavia, 2003). The extracts displayed both species-specific and broad-spectrum activity against assays organisms (de Nys *et al.*, 1996) and varied seasonally and geographically with species (Amade and Lemee, 1998; Wright *et al.*, 2000; Hellio *et al.*, 2004a). Screenings of crude extracts of whole organisms offer one route to identify putative natural product antifoulants if used in a bioassay-guided purification strategy. The vast majority of studies, however, did not identify the active compounds. Most research on purifying natural product antifoulants has been carried on Rhodophyta species and to a lesser extent on Phaeophyta.

22.3.1 Purified products

Red algae

Species of the genus *Laurencia* have been extensively investigated and are a well-known source of bioactive halogenated and non-halogenated secondary metabolites (Masuda *et al.*, 1997). More than 300 compounds have been isolated and classified in four groups: sesquiterpenoid, diterpenoid, triterpenoid and C₁₅ bromoethers. *Laurencia* species have been the most extensively studied for its potential AF potency. Antimicrobial compounds, elatol, and lembyne-A (Fig. 22.1a) from *Laurencia* spp., and iso-obtusol from *L. majuscula* exhibited activities towards marine bacteria with promising MIC values ranging from 5–30 µg/disc for elatol, 20–60 µg/disc for lembyne-A and 10–15 µg/disc for iso-obtusol (Vairappan *et al.*, 2001a), which is comparable to commercially available antibiotics. More recently, Da Gama *et al.* (2002, 2003), Pereira *et al.* (2003), Sudatti *et al.* (2006, 2008), and Salgado *et al.* (2008) performed a complete investigation of the ecological roles of elatol for the Brazilian specimens of *Laurencia obtusa*, including laboratory (barnacle larvae and juvenile mussel attachment) and field AF assays (using phytigel to control diffusion), herbivory assays, surface detection, quantification and testing, and finally, elucidating the storage, transport, and exocytosis. It was concluded that secondary metabolites, later identified as elatol (Da Gama *et al.*, 2003; Sudatti *et al.*, 2006, 2008; Salgado *et al.*, 2008), inhibited significantly the settlement of a natural assemblage of fouling organisms (Da Gama *et al.*, 2002). This same species from Australia was also analysed by de Nys *et al.* (1998) to determine the surface concen-



22.1 Examples of active compounds purified from red algae: (a) lembyne-A, (b) (6R,9R,10S)-10-bromo-9-hydroxy-chamigra-2,7(14)-diene, (c) yendolipin.

trations of aplysistanin and palisadin A. These compounds were found at very low concentration (ng cm^{-2}) compared to the whole plant extract concentration. Levels of halogenated furanones were also measured in the red alga *Delisea pulchra*, demonstrating the ecological role of these compounds in protecting against epibiosis (Dworjanyn *et al.*, 1999). Vairappan *et al.* (2001b) purified three compounds from *Laurencia* species: (6R,9R,10S)-10-bromo-9-hydroxy-chamigra-2,7(14)-diene (Fig. 22.1b) (brominated sesquiterpene) from *L. majuscula*, and laurintenol and iso-laurintenol from *L. nidifica*. All were active against various marine bacteria with MIC values $\leq 20 \mu\text{g disc}^{-1}$. Anti-settlement compounds have been isolated by Konig and Wright (1997) from *L. rigida* (elatol and deschloroelatol) that inhibited settlement of *Balanus amphitrite* and *Bugula neritina*, but showed high toxicity against the barnacle cyprids. An invasive species of *Laurencia* recently found in Brazil (*L. caduciramulosa*) is also known to produce

interesting antifouling compounds (Cassano *et al.*, 2008), active against the attachment of juvenile mussels (*Perna perna*).

Two previously reported halogenated monoterpenes, 3,7,11,15-tetramethylhexadec-1-en-3-ol and 3,7,11,15-tetramethylhexadec-1-phyten-3-ol, were purified from *Plocamium costatum* and were significantly active against cyprid settlement of the barnacle *B. amphitrite* (CH_2Cl_2 extracts, 10 to 1 $\mu\text{g}/\text{cm}^2$) (Konig *et al.*, 1999a,b). Isethionic acid and floridoside purified from *Grateloupia turuturu* (Simon-Colin *et al.*, 2002, 2003) were shown by Hellio *et al.* (2004a) to inhibit settlement of *B. amphitrite* cyprids. Floridoside had the advantage of preventing cyprid settlement at non-toxic concentrations (0.01 $\text{mg}\cdot\text{ml}^{-1}$).

An allelopathic polyunsaturated fatty acid, (5Z,8Z,11Z,14Z,17Z)-eicosapentaenoic acid, isolated from 4 *Neodilsea yendoana*, *Palmaria palmata*, *Chondrus yendoii* and *Ptilota flicina* and a lipobetaine, (2S)-2-[(3R)-3-[(5Z,8Z,11Z,14Z,17Z)-5,8,11,14,17-icosapentaenoyloxy]-(8Z,11Z,14Z)-8,11,14-icosatrienoylamino]-5 trimethylammoniopentanoate (Fig. 22.1c) (yendolipin) isolated from *N. yendoana*, displayed growth-inhibitory activity against the green alga, *Monostroma oxyspermum* (Suzuki *et al.*, 1996; Matsuo *et al.*, 1997).

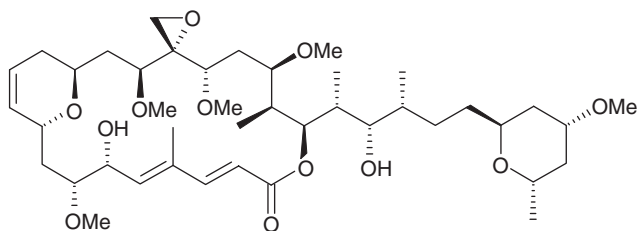
Brown algae

Concerning Phaeophyta, research effort has concentrated on the genera *Dictyopteris*, *Bifurcaria*, *Lobophora*, *Sargassum* and *Dictyota*. Kubanek *et al.* (2003) tested the hypothesis that *Lobophora variegata* is chemically defended against microbial pathogens, epiphytes and saprophytes. They purified a polycyclic macrolide, Lobophorolide (Fig. 22.2a), with strong antifungal activity. Similarly, Barbosa *et al.* (2007) isolated and tested the extract and a purified dolabelladiene diterpene from *Dictyota pfaffii*, in the laboratory and in the field, where it exhibited AF activity. Most recently, four structurally related diterpenes from *D. cervicornis* significantly inhibited the establishment of the mussel *Perna perna* in laboratory assays (Bianco *et al.*, 2008). This type of study with similar compounds is important to elucidate aspects such as structure-function, mainly in the context of antifouling technology development.

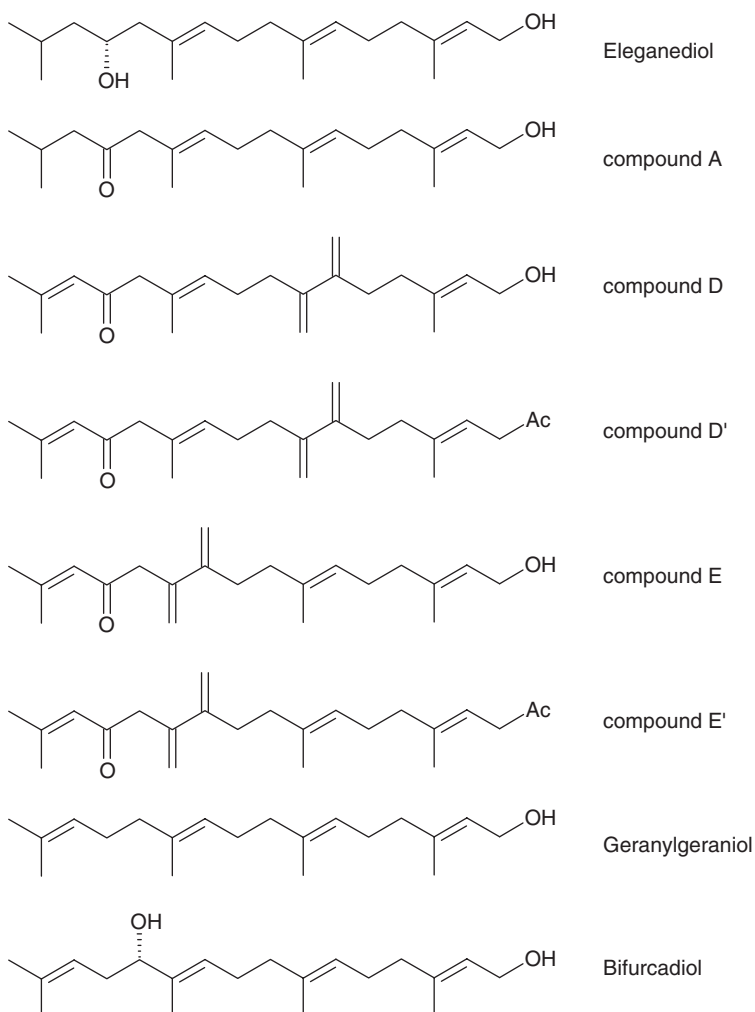
Secondary metabolites from the genus *Bifurcaria* (e.g., *B. bifurcata*) were identified as diterpenes (Fig. 22.2b) (Culioli *et al.*, 1999; Daoudi *et al.*, 2001) and used in chemotaxonomic studies. Their potential AF activities were investigated by Hellio *et al.* (2001a) and revealed some efficacy against several organisms involved in each step of the biofouling process (micro-organisms, algae and invertebrates).

Lau and Qian (1997) examined the complex antifouling action of *Sargassum tenerrimum* phlorotannins, phloroglucinol and tannic acid against

(a)



(b)



22.2 Examples of active compounds purified from brown algae:
(a) Lobophorolide, (b) Diterpenes.

Hydroides elegans settlement and antibacterial activity. They proposed that these compounds could interfere directly with larval settlement and survival and/or modify the primary bacterial biofilm, which in turn affects larval settlement of the polychaete.

22.3.2 Extracts of algae

A major screening effort of extracts of marine macroalgae has yielded many biologically active extracts against a range of fouling organisms (Walters *et al.*, 1996; Padmakumar and Ayyakkannu, 1997; Cho *et al.*, 2001; Hellio *et al.*, 2000a, b, 2001a, b, 2002). The results showed a wide range activity and specificity against the targets employed. From these screening results, it appears that among all the extracts tested, polar extracts of the class Rhodophyceae offer the best chance to discover compounds with AF activities. Recently, Da Gama *et al.* (2008a) determined that the activity of seaweed extracts, including pure compounds, observed in the laboratory was, in some cases, seen in the field. Their results appear to support known trends of secondary metabolite production among seaweeds, and suggest that research efforts should be focused on red macroalgae in the quest for new antifoulants.

Interestingly, seasonal variation in the activity of some extracts was highlighted (Hellio *et al.*, 2004a; Marechal *et al.*, 2004): Chlorophyceae species were mostly active throughout the year, in contrast to Phaeophyceae species, which were inactive for part of the year. The extracts from Rhodophyta species were the most active against bacterial strains and exhibited clear seasonal variation in antimicrobial activity but activity was present throughout the year. Moreover, Wright *et al.* (2000) highlighted some geographical differences in the concentration of furanones from *Delisea pulchra*. This was interpreted as a response to small-scale variation in environmental factors such as nutrients and light, and to genetic differences among individual plants.

Potin *et al.* (2002) reviewed the biotic interactions of marine algae and concluded that many chemical defences are induced following challenge by bioaggressors and that the defence strategies of algae have a major role in the structuring of marine communities. For example, Weinberger *et al.* (2002) demonstrated that a signal released by an endophytic attacker acted as a substrate for the rapid defensive reaction of *Chondrus crispus*. An amino acid oxidase that is able to produce elevated levels of H_2O_2 was required to fend off the pathogen. The release of another resource required for this defence was triggered by the host upon contact with the pathogen. This currently unique signal/response reaction demonstrated how independent principles have developed for signal reception and response in marine algae.

22.4 Marine invertebrates

Marine invertebrates comprise diverse phyla, with distinct phylogenetic and evolutionary origins, as well as physiology and ecology. They are benthic and sessile or equipped with slow motility, and must ward off natural enemies, competitors and epibionts by the use of a vast arsenal of chemical weapons, in many cases produced in cooperation with symbiotic marine microorganisms. Marine invertebrates are already sources of commercially important secondary metabolites and may become even more so as knowledge on NMPs and chemical ecology develops. Among the producers of these compounds, sponges, bryozoans and molluscs have received most attention of academic and industrial research and development. For all these groups, culture techniques have been developed encompassing *in situ*, laboratory and cell culture approaches for the production of natural products (Liebezeit, 2005). Potential applications of these are not restricted to pharmaceuticals but include marine cements, biominerals and antifouling compounds. All culture approaches require sound ecological knowledge about the organisms to be cultured and possible symbiotic interactions between host invertebrates and microheterotrophs (Liebezeit, 2005). We will review here the most well-known symbiotic organisms, marine sponges, but also include cnidarians, echinoderms, bryozoans, molluscs, ascidians, and worms.

22.4.1 Sponges

Sponges (Porifera) are among the oldest groups of multi-cellular animals (appeared on earth around 540 million years ago) (Müller, 1995) and more than 10000 species have been described (Zhang *et al.*, 2003). Among all investigated marine organisms, sponges produce the highest number of structurally-diverse natural products (Blunt *et al.*, 2006). A significant number of sponge metabolites have been shown to exhibit a wide array of bioactivities including antitumour/cytotoxic, enzyme inhibitory, receptor antagonist, antiviral and antifungal (Fusetani, 1996). Sponges are soft-bodied and sessile organisms, which seem to be very vulnerable to predation and epibiosis but often remain free of epibionts. It is also known that these organisms live with microbial symbionts (Lee *et al.*, 2001). Previous studies have shown they are rich in terpenoids and steroids, which can function in antipredation, competition for space and control of epibiont overgrowth (Becerro *et al.*, 2003; Burns *et al.*, 2003; Kelly *et al.*, 2003; Puyana *et al.*, 2003; Sera *et al.*, 2003; Thakur *et al.*, 2003; Tsoukatou *et al.*, 2003, 2007; Hellio *et al.*, 2005; Clavico *et al.*, 2006).

Pure compounds and extracts with AF properties, which have been obtained from sponges, are summarised respectively in Table 22.1a and 22.1b. Microbial compounds have been isolated from several species of

Table 22.1 AF activity from marine sponges
(a) NMPs

Porifera	Compound	Bacteria fungi	Micro- algae	Macro- algae	Invertebrate	Field	References
<i>Acanthella cavernosa</i>	Terpenoids				x		Hirota <i>et al.</i> 1996
	Kalihinene diterpenes				x		Okino <i>et al.</i> 1995
	diterpenoid compounds				x		Okino <i>et al.</i> 1996a
	kalihinol A				x		Fusetani 1997
	10-formamidokalihinene				x		Fusetani 1997
	15-formamidokalihinene				x		Fusetani 1997
	13-isocyanotheonellin				x		Fusetani 1997
	10-isocyano-4-cadinene				x		Fusetani 1997
	10 ss-formamidokalihinol A		x				Yang <i>et al.</i> 2006
	kalihinol A		x				Yang <i>et al.</i> 2006
<i>Agelas</i> sp. <i>Agelas mauritiana</i>	kalihipyran A				x		Okino <i>et al.</i> 1996a
	kalihipyran B				x		Okino <i>et al.</i> 1996a
	agelasine B				x		Sjögren <i>et al.</i> 2008
	Mauritiamine				x		Tsukamoto <i>et al.</i> 1996c
	Oroidin				x		Tsukamoto <i>et al.</i> 1996c
	epi-agelasine C		x	x			Hattori <i>et al.</i> 1997
	10-isothiocyano-11-axene				x		Hirota <i>et al.</i> 1996
	(+)-10(R)- isothiocyanoalloomadendrane				x		Hirota <i>et al.</i> 1996
	10 β -formamidokalihinol-E				x		Hirota <i>et al.</i> 1996
	10 β -formamidokalihinol-A				x		Hirota <i>et al.</i> 1996
	10 β -formamido-5-isocyanatokalihinol-A				x		Hirota <i>et al.</i> 1996
	10 β -formamido-5 β - isothiocyanoatokalihinol-A				x		Hirota <i>et al.</i> 1996
	Kalihinol A						Fusetani 1997
	10-formamidokalihinene						Fusetani 1997
	15-formamidokalihinene						Fusetani 1997

<i>Aplysina fitularis</i>	Aerothionin				x	Walker <i>et al.</i> 1985
	Homoaerothionin				x	Walker <i>et al.</i> 1985
<i>Aplysina glacialis</i>	1-methyladenine	x				Bobzin and Faulkner 1992
<i>Axinyssa</i> sp.	axinyssimides A, B, C					Hirota <i>et al.</i> 1998
	guaiane-type sesquiterpene peroxide				x	
<i>Callyspongia truncate</i>	Callytriol A, B, C, D, E				x	Tsukamoto <i>et al.</i> 1997b
<i>Crella incrustans</i>	1-O-palmityl-sn-glycero-3-phosphocholine (lyso-PAF)				x	Butler <i>et al.</i> 1996
<i>C. scalaris</i>	Dihydrofurospingin II				x	Hellio <i>et al.</i> 2005
<i>Dysidea herbacea</i>	Furanosesquiterpene				x	Sera <i>et al.</i> 1999a
<i>Ectyoplasia ferox</i>	triterpene glycosides				x	Kubaneck <i>et al.</i> 2002
<i>Eurylus formosus</i>	Formoside and triterpene glycosides	x		x	x	Kubaneck <i>et al.</i> 2002
<i>Geodia barrette</i>	bromine organic compound				x	Hedner <i>et al.</i> 2008; Sjögren <i>et al.</i> 2004
<i>Haliclona koremella</i>	ceramide compounds			x		Hattori <i>et al.</i> 1998
<i>Haliclona</i> sp.	haliclonamide C, D, E				x	Sera <i>et al.</i> 2002
<i>Ircinia oros</i>	ircinin I and II			x		Tsoukatou <i>et al.</i> 2002
<i>I. spinosula</i>	hydroquinone C				x	Hellio <i>et al.</i> 2005
	hydroquinone A	x		x		Tsoukatou <i>et al.</i> 2002
<i>Lendenfeldia chondrodes</i>	epidioxy sterols (mixture), and 3 sesterterpenes				x	Sera <i>et al.</i> 1999b
<i>Myrmekioderma dendyi</i>	(+)-curcuphenol				x	Tsukamoto <i>et al.</i> 1997a
	(+)-cucurdiol				x	Tsukamoto <i>et al.</i> 1997a
<i>Protophliptaspongia aga</i>	pyrimidine derivative				x	Hattori <i>et al.</i> 2001
	Zooanemonin				x	Hattori <i>et al.</i> 2001
	α -nicotinamide ribose			x		Hattori <i>et al.</i> 2001

(continued)

Table 22.1 Continued

Porifera	Compound	Bacteria fungi	Micro- algae	Macro- algae	Invertebrate	Field	References
<i>Pseudoceratina purpurea</i>	Halistanol sulphate				x		Tsukamoto <i>et al.</i> 1997c
	Pseudoeratidine				x		Tsukamoto <i>et al.</i> 1996d
	ceratinamine and moloka'iamine				x		Tsukamoto <i>et al.</i> 1996a
	Zamamistatin	x					Takada <i>et al.</i> 2001
	ceratinamide B				x		Tsukamoto <i>et al.</i> 1996b
	moloka'iamine				x		Tsukamoto <i>et al.</i> 1996b
	Psammaplysins A						Tsukamoto <i>et al.</i> 1996b
	Psammaplysins E				x		Tsukamoto <i>et al.</i> 1996b
<i>Pseudoceratina</i> sp.	5-bromoverongamine				x		Thirionnet <i>et al.</i> 1998
<i>Reniera sarai</i>	polymeric 3-alkylpyridinium salt				x		Faimali <i>et al.</i> 2003
<i>Smenospongia</i> sp.	Manoalide				x		Tsukamoto <i>et al.</i> 1997a
	seco-monoalide				x		Tsukamoto <i>et al.</i> 1997a
	monoalide 25-acetate				x		Tsukamoto <i>et al.</i> 1997a
	(4E,GE)-dehydromonoalide				x		Tsukamoto <i>et al.</i> 1997a
<i>Stelletta</i> sp.	stellettazole A	x					Tsukamoto <i>et al.</i> 1999a, b
<i>Stylotella aurantium</i>	styloguanidines 1-3				x		Kato <i>et al.</i> 1995
<i>Topsentia</i> sp.	halistanol sulphate				x		Tsukamoto <i>et al.</i> 1997a
	tri-2-aminoimidazolium halistanol sulfate				x		Tsukamoto <i>et al.</i> 1997a
Total		8	3	8	66	2	

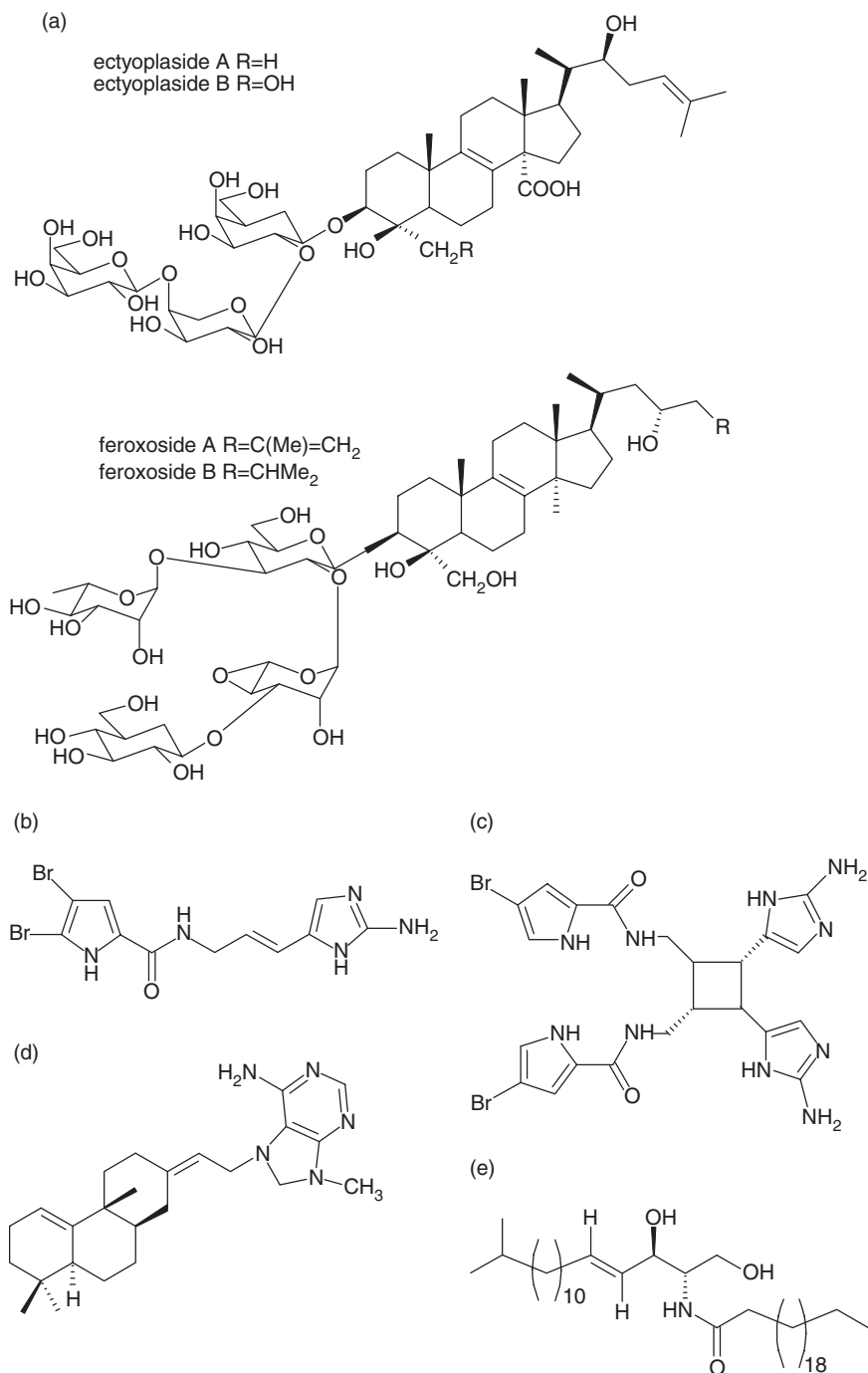
(b) Crude extracts

Porifera	Bacteria + fungi	Microalgae	Macroalgae	Invertebrate	Field	References
<i>Agelas conifera</i>	x					Kelly <i>et al.</i> , 2003
<i>Amphimedon compressa</i>	x					Kelly <i>et al.</i> , 2003
<i>A. viridis</i>	x					Kelman <i>et al.</i> 2001
<i>Aplysina fulva</i>	x					Kelly <i>et al.</i> 2003
<i>Callyspongia</i> sp.		x	x	x		Dobretsov <i>et al.</i> 2005
<i>Callyspongia pulvinata</i>		x		x		Dobretsov <i>et al.</i> 2004
<i>Crambe crambe</i>				x		Becerro <i>et al.</i> 1997
<i>Crella incrustata</i>				x		Butler <i>et al.</i> 1996
<i>Dercitopsis</i> sp.				x		Wilsanand <i>et al.</i> 1999
<i>Dysidea</i> sp.				x		Hellio <i>et al.</i> 2005
<i>Ectyoplasia ferox</i>	x		x	x		Kubaneck <i>et al.</i> 2002
<i>Erylus formosus</i>	x					Kelly <i>et al.</i> 2003
<i>Geodia berretti</i>				x		Sjögren <i>et al.</i> 2008
<i>G. corticostylifera</i>				x		Clavico <i>et al.</i> 2006
<i>Haliclona cymaeiformis</i>		x	x	x		Dobretsov <i>et al.</i> 2005
<i>Haliclona</i> sp.		x	x	x		Dobretsov <i>et al.</i> 2005; Devi <i>et al.</i> 1998
<i>Hemimycale columella</i>					x	Becerro <i>et al.</i> 1997
<i>Ircinia oros</i>	x	x	x	x		Tsoukatou <i>et al.</i> 2002
<i>I. spinulosa</i>		x		x		Tsoukatou <i>et al.</i> 2002
<i>I. variabilis</i>	x	x	x			Tsoukatou <i>et al.</i> 2002
<i>Mycale laxissima</i>	x					Lee <i>et al.</i> 2007
<i>Mycale adhaerens</i>	x					Lee <i>et al.</i> 2007
<i>Mycale microsigmatosa</i>				x		Pereira <i>et al.</i> 2002
<i>Plakortis halichondroides</i>	x					Kelly <i>et al.</i> , 2003
<i>Plakortis simplex</i>		x		x		Wilsand <i>et al.</i> 1999
<i>Ptilocaulis spiculifera</i>	x					Newbold <i>et al.</i> , 1999; Kelly <i>et al.</i> 2003
<i>Verongula gigantean</i>	x					Kelly <i>et al.</i> 2003
Total	13	8	6	15	1	

sponges. Kubanek *et al.* (2002) investigated triterpene glycosides (Fig. 22.3a) and formoside to determine if they contributed to the chemical defence of *Ectyoplasia formosus* and *E. ferox*. A mixture of these compounds and formoside deterred bacterial attachment at natural concentrations and below. AF assays were also carried out in the field using gels treated with the compounds and showed significant activity against invertebrates and algae after 14 days of field exposure. These results, coupled with the analysis of tissue concentrations and differential distribution in the body surface layer suggest that the compounds are actively involved in keeping the surface of the living sponge free of fouling. Similarly, Walker *et al.* (1985) stated that aethrothionin and homoaethrothionin, that are naturally exuded from the sponge *Aplysina fitularis*, inhibited surface overgrowth by microfouling organisms *in vitro* and *in vivo*. Promising levels of antimicrobial activity were detected while screening oroidin (Fig. 22.3b), 4,5-dibromopyrrole-2-carboxylic acid (BPC) and sceptrin (Fig. 22.3c) isolated from *Agelas* spp. and 1-methyladenine isolated *Aplysina glacialis* (Table 22.1a). These compounds did not show any anti-larval activity and could be used for specific formulation against marine biofilms.

Only a few studies have been dedicated to research on anti-algal activity of NMPs from sponges. The mixture of metabolites ircinin I and II from *Ircinia oros* and hydroquinone A isolated from *I. spinulosa* showed a high level of inhibition of macroalgal spores (Tsoukatou *et al.*, 2002). Hattori *et al.* (1997) isolated epi-agelasine C (Fig. 22.3d) from *Agelas mauritiana* (Hattori *et al.*, 1997) and a series of ceramide compounds (Fig. 22.3e) isolated from *Haliclona koremella* (Hattori *et al.*, 1998) inhibited spore attachment and germination of *Ulva conglobata*. Hattori *et al.* (2001) also isolated a nicotinamide ribose from *Protophilaspongia aga* which inhibited the germination and the attachment of *Ulva* spores. From the same sponge species, they purified a pyrimidine derivative and a zooanemonin, which exhibited antisettlement activity against *B. amphitrite*. The literature on antilarval compounds from sponges is large and many compounds have been described. We will review here only the most promising ones based on their levels of activity and absence of toxicity. They are listed in the Table 22.1a. Most of the bioassays of active substances have been tested against larval settlement of *B. amphitrite*, and to a lesser extent against *B. improvisus*, *Bugula neritina* and *Mytilus edulis*.

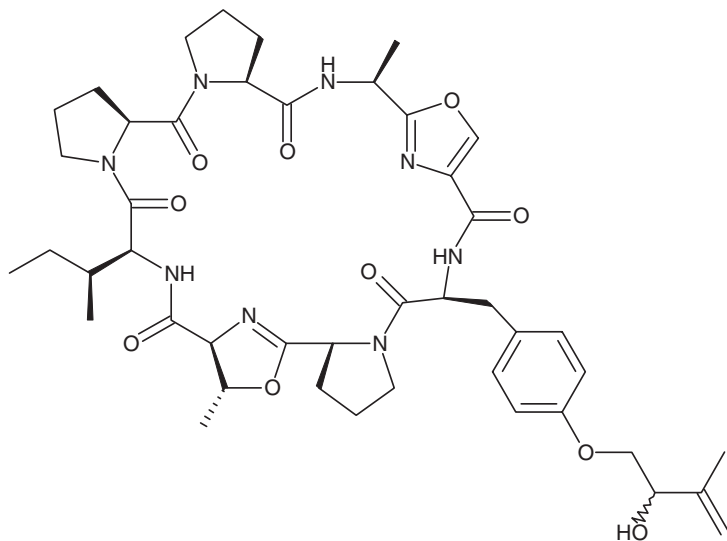
Three species have proved to be a very promising source of anti-larval compounds: *Acanthella cavernosa*, *Agelas mauritiana* and *Pseudoceratina purpurea*. They yielded a high diversity of compounds, the most potent of which were diterpenes, terpenoids, pseudoceratidine and psammaphysins. Strong activity against *B. amphitrite* was detected for kalihipyran A and B isolated from *A. cavernosa* (Okino *et al.*, 1996a) leading to inhibition of larval settlement and metamorphosis. Diterpenoids and sesquiterpenoids



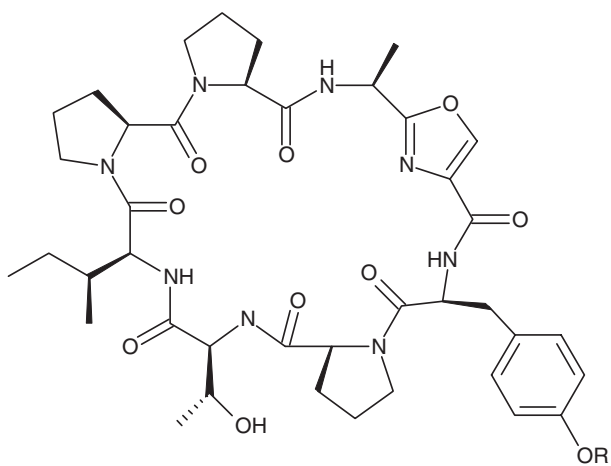
22.3 Examples of active compounds purified from sponges:

- (a) triterpene glycosides, (b) oroidin, (c) sceptrin, (d) Epi-agelasine C, (e) Series of ceramide compounds, (f) Haliclonamides C, D, and E (g) Furanosesquiterpene.

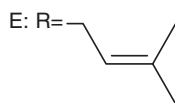
(f)



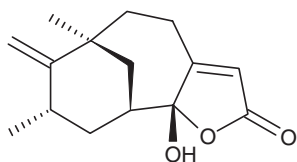
C



D: R=H



(g)



22.3 Continued

extracted from *A. cavernosa* had the great advantage of being non-toxic and thus can be considered to be promising AF compounds. Recently, two new interesting compounds were purified from *A. cavernosa*: a 10-formamido-4-cadienene (Nogata *et al.*, 2003), which was active at 0.5 $\mu\text{g ml}^{-1}$ against *B. amphitrite* settlement, and 10 ss-formamidokalihinol A and kalihinol A, which were capable of modifying the bacterial species composition of biofilm and modulating larval settlement (Yang *et al.*, 2006).

For most of the compounds, the mechanism of action remains unknown. Nevertheless, some information has been published relating to the following compounds. Styloguanidine 1–3, isolated from *Stylotella aurantium* inhibited moulting of cypris larvae of *B. amphitrite* (Kato *et al.*, 1995), an interesting biological activity with a direct bearing on AF activity since this larval stage precedes settlement of this crustacean. Dihydrofurospongins II from *C. scalaris* and hydroquinone C from *Ircinia spinosula* exhibited a non-toxic AF action against the settlement of *B. amphitrite* (Hellio *et al.*, 2005). Polymeric 3-alkylpyridinium salts (poly-APS), from *Reniera sarai*, inhibited settlement of *B. amphitrite* cyprids at a concentration of 1 $\mu\text{g ml}^{-1}$ (90% inhibition) with little toxicity (> 90% larval survival) (Faimali *et al.*, 2003). Moreover, cyprids were able to settle several hours after contact with poly-APS, supporting the non-toxic AF activity of this product. Several compounds isolated from *P. purpurea*, such as halistanol sulfate (Tsukamoto *et al.*, 1997a), pseudoeratinine (Tsukamoto *et al.*, 1996d), ceratinamine A and B, moloka'iamine, psammaplysins A and E, ceratinaminemoloka'iamine and pseudoceratinine (Tsukamoto *et al.*, 1996a, b) are also inhibited both the larval settlement and metamorphosis of the barnacle *B. amphitrite*.

Few assays have been conducted with other invertebrates. The most promising compound against the establishment of *Bugula neritina* is 1-O-palmityl-sn-glycero-3-phosphocholine (lyso-PAF) from *Crella incrustans* (Butler *et al.*, 1996). Two bromine organic compounds from *Geodia barretti* inhibited the settlement of *Balanus improvisus* cypris larvae (Sjögren *et al.*, 2004). Sera *et al.* (1999b, 2000) highlighted new compounds which were repellent against *Mytilus edulis*, isolated from *Lendenfeldia chondrodes* (two epidioxy sterols and 3 sesterterpenes), *Haliclona* (haliclonamides C, D, and E (Fig. 22.3f)) and from *Dysidea herbacea* (a new furanosesquiterpene, Fig. 22.3g).

Some compounds are active against several targets and could be interesting for the formulation of AF paints. For example, mauritiamine isolated from *Agelas mauritiana*, inhibited *B. amphitrite* metamorphosis and bacterial growth (Tsukamoto *et al.*, 1996c). *Pseudoceratina purpurea* was intensively studied and seven compounds with AF activity against *B. amphitrite* settlement were purified (Tsukamoto *et al.*, 1996a,b,c) and some of these compounds were active against marine bacteria.

Extensive work has been carried out recently on the screening of sponge extracts for AF efficacy (Table 22.1b). Activities have been recorded towards bacteria, fungi, microalgae, macroalgae and invertebrates from 27 species tested. The most promising species for further work appear to be *Callyspongia* sp., *Haliclona cymaeformis* and *Haliclona* sp. (active against microalgae, macroalgae and invertebrates) (Dobretsov *et al.*, 2005) and *Ircinia oros* and *I. variabilis* (active against bacteria, microalgae and invertebrates).

22.4.2 Cnidarians

Coral communities are known to be rich sources of natural products that are often implicated in allelochemical interactions. These biologically-active compounds have different functions according to the types of natural pressures that corals encounter, e.g. antimicrobial and antifungal activities (Jensen *et al.*, 1996; Koh *et al.*, 2002), and feeding deterrence (Kelman *et al.*, 1999). Problems with coral diseases and bleaching have led to intense research in the field of coral chemical defences against pathogenic organisms (Kelman *et al.*, 1998) and their relation with co-occurring bacteria (Peters *et al.*, 2003). Secondary metabolites from coelenterates have also been reported to be promising AF compounds, mainly against invertebrate larvae. Owing to repulsive activity, chemicals from cnidarians could be important components to be exploited in AF technologies.

Pure compounds and extracts with AF potency, which have been obtained from cnidarians, are summarised respectively in Table 22.2a and 22.2b. Among the pure compounds tested, it is of interest to note that none of them showed any antimicrobial activity, despite the fact that numerous crude extracts inhibited the growth of bacteria and microalgae. All the pure compounds reported in Table 22.2a showed specific activity towards only one type of organism (macroalgae or invertebrates), in contrast to the results obtained with crude extracts, the majority of which displayed activity towards several target organisms. This suggests that for Cnidaria, it may be better to consider the crude extracts for paint formulation rather than pure compounds, especially extracts from *Alcyonium paessieri* (Slattery *et al.*, 1995), *Dendronephyta* sp. (Harder *et al.*, 2003) and *Subergorgia suberosa* (Koh *et al.*, 2002). But the use of crude extracts can be restricted by regulatory issues. The most promising compounds for anti-settlement of larval invertebrates at non-toxic concentrations were isogosterones A, B, C and D (Tomono *et al.*, 1999) isolated from *Dendronephthya* sp. (active against *B. amphitrite*) and a furanoditerpene from *Phyllogorgia dilatata* (active against *Perna perna*) (Epifanio *et al.*, 2006). Cnidarians can also provide a very good source of algicidal compounds. For example, diterpenes 11-episulariolide and sinulariolide extracted from *Sinularia flexilis* inhibited the attachment of *Ceramium codii* in the laboratory (Michalek and Bowden, 1997).

Table 22.2a AF property from cnidarians

(a) NMPs

Cnidaria	Compounds	Macroalgae	Invertebrate	References
<i>Dendronephthya</i> sp.	D-secosteroids and Isogosterone A, B, D		x	Tomono <i>et al.</i> 1999
<i>Leptogorgia virgulata</i>	diterpenoid pukalide-compound		x	Gerhart <i>et al.</i> 1988
<i>Renilla reniformis</i>	Renilla foulins A, B, and C		x	Rittschof <i>et al.</i> 1996
<i>Phyllogorgia dilatata</i>	Furanoditerpene		x	Epifanio <i>et al.</i> 2006
<i>Sinularia flexibilis</i>	11-episinulariolide	x		Michalek and Bowden 1997
<i>S. flexibilis</i>	Sinulariolide	x		Michalek and Bowden 1997
<i>Sinularia</i> sp.	polyhydroxylated sterols		x	Mizobuchi <i>et al.</i> 1996
Total		2	9	

(b) Crude extracts

Cnidaria	Bacteria + fungi	Microalgae	Invertebrate	References
<i>Acanthogorgia turgida</i>			x	Wilsanand <i>et al.</i> 1999
<i>Alcyonium paessleri</i>		x	x	Slattery <i>et al.</i> 1995
<i>Cladiella pachyclados</i>			x	Devi <i>et al.</i> 1998
<i>Ctenocella umbraculum</i>	x			Koh <i>et al.</i> , 2002
<i>Dendronephthya</i> sp.	x		x	Mizobuchi <i>et al.</i> 1993; Wilsanand <i>et al.</i> 1999; Harder <i>et al.</i> 2003
<i>Echinogorgia complexa</i>	x		x	Wilsanand <i>et al.</i> 1999; Koh <i>et al.</i> 2002
<i>Gersemia antarctica</i>		x		Slattery <i>et al.</i> 1995
<i>Goniopora tenuidens</i>			x	Slattery <i>et al.</i> 1995
<i>Gorgonella sanguinolenta</i>			x	Wilsanand <i>et al.</i> 1999
<i>Gorgonia albellum</i>	x			Kim <i>et al.</i> 2000a; Koh <i>et al.</i> 2002
<i>Gorgonia ventalina</i>	x			Kim <i>et al.</i> 2000b; Koh <i>et al.</i> 2002
<i>Parerythropodium fulvum</i>	x			Kelman <i>et al.</i> 1998, 1999
<i>Sinularia compressa</i>			x	Devi <i>et al.</i> 1998
<i>Sinularia numerosa</i>			x	Devi <i>et al.</i> 1998
<i>Subergorgia suberosa</i>	x		x	Devi <i>et al.</i> 1998; Koh <i>et al.</i> 2002
<i>Xenia puertogaleriae</i>			x	Devi <i>et al.</i> 1998
Total	7	2	11	

22.4.3 Echinodermata

The external surfaces of echinoderms generally remain free of epibionts although there are subsurface symbiotic bacteria (McKenzie and Grigolava, 1996). The antibacterial defence strategy seems to be significant and target-specific. Echinoderms have developed a variety of defence molecules against pathogenic microorganisms (Haug *et al.*, 2002). Only a few investigations of AF compounds have been done on this neglected taxon. Pure compounds and extracts with AF properties, which have been obtained from echinoderms, are summarised respectively in Table 22.3a and 22.3b.

Table 22.3 AF property from Echinodermata
(a) Compounds

Echinodermata	Compounds	Bacteria + fungi	Macroalgae	References
<i>Acodontaster conspicuus</i>	acondontasterosides D-I, and steroids	x		De Marino <i>et al.</i> 1997
<i>Goniopecten demonstrans</i>	goniopectinosides A, B, C		x	De Marino <i>et al.</i> 2000
Total		9	3	

(b) Crude extracts

Echinodermata	Bacteria + fungi	Macroalgae	Invertebrate	References
<i>Asterias rubens</i>	x			Haug <i>et al.</i> , 2002
<i>Astrocyclus caecilia</i>		x		Iken <i>et al.</i> 2003
<i>Astropecten articulatus</i>		x		Iken <i>et al.</i> 2003
<i>Astrophyton muricatum</i>	x			Bryan <i>et al.</i> , 1996
<i>Curcumarina frondosa</i>	x			Haug <i>et al.</i> 2002
<i>Goniaster tessellatus</i>	x			Bryan <i>et al.</i> 1996
<i>Henricia downeyae</i>	x		x	Palagiano <i>et al.</i> 1996
<i>Luidia clathrata</i>		x		Iken <i>et al.</i> 2003
<i>Strongylocentrus droebachiensis</i>	x			Haug <i>et al.</i> 2002
Total	6	3	1	

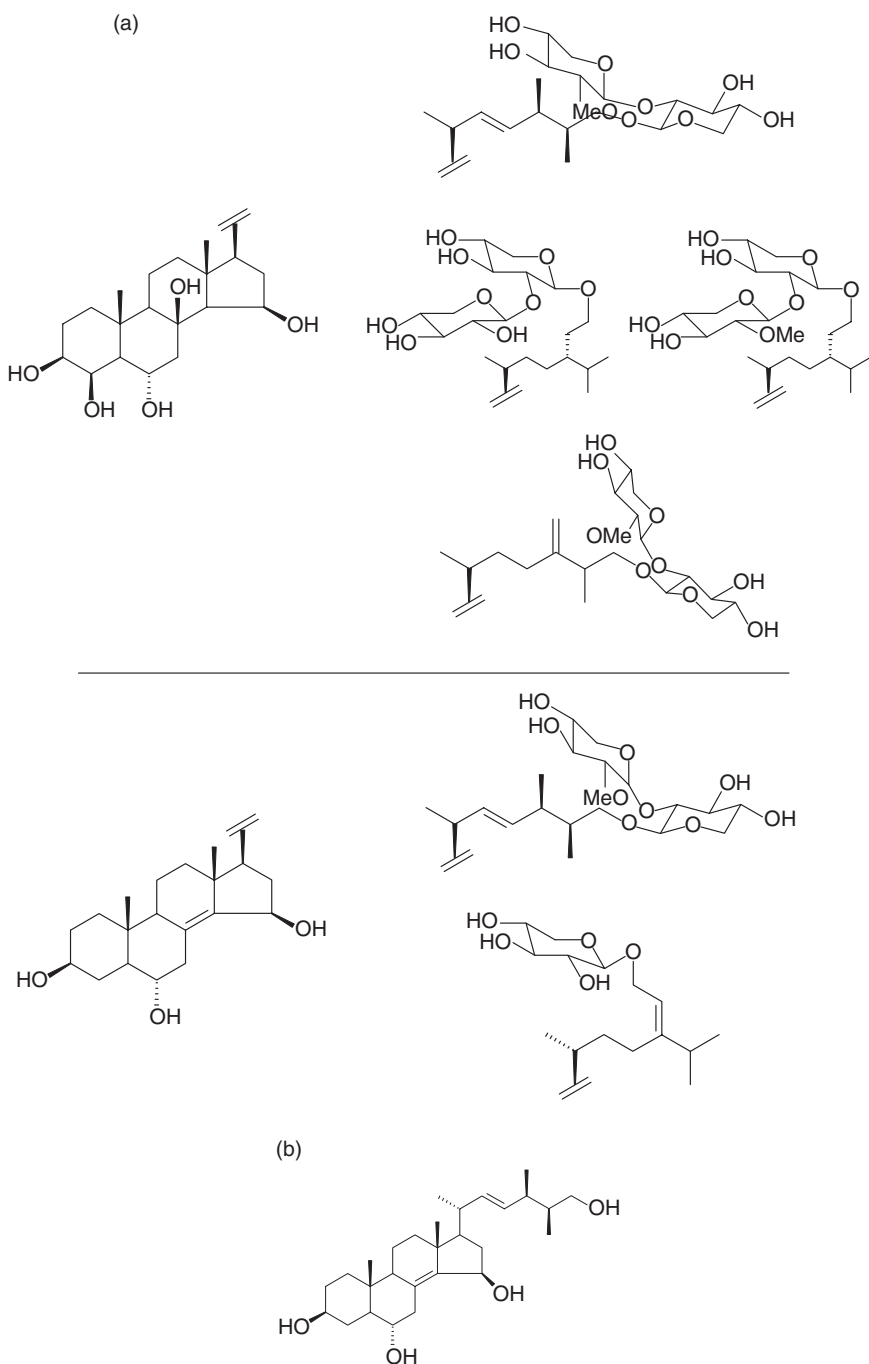
De Marino *et al.* (1997) demonstrated the antimicrobial activities of acodon-tasterosides D, E, F, G, H, I (Fig. 22.4a) and steroid compounds (Fig. 22.4b) from the Antarctic starfish, *Acodontaster conspicuosus*, suggesting that these compounds may play an ecological role in preventing microorganism fouling on surfaces (De Marino *et al.*, 1997). Later, De Marino *et al.* (2000) isolated three new asterosaponins, goniopectinosides A, B and C (Fig. 22.4c), from a deep sea starfish, *Goniopecten demonstrans*, which strongly inhibited spore settlement of *Hincksia irregularis*. Echinoderms appear to be a poor source of anti-larval settlement compounds, as among all extracts and pure compounds tested, only one extract from *Henricia doweyae* (Palagiano *et al.*, 1996) was active against *B. amphitrite* cyprids.

22.4.4 Bryozoans

Bryozoans are a great nuisance to the marine industry: over 125 species are known to grow on the bottoms of ships, causing drag and reducing the efficiency and manoeuvrability of the fouled ships. Bryozoans may also foul pilings, piers, and docks. The valorisation of this biomass could represent a way for a sustainable production of bioactive compounds. Yet although bryozoans produce a remarkable variety of chemical compounds, they have rarely been screened for AF activity. The available results from the literature are presented in Tables 22.4a and 22.4b for pure compounds and extracts respectively. Brominated alkaloids and one diterpene (Fig. 22.5) isolated from *Flustra foliacea* showed high antibacterial activity (Peters *et al.*, 2003). These compounds might act to regulate bacterial growth on the surface of *F. foliacea* (Peters *et al.*, 2003). One heterocycle compound, 2,5,6-tribromo-1-methylgramine, from *Zoobotryon pellucidum* exhibited promising activity in inhibiting settlement of the blue mussel *Mytilus edulis* (Konya *et al.*, 1994). Shellenberger and Ross (1998) showed that extracts of *Bugula pacifica* and *Tricellaria occidentalis* had strong antimicrobial properties.

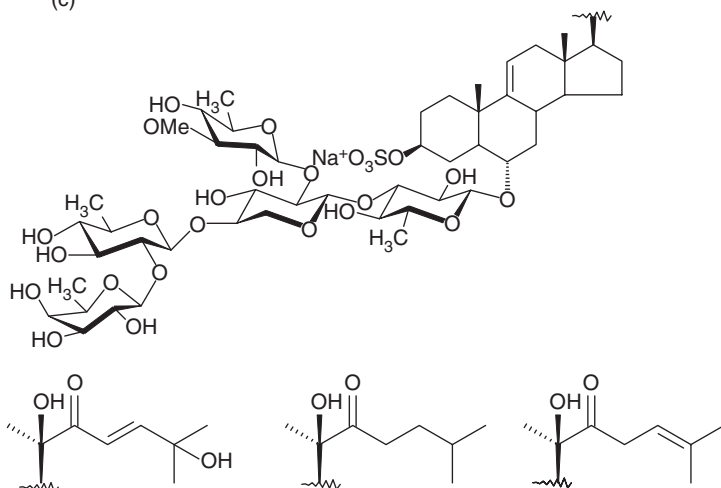
22.4.5 Molluscs

The utility of some natural products from molluscs has been known for centuries (Avila, 2006). However, only recently have modern technologies and advances in the fields of chemistry, chemical ecology, anatomy, histology, and laboratory culture allowed the exploitation of new, unprecedented applications of natural products. Recent studies have dealt with: (a) the natural role of compounds in protecting the animals (e.g., chemical defence), or in mediating their intraspecific communication (e.g., pheromones); (b) the geographical differences in similar or related species (and the implications of this in chemical ecology and phylogeny); and (c) the localisation of these metabolites in molluscan tissues (by means of the most modern



22.4 Examples of active compounds purified from Echinodermata:
 (a) Acodontasterosides D, E, F, G, H, I, (b) Steroid compound,
 (c) goniopectinosides A, B and C.

(c)



22.4 Continued

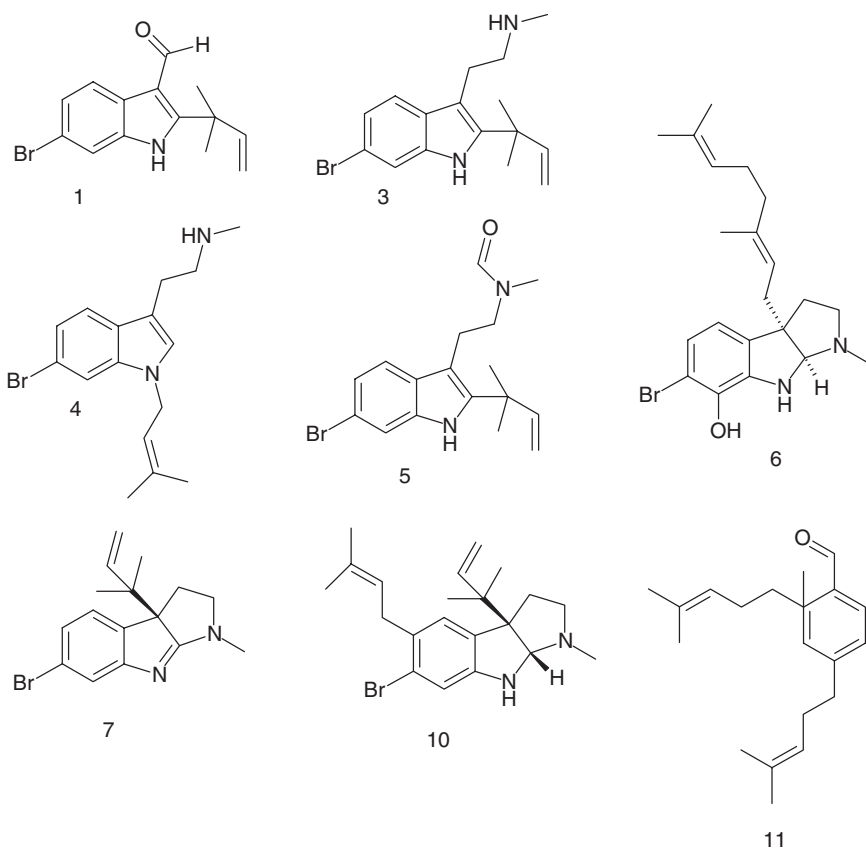
Table 22.4 AF property from Bryozoans

(a) Compounds

Bryozoa	Compounds	Bacteria + fungi	Invertebrate	References
<i>Flustra foliacea</i>	brominated alkaloids	x		Peters <i>et al.</i> 2002, 2003
	diterpene	x		Peters <i>et al.</i> 2002, 2003
<i>Zoobotryon pellucidum</i>	2,5,6-tribromo-1-methylgramine		x	Konya <i>et al.</i> 1994
Total		4	1	

(b) Crude extracts

Bryozoa	Bacteria + fungi	References
<i>Bugula pacifica</i>	x	Shellenberger and Ross 1998
<i>Tricellaria occidentalis</i>	x	Shellenberger and Ross 1998
Total	2	



22.5 Example of active compound (diterpene) from Bryozoa.

technologies), among others. The methodology for the laboratory culture of some species has also been established, thus offering new insights into this interesting field. Further applications of all these challenging studies are currently being developed. Pure compounds and extracts with AF properties, which have been obtained from molluscs, are summarised in Table 22.5a and 22.5b respectively.

Three selective pressures could potentially lead to the evolution of chemical defences in molluscan egg masses: predation, disease and surface fouling. Marine molluscs may rely on a range of alternative strategies to protect their egg masses from predators, including physical protection in leathery egg capsules, camouflage and rapid embryonic development, as well as behavioural mechanisms, such as brooding and the deposition of large aggregated egg masses. The gelatinous egg masses of marine molluscs also have no physical protection from predation. Thus, many marine

Table 22.5 AF property from Molluscs

(a) Compounds

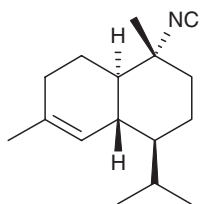
Mollusca	Compounds	Invertebrate	References
<i>Phyllidiopsis krempfi</i>	10-isocyano-4-cadinane		
<i>P. ocelata</i>	isocyanotheonelin	x	
<i>P. varicosa</i>	10-formamide-cadinene	x	Okino <i>et al.</i> 1996b
<i>P. pustulosa</i>	isocyanosesquiterpene alcohol	x	Hirota <i>et al.</i> 1998
	10- <i>epi</i> -axisonitrile-3		
	axisonitrile-3	x	
	10-isothiocyanato-4-amorphene	x	
<i>P. ocelata</i>	2-isocyanotrachyopsane	x	
<i>P. varicosa</i>	4-thiocyanatoneopupukeanane	x	
<i>Phyllidiopsis krempfi</i>	3-isocyanotheonellin	x	Okino <i>et al.</i> 1996a
<i>Trimusculus reticulatus</i>	labdane diterpenoid	x	Manker and Faulkner 1996
Total		9	

(b) Crude extracts

Mollusca	Bacteria + fungi	Invertebrate	References
<i>Aplysia juliana</i>	x		Benkendorff <i>et al.</i> 2001
<i>Chicoreus virgineus</i>	x	x	Benkendorff <i>et al.</i> 2001
<i>Bembicium nanum</i>	x		Benkendorff <i>et al.</i> 2001
<i>Cabestana spengleri</i>	x		Benkendorff <i>et al.</i> 2001
<i>Chicoreus virgineus</i>	x	x	Ramasamy and Murugan 2007
<i>C. ramosus</i>	x	x	Ramasamy and Murugan 2007
<i>Dicathais orbita</i>	x		Benkendorff <i>et al.</i> 2001
<i>Rapana rapiformis</i>	x	x	Ramasamy and Murugan 2007
<i>Planaxis sulcatus</i>	x	x	Devi <i>et al.</i> 1998
<i>Salinator fragilis juliana</i>	x		Benkendorff <i>et al.</i> 2001
<i>Sepioteuthis australis</i>	x		Benkendorff <i>et al.</i> 2001
<i>Stylocheilus longicauda</i>	x		Benkendorff <i>et al.</i> 2001
Total	13	5	

molluscs may have evolved mechanisms to avoid predation and surface fouling on their egg masses. Benkendorff *et al.* (2001) indicated that antibacterial compounds are common in both tough egg capsules and gelatinous egg masses, suggesting that chemical defence is the primary mechanism used to maintain an axenic environment for embryonic development. Studies on the antimicrobial properties of the marine molluscs are, however, scanty (but see Benkendorff *et al.*, 2001; Murugan and Ramasamy, 2007).

The compounds recently isolated were active specifically against invertebrate settlement. No activity against microorganisms or algae was reported. Three new sesquiterpene isocyanides, isolated from *Phyllidiopsis krempfi*, *P. pustulosa* and *P. varicosa* inhibited larval settlement and metamorphosis of *B. amphitrite* at non-toxic concentrations (Okino *et al.*, 1996b). Hirota *et al.* (1998) discovered a very active compound (isocyanosesquiterpene alcohol, Fig. 22.6) against *B. amphitrite* cyprid settlement from *Phyllidia pustulosa*. One labdane diterpenoid found in the intertidal pulmonate limpet *Trimusculus reticulatus* was repellent to larvae of the sabellarid reef-building tube worm *Phragmatopoma californica* (Manker and Faulkner, 1996). Besides being important to survival of this sessile filter-feeding mollusc (Manker and Faulkner, 1996), this compound could be a promising new AF agent. All the extracts tested (Table 22.5b) showed very promising levels of antibacterial and/or antifungal activities, demonstrating that molluscs are excellent sources for such compounds. The observation of antibacterial activity in egg masses substantiates several reports that a number of marine molluscs protect their eggs with natural products (Benkendorff *et al.*, 2000). The origin of these antibacterial compounds in the egg masses has been traced to hypobranchial glands. This could be substantiated from the observation of broad-spectrum inhibitory activity of the hypobranchial gland of *Chicoreus virgineus* against pathogenic bacteria (Prem Anand *et al.*, 1997). It is possible that the antimicrobial substances in the adult molluscs may be held in a nontoxic state and then enzymatically activated in the reproductive tract or egg masses (Benkendorff *et al.*, 2000) and that antimicrobial metabolites are likely to be more concentrated in freshly laid egg masses than in well developed or hatching egg masses.



22.6 Example of active compound (isocyanosesquiterpene alcohol) from Molluscs.

22.4.6 Ascidians

Ascidians are an interesting group because many of them have surfaces free of fouling organisms, and they seem to have several mechanisms to prevent fouling that are not mutually exclusive (Wahl and Banaigs, 1991). For instance, physical mechanisms such as sloughing off of external cuticle may remove epibionts (Turon, 1992). Inorganic defences such as low pH values were also reported as possible defence mechanisms in ascidians (Stoecker, 1978). As for the acidic substances, although their effectiveness in preventing predation is controversial (Pisut and Pawlik, 2002), their role as AF mechanisms cannot be ruled out (Davis and Wright, 1989). Finally, organic defences, such as secondary metabolites, have been held responsible for the lack of fouling in ascidian species (Clare, 1996), although experimental demonstration of an AF role is scarce (Teo and Ryland, 1995). Pure compounds and extracts with AF properties which have been obtained from ascidians are summarised respectively in Table 22.6a and 22.6b. Few examples of AF products are known for ascidians. Eudistomines G and H from *Eudistoma olivaceum* inhibited larval settlement of the bryozoan *Bugula neritina* (Davis and Wright, 1990; Davis, 1991). Promising extracts have been reported by Bryan *et al.* (2003), Becerro *et al.* (1997) and Wahl *et al.* (1994) with both antimicrobial and anti-invertebrates settlement activities.

Table 22.6 AF property from Ascidians

(a) Compounds

Tunicata	Compound	Invertebrate	Reference
<i>Eudistoma olivaceum</i>	eudistomines G and H	x	Davis and Wright 1990; Davis, 1991
Total		2	

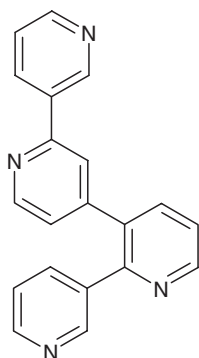
(b) Crude extracts

Tunicata	Bacteria + fungi	Invertebrate	References
<i>Amaroucium stellatum</i>	x	x	Bryan <i>et al.</i> 2003
<i>Botryllus planus</i>	x	x	Bryan <i>et al.</i> 2003
<i>Cystodytes dellechiaiei</i>		x	Becerro <i>et al.</i> , 1997
<i>Cystodytes lobatus</i>	x		Wahl <i>et al.</i> 1994
<i>Polysyncraton lacazei</i>		x	Wahl <i>et al.</i> 1994
Total	3	4	

Table 22.7 AF property from Nematoda

Compounds

Nemertida	Compound	Invertebrate	Reference
<i>Amphiporus angulatus</i>	nemertine pyridyl alkaloids	x	Kem <i>et al.</i> 2003
Total		3	



22.7 Example of active compound (Nemertelline) from Nematoda.

22.4.7 Nermerteans

No active extracts were reported in the literature. Active compounds are reported in Table 22.7. Pyridyl alkaloids which are known to be paralytic to some crustaceans and annelids were tested for inhibition of barnacle cyprid settlement (Kem *et al.*, 2003). Nemertine pyridyl alkaloids (e.g., Nemertelline (Fig. 22.7), isolated from the hoplohemertine, *Amphiporus angulatus*, inhibited cyprid settlement with an IC_{50} of $3.2 \mu M$. IC_{50} s for two other pyridyl alkaloids, 2,3'-bipyridil and anabaseine were $4.1 \mu M$ and $1.2 \mu M$ respectively. These compounds exhibited different levels of toxicity; nemertelline had particularly low toxicity and may be worth further investigation for antifouling use.

22.5 General issues in the use of natural antifoulants

22.5.1 The possibility of latitudinal patterns in the production of natural antifoulants

The tropical oceans represent the oldest marine habitats. Although it is true that the diversity of marine fauna peaks in tropical seas (Krebs, 2001; Willig

et al., 2003), the diversity of marine flora does not reach the maximum in tropical regions, but rather at temperate regions (Lüning, 1990; Kerswell, 2006). Many authors suggest that algae and invertebrates should be more chemically defended in the tropics, since the higher diversity of consumers (Floeter *et al.*, 2005), and their constant and intense predation pressure (as opposed to the seasonally variable activities of cooler-water, higher-latitude consumers) would exert a strong selective pressure for chemically defended species (e.g., Hay, 1996). However, the assumption that consumption pressure is – or was – the main evolutionary force driving the production of NMPs still needs to be more clearly demonstrated (Pereira and Da Gama, 2008). Alternative explanations, including other possible driving forces, such as epibiont pressure and space competition, still remain to be tested. To date, there is only one study that compared AF activity employing a standardised testing method along a latitudinal gradient (Da Gama *et al.*, 2008a). The authors screened 62 samples of seaweed belonging to 42 species from the southwestern Atlantic for AF activity, comprising a latitudinal range from 18°N to 27°S. The results did not provide evidence of any latitudinal pattern, but instead suggested a phylogenetic trend (see Section 22.5.3 ‘Phylogenetic trends in marine natural antifoulants’).

Since marine invertebrate diversity peaks in tropical latitudes (Krebs, 2001; Willig *et al.*, 2003), it is to be expected that a larger *number* of compounds with antifouling activity will be found in tropical species, although whether or not the *proportion* of invertebrates with AF compounds is higher at lower latitudes still remains to be demonstrated.

22.5.2 The possibility of seasonal patterns in the production of natural antifoulants

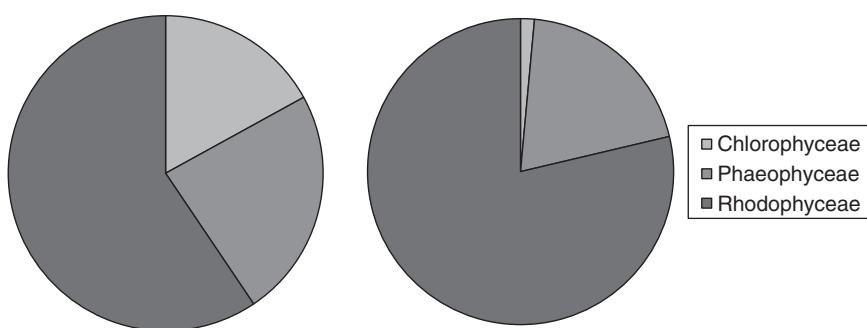
Hellio *et al.* (2004a) and Marechal *et al.* (2004) screened macroalgae from the Brittany coast (France) for antifouling activity, employing a diversity of fouling organisms. Of the extracts tested, 48.2% were active against at least one of the fouling organisms, and of these extracts, 31.2% were seasonally active with a peak of activity in spring and summer, corresponding to maximal values for water temperature, light intensity, and fouling pressure. Only 17% were active throughout the year. This study suggests that either season-related environmental fluctuations, or increased epibiosis in summer, could indeed lead to a higher production of AF defense compounds. Older studies (Phillips and Towers, 1982a, 1982b) investigated the seasonal variation in the production of halogenated compounds by the red alga *Rhodomela larix*, as well as the within-thallus variation in the concentrations of these compounds. This seasonality pattern has to be considered and monitored, especially if aquaculture of the source organisms for a sustainable production for AF compounds is considered. In order to reduce this complexity,

aquaculture in natural environments or outside ponds should be avoided, and controlled conditions should be preferred for temperate species. For tropical species, which are not susceptible to seasonal change, aquaculture could be done in open ponds and in the open sea and so would be less expensive and would represent a better investment.

22.5.3 Phylogenetic trends in marine natural antifoulants

The tropical oceans represent the oldest marine habitats, where red and green seaweeds dominate, while brown algae are more abundant towards cold temperate regions (Pereira and Da Gama, 2008). Sponges and corals are clearly more abundant in tropical than in colder oceans. Despite scant evidence, it appears that AF activity is not driven by latitudinal trends, but rather by phylogenetic constraints. Among macroalgae, screening studies such as Hellio *et al.* (2002) and Da Gama *et al.* (2008a) suggest that AF activity is higher and wider among red algae and lower among green algae, with brown seaweeds in an intermediate position (Fig. 22.8).

Similar results were found by Da Gama *et al.* (2008a), who screened 62 algal samples from the wide Brazilian littoral (southwestern Atlantic). Out of the 42 species tested, red macroalgae showed the highest proportion (55%) of active species (moderate or strong fouling inhibition), followed by brown macroalgae (14%). Green seaweeds never exhibited strong AF activity (> 80% inhibition of mussel byssal attachment relative to controls). Although the extensive review made by Bhadury and Wright (2004) covers



22.8 Proportion of antifouling activity among seaweeds belonging to the divisions Chlorophyceae, Phaeophyceae and Rhodophyceae according to data extracted from two screening studies, Hellio *et al.* (2002) [left] and Da Gama *et al.* (2008a) [right]. Only strong antifouling activity was considered (i.e., > 50% inhibition of microalgae – Hellio *et al.* 2002 or > 80% mussel byssus attachment – Da Gama *et al.* 2008a). N = 90 extracts from 30 seaweed species, and 62 extracts from 42 species, respectively.

greater numbers of papers in which brown algae produce AF compounds, this may be merely the result of more investigations using algae from this division. Comparative studies, however, provide evidence of more activity within the division Rhodophyceae, in accordance with the fact that among algae, they are the most prolific in terms of secondary metabolites, with a single genus (*Laurencia*) being known to produce *ca.* 700 natural products, particularly halogenated compounds.

Among marine invertebrates, sponges (phylum Porifera) are by far the most prolific marine organisms in terms of natural products (pure compounds and extracts) with AF activity (Tables 22.1–22.7). Chambers *et al.* (2006) pointed out that out of 160 species found to have some AF activity, 38.5% were sponges, 19.5% were algae, and 17.9% were cnidarians, with other invertebrate groups such as tunicates comprising 7% or less. It is opportune to mention that sponges have associated bacteria that may be prone to large-scale cultivation (e.g., Bringmann *et al.*, 2007) for mass production of natural AF compounds. From a production point of view, algae and microorganisms seem so far to be the best candidates for providing a sustainable source of AF compounds because they can be either cultivated for mass production or harvested (e.g., algal invasive species can be used for local production of AF compounds).

22.5.4 Activity spectrum of antifouling marine natural products

Fouling communities comprise a wide variety of species, from almost all known phyla, with variable growth forms, reproduction strategies, feeding modes, and environmental requirements. Hillman (1977) estimated that there are more than 2000 fouling species, and we now know that this number can be orders of magnitude higher. A new AF agent with practical applications for the marine environment should thus possess a wide spectrum of fouling inhibition, rather than inhibit just one or a few species. However, despite the fact that key organisms are widely used (e.g., *U. intestinalis* and *B. amphitrite*), studies of NMPs with AF activity sometimes lack standardisation, and the majority employ only laboratory assays, making assessments of the activity spectrum impossible (see Chapter 12 on bioassays). Surely field assays are the most appropriate to evaluate the activity spectrum of NMPs, but few studies used this approach due often to difficulties in obtaining enough pure compounds, formulating the compounds within a paint matrix or access to a raft (Bobzin and Faulkner, 1992; Henrikson and Pawlik, 1995, 1998; Da Gama *et al.*, 2002, 2003, 2008a; Barbosa *et al.*, 2007).

However, a few studies have assessed the spectrum of AF activity. This can be correlated to the geographical location of the laboratories. One example is a study by Hellio *et al.* (2002), that investigated the AF activity

of aqueous, ethanolic and dichloromethane (DCM) extracts from 30 seaweed species from Concarneau Bay, France (Northeastern Atlantic), against 12 species of benthic microalgae and spores and zygotes (attachment and germination rates) of three macroalgae species (*Ulva lactuca*, *U. intestinalis*, *Sargassum muticum*). In their study, two brown algae (*S. muticum* and *Pelvetia canaliculata*) and three red algae (*Chondrus crispus*, *Laurencia pinnatifida* and *Polysiphonia lanosa*) exhibited a broad spectrum AF activity against all microalgal strains, a promising result only comparable to the now banished antifoulant TBTO and the toxicological standard CuSO_4 used as controls. Also noteworthy is the fact that ecotoxicological tests were performed with non-target marine organisms (sea urchin and oyster larvae), which revealed that the red algae that exhibit AF activity are orders of magnitude less toxic than TBTO and CuSO_4 . From their results, they showed that fractions lost activity throughout the purification process, suggesting that some compounds gave better results in mixtures than alone, raising the question of whether it is best to work on a single compound or to start focusing on cocktails of several compounds which could work synergistically.

22.6 Future trends

Natural marine products are increasingly targeted for studies of structure–activity relationships (SARs), as templates for molecular modelling and also for their synthesis and that of their analogues. The total synthesis of the potent antifouling sesquiterpene elatol, originally isolated from the red seaweed genus *Laurencia*, was recently achieved (White *et al.*, 2008). Mass cultivation of seaweeds or marine invertebrates, isolated marine microorganisms or even genetically-engineered bacteria can provide commercial-scale amounts of compounds to overcome the difficulties of the testing and regulatory stages (e.g., Bringmann *et al.*, 2007). It seems reasonable to believe that pure natural products, co-biocides or combinations with other non-biocidal mechanisms, such as low adhesion/fouling-release coatings, or newer techniques, may lead us to employ natural products from marine organisms as new antifoulants in the future, despite the many reasonable constraints raised by some authors (Rittschof, 2000). Marine natural products have already reached the commercial stage in a number of cases (e.g., Haefner, 2003; Marris, 2006; see also the specialized journal *Marine Drugs*), and there is no reason to believe that this should not happen with their application as antifoulants as well. However, as public awareness concerning the environmental fate and effects of AF compounds grew in the last decades as a consequence of the use of TBTs, a number of steps must now be performed (Champ, 2003) in order to safely employ any compound as a new AF agent, regardless of its origin.

Even though no NMPs have made it to the market yet, the rationale for continued natural marine products discovery efforts is that the marine environment is rich in unexplored species (estimated at 1–2 million) that may have novel biosynthetic capabilities. Usually the really potent natural products are produced in very small quantity and thanks to the advances in technical abilities over the past two decades, the numbers of molecules have increased and quantities necessary for structure elucidation have decreased. Instrumental methods (NMR magnet sizes, probe technology, MS benchtop instruments, soft ionisation, FT-MS) have allowed for amazing advances in structure elucidation. Anticipated instrumental capabilities in 2020 are for (a) NMR: ‘Microcryophobes’ will allow structure elucidation of μg amounts of even very complex molecules and sensitivity gains in NMR spectroscopy will allow routine application of low-sensitivity-high information content experiments; (b) MS-MS n with high resolution and soft ionisation will continue to extend MS as a powerful structure elucidation tool. Thanks to these and other analytical methods, structural analysis will occur on increasingly small amounts of bioactive compounds from niche species and on microorganisms, depending on advances in culture methodologies. In order to maximise the discovery of new NMPs, several approaches should be considered: (1) the utility of marine biosynthetic investigations needs to be demonstrated and expanded via the development of ‘smart systems’ to select for molecules with desired properties from low-probability combinatorial biochemistry experiments; (2) more powerful genomic approaches need to be applied to the study of NP-rich complexes of symbiotic organisms; (3) more training opportunities for contemporary NMP research in developing countries are needed.

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Enzyme-based solutions for marine antifouling coatings

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Abstract: This chapter focuses on the application of enzymes as active ingredients in antifouling coatings. The various approaches reported in patents and articles during the past more than twenty years are considered. Based on the nature of the enzymes and substrates involved, the approaches are divided into direct and indirect enzymatic antifouling. Direct antifouling covers the application of adhesives-degrading or 'biocidal' enzymes, whereas indirect enzymatic antifouling is based on enzymatic generation of biocides from substrates present in seawater or coating-ingredients. It is evident that to function properly in an antifouling coating, enzymes must undergo some kind of modification to optimize their performance. The main challenges are to ensure compatibility of enzymes and substrates with coating ingredients, to establish adequate release rates or immobilization of the enzymes, and maintain enzyme activity for at least one year of seawater and biofouling exposure.

Key words: enzymes, antifouling coatings, controlled release.

23.1 Introduction

'Enzymatic antifouling' is here applied to describe antifouling coating approaches based on enzymes as the essential part of the fouling inhibiting property. Enzymes can be used as antifouling agents by a diversity of mechanisms. They can degrade the fouling organism, its adhesive, or produce other biocidal compounds.

Enzymes are catalytically active proteins, and as such they are omnipresent in nature. The catalytic activity of enzymes is utilized in several industries. Besides antifouling purposes, these include: food and drinks processing, analytics, treatment of textiles, detergents, modification of biological material and chemical reactors (Fullbrook 1996). Because enzymes are proteins, their application in industry is believed to be of little harm to the environment. One drawback of their application is that enzymes inevitably lose activity over time, typically according to a first order reaction mechanism

in enzyme activity (Fullbrook 1996). Therefore, a variety of biochemical methods have been developed to improve enzymatic stability and activity in artificial environments. These include: adsorption, encapsulation, entrapment, covalent immobilization and cross-linking (Bickerstaff 1997). Enzymatic activity and stability is affected by environmental parameters such as temperature, pH and salinity, but enzyme-stability can also be compromised by self-degradation (i.e., proteases degrade proteinaceous compounds). In non-sterile environments enzymatic stability can be lowered due to grazing by animals or bacteria.

The amount of literature available on enzymatic antifouling is not only limited, it is foremost in the form of patents. Patents are often published based on little scientific evaluation, and can therefore be of low technical value. The literature was recently reviewed in biofouling (Olsen *et al.* 2007). This text is based on the information provided in the review, and the reader is referred to the review for more information.

23.2 History of enzymatic antifouling

The first reporting of enzymatically mediated fouling control was, to our knowledge, concerned with paper mills and water towers (e.g., Hatcher *et al.* 1970). The enzymes applied were amylases that were added to the cooling water in solutions. As the enzymes were not enclosed in a coating, the application is of little technical interest to coating engineers.

In the first paper describing an enzymatic antifouling coating, several proteases were listed (Noel 1984), allegedly the invention outperformed the 'market leader' at the time during nine months in seawater. In the years between 1984 and 2005 16 independent patents and patent applications regarding enzymatic antifouling have been published. A timeline presenting the historical development of patenting within enzymatic antifouling is shown in Fig. 23.1. In the figure, the priority dates of the patents are shown. These are compared to selected dates regarding the regulation of tributyltin coatings.

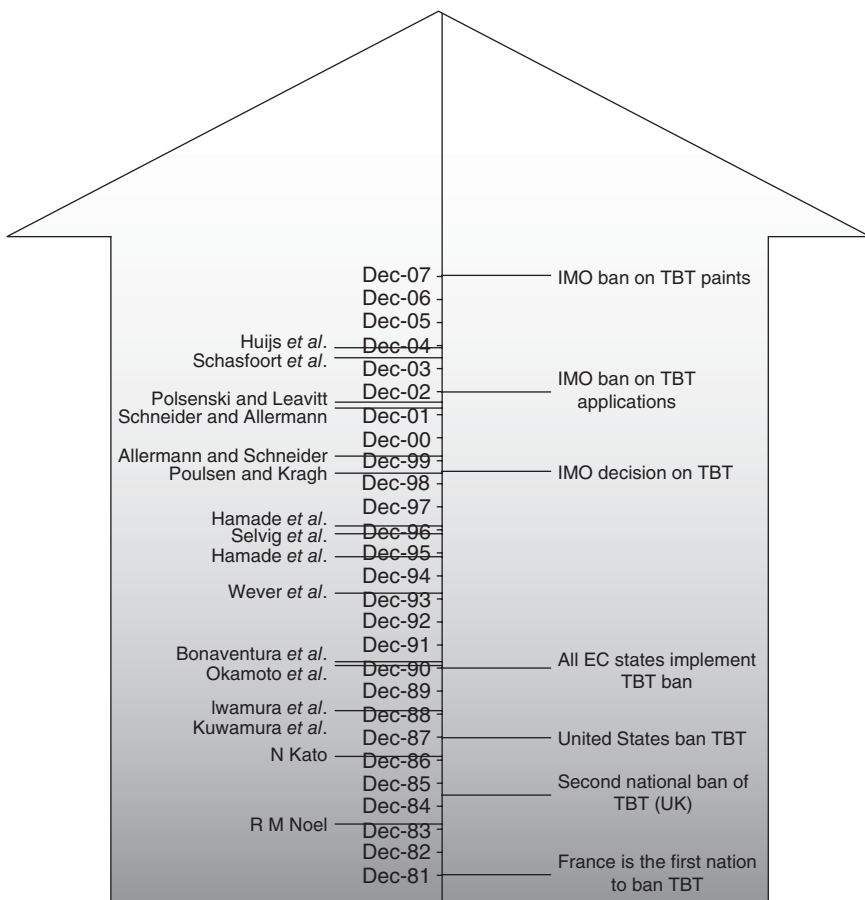
It is seen that the patenting is distributed evenly over the years, therefore the progress of the technology of enzymatic antifouling can be said to have been continuous since its origin in the 1980s. The enzymes mentioned in the patents are shown in Table 23.1. Also provided in the table is the general mechanism of the enzymes.

In at least one occasion, an enzyme-based antifouling coating has been commercialized. The product was introduced to the Danish yacht market during spring 2006. The coating is claimed to work by degrading biofouling adhesives, and is based on naturally occurring enzymes (Brejning 2006). Sales of the product, however, are still conducted on trial basis (Miljøstyrelsen 2007).

Table 23.1 Enzymes and their reactions, from patents on enzymatic antifouling. For more details see Olsen *et al.* (2007)

Inventor	Enzyme type	Reaction catalyzed
Noel (1984)	Protease	Protein degradation
	Endopeptidase	Protein degradation
Kato (1987)	Cellulytic	Cellulose degradation
	Proteolytic	Protein degradation
	'Cell-wall hydrolyzing'	
Iwamura <i>et al.</i> (1989)	Protease	Protein degradation
Kuwamura <i>et al.</i> (1989)	Protease	Protein degradation
Okamoto <i>et al.</i> (1991)	Protease	Protein degradation
Bonaventura <i>et al.</i> (1991)	Proteases	Protein degradation
	Amylase	Starch degradation
	Collagenase	Collagen peptide bond breaking
	Hyaluronidase	Hyaluronic acid degradation
	Carboxypeptidases	Protein degrading
Wever <i>et al.</i> (1994)	Haloperoxidase	$\text{H}_2\text{O}_2 + \text{Br}^- \rightarrow \text{HOBr} + \text{OH}^-$
Hamade <i>et al.</i> (1996)	Protease	Protein degradation
	Chitinase	Chitin degradation
	Lysozyme	Cell wall polysaccharide degradation
Selvig <i>et al.</i> (1996)	Amylase	Starch degradation
	Protease	Protein degradation
Hamade <i>et al.</i> (1997)	Esterase	$\text{RCOOR}' + \text{H}_2\text{O} \rightarrow \text{RCOOH} + \text{R}'\text{OH}$
	Amidase	$\text{RCONHR}' + \text{H}_2\text{O} \rightarrow \text{RCOOH} + \text{R}'\text{NH}_2$
	Alcohol dehydrogenase	$\text{RCOH} + \text{O}_2 \rightarrow \text{RCO} + \text{H}_2\text{O}_2$
	Chitinase	Chitin degradation
Poulsen and Kragh (1999)	Hexose oxidase	$\text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \rightarrow \text{C}_6\text{H}_{10}\text{O}_6 + \text{H}_2\text{O}_2$
	Amyloglucosidase (precursor)	Scissoring glucose units from starch
Allermann and Schneider (2000)	Protease	Protein degradation
	Amylase	Starch degradation
	Xylanase	Hemi cellulose (plant wall constituent) degradation
Schneider and Allermann (2002)	Oxidase	$\text{S} + \text{O}_2 \rightarrow \text{P} + \text{H}_2\text{O}_2^*$
	Precursor-enzyme	$\text{PreS} \rightarrow \text{S}^{**}$
	Protease	Protein degradation
Polsenski and Leavitt (2002)	Amylase	Starch degradation
	Cellulase	Cellulose degradation
Schasfoort <i>et al.</i> (2004)	Oxidase	$\text{S} + \text{O}_2 \rightarrow \text{P} + \text{H}_2\text{O}_2$
	Protease	Protein degradation
	Amylase	Starch degradation
	Cellulase	Cellulose degradation
	Lipase (etc.)	Hydrolysis of esterbonds in lipids
Huijs <i>et al.</i> (2004)	Glucose oxidase	$\text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \rightarrow \text{C}_6\text{H}_{10}\text{O}_6 + \text{H}_2\text{O}_2$
	Proteases	Protein degradation
	Amylase	Starch degradation
	Lipase	Hydrolysis of esterbonds in lipids

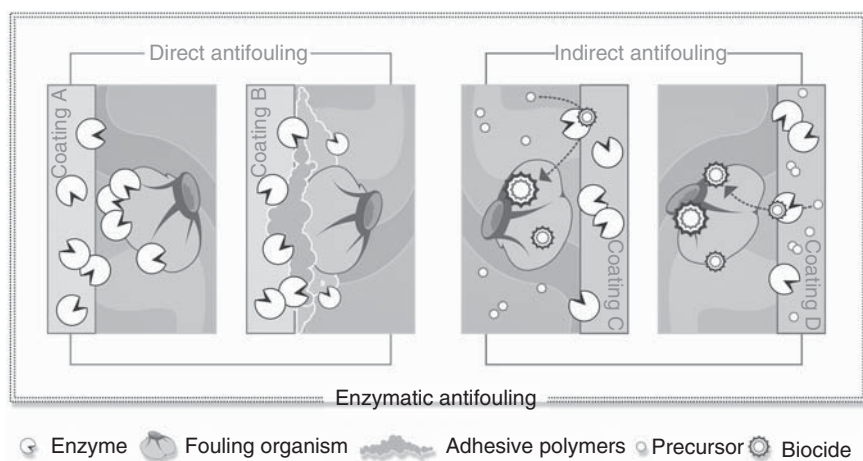
* S = substrate, P = product, ** Pres = precursor substrate.



23.1 Timeline on patents and patent-applications regarding enzymatic antifouling and the occurrence of tributyltin (TBT) ban in selected nations. Patent dates found in Olsen *et al.* (2007), legislation information found in Langston (2006).

23.3 Classification of enzymatic antifouling

Enzymatic antifouling can be classified based on the mechanism involved in the antifouling effect. If enzymes are applied to exert an antifouling effect, solely by their presence and activity, the approach can be classified as direct. If the enzymes, on the other hand, are applied to catalyze the *in-situ* production of antifouling active compounds, the approach can be labelled 'indirect'. The two diverging mechanisms can be further divided based on the nature of their substrates. The overall classification of the



23.2 Classification and proposed mechanisms of enzymatic antifouling. Coating A is based on biocidal direct antifouling. Coating B is based on adhesive degrading direct antifouling. Coating C is based on indirect antifouling with substrate in the environment. Coating D is based on indirect antifouling with the substrates provided from the paint. From Olsen *et al.* (2007).

mechanisms applied in enzymatic antifouling coatings is presented in Fig. 23.2; a schematic illustration of the different mechanisms is also provided in the figure. It is emphasized that the mechanisms proposed are based on the known catalytic effect of the enzymes as well as the descriptions found in the respective patents and thus the intentions of the inventions. Only a limited amount of data supports the alignment between theory and practice, intentions and reality.

23.3.1 Direct enzymatic antifouling

Direct enzymatic antifouling can be further divided into biocidal and adhesive degrading mechanisms. The former term describes the application of enzymes that 'attack' substrates vital to the fouling organisms, and the latter term describes the use of enzymes that degrade the adhesives of the fouling organisms.

Biocidal mechanism

Enzymes capable of direct biocidal antifouling mimics the mechanisms of commonly applied active ingredients. It is illustrated in coating A in Fig. 23.2. The mechanism is based on the release of enzymes that turn the

boundary layer next to the coating surface into a hostile environment for the fouling organisms. Enzymes capable of direct biocidal activity are seldom applied. However, cell wall degrading enzymes (Kato 1987), lysozyme (Hamade *et al.* 1996), chitinase (Bonaventura *et al.* 1991) and hyaluronidase (Bonaventura *et al.* 1991) can be expected to work in this manner. Lysozyme is commonly referred to as the body's own antibiotic as it is known to kill bacteria and is present in a number of human secretions (Madigan and Martinko 2006). Chitinase is known to catalyze the decomposition of chitin, which is an essential constituent of the barnacle shell (Bonaventura *et al.* 1991), and hyaluronidase hydrolyses hyaluronic acid (a cell tissue constituent), thereby increasing tissue permeability (Budavari *et al.* 1989).

In addition to the above-mentioned enzymes, catalase has been applied in one occasion (Bonaventura *et al.* 1991). Catalase is known to catalyze the decomposition of hydrogen peroxide to water and oxygen (Madigan and Martinko 2006). Since neither of these two products are toxic to fouling organisms, the use of catalase in antifouling coatings is expected to be rather inefficient. Only a biocidal effect of the protein (inactive or active) can be speculated (which is the reason for its placement in this category). However, catalase does not contribute to the antifouling activity of antifouling coatings at all (Bonaventura *et al.* 1991).

The effects of hyaluronidase (Bonaventura *et al.* 1991) and lysozyme (Hamade *et al.* 1996) have not been established, and the patent describing cell wall hydrolyzing enzymes has not been translated into English (Kato 1987). The only enzyme that is expected to contribute to fouling protection by a direct biocidal effect, and for which the antifouling efficacy has been tested, is chitinase. The effect of chitinase on barnacles is pronounced, but species specific. Whereas *Balanus* (a common barnacle) is significantly inhibited, the Tunicate, *Styela* is unaffected by the enzyme's presence in the coating (Bonaventura *et al.* 1991).

Antifouling coatings working by exposing fouling organisms to lethal or 'toxic' enzymes are challenged by the high diversity of fouling organisms that must be targeted. Species specific antifouling activity of the enzyme means that several different types of enzymes must be used. For each type of enzyme added to the paint system, difficulties regarding, e.g., paint compatibility and stability, are multiplied. Furthermore, an antifouling coating formulated with active ingredients that are both sufficiently potent and paint compatible needs to deliver these ingredients simultaneously at a rate close to the optimum. If the release rate of an individual enzyme deviates from the optimum, antifouling activity may be compromised irreversibly.

In summary; to achieve efficient antifouling based on biocidal enzymes, one must identify an enzyme or a combination of enzymes that attack the whole range of fouling animals, deliver the enzymes to the fouling animals

at a controlled rate close to the optimum rate, and still fulfill the general requirements regarding applicability, strength and stability of an antifouling coating.

Adhesive degradation

Fouling organisms apply different kinds of adhesives to attach themselves to a solid surface. Adhesives applied by fouling organisms can be divided into at least three groups; glycoproteins constitute the extra cellular polymer of algae (Callow and Callow 2006), diatom adhesives are based on carbohydrates (Chiovitti *et al.* 2006), and mussels and barnacles use proteins as their adhesives (Sagert *et al.* 2006, Kamino 2006) (cf. Chapter 24). The latter again emphasizes the need for more than one enzyme type if broad spectrum antifouling is sought.

Degradation of the adhesives as shown in Fig. 23.2 coating B can inhibit fouling in several ways. Besides repelling the organisms during settling, the enzymes may also inhibit settling by removing examining footprints, or revert settling by degradation of already cured adhesives.

Several biofouling organisms examine the substratum prior to settlement. This behaviour leaves adhesive materials called footprints. The footprint-adhesive can differ from the adhesive excreted during the irreversible settlement (see Bonaventura *et al.* 1991 for an overview of the adhesives). If the enzymes degrade these polymers as well, this may well contribute to the antifouling effect. Degradation of footprints by hydrolytic enzymes has been documented (Pettitt *et al.* 2004), but the antifouling effect can not be distinguished from the effect of degradation of irreversible adhesives.

To be effective in seawater, an adhesive should be released rapidly from the organism during attachment, wet the surface thoroughly, be insoluble in seawater, and exclude water from the matrix (Callow and Callow 2006). The co-substrate for hydrolytic enzymes is water, and it is therefore expected that hydrolytic enzymes have a greater activity against adhesives while they are still wet (i.e., during curing) than against cured (water-less) adhesives.

Based on the known adhesives used by only four common fouling organisms (proteins and polysaccharides, cf. above), it can be concluded that, as a minimum, two types of enzymes, proteases and amylases, must be used to achieve sufficiently broad antifouling activity. Several inventors failed to consider the broad diversity of adhesion-materials applied by fouling organisms by only applying one type of enzyme (Noel 1984, Iwamura *et al.* 1989, Kuwamura *et al.* 1989, Okamoto *et al.* 1991). Furthermore, enzymes are proteins that catalyse specific reactions. Proteases catalyse the degradation of proteins, including themselves and other enzymes. Therefore the presence of proteases will decrease the general enzyme stability. To prevent

proteases from exhibiting their proteolytic activity on themselves, the enzymes may be shielded from each other by encapsulating them in a carrier material (Jungbae *et al.* 2002). In fact, several benefits of encapsulation of common more simple biocides have been identified (Edge *et al.* 2001). Amongst others, improved control of the release rate is reported. In spite of this, no encapsulated biocides are commercially available at the moment. This indicates an added difficulty of applying encapsulation for active ingredients, independent of the nature of the ingredient.

For an enzyme to effectively remove fouling adhesives, it must be mobile enough to meet its substrate (the adhesive) on the site of its activity. If the active site is hidden, an adhesive can be applied in near proximity to the enzyme, and the enzyme will be unable to degrade it. The availability of the active site can be compromised due to immobilization of the enzyme in the coating matrix.

Enzymatic mobility may be caused by leaching of the enzyme, thereby creating an enzyme solution in the water layer next to the coating (Allermann *et al.* 2004, Bonaventura *et al.* 1991). Too fast leaching results in too short service life of the coating (Allermann *et al.* 2004). On the other hand, enzymes attached to the binder material of the coating (i.e., immobilized in the coating) have been reported to inhibit settlement effectively (Huijs *et al.* 2004). Accordingly, only limited mobility of the enzymes should be needed for the coatings to work efficiently. This is confirmed by the observation that polyurethane has been used as binder material in an enzyme based antifouling coating (Bonaventura *et al.* 1991). It is difficult to see how enzyme-release from thermoset polyurethane coatings can occur. Unless the coating was formulated close to or above the critical pigment volume concentration (CPVC: see also Chapter 12), the enzymes have most probably been effectively immobilized in the coating. As the adhesives will not penetrate into the film, the reported antifouling effect can only have been caused by immobilized enzymes situated in the outermost layer of the film (Bonaventura *et al.* 1991). Degradation of enzymes in such films will lead to fast loss of antifouling activity.

Very low leach-rate of enzymes must be compensated by a well defined and sufficient distribution of the enzymes on the surface of the coating (Bonaventura *et al.* 1991). Allegedly 1000 Å between adjacent enzymes is sufficient for good antifouling performance; however 100 Å was preferred (Bonaventura *et al.* 1991). One hundred Å between two adjacent enzymes corresponds to an enzyme PVC between 0.25 and 15 percent dependent on the enzyme diameter (2–20 nm applied in the calculations above). However, no technical investigations are provided to support the speculations regarding enzyme-distance and distribution, and the very low distances and 'pigment' diameters that are considered give the discussion a highly theoretic character.

During the investigation of degradation of pre-settlement adhesives (cf. above), Pettitt *et al.* (2004) became pioneers. According to our survey, they are the first to provide evidence substantiating the hypothesis that the presence of active hydrolytic enzymes actually decreases the amount of adhesives left on the substratum by fouling organisms. The presence of hydrolytic enzymes in bulk solution was also proven effective against a number of fouling species (Pettitt *et al.* 2004).

In the first paper covering the composition of an antifouling coating containing enzymes (Noel 1984), the enzyme-based coating outperformed two commercially available antifouling coatings. A test period of 9 months in natural seawater indicates long-term potential of the invention, but the mixture of enzymes and biocides (citric acid and natamycin as algacide and fungicide respectively) makes it difficult to interpret the results with respect to the effect of the enzymes. Another enzyme-based coating (Kato 1987) was claimed to efficiently prevent biofouling when applied to fishnets. However, the addition of surfactants and carboxylic acids to improve performance makes it difficult to determine the effect of the enzymes. The patented binder described by Iwamura *et al.* (1989) claims more antifouling efficacy than a commercial binder when enzymes are added to both. In this case, the effect of the enzymes is hidden in the overall effect of the coating. A patent by the same authors (Kuwamura *et al.* 1989) describes physical immobilization of protease onto a polystyrene polymer, and according to the English abstract, good antifouling efficiency is achieved when it is applied on nylon. Another patent (Okamoto *et al.* 1991) was issued based on the antifouling perspectives of proteases, but the English abstract provides little information about the antifouling effect, so it will not be discussed further here. In the early 1990s, the range of enzymes applied in antifouling coatings was expanded (Bonaventura *et al.* 1991). The majority of the enzymes contribute to the degradation of the extra cellular matrix of the biofouling community, but only a few have actually been tested for antifouling efficacy. Based on a study, in which the different coatings were exposed to seawater for 42 days, the authors concluded that protease and chitinase inhibited biofouling significantly. However, effects were reported to be species-specific (Bonaventura *et al.* 1991).

The actual antifouling effect of enzymes can be proven in laboratory assays, where the enzymes are present in the bulk-solution. Knowledge from such laboratory assays has been used to select enzymes for external test of antifouling coatings (Allermann and Schneider 2000). The effect of the enzymes in the coating was proven, but the coatings themselves carried a considerable antifouling effect. Shielding of hydrolytic enzymes by glucoside derivatives increases lipase activity in toluene significantly (Hamade *et al.* 1996). The covalently attached glucoside also increases the antifouling activity when enclosed in coatings. The treatment, however, increased the

polishing rate as well. Since the influence of the polishing rate on antifouling effect was not evaluated, it is impossible to preclude that the antifouling performance reported was due to faster polishing. Huijs *et al.* (2004) report that effective antifouling can be brought about by covalently immobilized enzymes on functional groups of binder constituents. The enzymes (glucose oxidase, proteases, α -amylase and lipase) applied in this manner contributed significantly to the antifouling effect of the coating, and immobilization improved the antifouling effect. Effective inhibition of the settling of common fouling organisms was achieved by Dobretsov *et al.* (2007). By retrieving proteases from deep-sea bacteria, the antifouling potency was significantly improved compared to commonly applied, commercially available proteases (Dobretsov *et al.* 2007).

In summary, for adhesive degrading enzymes contained in coatings to achieve antifouling performance that will satisfy the ship owners, one must identify enzymes capable of degrading all the adhesives applied by fouling organisms. The enzymes must be allowed to meet the adhesives, either by high mobility or a tight distribution. Furthermore, enzyme activity must be retained throughout the lifetime of the coating. Therefore the loss of enzyme activity should be compensated by the supply of active enzyme.

In addition to enzymatic antifouling coatings, microbial based antifouling has been reported. More specifically, a mix of hydrolytic enzymes and living micro-organisms has been applied in coatings for antifouling purposes (Selvig *et al.* 1996, Polsenski and Leavitt 2002). The addition of micro-organisms was intended to provide several benefits. Firstly, the added micro-organisms compete with biofouling micro-organisms on nutrients and food. Secondly, the 'benign' micro-organism may excrete antibiotics hindering the growth of biofilm. Third, the coating was supposed to contain hydrolytic enzymes, along with micro-organisms. These hydrolytic enzymes could ideally be provided by the micro-organism (Selvig *et al.* 1996). Microbial antifouling has been applied in combination with enzymes (Polsenski and Leavitt 2002), and spores and vegetative forms of the micro-organism have been used (Selvig *et al.* 1996). The presence of micro-organisms delayed the antifouling effect. Well performing coatings were generally more fouled after one month, than after four months. As the micro-organisms applied were rod shaped gram-positive bacteria, the delayed antifouling effect can possibly be explained by their dormant endospores. Endospores of the genus *Bacillus* can stay dormant for prolonged periods under stressful conditions (Madigan and Martinko 2006). The effect of the micro-organism containing coatings was unfortunately difficultly interpreted due to comparison with uncoated blanks (Selvig *et al.* 1996). However, coatings containing micro-organisms, and applied in layers, had increased hydrolytic activity (measured as starch hydrolysis), a tendency that increased with increasing number of coating layers (Polsenski and Leavitt 2002).

Furthermore, adding nutrients (sodium chloride saturated water) to the micro-organism in sub layers of the coating was found to be beneficial for the hydrolytic activity of the coating (Polsenski and Leavitt 2002).

Micro-organisms involved in paint production and application must, to survive that treatment, be in the form of dormant endospores. These are difficult to kill, and this is probably the only way to introduce living species into the coating. However, it is difficult to imagine how their nutrients can be added to a polishing coating without compromising the polishing behaviour and leached layer thickness due to their high water solubility (cf. Kiil *et al.* 2002a). Without nutrients present in their close environment, the micro-organisms enclosed in the antifouling coating must live in competition with the organisms originating from the marine environment. As the latter organisms are probably the most suited for the conditions, they will be the survivors and the added micro-organisms are therefore believed only to have a limited impact on antifouling performance when it comes to long-term stability.

23.3.2 Indirect antifouling

Considering indirect enzymatic antifouling; the origin of the substrates divides the concept into seawater originating and coating containing approaches. Generally speaking the enzymatic reaction involved in indirect antifouling can be presented as:



If all the precursor compounds are found in the marine environment, the mechanism is differentiated from the cases where one or more of the precursor compounds originate from within the coating. For instance hydrogen peroxide can be the antifouling agent intended as a product of enzymatic reactions within the coating. The substrates for the oxidases can be added to the coating (Poulsen and Kragh 1999) or produced from the surroundings (Schneider and Allermann 2002).

Substrates from surroundings

Enzymes in immersed antifouling coatings can act to convert compounds from their environment into potent antifoulants. The mechanism is illustrated in Fig. 23.2 coating C. Two patents have described this application of enzymes for antifouling purposes.

Haloperoxidase present on the surface of an antifouling coating can convert the seawater content of hydrogen peroxide and halide ions into hypohalogenic acid (Wever *et al.* 1994). Hypohalogenic acid (e.g., HOCl) is an oxidizing acid of halogens and a potent antifoulant. The antifouling effect of the acid exceeds that of a corresponding amount of hydrogen

peroxide (Wever *et al.* 1994). The major drawback of the application of haloperoxidase for antifouling purposes is the availability of hydrogen peroxide in natural seawater. In the experiment testing haloperoxidase for its antifouling potential, hydrogen peroxide was added in amounts of at least 10 μM (Wever *et al.* 1994). However, hydrogen peroxide content of surface seawater rarely exceeds 0.1 μM (Yuan and Shiller 2001).

Hydrogen peroxide may be produced from proteins or polysaccharides present in seawater. The mechanism involves a series of enzymatically catalysed reactions (Schneider and Allermann 2002). Precursor enzymes must be added to convert the precursor substrates into a suitable substrate for an oxidase. The precursor enzymes should most likely be of the type's protease or amylase, and the second enzyme therefore amine oxidase or hexose/glucose oxidase. A coating based on the production of hydrogen peroxide from precursor substrates has been reported antifouling efficient during 'monthly' inspections (Schneider and Allermann 2002).

When substrates are found in the surroundings of the coating, they must be present in amounts sufficiently high for the enzymes to continuously deliver an inhibiting release of the antifouling product. The concentration cannot fluctuate on a geographical scale nor should it be time dependent. This is believed to be a highly restrictive requirement, limiting the potential of the approach effectively. Furthermore, the inevitable build up of slime on the surface of the antifouling coating (Yebra *et al.* 2006a) will provide diffusion resistance to the seawater-dissolved substrates, lowering the active concentration of the substrates at the coating interface where the enzymes are situated.

Substrates from the coating

Enzymes can be applied as a way of facilitating a controlled release of active AF agents from the coating. This mechanism is here referred to as indirect antifouling where the substrates are a part of the coating (c.f. Fig. 23.2 coating D). In contrast to the direct approach, indirect enzymatic antifouling varies more in type of enzymes and reactants. The water-solubility of the precursor compound initially added to the coating is a parameter of concern. Even moderately water-soluble substrates will leach out of the coating rapidly, compromising the polishing rate of the coating (Kiil *et al.* 2002a).

A variety of enzymatic reactions producing possible antifouling agents have been proposed (Hamade *et al.* 1997) (cf. Table 23.1). A coating composition comprising tricaprin (C10 fatty acid trilipid) and lipase is the only test of indirect antifouling where the substrate comes from the coating, and from where data is provided (Hamade *et al.* 1997). The enzyme was intended to decompose the lipid in the coating, thus to continuously release capric acid from the coating. A coating was exposed to seawater for three months

after which 'no slimy biofouling' was found. A similar coating containing capric acid only was, however, 'fouled all over'. The difference in effects may be explained by the controlled release of capric acid from the former coating, and fast leaching of capric acid from the latter, but it may also indicate that the enzymes alone are antifouling active. Though still inconclusive, the comparison indicated that the antifouling effect was caused by the enzyme system, and not due to binder or surface properties (Hamade *et al.* 1997), a consistency seldom seen in the examples of the patent literature.

The composition of an enzyme-based antifouling coating was described in more detail by Poulsen and Kragh (1999). Continuous release of hydrogen peroxide from the antifouling coating was the aim. The enzymatic production of hydrogen peroxide is achieved in a one-step process, or a two-step process. The former is composed of the hydrogen peroxide releasing system by the combination of glucose and hexose oxidase, and the latter includes a precursor system producing glucose by amylase catalysing starch hydrolysis. Stable hydrogen peroxide release by a coating comprising starch, amylase and hexose oxidase was reported (Poulsen and Kragh 1999). The coating was also claimed to efficiently prevent fouling. However, data substantiating this claim was not provided.

When the substrate is supplied from within the coating, the initial amount of substrate in the coating must be high enough for the antifouling effect to last for the entire operational period (1 season for yacht purposes and 3–5 for trading ships). High amounts of substrates may be incompatible with other paint constituents or with paint production in general, potentially compromising paint properties, as applicability and structural integrity of the coating. A stable release of the substrate is needed for continuous antifouling effect. In the literature, this has been achieved by precursor enzyme-systems. In these cases (e.g., starch to glucose or proteins to amino acids), a complete turnover of the precursor product will be necessary to avoid any potential pro-fouling effect of the precursor compounds (e.g., glucose).

Because enzymes and substrates must be present together in the coating, they must be mixed at some point before paint application. It is of importance for the shelf-life of the product as well as the antifouling efficiency during operation to secure that no conversion of substrates occurs before the ship has been immersed. This may be done by eliminating one or more of the substrates needed in the conversion (e.g., O_2 , H_2O) in the paint can. In the absence of co-substrates, the content of the paint will be stable. Once immersed, penetration of these substrates into the coating will induce substrate conversion.

In summary, antifouling based on enzymatic conversion of precursor compounds in the coating into antifoulants, is dependent on a stable supply

of the active ingredient. The precursor compound must be compatible with the coating constituents, and the substrates have to reach the enzymes. More importantly, any enzyme activity must be restricted during paint manufacturing, storage and application.

23.4 Other aims of enzymatic activity in coatings

Enzyme-containing coatings have been used for other purposes than antifouling activity. In one case, enzymes were added to facilitate polishing of the coating (Schasfoort *et al.* 2004). In another scenario, phenol oxidizing enzymes were used to cure lignin (Bolle and Aehle 1993). The antifungal effect of the resulting coating would make it applicable for impregnation of wood (Felby and Hansen 1996). In another application, enzyme-containing coatings have been used for decomposition of a series of organophosphorus compounds used in chemical warfare (McDaniel *et al.* 2006).

23.5 Pitfalls in enzymatic antifouling coating development

Enzymatic antifouling is a technology that is more than twenty years old. Several patents have been granted based on the combination of enzymes and antifouling coatings. Yet the technology has only had limited commercial success. This indicates that the technology is challenged beyond the proof of concept stage.

23.5.1 Insufficient testing

When enzyme-based antifouling coatings are evaluated for their antifouling performance, not all the effects related to the presence of enzymes may be known. The enzyme itself may act on more than one substrate; additives in the enzyme product may possess antifouling properties; and coating parameters, such as polishing rate and surface properties, which may be affected by the addition of enzymes, constitute a considerable part of a coatings antifouling properties. It is therefore necessary to use a large number of references when examining the effect of an enzymatic antifouling system. Furthermore, the colour may vary between experimental coatings, and recently the effect of colour on short term antifouling activity was established to be a significant factor (Swain *et al.* 2006). A substantial time frame must therefore be used when assaying the antifouling ability of enzyme-based coatings in the marine environment (Chapter 16). As stressed in that chapter, the most difficult requirement for AF paints is not to show AF protection, but to keep it for prolonged periods of time.

23.5.2 Unknown effects of the enzymes

Distinction between indirect and direct enzymatic antifouling can prove impossible. Two very similar enzymes have been used for indirect antifouling (hexose oxidase, Poulsen and Kragh 1999) and direct antifouling (glucose oxidase, Huijs *et al.* 2004). In the latter case, the enzymes were immobilized, which improved the antifouling efficacy. The intended effect of an enzyme comprised in a coating constituent is therefore not necessarily in accordance with reality. It cannot be ruled out that the antifouling effect experienced by Poulsen and Kragh can be ascribed to a direct effect of hexose oxidase or vice versa.

For practical purposes, it may seem insignificant how an antifouling enzyme is working as long as it is efficient in preventing fouling, but manipulation and optimization of paint performance is highly dependent on knowledge about the influential parameters. Furthermore, evaluation of the environmental consequences of the enzyme in question is dependent on the activity of the enzyme. If the activity of an enzyme is restricted towards the extra cellular substances of the biofouling organisms, the environmental impact is limited. On the other hand, if the effect of the enzyme is toxicity towards the organisms, the environmental impact is also dependent on the enzymes' lifetime in seawater.

23.5.3 Limitations to enzyme activity and stability in coating matrices

Obstacles associated with the combination of active biochemical enzymes and common antifouling coating constituents may well contribute to the limitations of the technology. Despite the age of the technology and the amount of patents no commercial breakthrough has occurred, and it can be argued that mixing enzymes and paint is insufficient for long-term antifouling activity of the coating. Optimization of enzymatic stability in the paint solvent and matrix has been addressed (Hamade *et al.* 1996), but grazing of marine animals or bacteria may pose another threat to the proteinaceous compounds. Furthermore, as recognized by Poulsen and Kragh (1999), enzymes have narrow niches of activity within pH, temperature and salinity, niches not necessarily compatible with the marine environment.

A mix of enzymatic antifouling agents is often applied to address the diversity of environmental substrate, and often the enzyme blends contain proteases (Schneider and Allermann 2002, Allermann and Schneider 2000). Since enzymes are proteinaceous compounds, proteases will degrade enzymes as well, decreasing the stability of all enzymes in the coating matrix.

The impact on enzyme activity of inclusion into paint depends on the propellant of the paint. In most cases, enzymes can easily be added to water,

but for solvent-based systems, enzymatic denaturation may be accelerated by unfolding of the enzyme.

Based on the above, it can be stated that enzymes should be chosen, modified or improved to fulfil the following list of demands:

- miscible with paint
- stable in solvent
- possess niches of activity corresponding to natural seawater conditions
- protected from grazing and protease degradation
- stable in marine environment
- environmentally acceptable.

23.5.4 Active biochemical additives as novel coating ingredients

Enzymes are relatively new coating constituents, and in the cases where substrates must be provided from the coating as well (i.e., indirect antifouling), a large fraction of the coating may be novel coating ingredients.

The desired lifetime of an antifouling coating will often exceed the lifetime of an active enzyme situated at the surface of the coating. The enzymes at the surface should therefore be continuously replenished during operation. Replenishment of active enzyme can come about by means of polishing. Therefore, the enzymes should be a part of a self-polishing coating. For a coating to obtain or retain polishing behaviour it is necessary to evaluate the novel 'pigments' (substrates or enzyme containing particles). Kiil *et al.* (2002b) provide a straight forward graphical tool to determine the compatibility of a new soluble pigment and a self-polishing antifouling coating (i.e., obtainment of suitable polishing and leaching rates when adding novel pigments). Using physical data (seawater solubility and density) of the novel pigment and an approximate diffusion coefficient for the dissolved pigment in seawater, an estimate of the polishing and leaching rates of a tin-based, self-polishing antifouling coating can be easily obtained from dimensionless figures in the paper (i.e., without solving the mathematical model provided in the paper). The technique can also be used reversely to estimate the seawater solubility of a novel soluble pigment that one should aim for to obtain a certain polishing and leaching behaviour (Kiil *et al.* 2002a). For other types of paint, leaching behaviour can also be estimated roughly using a similar framework just by fixing the polishing rate (see also Yebra *et al.* 2006b).

23.5.5 Biocides in enzyme preparations

Commercial enzyme-additives can contribute to the antifouling effect of enzyme containing coatings. When enzymes are purchased, they will typi-

cally contain a large amount of stabilizing additives. The amount of enzyme is therefore only a small fraction of the material, and the additives may also possess biofouling inhibitive properties. Pettitt *et al.* (2004) experienced an equal effect of heat denatured enzymes and native enzyme solutions for several commercial enzyme products. This was the case for U. Linza settlement in solutions of Alcalase 2.5 L type DX® (a protease), and settlement and mortality of barnacles (*Balanus Amphitrite*) in solutions of AMG 300 L® (an amylase).

Alcalase 2.5 L Type DX® contains 5.7% protein (Pettitt *et al.* 2004). The additives consist amongst others of sorbitol and calcium formate (Allermann *et al.* 2004). AMG 300 L® contains 4.4% protein in a blend of potassium sorbate, sodium benzoate and sucrose/glucose (Allermann *et al.* 2004). Though both enzyme products are established not to be harmful to the environment (Allermann *et al.* 2004), the antifouling effect of benzoate towards the common barnacle, *Balanus Amphitrite*, has been established (Stupak *et al.* 2003). In the case of Alcalase®, formate may have a fouling inhibitory effect. Formic acid is reported toxic to mice and human skin (Budavari *et al.* 1989). Alcalase® 2.5 L and AMG 300 L® are used widely in the literature, (Allermann and Schneider 2000, Schasfoort *et al.* 2004, Pettitt *et al.* 2004). The results reported should therefore be interpreted with caution.

23.6 Legislation

The Biocidal Product Directive of the European Union (BPD-98/EC) governs, amongst 23 product groups, also the use of antifouling coatings. The directive covers biocides as defined in its article 2(1a): 'Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means' (EU 1998). A consequence of this definition of biocides is that all products used for antifouling purposes should be treated equally. This is pointed out in the Directives Manual of Decisions; an enzyme known to act on the adhesive of biofouling organisms is herein determined to be within the scope of the BPD (Manual of decisions for BPD-98/EC 2005; see Chapter 10 and Chapter 20 of this book).

23.7 Conclusions

Enzyme based antifouling approaches can be categorized based on the intended action of the enzymes involved in the antifouling activity. The different approaches are biocidal, adhesive degrading and AF agent-providing

(e.g., biocides). The two former can be said to be direct and the latter indirect. Indirect antifouling can be distinguished based on the origin of the substrates (i.e., from the seawater or the coating). The different mechanisms provide differing obstacles to be overcome. However, generally the technology is challenged by the compatibility of enzymes and coating matrices as well as the diverging timescales of their stability.

From a commercial point of view, antifouling coatings must be available to the consumer (e.g., fulfilling environmental legislation, sufficiently cheap, and possess stock stability), easily applied and prevent biofouling during the full service life of the coating (e.g., one year/season for yacht purposes). For the latter, any enzyme system utilized must have at least the following qualities:

- robust towards coating constituents
- non-destructive towards coating mechanisms
- broad spectrum antifouling effect
- stable activity in the coating upon exposure of the coating to seawater.

For these requirements to be met, the enzymes will undoubtedly have to undergo some kind of modification. Several methods in biochemistry can be applied to improve enzyme performance in coatings. Covalent immobilization (Huijs *et al.* 2004) and coating with hydrophobic material to increase solvent resistance (Hamade *et al.* 1996) have been described. Such methods add extra difficulties when tuning the AF performance of the coating, as discussed in this chapter.

It is necessary when establishing the effect of modified as well as native enzymes, that a sufficient amount of references are used, and that the coatings are tested in a substantial timeframe.

23.8 References

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Part IV

Surface approaches to the control of
marine biofouling

Advanced nanostructured surfaces for the control of marine biofouling: the AMBIO project

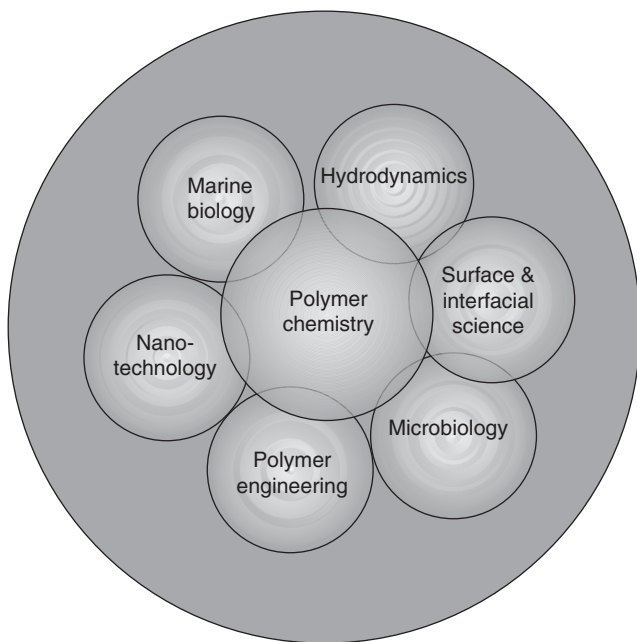
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Abstract: The AMBIO project ('Advanced Nanostructured Surfaces for the Control of Biofouling') is an Integrated Project funded by the European Commission under its 6th Framework Programme. The 5-year project, which started in 2005, is devoted to the knowledge-based development of antifouling coatings that function through their nanoscale properties, without the use of biocides or materials that are released into the environment. The principal knowledge-driven strategy of the project is to achieve structure/property/performance correlations for a wide range of nanostructured coating designs. State-of-the-art analytical methods are used to characterise the coatings in aqueous 'seawater-like' environments. A wide range of laboratory-based settlement and adhesion assays are used to explore the antifouling potential of coatings, selected examples of which will enter comprehensive field trials for a range of end-use applications. The project involves 31 partner organisations (industries, universities and research institutes) linking the scientific expertise of polymer chemists and engineers, surface scientists, and marine biologists with the experience and commercial perspectives of coating technologists and raw materials manufacturers, together with a wide range of end-users. The project has a strong stakeholder orientation involving a range of end-user industries and suppliers of raw materials and technologies to ensure relevance and take-up of the results. This chapter outlines scientific progress towards the project goals in the first three years.

Key words: nanotechnology, nanocomposites, adhesion, fouling-release coatings, biofouling.

24.1 Introduction

The AMBIO project ('Advanced Nanostructured Surfaces for the Control of Biofouling') is an Integrated Project funded by the European Commission under its 6th Framework Programme. The 5-year project, which started in 2005, is devoted to the knowledge-based development of antifouling coatings that function through their nanoscale properties, without the use of biocides or materials that are released into the environment. The project

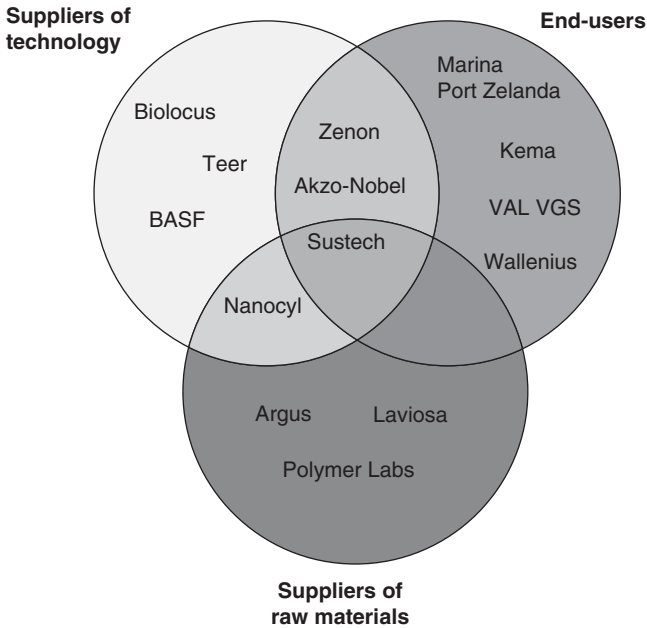


24.1 The interdisciplinary scientific base of AMBIO.

has a large critical mass and scale of resources: 31 partner organisations (industries, universities and research institutes) are linked as a legal consortium. The total value of the project is approx. €18 M, of which €12 M is provided by the EC. The project couples the scientific expertise of polymer chemists and engineers, surface scientists, hydrodynamicists, and marine biologists (Fig. 24.1) with the experience and commercial perspectives of coating technologists and raw materials manufacturers, together with a wide range of end-users (Fig. 24.2). The project has a strong stakeholder orientation involving a range of end-user industries and suppliers of raw materials and technologies to ensure relevance and take-up of the results.

24.1.1 Alternatives to biocides

Biocide-containing paints are the mainstay of the marine antifouling coatings industry and providing they meet relevant international legislation (e.g., in Europe the active ingredients have to comply with the Biocidal Products Directive EC 98/8/CE; Chapter 10), their use will continue for the foreseeable future. However, because the use of biocides is highly regulated, it is expensive to add new compounds to the list of allowed substances, which in turn means that there is restricted scope for innovation. The fol-



24.2 The company and supply-chain base of AMBIO.

following excerpt from the Biocidal Products Directive (BPD) is of particular relevance, a 'biocide' being defined as:

'Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means.'

Alternatives to biocide-based technologies are therefore urgently sought. The implication of the above wording of the BPD is that it does not cover coatings that rely on purely physical means to create a surface with properties that reduce biofouling. Therefore, the ideal 'green' technology should be biocide-free, should not release any toxic ingredients into the environment and its effectiveness should be based on the physical properties of the coating. It is in the latter context that the 'nanoscale' dimension becomes important (see Section 24.1.2).

There are, of course, current non-biocidal technologies, notably the commercial fouling-release silicone-based coatings. These are particularly successful for vessels characterised as 'high speed/high activity' but, are less successful for vessels that operate for most of their time at low-moderate cruising speeds, and which spend a significant proportion of their time at anchor, such as navy fleets. There are also issues of durability and the

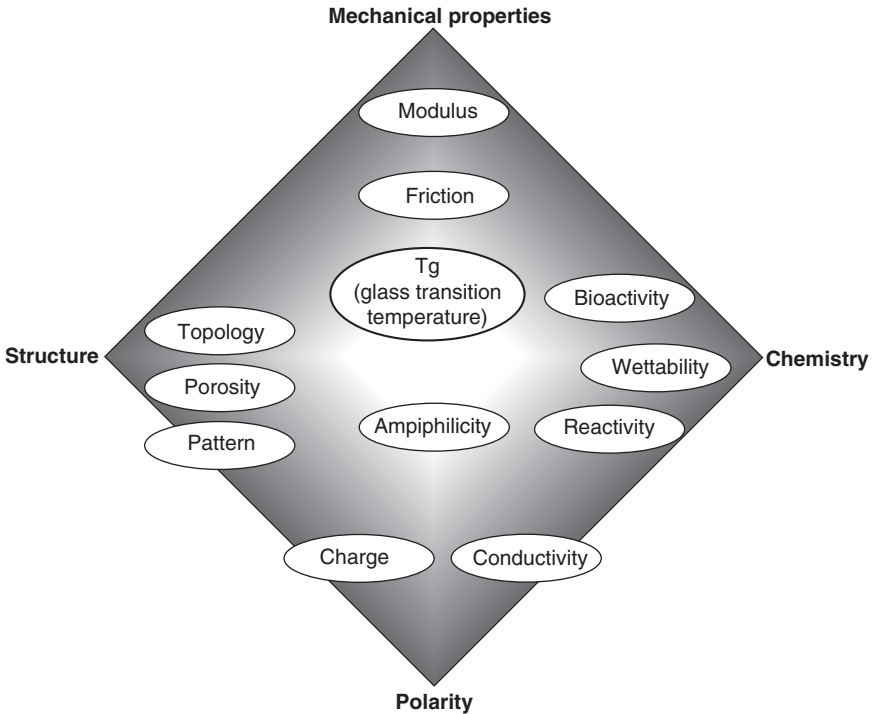
leaching of oils from some coatings. There is therefore considerable scope for either improving the properties of current fouling-release coating designs, or devising alternatives to the silicone-based materials in current commercial use. This is a key objective of the AMBIO project.

24.1.2 Why 'Nano'?

Nanotechnology is science and engineering at the scale of atoms and molecules. It deals with objects and materials that can be made, controlled, or manipulated on the scale of 1 to 100 nanometres. It studies the fundamental principles of these objects and the phenomena and laws that are particular to this length-scale, and is the natural scale of all fundamental life processes.

Biofouling is a prime example of a nanoscale interfacial process. Organisms that foul surfaces in aquatic environments, such as barnacles, algae, bacteria, do so through the secretion of adhesive polymers. The fouling process involves interactions determined within a few nanometres of the surface, the tenacity of adhesion being determined by the molecular interaction of specific structural elements in these adhesives (e.g., amino acid motifs or specific chemical groups) with the substrate, through a range of bonding mechanisms (Smith and Callow 2006). For example, in studies on the influence of topography on attachment of marine bacteria, it has been shown that the almost instantaneous adhesion is influenced by surface imperfections in the nanometre range, i.e. far smaller than the size of bacteria, suggesting that the results were due to a similarity between the size and shape of the imperfections and the size and configuration of the adhesive molecules (Kerr and Cowling 2003). Furthermore, fouling organisms, especially invertebrates and algae, are highly selective in their preferences for certain surfaces. 'Cues' presented by a surface initiate characteristic behaviours which either promote or inhibit attachment. Whilst these have been extensively explored at the micro-scale (e.g., Carman *et al.* 2006; Schumacher *et al.* 2007; Scardino *et al.* 2008) there is relatively little information on the influence of nanoscale features on the settlement of fouling organisms.

Adhesion may thus be regarded as a natural nanoscale process and therefore its ultimate control lies in modifying the properties of surfaces at the nanoscale through novel surface engineering technologies. Figure 24.3 summarises the range of interfacial properties that have been shown by experimentation to be relevant to anti-settlement and/or fouling-release performance of coatings against marine organisms. All of these interfacial properties may be influenced by the nanostructure of a coating, but as yet, few such influences have been systematically investigated. Whilst it is combinations of these parameters that will ultimately determine the overall



24.3 The range of physico-chemical coating properties that can influence settlement and adhesion of fouling organisms (modified after Rosenhahn *et al.* 2008).

performance of a coating for antifouling applications, the systematic manipulation of single interfacial properties to determine which exert the most important influences on fouling organisms is a worthwhile approach. This requires great precision and control over surface properties and in the AMBIO project this is provided by experiments on ‘model’ surfaces in which chemical and physical properties are patterned or tailored at different length scales through self-assembly, nanolithography, photolithography and micro-contact printing.

24.2 Aims of the AMBIO project

The primary aim of the AMBIO project is to explore the potential for innovatory, alternative coating solutions using a ‘knowledge-driven’ engineering strategy based on sound correlations between the chemistry of the coating, its physico-chemical and materials properties, and the biological performance. To achieve this ambitious aim requires the integration of a

wide range of scientific and engineering disciplines coupled to state-of-the-art approaches for:

- use of novel materials such as nanoparticles for structuring coatings
- characterising surface properties that are most relevant to biological performance, e.g. environmental AFM for *in situ* analysis of surface topology of fully hydrated coatings
- a biological evaluation programme that facilitates understanding of coatings that succeed or fail at a mechanistic level
- translation of coating concepts from the laboratory scale of the experimental scientist to the level of a formulated coating technology that can be produced and applied on a scale that will allow performance testing in real-world environments.

24.3 Structure of the project

24.3.1 Participants

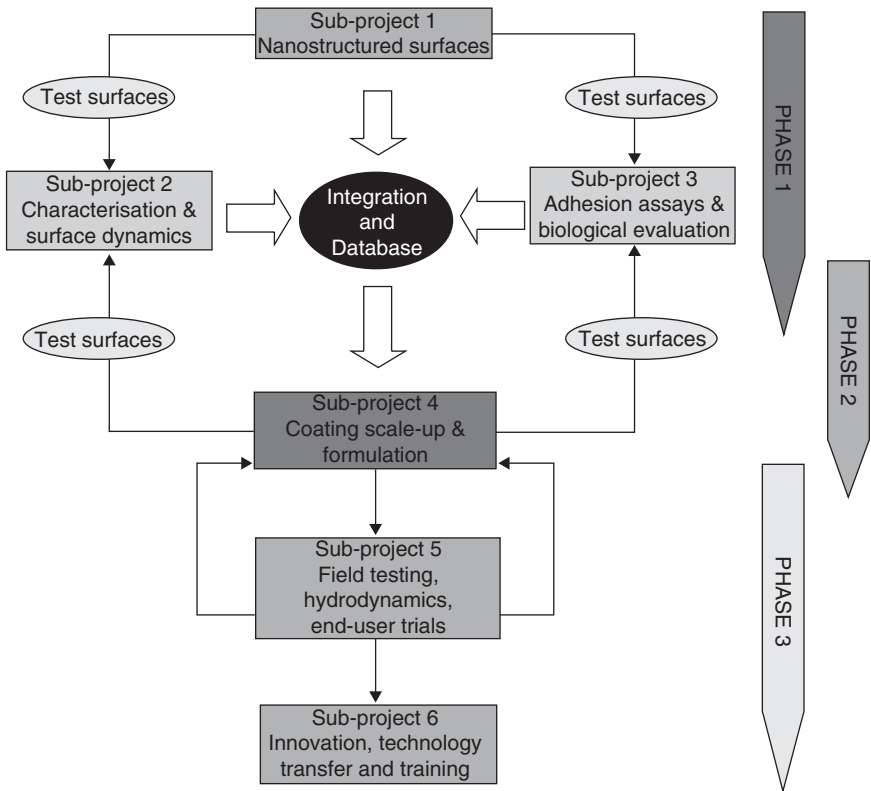
The project contains 31 'Partners' from 14 different nations within the EU, or which have some form of affiliation (Turkey, Norway, Israel). There are 11 universities, 5 research organisations and 15 companies (<http://www.ambio.bham.ac.uk/about/Partners.htm>), the latter including two large multinationals (BASF, Akzo-Nobel (International Paints)) and several SMEs (Fig. 24.2). 'Partners' are members of a consortium with formal contractual obligations to the EC. Intellectual property rights are formalised through a Consortium Agreement.

24.3.2 Work plan

Like all EC-funded projects, AMBIO is structured into a number of work packages with discrete objectives and activities, and these are organised into six sub-projects (Fig. 24.4).

Phase 1 ('Experimental') represents the main focus of activity in the first 3–4 years of the project in which the combination of novel coating and surface-structuring technologies, characterisation of interfacial and materials properties, and biological evaluation was anticipated to generate: a) candidate coatings for further work in the project, and b) new knowledge on the adhesion processes used by fouling organisms.

By the end of the third year it was anticipated that a number of candidate technologies with the appropriate combination of properties would be selected to progress to Phase 2 ('Scale-up and development'), in which experimental coatings will be scaled up and formulated by specialist partners (e.g., Akzo-Nobel) to a point where they are testable in field trials in



24.4 The structure of the AMBIO project.

Phase 3 (years 4–5). For coatings to progress, the following selection criteria were applied:

- good intrinsic antifouling and/or fouling-release properties against representative test organisms
- stable coatings on appropriate substrates in either marine or fresh-water conditions
- the coatings represent conceptual advances in nanoscale engineering for novel coating technologies
- there is a clear end-user application available for field-testing within the project.

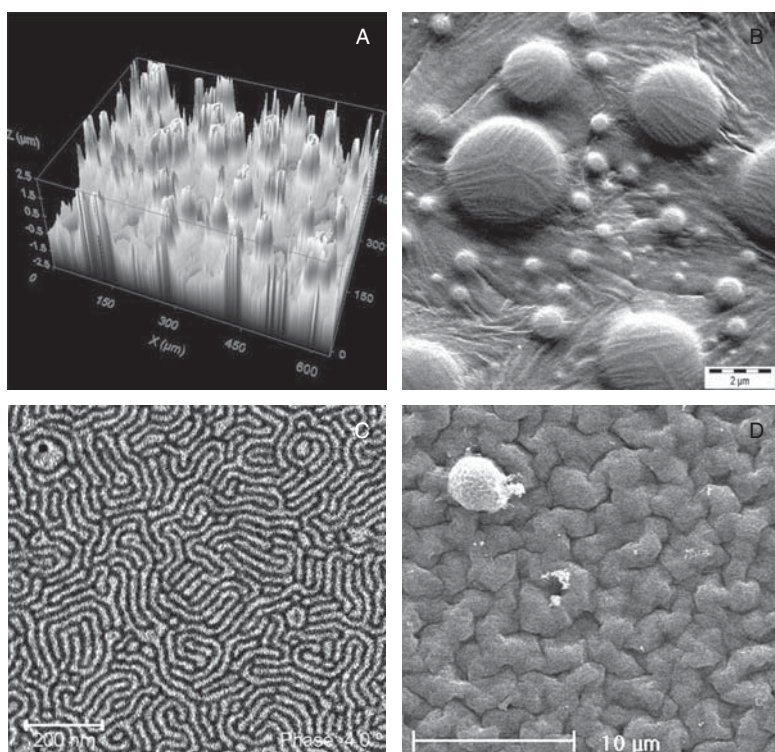
Phase 3 ('Field trials') due to start in March 2008, aims to make quantitative and comparative evaluations of the antifouling efficacy of promising nanostructured technologies through controlled field-scale testing. As a first approach, selected coatings/surfaces will be applied to a range of substrates representative of the range of end-user applications in the project (primed

steel for ship hull coatings, fibre-glass for pleasure craft, polypropylene for fish nets, titanium plates for heat exchangers, membranes for water purification, glass for optical windows, etc.). Replicate substrates will be immersed in suitable environmental conditions for a period of 2–18 months depending on their envisaged end-use. The performance of the technologies will be monitored by assessment alongside industry-accepted standards. For example, in-field adhesion measurements (ASTM D5618) will be used to measure the shear forces required for removal of adult barnacles from fouling release surfaces. Feedback on performance will be provided for further formulation/development work in SP4. The drag characteristics of promising nanostructured technologies will be tested hydrodynamically. Finally a ‘Demonstration Activity’ towards the end of the project will monitor the in-service performance of selected coatings alongside industry-accepted standards for a period of 2–10 months, depending on their envisaged end-use.

24.3.3 Methodological aspects: nanostructured coating designs

Nanostructured surfaces may be classified by their manner of preparation. ‘Bottom-up’ methods use chemical building blocks (molecules, nanoparticles) and self-organisation/supramolecular chemistry, for assembling surfaces with nanosized dimensions. Physical vapour deposition (PVD), chemical vapour deposition (CVD), and electrochemical deposition techniques are used to prepare thin films on top of solid substrates. Coatings of this type are particularly relevant for heat exchanger plates, where it is important that thin coatings are used for efficient thermal transfer, but other application areas for such coatings include optical windows (if they can bond effectively to glass) or water-filtration membranes (providing they do not occlude the pores). Since such methods are analogous to self-assembly of molecules on a surface from solution or the gas phase, it may be considered that these also represent a ‘bottom up’ approach. Bottom-up approaches, especially the polymer-based methods are particularly suited to those applications of AMBIO where a coating has to be applied to structures for aquatic environments, possibly on a very large scale. ‘Top-down’ approaches, e.g. nanolithographic methods, are only feasible for sophisticated, small area applications. Their use in AMBIO is limited to ‘model surfaces’ for exploring adhesion mechanisms.

The major approaches in AMBIO to the creation of novel coatings with tunable nanoscale properties are based on supramolecular chemistry, self-assembly, phase-separation of polymers, superlattices, or molecular building blocks. Appropriate polymers either alone, through spontaneous restructuring or self-assembly, or in combination with nanoparticulate materials, are



24.5 Selected examples of nanostructured coatings in the AMBIO project. A) Nanostructured sol-gel coating containing protruding nanoparticles viewed by confocal microscopy (courtesy TNO). B) SEM of nanostructured surface of solvent-cast polyolefins showing self-assembled surface topologies at micro- and nanoscales (courtesy Gebze Institute of Technology and BASF). C) AFM phase image of a block copolymer coating showing nanoscale phase-segregation of hard and soft blocks (courtesy University of Pisa and BASF). D) SEM image of self-assembled polyelectrolyte surface showing micro- and nanoscale topologies. This surface is highly resistant to the settlement of algal spores; the spherical object (top left) is a rare example of a settled *Ulva* spore (University of Heidelberg and University of Birmingham).

used to create a coating layer presenting a nanostructure to the surface (Fig. 24.5).

In the case of 'model' surfaces, the focus at the Universities of Heidelberg and Linköping is on the development of well-characterised tunable surfaces with which to explore the settlement and adhesion properties of fouling organisms. For example 'top-down' nanostructuring techniques such as various forms of lithography and self-assembly facilitate independent control of surface chemistry, topography and patterning.

24.3.4 Methodological aspects: evaluation of materials for antifouling technologies

Laboratory-based evaluations of the adhesion of organisms to surfaces have focused mostly on bacteria and have used settlement assays in either static or flow-through systems. Few studies have addressed the settlement and adhesion of higher organisms (algae and invertebrates) and evaluations of non-biocidal antifouling technologies have relied on higher level screening, e.g. raft panels.

A systematic, iterative approach to biological evaluation of surfaces and coatings is used within the AMBIO project. In the experimental phase, the main objective is to test the intrinsic anti-settlement and fouling-release performance of coatings on an experimental scale. Assays are performed on coated microscope slides with representatives of the main groups of fouling organisms, viz. bacteria, algae and barnacles. Coatings are selected for further study based on settlement and adhesion strength data compared to standard surfaces. Epoxy (Epikote) is considered as a negative standard for settlement (attachment) since it is known to foul readily. A silicone elastomer, Silastic®-T2 (Dow Corning) that has good fouling-release properties for higher organisms is the positive fouling-release standard. Assays of model surfaces typically include acid-washed glass as a standard.

Attachment and the adhesion strength of bacterial biofilms is studied at TNO with 3 species of marine bacteria (*Vibrio alginolyticus*, *Cobetia marina* and *Marinobacter hydrocarbonoclasticus*) presented either as a single species or a mixed consortium, and at the University of Dundee with the freshwater bacterium *Pseudomonas fluorescens*. The strength of attachment of the marine bacteria is determined by exposure to hydrodynamic shear provided by a rotor, operating at a speed equivalent to a ship moving at 10 knots. Biofilm is quantified by measurement of fluorescence in a plate reader after staining with a fluorescent dye such as Syto 13. The strength of attachment of *Pseudomonas fluorescens* is determined using a specially constructed apparatus that allows slides to be washed by a dipping process. Cells are quantified using agar plate dilution.

Assays with algae, performed at the University of Birmingham, employ zoospores and sporelings (young plants) of the green seaweed *Ulva* and a diatom, *Navicula*. *Ulva* (syn, *Enteromorpha*) is the most common macroalga that fouls ships and other submerged structures. Dispersal is mainly through motile, quadriflagellate zoospores (approximately 7–8 µm in length), which are released in large numbers and adhere through discharge of a glyco-protein adhesive (Callow and Callow 2006). The number of zoospores that settle on a surface provides information about the antifouling potential of a surface (Callow *et al.* 1997). The settled spores rapidly germinate into sporelings (young plants), which adhere weakly to silicone fouling-

release coatings (Chaudhury *et al.* 2005). The strength of attachment of sporelings is quantified by exposing slides to hydrodynamic forces generated either in a calibrated flow channel (Schultz *et al.* 2000) or from a water jet (Finlay *et al.* 2002). Those coatings that show comparable release of *Ulva* sporelings to that from Silastic® T2 are investigated for the release of hard fouling.

Two assays with the barnacle *Balanus amphitrite* (= *Amphibalanus amphitrite*, Clare and Høeg 2008) are employed at the University of Newcastle. The first provides information about the antifouling properties and quantifies the number of cypris larvae (cyprids) that settle on the test surfaces after 24 and 48 h compared to glass and epoxy (Hellio *et al.* 2004). The second barnacle assay is performed on those surfaces that show promise with the *Ulva* sporeling release assay. Juvenile barnacles are allowed to grow on the test surfaces until sufficiently large to give robust adhesion strength data, typically 12 weeks from settlement of the cyprids. The adhesion strength measurement is performed with a novel custom built apparatus that effectively automates the standard ASTM D5618 method that uses a hand-held force gauge (Conlan *et al.*, 2008). The force gauge automatically pushes the barnacle across the test surface at constant speed and can measure up to 10 N total force. The basal area of each barnacle being tested is calculated using digital scans analysed by image analysis software incorporated into the computer controlled force gauge's software. Adhesive strength per unit area is calculated to give adhesive strength in MPa.

24.3.5 Methodological aspects: digital in-line holography

One of the key ideas for the development of novel anti-fouling coatings is to exploit the 'choosiness' of the settling (adhesion) stages of fouling organisms by creating a deterrent surface. Whilst much can be done through simple assays of the ability of, e.g., motile larvae or spores of algae to settle on a surface, a more comprehensive understanding requires detailed observation of the behaviour of organisms as they explore different types of surface. However, the three-dimensionality of the motion of, for example, a swimming spore of an alga, makes it difficult for conventional optical microscopy to follow the cell throughout its exploration phase from a point far away from the surface to the close vicinity of the surface where sensing occurs. High-speed data acquisition in modern light microscopes allows algal cells to be traced, but the intrinsic two dimensionality of the technique provides only either the projection of the trace (large depth of focus) or the visualisation of one focal plane (small depth of focus). In general, a quantification of motion is difficult with this type of compound microscopy as all 3D coordinates are mandatory in order to perform an accurate calculation of, for example, swimming velocity.

In the AMBIO project a collaboration between the group at the University of Heidelberg and the University of Birmingham has demonstrated the feasibility of an entirely new approach based on digital in-line holography. An outline of the principles behind this approach is presented in Heydt *et al.* (2007) but in brief, the underlying concept is the formation of a diffraction pattern when coherent radiation is penetrating a sample. The undisturbed photons ('primary wavefront' or 'reference wave') and those scattered from the object ('secondary wavefront') interfere and form the observed diffraction pattern. Owing to the presence of the reference wave, it is possible to digitally reconstruct the holograms without phase retrieval, and obtain three dimensional real space information. Heydt *et al.* (2007) were the first to apply this technology to studies of swimming organisms that show surface exploration and settlement/adhesion types of behaviour. They showed that a range of 3D motion patterns could be detected when *Ulva* zoospores explore a surface, demonstrating the potential of digital in-line holography to image and quantify exploratory patterns of such organisms close to surfaces. More recently, Heydt *et al.* (in preparation) have shown that quite different patterns of exploration can be detected when zoospores explore surfaces on which they 'prefer' to settle (such as a hydrophobic fluorinated surface) compared with less congenial hydrophilic surfaces such as those presented by polyethylene glycols. An extension of this type of analysis to surfaces showing various types of surface topology is underway.

24.4 Selected emerging results

The project aims, as stated above, can be translated into 4 key questions:

- 1 Which nanodesigns show good antifouling properties against a range of test organisms?
- 2 How does nanostructure influence adhesion of organisms?
- 3 How do coatings respond to the aquatic environment and how do nano-fillers influence this?
- 4 Which design concepts show most promise as durable antifouling coatings for a range of 'real-world' applications?

At the time of the preparation of this article the project has just started its 4th year and for the first three questions notable progress has been made. However, most of the scientific results have yet to find their way into the primary literature. Furthermore, some results contain significant intellectual property and therefore cannot yet be disclosed. Therefore, in this section it is only appropriate to give a broad overview of emerging results suitable for the public domain.

24.4.1 'Practical' coatings

At the end of year 3 a total of 15 different coating types was selected for scale-up and formulation (and 3 provisional patents were filed). The details of the selected coatings cannot be presented here but, at a more general level significant advances made so far include the following:

- Silicone elastomers have been reinforced through the incorporation of multiwall carbon nanotubes (MWCNTs) and sepiolite nanoclays, (collaboration between the group of Philippe Dubois at the University of Mons-Hainault and Nanocyl, a specialist producer of carbon nanotubes). These nanocomposites showed enhanced fouling-release performance in laboratory assays with algae and barnacles compared with unfilled controls (Begbeider *et al.* 2008a) as well as in short-term field tests. The use of MWCNTs is particularly significant since the improved performance is obtained at low loadings of the nanofiller (0.05–0.2% by weight). At this level of loading, the bulk properties of the coatings (tensile moduli and cross-link density) appear to be unchanged but the coatings become slightly more hydrophobic. A significant change in shear-thinning behaviour has been detected (Begbeider *et al.* 2008b), which is particularly significant in terms of ease of application of such coatings to surfaces. The rheological properties have been attributed, on both theoretical and experimental grounds, to a strong molecular affinity between the siloxane chains and the MWCNTs, via CH- π interactions involving methyl groups of the PDMS and aromatic rings of the MWCNTs (Begbeider *et al.* 2008b). This affinity is also important in reducing the likelihood of MWCNTs being released into the marine (or any other) environment, which is relevant to the current debate on potential issues of nanoparticle toxicity. Current studies at the University of Mons-Hainault aim to understand how these molecular interactions generate the enhanced fouling-release performance with a focus on changes in surface topological properties.
- The University of Pisa has designed coatings based on fluorinated and siloxane-based block co-polymers, which through self-assembly and phase segregation generate a nanostructuring effect that influences surface energy and topology. These coatings show good fouling-release performance in laboratory tests with macroalgae and adult barnacles (Universities of Birmingham and Newcastle).
- Sustech and Linkoping University have designed coatings based on PEGylated and other hydrogel-type materials. These have some history of application as antifouling surfaces in biomedicine, but have not been systematically investigated for marine antifouling. Antifouling tests show some effectiveness against marine and freshwater bacterial biofilms

(TNO and University of Dundee) and the settlement and adhesion of green algal spores, diatoms and barnacle cyprids (Universities of Birmingham and Newcastle) (Ekblad *et al.*, 2008). Stability of hydrogels in seawater is an issue but field-application to end-windows of optical instruments will be explored, since for such applications an effective coating that lasts only a few months is still useful.

- Gebze Institute of Technology has designed superhydrophobic, polyolefin-based coatings and blends which produce surface topologies that can be adjusted by the deposition techniques used. These coatings are effective against soft fouling and have some potential for use in fresh-water environments.
- TNO has produced nanocomposite coatings based on sol-gel chemistry, in which the length scale of surface topology can be controlled by the chemistry of the cure reaction and the chemical functionalisation of the nanoparticles. These are effective against some of the fouling organisms tested.
- Good progress has also been made in developing bioactive model coatings using enzymes. The Institut für Polymerforschung, Dresden, has successfully explored strategies for the use of immobilised proteolytic enzymes to prevent marine biofouling by hydrolysing the adhesive proteins secreted by the fouling organisms. Subtilisin A, a proteolytic enzyme used in washing-powders, was randomly immobilised by covalent attachment to different maleic anhydride copolymer coatings. The enzyme-containing coatings reduce the settlement and the adhesion strength of spores of *Ulva linza* and the adhesion strength of diatom cells.
- Ultra-thin, low surface energy metal-polymer nanocomposites have been produced through chemical and physical vapour deposition techniques (TEER Ltd and University of Dundee) or electro-plating (CIDETC). These, typically low-energy surfaces, show efficacy in reducing bacterial biofilm formation and adhesion (e.g., Zhao *et al.* 2007) and in some cases show good release of macrofouling algae, which is unexpected for a non-elastomeric coating (Akesso *et al.* 2009). These surfaces are now being evaluated for use on heat exchanger tubes and plates where only thin coatings can be used in order to minimise the effect on heat transfer.

24.4.2 Model surfaces

Other fundamental advances in the project follow from the use of controlled, well-characterised model nanostructured surfaces (prepared, for example, by 'soft' lithography and self-assembly) that vary systematically in properties such as surface energy, charge density, friction and topology. Two good examples in AMBIO of the influence of molecular, or nanoscale

properties on the adhesion of fouling organisms are reflected in recently published work (Bowen *et al.* 2006; Schilp *et al.* 2007).

It is well known that protein resistance of self-assembled monolayers (SAMs) of hexa(ethylene glycols) (EG₆) is dependent on the alkoxy end-group termination of the SAMs, which in turn determines wettability (Herrwerth *et al.* 2003). Schilp *et al.* (2007) used the same series of hexa(ethylene glycols) to examine the correlation between protein resistance and the settlement and adhesion of zoospores of the macroalga *Ulva* and cells of the diatom *Navicula*, which adhere to the substratum through the secretion of protein-containing glues. Results showed that adsorption of fibrinogen, and attachment of both *Ulva* zoospores and cells of the diatom *Navicula* follow the same general trend and showed a general dependence of the wettability of the surfaces, i.e. the number of attached cells increased as wettability (hydrophobicity) increased. However, on closer inspection it was shown that the most hydrophilic SAM, EG₆OH, and to a lesser extent EG₆OMe, actually promoted settlement of *Ulva* zoospores but the settled cells were so weakly adhered that they floated off the surface as 'rafts' following even the slightest disturbance of the sample. The low numbers that adhere to this surface presumably reflects the fact that the secreted glycoprotein adhesive is unable to bond to the surface because water cannot be displaced from the interface, a condition which is essential to bring the bioadhesive molecules in direct contact with the SAM surface. The hydration of the SAM and adsorption of hydroxide ions to its end-group declines with increasing alkyl chain length and appears to moderate protein adsorption as well as the adhesion of higher organisms such as zoospores of *Ulva* and cells of *Navicula*.

In another example of the use of SAMs to provide a systematic variation in surface properties, Bowen *et al.* (2006) showed that frictional resistance of a surface is important in regulating detachment of *Ulva* spores and diatom cells under shear stress. A series of alkanethiol SAMs of different chain length (C8–C18) was prepared. These have very similar wettabilities but vary in their frictional properties. Results show that the adhesion strength of diatom cells and algal spores to the SAMs decreased with increasing alkyl chain length at C14 and above. This is the point of transition between the lower chain length, amorphous, less-ordered layers with high frictional coefficients, and the higher chain lengths which produce more crystalline, more lubricious structures less able to dissipate energy through-out the SAM, resulting in greater adhesive bond failure.

24.5 Acknowledgements

The AMBIO project (NMP-CT-2005-011827) is funded by the European Commission's 6th Framework Programme. Views expressed in this publication reflect only the views of the authors and the Commission is not liable for any use that may be made of information contained therein.

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Surface modification approaches to control marine biofouling

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Abstract: Surface modification is a novel, non-toxic method to reduce biofouling through physical mechanisms. Many marine organisms display surface-based characteristics and have relatively fouling-free surfaces. This chapter will review the potential of using surface modifications to control fouling. A variety of natural models will be outlined including bioinspired surface designs. Techniques used to create surface modifications will be presented as well as a discussion of the setbacks of using this type of fouling control.

Key words: biomimetics, microtextured surfaces, surface roughness, extreme wettabilities, nanoparticles, bioinspired surface design.

25.1 Introduction

25.1.1 The need for non-toxic alternatives

The control of biofouling has finally reached an important crossroad. No longer is it acceptable to use TBT or indeed any other toxins which will harm non-target marine organisms. It is extremely difficult to predict the impact biocides and heavy metals will have on marine life in the future. The precautionary principle suggests that non-toxic strategies are the safest approach to adopt. On the other hand, if there are no effective non-toxic alternatives available, the potential spread of invasive marine species around the globe could be just as ecologically devastating as toxic fouling control. It is therefore crucial that potential non-toxic antifouling technologies are scrutinised in detail and given the support they require to turn them into a commercial reality.

25.1.2 Advantages of biomimetics

Biomimicry is the copying of natural strategies to solve mankind's problems. For antifouling purposes there is a plethora of natural fouling resistant marine organisms that can be used to inspire novel surface design. While there has been extensive research into natural chemical deterrents (see

Chapter 22), there is comparatively little research on natural surface design and physical fouling deterrent mechanisms. Copying surface structures alleviates concern of release of toxins into the marine environment and potential harm to non-target species. The biomimetic approach to antifouling is the focus of this chapter, with an emphasis on physical fouling defence mechanisms and, in particular, surface modification approaches. Surface microtextures have been identified as potential fouling deterrent mechanisms on molluscan shells (Bers and Wahl 2004; Scardino *et al.* 2003; Scardino and de Nys 2004; Wahl *et al.* 1998), crustose coralline algae (Figueiredo *et al.* 1997; Vrolijk *et al.* 1990) and marine mammal (Baum *et al.* 2001; 2002; 2003) and shark skin (Carman *et al.* 2006; Schumacher *et al.* 2007a; 2007b). These natural models for bio-inspired antifouling design will be examined in detail along with the various techniques required to produce biomimetic surfaces. Modifying a surface alters the roughness, with the scale of roughness crucial to the settlement success of potential fouling organisms. This chapter will also review the various types of surface roughness and the importance of cut-off length and attachment points.

25.2 Surface roughness

25.2.1 Types of surface roughness

There are various ways to measure the roughness of a surface. For homogeneous surface topography the most common method is to report the width (R_{sm}) and height (R_z) of the topography. For more complex surface structures, or surfaces that do not have a uniform surface texture, the mean roughness of the surface is usually reported (R_a). Other surface roughness parameters often reported are the fractal dimension of a surface (F_d), which is a measure of complexity or self-replication, isotropy (Str), which measures directionality, skewness (R_{sk}), which measures the distribution of peaks and troughs, and waviness (R_w), which examines the overall shape or pattern of the surface. Table 25.1 is a glossary of common roughness terms used to characterise surfaces.

25.2.2 Microtextured surfaces

There have been several studies which have examined surface modifications to reduce fouling settlement (Andersson *et al.* 1999; Berntsson *et al.* 2000a; 2000b; Köhler *et al.* 1999; Petronis *et al.* 2000). These have generally involved etching microtextures into a substrate of varying widths and depths. Early studies examined textures in the millimetre range, however this scale of texture generally facilitates fouling by providing refuge from hydrodynamic forces (Walters 1992; Walters and Wethey 1996). For a summary of studies examining microtextured surfaces and fouling see Table 25.2.

Table 25.1 A glossary of common roughness terms used to characterise surfaces

Roughness parameter	Definition
Rsm	The mean width of the profile elements
Rz	The maximum height of the profile
Ra	The mean departure of the surface profile from the mean line
Rw	The average departure of the waviness profile from the mean line of the surface
Rsk	Indicates whether the surface consists of mainly peaks, valleys, or an equal combination of both
Fd	Quantifies whether irregular shapes have a common characteristic feature in that they are self-similar upon magnification. Low values = flat surface, high values = complex self-replicating surface
Str	Indicates the randomness or directionality of surface texture. Large values = isotropy, low values = anisotropy
Rdsr	Developed and projected surface area ratio
Rdva	Developed structure volume per projected area

Table 25.2 A summary of studies which have examined surface modification on artificial surfaces to control fouling. Studies are listed in chronological order, stating the scale of topography examined, the species tested and whether the surface modification enhanced (↑) or decreased (↓) fouling

Surface topography	Species examined	Outcome	Study
0,1,10,100 mm crevices	<i>In-situ</i>	1 mm preferred in first month, 10 mm in second month	Bourget <i>et al.</i> 1994
0,1,10,100 mm V-shaped grooves	<i>Balanus</i> sp., <i>Tubularia crocea</i>	<i>Balanus</i> sp. Preferred 1 & 10 mm, <i>T. crocea</i> preferred most exposed sites	Lemire & Bourget, 1996
Polyester tiles – smooth, 0.5 mm, 2 mm & 4 mm grain size	<i>Semibalanus balanoides</i>	↑ settlement on 0.5 mm tiles ↓ settlement on smooth tiles	Hills & Thomason 1998a, b
0.1, 0.5, 1, 5 mm	<i>Mytilus edulis</i> , <i>Polydora ciliata</i> , <i>Balanus improvisus</i> , diatoms, hydrozoa, bryozoa, ciliates	Species specific preference to varying rugosities, overall 0.5–1 mm preferred	Kohler <i>et al.</i> 1999
Microtextured mesh structures	<i>Balanus improvisus</i>	Reduced settlement on 50–100 µm compared to smooth controls	Andersson <i>et al.</i> 1999
0,1,10,100 mm	<i>In-situ</i>	Lowest abundances on 1 mm	Lapointe & Bourget, 1999

Table 25.2 Continued

Surface topography	Species examined	Outcome	Study
Microtextures – 20–500 µm	<i>Balanus improvisus</i>	↓ settlement on widths of 150–200 µm and depth of 30–45 µm	Berntsson <i>et al.</i> 2000a
Riblets	<i>Balanus improvisus</i>	↓ settlement on height 350 µm, width 134 µm	Berntsson <i>et al.</i> 2000b
Riblets & pyramids	<i>Balanus improvisus</i>	Optimal design – Riblets – Height 69 µm, width 97 µm, Pyramids – 33–97 µm	Petronis <i>et al.</i> 2000
5 µm wide pillars, spaced 5–20 µm, height 1.5 & 5 µm	<i>Ulva linza</i>	Attachment ↑ on valleys & pillars 5 µm deep and wide	Callow <i>et al.</i> 2002
Valleys 5 or 1.5 µm deep, floor size 5 & 20 µm			
10, 50, 100, 190 µm mesh with heights of 25, 36, 45, 100 µm	<i>Ulva linza</i>	Greatest removal of spores on 10 µm mesh with a depth of 25 µm	Granhag <i>et al.</i> 2004
5 µm wide pillars spaced 5, 10, 20 µm apart, depth 1.5 or 5 µm	<i>Ulva linza</i>	Greatest attachment to surfaces with a width, depth and spacing of 5 µm	Hoipkemeir-Wilson <i>et al.</i> 2004
Channels, ridges, pillars, and pits 5 µm wide and spaced 5, 10, and 20 µm apart, Sharklet AF™	<i>Ulva linza</i>	↓ attachment on Sharklet AF™, ↑ attachment on 5 µm columns	Carman <i>et al.</i> 2006
2 and 4 µm ripples and 4 µm projections on polyimide	<i>4 diatom species</i>	↓ attachment when texture smaller than cell width, ↑ attachment when texture larger than cell width	Scardino <i>et al.</i> 2006
2 µm ribs, ridges, circular pillars & 10 µm equilateral triangles on platinum-catalysed PDMS _e	<i>Ulva linza</i>	All topographies reduced settlement, ↓ on Sharklet AF™	Schumacher <i>et al.</i> 2007a
2 & 20 µm wide ribs with varying feature heights	<i>Ulva linza</i> , <i>Balanus amphitrite</i>	↓ settlement with ↑ aspect ratio. <i>Ulva</i> ↓ on 2 µm wide & 3 µm high ribs, <i>Balanus</i> ↓ on 20 µm wide & 40 µm high ribs	Schumacher <i>et al.</i> 2007b
4, 8, 16, 32, 64, 128, 256, 512 µm ripples on polycarbonate	<i>Amphora</i> , <i>Ulva</i> , <i>Centroceras</i> , <i>Hydroides</i> , <i>Bugula</i>	<i>Hydroides</i> – ↓ attachment on 128 µm, ↑ attachment on 256 & 512 µm; <i>Bugula</i> – ↓ attachment on 256, ↑ attachment on 512 µm	Scardino <i>et al.</i> 2008

Berntsson *et al.* (2000a, b) studied cyprid recruitment on artificial surfaces with varying Ra, Rsm and Rz. They found settlement was reduced on longitudinal textures rather than separate topographical structures such as pits and peaks. The scales were 20–500 μm and the mean width of spacing was 112 μm . Critical roughness values determined were Rz: 30–45 μm , Ra: 5–10 μm and Rsm: 150–200 μm . Petronis *et al.* (2000) examined artificial pyramids and riblets on cyprid recruitment. They measured Ra, Rz, Rsm and Rdsr and Rdva and hydrophobicity. Surfaces were characterised using SEM based on dimensions of measured microstructures. Barnacle density decreased with increasing Ra and Rdsr values and was positively correlated with hydrophobicity. While these surfaces were successful in reducing the settlement of one barnacle species, it is not known how the surfaces would perform against other species. These surfaces were only tested in laboratory bioassays; whilst this is undoubtedly important for initial investigations on surface design and optimisation, follow-up work on the performance of the surfaces in the field needs to be reported. Of course, in-situ, there are many organisms of varying shapes and sizes as well as the early colonisation of a biofilm which can change the surface properties.

In addition to attachment, surface characteristics can affect adhesion strength. Granhag *et al.* (2004) found that *Enteromorpha (Ulva) linza* spores adhered more strongly to surfaces with an Rz of 25 μm , compared to smoother (Rz 1 μm) and rougher (Rz 100 μm) surfaces. This type of surface roughness can aid the development of more successful foul-release coatings. Foul-release coatings could also be tailored to specific conditions if particular organisms are known to be more dominant, for example green algae or barnacles in certain regions. For further details on foul-release coatings refer to Chapter 26.

In one of the few studies which have explored the concept of fractals in relation to fouling, Hills *et al.* (1999) examined the influence of fractals on barnacle cyprid recruitment. Fractals were found to be positively correlated to cyprid recruitment, although it was believed cyprids were responding to potential settling sites rather than complexity (Hills *et al.* 1999). Letourneux and Bourget (1988) found higher fractal dimensions on preferred cyprid settlement sites. Fractal dimensions have also been correlated to changes in recruitment on artificial pond weed (Jeffries 1993) and macroalgae (Davenport *et al.* 1996; Gee and Warwick 1994), though causation was not demonstrated.

While the aforementioned studies have examined mean roughness, width and heights of artificial surfaces in relation to fouling, few studies have examined natural surface features. One such example of natural surface characterisation is by Holmes *et al.* (1997), who studied Ra and Rz values for rock types but were unable to predict cyprid settlement based on these

parameters. Other examples of natural surface characterisation include the dogfish eggcase (Marrs *et al.* 1995), gorgonian corals (Vrolijk *et al.* 1990), crustose coralline algae (Figureido *et al.* 1997) and mollusc shells (Scardino *et al.* 2003; Bers and Wahl, 2004; Scardino, 2006). These examples are covered in greater detail in Section 25.4.

25.2.3 Nano particles and extreme wettabilities

To date the properties investigated and manipulated to determine mechanisms affecting the settlement and attachment of fouling organisms have been on the scale of microns. However, with the development of new coatings technologies (Lamb *et al.* 1998; Sun *et al.* 2005) there is the possibility to decrease surface properties to the level of nanometres. This provides a new scale of surface that has yet to be investigated for its effects on the settlement and development of fouling communities. Clearly nanoscale surfaces will provide a new scale of roughness and fractal dimension to a surface and these will, in turn, affect the physical properties of a surface, in particular hydrophobicity (Guo *et al.* 2005; Zhang *et al.* 2005). Hydrophobicity is the degree of affinity a solid surface has for a liquid. Hydrophobic surfaces have a low affinity to liquids (water contact angle of 60–100°). In contrast, liquids spread over hydrophilic surfaces (water contact angles of < 30°). When a rough surface, in particular on the scale of nanometres, is combined with hydrophobic materials a superhydrophobic surface is formed (Zhang *et al.* 2005; Guo *et al.* 2005). Superhydrophobic coatings (SHCs) are surfaces with water contact angles of > 150°. Table 25.3 summarises the studies examining surface wettability and fouling.

Hydrophobicity and its influence on fouling have been investigated against a range of fouling organisms, often with mixed or contrasting responses. *Ulva linza* (Finlay *et al.* 2002a), *Balanus improvisus* (= *Amphibalanus improvisus*) (Dahlstrom *et al.* 2004) and *Nitzschia amphibia* (Sekar *et al.* 2004) prefer hydrophobic surfaces. In contrast, others fouling organisms such as *Balanus amphitrite* (= *Amphibalanus amphitrite*) cannot attach or adhere well to hydrophobic surfaces (Graham *et al.* 2000; Schultz *et al.* 1999; Tang *et al.* 2005; Thomason *et al.* 2002; Zhang *et al.* 2005). In some cases, such as the spores of *Ulva linza*, hydrophobic surfaces increase attachment but decrease adhesion strength (Finlay *et al.* 2002b), while some species of diatoms including *Amphora coffeaeformis* also have stronger adherence to hydrophobic surfaces (Finlay *et al.* 2002a; Holland *et al.* 2004). For species of *Amphora*, hydrophobic surfaces can also reduce motility (Finlay *et al.* 2002a; Wigglesworth-Cooksey *et al.* 1999). However, the effects of hydrophobicity often diminish with time (Zhang *et al.* 2005), or they are overshadowed by other surface properties such as porosity and the extent of cross-linking, in the case of sol-gel-derived xerogel materials

Table 25.3 Summary of studies examining surface wettability and fouling. Studies are listed in chronological order, stating the type of substrate examined and its wettability, the species tested and whether the surface modification enhanced (↑) or decreased (↓) fouling

Roughness	Contact angle	Deterrence	Study
Glass, polystyrene, Mylar, platinum & oyster shell	Cosine θ – 0.2–0.9	↑ <i>Bugula neritina</i> attachment on substrata with contact angles > 45°	Mihm <i>et al.</i> 1981
Glass with varying wettability	0–93	Reduced motility of <i>Amphora</i> cells above 40°	Wiggelsworth-Cooksey, 1999
Fluoropolymers	96–125	Bacteria, <i>Ulva</i> , cyprids reduced	Graham <i>et al.</i> 2000
Silicone foul-release coatings		Diatoms tenaciously adhere	Terlizzi <i>et al.</i> 2000
Patterned self-assembled monolayers (SAMS)	10–115	<i>Ulva</i> ↑ attachment, ↓ adhesion strength <i>Amphora</i> ↑ adhesion strength, ↓ motility	Finlay <i>et al.</i> 2002a
Highly hydrophilic and highly hydrophobic methylsilane-treated glass	4–81	<i>B. improvisus</i> attachment ↓ on highly hydrophilic	Dahlstrom <i>et al.</i> 2004
Hydrophobic PDMSE & glass	<15–109	↑ diatom settlement on hydrophobic surfaces	Holland <i>et al.</i> 2004
Titanium & stainless steel coupons with 120–800 grit roughness	Wettability coefficient of 22–66	↑ microalgal attachment to rough surfaces, no correlation to wettability	Sekar <i>et al.</i> 2004
Chemically modified micropatterned PDMS _e	30–110	↓ <i>Ulva</i> settlement on hydrophilic surfaces. ↑ settlement when 5 µm topography introduced	Hoipkemeir-Wilson <i>et al.</i> 2005
Hybrid sol-gel-derived xerogel films	Critical surface tensions 20–33.5	↑ fouling release of <i>Ulva</i> & <i>B. amphitrite</i>	Tang <i>et al.</i> 2005
Smooth & roughened superhydrophobic coatings (SHCs)	75–169	↑ fouling resistance on highly roughened surfaces	Zhang <i>et al.</i> 2005
Microtextured polydimethylsiloxane elastomer	108–135	↓ <i>Ulva</i> settlement on 2 µm ridges	Carman <i>et al.</i> 2006
Nano & micro SHCs	155–169	↓ <i>Amphora</i> , <i>Ulva</i> , <i>Polysiphonia</i> , <i>Bugula</i> & <i>Balanus</i> on nano SHC	Scardino, 2006

(Tang *et al.* 2005). Hydrophobicity is also impacted by initial colonisation processes under natural conditions (Becker *et al.* 1997).

Recent technological breakthroughs have enabled superhydrophobic surfaces to be developed by combining nano-scale roughness with hydrophobic materials. Superhydrophobic coatings (SHCs) with water contact angles of up to 175° (Guo *et al.* 2005; Zhang *et al.* 2005) can be formed using chemically modified nano-sized particles of hydrophobic silicon-based materials. Roughness-related antifouling properties have been limited to micron-scale hydrophobic surfaces (Callow *et al.* 2002; Carman *et al.* 2006; Hoipkemeier-Wilson *et al.* 2004; Verran and Boyd 2001), primarily due to the technological requirements to produce the smaller nano-scale of roughness.

One study which examined a nano-rough surface and superhydrophobicity in relation to fouling found that the surface, which was largely undefined, was unwetted after 6 months and reduced short-term bacterial attachment (Zhang *et al.* 2005). However, fouling resistance in the field decreased over a 2-month period (Zhang *et al.* 2005). The diminishing fouling resistance is attributed to the loss of superhydrophobicity, which can occur due to air dissolving in water over long periods of submersion. This was proposed to be due to the destruction of pores that may remove air physically from the water-solid interface, or the development of conditioning layers of macromolecules which alter the chemical and physical structure of the surface (Zhang *et al.* 2005).

Research on the response of fouling organisms to superhydrophobic surfaces is limited and mostly on surfaces with moderate range superhydrophobicity (100 – 135°). Fouling reduction by *Ulva linza* zoospores was reported in a recent study examining a range of microtopographical designs on 'ultrahydrophobic' PDMSe surfaces (Carman *et al.* 2006). The most successful surface (86% spore reduction) had the highest water contact angle (135°); however, it was the only surface with topographical widths smaller than the zoospore diameter (Carman *et al.* 2006). It appears topographical height and spacing are more important than hydrophobicity, as surfaces with similar 'ultrahydrophobicities' produced very different settlement responses for *U. linza* zoospores. This is consistent with Scardino (2006), in which superhydrophobic coatings ($> 155^\circ$) produced differing settlement responses that could not be attributed to hydrophobicity or roughness and is more likely to be due to micro- and nanoscale effects.

Amphora sp. cells adhere more strongly to hydrophobic surfaces than to glass (Finlay *et al.* 2002b; Holland *et al.* 2004). On patterned self-assembled monolayers (SAMs) of increasing hydrophobicity (water contact angles of 20° – 115°) *Amphora coffeaeformis* cells were removed in lower numbers on the most hydrophobic surfaces. Furthermore, Holland *et al.* (2004) found that adhesion strength by three common fouling diatoms, including

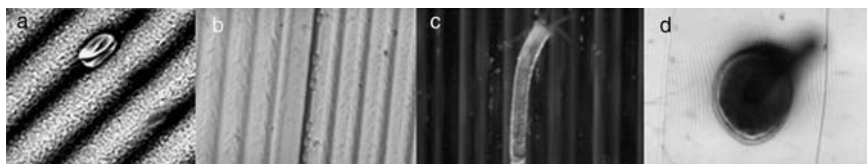
A. coffeaeformis, was stronger on a Polydimethylsiloxane elastomer (115°) (PDMSe) than glass. It had also been observed that diatom slimes often cover hydrophobic low surface energy antifouling coatings (Finlay *et al.* 2002b) and adhere tenaciously to them (Terlizzi *et al.* 2000). In contrast to these findings Scardino (2006) reported that all four superhydrophobic coatings examined had lower *Amphora* sp. attachment than glass.

Bryozoan larvae are known to avoid glass and other highly wettable (hydrophilic) surfaces in preference for non-wettable (hydrophobic) surfaces (Eiben 1976; Loeb 1977; Mihm *et al.* 1981). When *Bugula neritina* settlement on SHCs was examined by Scardino (2006), it was found that glass was avoided in preference for three SHCs. However, when compared to the only nanoscale SHC, glass was more attractive to *B. neritina* larvae. Therefore, strong repellent effects must be operating in the presence of the nanoscale SHC for *B. neritina* larvae to significantly prefer glass to the coating (Scardino 2006).

Nanoscale surface modifications for fouling control are in its infancy. As nanoscale features are sub-bacterial there is the possibility to combine nano- and microscale surface features to produce fouling defences against multiple-sized fouling species. Combining nanoscale surface modifications to produce extreme wettabilities may open up a new mechanism of fouling resistance through the generation of nanosized air incursions which could provide a physical barrier to settlement. These superhydrophobic coatings generate a layer of air at the water-solid interface due to having extremely rough surfaces (Attard 2003; Sun *et al.* 2005; Zhang and Jun 2004). There are several techniques to produce superhydrophobic surfaces which have been recently reviewed (Genzer and Efimenko 2006; Marmur 2006a, b). The implications of SHCs for biofouling control have previously been speculated and it is generally considered that minimising the wetted area of a surface or creating a solid-liquid gap will provide an impediment to fouling matter reaching a surface (Genzer and Efimenko 2006; Marmur 2006a). Continuing advances in nanotechnology indicate that this scale of surface modification will play a significant role in the development of novel anti-fouling surfaces in the near future.

25.2.4 Scale of roughness and attachment points

When fouling organisms are at the attachment phase they vary considerably in shape and size. Bacteria (~1 µm), diatoms (3–15 µm) and algal spores (5–10 µm) are considered to be the major micro-fouling organisms and tubeworms, bryozoans, ascidians and barnacle larvae (120–500 µm) are the major macrofouling organisms. Both types of foulers are influenced by a vastly different scale of roughness. A common mechanism for attachment preferences between the groups is the number of attachment points a surface



25.1 Attachment to microtextured surfaces – a) *Fallacia carpentariae* cell settled between 4 µm texture, b) *Ulva rigida* gametes settled between 16 µm texture, c) *Hydroides elegans* larvae settled on top of 256 µm texture, d) *Bugula neritina* larvae settled on 16 µm texture.

provides. When a cell/larvae can fit inside a surface feature, the number of attachment points are increased and attachment is greater. In contrast, if the size of the surface feature is slightly smaller than the size of the settling cell/larvae, the number of attachment points is reduced and settlement is generally decreased. This attachment point theory has been recorded for spores of the green algae *Ulva linza* (Callow *et al.* 2002; Hoipkemeir-Wilson *et al.* 2004; Carman *et al.* 2006), for four species of diatom (Scardino *et al.* 2006), and for the tubeworm *Hydroides elegans* and the bryozoan *Bugula neritina* (Scardino *et al.* 2008). It is therefore crucial to consider the dimensions of the fouling organism of interest in relation to the scale of surface modification (Fig. 25.1 depicts four fouling species on different sized microtextured surfaces.) For field based trials, it is essential to recognise the many sizes of fouling organisms and their different attachment preferences.

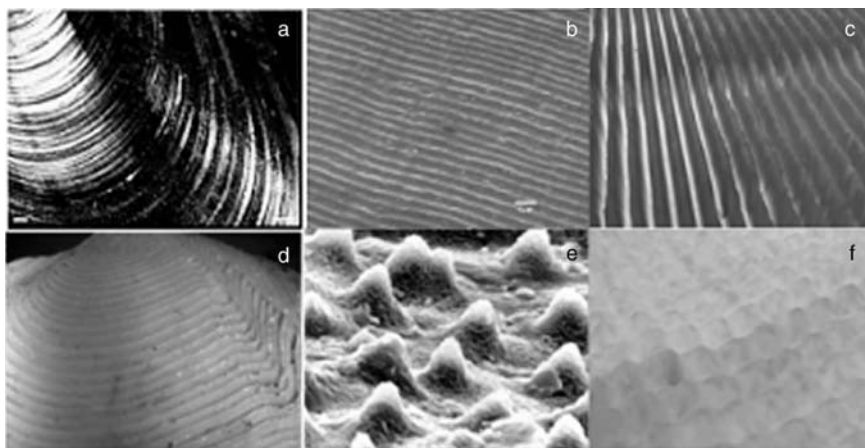
The causes and consequences of surface roughness in antifouling coatings are reviewed by Howell and Behrends (2006). This review points out the need for an international standard on surface characterisation, whereby biologists, chemists and fluid dynamicists can work together to fine tune antifouling capabilities. Some antifouling coatings create a surface roughness that is favourable to certain fouling organisms. It is therefore essential that all fields of marine coatings research measure and report surface roughness in a uniform way (Howell and Behrends 2006).

25.3 Surface modification techniques

The most common methods to produce microtextured surfaces are outlined below including substrates used, and integrity and application issues.

25.3.1 Laser ablation

This technique combines an excimer laser with a micro-photolithographic projection. Well defined and reproducible surface designs can be etched into a surface at the micron scale. A KrF excimer beam is projected through



25.2 Bio-inspired surface modifications: a) the mussel *Mytilus galloprovincialis* b) *M. galloprovincialis* surface microtopography, ripples 2 μm apart c) laser ablated surface micro-ripples d) the bivalve *Tellina plicata* e) the surface microtopography of *T. plicata*, projections 2 μm apart f) laser ablated surface micro-projections.

a metal photomask containing the desired surface pattern. A computer-controlled X-Y stage moves the laser beam precise distances to perform the microgroove. The advantage of excimer laser beams is that they produce little thermal effects, which results in a clean cut surface (Duncan *et al.* 2002). As the photo-mask is not in contact with the surface, there is no need for a photoresist (a radiation sensitive polymer film) which alleviates problems of adhesion to the surface, imperfect surfaces and excludes the need for organic solvents. This technique has been used in a variety of studies examining the attachment of cells and fouling organisms on microtopographical surfaces (Duncan *et al.* 2002; Scardino 2006; Scardino *et al.* 2008). Figure 25.2 depicts surface microtexture produced through laser ablation; the design was inspired by the microtopography found on bivalve shells.

Potential disadvantages of the laser ablation technique are possible alterations to surface chemistry, and changes in surface chemistry may influence fouling attachment. Therefore any changes to surface chemistry resulting from surface modifications must be carefully measured in order to separate the effects of topography and chemistry on fouling attachment.

25.3.2 Photolithography

Photolithography involves manipulating light to etch desired features onto a surface. This process involves transferring the pattern onto a mask on the surface, then etching away the areas of the surface unprotected by the mask.

A radiation sensitive polymer, or resist, is used as the primary mask; when irradiated at specific wavelengths it undergoes structural changes. The pattern is transferred by irradiating selected regions of the resist and dissolving them in the exposed areas. The pattern can be irradiated by drawing with a focused beam or irradiating the whole pattern at once. This technique allows production of surfaces with very high depth-to-width aspect ratios. The main problem is potential complications arising from removing the photoresist from the surface. For detailed descriptions of this method of surface modification see (Carman *et al.* 2006; Feinberg *et al.* 2003; Jelvestam *et al.* 2003; Petronis 2000; Petronis *et al.* 2000).

25.3.3 Moulds and casting

Another method to produce microtopographical surface features is to simply copy them from a surface which already contains the microtopography. When a natural surface is copied it is referred to as biomimicry. A common technique used involves taking a polyvinylsiloxane impression of the surface. This takes a negative copy of the surface features; the negative is then filled with a high-resolution epoxy resin to create a positive mould (Marrs *et al.* 1995). This technique allows copying of surface features to the nearest micrometre; it can also be used on wet samples and several replicates of the same surface can be produced by re-using the negative impression. Creating mimics of natural surface features separates physical and chemical effects which may be acting on the natural surface by testing only the surface microtopography. The disadvantage of this technique is that it is limited to a resin substrate, it is difficult to scale-up in size, and inaccurate curing of the surface will result in imperfections. Studies which have used this technique to create biomimics and examine their antifouling properties include: (Bers *et al.* 2006b; Bers and Wahl 2004; Marrs *et al.* 1995; Scardino and de Nys 2004).

Another technique commonly used to produce microtextured surfaces is to mould mesh or nets into the surface. Plankton nets or nets with fine mesh sizes are heated under pressure, cooled and soaked, leaving a microtextured imprint on the surface (Andersson *et al.* 1999; Berntsson *et al.* 2000a; Granhag *et al.* 2004). This technique is quick, cost-effective and it is easy to manufacture large quantities of microtextured surfaces. The drawback of this technique is potential damage or alteration to surface properties during the heating process. Small micron features and sub-micron features are difficult to produce and replicate using this technique.

25.3.4 Nano-particles

Using nano-particles, micro and nano topography can be built onto a surface. By using fumed silica particles (~50 nm), the hydrophobicity and roughness

of the surface can be manipulated. Surfaces can be designed to have extreme wettabilities (either superhydrophobic or superhydrophilic) and also have highly rough surfaces. Nano-rough surfaces are generally formed by sonicating the chemical mixture (primarily a siloxane copolymer and fumed silica), heating and adding a catalyst. Films can be applied to a surface by spray coating using a spray cane. However, the surfaces generally need subsequent annealing at high temperatures (Lamb *et al.* 1998). For full details of nano-rough and superhydrophobic coating preparations see (Jones *et al.* 2004; Lamb *et al.* 1998; Zhang and Lamb 2004). The advantage of this type of surface modification is that sub-nano features can be explored, a new scale of fractal dimension can be built into a surface, and the wettability of the surface can be altered at the same time. Although the coatings can be sprayed onto a surface, curing at high temperatures may make this technique impractical for some structures and applications. Although nano-particle coatings will be more expensive than fouling release coatings they may be applied as a thinner coat and have longer intervals between recoating.

25.3.5 Substrates used

A wide variety of substrates have been used to etch microtopographies. The most common substrate used is polydimethylsiloxane (PDMS), polyvinylchloride (PVC), polycarbonate and polyimide. Tables 25.2 & 25.3 list the substrate used for each study examining surface microtopography and wettability.

25.3.6 Integrity and application

The methods to produce surface microtopography and wettability will not be universally suitable for all applications. It is important to consider the ease of application onto the desired surface, the ability to scale-up if the surface is successful at deterring fouling organisms, and how durable the surface modification will be. Microtextured surfaces that are scratched or flawed will create an edge effect that is rapidly colonised by fouling organism, and could compromise the entire surface. It is important to consider the operational use of the modified surface, and the prospects of the surface being scratched or flawed. If possible the modified surface needs to be buffered from abrasions or contact with any other surfaces.

25.4 Bioinspired surface designs

The biomimetic approach has gained increasing momentum over the last decade with breakthroughs in materials science, which have allowed the copying of intricate surface details found on naturally fouling-resistant

marine organisms. Several natural models have been studied for biofouling control including gorgonian corals (Vrolijk *et al.* 1990), porpoise and killer whale skin (Gucinski and Baier 1983), pilot whale skin (Baum *et al.* 2001; 2002), shark skin (Ball 1999), echinoderms (Bavington *et al.* 2004; McKenzie and Grigolava 1996), red macroalgae (de Nys *et al.* 1998; Dworjanyn *et al.* 1999; Nylund *et al.* 2005; Nylund and Pavia 2005), crustose coralline algae (Figueiredo *et al.* 1997), the dogfish eggcase (Thomason *et al.* 1994; 1996), and bivalve molluscs (Wahl *et al.* 1998; Scardino *et al.* 2003; Bers and Wahl, 2004; Scardino and de Nys, 2004).

25.4.1 The dogfish eggcase

The dogfish eggcase was replicated to assess marine biofilms non-destructively (Marrs *et al.* 1995). The replication process involves using a dental impression material (polyvinylsiloxane; Kerr's extrude wash type 3 – Kerr UK Ltd), which is extruded onto the surface and peels off forming a negative impression. The negative is then filled with epoxy resin (Devcon 2-ton – Devcon, UK) that cures to create a positive mimic of the surface features at the sub-micron level (Marrs *et al.* 1995). Diatoms and cocci-type bacteria were successfully copied from the surface of the dogfish eggcase without damage to the original surface. The replication of the eggcase is one of the first reports at biomimicking a natural antifouling surface and led to the production of future biomimics on fouling resistant bivalves (Bers and Wahl, 2004; Scardino and de Nys, 2004) which used the same replication methodologies.

25.4.2 Molluscs, crustaceans and multiple defensive strategies

A consistent theme that occurs for organisms is to have multiple defensive strategies against biofouling and this approach of multiple, non-toxic defences was examined by Wahl *et al.* (1998) for the blue mussel *Mytilus edulis*, the gastropod *Littorina littorea*, the colonial ascidian *Cystodytes lobatus* and the crab *Carcinus maenas*. A variety of defence strategies were observed including grazing, burrowing, moulting, mucus secretion, cumulative filtration and unidentified surface properties (Wahl *et al.* 1998).

Potential physical defences including surface microtopography were examined by Bers and Wahl (2004) for *M. edulis*, the crab *Cancer pagurus*, the brittle star *Ophiura texturata* and the dogfish eggcase of *S. canicula*. These natural surfaces were mimicked using resin casts and compared against smooth casts without microtopography. The surface features ranged from 1–1.5 µm ripples on *M. edulis* to 200 µm circular elevations on *C. pagurus*, which are interspersed by 2–2.5 µm spicules. The dogfish eggcase

has longitudinal and lateral ridges spaced between 30 and 50 μm whilst the brittle star has 10 μm knobs on its surface (Bers and Wahl, 2004). All four mimicked surface microtopographies had some antifouling effects compared to the smooth control replicas; barnacle cyprids were repelled by *C. pagurus* mimics and were temporarily reduced by *M. edulis* and *S. canicula* replicas, whilst *O. texturata* deterred the microfoulers *Zoothamnium commune* and *Vorticella* sp. (Bers and Wahl, 2004). The antifouling properties of the mimicked microtopographies were species specific and diminished with time (< 4 weeks), which suggest other defences act in concert with topography on natural surfaces to provide long-term protection from fouling.

For bivalves and gastropods the natural microtexture of their shells is related to the outermost proteinaceous layer on the shell surface, the periostracum. Several studies have reported the significance of this layer in fouling deterrence (Wahl *et al.* 1998; Scardino *et al.* 2003) and the prevention of boring initiation by predators (Che *et al.* 1996; Harper and Skelton 1993). When the periostracum was physically removed from one valve of juvenile *M. edulis* shells, borings increased on the abraded valves (Harper and Skelton, 1993) as did algal and barnacle settlement (Wahl *et al.* 1998). The thick and hairy periostracum of the gastropod *Fusitriton oregonensis* has also been reported to reduce fouling by borers and epizoans (Bottjer 1981).

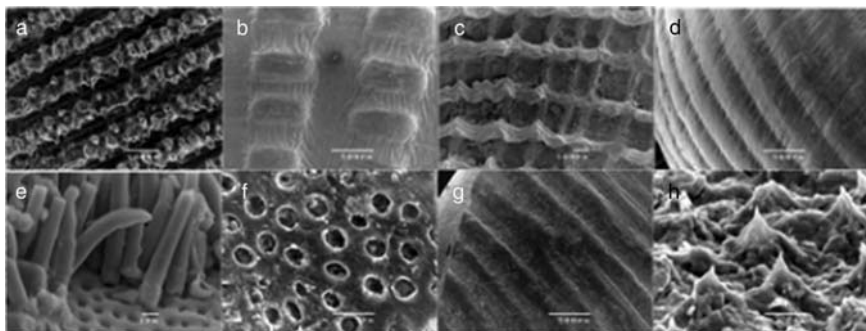
Scardino *et al.* (2003) demonstrated that *Mytilus galloprovincialis* shells, which had an intact periostracum, were significantly less fouled in the field than the oyster *Pinctada imbricata*, which had little periostracum cover. When the periostracum was removed from *M. galloprovincialis*, there was a strong preference by *Amphibalanus amphitrite* cyprids to settle in naturally abraded portions of the shell (Scardino *et al.* 2003). Intact portions of the shell had a homogeneous microtextured ripple of 1–2 μm , which was associated with periostracum presence (Scardino *et al.* 2003). However, other unidentified properties of the periostracum may contribute to fouling deterrence in molluscs, as resin replicates of *M. galloprovincialis* did not resist fouling to the same degree as the intact Mytilid shell (Scardino and de Nys, 2004). Recently Bers *et al.* (2006a) have demonstrated that crude extracts from the mytilid periostracum inhibit the attachment of micro and macrofoulers.

In a recent study examining the microtextures of two Mytilid bivalve species, *M. edulis* and *Perna perna*, resin replicas were less fouled by the barnacle *Semibalanus balanoides* than roughened mimics (Bers *et al.* 2006b). *M. edulis* species from varying geographical localities had similar settlement deterrent effects. Interestingly, the arithmetic mean roughness of both the mimicked shells and the roughened mimics were similar, indicating the isotropy of the texture is important to *S. balanoides* settlement (Bers *et al.*

2006b). Smooth control mimics were less fouled than roughened mimics, though not significantly different to natural mimics (Bers *et al.* 2006b). The antifouling performance of the Mytilid mimics were assessed over two weeks only, to coincide with peak cyprid settlement, hence the long-term potential of the mimics or broad spectrum antifouling effects are still unknown.

The potential antifouling effects of a broad range of mollusc surfaces were examined by Scardino (2006). Over 100 species of bivalves and gastropods were collected to determine if common microtopographical features existed. Whilst no common microtopography was discovered, a wide range of shapes and scales were found on molluscs shells (see Fig. 25.3). Thirty-six species were examined in greater detail to quantify underlying roughness parameters, including mean roughness and mean waviness, fractal dimension, anisotropy, skewness and wettability. Some of these roughness properties were found to correlate to the fouling resistance and fouling-release properties of the shells. These findings demonstrate that other roughness parameters are important to fouling control than simply surface topography. In depth profiling and characterisation of natural surfaces is required to optimise biomimetic design for fouling control.

Fouling resistant bivalves surfaces have been mimicked by resin casting (Scardino and de Nys 2004) and laser ablation of polymers (Scardino *et al.* 2006; 2008). Resin casts of *M. galloprovincialis* (2 μm ripples) and *Tellina plicata* (2 μm pyramids) shells resisted fouling for longer periods than smooth, untextured casts (Scardino and de Nys 2004; Scardino, 2006). However, the shell of *M. galloprovincialis* resisted fouling longer than the



25.3 Bioinspired surface designs: surface microtopographies on marine molluscs; a) *Diadema savignyi* scale – 100 μm b) *Gafrarium tumidum* scale – 500 μm c) *Nerita plicata* scale – 100 μm d) *Donax faba* scale – 500 μm e) *Gafrarium tumidum* scale – 1 μm f) *Lioconcha fastigata* scale – 5 μm g) *Semele crenulata* scale – 500 μm h) *Asaphis violascens* scale – 5 μm .

biomimics, indicating that other factors besides surface microtopography are important to fouling resistance. Recently Bers *et al.* (2006a) have identified fouling deterrent extracts from the mytilid periostracum (thin, surface bound layer of the shell) which indicates that mytilid shells have a multiple antifouling defence system (Bers *et al.* 2006a).

Biomimics of the microtextured bivalve surfaces were laser etched onto polyimide (Scardino *et al.* 2006) and polycarbonate (Scardino *et al.* 2008). When the biomimics were tested against a variety of fouling organisms (diatoms, red and green algae, tubeworms, bryozoans and barnacles) it was found that the number of attachment points in comparison to the size of the settling organisms was crucial to determining attachment success. Four diatom species with differing cell widths attached in higher numbers on microtextures slightly larger than the cell width. In contrast, cells attached in fewer numbers on microtextures slightly smaller than the cell width (Scardino *et al.* 2006).

On microtextured surfaces with scales ranging from 4–512 μm it was found that, when given a ‘choice’ (not all fouling organisms choose where to settle), attachment was highest on the texture slightly larger than the propagule/larvae size and lowest on the texture slightly smaller than the propagule/larvae size (Scardino *et al.* 2008). The effect of attachment points was strongest for larger fouling larvae (tubeworms, bryozoans, and cyprids) which have a more developed searching pattern. For the barnacle, *Amphibalanus improvisus*, the effect of microtexture was stronger than edge effects. Corner locations on a dish that are attractive to cyprids were rejected in favour of locations that maximise the number of attachment points (Scardino *et al.* unpublished data).

25.4.3 Marine mammal skin

Other early reports of biomimicry include the skin of porpoises and killer whales (Gucinski and Baier, 1983) which have critical surface tensions in the range for minimal bioadhesiveness (20–30 dynes/cm; Baier 1968). These surfaces provide low drag and have microtopographical features that may contribute to a fouling free surface (Anon 2002; Gucinski and Baier 1983). Surface texture replicas were made, and attempts to mimic the superficial layer of porpoise skin using tethered polymer chains were undertaken, but the antifouling potential of these replicas has not been documented (Gucinski & Baier, 1983).

Another study (not yet peer reviewed) of the surface of a fouling resistant marine mammal is on dolphin skin, which has a rippled topography in the nanometre range (Anon, 2002). This is similar to the micro-rippled surface of the mussel *Mytilus galloprovincialis*. The surface topography of the dolphin skin was mimicked using polymer crosslinking to produce nano-

sized 'treacherous terrain' which can be varied in scale of texture and degree of hydrophobicity (Anon 2002; Brown *et al.* 2004; Wooley *et al.* 2003). The nanoscale roughness is thought to interfere with protein binding of marine adhesives (Anon, 2002) and, combined with the high speed of dolphin movement, may produce fouling-release performance. The antifouling performance of the biomimicked dolphin skin has yet to be documented. While highlighting the potential importance of biomimetic approaches, confirmation of the effects of dolphin skin and treacherous terrain on biofouling are not yet described in the peer reviewed literature.

It seems many species of cetaceans have a remarkably clean surface free of all epibionts (Baum *et al.* 2001; 2002; 2003). In the case of the pilot whale, *Globicephala melas*, this is attributed to the nano-ridge pores on the surface of the skin (Baum *et al.* 2002). The nano-ridge pores ($0.1\text{--}1.2\text{ }\mu\text{m}^2$) are smaller than most fouling organisms and provide few microniches or contact points to adhere to. The skin surface properties, combined with the speed of movement, surfacing and breaching, may contribute to removing weakly adhered epibionts. The skin of the pilot whale also contains a zymogel which hydrolyses the adhesives of settling organisms (Baum *et al.* 2001), whilst attachment into the nano-ridged pores is prevented by heavy enzymatic digestion (Baum *et al.* 2002). The surface of the pilot whale skin and inter-cellular gel contain both polar and non-polar groups, and this chemical heterogeneity is thought to produce opposite wettability on the same surface, which could contribute to short and long-term fouling reduction (Baum *et al.* 2003). Biomimetic antifouling technologies based on the multiple defence principles of the pilot whale skin were reported as under investigation (Baum *et al.* 2003). However, there are no published reports of any potential technologies developed directly from this work.

25.4.4 Echinoderms

Most marine echinoderms appear to be remarkably free of fouling on their surface, even at the bacterial level (Kelly and McKenzie 1995; McKenzie and Kelly 1994). However, this has in most cases not been quantified, and there remains limited data on the presence and composition of fouling community on most marine organisms. Echinoderms such as sea urchins, sea stars and brittle stars are believed to use a combination of defence strategies to combat fouling (McKenzie and Grigolava, 1996). The pedicellariae of echinoids and asteroids are thought to contribute to cleaning and possibly removal of macrofouling organisms, but are unlikely to be effective against settling larvae or microfouling (McKenzie and Grigolava, 1996). Echinoderms also slough cuticle which removes fouling along with the old cuticle. However, this is a slow process (> 25 days) and cannot explain the

lack of bacterial and diatom fouling, which only requires hours to days to settle (Grigolava and McKenzie 1995). The cuticle of echinoderms also contains proteolytic enzymes and chondroitin sulphate which are thought to alter the adhesion of bacteria by preventing the formation of hydrophobic bonds between the bacteria and the surface (McKenzie and Grigolava, 1996). Mucus secretions are also believed to remove adhered bacteria, but shear forces of mucus on bacteria have not been quantified (McKenzie and Grigolava, 1996).

Recently Guenther *et al.* (2007) have demonstrated that the pedicellariae of the sea star *Acanthaster planci* are ineffective at preventing fouling. Guenther and de Nys (2007) also report that biomimetic moulds of four species of tropical sea stars are not effective in resisting fouling. The complex microtopographical surfaces of the sea stars alone were not efficient at preventing the settlement and growth of fouling organisms. This highlights the importance of other defence strategies in controlling fouling (Guenther and de Nys 2007; Guenther *et al.* 2007). It is important to recognise that not all microtopographical surfaces provide an effective defence against fouling. It is most likely that several strategies acting in concert help to maintain a broad spectrum fouling defence system.

25.4.5 Corals and crustose coralline algae

One of the earliest reports of the physical properties of a fouling resistant marine organism was for the gorgonian corals (sea fans) *Pseudopterogorgia americana* and *Pseudopterogorgia acerosa* (Vrolijk *et al.* 1990). The surface of the gorgonians had a surface free energy in the range for minimal bio-adhesiveness (20–30 dynes/cm; Baier 1968). This optimal surface free energy could be combined with mechanical sloughing, the production of secondary metabolites and sulphated polysaccharide mucus, similar to that documented for echinoderms (Bavington *et al.* 2004), to produce a surface free of fouling. The two gorgonian species had surfaces with arithmetic average roughness (vertical distance between peaks and troughs) values of between 2 and 4 μm , as measured by profilometry (Vrolijk *et al.* 1990). This is of a similar scale to the microroughness found on fouling resistant marine bivalves (Scardino *et al.* 2003; Scardino and de Nys, 2004). When visualised by scanning electron microscopy the surface of *P. americana* appeared smooth, but surface microtopographical features may have been masked by a mucus layer covering the entire surface. In contrast *P. acerosa* has spicules (Vrolijk *et al.* 1990) which may provide reduced numbers of attachment points to potential settling larvae/propagules (Scardino *et al.* 2008). Whilst marine bacteria were present on both gorgonian surfaces, there was no occurrence of microalgae or diatoms on the surface (Vrolijk *et al.* 1990). Interestingly dead gorgonians foul rapidly (Vrolijk *et al.* 1990), indicating

that surface mechanisms alone are not enough to maintain fouling resistance.

Crustose coralline algae have multiple defences against fouling including mechanical and chemical defences. Two species, *Phymatolithon lenormandii* and *P. purpureum*, were tested for algal settlement in the field and laboratory. Replicate and dead crusts were used for comparison (Figueiredo *et al.* 1997). *P. lenormandii* has a rough surface with 100 μm protuberances, whereas *P. purpureum* has a smooth surface (Figueiredo *et al.* 1997). In the field, fouling by *Ulva* spp. was higher on the rough *P. lenormandii* replicates than the smooth *P. purpureum* replicates, whilst in laboratory experiments the same trend was apparent for settlement by *Fucus* zygotes (Figueiredo *et al.* 1997). The surface roughness of *P. lenormandii* (100 μm) is larger than both *Ulva* (8–15 μm) zoospores and *Fucus* zygotes (35–50 μm) thereby providing microrefuges (Figueiredo *et al.* 1997). Overall dead crusts and replica crusts were more heavily fouled than live crusts, indicating other factors contribute to antifouling defence on live crusts (Figueiredo *et al.* 1997). Other potential antifouling mechanisms include thallus shedding and thallus sloughing, as well as chemical substances released by the crusts such as bromophenols (Figueiredo *et al.* 1997). Shading also plays a significant role in antifouling defences of coralline crusts, as *Fucus* settlement was greatly increased on live crusts under high light regimes (Figueiredo *et al.* 1997). Biomimetic studies on algae demonstrate that there are a variety of chemical, physical and mechanical defences to combat fouling. Biomimetic technologies will need to embrace all three strategies to successfully deter a broad range of fouling organisms. Biomimicry based solely on chemical defences will always suffer the criticism of release into the environment and effects on non-target species, unless they are rapidly biodegradable natural substances that are non-toxic and therefore resist fouling by other mechanisms.

25.4.6 Shark skin

Shark skin has also been studied for biomimetic technologies, initially due to the potential application of their drag reduction properties on aircraft (Ball 1999; Bechert *et al.* 2000) but more recently for their antifouling properties (Carman *et al.* 2006). A variety of shark skins have been analysed and have microtopographical ridges, with the number of ribs per scale and the length and spacing of the microtexture varying slightly between species (Bechert *et al.* 2000). However, the patterns are essentially the same across species (Bechert *et al.* 2000 and references therein). The surface micropatterns on shark skin have inspired the design of several antifouling films where surface patterns are etched into silicone wafers using photolithography (Hoipkemeier-Wilson *et al.* 2005; Carman *et al.* 2006). The design is

inspired from the shark skin, which has the same placoid pattern, but with different dimensions and with the tips of the ribs flattened. Hoipkemeier-Wilson *et al.* (2005) used a design termed 'sharklet', which is inspired from the placoids of fast moving sharks, and has microtextured ribs 4 μm high and 2 μm wide spaced 2 μm apart. The rib lengths vary with the middle of seven ribs being the longest at 16 μm and the first and seventh ribs the shortest (4 μm) (Hoipkemeier-Wilson *et al.* 2005). The Sharklet AFTM pattern is scaled down from the real shark skin topography size of between 100 and 200 μm (Bechert *et al.* 2000) to be less than the spore size of the ubiquitous fouling alga *Ulva*.

The Sharklet AFTM pattern reduced *Ulva* spore settlement compared to smooth films regardless of the surface chemistry. However, spores that did adhere to the Sharklet AFTM surface were less easily removed by flow than smooth surfaces (Hoipkemeier-Wilson *et al.* 2005). The Sharklet AFTM design has also been produced onto polydimethylsiloxane elastomers (PDMSe) and tested against *Ulva* zoospores (compared against a range of microtopographical patterns including pillars, pits, channels and ridges which were 5 μm wide and spaced by intervals of 5, 10 and 20 μm with feature heights of 1.5 and 5 μm (Carman *et al.* 2006)). The Sharklet AFTM design increased the wettability of PDMSe by 20% (108°) and had the highest water contact angle (135°) of all the microtopographical patterns. The Sharklet AFTM topography reduced *Ulva* spore settlement by 86% compared to smooth PDMSe. In contrast, ridges spaced 5 μm apart and 5 μm high increased *Ulva* settlement by 150% compared to the smooth surface. Spores which did settle on the Sharklet AFTM design were mostly confined to 'defects' in the pattern which create wider spaces for the spores to settle (Carman *et al.* 2006).

Bioinspired shark patterns are also successful in reducing barnacle settlement. Liedert and Kesel (2005) examined a range of microtextures etched onto silicone of differing complexions in the field over 3 months. The most successful surface, which had texture spacing of 76 μm and was composed of soft silicone material with a low surface energy, reduced barnacle settlement by 95% compared to smooth surfaces made of hard silicones (Liedert and Kesel 2005). This study has not yet appeared in the peer-reviewed literature.

The studies on bioinspired shark skin microtopographies demonstrate that fouling rates can be controlled to either promote or reduce settlement based on texture spacing for *Ulva* zoospores. The antifouling performance of the Sharklet AFTM design against a broader suite of fouling organisms and its longevity in field exposures remains to be determined. Several other studies have examined microtopographies which, although not bioinspired, have similar patterns to shark skin. Microriblets and pyramids reduced barnacle cyprid settlement by up to 92% compared to smooth surfaces

(Berntsson *et al.* 2000a; 2004; Petronis *et al.* 2000). The most successful surfaces are smaller than the cyprid body size and therefore reduce the number of attachment points. This may be a potential mechanism of action, but it is yet to be proven. Barnacle cyprids demonstrated a behavioural aversion to microtextures, spending less time exploring the surface than on smooth surfaces (Berntsson *et al.* 2000b).

Schumacher *et al.* (2007b) report 'Sharklet AFTM' surfaces specifically designed to be resistant to a particular fouling species. Two surfaces have been reported, one for the green algae *Ulva linza* and one for the barnacle *Balanus* (= *Amphibalanus*) *amphitrite*. This work is especially relevant to fouling regions which are dominated by one problematic species. However, it remains to be seen if preventing settlement of a dominant species will simply 'open the door' for other species to settle. Schumacher *et al.* (2007b) also highlight the importance of aspect ratio and tortuosity. This is important as biomimetic design is a complex process and previous studies focussed on finding the perfect topography have overlooked other surface roughness features. Schumacher *et al.* (2007b) also report initiation of trials on combining multiple scales of surface topography/roughness onto a surface. This type of fractal dimension approach to biomimetic design is crucial to producing a broad-spectrum antifouling surface.

25.5 Conclusions and future trends

Surface modification approaches to fouling control have provided the potential for species specific fouling deterrence. To achieve broad-spectrum fouling resistance, it appears clear that multiple strategies will need to be utilised, including nano-technology, natural products and surface chemistry. Surface modification design will need to include fractal dimensions as fouling organisms come in varied shapes and sizes. Biomimetic models will need to be studied and detailed surface characteristics analysed and correlated to fouling deterrence. There is still much to be learned from fouling resistant marine organisms. Ultimately an optimal surface design will need to be combined with foul-release materials or extreme wettabilities, along with an antibacterial agent to prevent biofilm adhesion. The greatest challenge in the future will be to maintain surface performance for long periods. This will require considerable advances in coating integrity, durability and abrasion resistance.

Although surface modification techniques are currently expensive and impractical for large vessels today this should not discourage research into this field. It is essential that more is learnt about natural fouling defence systems and surface selection and adhesion by fouling organisms. With increasing technological breakthroughs the ability to scale-up and keep costs down will increase. There are still considerable advances to be made

in self assembling structures, silicone imprinting and nanotechnology. Surface modification techniques will be most likely used on sensors and other specialised equipment first, then yachts and pleasure craft and finally large vessels when the technology is cost effective.

25.6 Sources of further information and advice

- AMBIO Research co-operative examining advanced nano-structured surfaces for fouling control (CH24) – <http://www.ambio.bham.ac.uk/>
- BASF – chemical company involved with designing nano-structured polymers. www.basf.com/
- Brennan Research Group – based at the University of Florida, this group examines micro-structured surfaces for fouling control. <http://brennan.mse.ufl.edu/>
- Centre for Bioinspired Design – Georgia Institute of Technology. This research centre focuses on biomimetic design to solve complex scientific and engineering problems. <http://www.cbid.gatech.edu/>
- MiniFAB – a microfabrication company which produces microtextured surfaces. www.minifab.com.au/
- Biomimetic books:
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Fouling control coatings using low surface energy, foul release technology

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Abstract: The ban on the use of organotin biocides in a fouling control coating led to a resurgence of interest in non biocidal measures, of which low surface energy coatings are the most practical. This chapter describes the physics and chemistry of such coatings, their smoothness and their effectiveness in preventing adhesion of fouling organisms. Some history of the development of foul release coatings is outlined and the chapter ends with an account of current and future developments, such as the coating of propeller blades and underwater cleaning.

Key words: low surface energy coatings, antifouling, foul release coating, poly-dimethyl-siloxane.

26.1 Introduction

Over the previous fifty years or so, there have been a number of investigations into the use of low energy surfaces to inhibit the adhesion of marine organisms. As detailed in Section 26.3, below, a serendipitous discovery by Milne in the early 1970s led to a patent (Milne, 1977). The patent concerned the enhanced foul release properties of commercially available silicone elastomers, by the addition of a low molecular weight silicone polymer. This discovery eventually led to the first commercially available silicone foul release systems. A detailed account of the development of foul release coatings, together with a comprehensive list of references, may be found in Anderson *et al.* (2002).

Despite the early start of research into fouling control by low surface energy coatings, the work was sidelined by the development of the self-polishing copolymer coatings (SPC), based on tributyl tin (TBT), as a biocide in conjunction with copper. Once again, much of the development was due to Milne, who filed a number of patents, especially (Milne and Hails, 1971). The outstanding success of the SPC variants, in terms of their effectiveness in eliminating hull fouling by shell, weed and slime, their longevity, and their smoothness in service, led many to think that fouling was

'yesterday's problem' and that only surface roughness remained as the ship performance penalty due to added frictional drag (Townsin, 2003).

TBT leaching from hull coatings, was, however, found to be an environmental threat to coastal marine life. A more intensive pollution was, no doubt, from the flushing of filter-less dry docks, in which TBT-loaded paints were applied or removed. An International Maritime Organisation regulation came into force in September, 2008, banning the use of TBT. Early notice of the impending ban led to the development of ablative coatings with the less virulent copper as the main biocide. Copper is permitted as a biocide, although it is not free of suspicion as a pollutant. Understandably, there was a resurgence of interest in non-biocidal measures to control fouling. Up to the present, low surface energy, foul release coatings, are seen to be the most practical.

Ship performance penalties are, and have always been, the prime reason for the considerable effort and cost incurred when developing fouling control coatings. A difficulty for the ship operator is to determine degradation of performance in terms of speed loss at constant power in standard conditions, or, more realistically, in terms of increased power, or fuel consumption, to maintain speed. Such monitoring should be able to indicate the effectiveness of a particular fouling control coating. Unfortunately, measuring speed and power and correcting to standard conditions is fraught with difficulty and inaccuracy. The issues are addressed elsewhere in this volume.

So far, the evidence from visual inspection by diver or in dry dock, and from performance monitoring, is that the first generation of foul release coatings effectively keep off weed and shell, but can suffer from persisting slime. A summary of research into the drag of slime films may be found in Townsin (2003). A wide range of drag penalty is recorded in the literature, but, as with all fouling types, it is difficult to devise a way of measuring slime film characteristics in order to correlate them with consequent drag.

26.2 The physics and chemistry of foul release coatings

The free energy of a surface, which is commonly referred to as 'surface energy' or 'surface tension', is the excess energy of the molecules on the surface compared with the molecules in the thermodynamically homogeneous interior. The size of the surface energy represents the capability of the surface to interact spontaneously with other materials (Brady, 1997; Vincent and Bausch, 1997). The surface energy and the critical surface tension of a surface are determined by comprehensive contact angle analysis, using a variety of diagnostic liquids, measuring the angles that the liquid droplets make with the coated surface.

The consideration of polymer surface properties as a means to reduce fouling was studied by Baier (1973). Baier proposed that a minimum in bio-adhesion occurs in a critical surface tension range between 20 and 30 mNm⁻¹. However subsequent studies have shown surface properties alone do not dictate the performance of foul release surfaces and that fracture mechanics plays an important role in the breaking of the surface marine adhesive joint. Chaudhury and co-workers' (1995) explained the observation that adhesive strengths of visco-elastic adhesives could not be predicted from the surface free energy of the substrate, by invoking a model of adhesion based on slippage at the interface. In studies by Brady (2000), relative bio-adhesion data, undefined by Brady, was shown to correlate with the square root of the product of surface energy and elastic modulus, and studies by Kohl and Singer (1999), on barnacle release mechanisms, showed that release forces decreased with increasing film thickness in accordance with the model developed by Kendall for the release of a rigid cylinder from a substrate.

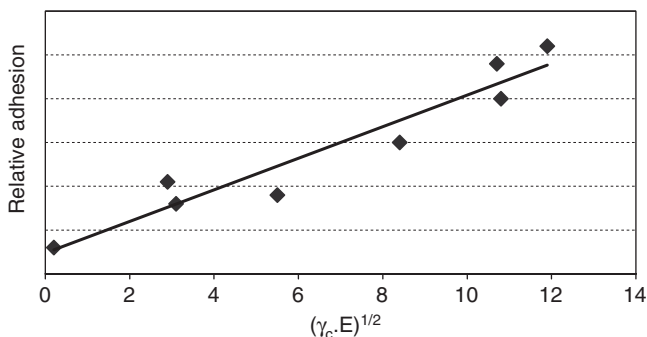
26.2.1 Elastic modulus

From a study of fracture mechanics it has been shown that elastic modulus is a key factor in bio-adhesion and hence the ability of organisms to 'release' from a coating, (Brady and Singer, 2000) and (Berglin *et al.*, 2003). The γ_c and E values and the calculated value for the square root of their product $(\gamma_c E)^{1/2}$ for a range of polymers is shown in Table 26.1.

From these data, a direct relationship between relative adhesion and $(\gamma_c E)^{1/2}$ has been established, as shown in Fig. 26.1. Despite some scatter in the data, Fig. 26.1 demonstrates that adhesion correlates better with $(\gamma_c E)^{1/2}$ than with either surface energy or elastic modulus on their own.

Table 26.1 Physical properties of some polymers (Brady and Singer, 2000)

Polymer	Relative adhesion (dimensionless)	Surface energy (γ_c) mJ/m ²	Elastic modulus (E) GPa	$(\gamma_c E)^{1/2}$
p-(hexafluoropropylene)	21	16.2	0.5	2.9
p-(tetrafluoroethylene) (PTFE)	16	18.6	0.5	3.1
p-(dimethylsiloxane)(PDMS)	6	23.0	0.002	0.2
p-(vinylidene fluoride)	18	25.0	1.2	5.5
p-(ethylene)	30	33.7	2.1	8.4
p-(styrene)	40	40.0	2.9	10.8
p-(methyl methacrylate)	48	41.2	2.8	10.7
Nylon 66	52	45.9	3.1	11.9



26.1 Relative adhesion as a function of the square root of the product of critical surface free energy (γ_c) and elastic modulus (E) (Brady and Singer, 2000).

26.2.2 Thickness

Thickness is another characteristic of low surface energy coatings that plays an important role in bio-adhesion. It has been found that if the foul release coating is too thin (below ~ 100 μm dry film thickness) barnacles can ‘cut through’ to the underlying coats and thus establish firm adhesion. A thin film does not have the ability to absorb the cutting force from the edges of the barnacles as they attach to the surface, and once the coating has been penetrated down to the underlying hard primer, the adhesion of the barnacles increases greatly. As the barnacles subsequently continue to grow, their sharp edges further undercut the foul release coating, and in this way the coating is forced up the sides of the barnacles.

The method of application of foul release coatings is important in order to achieve the minimum thickness required. This can be done relatively easily in a single coat by airless spray, so long as the coating is formulated to have sufficient hold-up on vertical surfaces, without any sagging. If application is by brush or roller at least two coats are required to achieve the necessary thickness everywhere.

26.2.3 Surface chemistry

The polymer systems that have generally shown the lowest adhesion and best foul release of bio-fouling are silicone (more accurately called polysiloxane) polymers, based on a backbone of the repeating (—Si—O—) unit, with saturated organic moieties attached to the two non-backbone valencies of the silicon. The (Si—O) bond is stronger than a (C—C) bond (108 Kcal/mole compared to 83 Kcal/mole) and is extremely durable. This means that silicone polymers have the capability to provide long-term control of fouling.

The silicone polymers used in foul release coatings are generally formed by a condensation mechanism in which OH-terminated telechelic silanol polymers are reacted at ambient temperature with a tetraalkoxysilane cross-linking agent. The most commonly used silicone polymers are those based on poly-dimethyl-siloxane (PDMS). It has been found that incorporation of low molecular weight polymers ('oils') can enhance foul release properties of PDMS polymers (Milne, 1977; Stein *et al.*, 2003). Oils by their nature are lubricants and therefore can decrease the coefficient of friction, but this is not the main reason for their efficacy. This is thought to be due to the surface tension and hydrophobicity changes that the oils effect during the curing process and after immersion (Milne and Callow, 1985; Truby *et al.*, 2000). The most commonly used oils are non-reactive siloxanes or fluoropolymers.

Contrary to popular opinion, it has been shown that foul release coatings do not rely on leaching of oils for their releasing properties. Radio labelled ^{14}C laboratory studies have demonstrated this (Truby *et al.*, 2000), and the fact that fouling control performance is maintained for over 10 years in service on ships confirms this: see for example, the '*Tropic Lure*', as quoted later on.

26.3 Fouling adhesion characteristics on foul release coatings

Representatives of all the phyla living in the sea, from bacteria, through algae, to invertebrates, use sticky materials with permanent or temporary adhesive capabilities at some point in their life histories, to attach to substrata. Larvae of invertebrates and spores of algae need quickly to find and attach to a surface in order to complete their life history. This 'first-kiss' adhesion (Wetherbee *et al.*, 1998) takes place often within minutes, under water, to a wide range of substrates, over a wide range of temperatures, salinities and conditions of turbulence. Adhesion may be permanent or reversible if the organism needs to move around on a surface to find the most appropriate settlement site (Callow and Callow, 2002). Controlling fouling without the use of toxins is essentially a problem of controlling adhesion, and nature, unlike technology, has very effectively conquered the problem of sticking to wet surfaces.

There are three main types of fouling that are most commonly found on underwater hulls: diatom slimes, macro-algae (e.g., ulva) and shell fouling (e.g., barnacles, tube worms). A summary of the mechanisms by which these three fouling types adhere to submerged surfaces has been previously summarised (Anderson *et al.*, 2002).

The discovery of the fouling control properties of silicone systems came about through observation of an unexpected result during a routine R&D

experiment (Milne and Callow, 1985). Milne was investigating the antifouling properties of pre-cast, elastomeric (usually neoprene), rubber panels in the early 1970s, into which biocides were incorporated. Since it was inconvenient to handle these panels in the laboratory as solid rubber sheets, Milne sought an alternative method of application, in liquid form. The only materials readily available to him were a limited number of ambient temperature curing liquid elastomers, amongst them urethane elastomers and room-temperature vulcanising (RTV) silicone rubbers. He then observed that the non-biocide containing control for this series was a better antifouling than the experimental material that contained biocides. He also noted that the slime fouling, which the control accumulated, was remarkably non-adherent compared with other surfaces.

Following Milne's observation, a detailed laboratory development programme commenced, testing a series of commercially available silicone elastomers for foul release properties, with different additives, but without biocides. It was found that the addition of a low molecular weight silicone polymer (often referred to as a 'silicone oil') greatly enhanced the foul release properties, and this was the key invention incorporated into the Milne patent (Milne, 1977). This remains the basis of most silicone foul release systems commercially available today.

In the early 1980s, when the environmental problems associated with TBT SPC antifoulings were starting to appear, research and development on foul release technology focussed on finding solutions to the following 'problems':

- Suitable priming systems to which the 'non-stick' silicone elastomer finish would adhere, and which themselves would protect the substrates (such as steel) from corrosion.
- Toughness and durability for use in the marine environment.
- Practicality of application (long pot-life and shelf-life).
- Prevention of sagging when applied on vertical surfaces.
- Maintenance and repair of damaged areas.
- Suitable market end-uses.

The first recorded small boat application of a foul release coating was on the 34' sailing yacht 'Artemis', based in Southampton, in 1987. The application was carried out by roller, but this produced a relatively rough surface, which rapidly succumbed to unsightly slime fouling. This slime fouling was quickly deemed unacceptable by the skipper of the yacht and the trial was terminated. Since then, little progress has been made with foul release technology in the yacht market.

The first recorded large vessel trial application of a foul release coating was also carried out in 1987, as a test patch on 'Selandia', a US Military Sealift Command container vessel. When 'Selandia' dry-docked again in

1990, after three years in service, it was found to be in remarkably good condition. This gave encouragement to further trials on marine vessels, and once a proven full scheme had been found (including the anticorrosive system) a good many aluminium US Coastguard vessels were subsequently coated. A particular benefit of foul release products for aluminium vessels, is the absence of copper, as used in SPC and most conventional antifoulings, meaning that there is no risk of galvanic corrosion.

The first full vessel application of a foul release coating took place in Jacksonville, Florida, in May 1993 (Fig. 26.2). The vessel was 'Tropic Lure', a small (2563 dwt) Ro-Ro owned by Tropical Shipping. The vessel operated between Palm Springs, Florida and the Caribbean Islands.

At the first dry-docking of this vessel in 1995, after two years, the only fouling on the vessel was slime. This was found to be the case when she docked again in 1998 (after 5 years in service) and again in 2003 (after 10 years in service) (Fig. 26.3).



26.2 'Tropic Lure', first full vessel foul release application, May 1993, Florida.



26.3 'Tropic Lure', July 2003, Florida.

In 1996, a foul release coating was formally introduced for the rapidly expanding fast ferry market. The high speeds of these vessels (> 30 knots) made them ideal for 'self-cleaning' and in addition there were both speed increases and fuel savings reported (Millett and Anderson, 1997). These vessels use large quantities of expensive fuel per tonne/mile and small improvements to hull smoothness and the prevention of fouling can have immediate beneficial effects on operating costs. For this reason many of the world's leading fast ferry operators now use foul release coatings.

In 1996 the well-known cruise liner 'Norway' (formerly the 'France'), was fully re-blasted in Southampton and a foul release coating applied. This vessel was operating in the high fouling area around Florida, and the foul release coating proved to be equally successful on 'Norway' as on 'Tropic Lure'. Other successful scale-up trials were carried out on other vessel types. By the turn of the century, several foul release products, from a number of the major marine paint companies became available, targeted at the high activity, high speed (> 15 knot) scheduled ship market, such as container ships, gas carriers, vehicle carriers, reefers, cruise liners and large Ro-Ro vessels.

It was when the IMO ban on application of TBT antifoulings was implemented at the start of 2003 that sales of foul release coatings started to rise significantly, initially from interest in the environmental attractiveness of being 'biocide-free'. However it was not long after this that the price of fuel started to rise dramatically, and it was the possibility of reduced fuel consumption that attracted ship owners and operators to use foul release technology, again increasing sales. Table 26.2 lists the currently available foul release products from marine paint companies.

Table 26.2 Commercially available foul release coatings

Product type	Market	Company	Product name	Target market
Silicone (1st generation)	Marine	PPG	ABC Release	All vessel types
			SigmaGlide 890	Fast vessels
			Intersleek 425	High speed vessels, > 30 kts
		International Paint	Intersleek 700	High activity vessels, > 15 kts
			Hempasil 77100	Activity > 50% and > 25 kts
			Hempasil 77500	Activity > 75% and > 15 kts
		Jotun	SeaLion	High speed, high activity vessels
		Chugoku	Bioclean	Ocean going vessels, > 15 kts
			Bioclean S	Coastal vessels
			Bioclean C	High speed aluminium craft, > 30 kts
Fluoropolymer (2nd generation)	Pleasure craft	Kansai KCC	Biox	Fast vessels
		Transocean	Lo-Frick A/F100	Fast vessels
		Oceanmax	Ultima System	Fast vessels
Hybrid foul release	Pleasure craft	Chugoku	Propspeed	Propellers
			Bioclean S	Hulls
Hydrogel silicone	Marine	International Paint	Seajet Pellerclean	Propellers
			Intersleek 900	All vessel types, > 10 kts
Hybrid foul release	Pleasure craft	Microphase Coatings Inc.	Phasecoat UFR	Propellers and outdrives
			Phasecoat UFR	Propellers and outdrives
Hydrogel silicone	Marine	Hempel	Hempasil X3	All vessels above 8 knots
			87500	All vessels above 8 knots

26.4 The roughness of the applied foul release coating

The roughness of an applied foul release coating is of interest in relation to its fluid drag. Do the roughness characteristics of foul release coatings differ from those of earlier types of fouling control coating, e.g. SPC, either TBT or tin-free? Do any such differences affect their drag?

Since the early work of Musker (1977), and others, it is generally understood that two statistical parameters are needed to define a surface in order to correlate it with its fluid drag. The required descriptors are a height measure and a texture parameter. Generally, the height is a reflection of the quality of application and the roughness of the substrate, whereas the

texture relates more to the rheology of the coating. In the case of earlier coatings, their similar rheologies resulted in a close relationship between height and texture for moderate roughnesses, so that only the one parameter, viz. height, was needed in order to characterise the roughness and correlate it with its fluid drag.

One obvious candidate roughness height parameter to characterise the coated surface is a statistical measure devised for the *Lucy Ashton* trials (Conn *et al.*, 1953). The parameter is Rt(50) and is the maximum peak to the lowest trough in a 50 mm sample length, along the rough surface. The roughness height distribution is random and therefore it is necessary to use an average value of this parameter over a measurement position (mean hull roughness, MHR), and then MHR is averaged over the hull (average hull roughness, AHR). There is a standard procedure for measurement of a ship hull surface Townsin *et al.* (1981). There is also a special stylus instrument for taking the measurements of Rt(50), viz. the Hull Roughness Analyser.

As a concession to simplicity, it was found that, providing the data was restricted to values of Rt(50) < 230 μm , which is the moderately rough ship range, then the roughness drag correlated well enough with height characterised by Rt(50), for all the paint surfaces tested (Townsin and Dey, 1990). The way was now open to correlate Rt(50) directly with roughness added resistance values, as measured by a number of authorities. Finally, a simple formula for the added ship resistance in terms of average hull roughness, AHR, was the outcome:

$$1000 \Delta C_F = 44 \left[(AHR/L)^{1/3} - 10R_n^{-1/3} \right] + 0.125 \quad 26.1$$

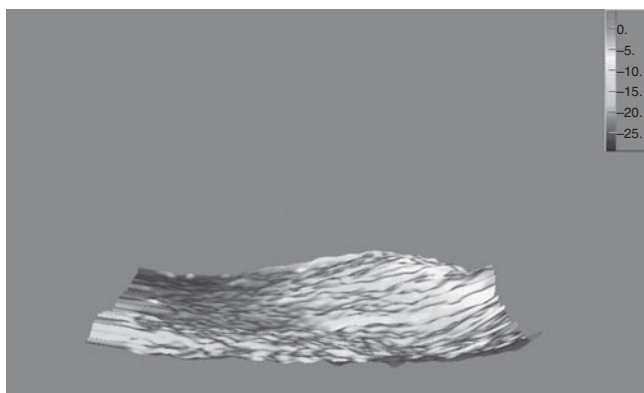
where the added resistance, ΔR , in coefficient form, is, $\Delta C_F = \Delta R / 0.5\rho SV^2$, where the ship length is L, R_n is the ship Reynolds Number at speed V and the ship wetted surface is S.

Early in the commercial development of foul release coatings, it was realised that the nature of the silicone elastomer surfaces might mean different roughness characteristics and consequent differences in roughness drag penalties. Accordingly, an extensive research programme was set up at Newcastle University. Candries (2001) measured the resistance of coated flat planes in the towing tanks of the university and at a Spanish facility, CEHIPAR. Also a coated catamaran model was tested at the Denny ship model testing tank in Dumbarton. Laser-doppler anemometry was used to measure the boundary layer characteristics of the flow over foul release and other surfaces, in the cavitation tunnels at Newcastle and in CEHIPAR. These measurements were coupled with an extensive study of the roughness of variously coated surfaces, using an optical measurement system. A principal aim of the work was to compare the roughness and drag of a foul release coating with that of a tin-free SPC.

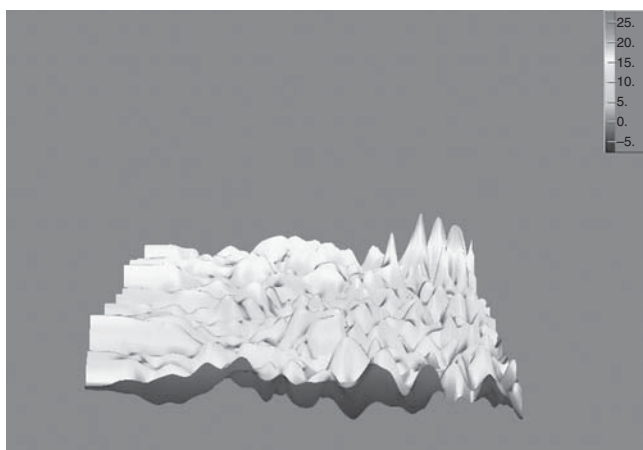
Two difficulties arise in attempting to apply the Hull Roughness Analyser techniques to foul release coatings: one is practical and the other theoretical. A mechanical, stylus instrument is difficult to use on a foul release surface. When the surface is dry, e.g. in dry dock, the rubbery nature of the surface tends to cause the stylus to judder and snag in transit, whereas, if the surface is wet, e.g. underwater measurement by diver, then the drive wheels of the transitting carriage tend to slip. With great care, the instrument may be used on a dry surface by carefully sprinkling water around the stylus during a transit. No doubt, an improved instrument could be devised, but the second of the two difficulties is more profound.

In the laboratory, a non-contact, laser profilometer has been used to display and measure, in three dimensions, surfaces with various coatings (Anderson *et al.*, 2002). Two typical profilograms are shown in Figs 26.4 and 26.5: one is of a foul release coating and the other is of a tin-free SPC coating. Whereas the SPC surface displays a spiky, 'closed' texture, the wavy, 'open' appearance of the foul release coating results from less short wavelength roughness. Measured texture parameters, such as the mean absolute slope and the fractal dimension, confirm this appearance. From measurements on a number of surfaces, including eight foul release coatings, Candries (2001) found that a best correlation with added roughness drag occurred when the surface was characterised by the product of the average roughness height, R_a , and the mean absolute slope, Δa , so that a height measure alone proved inadequate.

A difficulty faced by Candries early on in the study, was that of ensuring some parity in airless spraying of two candidate surfaces for drag comparison. Many practical application issues affect the final roughness outcome of an antifouling coating. By inter-coat measurements, Candries showed that the appropriate anticorrosive and tie coats for the alternative final



26.4 Profilogram of a foul release surface (applied by spraying, μm).



26.5 Profilogram of a tin-free SPC surface (applied by spraying, μm).

coatings all affected the final roughness outcomes. Despite these difficulties of comparison and because of the extensive nature of the study, it is clear that foul release surfaces are inherently smoother than their predecessors, resulting in a lower drag, at least initially. It must be recognised, of course, that any fuel saving due to smoothness can be negated if a coating becomes fouled.

26.5 Recent developments and advances

26.5.1 Propeller coating

It has been recognised for some time that typical deterioration of propeller blade surfaces in service, can result in marked increases in propulsive fuel consumption. Good husbandry of propellers requires grinding and polishing of the surfaces, obeying the principle, 'little and often'. Underwater maintenance is common and convenient. Diver assessment of blade surface roughness can be adequately made using the Rubert comparator gauge, which allows a tactile comparison of a blade surface with six replicas of blade roughness, ranging from best possible new finish to serious degradation. A detailed account of propeller surface roughness, its measurement and a method for calculating resulting power penalties, may be found in Townsin *et al.* (1985), as an outcome of work sponsored by SNAME, at Newcastle University. During the first commercial applications of foul release coatings, a trial of propeller coating was made. It was noted that blade surface areas are small compared with the hull surface, thus making application relatively economical. Also, it was realised that the high fluid

shear towards the blade tips should be helpful in removing any fouling that had adhered whilst stationary. Concurrently, a research programme was set up at Newcastle University and a principal outcome was reported by Atlar *et al.* (2002).

Apart from the prime issue of the effectiveness of the blade coating as an antifouling, two other questions arose. Will the coating be as 'smooth' as a typical new finish of the propeller blade surfaces, and, can this 'smoothness' be maintained over the longer term in service? From previous laboratory tests on five foul release coated surfaces Candries (2001) and Atlar *et al.* (2002) give a clear, affirmative answer to the first question. As to the second question, and also to the antifouling effectiveness over the longer term, practical experience of coated propellers in service must be awaited.

26.5.2 Hardness

The elastomeric nature of current foul release coatings means that they are readily susceptible to scraping or gouging damage, which can frequently happen on the sides of ships as they are moored alongside, and this is a perceived drawback for current foul release coatings. Conventional biocidal antifoulings suffer the same fate, but since they do not rely solely on their surface properties for their efficacy, this is not considered as significant an issue as with foul release coatings, whose efficacy relies solely on the special nature of the surface.

In order to overcome this perceived weakness, attempts have been made to harden or toughen foul release coatings, either by using reinforcing pigmentation or by incorporating alternative binders, such as polyurethanes. However, neither of these changes has been successful. The increased hardness results in a reduction of the modulus of the coating, which in turn reduces foul release properties and thus leads to premature failure.

Some improvement in surface toughness has been achieved with newer generation foul release coatings, by incorporation of fluoropolymer technology. This introduces amphiphilic (a combination of hydrophobic and hydrophilic) networks to the polymer system, thus providing compositional and topographical heterogeneity that not only reduces adhesive interaction with complex marine adhesives but also increases toughness.

26.5.3 Slime control

Slime films on foul release coatings consist largely of 8–10 types of diatoms (Molino, 2008). These are microscopic uni-cellular organisms that exude a mucilaginous glue that enables them to adhere to foul release surfaces even on vessels at speeds in excess of 50 knots. They remain within the surface

boundary layer and are thus unaffected by the turbulence effects next to the hull outside this layer.

The effect of these slime films on ship performance has been difficult to quantify precisely, but it is generally acknowledged that they do contribute to increased drag. This has been highlighted by one major shipping company as the reason for their switching back to biocidal antifoulings from foul release coatings. The increase in fuel consumption and subsequent air emissions due to the presence of slime on first generation foul release coatings was evaluated by them as having a higher environmental impact than the increase in toxicity of the biocide based paint (Maersk, 2007).

There is some evidence that the newer generations foul release coatings have improved resistance to slime and this is now the focus of much academic and industrial research and development.

26.5.4 Coating maintenance

A current preoccupation of marine coatings chemists is to improve the hardness of foul release coatings as well as to increase their ability to slough off slime, as referred to above. Meanwhile, the techniques of underwater hull surface cleaning by diver have made progress over the years and now offer special methods for foul release coatings, taking account of their somewhat softer nature. Machines and brushes have been developed which are less abrasive than their predecessors and better suited to the removal of slime films (Richards and Blair, 2007). The further development of robotic cleaners would encourage their deployment voyage by voyage. A current EU funded co-operative project HISMAR, led by Roskilly (2007), at Newcastle University, aims to map a hull surface accurately and in great detail, to be followed by a magnetic cleaning head.

Small craft present special problems. Many boats are stationary for long periods, usually in marinas, and if the hulls are foul release coated, they will quite rapidly accumulate slime and other fouling organisms, thus necessitating regular cleaning. An extensive list of companies specialising in small craft cleaning may be found on the web. The non-toxic nature and slipperiness of foul release coatings offer advantages to the diver when cleaning a boat hull.

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Non-silicone biocide-free antifouling solutions

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Abstract: The prevention of biofouling attachment and growth on vessel hulls and other immersed surfaces has, and continues to be, primarily achieved through the use of antifouling paints, which continuously release one or more biocides through the paint surface. However, the environmental impact of antifouling biocides is of increasing concern and the search is ongoing for non-toxic methods to prevent biofouling. In addition to silicone fouling-release coatings and surface modification (considered elsewhere in this volume), fluorinated polymer coatings, smart polymers, hydrophilic surfaces, fibre coatings, scrubable and inert coatings and non-leaching active coatings have all been investigated and considered as alternatives to biocidal antifouling paints. While most of these technologies do inhibit biofouling attachment and/or growth in various ways, none has yet provided a practical, widely applicable alternative for vessels. This is because either they have not been developed into a functional coating system or, if they have, they do not provide the long-term or broad spectrum efficacy needed to maintain the clean hull needed to optimise vessel performance and no translocation of harmful marine species.

Key words: antifouling, biocide-free, fouling release, non-toxic, coatings.

27.1 Introduction

Antifouling paints that continuously release one or more biocides through the paint surface have been the primary method of antifouling prevention on ships and other marine vessels for more than a century. However, by necessity, antifouling biocides are toxic, and can cause secondary environmental impact if the biocide does not quickly degrade after release and maintains its toxicity and bioavailability. Many antifouling biocides, such as mercury, arsenic, DDT and tri-organotin compounds, have been widely banned, and others, including copper, continue to be under scrutiny (Anonymous, 2002; Warnken *et al.*, 2004; Jones and Bolam, 2007; Schiff *et al.*, 2007; Chapter 19, this volume). Maintenance of vessels painted with biocidal coatings can also contaminate inshore environments (Schiff *et al.*, 2004; Turner *et al.*, 2008). The search therefore continues for antifouling solutions that do not depend on the release of biocides.

A broad range of approaches have been proposed as non-toxic methods for biofouling prevention, and several of these are discussed elsewhere in

this volume; for example, silicone fouling-release coatings in Chapter 26 (Townsin and Anderson), and surface modification in Chapter 25 (Scardino). In this chapter, other non-toxic coatings and chemistries that have been proposed for antifouling applications are discussed.

27.2 Fluorinated polymer coatings

Considerable attention in recent decades has focussed on the concept of non-biocidal and non-toxic coating systems which have surface properties that prevent the secure adhesion and attachment of fouling organisms; i.e., non-stick coatings. Such coatings are often referred to as 'fouling release' (Swain *et al.*, 2000), 'foul release' (Anderson *et al.*, 2004; Nendza, 2007) or 'minimally adhesive' (Wynne *et al.*, 1993) coatings. The objective for these coatings is to create surface characteristics that reduce the adhesion strength of attaching organisms to the point where the organism detaches under its own weight as it grows, or is dislodged by water movement as, for example, when a ship moves through the water.

The major class of candidate fouling release coatings is a group of synthetic polymers and copolymers generally called low surface energy (Baier and Meyer, 1994). The surfaces of these are free of groups which, at oceanic ionic strengths, are charged either positively or negatively. The initial focus in the development of fouling release coatings was on fluorinated coatings, but interest subsequently moved to silicone polymers (Brady *et al.*, 1987; Bultman and Griffith, 1994; Townsin and Anderson, Chapter 26). In recent developments, silicone-based fouling release coatings have been modified by incorporation of fluorinated oils in place of silicone oils, but the focus of this chapter is on coatings based on fluoropolymer resins.

To achieve effective fouling release from a fluorinated coating, initial bonding of marine organisms must be discouraged (Brady, 2000a–c). This requires construction of a well-organised surface of closely-packed fluorinated groups, to achieve as low a surface energy as possible, and cross-linking or otherwise stabilising the surface so that it resists rearrangement and penetration of marine adhesives. To minimise fouling, fluorinated coatings must meet five conditions (Brady, 2000a–c, 2005):

- the surface must be very smooth;
- the surface must be composed exclusively of fluorinated groups;
- there must be sufficient fluorine in the bulk of the coating to ensure the presence of a sufficient amount of fluorine at the surface;
- fluorinated groups must be large enough to cover polar groups and dipoles; and
- the surface must be cross-linked in order to hold fluorine in place, resist rearrangement and infiltration of marine adhesives, and maintain stability in the marine environment.

Poly(tetrafluorethylene) (PTFE) was initially considered to offer promise as a fouling release or non-stick coating because of its low surface energy. However PTFE is not easily processed because it is not dissolved, softened or wetted by commercial solvent. In their liquid state, conventional epoxy and polyurethane resins, which produce the toughest marine coatings, also do not wet PTFE (Brady *et al.*, 1987).

As adsorbed monolayers of perfluorinated surfactants were considered to have the lowest known surface free energies, monolayers were simulated by mixing compounds into an epoxy coating and by synthesising acrylate, methacrylate and siloxane polymers with long perfluorinated sidechains (Lindner, 1992, 1994). These polymers showed much lower surface free energies than Teflon®-type perfluorinated polymers with no side chains, and showed excellent antifouling properties. Fluorinated diols have also been considered to have potential for creating minimally adhesive surfaces (Ho and Wynne, 1992).

Fluorinated epoxy and polyol resins were synthesised with low surface energies similar to PTFE and these enabled powdered PTFE to be used as commercial paint pigment. However, the usefulness of PTFE coatings was still found to be limited. PTFE surfaces rapidly accumulated biofouling because irregularities in the surface enabled adhesives to invade and cure in microcavities and create a secure mechanical interlock (Brady, 1994, 1997; Davis, 1996). Also, the ability of barnacles to adhere securely to fluorinated surfaces, despite their low critical surface tensions, was considered to possibly relate to a highly localised polarity to the carbon-fluoride bond which allowed very polar groups in barnacle cement to develop a close association over time (Griffith and Bultman, 1997).

Concealing dipoles such as $\text{—CF}_2\text{—CH}_2\text{—}$ well beneath the surface, having a surface composed exclusively of fluorinated groups, and ensuring there is sufficient fluorine in the bulk of the coating to effectively control the organisation of fluorine at the surface can minimise fouling accumulation (Brady, 2001). Constructing a well-organised surface that resists rearrangement and infiltration of marine adhesives, reducing surface energy to the minimum achievable value ($6\text{--}12 \text{ mJm}^{-2}$), and crosslinking or otherwise stabilising the surface in that arrangement, can also discourage the initial bonding of marine organism (Brady *et al.*, 1999).

As biofouling attachment cannot be prevented on non-toxic coatings, the aim is to allow no more than a weak or imperfect joint to form between the organism and the coating, to then cause the early and easy failure of the joint (Brady, 2001). The fouling release mechanisms for fluoropolymers and silicone elastomers are quite different. For silicones, release is facilitated by peeling of the organism from the surface, and this is optimised by control of the thickness and elastic modulus of the silicone coating. Application of force to the joint deforms the rubbery silicone, and the resin peels away from the marine adhesive.

For the hard, glassy fluoropolymer coatings, because their surface is smooth, non-porous and low energy, a weak interface forms with the marine adhesive (Brady, 2001). The high resistance of the surface to molecular interdiffusion and rearrangement gives a sharp, well-defined interface, which is easily snapped by in-plane or out-of-plane shear. However, because the bulk modulus of fluoropolymers is higher than that of elastomers, the joint fails at a higher critical stress.

An alternative approach to PTFE coatings was to polymerise perfluorooctyl acrylate or perfluorooctyl methacrylate esters with monomers containing ionising functional groups to create a fluorinated polymeric surfactant (Brady, 1994; Schmidt *et al.*, 1994). The polymer was reacted with a water-reactive cross-linking polymer to form a sturdy cross-linked film when applied to a surface and cured. The coating had a surface energy of 11–16 mJm⁻², synthetic adhesive did not stick to the film, and it was not wetted or attacked by common solvents.

However, a fluorinated coating considered to achieve Brady's five conditions for an effective fluorinated coating contained an array of closely-packed, surface-oriented CF₃-terminated perfluoroalkyl groups on the surface (Brady, 2000b). This coating was found to resist the attachment of fouling more effectively than any other fluorinated coating (Brady *et al.*, 1999; Brady, 2000a–c). Detailed study of the relationship between contact angle hysteresis, adhesion and marine biofouling with this coating found that the best overall release properties correlated with the highest cross-link density and highest receding contact angles and the lowest contact angle hysteresis (Schmidt *et al.*, 2004). An optimised coating with both low water contact angle hysteresis and high receding contact angle showed 'unprecedented' resistance to marine biofouling.

The perfluoroalkyl coating developed by Brady and his colleagues (Brady *et al.*, 1999) was considered to achieve its good antifouling performance because of its high cross-link density and the immobilised, oriented perfluoroalkyl groups resisting both the infiltration of adhesive molecules and adhesive-induced molecular rearrangement (Brady, 2005).

Reduced settlement of bacteria, algal spores and barnacle larvae has been observed on fluoropolymer films (Graham *et al.*, 2000). Bacteria attached more weakly to the films than to control surfaces, algal zoospores tended to settle at surface faults and cracks and showed some sensitivity to fluorine content, and barnacle cyprids could find only occasional sites for attachment on the films. The overall conclusion from this study was that, due to their low surface energies, smooth films of perfluoropolymers did show considerable promise as fouling resistant coatings. However, these authors considered further work was necessary to achieve adequate smoothness over large areas of film, freedom from cracks and surface blemishes, and strong attachment to underlying substrates.

27.3 Smart polymers

The potential of 'smart polymers' as biofouling-release agents has been explored (Ista and Lopez, 1998). Examples of smart polymers are those that undergo rapid, reversible phase changes in response to small changes in environmental conditions. Ista and Lopez employed poly(*N*-isopropylacrylamide) (PNIPAAm), a polymer that is soluble in water below, but insoluble above, 32°C. PNIPAAm was viewed as a suitable model for other polymers that could be used as fouling-release agents through design to exhibit critical solubility transitions at desired temperatures, or in response to desired stimuli. Initial experiments using thin films of PNIPAAm demonstrated the potential of this, with dissolution of the coating releasing over 90% of attached biofouling, and suggested that tethered PNIPAAm might be useful as regenerable fouling release surfaces.

27.4 Hydrophilic surfaces

Hydrophobic surfaces have been found to favour the formation of biofilms, and hydrophilic surfaces have therefore been investigated for their antifouling potential. Hydrogels are a special class of hydrophilic material that absorb large amounts of water into their structure (see Cowling *et al.*, 2000). They are used in biomedical applications and generally show minimal protein interaction. On this basis, the antifouling behaviour of PHEMA hydrogels, based on 2-hydroxyethyl methacrylate (HEMA), with and without the incorporation of active substances was investigated (Cowling *et al.*, 2000). The research was primarily directed at finding a method of keeping oceanographic sensors free of fouling and PHEMA hydrogel containing benzalkonium chloride (BCL) was successful in keeping surfaces visually clean for a period of twelve weeks, longer than the requirement for the sensors. One sample remained free of fouling for five months.

As a potential antifouling agent, toxicity studies assessed BCL as being, at worst, two orders of magnitude less toxic to oysters than TBT and one order of magnitude less toxic than copper (Cowling *et al.*, 2000). At laboratory scale production, the costs of the PHEMA/BCL combination was of the order of three times the cost per square metre of premium quality proprietary copper-based antifouling paints, purchased in volumes typically required for family-sized boats. However, this cost differential was considered likely to be eliminated if the hydrogel was produced in a commercial production process. Other practicalities, such as formulating a system to maintain efficacy for years, rather than months, physical durability, and ease of application would have to be addressed if these systems were to be a viable alternative to conventional antifouling coatings.

The creation of hydrophilic non-stick coatings by the 'tethering' of drag-reducing molecules such as polyox (polyoxyethylene) to a surface was proposed by Gucinski *et al.* (1984). This has not been developed further in the marine area. However, hydrophilic polymers and zwitterionic polymers are used in the biomedical area to prevent or reduce the adhesion of microbial cells, some of which may have antifouling applications (Milne, 1991).

27.5 Fibre coatings

Flocking is a process for making smooth surfaces into fibrous ones using an adhesive and electrostatically-charged fibres (Phillippi *et al.*, 2001). The fibres are attached vertically and create a three-dimensional surface. Flocking can be done to most surfaces and with a variety of adhesives (Müller, 1995; Phillippi *et al.*, 2001). PVC and nylon nets have been flocked for study and antifouling paint has been used as an adhesive for the flock fibres (Phillippi, 1999).

Forsberg (1994) reported that larvae of mussels did not grow on fibre-flocked surfaces and barnacles appeared to be deterred from settling. Gyllenhammer (1997) found that flocked surfaces had a lower abundance of barnacles and green algae than untreated surfaces. Fibre characteristics were also found to be important in determining the effectiveness of flocking. Gyllenhammer (1997) found that fibres longer than 1 mm were more effective at controlling hydroid and barnacle fouling, while shorter fibres were more effective against mussels, tunicates, and brown and red algae.

Alm and Gyllenhammar (1999) report that a fibre-flock surface gave good protection against hard fouling organisms like barnacles, mussels and tubeworms. There was slight attachment of soft fouling organisms, such as hydroids, tunicates and seaweed, on static vessels, but this growth was claimed to be loosely attached and would be washed off a vessel underway. However, Gyllenhammer (1997) found that although flocked surfaces reduced the recruitment of barnacles and green algae, red and brown algae were more abundant on the flocked surfaces. Larsson (1997) similarly found that barnacle recruitment was reduced on flocked surfaces, but recruitment of mussels, green algae and the solitary ascidian *Ciona* were increased.

Phillippi *et al.* (2001) reported the results of a further investigation into the effects of flocking on the recruitment of fouling organisms. They found that flocking surfaces resulted in lower recruitment of green (*Enteromorpha* (= *Ulva*) spp.) and brown (*Feldmannia* sp., *Petroderma* sp.) algae, but had no effect on red algae (*Polysiphonia* spp.). For invertebrates, flocking was effective at inhibiting the recruitment of encrusting animals (encrusting bryozoans, encrusting ascidians, spirobid tubeworms), had no effect on stoloniferous animals (hydroids, stoloniferous bryozoa), and increased the abundance of tube-building polychaetes (*Polydora ligni*) and solitary ascid-

ians. An exception was the encrusting sponge, *Halichondria* sp., which recruited equally to flocked and unflocked surfaces. The settlement processes of the various organism groups were examined in an attempt to explain the variable settlement. For example, the motile spores of green and brown algae were considered more likely to respond to the stimuli of the fibres than those of red algae, which settle passively. Alternatively, flocked surfaces were considered to possibly provide a favourable habitat for small grazers such as copepods, amphipods and nudibranchs, which could graze on settled spores.

Phillippi *et al.* (2001) concluded that flocking could be a cost-effective strategy for aquaculturists if the organisms causing problems were those inhibited by flocking, i.e., green and brown algae or encrusting invertebrates, including barnacles. The major disadvantage of flocking was considered to be wet weight gain in areas where solitary ascidians were abundant because of their high water content and affinity for flocked surfaces. Flocked surfaces also gained mass from the detritus and sediment they trapped.

The fibre-flock coating tested in the WWF-Germany coastal vessel trials performed well, with selective effectiveness against barnacles even on slow vessels with long periods in harbour (Cameron, 2000). Algal growth was also negligible. However, the system was only tested over one year. Fibre coatings were further evaluated as part of the German trial of biocide-free antifouling paints on deep-sea vessels (Watermann *et al.*, 2001). The conclusion from these trials was that fibre coatings were effective against barnacles, as long as the coatings were applied under good conditions and were undamaged (Watermann *et al.*, 2003). However, there was no observed effect against macroalgae, and algal spores appeared to become entrapped by the fibre layer.

In laboratory tests, using barnacle cyprids, settlement onto fibre coatings was significantly less than on controls (Watermann *et al.*, 2005). No toxic effect of the fibres was detected on either luminescent bacteria, or the barnacle cyprids.

Fibre technologies have been developed as commercial products (Forsberg, 1994), and marketed for both shipping (www.sealcoat.com) and aquaculture (www.micanti.com) applications.

27.6 Biocide-free self-polishing coatings

Biocide-free self-polishing coatings have been developed which utilise similar chemistry and technology to the TBT and tin-free, copper-based, self-polishing coatings (Watermann *et al.*, 2001). In non-toxic SPCs, a non-toxic compound is substituted for the copolymer bound biocide (i.e., the TBT monomer in the TBT copolymer). In a similar manner to TBT and copper-based SPC coatings, the hydrolysis process splits the substitute side

group from the polymer backbone, which then becomes soluble. Methacrylate and several specially designed epoxies have been used to create non-biocidal self-polishing coatings. The objective of these is to create an active, polishing surface that would be too unstable for fouling to remain attached.

Biocide-free self-polishing coating tested patch trials on German ferries showed variable performance (Watermann *et al.*, 1997). On one vessel the coating was completely fouled by macroalgae after 4 months and, on a second, fouling levels were higher than the silicone coatings. Some fouling loss between inspections was noted, and this correlated with ship speed. In trials on coastal vessels, one of the two self-polishing coatings performed well, especially on fast moving vessels (Cameron, 2000). Adhesion of barnacles and total length of algae were reduced and, when green weed developed, it was easily removed.

Nine biocide-free self-polishing coatings were included in the German trial of biocide-free coatings on deep-sea vessels (Watermann *et al.*, 2001). Results were variable, with the conclusion that these coatings may be an alternative for coastal operating ships with a dry-dock interval of 12–24 months (Watermann *et al.*, 2003). However, the dry film thickness applied was considered critical to performance, and polishing rate must be tailored to the activity level and service speed of the ship.

Bioassay and chemical leachate testing of eroding coatings found them to exhibit toxic properties, with barnacle cyprid settlement reduced and high larval mortalities (Watermann *et al.*, 2005). The source of the toxicity was uncertain. Elevated levels of nonylphenol and bisphenol A were detected in leachates of one coating, and zinc in them all, but the latter was considered likely to promote and facilitate the eroding process rather than antifouling efficacy. Enabling polishing without soluble pigments, such as zinc or copper oxides, is difficult (Kiil *et al.*, 2002; Kiil and Yebra, Chapter 14).

27.7 Scrubbable and inert coatings

A non-toxic antifouling strategy, combining a nontoxic boat bottom coating with a companion strategy, has been advocated in California as an alternative to copper-based antifouling paints for recreational craft (Johnson *et al.*, 2006). Frequently cleaning the coating is given as one example of a companion strategy, and epoxy and ceramic-epoxy coatings proposed as very durable, corrosion and abrasion resistant, and able to be scrubbed hard by divers.

In 2002–2003, the University of California Cooperative Extension – Sea Grant Extension Program conducted a field demonstration of non-toxic bottom coatings (Johnson and Gonzalez, 2004). The study tracked the performance of three types of non-toxic bottom coatings, epoxy, ceramic-epoxy,

and silicone rubber, on six recreational boats for a year. For the epoxy and ceramic-epoxy coatings, in-water cleaning by divers was undertaken at mean intervals of between 15 and 18 days, but the life of the coatings was considered to offset the costs of the frequent cleaning and converting from copper-based coatings. The ceramic-epoxy coatings applied in this study were still on the boats in the fall of 2007 (L T Johnson, pers. comm.).

Glass flake lining technology has been applied to the development of an antifouling protective coating by incorporating glass particles into a matrix of tough carrier resins and the Belgian company *Subsea Industries* have developed and marketed a product based on this technology (Anonymous, 2005). When applied in the form of a coating, the carrier resins cure to create a tough glass-like product that strongly bonds to a range of substrates and has an exceptionally smooth surface finish. The coating is claimed to be almost totally impervious and prevents the ingress or permeation of seawater. The coating is not claimed to prevent biofouling, but to offer very low flow resistance and a high resistance to biofouling, and that any fouling that does occur can be easily removed (Anonymous, 2007).

The Belgian Navy trialled this coating on a minehunter, in 2004. After application and launch the vessel remained alongside and inactive for several months and, during this time, biofouling accumulated on the coated hull. This fouling was removed prior to the vessel entering a period of active duty, with only a short two week layover midway through the duty cycle of around six months. Immediately after the activity, the vessel was drydocked and found to have only a light biofilm on the hull which was easily cleaned with water jets (Anonymous, 2005).

Watermann (1999) discusses the option, proposed by David Jones (UMC International, UK), of not applying an antifouling coating at all, but instead applying a hard, smooth anticorrosive system and to maintain it in this condition by regular underwater cleaning for several years. Epoxy, ceramic-epoxy, and glass flake coatings would appear to be likely candidates for such an approach. Watermann and his colleagues determined that special coatings were needed to extend cleaning intervals up to several months. For such a system to be economic, Watermann (1999) considered that sophisticated, possibly robotic, cleaning systems were necessary to support this approach. A network of hull cleaning stations on all important trade routes would also be required, and cleaning should be automatic, either by means of a car wash system or remotely operated vehicle. Even then, awkward areas such as bilge keels, rudders and stern arches would still require manual cleaning.

This approach extends the in-water cleaning practices commonly used in recent years to extend the time between an antifouling losing effectiveness and dry-docking. Diver operated machines with rotating brushes were utilised for this purpose, and vessels cleaned while loading or unloading in

port. Such practices have become increasingly restricted because of concerns about the pulse release of antifouling biocides during the scrubbing of the paint. In Australia, ANZECC have recommended that in-water cleaning be banned by ports because of a perceived risk that the practice could facilitate spread of marine pests (ANZECC, 1997; Parliament of Victoria, 1997).

Swain (1999) observed that many, particularly larger, marine creatures frequently adopt behavioural activities which prevent biofouling. These can include spending extended periods of time out of the water (seals, sea lions, sea otters, etc.), migrating into fresh water or, as is seen on coral reefs, attending cleaning stations where fish or shrimp pick off attached fouling organisms. Such practices are mirrored by boat owners using dry boat storage, freshwater soaks, and hull cleaning.

27.8 Non-leaching active coatings

Biological and biochemical processes with potential for preventing fouling settlement have been proposed as an alternative to using toxic compounds. These include the dissolution of adhesive substances by enzymes, intervention in metabolic processes, adhesive synthesis or calcium transport, competitive inhibition of receptors, and negative chemotaxis (Abarzua and Jakubowski, 1995). Enzymatic antifoulants are considered to fall in the category of coatings releasing chemically bioactive compounds, rather than nontoxic coatings (Olsen *et al.*, 2007; Chapter 23, this volume).

The potential of non-leaching biocides has also been suggested (Clarkson and Evans, 1993). These are attached to a surface and exert a toxic effect on organisms that contact the surface, but without the biocide being released into the surrounding environment. If this approach proved effective, the total quantity used would be small, and the amount released into the environment greatly reduced compared to conventional antifoulants, thus creating a cost-effective, environmentally acceptable system.

Non-leaching biocides (NLBs) are toxins held onto the surface to deter fouling organisms, and are not leached into the surrounding environment (Clarkson and Evans, 1993). The antifouling potential of quaternary ammonium salts as NLBs has been investigated (Mellouki *et al.*, 1989). The salts were grafted on to a vinyl copolymer by means of a covalent non-hydrolysable bond, and did not diffuse into the environment. After four months immersion only bacteria were present in the microbial films; diatoms and cyanobacteria were absent.

Clarkson and Evans (1993, 1995) assessed DC5700 as a potential non-leaching biocide. However, the compound leached from the surfaces, whether bonded via a silane coupling agent or incorporated into a silicone elastomer. These authors concluded that, with the current state of knowl-

edge, the concept of NLBs, although theoretically desirable, was found in practice impossible to achieve.

The use of leaching non-toxic organic repellents as antifoulants has also been proposed (see also Chapter 21, this volume). Sodium benzoate has been found to be effective in preventing barnacle settlement on soluble matrix paints (Vetere *et al.*, 1999). The compound also retarded the growth of the green macroalga *Enteromorpha* (= *Ulva*), and diminished the settlement of tube building amphipods. The effect of barnacles was considered to be the larvae either being repelled and swimming away, or a narcotic effect of the benzoate causing the larvae to become sluggish.

Some promising research has also been undertaken on neurotransmitter blockers to prevent the settlement and metamorphosis of barnacles and mussels (Yamamoto *et al.*, 1998). Larval attachment and metamorphosis is influenced by a range of environmental factors and larval signal transduction and neurotransmission systems are integral to this process. Interference with neurotransmission is seen as a potential non-toxic antifouling mechanism.

27.9 Synthesis and discussion

The prevention of biofouling attachment and growth on a surface that does not leach a biocide requires coatings or treatments that will prevent attachment, cause attached biofouling to dislodge, or reduce the strength of attachment to facilitate dislodgement either by water flow across the surface as a vessel moves, or by mechanical cleaning. Of the technologies discussed in this chapter, those intended to prevent attachment are fibre coatings, hydrophilic coatings and non-leaching active coatings, smart polymers and biocide-free self-polishing coatings are intended to cause attached biofouling to dislodge, fluorinated polymer coatings to facilitate fouling release, and inert and scrubbable coatings to withstand regular in-water cleaning. Hydrophilic coatings, non-leaching active coatings and smart polymers have shown promise in laboratory or small-scale field experiments, but have yet to be developed into coating systems for trial on vessels.

However, the challenge does not end with demonstration of biofouling prevention or minimisation in small-scale trials. Widespread application in the maritime world requires antifouling systems not only to be long-lasting, which for shipping means 3–5 years, but to be competitively priced, easy to apply in a timely manner under the broad range of shipyard conditions, and physically durable, both in sound adhesion and resistance to the mechanical impact and abrasion experienced by ships in the course of their normal operation. Not an easy task.

The performance of fibre coatings in field trials and on vessel hulls has shown that, although effective against barnacles, not all organisms are

deterred from settlement and the coatings appear to promote the settlement of other organisms including macroalgae, hydroids and solitary ascidians. Similarly, biocide-free self-polishing coatings are not able to keep hulls completely free of fouling, with surface coverage of fouling invertebrates generally greater than 20% within 12 months.

Of the non-biocidal antifouling technologies, most research and development has been on fluorinated foul release coatings. Although fluorinated coatings can be formulated with the surface energy critical to minimise wettability and adhesion, organisms are often able to key adhesive into the surface microtexture of these materials. Silicone foul release coatings have been found to have better foul release properties. However, silicone coatings are susceptible to abrasion damage which can compromise their durability. Also, particularly if applied to vessels with periods of inactivity or that do not maintain operating speeds greater than 15 knots, hydrodynamic forces are insufficient to completely remove fouling organisms and cleaning may be required to keep the hulls fouling-free and maintain performance (Swain, 1999; Holm *et al.*, 2003). Conventional mechanised in-water cleaning equipment utilises stiff, rotating brushes, and these damage the soft top coats and compromise future performance of silicone foul release systems, requiring the development of modified brushes and in-water cleaning techniques (Holm *et al.*, 2003). The greater durability of fluorinated coatings may suit their application in some niches that do need regular cleaning, with the reduced strength of biofouling adhesion allowing quicker and easier cleaning than for epoxies, urethanes or other inert coatings.

In the marine environment the purpose of underwater hull coatings is generally two-fold: to prevent corrosion and degradation of the structural material, and to prevent biofouling growth. Ice breakers and other polar ships often do not have an antifouling coating, as this is quickly abraded by the ice, and the ship hull is coated with only a high performance, abrasion resistant anticorrosive coating. This approach is emulated by use of epoxy or ceramic-epoxy coatings and regular hull scrubbing, as trialled in the San Diego studies (Johnson and Gonzalez, 2004; Johnson *et al.*, 2006). However, these coatings may need to be cleaned every 2–3 weeks to keep them free of significant biofouling. Such an approach may be feasible for small craft, but would be impractical on ships and other large vessels.

The spread of invasive marine species is now recognised as a global issue causing widespread economic and environmental impact, with vessel biofouling known to be a major vector for species spread (Johnson *et al.*, 2006; Lewis and Coutts, in press). Should a non-toxic antifouling strategy be adopted, particularly one dependent on regular in-water cleaning, the measures need to be implemented to ensure that the strategy does not facilitate the translocation and release of exotic marine species beyond their native or established range. Such measures could include ensuring capture of all

biological debris dislodged during the cleaning process, or that cleaning takes place before a vessel departs a port, so that species that settled on the vessel in that port stay in that port.

27.10 References

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Abstract: The benefit of being the last chapter in a book on fouling is those readers that have followed the logical progression have an appreciation for the complexity of fouling and for the myriad of threads of fouling research which are the foundation for the future. This chapter highlights a few research areas that illustrate concepts that give hope for the future of fouling management. In the best of circumstances new environmentally benign approaches are a minimum of two to three decades from commercialization. The more applied approaches, which all have unknown environmental impacts, should be available in niche applications in less than a decade. To continue with the hypothesis that progress in fouling management will be through a 'business as usual' model is untenable. Development and implementation of effective solutions to fouling and other global problems will require that science and society develop new paradigms that combine competition and cooperation in effective and relevant ways.

Key words: fouling, antifouling, foul release, biomimetics, pharmaceuticals, natural products, materials research.

28.1 Introduction: existing business models

If one desires to understand the practical future of antifouling research, then one should have a basic understanding of the business models that drive the industry. Business does not publish its models or discuss them freely. As a result, one has to guess the plans. In my estimation, there are two general business models, one for antifouling coatings and one for foul release coatings. Antifouling coatings are based upon specific polymer systems that are compatible with existing anticorrosion systems and that have the essential properties of applicability, physical and mechanical properties. A detail is there are ablative antifouling coatings and non-ablative controlled released based coatings. The polymer and anticorrosion systems are slowly evolving as government regulations change. For example, as regulations reduce discharge of volatile compounds, water-based coatings are being introduced. As regulations require reduction of copper release from coatings, additional broad spectrum biocides are being introduced. The antifouling coating business model tolerates relatively small changes in mixtures of the components paint companies purchase to generate the

coatings and tolerates only toxic additives whose chemistry is compatible with delivery and that does not interfere with the application, physical, mechanical and anticorrosive properties of the coating.

The foul release coating business model is evolving much more quickly than the established antifouling business model. This model uses silicone-based polymers and additives that improve foul release properties. In reality, most foul release coatings contain toxic additives such as organometal catalysts and surface active compounds, and release into the environment compounds, including a variety of silicones, with unstudied environmental impacts. If and when the foul release approach gains market share, one can expect that environmental consequences will become apparent. At present, people in this industry are ignoring the issues of silicone leachates and their impact because there are no government requirements to study them.

28.2 The context for research in biofouling management

Unless the reader started at this chapter and has no experience, it should be abundantly clear that biofouling is a multifaceted problem that has inorganic, organic, microbial, and macrobiological components. Fouling must be managed to minimize our carbon footprint and introduction of invasive species. Environmentally benign solutions will involve chemists, engineers, biologists, governmental regulators, the coatings industry and a novel societal attitude. The multifaceted nature of fouling improves the possibilities for partial solutions in specific applications. Fouling is associated with virtually all inert and biological surfaces in any environment from the deep sea to the international space station. Fouling management adds environmental health and societal complexities. It is within these combined contexts that future research should be considered.

Research on fouling of surfaces includes impractical but essential basic studies such as self assembling monolayers on gold surfaces and the prevention of adhesion to theoretical surfaces. More practical and applied research is literally of life and death importance; for example, managing fouling in nuclear power plant heat exchangers or fouling of stents used to expand heart arteries. Every chapter in this book represents active research streams that will continue into the foreseeable future. Although the schism between academic antifouling research and applied fouling management is shrinking, it is still extreme. Ten years ago, the idea of technology transfer from basic research studies to practical commercial research and development was unimaginable for other than, for example, medical applications which have attractive business potential. However, as globalization proceeds, the importance of fouling and fouling management is becoming increasingly obvious. With awareness may come changes in societal approaches that

improve communication and interaction between interest groups toward solving fouling issues to meet critical societal needs.

In contrast to practical solutions to fouling management which are in large part driven business models and adversarial rather than cooperative government/industry interactions (Rittschof, 2008b), research into basic antifouling mechanisms, foul release (surfaces that foul but are easy to clean) and combinations of antifouling with foul release are exciting areas of research combining cooperative and competitive approaches driven by supportive government funding for studies on the forefront of theory and technology. Most of the theoretical constructs can be classified into one or more categories that are extensions of existing research lines.

28.3 Novel mechanical cleaning approaches and organic broad spectrum biocides

One potentially environmentally benign fouling management strategy based in engineering expertise is hull husbandry. The idea is simple; clean hulls robotically. This approach will probably be used in conjunction with existing chemical and foul release management systems. Technical problems include guidance of the robot, attachment to the hull, collection of wastes, changes in coating performance and disruption of physical properties. In the United States, these and other problems are systematically being solved by dedicated researchers supported by the US Navy over the last 15 years. Once a robot that can clean a large portion of a ship hull in an environmentally benign way is developed, then problems such as cost and versatility can be addressed.

Organic broad spectrum biocides

This refers to replacing heavy metal and covalent organometal biocides such as tributyl tin in antifouling coatings with more degradable organic biocides. It might be great if biologically necessary metals like zinc could function as organo tins or bismuths do, but the chemistry is unfavorable. These approaches describe the new additives as 'booster' biocides, when in fact they are approximately as toxic as the metal biocides. Broad spectrum organic biocides are the most likely next generation of fouling management because they are consistent with existing business models. Eventually, probably in the next 20 years, rapidly degradable organic biocides will begin to replace metal and long-lived organic biocides.

28.4 Biomimetics

Biomimetics is an encompassing biophysical approach that involves copying any aspect of biology that is used to manage fouling. Examples of biomimetics approaches are copying natural physiochemical and chemical

phenomena that have antifouling or foul release properties. Most broad spectrum biocides are not biomimetics because they are not biologically derived. Classic research streams in this area include (a) natural products antifoulants; (b) pharmaceuticals as antifoulants; and newer research streams (c) textured/modified antifouling and foul release surfaces and (d) polymerization and de-polymerization processes in natural glues.

28.4.1 Natural products as antifoulants

This has been an active research area for the last 30 years (i.e., Hadfield and Ciereszko, 1978). As long as there are biologists and chemists studying fouling and antifouling, there will be a research thread that follows an attractive suite of evolutionary based hypotheses that secondary compounds produced by organisms function to control fouling of their surfaces. There are four fundamental flaws or pitfalls in developing natural products as antifoulants: 1) The chemistry of the natural product is unlikely to be compatible with existing coatings technology; 2) natural products routinely have complex chemistry, which makes commercial synthesis difficult; 3) the half life of natural products may be very long; 4) environmental fates and effects of natural products are unstudied and unknown. It is likely that any natural product will manifest at least two and potentially all four of these issues. Although the kinds of approaches used with pharmaceuticals are applicable to natural products, starting with the unstudied and unique natural product adds about a decade to the development process.

28.4.2 Pharmaceuticals as antifoulants

This area is an extension of the concepts of natural products effectiveness to compounds with correct chemistry and some understanding of fates and effects by antifouling researchers who are attracted by the ideas of capitalizing on trillions of dollars of drug research and using existing infrastructure related to drug production to generate 'value added products' (Rittschof *et al.*, 2003).

28.4.3 Novel materials

This area is a logical extension of the biomimetics concept to materials that have no known counterparts in nature. The research is usually at the extremes of chemical or physiochemical possibilities. Exciting work in this area is with low surface energy and nanostructured surfaces. Major stumbling blocks in novel materials are developing materials with antifouling or foul release properties that have the requisite physical and mechanical properties and being able to generate the materials on a large scale.

28.4.4 Disrupting natural glues

Historically, natural glues are an enigma. Most research was done on polymerized glues. Extraction products of polymerized glues (highly cross-linked biopolymers) are at best indirectly related to glue precursors and biochemical processes that generate the glues. Future work with unpolymerized and polymerizing glues provides potential for control by inhibition of key enzymatic steps.

28.5 Other approaches

Combinations of biocidal and materials approaches

As basic and applied researchers learn to communicate, a research stream is evolving that is based upon practical assumptions, such as business models and the reality of fouling in nature. Most researchers that work in this area are convinced that many practical fouling management solutions will require toxic 'environmentally benign' and easy clean components.

Research involving new societal paradigms

This area is virtually unpopulated but has its genesis in the European Union AMBIO project and in the US ONR projects and in the United Nations ballast water treatment program. The basic premise is that effective fouling management requires international cooperation as well as cooperation between governments, industry and researchers (Rittschof, 2008a,b,c).

28.6 Future research

28.6.1 Organic broad spectrum biocides

The most immediately practical avenue of future research in antifouling is the development of new additives that can be used as co-biocides or primary biocides in existing antifouling coatings. The substitution of one biocide for another is compatible with existing business models and requires no new infrastructure for implementation (Rittschof, 2008a,b,c). In just the last several years coatings with broad spectrum organic biocides are gaining increased market share. Because registration of biocides is costly and slow, industry takes advantage of existing registered biocides (Rittschof, 2000). Industry has been pursuing these approaches for at least three decades (Law and Willingham, 1992; Callow and Willingham, 1996; Willingham and Jacobson, 1996; Thomas, 1999; Boxall *et al.*, 2000; Jacobson and Willingham, 2000; Thomas *et al.*, 2000; Turley *et al.*, 2000; Maraldo and Dahllöf, 2004; Petersen *et al.*, 2004) and several groups (e.g., Rhom and Haas and Arch

Chemical) are targeting what this author has labeled 'environmentally benign toxins'. Environmentally benign toxins are those that degrade rapidly to simple molecules that can be consumed by microbes.

Biocides released from a coating kill by entering target organisms and disrupting vital life processes like oxidative phosphorylation or photosynthesis. Ideally an environmentally benign toxin would be protected while entrapped in the coating and have a half life in the environment that was slightly longer (minutes) than the time it spent diffusing from the boundary layer where flow would carry it away from the surface. In reality metal biocides are biologically recycled and most organic biocides have half lives of hours to years. In practice, most biocides bind to organic and inorganic particulates which inhibit degradation and the biocides remain in the water column or in sediments for periods of time many times longer than their theoretical time to breakdown. When bound to particulates, biocides are often consumed by filter feeding animals including commercially important bivalves which are in turn impacted.

Research with biocides can be expected to be a very active future research area. Many foulers, for example propagules trapped in marine snow, settle passively (Clare *et al.*, 1992) and many microfoulers have weak adhesives, but stick to any surface. Thus, for many fouling control applications, at least one biocide may be required in fouling control (Rittschof, 2000). Copper biocide use is restricted because release rates generate levels of copper that exceed regulations like the US clean water standards. As the long lived organic biocides (cf. irgarol, diuron, and shorter-lived but still too long-lived isothiazalones and pyridiones (93 day half life) Gough *et al.*, 1994; Irgarol, 1996; Tolosa and Readman, 1996; Tolosa *et al.*, 1996; Liu *et al.*, 1999; NRA, 2001; Isothiazalone, 2005) gain market share, one can expect their environmental impacts will result in restrictions on their use as has happened to date with all previous biocides. As a result, there will be a need for new biocides until new technology and business models are generated. This particular area is one that can place new products in some market in less than a decade. As the industry better understands environmental issues associated with biocides and plans accordingly, in the future one could see biocides that are designed to be environmentally benign.

Because registration of biocides is expensive and slow, especially in the United States, where biocide use is still condoned (Rittschof, 2000), one can predict that work with existing registered biocides with the correct chemical properties for coating compatibility will be exhausted before new biocides are commercialized. Active research will probably include controlling release of biocides by tethering (Thomas *et al.*, 2004; Boudjouk *et al.*, 2007; Chisholm *et al.*, 2007). In order for biocides to kill organisms, they must get into the target organisms which implies they must be released. Having fouling pressure or a proxy for fouling pressure (temperature or day length, for example) impact release might be an avenue in the future. De-engineering

biocides including pharmaceuticals to maintain toxic effects while speeding up degradation (Teo *et al.*, 2008) is another attractive strategy. As a consequence of the regulatory structure, if a new environmentally benign biocide compatible with existing coatings were identified today, it would probably be about 12 years in much of the world and about 20 years in the US before the product would appear on the market.

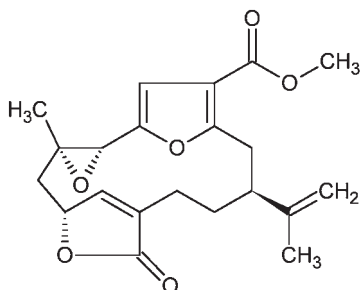
28.6.2 Biomimetics

Biomimetics began as an antifouling concept with studies of how organisms defend themselves from fouling by chemical and physical means (Hadfield and Ciereszko, 1978; Standing *et al.*, 1984; Vrolijk *et al.*, 1990; Targett, 1997; Afsar *et al.*, 2003; Stein *et al.*, 2003). The concept has expanded to include virtually all aspects of adaptive biology including burying behavior that is mimicked by robotics (Nekton Research, 2008). Active lines of classic biomimetics research involved in antifouling and foul release research includes antifouling natural products, non-stick surfaces and textured surfaces and the mechanics and biochemistry of natural glues.

Natural products antifoulants

Natural products are defined as products of secondary metabolism in organisms. Although the concept of natural products antifoulants has been around for almost 40 years and literally hundreds of compounds (Clare, 1996, 1997; Rittschof, 2000) have been identified, antifouling natural products studies continue to be popular (Chen *et al.*, 2008; Toth and Lindeborg, 2008). This is in spite of the fact that there are fundamental disconnects between the chemical nature of most natural products antifoulants and their potential for practical use in antifouling coatings (see above and Rittschof, 2000, 2001).

Most natural products have very complex synthetic chemistries and structures with multiple chiral centers. Pukalide, the first natural product reported to have antibarnacle settlement activity is an example of a complex natural product (Fig. 28.1). Synthesis of compounds like pukalide with multiple chiral centers is impractical on a commercial scale. Fates and effects of complex natural products have not been studied. Compatibility of natural products with coatings is unlikely as compatibility requires specific chemistries that are unrelated to natural product synthesis pathways. In practice, most natural products are oils that modify the composition of coatings to the extent that they interfere with polymer film formation and properties, alter the physical properties of the coating and/or cannot be effectively released from the coating. Because of the multiplicity of functions performed by hull coatings, extensive reformulation of coatings is not compatible with existing business models.



28.1 An example of a natural product with antifouling activity. The molecule depicted is Pukalide, a sesquiterpene isolated originally from a Hawaiian soft coral in the 1970s (Missakian *et al.*, 1975). A bioassay (inhibition of barnacle settlement) directed purification of molecules found in a temperate estuarine soft coral *Leptogorgia virgualata* found pukalide (Keifer *et al.*, 1986). Pukalide is typical of most natural products with antifouling activity in that it is a complex molecule with several chiral centers and would be very difficult to synthesize on a commercial scale. Nothing is known of its environmental fates and effects.

Encapsulation is often a basic research strategy of choice for effective molecules with chemistry that is incompatible with the polymer film chemistry. Although encapsulation may solve chemical incompatibility and enable delivery, it does not address synthesis, environmental fates and effects and registration hurdles. To date no commercial antifouling coatings use encapsulation technology.

Routinely, the mechanism of antifouling action for natural products and the relation to potential human and environmental health impacts are unknown. Given the time horizon for registration of a new biocide in the US is approximately 10 years and the cost is in the millions of dollars (Rittschof, 2000), it is unlikely that any but simple natural products, for example those that resemble molecules such as homoserine lactones bacterial quorum sensing signals (Givskov *et al.*, 1996; Maximilien *et al.*, 1998; Manefield *et al.*, 1999; Rasmussen *et al.*, 2000; Manefield *et al.*, 2002; Hentzer *et al.*, 2003), will be commercialized as antifoulants.

Owing to the issues for natural products that have just been discussed, most research groups with the goal of practical solutions to antifouling coatings development have abandoned complex natural products concepts as a viable approach. However, basic researchers continue to pursue natural products research and concepts. In the future, some of these groups may pursue structure function studies such as those initiated in the 90s (Gerhart *et al.*, 1993a,b,c, 1994) which yield simple molecules with potential. The most successful and advanced research in this area is the Steinberg/Kjelleberg/DeNyes research group in Australia (School of Microbiology

and Immunology & Centre for Marine Biofouling and Bio-Innovation, University of New South Wales). This group has basic research and strongly applied components that show promise for specific fouling management options.

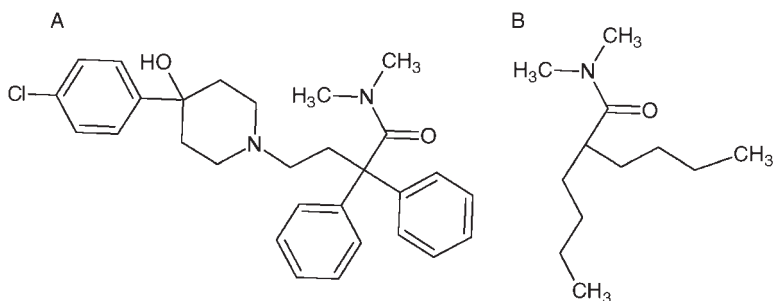
Even though natural product antifoulants have little practical value, bioassay directed purification will continue to be attractive and have value to researchers with basic interests in biodiversity and natural product chemistry. Bioactive natural products will continue to be an active area of research into the foreseeable future.

As time has passed the natural products area has expanded to include signal molecules such as anti-aggregation pheromones (Gerhart *et al.*, 1993b), those used in quorum sensing (Rasmussen *et al.*, 2000; Manefield *et al.*, 2002; Hentzer *et al.*, 2003) and biogenic amines (Yamamoto *et al.*, 1998). As these molecules generally work quickly by impacting microbe physiology or sensing and/or macrofouler sensing or transduction cascades, there is potential for short-lived environmentally benign alternatives as long as the molecules are protected from degradation while in the polymer film. Again, the regulatory structure is such that these kinds of molecules will require decades for commercialization.

Pharmaceuticals as antifoulants

The use of pharmaceuticals as antifoulants probably has its origin in the use of drugs to probe molecular mechanisms of metamorphosis (Baloun and Morse, 1984; Rittschof *et al.*, 1986; Yamamoto *et al.*, 1998; Fusetani, 2004). Pharmaceuticals are more attractive as antifoulants than natural products because they have known and practical synthesis, known mechanisms and fates and effects in vertebrates and other biological systems. Perhaps most important is they have known physical and chemical properties that can be used to predict compatibility with existing coatings and existing business models (Rittschof, 2008a,b). However, like any other chemical, even the perfect pharmaceutical probably faces several decades to be commercialized.

At present, research groups in Europe and Asia (Yamamoto *et al.*, 1998; Rittschof *et al.*, 2003; Teo *et al.*, 2006; Rittschof *et al.*, 2007) are actively pursuing these concepts. Step one in the process is to select pharmaceuticals that have properties of interest that are commercially available and to test them. Most pharmaceuticals have been engineered to be chemically robust for oral route of administration. A consequence is the pharmaceuticals are so stable that they are guaranteed to build up in the environment. Thus, the second phase of this research is to de-engineer the molecules so they are more readily degraded. Figure 28.2 is an example of a candidate pharmaceutical before and after the de-engineering process. It is fascinating that



28.2 Environmentally benign molecules are those that do not build up in the environment and whose degradation products are simple. Structure A is an example of a complex bioactive molecule that is stable in the environment and which has a variety of potential breakdown products with unknown effects. Structure B is derived from A by a bioassay directed structure function studies meets goal of a simple structure with reduced complexity of breakdown products. None of the breakdown products of Molecule B are biologically active or of environmental consequence once the amide bond is broken.

the de-engineered molecule is approximately as active as the parent compound in the target biological assays. The third phase of development will be re-engineering to improve compatibility and delivery from commercial coatings systems. One hope for funding this process is de-engineering results in novel patentable bioactive molecules that are easy to synthesize and that still have functions as pharmaceuticals.

Thus, the immediate future of antifouling research with pharmaceuticals is in studies of compatibility with existing coating technology, addressing environmental impacts and breakdown and approval by regulatory agencies (Rittschof, 2008a). Two areas of potentially very active research in the future are de-engineering of pharmaceuticals to reduce their residence time in the environment and re-engineering of pharmacophores to control compatibility and delivery from the coating.

De-engineering is the major topic of recent patent applications (Teo *et al.*, 2006, 2008). In these applications, complex refractory drugs which contain multiple aromatic rings and halogens are reduced to biologically active molecules which contain no aromatic rings and halogens and small numbers of amide linkages. The re-engineering concept is one that is an active area of research, but that has yet to see the first complete patent or publication.

Textured surfaces

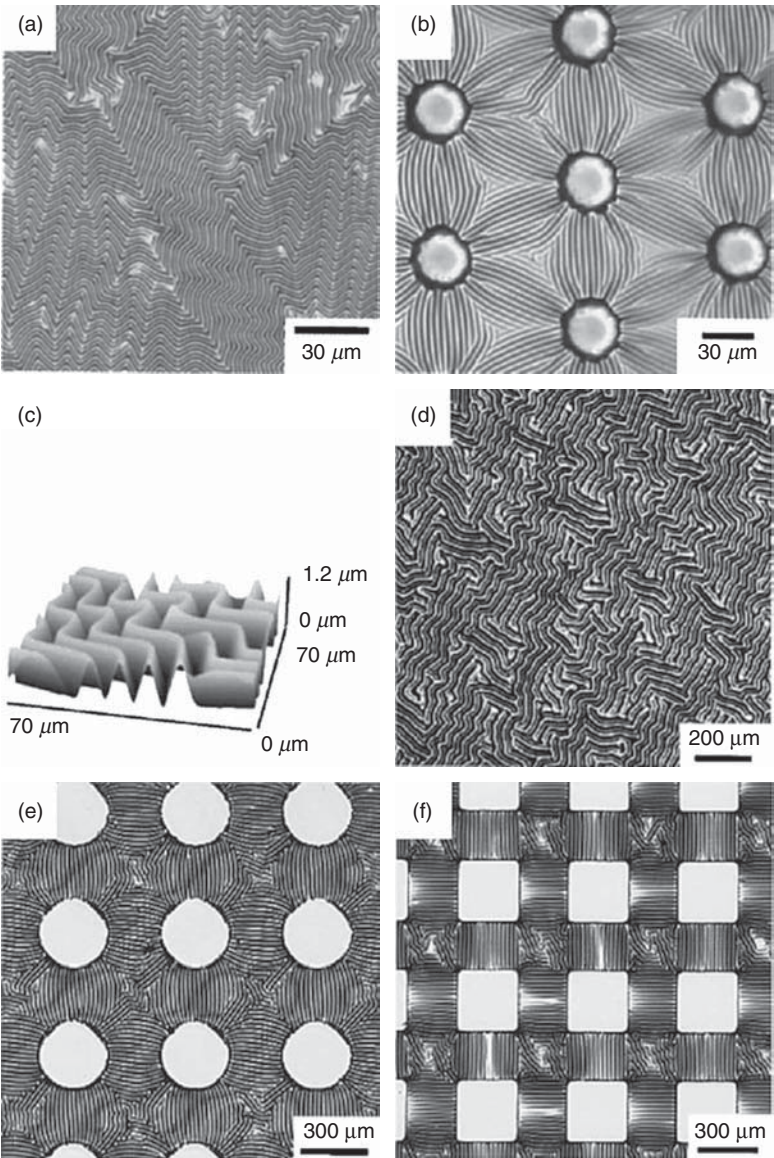
An exciting, relatively new area of antifouling research is materials science approaches to biomimetics in which synthetic polymers are generated which mimic the physiochemical and mechanical properties of natural sur-

faces that do not foul. The concept of using sophisticated polymer systems in fouling management is represented in the most recent book, *Interdisciplinary Globally-Leading Polymer Science and Engineering* (edited by Ober). The book was generated at the 2007 US National Science Foundation workshop and highlights drug delivery from stent polymers. Although anti-fouling isn't specifically addressed, it is clear that polymer science is essential to future development of environmentally benign coatings.

Perhaps the most well known of the recent polymer processes that may have antifouling potential is a shark skin mimic (Carman *et al.*, 2006) comprising molded polydimethyl siloxane generated by photolithography techniques which are capable of generating nanoscale surface modifications. Although it is unlikely that textured surfaces will remain free of all fouling and there are enormous issues with scale up and no studies of environmental impacts of molecules leaching from these kinds of surfaces, there may be a niche market for this concept.

A second polymer research line uses self assembling monomers (SAMs, Ruiz *et al.*, 2000; Brovelli *et al.*, 1999), block copolymers (Wooley *et al.*, 1993; Huang *et al.*, 1999; Ma and Wooley, 2000; Wooley, 2000; Hawker and Wooley, 2005), or nanofillers and phase separation (Ober and Wegner, 1997) to generate nanometer molecular level patterned surfaces with specific surface properties. Some of these surfaces mimic lotus leaves or gecko foot pads and are super hydrophobic (DeSimone *et al.*, 1997; Buhler *et al.*, 1998; Federle *et al.*, 2002; Lau *et al.*, 2003; Monge *et al.*, 2003; Marmur, 2004; Zhai *et al.*, 2004; Cheng and Rodak, 2005; Zhao *et al.*, 2005; Cheng *et al.*, 2006; Drechsler and Federle, 2006; Federle *et al.*, 2006; Zhai *et al.*, 2006; Bhushan, 2007; Bhushan and Sayer, 2007a,b; Ge *et al.*, 2007; Kustandi *et al.*, 2007; Qu and Dai, 2007). These surfaces are interesting because they are not usually wetted by water, which for most surfaces rolls off like a ball of mercury on a glass surface. The concept is, if the surface cannot be wetted, then it cannot easily be fouled. The flaw in this argument may be the ability of amphiphilic glue molecules to rearrange to match surface characteristics (Naldrett and Kaplan, 1997).

A fascinating final way to generate textured surfaces is to simultaneously stress (temperature or stretching) and oxidize polymers such as polydimethylsiloxane which wrinkle with predictable patterns of wrinkling upon removal of the stress (Efimenko *et al.*, 2005; Genzer and Groenewold, 2006; Fig. 28.3). The resultant high wrinkling can be overlaid upon existing macro or micro-ordered patterns to provide an additional novel level of surface complexity. As with the other structured and patterned surfaces, the impacts of these man-made surfaces on biofouling are in the preliminary phases of investigation. As with all the novel polymer approaches, progress toward effective antifouling that could be used on ships and other submerged surfaces is at least three decades away and would require a new business model and total retooling of the antifouling coatings industry.



28.3 (adapted from Fig. 4. Genzer and Groenewold, 2006). Micrographs of wrinkled surfaces prepared by oxidation of heated polydimethylsiloxane (PDMS sheets); a) homogenous PDMS; b) PDMS with 5 μm high 30 μm diameter separated by 70 μm. Wrinkles form spontaneously when the samples cool to room temperature. c) scanning force microscopy of disordered wrinkles; d–f) patterns formed by depositing a thin layer of gold onto warm PDMS. As in b), the clear areas in e) and f) are posts. From Bowden *et al.*, 1998 and Bowden *et al.*, 1999.

Other experimental surfaces are highly hydrophilic and based upon polyethylene glycol copolymers. The concept here is if the surface resembles bulk water, then molecules and organisms in bulk water will not recognize and bind to the surface. Preliminary data indicate that this may be a viable concept. The challenges at this point are developing hydrogels that do not spontaneously degrade in sea water, that can be effectively applied, and whose associated polymers have essential physical and mechanical properties.

Still other surfaces, and many yet to be conceived, will be combinations of the two major kinds of techniques, photolithography and self-assembling block copolymers described above. The spectrum of possibilities appears vast and this should be a fascinating and frustrating area of research into the future. The challenges here are to understand the basic phenomena, measure them, and when viable options are generated, find a practical way to generate the surfaces. An aspect essential to the success of these approaches is effective communication between the polymer chemists and biologists, as both areas require extensive expertise.

Natural glues

Natural glues such as mussel and barnacle glues have entertained researchers for about 50 years. Historically, researchers have worked with polymerized glues which are secondarily cross-linked biological polymers (Larman *et al.*, 1982; Gabbot and Larman, 1987; Waite and Qin, 2001; Nakano *et al.*, 2007). Resolubilizing cross-linked biological polymers is difficult and confusing because the components that are recovered are different from the precursors. Starting from polymerized end product, Kamino (Kamino, 2001; Nakano *et al.*, 2007) made heroic strides in understanding barnacle glues. His most recent accomplishment is a synthetic protein that spontaneously polymerizes (Nakano *et al.*, 2007).

However, with respect to glues, the most powerful experimental approaches are those that combine modern analytical chemistry tools with studies with small amounts of prepolymerized and polymerizing glues. This process was recently revisited with new studies of unpolymerized barnacle glue based upon techniques reported almost 40 years ago (Saroyan *et al.*, 1970a,b). When studies using unpolymerized glues (Rittschof *et al.*, 2008b) are combined with high powered modern analytical chemistry and materials science approaches, the results are exciting (Rittschof *et al.*, 2008b). These techniques are so powerful that new research directions for study of other glues will be driven by comparative approaches and by developing technology to work with unpolymerized glues.

Recent experimental advances with barnacle glues includes genetic lines of barnacles with heritable glue phenotypes (Holm *et al.*, 2005) and the ability to collect and work with prepolymerized barnacle glue (Dickinson,

2008; Rittschof *et al.*, 2008a,b). The ability to work with prepolymerized glues enables rigorous experimental approaches based upon established evolutionary theory as well as sophisticated enzymology, antibody visualization techniques (western blots), atomic force microscopy, fourier transform infrared spectroscopy, circular dichroism and tandem mass spectrometry studies of glue precursors and the chemistry of cross-linking. Once the power and potential of working with unpolymerized glues is appreciated, researchers will focus on developing techniques to work with other glues in their prepolymerized state. This area has potential if there are just a few common pathways that natural glues use for polymerization. Recent findings that pathways, even after separation by approximately one billion years of evolution (Dickinson, 2008), barnacle glue polymerization and vertebrate blood clotting, share two central enzymatic pathways, support the idea of common pathways.

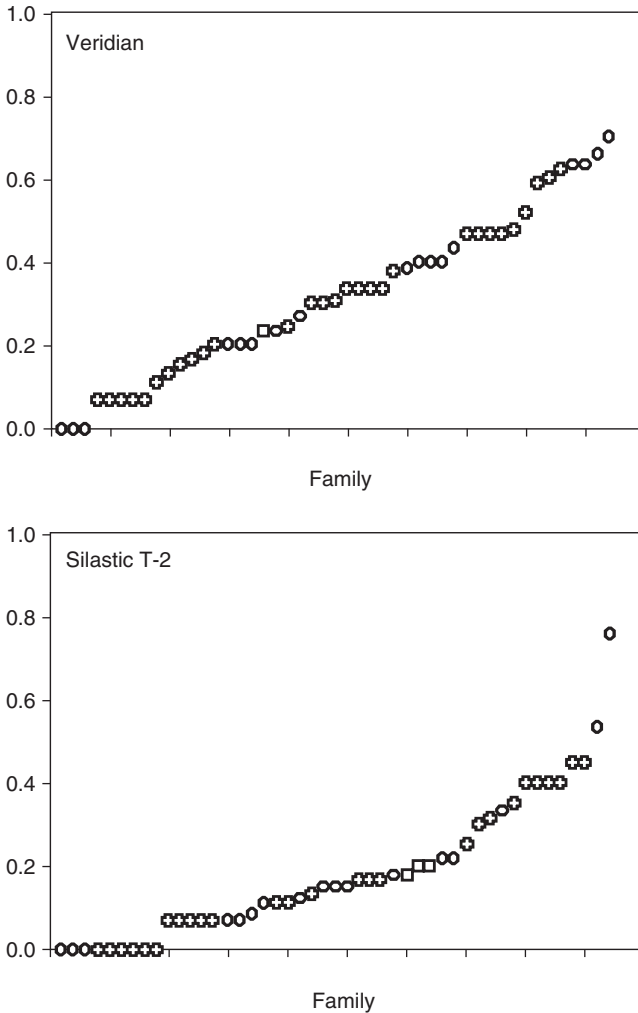
One obvious new line of research will be developing basic biochemical understanding of natural degradation of glues (see chapter 18). This particular area has immediate practical potential in supplementing mechanical cleaning procedures. As an antifouling technology in its own right the process is decades away from an environmentally benign commercial product because there have been no studies of environmental impacts of these kinds of approaches. Biochemical research is another kind of research that promotes the growing and very productive interaction between biologists, chemists and materials scientists and engineers in multidisciplinary teams. As these new interactions are fostered one can expect the synergism will result in novel concept development.

Polymers that change properties

One especially intriguing area is that of polymer films that can drastically change physical properties (Varineau and Buttry, 1987; Jacobson and Comiskey, 1999). The change in properties is attractive because it is an easy and effective way to dislodge fouling from a surface. Although the present experimental examples are polymers that require a change in conditions not easily accomplished in estuarine and oceanic environments, future research could provide exciting novel materials.

28.7 Biological responses to new technology

Biology is notorious for evolving around man's attempts at control. Historically, controls that remain effective are so environmentally damaging and dangerous to humans that they are discontinued. It is clear that natural variability for impacts of even toxic compounds within a fouling



28.4 Proportion of *Balanus* (= *Semibalanus*) *amphitrite* individuals producing a thick adhesive plaque on Veridian® and Silastic T-2® silicone elastomers. Data are re-plotted from Fig. 1 of Holm *et al.*, 2005. See Holm *et al.*, 2005, for details on methods. Each point represents the mean proportion of thick plaque for one family. The order of the families on the x-axis differs for each graph. Family responses are strongly surface dependent reflecting the complexity of the barnacle glue polymerization process. Error bars have been omitted for clarity.

species such as barnacles is impressive (Fig. 28.4). So, the question arises; will biology evolve in response to new technology? The answer is most assuredly yes, but we can learn from our efforts to control diseases like HIV and, for example, the AIDS cocktail approach. The plan would be to take

approaches specifically designed to minimize the ability of natural selection on organisms to come up with chance solutions. A new approach that uses a broad spectrum toxin that is safe to apply and remains in the environment for microseconds after release is one extreme for a successful solution. The other extreme would be surfaces that cannot foul. It is likely that practical solutions will be somewhere between these extremes.

28.8 Research restructuring societal approaches to fouling management

The keys to progress in any research area are governmental approvals and legislative support from international to local governments which includes consistent provision of funding. As society and governments become more aware of the disastrous environmental consequences of existing fouling management approaches, funding for training new researchers and supporting more novel approaches should increase. Existing funding by the European Union, Japan, Australia and the United States will ensure research progress in the existing research lines and result in opening of new basic and applied lines of research. However, it is clear to those who have thought about the scope of fouling management that new societal paradigms are needed if environmentally responsible fouling management concepts are to be developed and implemented.

The research area involved with changes in policy and perception is as complex as fouling itself and a reflection of how governments, businesses, academia, societies and human beings are structured and function. In the United States, the precursor to future approaches to meeting societal needs through cooperation rather than just competition is exemplified by the California agricultural extension program headed by Johnson and Gonzalez (Johnson and Gonzalez, 2005, 2006, 2007a,b, 2008; Gonzalez and Johnson, 2007; Johnson, 2008). These are the first steps in developing the conceptual infrastructure necessary for society to move forward.

However, one need only look to the problem of global warming and global climate change to appreciate how difficult it is to work within the existing political and economic structures toward practical solutions. It is fascinating that a major impediment to progress is due to perceived conflicts between business and scientists and is modulated by government administrators who are operating on belief rather than logic. Thus it may be humanity's downfall that the variety of perspectives based upon belief systems and individual perceptions preclude reaching consensus on complex and pressing problems. If society is to prosper in the future, it is this sociopolitical research area that is the most pressing.

28.9 A new business model

If business as usual is not an alternative in the future of fouling management, then what might the form of a new business model be? One alternative might be recognizing that free market solutions to global societal problems are routinely flawed by the inability to know actual human, societal and environmental costs. Maybe the business model that would work in the future would be a model in which the governments of the world reward companies that minimize the human cost of fouling management, minimize carbon footprints, minimize environmental damage, minimize transport of invasive species and maximize ship performance. Given that most of the world doesn't realize the scope of the issues, this kind of change seems very unlikely.

28.10 Acknowledgements

ONR and TMSI, NUS provided funding. Thanks to authors and publishers of figures, Ruth McDowell, and reviewers.

28.11 References

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