

10th International Congress on Marine Corrosion
and Fouling,
University of Melbourne, February 1999

Additional Papers

John A Lewis (Editor)

Maritime Platforms Division
Aeronautical and Maritime Research Laboratory

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ABSTRACT

This volume contains nineteen papers from the 10th International Congress on Marine Corrosion and Fouling, held at the University of Melbourne in Melbourne, Australia, in February 1999. The scope of the congress was to enhance scientific understanding of the processes and prevention of chemical and biological degradation of materials in the sea. Papers in this volume range across the themes of marine biofilms and bioadhesion, macrofouling processes and effects, methods for prevention of marine fouling, biocides in the marine environment, biodeterioration of wood in the sea, and marine corrosion.

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10th International Congress on Marine Corrosion and Fouling, University of Melbourne, February 1999

Additional Papers

Executive Summary

The fouling and corrosion of vessels and structures immersed in the sea continues to pose significant economic and operational costs to the owner. Fouling growth can interfere with the operation of submerged equipment, impose increased loading stresses and accelerate corrosion on marine structures, and adversely affect the performance of ships by increasing hydrodynamic drag, which necessitates the use of more power and fuel to move the ship through the water. Similarly, marine corrosion and biodegradation of materials can compromise the operation and structural integrity of vessels, structures and other immersed equipment. To enhance protection against fouling and corrosion would generate significant savings in both the maintenance and operation of maritime platforms and equipment.

The first *International Congress on Marine Corrosion and Fouling* was held in France in 1964, and the Congress has continued to be held at approximately four year intervals since. Over this time the Congress has become the foremost international scientific conference on the chemical and biological degradation of materials in the sea, and brings together scientists from academia, industry, defence and other government organisations to present and discuss recent scientific developments in understanding and combating the degradation of materials, structures and the performance of vessels in the marine environment.

The inaugural *U.S./Pacific Rim Workshop on Emerging Non-Metallic Materials for the Marine Environment* was held in Hawaii in 1997. Recognising the increasing pressures to reduce the costs of building and operating ships and the need to reduce or eliminate materials potentially toxic to shipbuilders, ships' crews, and the environment, the workshop was organised to highlight the problems to be solved, the new materials available to address these needs, and areas where further research was needed

The 10th *International Congress on Marine Corrosion and Fouling*, and the 2nd *U.S./Pacific Rim Workshop on Emerging Non-Metallic Materials for the Marine Environment*, were brought together at the University of Melbourne, in Melbourne, Australia, in February 1999. Close to 200 delegates from 24 countries attended, and 118 papers were presented in sessions on Biofilms and Bioadhesion, Chemical Mediation of Fouling in

Natural Systems, Macrofouling and Macrofouling Processes, Prevention of Fouling, Regulation of Antifouling Practices, Antifouling Biocides in the Environment, Transport of Marine Species on Ship Hulls, Biodeterioration of Wood, and Marine Corrosion and Corrosion Control.

Twenty-two selected papers from the congress were published in a special issue of the journal *Biofouling* in June 2000. An additional 19 papers are presented in this publication.

Editor

John A. Lewis

Maritime Platforms Division

John Lewis graduated from the University of Melbourne with a BSc (Hons) degree in 1975 and a MSc degree in 1977, both in marine biology. In 1977 he was recruited by DSTO to work within the Marine Environment Group of the then Materials Research Laboratory in Maribyrnong. He has remained with DSTO since, with primary research interests in marine biofouling and its prevention, and the effects of RAN activities on the marine environment. John is currently a Senior Research Scientist within the Maritime Platforms Division of DSTO's Aeronautical & Maritime Research Laboratory, and manages tasks addressing new, environmentally acceptable methods of biofouling control, environmental compliance of naval vessels, and other environmental aspects of navy operations.

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1. Preface

The 10th *International Congress on Marine Corrosion and Fouling*, incorporating the 2nd *U.S./Pacific Rim Workshop on Emerging Non-Metallic Materials for the Marine Environment* was held at the University of Melbourne from 7-12 February 1999. This volume is the second volume of papers to arise from the Congress. A special issue of the journal *Biofouling* was published in June 2000, which contained a series of 22 papers selected from across the conference program. The themes represented were biofilms and bioadhesion, biocorrosion, fouling settlement processes, methods for the prevention of fouling, and deterioration of wood in the sea. Details of these papers appear at the end of this preface. The current volume contains an additional 19 papers submitted for publication by congress participants. Themes addressed herein encompass biofilms and bioadhesion, macrofouling processes and effects, prevention of fouling, biocides in the marine environment, biodeterioration of wood, and marine corrosion.

I feel it is important to once again thank the many individuals who assisted me in the organisation of the conference and to all who participated. I must also repeat my thanks to the organisations whose financial support made the conference possible: the Comité International Permanent pour la Recherche sur la Préservation des Matériaux en Milieu Marin (COIPM), U.S. Office of Naval Research International Field Office (Europe and Japan*), Defence Science and Technology Organisation (Australia), Akzo Nobel (Australia and UK), Jotun Paints (Australia and Norway), Hempel's Marine Paints A/S (Denmark), Kansai Paint Co. (Japan), Department of State Development (Victoria, Australia), Radiometer Pacific (Australia) and the CSIRO Centre for Research on Introduced Marine Pests (Australia). Coasts and Clean Seas, an initiative of the Australian Federal Government's Natural Heritage Trust, also supported the Congress. I would also like to thank the following colleagues who acted as referees for the papers in this volume: Graeme Batley, Simon Cragg, Helen Dalton, Rocky de Nys, Lyn Fletcher, Emma Johnston, Peter Mart, Brian Moore, Raman Singh, and Don Wright.

Planning is now well underway for the 11th *International Congress on Marine Corrosion and Fouling*. This event will be held San Diego State University, San Diego, California, U.S.A., from July 28 to August 2, 2002. Details are available on the World Wide Web at: <http://www.marine2002.org>.

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Details of the first volume of proceedings from the Congress are as follows:

- Biofouling:** The Journal of Bioadhesion and Biofilm Research (ISSN 0892-7014)
 Volume 15, Numbers 1-3 (June 2000) , Harwood Academic Publishers
 SPECIAL ISSUE: Papers from the 10th International Congress on Marine Corrosion and Fouling, University of Melbourne, February 1999. Edited by Maureen E. Callow and Peter Steinberg
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- Substratum location and zoospore behaviour in the fouling alga *Enteromorpha*
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- Bacteria immobilised in gels: improved methodologies for antifouling and biocontrol applications
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2. Biofilms & Bioadhesion

Nano-indentation measurements of the marine bacteria *Sphingomonas paucimobilis* using the atomic force microscope

Ian Penegar, Catherine Toque, Simon D.A. Connell, James R. Smith and Sheelagh A. Campbell

Nano-indentation Measurements of the Marine Bacteria *Sphingomonas paucimobilis* using the Atomic Force Microscope

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ABSTRACT

The elastic properties of copper-resistant marine bacteria *Sphingomonas* (formerly *Pseudomonas*) *paucimobilis* grown in different concentrations of copper(II) ions have been investigated using atomic force microscopy nano-indentation measurements. Analysis of the force *versus* distance data using Hertzian Mechanics models allowed the determination of cellular compliance. The Young's Modulus of *Sphingomonas paucimobilis* was found to be significantly reduced when grown in a copper-rich medium ($E = 50 \pm 28$ kPa) compared with that observed in the absence of copper(II) ions ($E = 82 \pm 39$ kPa).

INTRODUCTION

Atomic force microscopy (AFM) has proven to be a useful technique for imaging surfaces at atomic or molecular resolution (Binnig *et al.*, 1986; Rugar & Hansma, 1990). Since the technique does not require specimens to be metal coated or stained, non-invasive imaging can be performed on surfaces in their native states and under near physiological conditions. AFM has proven to be particularly successful for imaging biological samples, such as proteins, DNA and whole cells (Hansma & Hoh, 1994; Henderson, 1994; Radmacher *et al.*, 1992; Zhang *et al.*, 1999).

AFM utilises a small, square-pyramidal silicon nitride, or silicon, tip (base dimensions *ca.* $4 \times 4 \mu\text{m}^2$) mounted on a cantilever that is raster-scanned across the surface of the specimen. In contact mode, a constant force is applied between the tip and the sample through the use of a feedback voltage that is applied across the piezo-electric scanner on which the sample is mounted. This feedback signal is used to generate the height (*z*)-data. In addition to topographic imaging, AFM can also be used to measure the forces of interaction between the tip and a surface. These can be accomplished through the acquisition of force *versus* distance curves (Fig. 1), where the deflection of the cantilever

is monitored as a function of tip-sample distance. The gradient of a force-distance curve, after the tip is brought into contact with the surface, is a measure of sample compliance or stiffness. Adhesion between the AFM tip and the sample can also similarly be obtained by measuring the 'pull-off' forces. By raster-scanning the tip while acquiring these force data, force maps displaying either variations in compliance or adhesion can be obtained. Force mapping has been used in biological studies to resolve the cytoskeleton of a fibroblast cell (Ricci & Grattarola, 1994), and to measure local visco-elastic properties of gelatin (Radmacher *et al.*, 1995) and cells (Hoh & Schoenenberger, 1994; Radmacher *et al.*, 1993, 1994, 1995; Tao *et al.*, 1992; Weisenhorn *et al.*, 1993).

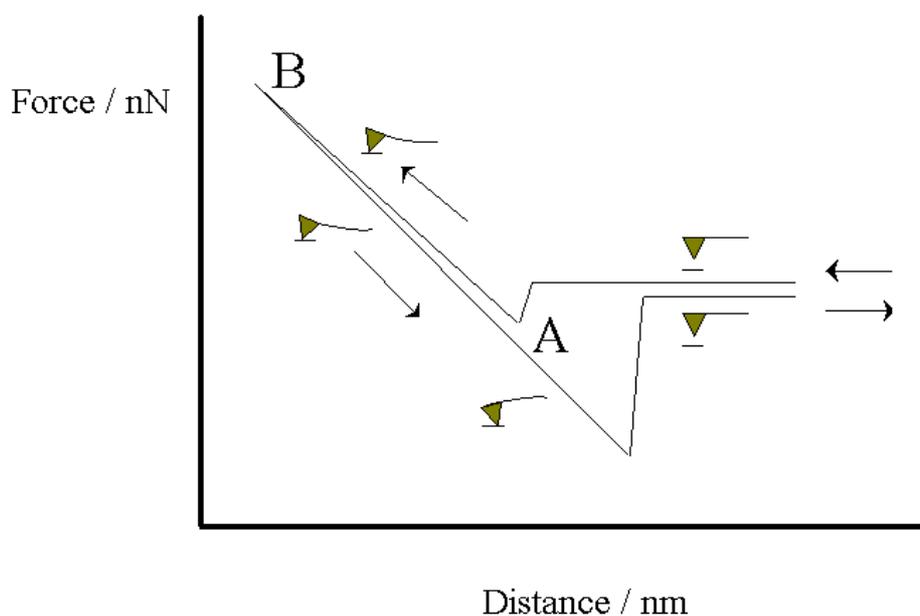
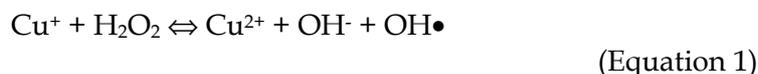


Figure 1 Schematic diagram of an idealised force-distance curve. Points A - B show the region where compliance information can be extracted. Relative elasticity of a sample can be recorded by monitoring the cantilever deflection as a function of the applied force. In this type of experiment, the tip is pushed into the sample surface at a specified distance while the cantilever deflection is measured at regular intervals.

This communication reports some preliminary results of the use of AFM to measure the elastic properties (Young's Moduli) of bacterial cells. Of particular interest in these studies are the copper resistant bacteria, *Sphingomonas* (formerly *Pseudomonas*) *paucimobilis*, which are known to be able to withstand high levels of copper(II) ions in a marine environment; the mechanism through which they achieve this remains uncertain (Cervantes & Gutierrezcorona, 1994; Cooksey, 1994; Ji & Silver, 1995).

Copper is an essential trace metal required for the synthesis of metalloproteins, mainly oxygenases and electron transport proteins. However, high concentrations of copper ions are toxic to microorganisms suggesting that mechanisms must exist for maintaining essential supplies of copper for metalloprotein biosynthesis whilst simultaneously protecting cells from toxic concentrations of these ions.

Under physiological conditions, copper can undergo redox reactions between Cu(I) and Cu(II), and thus can act as an electron donor and acceptor in the electron transport chain. It can also catalyse adverse redox reactions in the cell such as the generation of hydroxyl ions and radicals (Equation 1).



The hydroxyl radicals formed are highly reactive and can participate in a number of deleterious reactions, such as the peroxidation of lipids, which cause membrane disruption.

The toxicity of copper to microorganisms has been exploited in the production of corrosion resistant antifoulant coatings, including copper-based antifouling paints and copper/copper-nickel sheathing. Whilst these coatings are highly effective in the prevention of fouling and corrosion, copper-resistant organisms implicated in biocorrosion have been isolated from such copper-rich surfaces (Penegar, 1999; Rogers, 1948). The first report implementing copper-resistant bacteria in corrosion was that of Grant (1921) who showed that the pitting of copper condenser tubes in power stations was due to bacterially produced ammonia. Copper biodeterioration in water distribution systems has also been attributed to microbially influenced corrosion (Wagner *et al.*, 1992). Other studies of copper-resistant bacteria (*Sphingomonas spp.*, *Micrococcus spp.* and *Corynebacterium spp.*) revealed gelatinous deposits on the inside walls of copper alloy condenser tubes that had been in contact with seawater (Schiffirin & DeSanchez, 1985). In the latter case, the presence of copper-tolerant film-forming *Sphingomonas sp.* resulted in a 20-fold increase in the corrosion rate of 90/10 Cu-Ni and Al-bronze alloys. The mechanism by which bacteria and other microorganisms (Cooksey, 1990; Mellano & Cooksey, 1988) tolerate and propagate in copper-rich environments is therefore of considerable commercial relevance.

A method by which copper-resistant microorganisms are able resist high levels of copper is by the accumulation of copper ions. A number of different resistance mechanisms have been proposed. For example, *Klebsiella aerogenes* are able to uptake copper ions through complexation with metal-binding sites in the extra-cellular polymeric substances (EPS), secreted by the bacteria (Bitton & Freihofer, 1978; Geesey *et al.*, 1988). EPS play an important role in the adhesion of bacterial cells to surfaces and in subsequent biofilm formation (Jucker *et al.*, 1998). In *Mycobacterium scrofulaceum*, copper-tolerance is facilitated by the formation of a CuS precipitate by the reaction with H₂S (Eradi *et al.*, 1987). The cyanobacterium *Synechococcus* has been shown to contain a metallothionein, the transcript for which increases in the presence of copper (Robinson *et al.*, 1990). The sorption of heavy metals, such as copper, zinc and nickel, in the filamentous bacterium *Thiothrix strain A1* has been proposed as an ion-exchange mechanism since 66% to 75% metal ions could be desorbed by placing metal-laden cells in a solution of CaCl₂ (Shuttleworth & Unz, 1993).

The interactions of copper species with cellular or extracellular components, as described above, may cause modifications to the surface properties or morphology of the cells. To establish the effect of copper on cell surface structure, AFM topographic investigations and nano-indentation measurements were carried out on *Sphingomonas paucimobilis*.

MATERIALS AND METHODS

Microbiological methods

A modified mannitol-glucamate (MG) medium was used as the bacteria were to be isolated from a marine environment. The modified MG medium consisted of mannitol (10.0 g dm⁻³), L-glutamic acid, sodium salt (2.0 g dm⁻³), KH₂PO₄ (0.5 g dm⁻³), NaCl (30.0 g dm⁻³), MgSO₄·7H₂O (0.2 g dm⁻³). For the solid medium, agar (15 g dm⁻³) was added. The pH was adjusted to 7.0 with NaOH prior to autoclaving. For the MG medium containing Cu(II), the required concentration of CuCl₂ was added aseptically through a 0.45 µm filter.

Bacteria were isolated from copper panels immersed in Langstone Harbour, Portsmouth, by swabbing the plates in several places and placing the swab in sterile water (1 cm³). Aliquots (0.1 and 0.01 cm³) of this solution were transferred onto solid MG media plates containing the required concentration of CuCl₂. The plates were incubated at 28 °C and those that grew well were subcultured after seven days. Using successive subculturing, a pure culture of *Sphingomonas paucimobilis* was achieved as determined using Analytical Profile Index (API) methods. This culture was stored on agar plates of MG media at 4 °C. When sufficient growth had occurred, a loop of bacteria was transferred into MG media (200 cm³) and grown at 25 °C on an orbital shaker at 120 rpm. The total cell count of the cultures was estimated using a Neubauer haemocytometer. These cultures were then used as a source of inoculum.

Biofilms were grown on AISI 304 stainless steel and glass coupons (1 cm²). These were held vertically in PTFE stubs that were placed at the bottom of 100 cm³ conical flasks. These were filled with MG media (50 cm³) and autoclaved prior to inoculation. For the MG medium containing Cu(II), CuCl₂ was added as described above. After 2 days, coupons were removed from the medium, washed with double-distilled water and allowed to air-dry briefly prior to immediate AFM investigations.

AFM studies

AFM was performed in air under ambient conditions using a TopoMetrix TMX 2000 Discoverer Scanning Probe Microscope (SPM, ThermoMicroscopes, Bicester, UK) in either contact or non-contact modes using a tripod scanner with a maximum *x,y,z*-translation of 70 × 70 × 12 µm. Topographic images were acquired in contact mode using 'V-shaped', silicon nitride cantilevers, of length 200 µm and nominal spring

constant 0.032 N m^{-1} , bearing standard-profile, square-pyramidal tips (Part no. 1520-00, ThermoMicroscopes, UK). For nano-indentation studies, non-contact, 'I-shaped' silicon cantilevers, of length $225 \mu\text{m}$, with integrated silicon pyramidal-profile tips (Part no. 1660-00, ThermoMicroscopes, UK) were used. A non-contact image was obtained prior to nano-indentation measurements over the same scan area. The force constant of each 'I-shaped', silicon cantilever used was calculated as 92 N m^{-1} using their measured unloaded resonant frequency (210 kHz) and bulk material properties of silicon (Cleveland *et al.*, 1993). The radius of curvature (R) was assumed to be 20 nm , as quoted by the manufacturers. Topographic imaging was acquired at a scan resolution of $500 \text{ lines} \times 500 \text{ pixels}$ and height (z)-data were displayed at a resolution corresponding to $256 \text{ grey-scale levels}$.

Nano-indentation measurements were obtained over a $10 \times 10 \mu\text{m}$ scan range using a lateral scan rate of $1 \mu\text{m s}^{-1}$. The scan resolution was reduced to $100 \text{ lines} \times 100 \text{ pixels}$ ($10\,000$ force curves) to reduce the vast computational storage demand and to allow an acceptable time frame for acquisition. This resulted in a spacing between each force curve of 10 nm .

The nanoindentation of the cell at each point was obtained by subtracting the cantilever deflection measured when the tip was in contact with the cell surface from the mean response recorded on the substratum. Cantilever deflections were converted into distances, recorded in nanometres, by dividing by the sensor response. This value was equal to the gradient of the approaching force curve obtained from the hard, substratum curve.

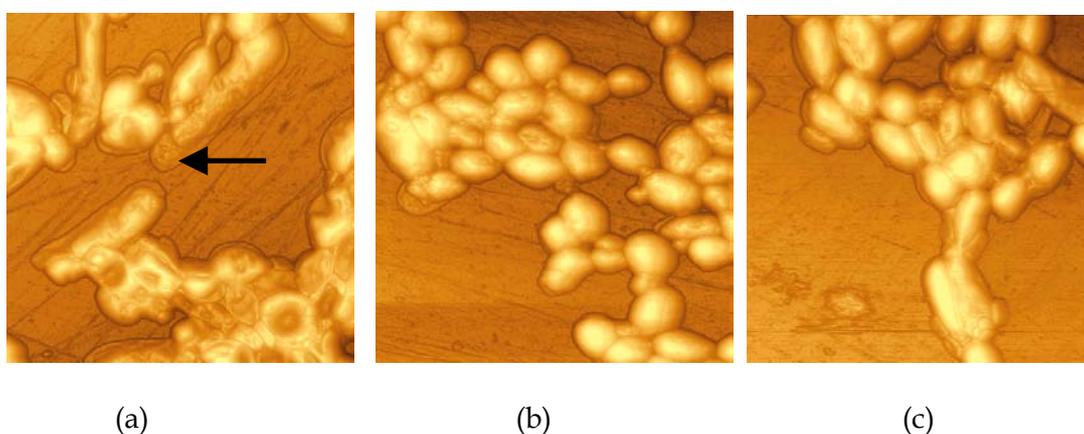


Figure 2 Typical contact-mode AFM images of *Sphingomonas paucimobilis* grown on stainless steel substrates in modified MG media containing various concentrations of Cu(II) ions: (a) 0 ppm , (b) 10 ppm , and (c) 50 ppm . $x = y = 10 \mu\text{m}$. In the absence of Cu(II) ions, EPS can be seen extending from the edges of the microorganisms onto the steel substrate (arrowed).

RESULTS AND DISCUSSION

Prior to nano-indentation-studies, the topography of cells that had been grown on AISI 304 stainless steel and glass in the absence and presence of various concentrations of Cu(II) ion was investigated. Figure 2 shows typical, contact-mode AFM images of the bacterial biofilms grown on stainless steel. Thick layers of EPS were seen at the periphery of many cells, as has been reported elsewhere (Beech *et al.*, 1996). The apparent smoothness of the EPS layers is likely to have resulted from coverage with a thin film of water. The presence of EPS was more prominent with cells that had been grown in the absence of Cu(II) ions (Fig. 2a), where regions of EPS could be seen extending from the edges of the microorganisms onto the steel substrate.

Table 1 Measured dimensions of *Sphingomonas paucimobilis* grown in modified MG media containing various concentrations of Cu(II) ions.

[Cu ²⁺] (ppm)	Measured width (μm)	Measured length (μm)	Measured height (μm)
0	2.3 ± 0.3	3.4 ± 0.4	0.63 ± 0.06
10	2.8 ± 0.3	3.9 ± 0.5	0.82 ± 0.11
20	2.7 ± 0.2	3.9 ± 0.3	0.82 ± 0.07
50	2.8 ± 0.4	4.0 ± 0.3	0.84 ± 0.09

For cells grown in the presence of Cu(II) ions (>10 ppm), the EPS appeared to be more localized and closely followed cell boundaries. The AFM images of bacteria grown in copper-free media (Fig. 2a), exhibited central depressions whereas those cultured in copper-containing media did not. This may be indicative of some degree of dehydration of the cells grown in copper-free media. This is reflected in the cellular dimensions (Table 1), which show a significant increase in width, length and height of the cells at a Cu(II) concentration of 10 ppm ($N = 25$, $p < 0.05$) compared to those grown in the absence of copper. For the cells grown in copper-rich media, no further increases in size were observed at increased concentrations of 20 and 50 ppm.

Having noted differences in cell structure according to exposure to copper ions, the AFM studies were extended to nano-indentation measurements. These were obtained from force-distance curves of randomly selected positions on bacterial cells and the underlying substratum (either stainless steel or glass). AFM force-indentation curves were modelled using Hertzian mechanics, which describes the elastic deformation of two surfaces touching under load (Hertz, 1881). This theory assumes that there is no adhesion between the contacting surfaces. It was found that the Hertz Model for a sphere was the most suitable geometry to describe the indentation of an AFM tip with bacterial cells (Penegar *et al.*, 1999). For an infinitely hard sphere of radius R touching a soft planar surface, the Hertz model gives the relation between the loading force F and the indentation δ , as:

$$F_{\text{sphere}} = \frac{4}{3} \frac{E}{(1-\nu)} \sqrt{R} \delta^{3/2}$$

(Equation 2)

where, E is the Young's Modulus and ν is the Poisson ratio of the soft material, which relates to the compressibility of the material, taken to be 0.5 (Hofmann *et al.*, 1997).

Thus, the Young's Modulus was calculated from the gradient of many plots of loading force versus indentation^{1,5}. Fig. 3 shows selected AFM images of cells grown in copper-free and 100 ppm copper-containing media, together with line profiles indicating the points at which Young's Moduli were calculated. Typically, measurements were made at eight sites on each cell, but to avoid irregularities associated with edge effects, only data from the central regions were selected for compliance data extraction. The spatial variation of the Young's Moduli on cell surfaces show that measurements at cell peripheries may be misleading. At these positions, lateral displacement of the cell may occur in response to the force applied invalidating the use of the Hertz model for obtaining compliance data. Alternatively, the values measured may be influenced by contributions from the Young's Modulus of the underlying substratum. This can be observed in Fig. 3b, where the compliance calculated from measurement 8 is 150 kPa, more than double that measured at the centre.

Young's Moduli of 50 ± 28 kPa and 82 ± 39 kPa were calculated for cells grown in the presence and absence of Cu(II) ions respectively, values comparable with those measured for living cells and other biological materials (Laney *et al.*, 1997; Radmacher *et al.*, 1994). The reasons for the differences in compliance between cells cultured in copper-free media and those exposed to copper ions are unclear, but the lower compliance exhibited by the cells grown in the copper-free media may be a consequence of a greater degree of dehydration than that observed in copper rich media where EPS and cell membrane proteins may complex copper ions altering the cell turgor pressure.

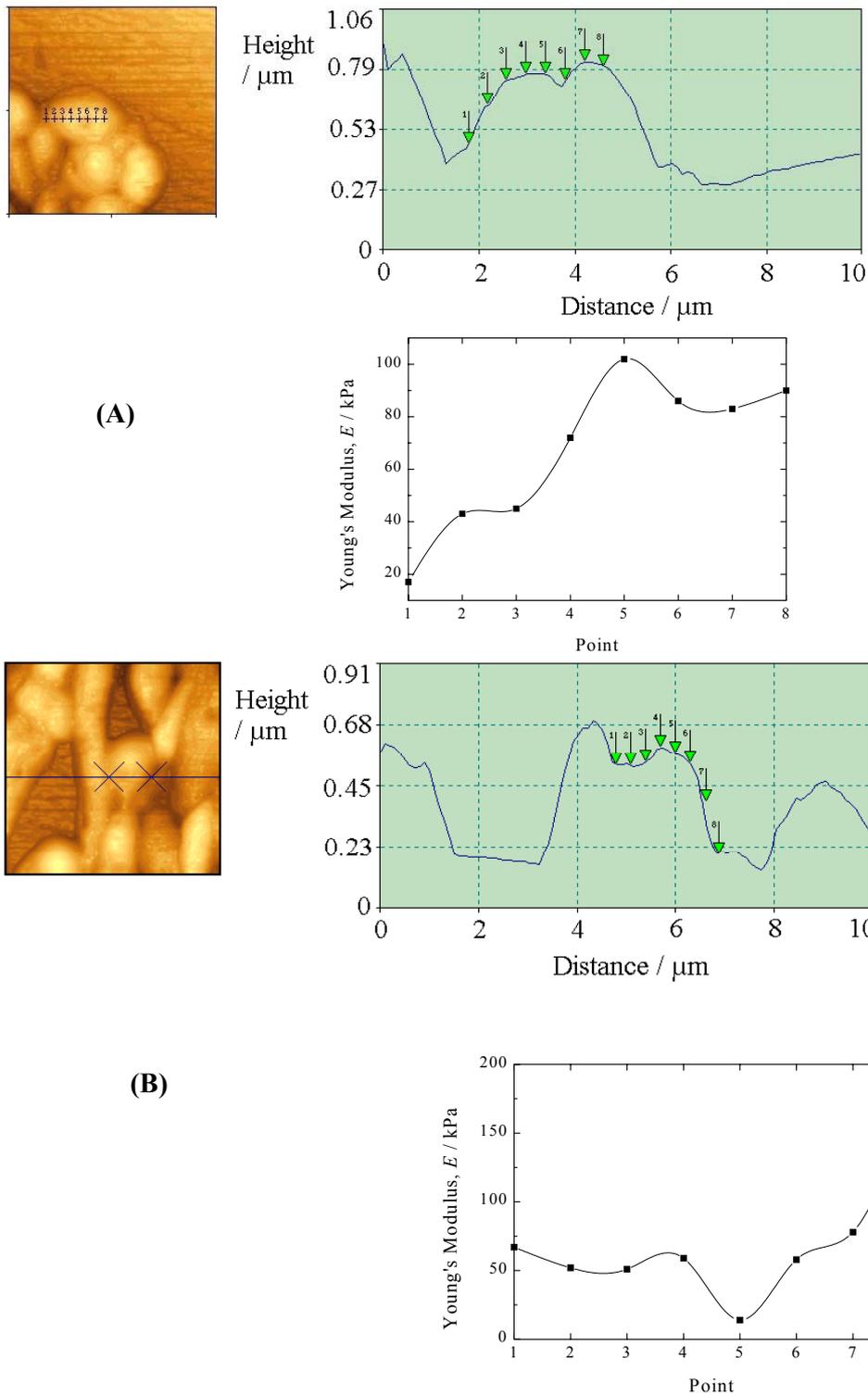


Figure 3. AFM profiles showing the variation of Young's Moduli across bacterial surfaces of *Sphingomonas paucimobilis* grown on stainless steel substrates in modified MG media: (a) no Cu(II) ions present, and (b) 100 ppm of Cu(II).

Acknowledgements

The authors would like to thank C A Powell at the Nickel Development Institute (NiDI), Birmingham, UK and the EPSRC for financial support. Appreciation is also expressed to Dr Iwona Beech for the use of microbiology facilities at the University of Portsmouth.

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3. Macrofouling Processes

Macrofouling Role of Mussels in Italian Seas: A Short Review

Giulio Relini and Manuela Montanari

Macrofouling of an Oceanographic Buoy in the Ligurian Sea (Western Mediterranean)

G. Relini, M. Montanari, P. Moschella and A. Siccardi

Effects of Fouling Organisms on the Water Quality of a Nuclear Power Plant Cooling System

K.K. Satpathy and R. Rajmohan

Macrofouling Role of Mussels in Italian Seas: A Short Review

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ABSTRACT

A mussel (*Mytilus galloprovincialis* Lam.) dominated community represents the climax fouling assemblages on most structures and facilities in Italian seas and enclosed brackish environments. On offshore structures mussels account for 80% to 98% of the total fouling wet weight. In the Adriatic Sea (Ravenna platforms), mussels near the surface can achieve a wet weight of more than 90 kg/m² and a shell length of 6-7 cm within one year, and on artificial reefs up to 120 kg/m². In the Ionian Sea (Crotone platform) mussels reach wet weights of 6 kg/m² and lengths of 3.5 cm near the water surface, and in the Ligurian Sea (Genoa platform), 26 kg/m² and 6.2 cm.

INTRODUCTION

Fouling communities in Italian waters are the best known in the Mediterranean Sea and more than 70% of the papers published appear in Italian literature. These studies have been undertaken over 35 years, with more than 300 papers published, most of them in the period 1970-1985 (Relini, 1993). Species composition, settlement and development of the fouling communities have been studied in different environments (ports, estuaries, lagoons, shallow and deep, inshore and offshore waters) and on different structures (wharves, power stations, intakes, platforms, ship hulls, etc.) all around Italy (Relini, 1993).

In most marine and brackish environments, the fouling community is dominated by mussels (*Mytilus galloprovincialis* Lam) which form a final and quite stable climax association at depths up to 20 m. However, this climax community is not always reached because of local environmental characteristics or pollution. In the past the

distribution of mussels in Italian waters was much more localized but, due to mariculture and the fouling transport, the species has spread over the last 50 years.

The aim of this paper is to collate and summarize available data on fouling by the Mediterranean mussel species *M. galloprovincialis*. Emphasis is placed on mussel fouling on offshore platforms (Relini *et al.*, 1976, 1998).

REPRODUCTION, SETTLEMENT AND GROWTH

Mussels are ripe throughout the year, but there are two distinct settlement periods: one in spring and one in autumn (Ravano & Relini, 1970; Relini and Ravano, 1971; Tursi *et al.*, 1990). The first one is the more significant and coincides with increasing sea temperatures and the spring bloom of phytoplankton. Normally, mussels are not pioneer settlers, but require a substrate colonized by other foulers, as shown in the classical diagram of Scheer (1945). Nevertheless in eutrophic environments mussels can settle on newly exposed substrata if that substrate is rough or is colonised by filamentous algae or stoloniferous hydroids (Relini *et al.*, 1976).

Detailed studies were undertaken on Ravenna platforms in the Adriatic Sea in 1975 and 1976, during which time panels were immersed at various depths for periods of one month, three months, six months and one year. Two platforms were considered: PCW-A sited on a seafloor of 12 m and AGO-A on a seafloor of 23 m depth. Results from these are presented as an example of mussel fouling characteristics (Tables 1, 2). The following conclusions could be drawn from this data.

For the AGO-A platform:

- Maximum settlement occurred near the surface (93% on 1 month panels), with only occasional settlers at the third level (-20 m)
- The total number of mussels settled on the three-month panels was about 4 times higher than that found on the one-month panels
- The total settlement on the two six-month panels is about six times higher for the first level than for the second level (Table 1).
- Among the one-year panels, the higher settlement was found on surface panel (first level) with 9724 individuals/dm². The maximum size (70 mm) was reached only on the second level (-9m). Also, monthly panels immersed near the surface (first level) had the highest settlement (79.5%), while those at -11m (third level) were very thinly populated
- Among three-month panels, those immersed in the period from May to July: (1500 individuals/dm² at the first level, 763 at the second level) showed the higher settlement.

Period	Month	I level 0 m						II level -9 m							III level -20 m						Total					
		1 ^a	2	3	4	5	6	Total	1	2	3	4	5	6	7	Total	1	2	3	4		5	Total			
1M	May	1						1								1						1				3
1M	Jun	489						489								33										522
1M	Jul	1						1								1										2
1M	Aug	+						+								+										+
1M	Sep	+						+								+										+
1M	Oct	+						+								+										+
1M	Nov	11						11								+										11
1M	Dec																									
1M	Jan																									
1M	Feb																									
1M	Mar																									
1M	Apr	501						501	35							35										538
Total		1822	45	2				1869	239	7						246										2115
3M	Jul	+						+								+										+
3M	Oct	59						59	6							6										65
3M	Jan	+						+								3										5
3M	Apr	1881	45	2				1928	248	7						255										2185
Total		2082	276	94	10			2462	111	73	62	21	1			268										2638
6M	Oct	115						115	159	3	+					162										287
6M	Apr	2197	276	94	10			2577	270	76	62	21	1			430										2925
Total		9269	254	120	51	29	1	9724	359	4	5	10	18	20	1	410										10170
12M	Apr																									36

Table 1 Settlement of mussels (no/dm²) of different size-classes at AGO-A platform (Ravenna)

^a 1,2,3,..... represent shell length classes: 1 = 0-10 mm, 2 = 10-20 mm, 3 = 20-30 mm, etc.
 "+'' = no/dm² < 1

Period	Month	I level 0 m						II level -5 m							III level -11 m					Total			
		1 ^a	2	3	4	5	6	Total	1	2	3	4	5	6	7	Total	1	2	3		4	5	Total
1M	May	3						3								13	+					+	16
1M	Jun	320						320								52	3					3	375
1M	Jul	+						+							+	+						+	+
1M	Aug	2						2							+	+						2	+
1M	Sep	+						+							+	+						+	+
1M	Oct															10							+
1M	Nov	1						1							10								11
1M	Dec	19						19							11								30
1M	Jan														+	+						+	+
1M	Feb																						
1M	Mar																						
1M	Apr	345						345	86	116	18	+			86	3						3	434
3M	Jul	1429	65	6				1500	629						763	16	+				16	2270	
3M	Oct	+						+	+						+	+					+	+	
3M	Jan	747						747	291						291								1038
3M	Apr	6						6	10						10	1					1	17	
Total		2182	65	6				2253	930	116	18	+			1064	17	+				17	3334	
6M	Oct	1809	421	105	9			2344	53	12	22	22	3		112	3	4	11			19	2475	
6M	Apr	3604	64	+				3668	800	95	4				899	2					2	4569	
Total		5413	485	105	9			6012	853	107	26	22	3		1011	5	4	1			21	7044	
12M	Apr	4701	140	90	34	29	3	4997	2943	56	12	15	11	17	3069	1	2	5	14	4	61	8129	

Table 2 Settlement of mussels (no/dm²) of different size-classes at PCW-A platform (Ravenna)

^a 1,2,3,.... represent shell length classes: 1 = 0-10 mm, 2 = 10-20 mm, 3 = 20-30 mm, etc.
 "+", " " = no/dm² < 1

In contrast to the AGO-A, at the PCW-A platform the six-month panels immersed in the second period of six months had a higher population (3688 individuals/dm²) than those immersed in the first semester (2344/dm²). Settlement results from the one year panel exposed at the first level on PCW-A (4999 individuals/dm²) underestimate actual settlement because some of the community was lost during recovery. The maximum size (seventh class) was reached only at the second level.

Data on mussel settlement are also available from the Ionian Sea, off the coast at Crotona on a platform named "LUNA-A". Here, fouling was studied at four depths (0, -14, -20 and -65 m, seafloor at -70 m) between July 1975 and June 1976 (Relini *et al.*, 1976). Features of this community were:

- On one month panels mussels appeared only at the first and second level during the spring months
- Settlement on three month panels was still low on the first level (37 individuals/dm²)
- Only the panels of the second six-month period showed a considerable number of mussels at first level (105 individuals/dm²) and second (61/dm²) levels.
- On one year panels, an appreciable settlement of mussels was recorded only at the first level (379 individuals/dm², with valves reaching 35 mm). At the second level the mussel density was down to 93/dm² and at the third level to 25/dm². At the fourth level the panel was completely covered by the oyster *Neopycnodonte* and no mussels were present.

In the Ligurian Sea, fouling was examined on December 1977 at the landing platform (oil pipe terminal) for super oil tankers situated offshore at the oil port of Genoa-Multedo (Relini, 1979). All the data concerning mussels is collated in Table 3. The lowest limit for the presence of mussels was 34 m, while the seafloor is at 40 m.

Table 3 Mussel samples from 12 dm² surfaces at different depths on the Genoa platform (a 1, 2, 3...represent length classes: 1= 0-10 mm, 2= 10-20 mm...)

GENOA OIL LANDING PLATFORM (G.O.L.P.)															
Depth	Mussel Density No/12dm ²	Length classes											% of most common length classes	Weight of mussels	
		1 ^a	2	3	4	5	6	7	8	9	10	11		g/12 dm ²	kg/m ²
0 m	1,059	1	236	398	278	112	33	1	-	-	-	-	37.5% (20-30 mm)	3,129	26.1
5 m	434	-	69	210	94	11	30	15	5	-	-	-	48.2% (20-30 mm)	2,123	17.7
11 m	34	-	-	2	-	-	-	8	12	10	1	1	35.3% (70-80 mm)	1.2	10
34 m	21	-	-	1	-	-	-	3	-	2	10	2	52.4% (90-100 mm)	1.24	10.3

Mussels at tide level could grow to a length of 62 mm with one year, although 37.5% of the population was only 20 to 30 mm in length. The presence of large individuals below 5 meters was due to there being no periodical cleaning operations of the platform.

Additional data on fouling in the Ligurian Sea was collected from a meteorological buoy of the series ODA (Oceanographic Data Acquisition System). Mussels were abundant on this structure despite it being 37 nautical miles from the coast.

BIOMASS

Mussel growth can be fast and a shell length of 6-7 cm can be achieved within one year (Relini, 1977a; Tursi *et al.*, 1985a, 1985b; Relini M., 1990). The contribution of mussels to the total biomass of fouling is shown in Tables 4 and 5. Biomass values reflect the increase in accumulation of fouling with time. The highest weights (i.e. above 10 kg) were recorded on panels positioned at the uppermost level of the platform AGO-A, and on the first and second levels of the platform PCW-A after 12 months of exposure. The decrease in fouling biomass with depth is due to the dominance of mussels on upper levels, especially on multi-month panels. On substrata placed near the sea floor, the fouling community consisted predominantly of barnacles, serpulids and hydroids. These latter organisms contribute little biomass due to their small individual size and abundance.

Table 4 Biomass (wet weight in g per panel) of total fouling and of mussels at different depths on panels immersed for 3, 6, 9 and 12 months at platform PCWA-A.

Period	Month	I LEVEL 0 m			II LEVEL - 5 m			III LEVEL - 11 m		
		Total fouling weight	Weight of mussels	% of total	Total fouling weight	Weight of mussels	% of total	Total fouling weight	Weight of mussels	% of total
3 M	Jul	1395	1066	76%	2140	1450	67%	174	3	1.70%
3 M	Oct	379	1	+	330	+	+	68	+	+
3 M	Jan	179	+	+	180	+	+	95		
3 M	Apr	25	+	+	18	+	+	16	+	+
6M	Oct	7041	6710	95%	3074	2600	84%	434	313	72%
6M	Apr	1197	907	76%	1534	1168	76%	280	1	+
9M	Jan	6802	6295	92%	5621	5147	91%	1306	1154	88%
12M	Apr	10909	10391	95%	10601	10100	95%	2426	1398	57%

The biomass accumulated on a surface immersed for longer periods of time was found to be greater than on panels immersed for successive shorter periods over the same period. For example, the biomass of fouling on a panel immersed for 3 months near the surface of AGO-A from May to July was 795 g, whereas the summed biomass on one-month panels immersed in May, June and July was 315 g (5+170+140).

There was no great difference between the two platforms in the accumulation of fouling on the panels immersed at the upper level, while at the other levels the differences were considerable, possibly because the two platforms are in different depths of water (Table 4, 5).

The contribution of mussels to fouling biomass increases with the duration of the immersion, especially during the summer months. On 9 and 12 month panels, mussels can account for over 90% of the biomass (Table 4, 5). As already reported for the wet weight, the mussel biomass was almost equal on the partially immersed substratum of the two platforms. The trend with increasing depth is consistent, i.e. the greater the depth, the lesser the biomass.

Table 5 Biomass (wet weight in g per panel) of total fouling and of mussels at different depths on panels immersed for 3, 6, 9 and 12 months on platform AGO-A

Period	Month	I LEVEL 0 m			II LEVEL - 9 m			III LEVEL - 20 m		
		Total fouling weight	Weight of mussels	% of total	Total fouling weight	Weight of mussels	% of total	Total fouling weight	Weight of mussels	% of total
3 M	Jul	795	500	63%	386	85	22%	37	+	+
3 M	Oct	243	+	+	144	+	+	11		
3 M	Jan	23	+	+	120	+	+	10	+	+
3 M	Apr	< 1	+	+	11	+	+	16	+	+
6M	Oct	5010	3713	74%	2389	1763	74%	320	45	14%
6M	Apr	60	15	25%	200	40	20%	61	1	1.60%
9M	Jan	6338	6212	98%	3491	3256	93%	397	257	65%
12M	Apr	11916	11600	97%	5949	4797	81%	820	584	71%

A further study was carried out 18 years later (Relini *et al.*, 1998) in the same area of Ravenna, Central Adriatic Sea, at platforms PCWA and ANTARES. The fouling which settles on gas platform piles was studied using samples taken in 1993 from the PCWA and ANTARES platforms, at 7 and 10.5 km from the shore and in water depths of 12 m and 14 m respectively. In March and September 1993 samples were obtained by scraping an area of 600 cm² from three or four different aspects (North, East, South, West), at the same depth on one pile. Samples were taken at depths of 0.5, 5.5 and 12 m on PCWA and 0.5, 7 and 12 m on ANTARES.

On both platforms the macrofouling was dominated by mussels from the surface to a depth of about 10 m. Near the seafloor the bivalve *Crassostrea gigas*, barnacles, hydroids and serpulids were dominant. Fouling biomass on the two platforms was similar for the two seasons. The highest biomass was measured in September on PCWA (1 m) with 1561.3 g/dm², of which 1553 g/dm² comprised mussels. In table 6 biomass is reported as mean values of four samples per depth and season.

At Crotona (LUNA-A), the highest biomass occurred from March to September, although settlement was less abundant than on the other platforms. Adding together all the monthly weights obtained at Crotona at different depths, gives a total of 215g, less than that reported in only one month at Ravenna (August, PCW-A, first level = 300

g/panel). On the three-month panels at Crotona the maximum biomass of fouling was 106 g per panel, while at Ravenna the maximum was 2140 g/panel. The panels immersed for 9 months showed the greatest biomass at the two intermediate levels, while the one-year panels showed equally high weights at the upper three levels.

The influence of the mussels on weight was only evident at the first level for panels immersed for nine months or more. In particular, for the one-year panel, of 816 grams of fouling, 492 grams were mussels (60.2%). Converting these values to kg/m² gives 6.8 kg/m² (fouling) and 4.1 kg/m² (mussels). Mussel settlement at the platform in Genoa-Multedo (Ligurian Sea) reached 26 kg/m² at the surface, but diminished with depth.

Table 6 Mean values and Standard deviation of four samples taken at different orientations (N,S,W,E) in two platforms at three levels, during March and September 1993

PCWA (March)		mussel density (no/dm ²)	mussel wet weight (g/dm ²)	mussel dry weight (g/dm ²)	mussel ash weight (g/dm ²)	fouling wet weight (g/dm ²)	mussels fraction %
Depth							
0.5m	Mean	189.25	791.86	378.22	306.13	808.33	97.96
	St Dev	134.54	297.84	137.76	109.88	286.26	
5.5m	Mean	62.50	355.90	153.85	122.77	358.33	99.32
	St Dev	16.90	48.64	27.83	22.53	48.11	
11m	Mean	0.27	33.10	17.50	15.07	83.29	39.75
	St Dev	0.25	40.21	21.37	18.44	34.25	
ANTARES (March)							
0.5m	Mean	81.00	582.49	450.97	249.50	588.89	98.91
	St Dev	50.69	273.12	231.98	135.46	269.95	
7m	Mean	56.00	752.64	418.81	356.43	760.70	98.94
	St Dev	31.38	87.27	65.85	58.55	87.43	
12m	Mean	0.98	10.05	4.63	3.82	122.25	8.22
	St Dev	1.36	16.44	7.54	6.16	65.58	
PCWA (September)							
1m	Mean	510.25	1142.15	710.66	527.66	1150.79	99.25
	St Dev	271.58	447.73	279.56	193.12	445.20	
5.5m	Mean	36.35	274.85	181.72	142.13	279.56	98.31
	St Dev	17.18	162.44	94.60	92.37	159.24	
11m	Mean	0.08	0.11	0.06	0.05	45.05	0.23
	St Dev	0.10	0.20	0.11	0.10	31.08	
ANTARES (September)							
0,5m	Mean	442.33	627.60	493.29	330.21	639.31	98.17
	St Dev	174.05	252.11	187.70	135.75	260.17	
7m	Mean	21.53	111.05	86.33	58.14	138.90	79.95
	St Dev	10.91	60.39	48.29	31.64	57.61	
12m	Mean	6.28	30.91	16.80	14.44	119.16	25.94
	St Dev	2.03	22.42	14.35	12.41	11.75	

Some data on mussels found on different offshore structures (Relini & Relini,1994) are presented in Table 7. The large difference between the fouling (mainly mussels) accumulation in the central and north Adriatic, in comparison to other Italian seas is due to different characteristic of the sea water, particularly nutrient content (Table 8).

DIFFERENT ENVIRONMENTS

Mussels contribute to the fouling of harbours of Liguria, in particular Genoa (Relini, 1964, 1966, 1993; Montanari & Relini, 1973), Ischia (Idato *et al.*, 1980, 1981), Civitavecchia (Chimenz, 1965; Chimenz & Taramelli, 1973; Taramelli & Chimenz, 1965; Taramelli *et al.*, 1977), Catania (Cantone *et al.*, 1980, 1981), Taranto (Tursi *et al.*, 1979, 1982a, 1982b, 1983/1984, 1984a, 1984b, 1985a, 1985b, 1990), Brindisi (Lepore & Gherardi, 1977; Scalera *et al.*, 1976), Bari (Marano *et al.*, 1976, 1980), Manfredonia, (Gherardi *et al.*, 1974), Venice (Barbaro & Francescon, 1975, 1976; Relini, 1995; Relini *et al.*, 1972). Mussels were not found in the fouling of Palermo harbour (Riggio, 1979; Riggio & Mazzola, 1976). Mussel fouling has also been described from the intakes of cooling systems of power plants in Genoa (Relini, 1977b), La Spezia (Relini, 1976, 1977b), Vado Ligure (Relini 1977b; Relini & Romairone, 1976) and in Torrevadali (Relini, 1984; Relini *et al.*, 1980).

Table 7 Summary of data on fouling of offshore structures in Italian Seas

Offshore Structure	PCW-A	AGO-A	LUNA-A	G.O.L.P.
Sea	Adriatic	Adriatic	Ionian	Thyrrhenian
Locality	Ravenna	Ravenna	Crotone	Genova
Distance from coast (km)	7	18	6	2.8
Depth bottom (m)	12	23	70	50
Level of sample	surface	surface	surface	surface
Substrate	panels	panels	panels	girders
Maximum age of mussels	12 months	12 months	12 months	12 months
No of mussels/12 dm ²	59,988	116,688	4,549	1,059
Maximum length (mm)	60	60	35	62
% of most common	94%	95.30%	78%	37.50%
Length class	(0-10 mm)	(0-10 mm)	(0-10 mm)	(20-30 mm)
Weight of mussels (g/12 dm ²)	10,391	11,600	654	3,129
Weight of mussels (kg/m ²)	86.6	96.6	5.4	26.1
Mussels as % of total fouling weight	95%	97%	80%	90%

A very high biomass of mussels has been reported from artificial reefs of the Adriatic Sea. On concrete blocks forming an artificial reef near Ancona (Central Adriatic), up to 120 kg/m² of mussels with shell lengths of 5-6 cm were harvested after one year at 7-8 m depth (Bombace, 1981, 1989; Relini, 1979). In the Tyrrhenian Sea, up to 70 kg/m² of mussels with an average valve length of 6.2 cm were collected from concrete blocks forming an artificial reef off the mouth of the river Tiber (Ardizzone *et al.*, 1989). In contrast, mussels were absent from the artificial reefs in Sicily and Liguria.

As previously discussed, mussels are the main foulers of offshore structures near Ravenna, Crotone and Genoa. Additional information is available for a small platform offshore of Venice (Dalla Venezia, 1976, 1981; Montanari *et al.*, 1992) where 62 kg/m² of mussels were recorded on panels immersed for one year near the sea surface. In the

Tyrrhenian Sea, Ardizzone *et al.* (1980) reported an average fouling wet weight of 37.7 kg/m², most of which was attributable to mussels, on a platform 3.5 miles off the coast, at Fiumicino.

In brackish environments, in particular lagoons, mussels may be the dominant fouling organisms if the salinity is not less than 7-8 ‰. Studies have been undertaken on the Delta Po River lagoons (Matricardi *et al.*, 1980; Relini *et al.*, 1983-84, 1985) and the Venice lagoon (Barbaro & Francescon, 1975; Francescon & Barbaro 1976; Relini & Patrignani, 1993).

Table 8 Environmental parameters measured at the platforms at Ravenna and Crotona. The three values presented for each parameter are the minimum, mean and maximum of monthly measurements taken over a period of one year (1975-76).

	RAVENNA				CROTONE			
	AGO A		PCW A					
	2m	12m	2m	12m	2m	15m	30m	60m
Temperature °C	5.3	8.3	4.4	7.8	13.7	13.6	13.6	13.7
	13.9	12.8	13.3	13.1	16.4	16.4	16.0	15.6
	25.0	21.2	23.7	22.4	21.9	21.8	20.0	18.9
Salinity ‰	31.2	35.7	30.3	34.1	38.0	38.0	38.0	38.1
	33.7	36.9	32.8	35.4	38.2	38.2	38.2	38.3
	35.8	37.6	36.4	36.6	38.4	38.4	38.4	38.4
O ₂ mg/l	6.8	2.5	7.4	3.9	6.9	7.0	7.3	7.0
	9.1	7.3	9.4	7.1	7.4	7.3	7.4	7.3
	13.1	9.2	13.0	9.4	7.6	7.4	7.5	7.6
pH	8.1	7.9	8.1	8.1	8.0	8.0	8.1	8.1
	8.2	8.1	8.3	8.2	8.1	8.1	8.1	8.1
	8.4	8.3	8.5	8.4	8.2	8.2	8.2	8.2
N - NO ₂ µg/l	0.5	0.4	0.7	1.5	1.8	2.5	2.1	2.1
	4.7	2.7	6.5	6.1	4.2	4.5	3.4	4.1
	10.7	5.3	14.2	13.4	8.2	6.7	4.9	5.1
N - NO ₃ µg/l	11.4	9.9	13.7	15.4	7.7	10.3	10.6	8.9
	76.2	25.2	103.4	61.1	24.6	26.7	22.8	27.2
	215.4	56.2	299.1	141.8	41.4	40.1	44.3	55.8
P - PO ₄ µg/l	1.5	0.6	1.8	2.4	2.1	1.6	1.5	1.5
	4.4	6.2	4.3	6.4	4.5	2.8	2.6	4.5
	9.9	21.7	7.4	16.1	7.8	5.3	4.8	8.0

DISCUSSION

The biomass values and growth rates recorded for mussels in the Adriatic Sea are the highest reported in the literature (see Seed, 1976). Barker Jørgensen (1990) reports growth rates of shells of *M. edulis* of 7 to 11 mm per month in different localities. On offshore platforms in the North Sea and Morecambe Bay, Richardson & Seed (1990) report mussel (*M. edulis*) biomass of 8.16 kg/m² and shell length of 33.3 mm after 1.5 year and 19.98 kg/m² and 71.6 mm after 7.5 years at mean low water. At a depth of 25 m the values were 8.67 kg/m² and 28.5 mm after 1.5 years, and 24.58 kg/m² and 65.2 mm after 7.5 years. At the offshore platform "Holly", in the Santa Barbara Channel, California, the time to achieve a shell length of 50 mm was estimated as 6-8 months from recruitment (Page & Hubbard, 1987).

In eutrophic waters, in particular in the Central and Northern Adriatic Sea, the growth and biomass are very high, exceeding 100 kg/m² wet weight after one year. This can be turned to economic gain, such as on platforms in the Adriatic Sea. On these platforms it was decided not to use any antifouling system and, through an agreement between the oil company "Agip" and fishermen, the jackets are cleaned at least once a year and the harvested mussels (100 kg/m²) are marketed for human consumption.

In one year the accumulation of mussels on a single platform (limited to the calculation of 20 girders with a diameter of 80 cm and 10 meters of depth, and not considering the beams) is about 50 tonnes. A total estimate for all the structures used for the extraction of natural gas off the coast at Ravenna (probably about 360 girders with an average diameter of 80 cm) suggests that in one year there is an accumulation of about 900 tonnes of mussels. In 1975-76, when our research was initiated, this enormous mass of mussels was removed annually by divers who scraped the surfaces and then threw the mussels back into the sea with significant environmental consequences. These are now collected and marketed with considerable advantage, turning a problematic fouling organism into a valuable fishing product.

CONCLUSIONS

Data on fouling within different environments, and on different structures and facilities in Italian seas, shows that the mussel *Mytilus galloprovincialis* is the predominant fouler, at least down to depths of 10 m. High growth rates, high densities and large biomass present a significant fouling problem. Mussel biomass and growth rates exceed those reported from offshore structures elsewhere in the world. However, this fouling problem can be turned to economic gain by allowing the harvesting of mussels for human consumption

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Macrofouling of an Oceanographic Buoy in the Ligurian Sea (Western Mediterranean)

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ABSTRACT

On the oceanographic buoy ODAS Italia 1, moored in the Ligurian Sea (43°48.90' N, 09°06.80' E) at over 1270 m depth, macrofouling was studied in different time periods. The fouling that had directly settled on the buoy was studied on April 15, 1991, when the buoy was recovered after 52 months at sea. A total of 79 taxa, of which 71 were species, were identified. Along the body buoy, from the surface down to -34 m, biomass wet weight values varied without any consistent trend with increasing depth. The highest value (20.8 g/dm²) was recorded at -20 m, while on the horizontal surface of the disk, at 37 m depth, the highest value reached was 42.4 g/dm². Investigations on macrofouling started on June 28, 1992 and are still in progress, covering a total period of 70 months. They featured panels fixed to the body of the buoy, at -12 m and -33 m, and removed at progressively longer intervals. A total of 55 taxa, of which 51 species, were identified; at -12 m, 31 species were observed, while at -33 m, 47 species, 20 of which only at this depth, were identified. No acorn barnacles, very common species in coastal fouling, were found. Mussels and other bivalves, soft bryozoans, hydroids, serpulids were the main components of macrofouling assemblages. After 70 months, the fouling biomass, in terms of wet weight, reached 28.1 g/dm² at -12 m and 47.4 g/dm² at -33 m. Possible ways of buoy colonization are discussed.

INTRODUCTION

Knowledge on the severity of marine fouling on offshore structures is important for hydrodynamic loading (Wolfram & Theophanatos, 1985) and corrosion rates calculation (Edyvean *et al.*, 1988; Forteath *et al.*, 1984). However, these communities also bear an ecological interest as they act as artificial reefs on which community development takes place remotely from established fouling populations (Scarborough Bull & Kendall, 1994; Picken *et al.*, 2000). Also, there is increasing interest for offshore aquaculture in the Mediterranean, and fouling is one of the problems to be solved.

While macrofouling data on offshore structures in the Mediterranean Sea are not abundant (see Relini G, 1993; Relini G & Relini M, 1994), there are even less data on buoys or floating structures. Most of the papers on offshore fouling in the Mediterranean are concerned with platforms generally placed in neritic waters (Relini G, 1993; Relini G & Relini M, 1994). Fouling settled on floating or suspended objects and structures like oceanographic equipment (Aliani *et al.*, 1998; Aliani & Meloni, 1999; Tsikhon-Lukanina *et al.*, 1977) has been described only after short exposure periods. Papers on macrofouling of buoys are also rare in the literature worldwide (Redfield & Hutchins, 1952; Thompson, 1977 - quoted in Haderlie, 1984; Yan *et al.*, 1994; 1999; 2000)

Fouling on buoys is likely to be different, because of the floating nature of the substratum which means that the community is always at a constant depth and not exposed to tidal movement. This, however, could be important in the North Adriatic Sea, where the tide can exceed 1 m, not in the Ligurian Sea, where the tide range is about 30 cm. Conversely, the distance from the coast and sea currents are more important factors.

The presence in the Ligurian Sea (N-W Mediterranean Sea) of an oceanographic buoy (ODAS Italia 1, Oceanographic Data Acquisition System) moored at 37 miles from Genoa in a site where the sea bed is over 1270 m deep, has offered the opportunity to study fouling on this structure since 1987.

Fouling organisms in the Gulf of Genoa (central part of Ligurian Sea) are the best known in the Mediterranean, since investigations first began back in the sixties. Fouling of harbours, coastal water, deep water (-200 m), artificial reefs, power station intakes, platforms, warf piling was described (for the long list of references see Relini G, 1993). In surface water (up to 10-15 m depth), a "climax" community is reached with the dominance of mussels, but, for different reasons, mussels do not settle in many sites, hence barnacles, serpulids, bryozoans and sometimes sponges are the dominant foulers. In some sites, fouling was studied over long periods of time, (for example for more than 30 years in Genoa harbor), but always for short immersion periods - from 1 month to 12-24 months - of experimental substrata. The same occurs in other parts of the Mediterranean. Some long-term observations are available for colonization dynamics of artificial reefs by macrobenthos. Ardizzone *et al.* (2000) have studied fish and benthic settlement in the Fregene artificial reef, central Tyrrhenian Sea, 9 km from the mouth of the river Tiber. Over the 11 years of investigations, the reef fauna has changed from pioneer stage to a mussel dominated community. Mussels were not harvested and they declined over time, since further mussel settlement was prevented by siltation and lack of colonization .

Relini G *et al.* (1994) have described patterns of sessile macrobenthos development on an artificial reef in Loano (Gulf of Genoa) from 1986 onward, by means of panels (1 to 24 months), samples, photos, and scuba diver observations. After five years, succession in the colonization process was still going on. There are two main communities, one exposed to light which tends to a mature stage and is dominated by Cystoseiraceae

(brown algae), the second assemblage - settled on shaded surfaces - is composed of typical sciophilic organisms. Sponges, coelenterates, large bryozoans, such as *Pentapora octomilleriana*, are dominant. The process of reaching a steady state on Loano artificial reef is complicated and slackened because of some unpredictable factors such as water dynamics, sedimentation (siltation), and predation. In particular, the cleaning grazing activity of sea urchins is very important. And yet, after 10 years from the immersion of the concrete blocks, the biotic assemblages look like those living on natural rocks (Relini G, 2000).

On a Monaco (MC) artificial reef, benthic community development was studied for six years by different methods and in particular by long-term photographic records (Balduzzi *et al.*, 1985; 1986). In Sicilian artificial reefs biotic succession converges towards natural reefs after 60 months if the water is clear; in turbid water, the convergence process leads to an impoverished biotic assemblage made up of taxa typical of non-consistent substrata (D'Anna *et al.*, 2000).

In the world literature, very interesting data and comments for long-term studies on fouling, although with regard to coastal waters, are reported by Butler & Connolly, (1996; 1999), who studied the macrofouling on Port Bonython pier, South Australia, 6.5 and 13.5 years after the initial immersion of piles. They used photographs of fixed positions and direct observation by divers. The main conclusion is that the fouling assemblages had not yet converged to a "stable" condition by 6.5 years and more sampling data would be needed to determine whether it had done so by 13.5 years.

In previous papers, macrofouling of the ODAS buoy was described on the basis of scuba diver observations, photos, and samples scraped away from the buoy (Relini G *et al.*, 1990). A high growth rate for mussels (*Mytilus galloprovincialis*) was reported (Relini M, 1990), which is quite interesting for a substrate positioned so far off the coast. Also, the FAD (Fishing Aggregating Device) function of the buoy was described. Some benthic fish such as Blennidae belong to the fouling community of the buoy, and some pelagic fish can find food in the buoy fouling community (Relini M *et al.*, 1994; Relini G *et al.*, 2000).

This paper reports our observations on macrofouling, made on April 15, 1991 when the buoy was recovered after 52 months at sea, and with investigations carried out with the use of panels, from June 28, 1992 to April 24, 1998. An additional sampling was devoted to algae. The buoy is still in operation and the monitoring of macrofouling is in progress as part of a larger research team work on the buoy and the surrounding environment (Siccardi & Montanari, 1997).

MATERIALS AND METHODS

The Buoy System

The buoy, which is operated by IAN-CNR (Institute for Ship Automation of the Italian National Research Council) consists of a tubular steel structure (diameter 600 mm), 52 m long; 37 m are submerged and 5 m are above water. Figure 1 shows a full picture of the structure: on top of it, there is a laboratory unit, at the bottom, at -37 m, there is a large stabilizing disk (diameter 2000 mm) designed to dampen the buoy vertical motion. No antifouling paint was applied, only an epoxy-tar product was used. The buoy is anchored on a 1270 m deep sea bed, through an elastic 1900 m mooring cable which allows the buoy to move around the mooring for about half a mile and keeps it afloat even under strong side thrusts (Siccardi & Montanari, 1997).

By means of hourly sampling, the buoy measures meteorological parameters, wave motion, sea temperature, and currents. Collected data are used for real time application as well as for historical series with a view to developing a valuable reference framework (Siccardi & Montanari, 1997).

Working Area

The working area was originally selected because the buoy was supposed to operate only as a navigation aid unit. The selected position is at the crossroads of different routes - i.e. from Genoa to the South, to Corsica, Sardinia and Sicily - and the routes crossing the Ligurian-Provence basin. It is an area highly exposed to winds and waves. This area was later found to be very interesting for the study of ecological phenomena in the Ligurian-Provence basin. This is a Mediterranean region with well-defined hydrodynamic patterns and a cyclonic circulation along the coast. Currents move northward, on the west and east side of Corsica respectively (currents originating from the Atlantic and from the Tyrrhenian sea) later joining in the Gulf of Genoa in a turbulent way which gives origin to the Ligurian current.

This current is a 30 km wide and 150 m deep water stream with an average annual flow of $1.4 \times 10^6 \text{ m}^2\text{sec}^{-1}$ (Bethoux *et al*, 1982). Deep water currents are formed in this basin under the action of cold and dry winds in winter. The much higher density of surface water in the Ligurian-Provence basin than in the Tyrrhenian Sea forms a gradient whereby Tyrrhenian waters are forced to penetrate into the Ligurian Sea. A major frontal zone (Figure 1) is also developed in the basin with divergence and convergence phenomena which, with the upwelling of deep water layers, enrich euphotic water with nutrients.

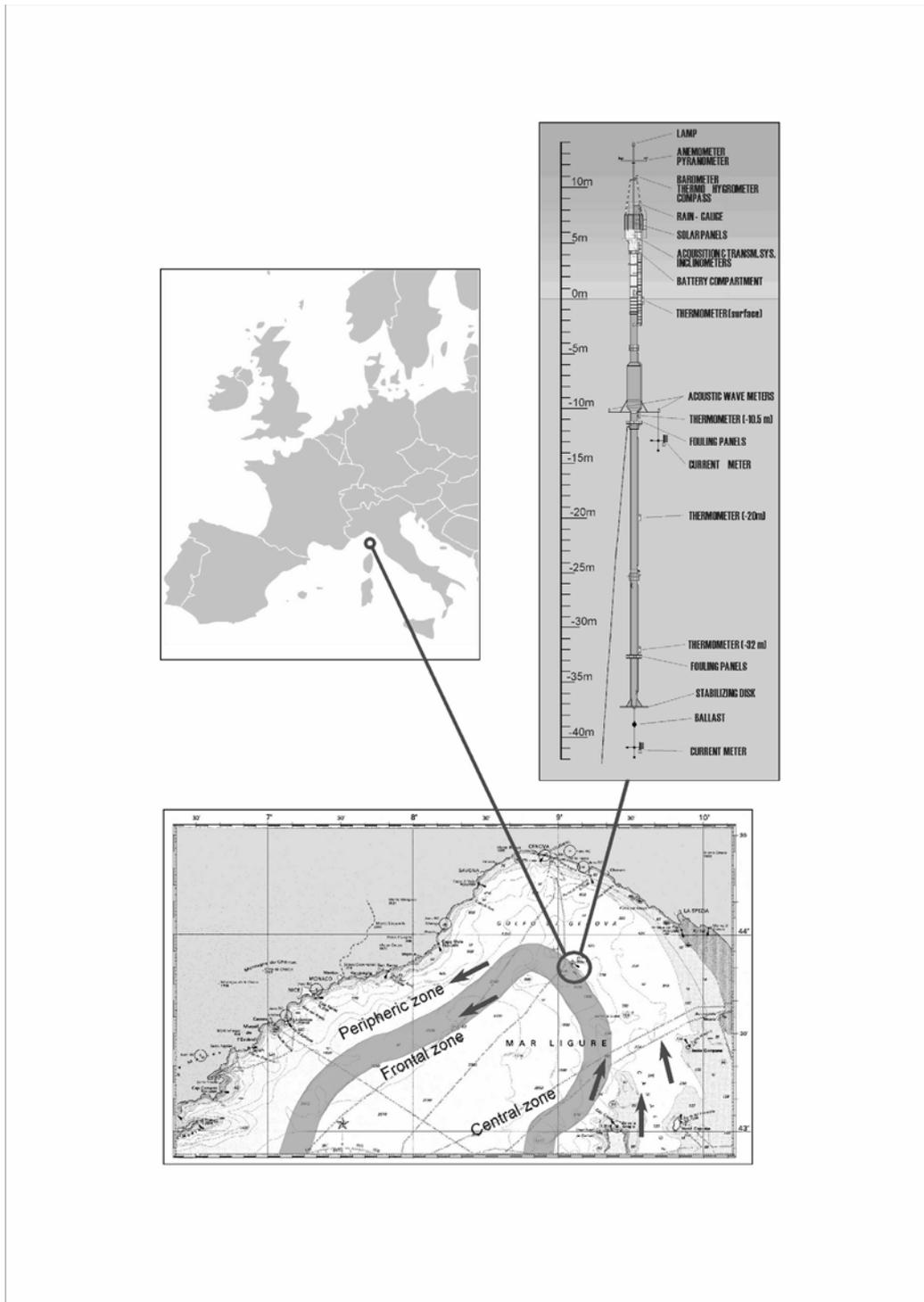


Figure 1 The ODAS Italia 1 buoy is currently moored at the centre of the working area, at 43°48.90' N, 009°06.80' E, at 37 nm from Genoa, on a 1270 m deep sea bed.

Several water sampling campaigns have been carried out in the area surrounding the buoy during the study period. The maximum values obtained in the lab of Istituto Corrosioni Marine for nitrites, nitrates and phosphates are respectively: 0.34 (NO_2^-), 1.98 (NO_3^-), 0.68 (PO_4^{3-}) $\mu\text{g at/l}$. Salinity values, also measured during the same period but in situ, range between 37.8 and 38.1 ‰.

As shown by the data recorded by the buoy measuring system, in the period of fouling observation, temperature values measured at 0, -10 and -42 m have changed, following a seasonal cycle, from a common winter minimum of slightly less than 12°C, to summer peaks during maximum insolation hours of 28°C in surface waters, 26°C at -10 m and 17°C at -42 m.

The dominant current, as measured by current meters installed on the buoy at -10 and -42m, over the same period, had a 330° and 345° orientation, with average values of around 20 cm/sec, and maximum winter peaks of up to 60 cm/sec.

Macrofouling Study

Evaluation of macrofouling settled directly on the buoy after 52 months of immersion

The buoy, moored in the center of the Ligurian sea on January 9, 1987, was recovered after 52 months, on April 15, 1991, and was towed to the Genoa harbor at a speed of 3 knots and then transferred on a dockyard wharf (Figure 2).



Figure 2 Picture of the buoy laying in dry dock

The fouling settled along the full 37 m length of the immersed part of the buoy was sampled along one side every 2-3 m, scraping a 50 x 50 cm square surface. This

sampling surface was selected for its good amount of fouling, since normally a 20 x 20 cm surface is considered as the “minimum area” for this kind of samples; one side of the cylinder was deemed to be representative of the whole fouling, because the buoy, when in the water, was rotating on its own axis, at least partially.

Additional samples were taken from both horizontal sides (upper, lower) of the stabilizing disk (Figure 3).



Figure 3 Mrs Montanari pointing to the fouling on the bottom side of the damping disk of the buoy

Evaluation of the development of macrofouling settled on panels immersed for increasing periods of time

Fouling settlement and colonization patterns over time were studied using inert material panels (20 x 30 x 0,4 cm in size) which were placed in round panel-holding racks secured to the buoy body at two depths: 12 m and 33 m (Figure 4a).

Twelve panels on each rack were removed by scuba divers after progressively longer periods of immersion and replaced with new ones. Unfortunately it was not possible to have replicates for long time exposure. Owing to the position of the panels (Figure 4a), the two sides of each panel can act as replicates, however in this paper the two sides are not considered separately. This monitoring of macrofouling started on June 28, 1992 and is still in progress. The available substrata used in this study were recovered after 3, 9, 14, 18, 24, 40, 51, 57, 70 months of immersion, the last one was brought in the Lab on April 24, 1998, about 70 months after initial immersion. Fouling of panels replaced and recovered for other intermediate exposure periods is not considered in this paper. It is important however to confirm fouling settlement patterns during the first 12-24 months and to give some seasonal information.



Figure 4 a. Panel holding rack, mounted on the buoy body
 b. Panel immersed for 51 months (June 28, 1992 through September 18, 1996) at -33 m

Evaluation of the presence of different algae species on the buoy body

At the beginning, algae were neglected, also because of difficulties in identifying them due to poorly preserved material. Algae were not separated when the material was fixed. Because of this and also because few of them had been listed in previous papers, an "ad hoc" collection was taken at different depths on October 2, 1995, in order to have a full identification of all macroalgae on the buoy. A careful macrofouling examination was carried out by two biologist divers and the macroalgae were randomly picked up along the buoy body, at 3, 5, 10, 15, 20, 25, 30 m depth.

Qualitative and quantitative evaluation procedures

Samples and panels were photographed before collecting them. On the basis of photos and direct examination, the settlement of organisms was defined.

According to Boudouresque (1971) definition, the Covering Index is evaluated, using Braun-Blanquet scale of the abundance-dominance coefficient, according to phytosociological method:

+	=	negligible presence
1	=	< 5% surface cover
2	=	5-25% surface cover
3	=	25-50% surface cover
4	=	50-75% surface cover
5	=	>75% surface cover

The share comprising free-living species was evaluated by the number of individuals for each panel (Numeric Index). The following arbitrary scale was used:

+	=	few individuals;
1	=	<10 individuals
2	=	11-25 individuals
3	=	26-75 individuals
4	=	76-150 individuals
5	=	>150 individuals.

For Polichaeta and Bivalvia, also data on density (no/dm²) and sizes are available. Biomass values were measured, by non decalcified wet, dry, and ash weight, following the standard methods.

RESULTS

Macrofouling Settled Directly on the Buoy (1987-1991)

While macrofouling assemblages differ at different depths, no regular change pattern can be observed with depth, in particular for biomass (Figure 5). Nevertheless, some similarities are evident (Figure 6). Samples at 1, 3, 7, 9 m depth show a high similarity to the group of samples collected between 14 and 21 m depth.

The latter group presents the highest biomass values along the vertical surfaces. A schematic representation of biotic assemblages at some of the investigated depths is shown in Figure 5. Covered surface percentage values by different systematic or functional groups are sketched.

The list of species found at different depths is reported in Table 1 with covering index or numeric index, for each species and each sampling depth. In the samples collected on the recovered buoy, 79 taxa, of which 71 species (also the genus of non identified species was considered when there was no other congeneric species identified), have been described and evaluated.

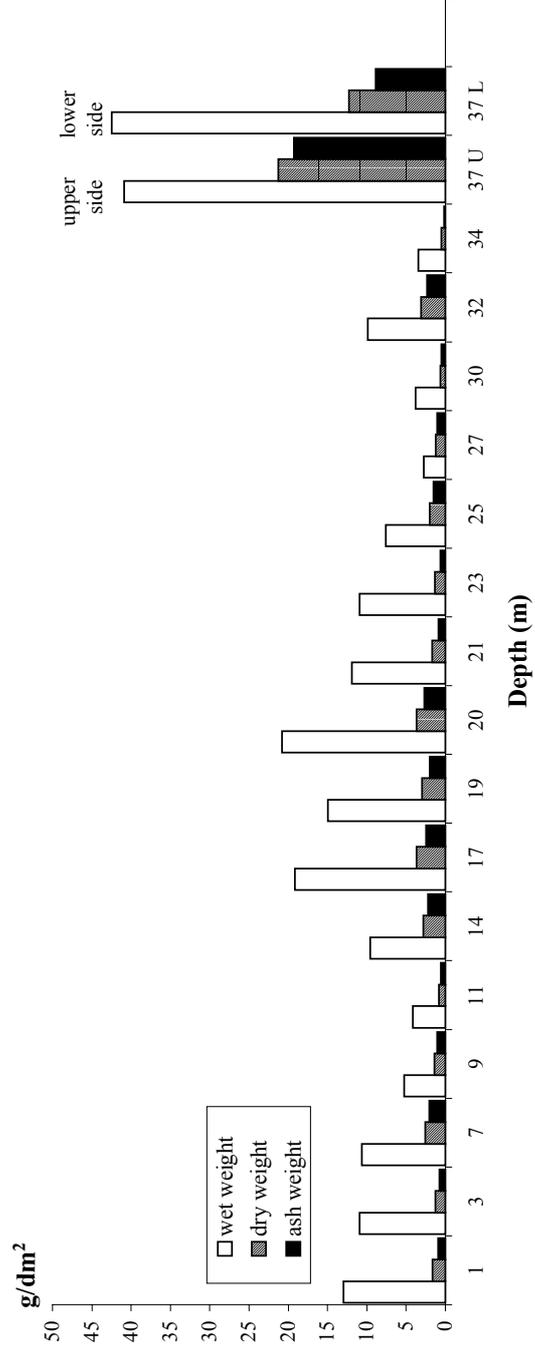
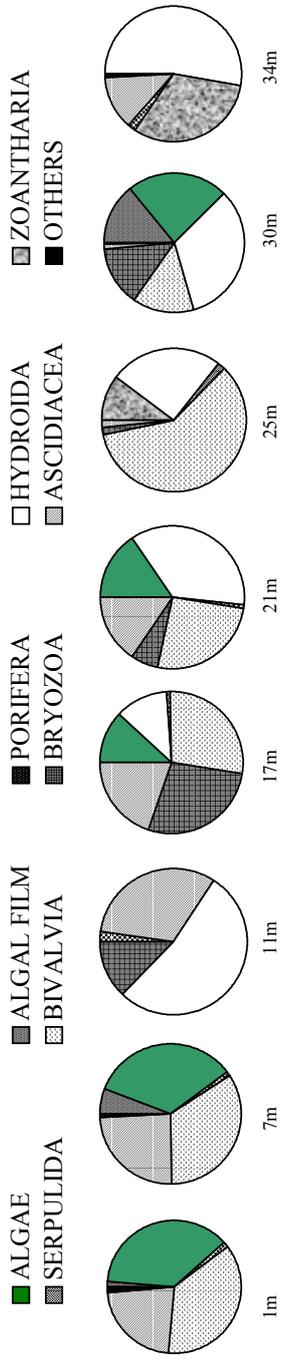


Figure 5 Buoy macrofouling characterization versus depth. Samples have been collected from the buoy vertical body, from 1m of immersion down to 34 m, and from the upper and lower side of the stabilizing disk. (Upper part of the figure: schematic representation of the main taxa present in samples collected at some depths on the buoy vertical body. Lower part: ash weights of macrofouling settled in each sampled zone of the buoy body and of the disk)

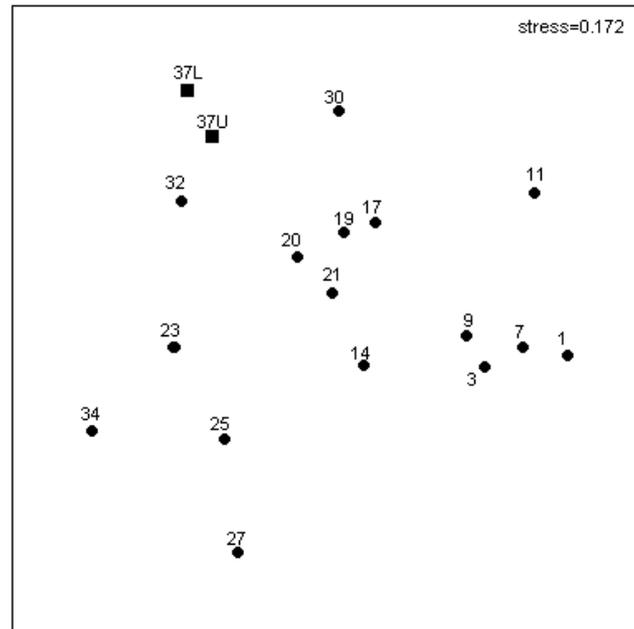


Figure 6 Nonmetric multidimensional scaling plot in two dimensions of the similarity (Bray-Curtis) between macrofouling assemblages sampled at different depths along the buoy when it was recovered after 52 months at sea. Circles: samples from the buoy body; squares: from disk (L: lower side; U: upper side).

25 species were found only on the buoy body and 8 only on the stabilizing disk. On the latter structure, 46 species in total were found, 34 on the upper side and 36 on the lower side. On the cylinder of the buoy, the highest number of species was found at -19 m (30 species); the lowest at -1 and -3 m (11 species).

The most important systematic groups, namely those representing taxa with the highest number of species, are bivalves (22 species), polychaetes (20 species), followed by hydroids (8 species), gastropods, bryozoans, and echinoderms (3 species).

Some species are found at all depths, while others only in one specific site of the stabilizing disk. The following species were found only on the latter structure: polychaete *Chaetopterus variopedatus* and endoprocta (which were present on both sides of the disk), polychaete *Sabella spallanzani*, bivalves *Ostrea edulis*, *Plagiocardium papillosum* and gastropods *Epitonium pulchellum* (found only on the upper side) and alcionarion *Alcyonum palmatum*, sea anemone *Condylactis aurantiaca* and bivalve *Neopicnodonte cochlear* (found only on the lower side).

The stabilizing disk is the part of the buoy with the highest settlement rate, in terms of number of species, covering and numeric index (Table 1), and biomass (Figure 5).

Table 1 List of taxa and corresponding covering and numeric index at different sampling depths on buoy body and at two sides, upper and lower, of stabilizing disk

	BUOY BODY																DISK	
	Depth (m)																Up.	Low
	1	3	7	9	11	14	17	19	20	21	23	25	27	30	32	34	37	37
ALGAL FILM	1		2		+									3	4			
ALGAE	4	4	5	4		2	3	4	4	3	1		+	4				
PROTOZOA																		
FORAMINIFERA																		
<i>Miniacina miniacea</i> (Pallas)						1	+	+	+	+	+	+	+	+	1	+	+	1
Unident. sp.		1	1	1	+	1	1	1	+	1	+	+	+	+	1	1	+	+
PROTOZOA					+		+	1	+	+	+			+			+	+
FOLLICULINIDA																		
PORIFERA																		
<i>Sycon</i> sp.								1	1				+	1			1	2
HYDROIDA																		
<i>Eudendrium capillare</i> Alder					4		1	3						4			+	
<i>Obelia dichotoma</i> (L.)												1		1		1	3	2
<i>Obelia bidentata</i> Clarke								1				1	2	1				
<i>Bougainvillia ramosa</i> (Van Beneden)					+			3	5	5	5		1			3		
<i>Lafoeina tenuis</i> M. Sars										1							+	
<i>Tubularia crocea</i> Agassiz				+		2	3	2	3	2	2			4	4	2	2	4
<i>Garveia franciscana</i> (Turrey)												3						
<i>Hydranthea magarica</i> (Hincks)							+	2	2				1	3		1	1	
ALCYONARIA																		
<i>Alcyonium palmatum</i> Pallas																		2
ZOANTHARIA																		
<i>Condylactis aurantiaca</i> (Delle Chiaje)																		2
<i>Corynactis viridis</i> Allman						3					3	2	2		1	3	1	3
ENDOPROCTA																	1	2
POLYCHAETA																		
<i>Pomatoceros triqueter</i> (L.)				1		1				+				+	1			+
<i>Pomatoceros lamarckii</i> (Quatrefages)			1	1														1
<i>Spirobranchus polytrema</i> (Philippi)	1		1	2	1	1	1	2	1	1	1	1	+	+	2	1	1	1
<i>Hydroides stoichadon</i> Zibrowius				+														
<i>Hydroides</i> sp.	+							+										+
<i>Serpula concharum</i> Langerhans						+					+						1	1
<i>Serpula vermicularis</i> L.														+	1	+		1

Table 1 (cont.)

	BUOY BODY															DISK		
	Depth (m)															Up.	Low	
	1	3	7	9	11	14	17	19	20	21	23	25	27	30	32	34	37	37
POLYCHAETA (cont.)																		
<i>Serpula</i> sp.		1			+		1		1	1			1			1		
<i>Spirorbis</i> sp.							1			1								1
<i>Sabella spallanzanii</i> (Gmelin)																	1	
<i>Polyophthalmus pictus</i> (Dujardin)	+	1	1			1		+		1				1				
<i>Chaopterus variopedatus</i> (Renier)																	1	1
<i>Harmothoe</i> sp.				+												1		
<i>Eunice harassii</i> Audouin & Milne-Edwards							+		+							1		
<i>Eunice schizobranchia</i> (Claparède)											+							
<i>Lysidice ninetta</i> Audouin & Milne-Edwards							+											
<i>Marphysa sanguinea</i> (Montagu)									1									
<i>Syllis gracilis</i> Grube			1			1												
<i>Adyte pellucida</i> (Ehlers)															+		+	
<i>Lumbrineris</i> sp.													+					
<i>Scoletoma funchalensis</i> (Kinberg)											+							
<i>Nereis rava</i> (Ehlers)												+						
Nereididae				1		+		1										
Polynoidae											+							
PYCNOGONIDA																		
<i>Achelia echinata</i> Hodge							4	3	3	3	+	3		2	1		+	2
Unidentified sp.																		2
CIRRIPEDIA																		
<i>Lepas anatifera</i> L.	1																	
DECAPODA																		
<i>Pilumnus hirtellus</i> (L.)				+			+											+
<i>Eriphia verrucosa</i> (Forsskal)									+									
TANAIDACEA			1	+		+	+											
AMPHIPODA																		
<i>Caprella andreae</i> Mayer	3	2	2	+	4	1	3	2	1	+				1			+	2
<i>Jassa marmorata</i> Holmes	4	3	2	1	5	1	5	5	5	5	4	1	+	+	3	2	2	1
GASTROPODA																		
<i>Bittium reticulatum</i> (Da Costa)			1				1	1	1									+
<i>Doto</i> sp.							+		1	+			+					
<i>Epitonium pulchellum</i> (Ant. Bivona)																		+

Table 1 (cont.)

	BUOY BODY															DISK		
	Depth (m)															Up.	Low	
	1	3	7	9	11	14	17	19	20	21	23	25	27	30	32	34	37	37
BIVALVIA																		
<i>Mytilus galloprovincialis</i> Lamarck	2	1	2	1	+		2	2	1	1		1			1		3	2
<i>Anomia ephippium</i> L.	2	2	3	1		3	2	2	2	2		3	2		1		1	
<i>Hiatella rugosa</i> (L.)	+		2			1											1	+
<i>Hiatella arctica</i> (L.)							1	1	1	1					1		+	1
<i>Aequipecten opercularis</i> (L.)															1			
<i>Chlamys varia</i> (L.)						1												1
<i>Chlamys multistriata</i> (Poli)							1	1	+						1		1	1
<i>Palliolium incomparabile</i> (Risso)																+		+
<i>Modiolarca subpicta</i> (Cantraine)	2	2	2	1		1	1	1	+	+		1		1			1	2
<i>Pteria hirundo</i> (L.)							1		+	1				2	2		2	2
<i>Lima hians</i> (Gmelin)		1					1											
<i>Arca noe</i> L.													+					
<i>Arca tetragona</i> Poli							+	1	+					+	1		1	1
<i>Modiolus barbatus</i> (L.)						1	1	1	1	+		1	+	1	1		1	1
<i>Pododesmus patelliformis</i> (L.)									1		1	1		1	2		1	1
<i>Neopicnodonte cochlear</i> (Poli)																		1
<i>Ostrea edulis</i> L.																	1	
<i>Corbula gibba</i> (Oliv)								+									+	2
<i>Chama gryphoides</i> L.						+											+	
<i>Pecten jacobaeus</i> (L.)								+	2									
<i>Parvicardium exiguum</i> (Gmelin)							+		+									
<i>Plagiocardium papillosum</i> (Poli)																	+	
BRYOZOA																		
<i>Aetea truncata</i> (Landsborough)		1	1	2	2	2	4	2	3	2	1	1	+	3	2		3	2
<i>Scruparia ambigua</i> (D'Orbigny)		1		1	1	1	3	1	1	1	1		1	1			2	2
<i>Scruparia chelata</i> (L.)												1						1
ECHINODERMATA																		
<i>Sphaerechinus granularis</i> (Lamarck)														+				
<i>Arbacia lixula</i> (L.)					+													
<i>Psammechinus microtuberculatus</i> (Blainville)							+											
ASCIDIACEA																		
<i>Diplosoma listerianum</i> (Milne-Edwards)	3	4	4	4	3	5	4	2	2	3	2	1	2	1	3	2	1	2
NUMBER OF SPECIES	10	10	14	14	10	25	26	27	26	23	13	17	15	24	22	11	34	35

Interestingly, there are no important differences between the two sides (upper and lower) of the stabilizing disk, which is opposite to what normally happens to horizontal surfaces. On the upper side, wet weight reached 40.9 g/dm², whereas on the lower side it was slightly greater, e.g. 42.4 g/dm². Conversely, dry weight is higher on the upper side, due to a significant presence of bivalves.

Along the buoy body, from the surface down to -11 m, there was a decreasing trend in the wet weight of fouling with a high water content, up to 80% lower in terms of dry weight. From -11 to -20 m this value increased up to a maximum absolute value of 20.8 g/dm², followed by a slight decrease down to -27 m. Between -27 and -34 m wet weight values were variable.

Macrofouling Settled on Panels (1992-1998)

There is a clear change in fouling assemblages settled on panels exposed at two depths and for increasing time of immersion (Figure 7, 8, 9).

On panels immersed from 3 to 70 months at 12 and 33 m depths, 55 taxa, of which 51 species, were identified. The seven species indicated with an * in Table 2 were found only on panels and not in the material collected directly on the buoy.

With the exception of 3 and 9 months periods, the highest number of species was always found at -33 m. At -12 m, 32 species were found, only 4 of which exclusive ones, while at -33 m, 47 species were found, 20 of which exclusive of this depth. Only two species occurred on substrata of 9 months or less, while the number of species increased on panels with more than 24 month exposure; a 51 month panel, offering a nice picture of differentiated settlement, is shown (Figure 4b). An idea of the importance of different settlers can be obtained by examining the covering and numeric index (Table 2).

Settling density over the whole plate is normally very low, as confirmed by the count of serpulids and bivalves. The highest values were 9 ind./dm² for serpulids and 15 ind./dm² for molluscs, while normally they do not exceed 6 ind./dm² or 5 ind./dm², respectively.

If we look at biomass data, there is a clear correlation between biomass increase and immersion period. This correlation however is lost, because some of the settled organisms come off, as has most likely happened to the 70 month panel placed closer to the sea surface. The histogram (Figure 7) shows that biomass is always the greatest on the panels at -33 m, with a maximum of 47.4 g/dm² wet weight on the 70 month panel.

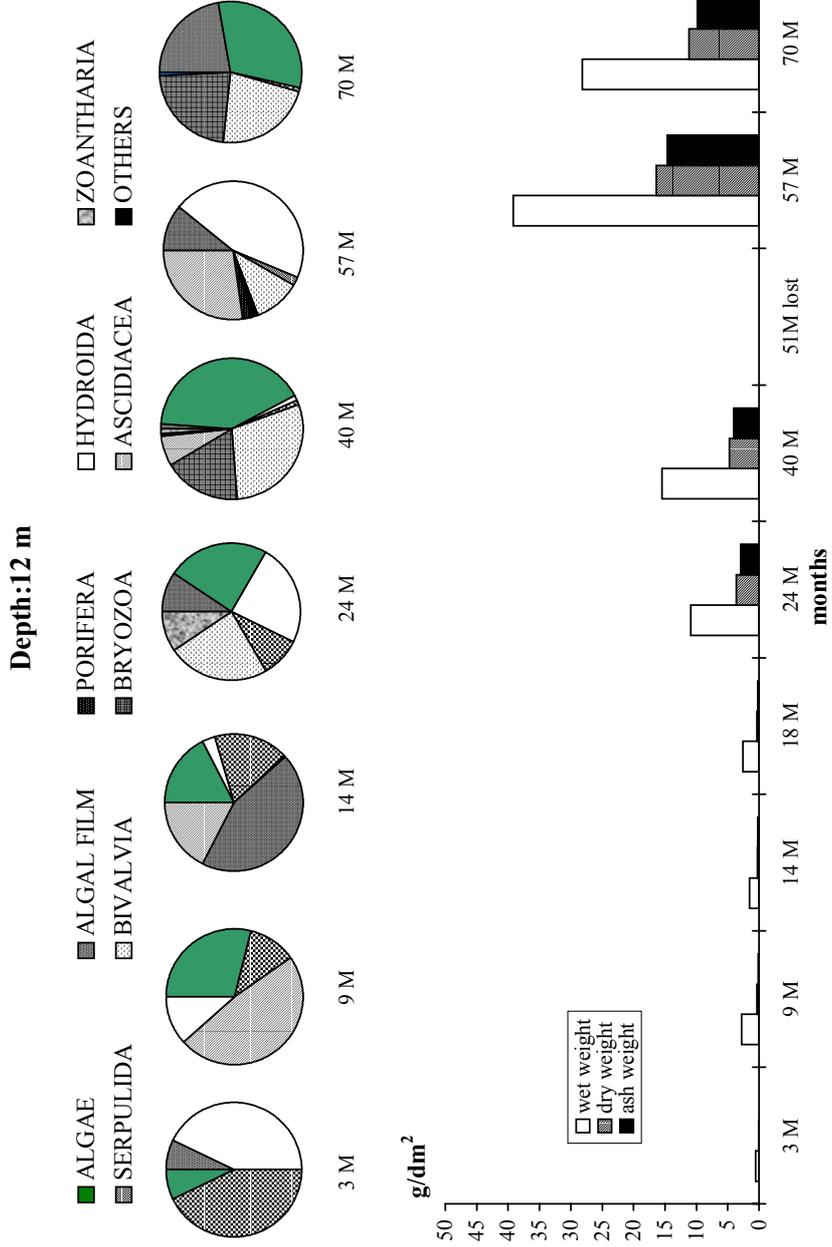


Figure 7 Buoy macrofouling characterization versus time. Upper part of the figure: schematic representation of the main taxa on some of the panels exposed for increasing immersion time (from 3 to 70 months) at 12 m depth. Lower part: wet, dry, ash weights of macrofouling settled on all panels exposed for increasing immersion time (from 3 to 70 months) at 12 m depth.

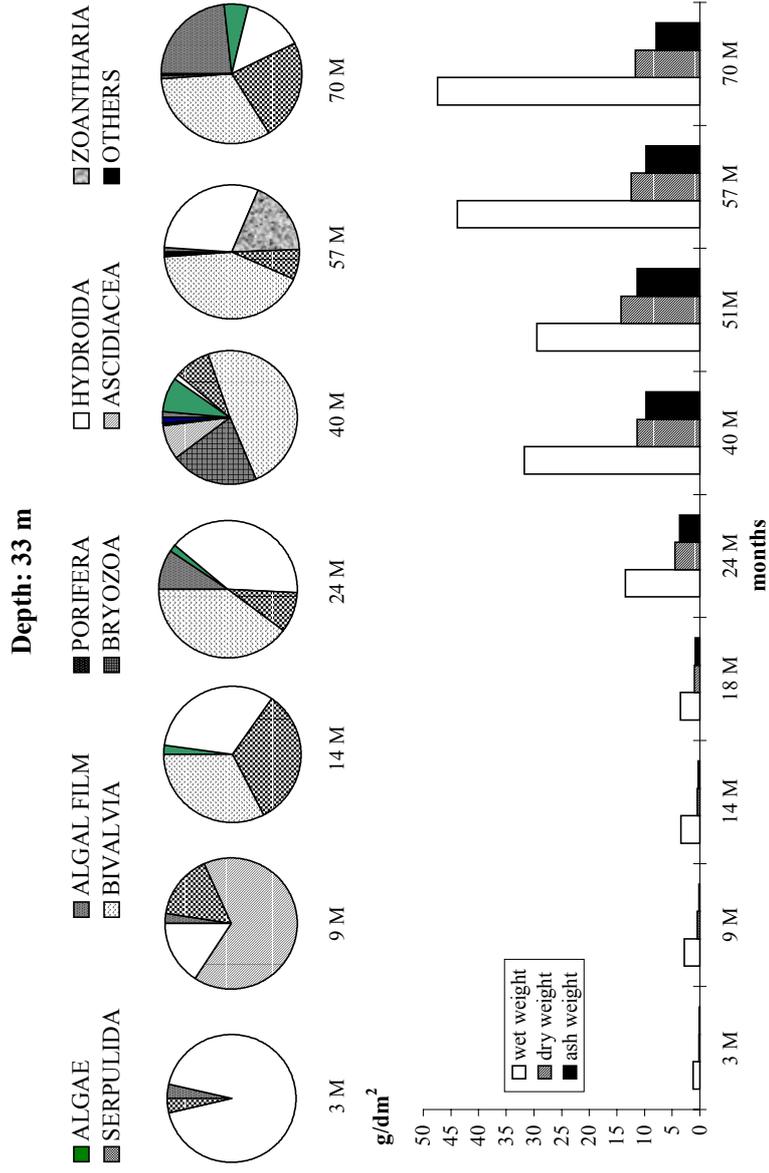


Figure 8 Buoy macrofouling characterization versus time. Upper part of the figure: schematic representation of the main taxa on some of the panels exposed for increasing immersion time (from 3 to 70 months) at 33 m depth. Lower part: wet, dry, ash weights of macrofouling settled on all panels exposed for increasing immersion time (from 3 to 70 months) at 33 m depth.

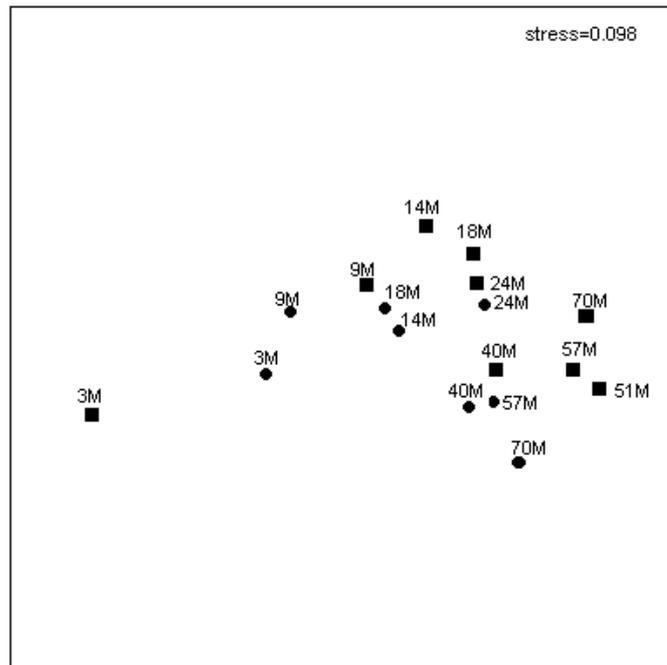


Figure 9 Nonmetric multidimensional scaling plot in two dimensions of the similarity (Bray-Curtis) between macrofouling assemblages settled on panels immersed for increasing periods of time. Circles: panels exposed at -12 m; squares: panels exposed at -33 m.

Evaluation of the Presence of Different Algae Species on the Buoy Body (1995)

In total, 33 taxa, of which 32 at species level were identified, 12 Chlorophyta, 7 Phaeophyta and 13 Rodophyta. The list of algae and depth range are recorded in Table 3. Only brown algae were present up to 30 m depth, while red algae reached a maximum depth of 20 m.

DISCUSSION

This study was carried out on a single large navigational buoy immersed in the Ligurian Sea. As such, we do not attempt to generalise our findings to other sea areas nor to other buoys in the Ligurian Sea, also because fouling could be affected by the different position visavis the main current or frontal region (Figure 1). However, the data set provided is probably the only available for the Mediterranean and also for other European seas based on a long observation time, and in particular on an artificial

Table 2 List of taxa found on panels immersed at two depths (12m and 33m) from 3 to 70 months of exposure. Two indices, one for each side of panels, are recorded. (L: Lost; * : Species found only on panels)

Months of immersion	3		9		14		18		24		40		51		57		70	
Depth (m)	12	33	12	33	12	33	12	33	12	33	12	33	L	33	12	33	12	33
ALGAL FILM	1.1		3.3		3.3			2.2	2.2	2.2	1.1	1.1		+.+	2.2	1.+	4.3	4.3
ALGAE	1.1			1.1	1.2	1.1	2.3		2.3	1.1	5.5	2.2			4.4		5.4	-2
PROTOZOA FORAM.																		
<i>Miniacina miniacea</i>									-.+		+.+	+.+		+.+		+.+	+.+	+.+
Unident.sp.	+.+	+.+	+.+	+.+	+.+	+.+	+.+	+.+	+.+	+.+	+.+	+.+		1.1	+.+	1.1	1.1	+.+
PROTOZOA FOLLIC.												+.+		+.+		+.-	+.+	1.+
PORIFERA																		
<i>Sycon</i> sp.											-1	1.1		1.+	1.2	+1	+1	+.+
HYDROIDA																		
<i>Eudendrium capillare</i>				2.1	1.+	3.3	1.1	2.2	3.3	3.3	1.-			1.+	4.3	4.2		3.2
<i>Clytia hemisphaerica</i> *	2.2	4.3	1.1															
<i>Obelia dichotoma</i>			1.1	1.1														
<i>Obelia bidentata</i>							1.1					1.1		1.1				1.1
<i>Lafoeina tenuis</i>							3.3		2.2	1.-								
Unident.sp.																		+.+
ZOANTHARIA																		
<i>Corynactis viridis</i>							-1	2.-				1.1		1.2	+.-	3.3	+.+	4.5
POLYCHAETA																		
<i>Pomatoceros triqueter</i>	1.1		1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	+.-	1.1		+.+		+1	+.+	+.+
<i>Pomatoceros lamarckii</i>	-1						1.-		1.1	1.1							-.	+.-
<i>Spirobranchus polytrema</i>				1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	2.2		3.2	1.1	1.1	1.1	2.1
<i>Hydroides elegans</i> *		-1		-1		1.1												
<i>Hydroides norvegica</i> *		-1																+.-
<i>Hydroides</i> sp.		-1	1.1	1.1	1.1	1.1	1.1	1.+						+.+		2.1		
<i>Serpula concharum</i>	-1	-.													+.-			1.-
<i>Serpula vermicularis</i>						-.								1.1				+.-
<i>Spirorbis</i> sp.			-1			-1			-1	1.1	1.1	1.1			+.+		+.+	-.
<i>Sabella spallanzani</i>																-2		2.-
<i>Adyte pellucida</i>														+.-				
<i>Marphisa sanguinea</i>														+.-				
<i>Chaetopterus variopedatus</i>														1.-				

Table 2 (cont.)

Months of immersion	3		9		14		18		24		40		51		57		70		
Depth (m)	12	33	12	33	12	33	12	33	12	33	12	33	L	33	12	33	12	33	
POLYCHAETA (cont.)																			
<i>Glycera</i> sp. *														-1					
Nereididae					-.+						1.1	1.1		+-		+-			
PYCNOGONIDA																			
<i>Achelia echinata</i>										-.+		++.		-3		1.1			1.+
<i>Endeis spinosa</i> *							+-				++.	++.							
DECAPODA																			
<i>Pilumnus hirtellus</i>																+-			
AMPHIPODA																			
<i>Caprella andreae</i>							1.1	1.1		++.	1.1	++.			5.5	3.3	4.4		
<i>Jassa marmorata</i>																	3.5	-.+	
GASTROPODA																			
<i>Bittium reticulatum</i>												+-							
<i>Doto</i> sp.															1.1		-.+		
BIVALVIA																			
<i>Mytilus galloprovincialis</i>					+-	++.	+-		1.1		2.1	1.1		2.1	2.3	2.2	3.2		
<i>Anomia ephippium</i>					+-	1.-		1.1	1.1	1.-	-1	1.1							+-
<i>Hiatella rugosa</i>							+-	1.1	1.1			1.1							1.1
<i>Hiatella arctica</i>											1.1			1.1	+-	1.1	++.		
<i>Aequipecten opercularis</i>						1.1		1.1	1.1	1.1				1.-					
<i>Chlamys varia</i>								1.+	1.1	1.1				2.-					
<i>Chlamys multistriata</i>														2.2		1.1			1.1
<i>Chlamys</i> sp.																			+-
<i>Pallium incomparabile</i>							1.-		++.		1.-								
<i>Modiolarca subpicta</i>			-.+			+-		-.+	+-	1.1	1.-	1.1	1.1		1.1	1.1	1.1	2.1	+1
<i>Pteria hirundo</i>														-2	1.1		-2		2.-
<i>Modiolus barbatus</i>															+1	+-	++.	++.	++.
<i>Pododesmus patelliformis</i>															-1	-.+	1.1	-1	-1
<i>Lima hians</i>														+-					
<i>Arca noe</i>															-.+		-1		
<i>Arca tetragona</i>															1.+		1.-		1.1
<i>Neoplicnodonte cochlear</i>															-2		1.-		2.-
<i>Ostrea edulis</i>															-.+				-1
BRYOZOA																			
<i>Scruparia ambigua</i>										-2	2.2	2.2	2.2			1.1	1.+	3.2	2.1
<i>Aetea truncata</i>												3.3	2.2		1.2	1.1	1.+	4.4	2.1
ECHINODERMATA																			
<i>Arbacia lixula</i>																			+-

Table 2 (cont.)

Months of immersion	3		9		14		18		24		40		51		57		70		
Depth (m)	12	33	12	33	12	33	12	33	12	33	12	33	L	33	12	33	12	33	
ASCIDIACEA																			
<i>Styela canopus*</i>						1.-				1.-									
<i>Botryllus schlosseri*</i>											1.-								
<i>Diplosoma listerianum</i>			4.3	4.4	2.2		4.4				2.2	2.2		-1+	3.1				1.-
NUMBER OF SPECIES	4	4	6	6	7	11	10	13	14	15	18	20	-	28	16	22	18	28	

offshore structure moored far from the coast, where the bottom is more than 1000 m deep.

The main characteristic of buoy macrofouling is the absence of acorn barnacles and of some encrusting bryozoans, as well as the irregular settlement and growth of mussels. The latter constitute the main "climax" fouling community in Italian inshore and offshore waters. The presence of *Mytilus galloprovincialis* mussels among the buoy foulers is intriguing, because it seems that new individuals are able to settle only in certain years. Also larvae produced by adult mussels attached to the buoy seem to have been washed away by currents. The complete absence of barnacles, in particular of *Balanus* genus (4-5 species are common foulers) and cheilostomata bryozoans is very strange because some of these species can be found on drifting objects. Furthermore, encrusting organisms never occur in a dense and crowded cover, after a 5-6 year immersion there is still bare surface available for settlement. We are probably far from a stable benthic assemblage, differently from the fish assemblage which is composed by the same species, in spite of seasonal changes. In total, in the buoy macrofouling, including panels, 86 taxa, of which 78 at species level, were identified during 6 years of studies. The species recorded are all animals, while 32 species of macroalgae were listed during a special sampling campaign devoted only to algae study.

Fouling of the different parts of the buoy is quite different also in relation with depth and changes over time. Some differences were also found between macrofouling of panels exposed up to 70 months and the one collected on the buoy when recovered after 52 months of immersion. 51 species were described on the panels, 7 of which were not found in the samples collected on the buoy. This however could just be fortuitous and due in part to the season when the buoy was recovered. On the buoy, 71 species were found. The highest number of settling species and biomass was recorded on the stabilizing disk, which is horizontal to the buoy body. In general, macrofouling consists of a lot of species that are typical of shallow coastal waters, like *Mytilus galloprovincialis*, *Sphaerechinus granularis*, *Arbacia lixula*, *Psammechinus microtuberculatus*, some living in soft bottoms like some pectinidae, while others are common harbour fouling organisms in the Mediterranean Sea (Relini G, 1980).

Table 3 List of algae collected along the buoy body, with depth range

	Depth (m)			
CHLOROPHYTA				
<i>Enteromorpha torta</i> (Mertens) Reinbold				15
<i>Enteromorpha crinita</i> (Roth) J. Ag.		5		
<i>Enteromorpha compressa</i> (L.) Grev.	3	5		20
<i>Enteromorpha</i> sp.	3			
<i>Rhizoclonium</i> sp.			10	20
<i>Chaetomorpha aerea</i> (Good. ex Dill) Kuetz.			10	20
<i>Cladophora dalmatica</i> Kuetz.		5	10	15
<i>Cladophora sericea</i> (Huds.) Kuetz.	3			25
<i>Cladophora albida</i> (Nees) Kuetz.			10	20
<i>Cladophora</i> cfr. <i>albida</i> (Nees) Kuetz.				20
<i>Cladophora liniformis</i> Kuetz.	3	5	10	20
<i>Cladophora socialis</i> Kuetz.				20
<i>Cladophora</i> cf. <i>socialis</i> Kuetz.				20
<i>Cladophora</i> sp.				20
<i>Ullothrix flacca</i> (Dill.) Thuret				15
PHAEOPHYTA				
<i>Feldmania globifera</i> (Kuetz.) Hamel	3	5	10	15
<i>Feldmania</i> sp.	3	5		15
<i>Sphacelaria cirrosa</i> (Roth) C. Ag.	3	5	10	15
<i>Sphacelaria tribuloides</i> Kuetz.		5		30
<i>Sphacelaria</i> sp.				
<i>Dictyopteris membranacea</i> (Stackh.) Batters				30
<i>Myriactula rivulariae</i> (Suhr) Feldm.			10	
<i>Acinetospora crinita</i> (Charm. ex Harv.) Sauv.			10	
<i>Ectocarpus confervoides</i> (Roth) Kjell.			10	15
				30
RHODOPHYTA				
<i>Ceramium fastigiatum</i> (Roth) Harv.	3	5		
<i>Ceramium circinatum</i> (Kuetz.) J. Ag.		5		
<i>Ceramium codii</i> (Rich.) Feld.-Mazoyer		5		
<i>Ceramium diaphanum</i> (Lightf.) Roth		5		
<i>Dasya rigidula</i> (Kuetz.) Ardiss.	3	5		
<i>Bangia atropurpurea</i> (Roth) C. Ag.	3			
<i>Aglaothamnion bipinnatum</i> (Cr. & Cr.) Feld.-Mazoyer	3			
<i>Polysiphonia elongella</i> Harv.	3		10	15
<i>Polysiphonia denudata</i> (Dill.) Grev.		5		20
<i>Polysiphonia elongata</i> (Huds.) Spreng.			10	15
<i>Polysiphonia furcellata</i> (C. Ag.) Harv.			10	
<i>Polysiphonia</i> sp.			10	
<i>Polysiphonia atlantica</i>				15
<i>Lophosiphonia subadunca</i> (Kuetz.) Falkenb.			10	

Though the panels were recovered in different seasons, the difference found in the plate assemblages after a 24 month immersion is partly due to seasonal variation of settlers, because the community is mainly composed of long living species (more than 1-2 years).

The settling community composition and structure, as is well known, depends on the arrival of larvae, on recruitment and settlement success, and also on transport mechanisms to the site of investigations (Butman, 1987; Pechenik, 1990) in the form of larvae, adults, or other resistance forms such as cysts and propagules.

Giangrande *et al.* (1994) have examined different larvae strategies and life-cycles in the dispersal of marine invertebrates. They have observed that the length of pelagic existence increases from lecithotrophic to teleplanic larvae with subsequent dispersal

capacity increase. Free spawning with planktotrophic development seems to be the most widely distributed strategy in marine invertebrates with a known life-cycle. The length of larvae life for most species is extremely flexible (Scheltema, 1992). Some larvae also have the capacity to delay metamorphosis if they do not find suitable substrata, and this delay can last from a few days up to several months (Pechenik, 1985; 1990).

However, it should be pointed out that dispersal mechanisms of these organisms are not only put in place by larvae themselves. There are also other mechanisms having an impact on larvae dispersal, such as human activities, like shipping and the transport of adult individuals on floating objects, pieces of plastic, wood, and mainly algae (Highsmith, 1985), helped by currents.

Currents, in our case, have a double function: although the buoy is exposed to the current from Corsica, the strong parallel current from the Ligurian coast (Figure 1) exercises a barrier effect thus preventing organisms from the Ligurian coast from reaching this study station. Hence, it has been assumed that the places of origin of larvae are selected by currents. The role of water currents in the transport of benthic invertebrates was described in the Corsica Channel by Aliani & Meloni (1996) who studied fouling on oceanographic devices.

In conclusion, it can be said that the ODAS Italia 1 buoy, being a solid substrate in an offshore environment comparable to a “miniature island”, is an aggregation point for a peculiar community which ranges from pelagic fish species, reacting to the FAD effect of the structure (Relini M *et al.*, 1994; Relini G *et al.*, 2000), to benthic organisms characterized by their remarkable specific richness and reaching a high settlement rate in a short period of time, though they have not yet reached a stable stage. Therefore, the buoy is an extremely interesting site, not only for studies on fouling but also to investigate other issues like colonization and dispersal of marine organisms, and changes in biodiversity in this peculiar environment.

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Effects of Fouling Organisms on the Water Quality of a Nuclear Power Plant Cooling System

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ABSTRACT

Intake tunnels carrying seawater for cooling purposes are prone to infestation by fouling organisms. The Madras Atomic Power Station (MAPS) draws seawater through a sub-seabed tunnel for extracting heat from condensers and for other auxiliary cooling systems. The impact of fouling organisms inside the tunnel on water quality was studied. Results showed that activity of biofouling organisms cause changes in cooling water characteristics including an increase in pH, suspended matter, ammonia content, chlorine demand and depletion of dissolved oxygen and chlorophyll 'a' content. Increase in DO consumption, ammonia production, chlorophyll reduction and chlorine demand was found to be correlated with the amount of flow of cooling water. During a low flow / stagnation condition it was observed that DO content at the bottom of the tunnel was reduced to a very low level. It is proposed that this anaerobic condition could be used as an alternative to chlorination and heat treatment for biofouling control.

INTRODUCTION

Power stations are frequently located near coastal sites to make use of seawater for cooling purpose. In India power plants draw seawater through an open canal, a pipe line or a tunnel and these are generally infested with marine organisms, despite the use of certain control measures. Biofouling in the cooling system of seawater cooled power plants is a universal problem (Brankevich *et al.*, 1988; Chadwick *et al.*, 1950; Collins, 1964; Dobson, 1946; Holes, 1967; James, 1967; Relin, 1984; Satpathy, 1990) and can impose penalties on power production, impair the integrity of the cooling system components and even precipitate safety problems in nuclear power plants (Henager *et*

al., 1985; Imbro & Gianelli, 1982; Rains *et al.*, 1984). Different aspects of biofouling in the cooling conduits of coastal water power plants, both from tropical and temperate regions, have been studied by several authors (Brankevich *et al.*, 1988; Chadwick *et al.*, 1950; Collins, 1964; Dobson, 1946; Holesms, 1967; James, 1967; Rajgopal, 1991; Relin, 1984; Sasikumar, 1991; Satpathy, 1990). However, a few studies have investigated the effects of fouling organisms inside a cooling system on cooling water quality. (Venugopalan & Nair, 1990). This paper reports on a study of the effects of presence of biofouling community on cooling water quality of a coastal power plant.

The Madras Atomic Power Station (MAPS), draws seawater for cooling purposes through a sub-seabed tunnel. Biofouling has been a serious problem in the MAPS cooling system, which has adversely affected the performance of the plant (Nair, 1990; Rajgopal, 1991; Sasikumar, 1991; Satpathy *et al.*, 1994). Investigations have indicated that extensive fouling inside the tunnel can cause severe pressure drops in the cooling circuits (Nair *et al.*, 1988; Rajgopal *et al.*, 1991a;). Similarly, preliminary post operational studies in the coastal water adjoining the power plant revealed a marginal increase in suspended matter and wide fluctuations in nutrient contents as compared to the pre-operational period (Nair, 1985; Satpathy & Nair, 1990, Satpathy, 1996). Moreover, a recent study of the biology of this tunnel showed an increase in the reduction of diatom, chlorophyll and DO content in the tunnel at increasing volume of water flow (Venugopalan & Nair, 1990).

Though, various kinds of barriers, like trash racks and traveling screens, are provided to prevent the entry of large marine life, larvae of organisms such as barnacles and mussels can enter the tunnel, settle and grow. Growth rates of organisms inside the tunnel have been found to be higher than that in the natural habitat immediately outside the tunnel (Rajgopal *et al.*, 1991). The biomass of fouling in the tunnel has been estimated to be 580 tones (Nair *et al.*; 1988). In the present work, changes in seawater quality, (including pH, dissolved oxygen, suspended matter, ammonia, chlorine consumption and chlorophyll content), between the intake and forebay were studied over a period of one year.

MATERIALS AND METHODS

The Madras Atomic Power Station (MAPS), a pressurized heavy water reactor (PHWR) consisting of two units, each of 235 MW(e) capacity, is situated at Kalpakkam (12° 33'N, 80° 11'E), about 65 km south of the city of Madras, on the east coast of India. Seawater at a rate of 35 m³/sec is used as a medium for cooling the condenser and the process cooling water systems (Satpathy, 1992). Seawater is drawn through a sub-seabed tunnel 468 m long and 3.8 m in diameter, built 53 m below the seabed (Fig. 1). The tunnel is connected at the landward end to the pump house through a vertical shaft called the forebay. Similarly, at the seaward end it is connected through a vertical shaft

called the intake. Seawater enters the intake through 16 windows located radially on the intake and 1 m below the level of the lowest low water tides. The cooling water circuits of MAPS are described in more details elsewhere (Satpathy, 1996a).

Surface seawater samples were collected from the intake and forebay at fortnightly intervals (when different flow rates were existing) for a period of one year and analyzed in duplicate for the following parameters: pH, DO, suspended matter (SM), ammonia, chlorine demand and chlorophyll 'a'. The relationship between cooling water flow and amount of dissolved oxygen depletion, chlorine consumption, suspended matter increase, chlorophyll reduction and ammonia increase was analyzed by using linear regression. The mean values of various parameters were compared by using a t-test (Scalzo *et al*, 1978). The reduction and increase in different parameters were calculated by multiplying the flow with the difference between the intake and the forebay values. Regression plots between different flow rates and the increase or decrease in different parameters were thus made.

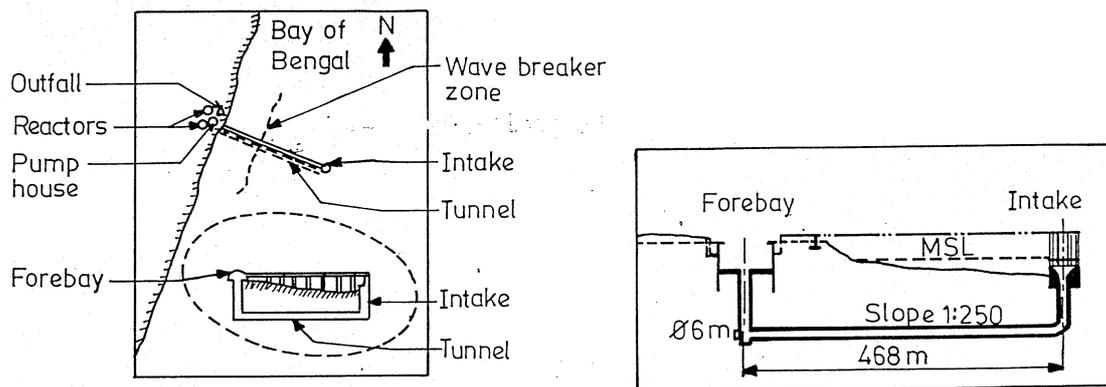


Figure 1 Schematic diagram showing the intake and the forebay of the MAPS cooling conduits.

pH was measured by using a calibrated pH meter (KENT, EIL 3055 model). For suspended matter estimation, 500 ml of seawater was filtered through a 0.45 μ previously weighed Millipore paper. The paper was then dried at 80 °C to a constant weight and re-weighed, with the difference in weights taken as the weight of the suspended matter and converted to mg/l. For DO determination, samples were treated with manganese sulfate, potassium hydroxide and potassium iodide and subsequently acidified to release iodine (Parsons *et al.*, 1984). The released iodine, which is stoichiometrically equivalent to the dissolved oxygen in the sample, was then titrated against standard sodium thiosulphate using starch as the indicator. For ammonia estimation, the sample was allowed to react with alkaline phenol and hypochlorite to form indophenol blue, which is quantitatively proportional to the ammonia concentration in the sample. The blue colour formed was intensified by the

addition of sodium nitroprusside, and the absorbance measured at $\lambda=600$ nm. For chlorophyll estimation, 500 ml of seawater was filtered through a 0.45μ Millipore membrane filter paper. The filter paper was put in a dark test tube (wrapped with aluminium foil) containing 10 ml of 90 % acetone and kept overnight in a refrigerator. The sample was then centrifuged (3000 rpm for 15 min) and the absorbance was measured at $\lambda=750, 665, 647$ & 630 nm by using a double beam Chemito UV 2500 spectrophotometer. The amount of chlorophyll 'a' in the sample was calculated by following the procedure of Parsons *et al.* (1984):

$$\text{Chl 'a'} = 11.85 E_{665} - 1.54 E_{647} - 0.08 E_{630}$$

where E is the corrected absorbance value.

For chlorine demand, one-litre samples from both the intake and forebay were chlorinated in the laboratory with NaOCl stock solution to yield an initial concentration of 1 mg/l of chlorine. Total residual chlorine was estimated after 5 minutes following the Diethyl Paraphenyldiamine (DPD) method (White 1972).

During another inspection of the tunnel (spanned only for 7 days), on one occasion a very low flow ($2.7 \text{ m}^3/\text{s}$ as compared to a normal flow of $35 \text{ m}^3/\text{sec}$) was maintained inside the tunnel, and on another all the pumps were shutdown for about 8h resulting in total stagnation. Advantage was taken of these opportunities to collect samples at different depths to study the impact of the fouling community on DO & SM during stagnation and low flow. Samples were collected at the surface, 5, 10, 20, 30, 40 & 50 m in the forebay using Nansen plastic reversing water bottles (Hydrobios, Germany). Surface samples, were collected immediately after the stoppage of pumps and only DO and SM were analysed on these two occasions.

RESULTS

Changes between intake and forebay

DO (Fig.2A) and chlorophyll 'a' (Fig. 5A), levels decreased from the intake to the forebay whereas pH (Fig.3A), suspended matter (Fig. 3B), ammonia (Fig.4A) and chlorine demand (Fig.6A) increased (Table -I). A comparison of mean values of various parameters between intake and forebay indicated that the variations in SM, chlorophyll 'a' and chlorine demand were significant (Table-1). However, the variations in pH, ammonia and DO were significant only at the level of 10%. When change in various parameters was correlated with the amount of water flow the following trend emerged. An increase in SM was generally associated with higher flow rate; however, there was no definite correlation between the two. Thus the values are not reported for different flow rates. Values for oxygen depletion (Fig.2B), chlorophyll 'a' reduction (Fig.5B),

chlorine demand (Fig.6B) and ammonia increase (Fig.4B), increased with flow. Regression analyses showed that oxygen depletion ($r=0.887$, $p<0.01$), chlorine demand ($r=0.819$, $p<0.01$) and ammonia ($r=0.767$, $p<0.01$) increase, were reasonably correlated with flow rate, whereas chlorophyll 'a' reduction showed a poor correlation ($r=0.381$, $p<0.05$). A close comparison of chlorine demand data for intake and forebay water over the whole year (Fig. 6A) shows that the values were always higher in the forebay than in the intake water reflecting higher organic content in the forebay water than the intake water, which could possibly be generated by the large congregation of marine organisms inside the tunnel.

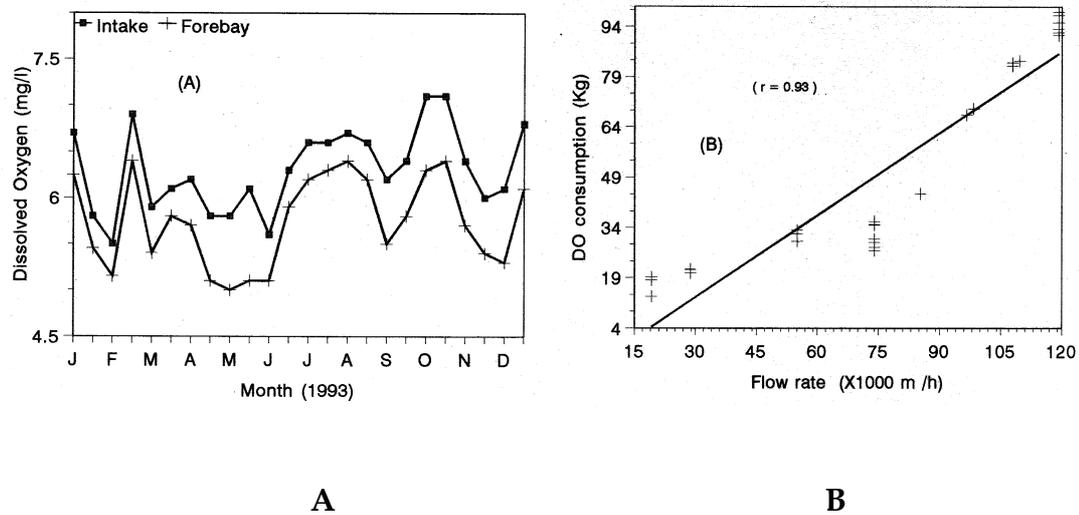


Figure 2 Variations in DO content between the intake & forebay (A) and its variation at different flow rates (B).

Table 1 Cooling water characteristics measured in the intake and forebay of the MAPS cooling system.

Parameters	Intake			Forebay			t-Values
	Range	Mean	SD	Range	Mean	SD	
pH	7.70 - 8.30	8.08	0.19	7.85 - 8.35	8.15	0.14	1.668**
Ammonia ($\mu\text{g.N/l}$)	0.35 - 9.92	2.81	2.11	1.14 - 12.0	4.0	2.39	1.757**
Chlorophyll "a" ($\mu\text{g/l}$)	1.2 - 15.3	8.08	3.87	0.57 - 12.9	4.68	3.02	3.899*
Chlorine demand (mg/l)	0.4 - 0.9	0.6	0.2	0.7 - 1.0	0.9	0.1	7.771*
Suspended matter (mg/l)	21.6 - 46.7	32.2	7.2	26.8 - 61.8	39.4	8.4	3.478*
DO (mg/l)	5.5 - 7.1	6.0	0.9	5.0 - 6.4	5.5	0.9	1.978*

Significance level: ** = $p<0.1$, * = $p<0.05$

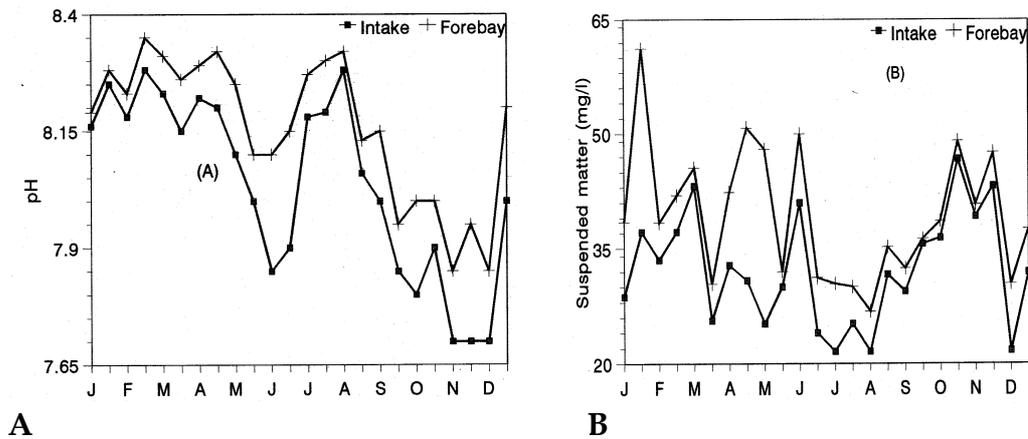


Figure 3 Variations in pH (A) and suspended matter content (B) between the intake and forebay.

Changes during low flow and stagnation

DO values on the first and second days showed a decrease with depth (Fig. 7A). At 50 m the DO values were 3.1 and 1.9 mg/l for 1st and 2nd day respectively. These are low values when compared to levels observed in coastal waters at this site. Even the surface water values at the forebay were lower (by 0.5 to 1.5 mg/l) than those of coastal waters. The reduction of DO at 50 m is attributed to the consumption of oxygen by fouling communities in the sub-seabed tunnel. DO levels on the 2nd day showed an even a greater decrease with depth than on the first day from 5.1 mg/l at the surface to 1.9 mg/l at 50 m.

Suspended matter values on both days showed an increase with depth (Fig. 7B). Values obtained at forebay were much higher at all depths than those obtained during this period for coastal waters. Even the surface values observed in the forebay water during normal period were higher than the values generally encountered in the coastal waters (Fig. 3B).

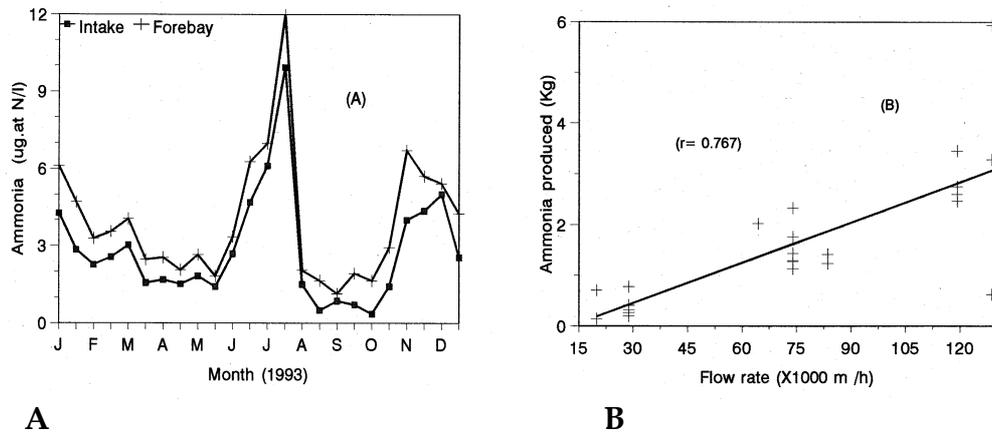


Figure 4 Variations in ammonia content between the intake & forebay (A) and its variation at different flow rates (B)

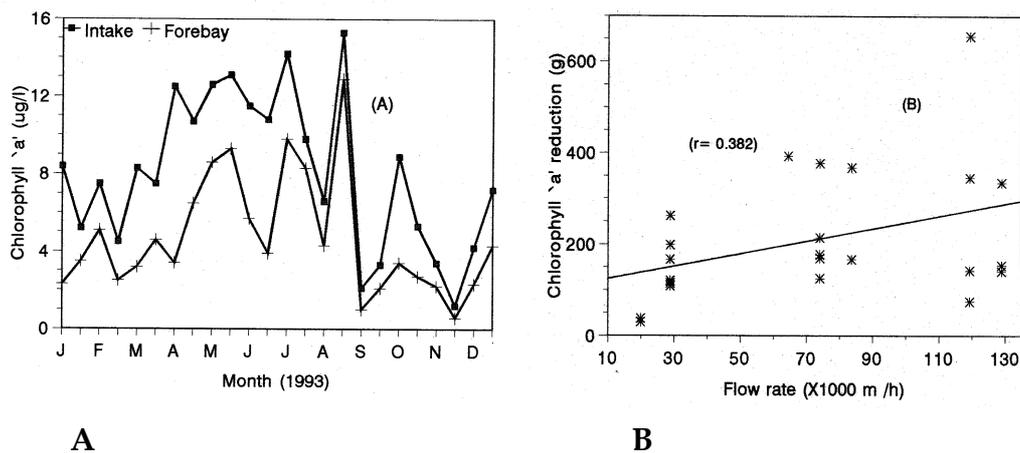


Figure 5 Variations in Chlorophyll 'a' content between the intake and forebay (A) and its variations at different flow rates (B).

DISCUSSION

The subsea-bed tunnel of MAPS nurtures a well-established fouling community, dominated by the green mussel *Perna viridis* [Rajgopal, 1991] and barnacles [Sasikumar, 1991]. The fouling biomass within the tunnel has been estimated to be about 580 tonnes [Nair *et al*, 1988] and the biomass of green mussel alone contributed 410 tonnes [Nair, 1990]. Biological processes such as consumption of oxygen and detritus from the water, and release of faeces, pseudofaeces and ammonia, would result in significant changes

in water quality. These changes, in turn, have adverse implications, for the operation of power plant. Mussels the dominant species inside the tunnel are known to be the most actively metabolizing species among various marine organisms (Nixon *et al*, 1971). Continuous flow of relatively high volumes of seawater inside the tunnel provide a continuous supply of oxygen and food to these organisms and water flow also removes the metabolic wastes produced by the organisms. Enhanced growth at higher flow rates have been reported by Jenner (1980) Perkins (1974), and Rajagopal (1991).

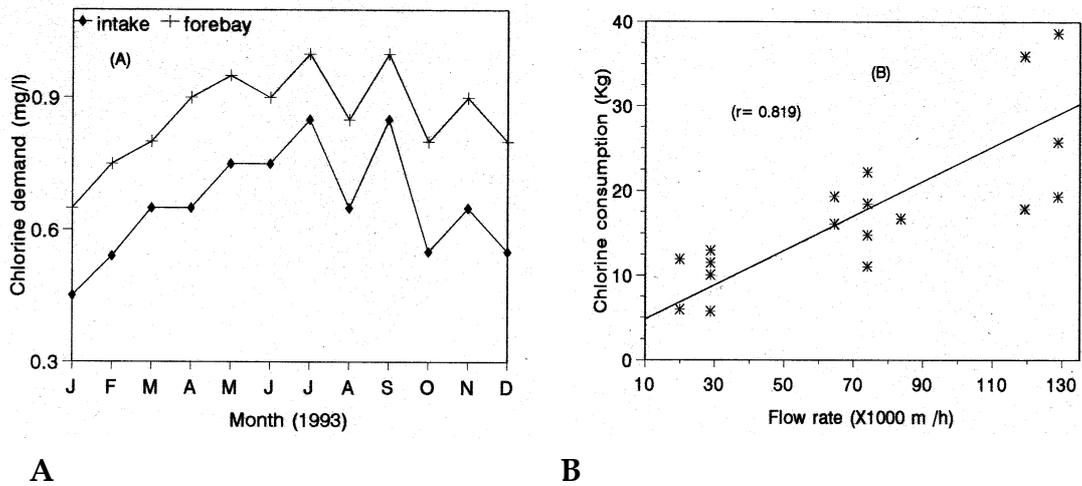


Figure 6 Variations in chlorine demand between the intake & forebay (A) and its variation at different flow rates (B).

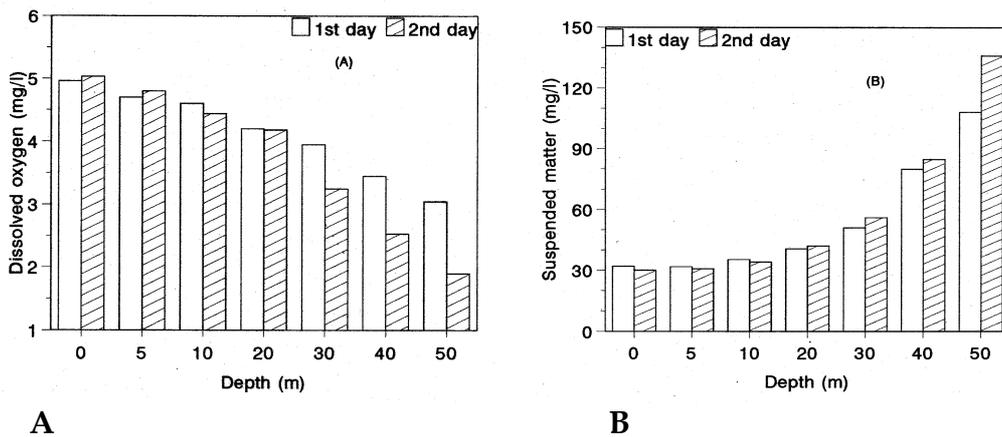


Figure 7 Variation in DO (A) and suspended matter (B) content in the forebay during low flow and shutdown period.

In the present study regression analyses showed that ammonia increase, DO depletion, and increase in chlorine demand were significantly correlated with flow. Increased oxygen reduction at high flow rates possibly reflects increased metabolism and activity of the fouling organisms. At low flow velocities reduced food and oxygen supply may slow down the metabolic process as well as removal of metabolic wastes.

The changes in the water quality as the water passes through the tunnel have been found to have some impact on the operation of the power plant. For example the suspended matter generated in the tunnel probably explains the observed high siltation rate in the pump chambers in the forebay causing problems for smooth operation of pumps. It is assumed that organisms settled inside the tunnel aggregate the smaller particles into larger particles during the filtration process, as a result of which these heavier particles settle down easily at the bottom of the forebay. In fact the chambers have to be desilted at very frequent intervals. In addition to siltation problems, suspended matter may get trapped in the biofilm formed inside the condenser tubes resulting in heat transfer losses (Rippon, 1979). There have been reports of increase in back pressure and loss of vacuum in the condenser tubes of MAPS and increased level of suspended matter in the forebay water may possibly enhance such event. Similarly it is reported that suspended matter provides a large area to the bacteria and fungi for their growth thereby enhancing the possibility of biocorrosion in the cooling system (Venkataswarlu & Sriraman, 1985; Bhosle *et al.*, 1990).

All organisms produce reduced nitrogen compounds as by-products of metabolism and they are eliminated by excretion (Chen *et al.*, 1994). In most marine invertebrates the majority of the nitrogen is excreted as ammonia (Clarke *et al.*, 1994). In the present study it was found that the ammonia level in the seawater increased between the intake and the forebay. This ammonia could reduce the efficiency of chlorination by reacting with hypochlorous acid (HOCl) to produce chloramines. These chloramines are known to have relatively low biocidal efficiency, 150 to 200 times less than free chlorine (Venkateswarlu, 1990). Furthermore, ammonia is known to accelerate the corrosion rate of copper-based alloys which are extensively used as condenser tubes in seawater cooled power plants in India (Venkateswarlu, 1990). In one plant, a high level of ammonia attributed by bacterial growth inside the condenser cooling section was considered responsible for the corrosion of condenser tubes which resulted in heavy leakage of cooling water into the boiler feed water (Satpathy *et al.*, 1997). A concentration of only 1 mg/l of ammonia is considered sufficient to induce stress corrosion cracking (SCC) in copper based alloys (Behrens, 1988). Although corrosion problems have not been a major problem in MAPS the possibility of such failure exists. The increase in pH from intake to forebay is apparently due to the production of ammonia by the micro and macro organism. A substantial increase in chlorine demand has been observed from intake to forebay. Presence of enhanced SM content and organic matter in the cooling conduit apparently explains this increased chlorine

demand. On ignition (at 400°C) of the silt obtained from the pump chamber, 30 % loss in weight was observed indicating relatively high organic content in the silt.

Reduction in oxygen from intake to forebay and its reduction to very low levels at times of low flow rates could be of significance to power plant operation. Chlorination or heat treatment the two most common fouling control measures pose many difficulties, both from an operational and an environmental point of view. In this context depletion of oxygen in a cooling water system as an antifouling strategy assumes importance. Little & Depalma (1988) have reported that depletion of oxygen in the cooling system, infested with biofoulants, particularly during stagnation could be used as a method to combat biofouling. A few hours of stagnation, possibly 10 to 12, hours may totally deplete oxygen inside the cooling system, thereby killing the organisms. In the present study, an 8 h period of stagnation reduced DO to 1.9 mg/l and a few more hours of stagnation could bring DO levels down sufficiently to kill the fouling organisms. However, this approach has to be implemented with caution, as this anaerobic condition may be conducive for the prolific growth of anaerobic bacteria such as sulphate reducing bacteria (SRB) which can promote biocorrosion.

CONCLUSION

The study found that biological processes such as feeding and excretion by marine organisms inside the tunnel altered the quality of cooling water (increase in SM, Chlorine demand and ammonia) both chemically and biologically as it passed through the tunnel, despite the time of contact being relatively short (5 to 40 minutes). And these changes affect the plant operation in various ways. However, additional studies are necessary to prove the hypothesis concluded here .

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4. Prevention of Fouling

Controlling Biofouling on Ferry Hulls with Copper-Nickel Sheathing
Leslie H. Boulton, Carol A. Powell and W. Bruce Hudson

Antifouling from Nature: Laboratory Test with *Balanus amphitrite* Darwin on Algae and Sponges
Sebastiano Geraci, Marco Faimali, Louis Piovetti and Guido Cimino

Electromagnetic Antifouling Shield (EMAS) - A Promising Novel Antifouling Technique for Optical Systems
Thomas Leya, Annette Rother, Torsten Müller, Günter Fuhr, Martina Gropius and Burkhard Watermann

Properties of a Titanium Nitride Electrode and its Application for Electrochemical Prevention of Marine Biofouling
Tsuruo Nakayama, Hitoshi Wake, Mina Okochi, Kinichi Ozawa and Tadashi Matsunaga

Electrochemical Prevention of Diatom Adhesion and Direct Estimation of Diatom Viability using TO-PRO-1 Iodide
Mina Okochi, Tomoyuki Taguchi, Makoto Tsuboi, Reiko Sato and Tadashi Matsunaga

Development of a New Antifouling Paint Based on a Novel Zinc Acrylate Copolymer
Yoichi Taki, Yoichi Yonehara, Fumihiko Nakakita and Mahamedi H. Jadhwal

Controlling Biofouling on Ferry Hulls with Copper-Nickel Sheathing

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ABSTRACT

Copper-nickels are alloys which possess a combination of excellent corrosion resistance and a high natural resistance to biofouling in seawater. They have a proven performance record over many years in applications such as seawater piping, intake screens, water boxes, and for cladding of offshore structures. The property combination also makes copper-nickel an attractive material for boat hulls, either as the hull material itself or applied as a sheathing. A more recent innovation has been the application of 90-10 copper-nickel sheathing as an adhesive-backed thin foil.

This paper principally describes trials and evaluation of 90-10 copper-nickel sheathing on the hulls of two commercial passenger ferries, in service on the Auckland harbour, Auckland, New Zealand. One vessel is a slow ferry (10 knots), constructed of fibreglass reinforced polymer (FRP), which was retrofitted with copper-nickel sheathing in 1993. The other vessel is a fast catamaran ferry (22 knots) with a FRP hull, which was sheathed during construction in 1994. The older monohull vessel MV *Koru* is kept in reserve most of the time, whereas the catamaran MV *Osprey* has been in service for about 30,000 nautical miles since construction.

In addition, biofouling results of parallel testing programmes exposing test panels in harbour environments in New Zealand, Singapore and the United Kingdom are described.

INTRODUCTION

Marine biofouling is commonplace on immersed marine structures, including the hulls of vessels such as harbour ferries, which are in routine commercial service. Regular removal of biofouling is an operational requirement on passenger ferries, otherwise the vessels suffer frictional drag problems during service and fuel consumption rises proportionately. The cost associated with removal of macrofouling from a ship's hull can be high, particularly when a fleet of ferries requires regular dry-docking for maintenance. Even when antifouling paints are employed routinely on the hulls, slipping the vessels for hull cleaning and re-painting is a regular operational requirement.

The hull cleaning process usually involves mechanical scraping and pressure water blasting to remove tightly adherent macrofouling, such as barnacles. The employment of manual labour to carry out the cleaning operation, substantial periods of unproductive downtime for cleaning, and the application of expensive antifouling coating systems to a ship's hull, add substantial costs to the total operational expenditure. Maintenance of cathodic protection (CP) systems for corrosion control is also a frequent expense on the hull. In addition, antifouling paints often contain toxic chemicals, which may be considered environmentally unfriendly, due to overspray during application and leaching into the seawater during service. A system to eliminate or significantly reduce such processes clearly offers significant advantages.

90-10 copper-nickel (UNS C70600) has been commercially available in many product forms and has been used for many applications related to seawater since the 1950's. The alloy has excellent resistance to corrosion and has a high inherent resistance to biofouling. It was first used as a boat hull material in 1971 and a survey in 1994 of vessels built since that time showed good performance (Powell, 1994a).

Observations of the biofouling resistance of the 90-10 copper-nickel alloy under normal exposure conditions on vessels and offshore platforms suggest that slime (microfouling) does not build up sufficiently to allow macrofouling to become established. Under quiescent or stagnant conditions macrofouling can eventually occur (Efird, 1975), but larger marine growths will periodically slough off during service as seawater flow conditions are established. In addition, biofouling which does accumulate on a hull, can be easily removed by wiping, or with a gentle scraping action. For optimum fouling resistance, 90-10 copper-nickel must be freely exposed and not subject to galvanic contact with less noble metals, such as zinc anodes, or skin fittings. Thus, cathodic protection (CP) can significantly reduce the biofouling resistance of the 90-10 copper-nickel and the alloy must be electrically isolated from any on-hull CP system (Powell, 1994b).

About 20 years ago, Mitchell¹ began to develop a proprietary adhesive-backed copper nickel foil in the United Kingdom for application to wood, glass-fibre and steel boats. This was applied to about forty boats and, when correctly applied, the performance was very encouraging. In 1992, work began to develop the process further in New Zealand. Copper-nickel foil has now been applied to a medium size pleasure craft and two commercial fleet ferries, by Copper Mariner Ltd, enabling the observation of more exacting service conditions and allowing a more detailed evaluation to be documented, than had previously occurred.

In addition, parallel trials have been performed using test panels prepared using the copper-nickel cladding immersed in seawater at Singapore Harbour (1 year), Langstone Harbour, UK (3 years), and in the Auckland Harbour, New Zealand (5 years). The ongoing seawater tests in Langstone and Auckland Harbours are to assess the 90-10 copper-nickel cladding performance on materials such as steel and aluminium alloys, as well as on FRP and painted wood.

The information provided by this paper documents the outcomes of the above trials. The results presented combine the experience of Copper Mariner Ltd who produced and applied the sheathing, Fullers Auckland the ferry operator, investigations by Mr V. Linkhorn a consultant to Copper Mariner Ltd, and visual inspections by the Nickel Development Institute.

EVALUATION OF 90-10 COPPER-NICKEL SHEATHING

The Sheathing and Evaluation Programme

The foil *Mitchell Mariner 706 System* involves the application of adhesive-backed panels (approximately 210mm x 500mm) to the prepared hull, allowing about 15mm overlap and, normally, positioned in the direction of the water flow over the hull. The copper-nickel foil thickness chosen depends upon the severity of service and is about 0.15mm thick or greater. The panels are easily cut and manipulated even over the most difficult contours.

Currently the system is readily applied by hand, but it lends itself to automation if large vessels are considered. The bonding system acts as a barrier and as an insulator, which protects the hull from the corrosive actions of seawater on its own. An advantage of the system has been that if impact occurs and some panels are damaged, the repair process is simple and quick to perform in order to repair the sheathing. The system can be applied to hulls on new vessels and as a retrofit.

¹ F. J. Mitchell, *Mitchell Mariner 706 System*, UK, (1989).

An evaluation programme on the installation and performance of the foil sheathing commenced on three vessels in August 1993 in Auckland. The second vessel, the yacht *Intrepid*, was sheathed in 1994. However, several months after its launch the yacht was sailed into the Pacific Ocean and its present whereabouts and condition are not known. This paper therefore concentrates on the first and third boats to be sheathed, the *Koru* and the *Osprey*; both of which are still in service in Auckland. In addition, three test programmes involving assessment of immersed panels were initiated at that time.

MV Koru

An old 48 tonne motor vessel, the ferry MV *Koru* (Figure 1) had the FRP hull sheathed with the *Mariner 706* copper-nickel foil system in 1993. *Koru* is 17 metres long and 5 metres in the beam. *Koru* is a slow ferry, which travels at about 10 knots. It is held mostly in reserve for emergencies. The fouling and corrosion performance of the sheathed hull was monitored by inspection at dry-docking intervals during the following year.



Figure 1 *MV Koru*, at berth, in the Waitemata Harbour, Auckland, 1998



Figure 2 Water blasting of biofouling off the 90-10 copper-nickel hull sheathing of FRP ferry *MV Koru* on the slipway at Auckland, 1998, after a full year's service in Auckland Harbour.

Several problems occurred due to galvanic action between the new hull protection system and some existing hull fittings. The nature of these problems were as follows:

A 70-30 copper-nickel solid rubbing strip had been installed along the keel, which inadvertently made electrical contact with some adjacent 90-10 hull panels. Unexpectedly, this design promoted sufficient galvanic corrosion of several 90-10 panels to corrode their surfaces. The solution chosen to overcome the galvanic effect was to replace the keel shoe with a full length FRP rubbing strip up to the waterline at

the bow. Several 90-10 foil panels had to be replaced adjacent to the keel and this was readily achieved. The replacement panels remained in good order.

Fasteners holding the copper-nickel keel shoe also corroded. These were found to be silicon bronze fasteners, a slightly more anodic material than 70-30 copper-nickel. The silicon bronze was replaced with type 316 stainless steel, which is a more noble alloy.

The rudder shoe and bearing attachments were made from manganese bronze and were showing signs of dezincification, as was the propeller. Cathodic protection had been removed to achieve the optimum biofouling of the copper-nickel and thus these components were no longer protected. Zinc anodes were then installed on the rudder shoe in such a way that they were electrically insulated from the copper-nickel panels on the hull.

In the second stage of the project the above factors were all taken into consideration and modifications were carried out to the sheathing system during dry-docking periods.

Green slime formation was generally slight to medium in severity. This tended to be heavier on sides exposed to sunlight when moored. Some patches of small barnacles had formed, which seemed to have occurred preferentially on the adhesive at the overlap of the panels. This emphasised the importance of minimising this gap during application. Barnacles on the copper-nickel were easily removed by finger pressure.

More recent inspections of the *Koru's* hull during dry docking (1995-1998) have revealed that the early (1993-1995) problems encountered with hull sheathing have been overcome. Consecutive inspections of the *Koru's* hull over a period of three months in seawater (much of the time out-of-service) showed that there is only green algal slime present on the hull. The green slime observed is always more dense at the waterline due to higher exposure of the hull at the waterline to sunlight. The slime easily wipes off and can be removed easily with low pressure water blasting (Figure 2) in a short time (typically 1½ hours on the slipway).

The service performance of the adhesive has lived up to expectations throughout, and the overall performance of the foil attachment system on the *Koru* has been good.

MV Osprey

The 21 metre catamaran MV *Osprey* (Figure 3), built as a fast passenger ferry, was constructed at Whangarei (NZ) and launched in December 1994. The ferry has since been in passenger ferry service for about 30,000 nautical miles on the Auckland harbour. Both FRP hull pontoons were sheathed from the waterline down with 90-10 copper-nickel foil panels during construction, but deviated from the established Mitchell system in using a different hull preparation coating and panel alignment from the two earlier boats.



Figure 3 The FRP catamaran ferry *MV Osprey* with 90-10 foil sheathing, being slipped from Auckland harbour for maintenance, 1998.



Figure 4 Relatively clean internal surface of 90-10 sheathed hull pontoon on the *Osprey* after slipping 1998, following one year's continuous service in Auckland Harbour.

During slipway inspections of the *Osprey* hull over the next two years, it was found that this vessel experienced the same galvanic corrosion problems with the keel strip encountered earlier with the *Koru*. Again, this required a replacement with FRP to solve the problem. The adhesive performed very well on the FRP hull. The other parts of the hull appeared in good condition, apart from the leading edges of the two rudder skegs which showed evidence of erosion-corrosion (impingement) after six month's service. Although occurring quite rapidly, the erosion-corrosion problem on the rudder is commercially acceptable and the affected panels are periodically replaced during a maintenance haul-out.

Fouling again consisted of moderate green slime growth. The growth was heavier on the exterior sides of the catamaran pontoons exposed to more sunlight. On the sheathed hull surfaces between the pontoons, where seawater velocity/turbulence is higher and sunlight exposure is less, the development of surface slime was minimal (Figure 4).



Figure 5 Repair of physical damage to the copper-nickel sheathing on FRP hull of *Osprey* by cutting out copper-nickel foil on the FRP hull and replacing with new adhesive-backed panels.



Figure 6 Some 90-10 foil panels on the hull of the *Osprey* applied in two different configurations. Biofouling and adhesion to the hull is the same for both geometries, after four years' service.

Another factor, which was found to be consistent on both the *Koru* and the *Osprey*, was that mechanical damage to the copper-nickel panels (sustained through collisions with floating debris) was minimal. The foil panels were not only durable, but they also exhibited excellent mechanical properties, such as toughness and impact resistance. Additionally, any significant mechanical sheathing damage sustained on both vessels was quickly and easily repaired during normal maintenance time on the slipway. Sections of the damaged foil on the hull are easily cut out and replaced with new adhesive-backed foil panels (Figure 5). To date, application of the copper-nickel foil panels on the *Osprey* at either 45° to vertical, or horizontal, has made little difference to the attachment performance (Figure 6).

Auckland Test Panels

In August 1993, two wooden frames (Frame 1 and Frame 2) each containing five 90-10 copper-nickel sheathed panels, were immersed in the harbour, at the National Maritime Museum, Auckland. The test site was adjacent to a concrete pontoon about 40 metres from the shore, in deep, relatively unpolluted, quiescent seawater. The sheltered test location was chosen to avoid bright sunlight reaching the frames through the seawater, and seawater flow rate was only due to tidal action and wind. The test panels were initially inspected routinely at intervals of a few months.

Frame 1 was constructed of plain painted wood, supporting marine grade aluminium alloy panels coated with epoxy paint and sheathed with 90-10 copper-nickel foil, exposing various size areas of the copper-nickel foil to seawater contact. Frame 2 was constructed of a painted wooden frame, supporting steel panels coated with the same epoxy paint as Frame 1 and sheathed with copper-nickel foil.

After eight months' exposure, light marine growth was present on the copper-nickel panels, consisting of small barnacles, filamentous bryozoans, and algae. The marine growth was easily wiped off with finger pressure. Heavy marine growth quickly developed upon the wooden support frames, and this growth steadily increased at each inspection thereafter. The very dense marine growth on the wood (and the support ropes) consisted of various types of organisms including barnacles, seaweeds, tunicates, serpulids, coral worms and bryozoan colonies.

In March 1994, Frame 2 was withdrawn, cleaned of fouling, and the wooden frame was completely sheathed with 90-10 copper-nickel foil. This test panel then became the *new* Frame 2. At the same time a third test panel (Frame 3) was installed at the testing site. This was constructed of wood framing supporting aluminium alloy panels, with the entire frame and the alloy panels sheathed in 90-10 copper-nickel foil.

After approximately two years' exposure immersed in seawater, the following accounts of the three submerged test panels were recorded. The observations were made before and after low pressure waterblast cleaning had been performed:

Frame 1: The attachment ropes, painted wood frames, and the foil panels coated with paint, were all heavily encrusted with various types of marine growth, ranging from heavy slime to large tunicates, barnacles and bryozoans. The exposed 90-10 copper-nickel foil was coated with a medium slime and some serpulids, which were easily removed with finger pressure.

After the panel was water blasted, the copper-nickel foil was very clean with a speckled appearance due to different copper-nickel oxides forming in the surface film. The painted areas and the frame were extremely hard to clean and attached barnacles could only be removed by scraping.

Frame 2: The frame and rope were encrusted with marine growth which encroached onto the copper-nickel panels, but this was not firmly attached and was easily removed by water blasting. The exposed foil was covered with medium density marine growth and this wiped off with finger pressure. Small barnacles were prised off the copper-nickel foil with a fingernail. Light abrasion of one area of the foil with Scotchbrite® returned the surface to its original clean golden colour.

Frame 3: The panels and the frame showed similar appearances to Frames 1 and 2, with light fouling which was easily wiped off the surfaces of the frame and the panels.

Other than some small areas of unexplained discolouration, which were observed in crevices at the bottom of several foil-over-steel panels on Frame 2, the inspections did not reveal any evidence of galvanic corrosion occurring between the copper-nickel foil and the steel or aluminium alloy panels. The carefully applied adhesive-backed foil cladding isolated the substrate alloys from contact with seawater, thereby eliminating the prospect of corrosion occurring.

There was no evidence observed on any of the test panels of detachment or delamination of the adhesive, either from the panel substrate or from the foil. Neither was there any evidence observed of degradation of the adhesive material in seawater. When the adhesive is exposed to seawater for long periods, it becomes fouled with marine growth at about the same rate as paint or other organic materials.

Recent inspections of the test panels (including Frame 3) at the Maritime Museum (1998) by the authors confirmed that after five years' submersion in the harbour, the test panels have continued to exhibit the same trends described after two years' immersion (Figure 7).



Figure 7 Two test panel frames removed after five years' seawater immersion at the Maritime Museum site, Auckland, 1998. The differing degrees of fouling on the wooden frames are visible. The near wooden frame is painted, while the farther frame is clad with 90-10 copper-nickel foil.



Figure 8 A copper-nickel sheathed FRP test panel after 2 years 9 months' exposure in Langstone Harbour (United Kingdom). Finger pressure was sufficient to remove biofouling off the panel surface revealing the original 90-10 copper-nickel substrate.

Singapore Test Panels

In September 1994, a test panel assembly was submerged in the harbour at Singapore in a 1-2 knot current. This location was chosen because experience was required with the adhesive-bonded 90-10 copper-nickel foil panels in a warm, probably polluted seawater environment. The test panels were withdrawn and inspected monthly for a period of one year, from 1994 to 1995.

Generally, marine growth and fouling on the wooden support frames was very rapid, with heavy barnacle encrustation evident within three months' exposure. This fouling was scraped off the wood framing regularly thereafter, as it was very dense and threatened the test panels' longevity.

During the same time, there was some marine growth on the copper-nickel panels, but it underwent little further change after the first three months. The copper-nickel panel fouling consisted mainly of small barnacle growth, tubeworms and soft corals. These were easily wiped off with finger pressure.

Langstone Harbour Trials

Nine exposure panels were immersed from rafts in the tidal flow of Langstone Harbour in the UK, in August 1996. They represented triplicate panels of copper-nickel sheathed steel, FRP and aluminium. The substrate materials were sheathed on both sides (*Mariner 706 System*) for the bottom two thirds of the panel length and positioned in the water such that the seawater level was below the upper level of the copper-nickel sheathing. The upper portion of each test panel was coated with a proprietary primer, part of the *Mariner 706* system.

After 3.5 years' exposure there are medium slime levels on the copper-nickel and minimal levels of macrofouling. Figure 8 shows the FRP panel with copper-nickel sheathing after 2.75 years' exposure in Langstone Harbour. This illustrates the low level of macrofouling on the copper-nickel for this length of exposure time, and the ease with which the biofouling is wiped off with finger pressure.

It is proposed to evaluate the copper-nickel foil adhesion to the base materials, and the presence of any potential metal substrate corrosion, at a later time when destructive testing of the panels will be performed.

DISCUSSION

The principal commercially available copper-nickel alloys, 90-10 (UNS C70600) and 70-30 (UNS C71500), are solid solution alloys which exhibit excellent fabrication characteristics, useful mechanical properties and a high level of corrosion resistance in seawater (Jordan and Powell, 1998). Forming and welding of these alloys is relatively uncomplicated and they have enjoyed many successful applications in the marine industry.

The corrosion behaviour and corrosion rates of 90-10 copper-nickel alloy in seawater have been researched and recorded in the literature (Powell, 1994b). The 90-10 alloy has an acceptably low corrosion rate in seawater, except when the water is heavily polluted with sulphides (Alhajji, 1998), and it can sustain long periods of seawater exposure without degradation.

Long term research at the LaQue Center for Corrosion Technology (Int. Copper Assoc., 1998) has shown that 90-10 copper-nickel alloy has good characteristics for the sheathing of marine steel structures in the splash, spray and tidal zones, where corrosion can otherwise be very severe. The accumulation of biofouling on insulated 90-10 sheathing was also shown to be less than 2% of that occurring on corresponding areas of bare steel. Thus, 90-10 copper-nickel alloy has a proven record for use as a sheathing material on marine structures, whether they be static, such as offshore platform piles, or dynamic, as on sea going ships.

Biofouling resistance on 90-10 copper-nickel exists if the alloy is freely corroding, albeit at a very low rate. As copper-nickel loses at least part of its biofouling resistance when coupled to less noble materials (International Copper Association Ltd., 1998), copper ions within the surface film are suggested as being partly responsible for the biofouling behaviour. However, Efirid (1975) suggested from his observations that this is not the full mechanism. The initial film formed in seawater, probably red-brown cuprous oxide, is inhospitable to marine organisms. In time the cuprous oxide (Cu_2O) film oxidises to form greenish cupric hydroxychloride $[\text{Cu}(\text{OH})\text{Cl}]$, which is considered to be less toxic to marine organisms. This copper compound does not adhere as tightly to the metal surface, and it can be eroded away in flowing seawater

relatively easily. The sloughing action prevents secure biofouling attachment for any length of time. The cuprous oxide film is then re-formed and the cycle repeats itself. There is ongoing debate about the copper-nickel antifouling mechanism, and further research is required to establish the full mechanism.

The presence of any sulphide compounds in seawater, as found in polluted waters, can form stable surface copper sulphide on copper-nickel. This surface film is not so protective and copper-nickel can experience higher corrosion rates and pitting. However, once the exposure to sulphides is eliminated, for instance when the vessel is moved and moored in open unpolluted water, the copper sulphide is in time replaced by cuprous oxide again.

It is perhaps a natural progression of copper-nickel alloy technology that development of an adhesive-backed sheathing system for the hulls of small and medium size pleasure and commercial craft should occur. A system that was originally developed in the United Kingdom has now been applied and given rigorous service experience in New Zealand.

The overall assessment of the evaluations to date is that it has proved to be a viable system and the adhesive has shown excellent adherence to the FRP boats examined. The observed biofouling resistance has been consistent with documented accounts that green algal slime (microfouling) does form on copper-nickel but colonisation of macrofoulers is restricted. If macrofouling does attach, a wipe or finger pressure can readily remove it, and a light waterblast will quickly remove any growth. The turnaround time for cleaning the *Koru* on the slip by this method is about 1.5 hours. In comparison, removing fouling from the equivalent painted vessels in the fleet could take up to one day per vessel.

The evaluation has indicated that there are a number of factors that must be taken into account when considering the response of copper-nickel to biofouling, namely:

- Seawater temperature;
- Access to sunlight;
- Velocity of the seawater;
- Avoidance of galvanic action.

Temperature

The effect of seawater temperature remains somewhat uncertain. However, a review of the test panel results obtained in Singapore and Auckland Harbours indicated that biofouling of the copper-nickel alloy foil in warm tropical seawater after one year greatly exceeded the marine growth in cooler waters, although any fouling was still loosely attached and could easily be removed.

Sunlight

The experience on *Koru* and the *Osprey* showed that green slime formed predominantly on the copper-nickel foil at, or just below, the waterline on both vessels. In addition, more green slime was observed on the side of the *Koru* hull facing the sun during out-of-service time.

The green algal slime was easily removed by brushing, but the growth became firmly attached and more difficult to remove if it dried when the vessel was on the slipway. It was also observed that small, lightly attached barnacles, grew adjacent to the waterline on the foil when the *Koru* was left moored and unused in the harbour for longer periods of time.

Velocity

The velocity of the seawater had a substantial effect on the degree of fouling resistance of the copper-nickel foil. Areas of the *Koru* and *Osprey* hulls were almost entirely free of biofouling where the velocity of seawater experienced by the alloy exceeded some undetermined speed. Turbulence was possibly a factor that also contributed to this observation. Typically, the stern and waterline tended to show earlier signs of fouling than other hull areas.

The effect of water velocity can possibly be related to the sloughing of microfouling from the hull surface. Macrofouling on the hulls of both vessels only resulted after microfouling had been well established on the foil during quiescent periods. This experience was also recorded in the early copper-nickel work with the first solid copper nickel hulled vessel built, the *Copper Mariner* (Powell, 1994a). Further research and testing is required in this area, to evaluate the conjoint effects of velocity and turbulence upon the biofouling characteristics of the copper-nickel foil.

Galvanic Action

It has been confirmed that the 90-10 copper-nickel alloy foil must be freely exposed to seawater to achieve maximum biofouling resistance, and it must not be subject to cathodic protection, or any other type of galvanic coupling.

In the first few years of the sheathing trials (1994-95) on the *Koru* and *Osprey*, there was electrical contact occurring between 90-10 copper-nickel foil panels on the hulls and 70-30 copper-nickel alloy keel shoes. This bimetallic contact resulted in galvanic corrosion of the 90-10 hull sheathing panels (anodic) adjacent to the keels, where the 70-30 alloy foil behaved as the cathode. The corrosive action, possibly releasing copper ions into the seawater at the 90-10 copper-nickel surface, produced clean metallic areas of foil cladding free from any biofouling.

After replacement of the corrosion-damaged 90-10 foil panels and installation of FRP keel shoes instead of 70-30 copper-nickel strip, the galvanic corrosion problem immediately subsided on both vessels.

CONCLUSIONS

A five year feasibility study on using 90-10 copper-nickel adhesive-backed foil sheathing on the hulls of commercial passenger craft, to minimise corrosion and biofouling, has been undertaken.

Copper-nickel adhesive-backed foil has been found to effectively form part of an *antifouling regime* on the ferry hulls, providing decreased biofouling levels and reduced downtime on the slipway. Corrosion performance of the copper-nickel foil on FRP ship hulls is very good in seawater.

Specific observations can be summarised as follows:

The adhesive-backed copper-nickel foil sheathing, trialled on two ship hulls and on test panels, is readily applied. The system has shown excellent adherence to the FRP hull substrate in the test programme and is suitable for new-build and retrofit.

On retrofit, care should be taken to establish the alloy identity of hull fittings to avoid galvanic corrosion. Steps may need to be taken to replace metallic fittings, or to ensure that the fitting corrosion resistance is maintained and is compatible with the new copper-nickel foil system on the hull.

No evidence of the detrimental influence of copper sulphide formation, due to pollutants, was observed on the corrosion performance of the copper-nickel sheathing in the seawater immersion trials.

Copper-nickel has a good resistance to macrofouling, but green algal slimes can form on the 90-10 copper-nickel foil during service.

If conditions exist that some macrofouling eventually starts to colonise, the macrofoulants tend to be loosely attached and easily removed.

An annual low-pressure waterblast easily removes the biofouling from the ship's hull. This haul-out process is not necessary however, with periodic underwater hull cleaning using brushing.

Factors influencing resistance to biofouling during the trials were:

- The degree of exposure to sunlight in seawater;
- The ambient seawater temperature;

- Galvanic coupling to less noble materials;
- The velocity of seawater along the hull.

Regular inspection of the hull during dry-docking, to check for mechanical damage, erosion-corrosion and galvanic action, is an advisable precaution for maintenance of the system. The sheathing system is easily repaired if physical damage occurs during service. The cladding shows very good impact resistance and it can add integral strength to the vessel's hull.

The test programme results and experience gained during the five year evaluation period of the system in Auckland harbour, confirms similar performance results in the USA and Europe during the past 27 years of trials and testing using 90-10 copper-nickel alloy for the fabrication of ship hulls.

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Antifouling from Nature: Laboratory Test with *Balanus amphitrite* Darwin on Algae and Sponges

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ABSTRACT

Crude extracts and isolated molecules of five species of algae and three sponges have been tested using *Balanus amphitrite* laboratory settlement test to assess their potential antifouling efficacy. In some cases, samples were collected at different times and locations (summer and winter, Atlantic Ocean and Mediterranean Sea). Crude extracts of the algae *Caulerpa taxifolia* and *Bifurcaria bifurcata* and of the sponge *Raspaciona aculeata*, showed a 72 hour EC₅₀ of less than 1 µg·ml⁻¹. Toxicity of extracts and fractions has been tested against *B. amphitrite* nauplius II, to assess whether antifouling activity occurs through either a toxic or non toxic mechanism. Cyprids EC₅₀ are therefore compared with nauplius II toxicity threshold values (LC₁) and the ratio used to concurrently assess antifouling efficacy with or without toxic effect of the various molecules.

INTRODUCTION

Many organisms, from bacteria to algae, invertebrates and marine phanerogams, have been studied during the last 15 - 20 years in order to determine the presence of bioactive antifouling substances (Clare, 1996). However, antifouling research on substances derived from nature is only beginning, and only few marine organisms which maintain their surface free from epibionts have been extensively studied and tested for the presence, or production, of bioactive molecules with antifouling properties (Targett *et al.*, 1983; Ware, 1984; Standing *et al.*, 1984; Rittschof *et al.*, 1985, 1987; Keifner & Rinehart, 1986; Gherard *et al.*, 1988; Sears *et al.*, 1990; Davis *et al.*, 1989, 1990, 1991; Uritz *et al.*, 1992; Willemsen & Ferrari, 1993a, 1993b; Clare, 1996). A large amount of work has been undertaken within specific research projects such as the

“Fusetani Biofouling Project ERATO” or is still in progress in programs such as the “Marine Biofouling Thematic Programme” currently under way in the UK. Nevertheless, much remains to be done despite a perceived decline in interest from antifouling paint manufacturers. This loss of interest is in part due to the difficulty in applying research results to immediate use: i.e., improving their commercial antifouling products and marine paints. It is also due to the difficulty in supplying the large quantities of raw material required by industry.

Some work is also needed to inter-calibrate the test methods in order to assess antifouling efficacy in the laboratory, as present laboratory data are often difficult to compare. Moreover, more work is needed to assess environmental impact tests on non-target organisms.

In this study we report data on antifouling efficacy of natural extracts of some marine algae and sponges, using the *Balanus amphitrite* Darwin cyprid settlement test. According to the literature, these two groups of marine organisms have some interesting potential as new sources of natural antifoulants (Willemsen, 1994; de Nys *et al.*, 1995). For these natural compounds, acute toxicity tests have been carried out on nauplius II to get a general screening, taking into account both antifouling efficacy and possible environmental impact on non-target organisms (Rittschof *et al.*, 1992). The meaning of this test is clear, assuming that barnacle naupliar stage belongs to the zooplanktonic community. For this reason, together with the efficacy test on *B. amphitrite* cyprids, we have obtained LC₅₀ and LC₁ values on nauplius II of the same barnacle species. This species, with its two larvae, will show antifouling efficacy due both to toxicity and repellence of natural compounds.

MATERIALS AND METHODS

Five species of algae, *Caulerpa taxifolia* (Valh) C. Agardh, *Bifurcaria bifurcata* Ross, *Dictyopteris membranacea* (Stackhouse), *Laminaria digitata* (Hudson), *Cystoseira amentacea* (C.Agardh) var. *stricta* Bory Montagne, and three sponges, *Cacospongia mollior* Schmidt, *Raspaciona aculeata* (Johnston) and *Reniera sarai* Pulitzer-Finali, have been tested as crude extracts (Table 1). Some purified metabolites have also been tested: Eleganolone and Eleganediol, isolated from *B. bifurcata*, and Caulerpenyne isolated from *C. taxifolia*. In addition, two crude extracts of *D. membranacea* have been tested: DMB2, collected in the Mediterranean sea and DMQ, collected along Atlantic coast of France. Crude extract (BB) and two molecules, Eleganolone (EL) and Eleganediol (EDIAL), have been isolated from *B. bifurcata*. Two seasonal crude extracts (CT during winter and TC during summer) of *C. taxifolia* have been tested, as well as the isolated Caulerpenyne (CP). Only crude extracts of *C. amentacea* (CSS), *L. digitata* (LDP) and the three Mediterranean sponges, *C. mollior* (CM), *R. aculeata* (RA) and *R. sarai* (RS), have been tested.

Preparation of Extracts

Algae were rinsed in fresh water and air-dried in the shade. Dried material was extracted three times in diethyl ether and the extract was then allowed to dry. *R. aculeata* and *C. mollior* were also extracted by ether and *R. sarai* with butyl alcohol. Some of the algae extracts were separated in different fractions.

A preliminary solubility test showed low solubility in seawater, also with a 1% DMSO solution. For this reason, a solution of 50% Ethanol in distilled water was used to dissolve the extracts for laboratory activity tests. Two stock solutions with 100 and 1 $\mu\text{g}\cdot\text{ml}^{-1}$ of extract were used to prepare the different concentrations. The aliquots of the above solution were put into a 3.5 cm diameter petri dish, thus obtaining a total volume of 5 ml. Petri dishes were kept for 12 hours at 35°C and then for 15 minutes at 50°C to ensure complete evaporation of the ethanol solution. Tested concentrations before full solvent evaporation ranged from 0.01 to 100 $\mu\text{g}\cdot\text{ml}^{-1}$ dry extract.

Table 1 Species extracts abbreviation and sampling sites location

DMQ	<i>Dictyopterus membranacea</i>	Quiberon - Atlantic Ocean
DMb2	<i>Dictyopterus membranacea</i>	Boulouris (Saint Raphael) - Mediterranean sea
CSS	<i>Cystoseira amentacea</i> var. <i>stricta</i>	Sausset Les Pins (Marseille) - Mediterranean sea
LDP	<i>Laminaria digitata</i>	Bretagne - Atlantic Ocean
TC	<i>Caulerpa taxifolia</i>	Le Brusc (Toulon) - Mediterranean sea - winter
CT	<i>Caulerpa taxifolia</i>	Le Brusc (Toulon) - Mediterranean sea - summer
CP	Caulerpenyne (from CT)	Le Brusc (Toulon) - Mediterranean sea - summer
BB	<i>Bifurcaria bifurcata</i>	Quiberon - Atlantic Ocean
EL	Eleganolone (from BB)	Quiberon - Atlantic Ocean
EDIAL	Eleganediol (from BB)	Quiberon - Atlantic Ocean
RA	<i>Raspaciona aculeata</i>	Thyrrhenian sea
RS	<i>Reniera sarai</i>	Thyrrhenian sea
CM	<i>Cacospongia mollior</i>	Thyrrhenian sea

Bioassays

Bioassays were performed using stage II nauplius and cypris of *B. amphitrite* collected in the Genoa harbour and maintained in the lab at a temperature of 25°C. Larvae were normally released by adults when the sea water was changed. They were reared at a temperature of 25°C and fed with the microalgal species *Pavlova lutheri* Droop and *Tetraselmis suecica* Kilim at approximately $5\text{-}6 \times 10^5$ cells. ml^{-1} every 2 days. After 5-6 days, cypris observed in the culture were pipetted into a vessel where they stayed at 6°C until beginning of the settling test, two or three days later.

Naupliar Toxicity Test

After ethanol solution evaporation, a dry extract film remained on the bottom of the Petri dish. 100-200 stage II nauplii, whose stage determination was done following Geraci and Romairone (1986), were added together with 5 ml of 0.22 μm filtered natural seawater. Another series of cleaned dishes with filtered natural seawater and larvae were added as controls. All dishes, in four replicates, were kept in the dark in a thermostatic room at a temperature of 20°C. After 24 hours, completely motionless larvae were counted as dead organisms. LC₅₀ and LC₁ after 24 hours was obtained for all the extracts and fractions, using the "Probfis II" program (Puddu, 1989).

Cyprid Settlement Test

After solvent evaporation from the dishes containing the extract dilutions, 30-50 *B. amphitrite* cyprids obtained from cultures reared in the laboratory since fertilisation, were added to the test dishes together with 5 ml of filtered seawater. Petri dishes were kept for 72 hours in a thermostatic room with a 12h:12h L:D cycle at 1400 Lux at 25°C. At the end of the test, a 5% formol solution was added which simultaneously stopped the metamorphosis in all the replicates (4 for each concentration and control). The percentage of metamorphosed cypris was calculated and EC₅₀ 72 hours values were derived for each product with the same "Probfis II" program. A 72 hour contact time was selected, because cypris settlement has been observed (Satuito *et al.*, 1996) to reach its peak at this time and then to gradually slow down.

Table 2 Extracts threshold concentration against *B. amphitrite* nauplius II (LC₁) after 24 hours and 50% antifouling efficacy concentration against *B. amphitrite* cyprid (EC₅₀) after 72 hours

Species	Extracts	LC ₁ μg/ml	Conf. Lim 95%	EC ₅₀ μg/ml	Conf. Lim 95%
<i>Dictyopteris membranacea</i>	DMB2	0.13	(0.02-0.41)	0.97	(0.17-3.14)
	DMQ	3.11	(0.04-5.25)	61.83	(18.77-986.90)
<i>Cystoseira amentacea</i>	CSS	0.68	(0.05-2.20)	5.83	(2.30-10.60)
<i>Laminaria digitata</i>	LDP	4.56	(0.15-13.08)	3.32	(0.34-11.20)
<i>Caulerpa taxifolia</i>	TC	2.20	(1.22-3.4)	0.24	(0.18-0.56)
	CT	5.54	(0.13-15.61)	0.10	(0.02-0.32)
	CP	5.57	(3.07-8.19)	28.30	(13.93-47.94)
<i>Bifurcaria bifurcata</i>	BB	0.64	(0.26-1.14)	0.43	(0.08-1.26)
	EL	3.48	(2.53-4.52)	2.14	(0.88-3.9)
	EDIAL	7.31	(3.84-8.78)	40.37	(11.89-62.93)
<i>Raspaciona aculeata</i>	RA	0.06	(0.003-0.3)	0.36	(0.06-1.23)
<i>Reniera sarai</i>	RS	2.62	(2.28-2.94)	1.46	(1.11-2.93)
<i>Cacospongia mollior</i>	CM	NC		8.81	(7.57-10.01)

RESULTS

Table 2 summarises the results obtained both on efficacy (EC_{50} 72 hours values) and toxicity (LC_{50} and LC_1 24 hour). Figures 1 and 2 show the relative antifouling efficacy obtained with *B. amphitrite* laboratory test and the cyprids EC_{50} versus nauplius LC_1 ratio, respectively. This R ratio was obtained averaging the EC and LC values of the four replicates.

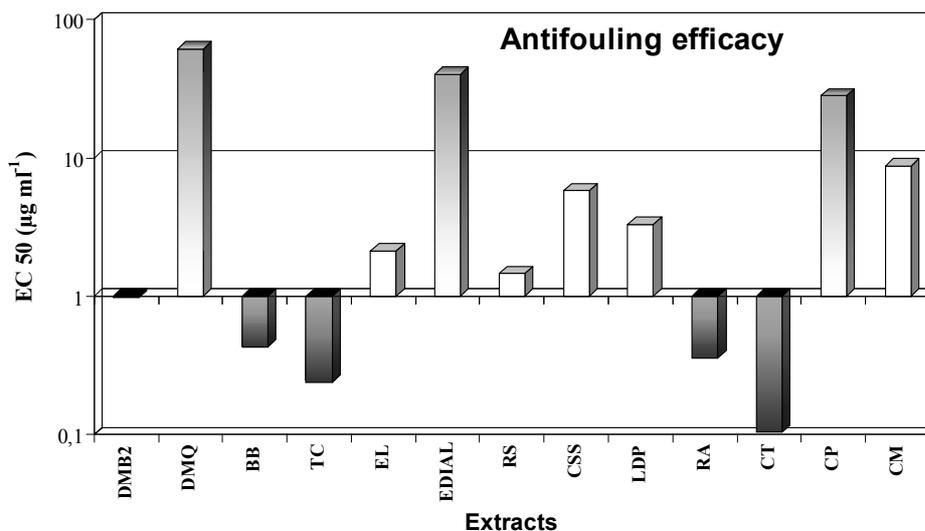


Figure 1 Antifouling efficacy range of crude extracts and isolated molecules

We can distinguish the results obtained in three categories: the first one, comprising EC_{50} values of less than $1 \mu\text{g}\cdot\text{ml}^{-1}$: *D. membranacea* taken from the Mediterranean, *C. taxifolia* collected both in winter and in summer, *B. bifurcata* and the Sponge, *R. aculeata*. The second group, with middle-range antifouling efficacy, includes the extracts or their isolated molecules with an EC_{50} of between 1 and $10 \mu\text{g}\cdot\text{ml}^{-1}$: *L. digitata*, *C. amentacea* and Eleganolone. Sponges included in this group are: *R. sarai* and *C. mollior*. The third group includes extracts or molecules with a low level of antifouling activity: *D. membranacea* from the Atlantic, Caulerpenyne, and Eleganediol.

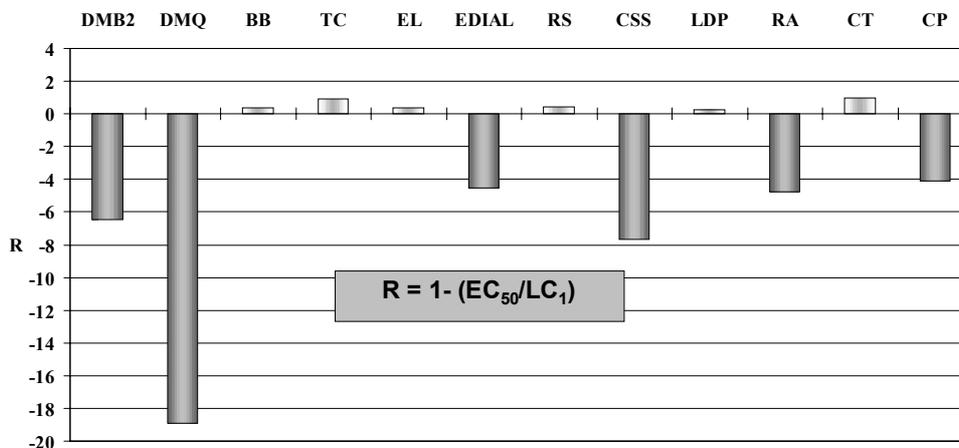


Figure 2 Antifouling efficacy versus threshold concentration ratio. R positive values show a more or less repellent action, R negative values indicate a toxic action against not target organisms.

DISCUSSION

This arbitrary subdivision is also suggested by literature data comparable with ours with regard to the organism test and the methodology. Hirota *et al.* (1996), in their screening of fourteen terpenoids including six new compounds, isolated from the marine sponge *Acanthella cavernosa*, found that isocyanate and isothiocyanate derivatives from kalihinanes were «highly antifouling» with an EC_{50} of ca $0.05 \mu\text{g}\cdot\text{ml}^{-1}$. The first group of our extracts showed higher EC_{50} values. In our case, however, the results refer to the whole extract. Purification and isolation of bioactive molecules is likely to improve antifouling efficacy. Tsukamoto *et al.* (1996) define the antifouling activity of two dibromotyrosine derivatives of the sponges *Pseudoceratina purpurea* as “particularly potent”, with ED_{50} of 0.10 and $0.27 \mu\text{g}\cdot\text{ml}^{-1}$. The same definition is given to the antifouling activity of three new kalihinenes by Okino *et al.* (1995). These 3 kalihinenes have an EC_{50} of 0.49 , 0.45 and $1.1 \mu\text{g}\cdot\text{ml}^{-1}$. Kalihinol A and 10-formamidokalihinene - with an EC_{50} of 0.08 and $0.09 \mu\text{g}\cdot\text{ml}^{-1}$, respectively - are more active than CuSO_4 ($EC_{50} = 0.15 \mu\text{g}\cdot\text{ml}^{-1}$). These values are in the same order of magnitude of those in our first group. The best values of EC_{50} with a *B. amphitrite* cyprid test were recorded by de Nys *et al.* (1996) on red alga *Delisea pulchra* showing an antifouling activity of its purified metabolites ranging from $10 \mu\text{g}\cdot\text{ml}^{-1}$ to $1 \mu\text{g}\cdot\text{ml}^{-1}$. These values however are not fully comparable to those from Japan, since ours have been obtained with metabolite solution and not with a dry film.

During our work, still in progress, we checked whether the interesting antifouling activity recorded for *C. taxifolia* (a tropical green alga recently introduced accidentally in the Mediterranean), is due to Caulerpenyne, its main secondary metabolite, considered to be responsible for a moderate antiviral activity (Rana, 1996) and to be

embryo and larvotoxic against *Paracentrotus lividus* (Pedrotti *et al.*, 1996). It seems that Caulerpenyne is not responsible for the high level of antifouling activity of Caulerpa given that extracts of this species collected during winter were low in this metabolite (TC in Table 1). A good level of antifouling efficacy, only a little lower than the extracts coming from a summer collection (CT in Table 1) and rich in Caulepenyne, was observed. CP is the Caulerpenyne obtained from an ether extract of CT showing very limited antifouling activity. This means that we have to search for another metabolite accounting for Caulerpa antifouling activity.

As far as *B. bifurcaria* is concerned (the other “Atlantic” alga whose extract shows a high level of antifouling activity), we found a middle-range activity in the diterpenoid Eleanolone (EL in the table, $EC_{50} = 2.1 \mu\text{g}\cdot\text{ml}^{-1}$, but practically no activity in another diterpenoid Eleanediol (EDIAL), obtained from the ether extract of this alga. Which means that either another metabolite or some synergy with a different substance must be identified.

With regard to sponges, the screening carried out by Willemsen (1994) on 44 samples of 35 different species with *B. amphitrite* cyprid bioassay, showed that 50% of the extracts had antifouling activity at the lowest concentration of $0.01 \text{ mg}\cdot\text{ml}^{-1}$, corresponding to $10 \mu\text{g}\cdot\text{ml}^{-1}$. This means that the best extracts of that screening would belong to our middle category (antifouling activity from 1 to $10 \mu\text{g}\cdot\text{ml}^{-1}$). Our data show that the sponges *R. sarai* and *C. mollior* belong to the same category of the best extracts of Willemsen’s work, while *R. aculeata* displays an antifouling activity which is about 10 times higher.

Concerning the nauplius toxicity test, we decided to compare EC_{50} (of cypris) and LC_1 (of nauplius) in a sort of index R which takes account of the antifouling efficacy of a compound as well as of (LC_1), the concentration that theoretically kills only one nauplius II, a planktonic organism. Rittschof *et al.* (1992), using nauplius LC_{50} to assess toxicity of some antifouling compounds, stated that: “Compounds of low or negligible toxicity that inhibit settlement are believed to be potentially a more environmentally acceptable solution to fouling”. In our opinion, LC_1 , very near to the acute toxicity threshold, better than LC_{50} , shows differences between antifouling efficacy and a potential risk for planktonic community. We are aware that a meroplanktonic larva, like *Balanus* nauplius II, cannot be representative of the entire planktonic community, but the higher or lower amplitude of concentration interval between EC_{50} and LC_1 is a first signal of a more or less potential risk for non target organisms. In other words, if $1 - EC_{50}/LC_1$ is positive, it means that in its antifouling action, that compound acts more as a repellent than as a toxic agent.

Table 2 shows that both extracts of *C. taxifolia* (CT and TC) have a toxic threshold value much higher than EC_{50} . This means that the antifouling activity of these extracts is evident before any evidence of mortality is found in the planktonic larvae. If we consider this as a tool for assessing a potential risk for the environment, we can also see (Figure 1) that the whole extract of *B. bifurcata* and Eleanolone, as well as that of *L. digitata*, among algae, display a similar behaviour although to a lesser degree than

Caulerpa. With regard to sponges, the toxic threshold of *R. sarai* is about twice as high as its EC₅₀.

These preliminary data suggest that at least two algae, *B. bifurcata* and *C. taxifolia*, especially the samples of this latter collected during summer, and one sponge, *R. aculeata*, have a good antifouling activity (Figure 1). Moreover, the two algae, having a positive R (Figure 2), are potentially more environmentally acceptable than the sponge. Our work on separation and testing of the different extract fractions is still in progress in order to identify their bioactive molecules.

Acknowledgement

We wish to thank Monica Andrenacci for her help in laboratory cultures and toxicity tests.

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Electromagnetic Antifouling Shield (EMAS) - A Promising Novel Antifouling Technique for Optical Systems

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ABSTRACT

We have tested the effect of high frequency alternating current (H.F.-AC) electric fields, created by ultramicroelectrodes on glass substrata, on potential fouling organisms such as cyprid larvae of the barnacle *Balanus amphitrite amphitrite* Darwin and zoospores of the green alga *Ulva lactuca* Linnaeus. High strength electric field pulse experiments on the brine shrimp *Artemia salina* Linnaeus and cyprid larvae induced electric breakdown of membranes and damage at the cell level observed by fluorescence staining with propidium iodide. Settlement assays reveal that the damage may be reversible or irreversible, and that periodically applied H.F.-AC fields negatively interfere with the settlement process of cyprid larvae. Damage results from the electric fields created between ultramicroelectrodes interfering with the fouling organisms' cell membrane potentials. Effects on the membrane potential, rather than electrochemical forces, are responsible for the observed effects.

INTRODUCTION

In 1991, the IOC (Intergovernmental Oceanographic Commission) established an international program called GOOS (Global Ocean Observing System). In cooperation with the WMO (World Meteorological Organization), UNEP (United Nations Environment Programme) and ICSU (International Council of Scientific Unions), the program aim is to acquire long-term oceanic data to describe the present status and future changes in the sea with regard to energy and food resources and the influence of

the ocean on global climate change (<http://www.ioc.unesco.org/goos/>). To achieve the required goals, committees of the GOOS have agreed that sensors need to be in service for a period of at least 30 days without maintenance. In contrast to the fouling of ship hulls, the fouling of optical sensors and sensor surfaces for obtaining long-term data on the ocean's chemical, physical and oceanographical status is of scientific rather than commercial importance. These sensors are used to measure optical density, currents, pH, conductivity and for long-term video observation. Conventional and pigmented antifouling paints are not suitable for obvious reasons.

Experiments with H.F.-AC electric fields have shown that objects near the electrodes are repelled from the electrodes by dipole forces (Pohl, 1978; Fuhr *et al.*, 1994). The aim of this study was to determine whether these dielectric forces (F_{DEP}) alone, or in combination with additional H.F.-AC or intense field pulses may act as a non-toxic antifouling microsystem for small scale and long-term applications. We assigned the name ElectroMagnetic Antifouling Shield (EMAS) to our technique because of the electromagnetic field that develops between the ultramicroelectrodes, and which acts as a shield to protect the sensor or optical surface behind from being fouled.

MATERIALS AND METHODS

Biophysical background

An electric field develops between two electrodes connected to an alternating current (AC) source or during the application of uni- and bidirectional field pulses (Figure 1a-c). Any

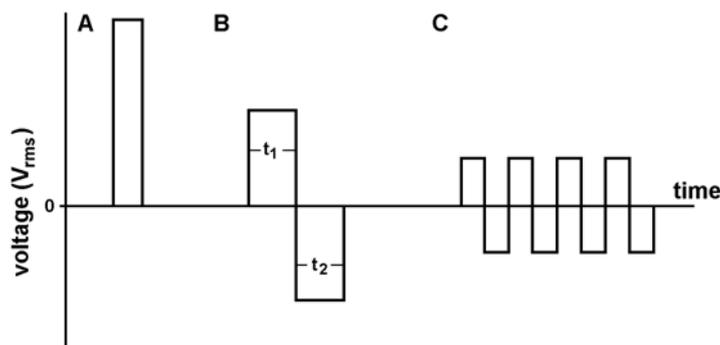


Figure 1 Different pulse types: (A) unidirectional field pulse, (B) bidirectional field pulse with variable pulse widths (= duration, t_1 and t_2) and/or variable voltages, (C) AC-pulse.

dielectric, e.g. a membrane surrounding a cell or organelle, is polarized when placed in an electric field because of the accumulation of charges at membrane surfaces. Field pulses and AC-fields at extreme low frequencies (ELF = < 3 kHz) lead to electrophoresis, whereas at high frequencies inhomogeneous AC fields result in dielectrophoresis (Pohl, 1978). Intense field pulses can destabilize membranes and induce the formation of pores (electroporation) when a threshold value for the total transmembrane potential of approximately 1 V is exceeded (for reviews see Zimmermann, 1982; Chang et al., 1992). At the same field strength (E) but with rising frequency, the induced membrane potential decreases, which means that AC-fields are less stressful for the cell membrane. In an inhomogeneous field, and in a low conductive medium, a polarized body is attracted into the area of higher field strength (Figure 2a). In general this is the case at frequencies between 3 kHz and 3 MHz (Fuhr & Shirley, 1995). The situation is different in seawater, when the concentration and mobility of ions in the cytoplasm is lower than in the surrounding medium. The body is again polarized but positive charges accumulate opposite the anode on the outside of the membrane (and negative charges vice versa) and consequently the body is subject to negative dielectrophoresis, i.e. it is repelled from the electrode (Figure 2b) (Fuhr *et al.*, 1996). In media of high conductivity negative dielectrophoresis can be observed at all frequencies but with maximum effects below 3 MHz (Figure 3).

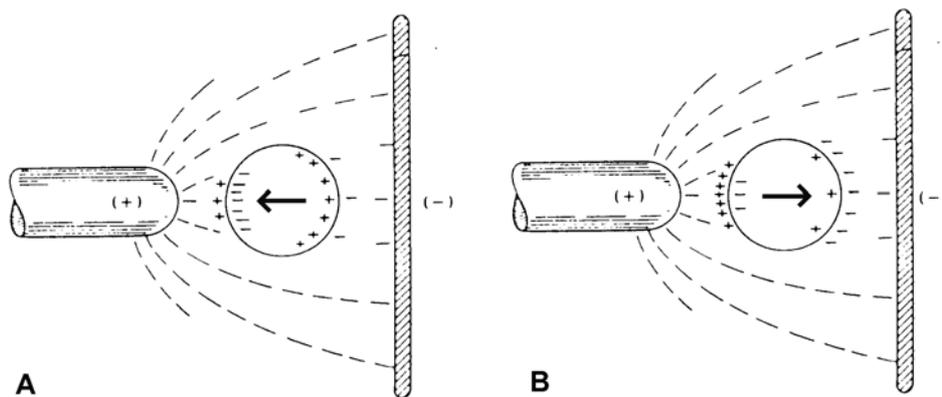


Figure 2 The influence of an inhomogeneous electric field on a microparticle. (A) In medium of low conductivity the bead is being polarized and moves towards areas of higher field strength, towards the anode (arrow). (B) In media of high conductivity positive charges accumulate opposite the anode and the cell is subject to negative dielectrophoresis and thus is being repelled from the electrode (arrow) (adapted from Pohl, 1978).

Both, electroporation and negative dielectrophoresis may severely interfere with the settlement of unicellular (e.g. algal spores) or multicellular (e.g. cyprid larvae) fouling organisms due to a breakdown of the transmembrane potential and a repulsion from the electrode plane respectively. Depending on the duration of the field pulse, electroporation may have reversible effects or be fatal to the organism. However, a

continuously or periodically applied H.F.-AC field will repel fouling organisms with less damaging effects. Additionally H.F.-AC pulses reduce electrode processes, such as reduction and oxidation reactions at the electrodes, due to the symmetry of the pulses. Electrolysis may be observed at the electrodes during field pulses, depending on the voltage applied, the duration of the field pulse and the conductivity of the medium. The total transmembrane potential (V_{tot}) is the sum of the induced membrane potential (V_{ind}) and the membrane potential (V_{m}). V_{ind} can be approximated by using the equations

$$V_{\text{ind}} = 1.5 \cdot E \cdot r \cdot \cos(\alpha) \quad [\text{V}] \quad (1)$$

$$\text{or} \quad V_{\text{ind}} = 1.5 \cdot U \cdot d^{-1} \cdot r \cdot \cos(\alpha) \quad [\text{V}] \quad (2)$$

where E = electric field strength; r = radius of the cell subjected to the field; α = angle of the cell to the field vector; U = voltage applied; d = distance between electrodes (Zimmermann, 1982). V_{m} in living cells is approx. -100 mV. Depending on the ion composition of the outer medium, and the cell's cytoplasm, an applied electric field will induce an additional peak membrane potential (V_{ind}). If

$$V_{\text{ind}} + V_{\text{m}} > \text{approx. } 1 \text{ V} \quad (3)$$

the transmembrane potential exceeds the critical value and no reversible dielectric breakdown occurs, i.e. persistent pores form at membrane structures (Bryant & Wolfe, 1987), transmembrane metabolism will fail. If the membrane pores are formed briefly only, cell damage may be reversible. For our application, in seawater, damage will be induced if intense field pulses are applied and, typically, short pulses of 40 μs are used (see Zimmermann & Neil, 1996). A range of abiotic and biotic variables influence the breakdown potential, including:

- voltage / frequency / number of pulses
- electrode distance / shape / material
- conductivity of outer medium
- temperature
- conductivity of cytoplasm
- membrane structure
- shape and size of the cell / organism
- existence of cell wall
- composition of exoskeleton (chitinised, incrustations)

Figure 3 shows V_{ind} and the dielectrophoretic force acting on a cyprid larva of *Balanus amphitrite*, calculated on the basis of average parameters for our application (Schnelle et al. 1999b). The parameter set was chosen according to histological cuts and literature (Schnelle et al., 1999a). Chitin layer: thickness (t) = 200 nm, permittivity (ϵ) = 5, conductivity (σ) = 10^{-5} S m^{-1} ; membrane: t = 8 nm, ϵ = 8, σ = $3 \cdot 10^{-6} \text{ S m}^{-1}$; cytoplasm: ϵ =

50, $\sigma = 1 \text{ S m}^{-1}$; external medium: $\epsilon = 78.5$, $\sigma = 5.7 \text{ S m}^{-1}$; average cyprid larva diameter: $300 \text{ }\mu\text{m}$; field strength = 13 kV m^{-1} .

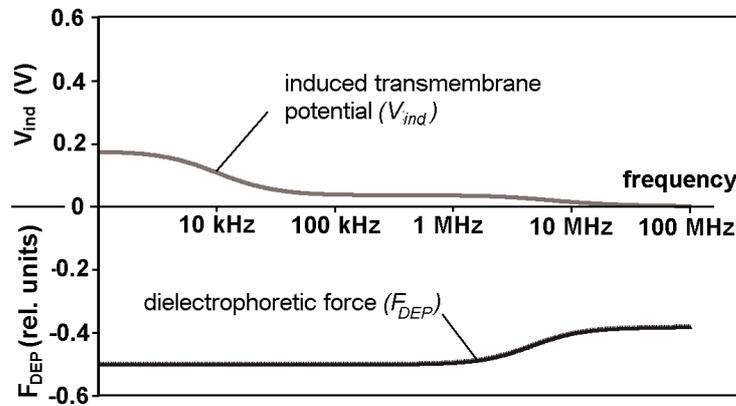


Figure 3 Transmembrane potential V_{ind} (top) and dielectrophoretic force F_{DEP} (bottom) acting on a cyprid larva of *Balanus amphitrite* as a function of the frequency of the electric field.

Test organisms and electric generators

Artemia salina Linnaeus was reared in the laboratory in 9 ‰ seawater. Ten day old cyprid larvae of *Balanus amphitrite amphitrite* Darwin were obtained from TNO, Delft, Netherlands and stored in seawater (28 ‰) at 4 °C in darkness. *Ulva lactuca* Linnaeus was cultured in Provasoli enriched seawater (PES) (15 ‰) in our laboratory in Berlin. For these cultures, thalli of *U. lactuca* were obtained from the BAH/AWI (Biologische Anstalt Helgoland / Alfred-Wegener-Institute for Polar- and Marine Research in List/Sylt, Germany). Those thalli were collected in the field in the vicinity of the marine station of the BAH/AWI in List. For assays, spores released from these thalli were used. Cultures and assays were run in artificial seawater (hw-Meersalz professional, Wiegandt GmbH, Krefeld, Germany) and under a 15:9 light/dark cycle (Lumilux Plus fluorescent tubes, cool white, Osram GmbH, Munich, Germany). *U. lactuca* was cultured at 15 °C; cyprid assays were performed in a water bath (Sigma Techware, USA) at $26 \pm 1 \text{ }^\circ\text{C}$.

For field pulse experiments, a commercial porator with pulse cuvettes with aluminum electrodes (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) was used. For cyprid settlement assays and material tests, an HP 33120A generator (Hewlett Packard Co., Loveland, CO, USA) supplied a rectangle AC-voltage. *Ulva* settlement assays were conducted with a laboratory-made 2-channel generator. All voltages are stated in V_{rms} and were measured using a digitizing oscilloscope (TDS 210, Tektronix Inc., Beaverton, OR, USA). Bright light and fluorescence photomicroscopy was performed using a Zeiss Axioskop and a Leitz Aristoplan with CLSM (Confocal Laser Scanning Microscopy) feature.

Field pulse experiments

Young larvae of *Artemia salina*, one to three days old, were used for pulse experiments. Single larvae were added to a pulse cuvette (electrode distance = 2 mm) containing 400 μ l distilled water and propidium iodide (PI, Sigma-Aldrich Co., USA, order no. P 4170). Single field pulses of various voltages and durations were applied. After pulsing, the *Artemia* larvae were then incubated for 5...10 minutes and thereafter examined for cell damage using fluorescence microscopy (Zimmermann & Neil, 1996).

Cyprid larvae were pulsed with two 100 μ m diameter gold wire electrodes, glued into separate glass micropipettes and fixed onto a micromanipulator with the electrodes 200 μ m apart. For pulsing, larvae were placed in a microscope trough in their culture medium with PI added. The gold electrodes were manipulated so that the first antennae were directly between the electrodes and single field pulses were applied. Larvae were then incubated for 5...10 minutes and examined for cell damage using fluorescence microscopy as above.

Settlement assays

For settlement assays and material tests, glass chips with microelectrode structures processed by photolithography (GeSiM mbH, Großerkmannsdorf, Germany) or laser ablation (our workgroup) were used (Figure 4). Platinum (Pt), Titanium (Ti) and Tantalum (Ta) were used as electrode material. 0.5 mm thick glass squares (2 x 2 cm) provided the base for the electrode chips. For Ti/Pt- or Ta/Pt-electrodes, a 10 nm thick layer of titanium or tantalum was applied as a plating base, then the platinum electrode (100 nm) was processed on top. The electrode design had a comb-like structure with single comb teeth of one electrode interdigitating with those of the opposite electrode. Opposing electrodes were 50 μ m wide and 300 μ m apart. An array of 48 comb-electrodes was arranged on the 4 cm² glass chip and covered the surface. A computer controlled time-relay admits a switch regime to connect only a certain number of electrodes to the generator at one time for a certain period. After that period the relay switches to the next set of electrodes etc.. An oscilloscope was used to check the voltage applied to the charged electrodes. The petri dish, together with unfielded control dishes, was placed in a large petri glass dish in a water bath to ensure proper culture temperatures. All parts of the electrode chip and electric connections, other than the active electrode area, were insulated against seawater with a two component epoxy glue EPO-TEK 302-3M (Epoxy Technology, Billerica, MA, USA). Extensive glue areas were covered with coverglasses. The basic cyprid settlement assay technique followed Rittschof et al. (1992).

Single field pulse settlement assay

Single cyprid larvae were pulsed once for 40 μ s at 50 V or 100 V. After pulsing, 25 to 35 larvae were placed in single petri dishes with 10 ml of sterile culture medium and cultured for 44 hours. Settled and non-settled larvae were then counted. Controls were run in triplicate.

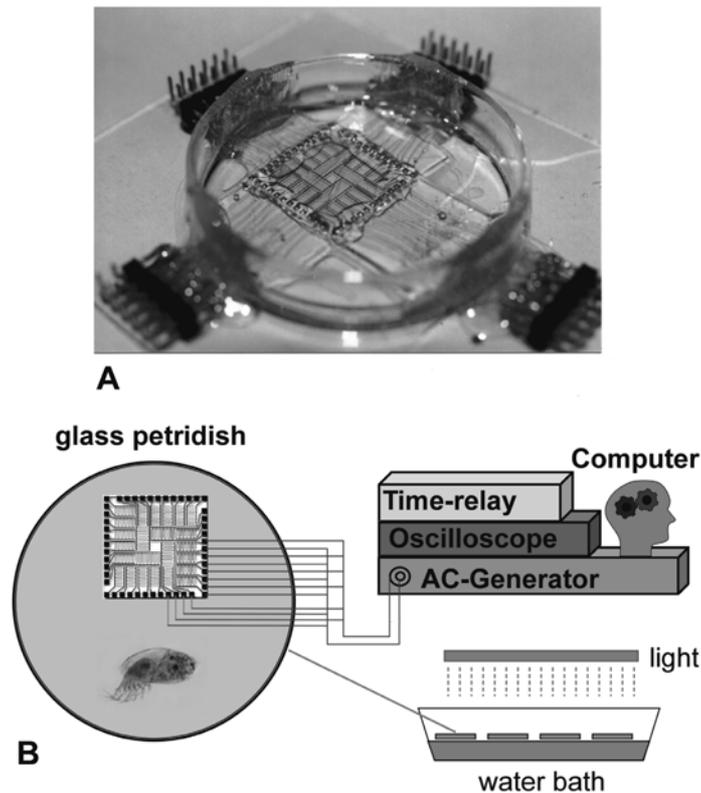


Figure 4 Experimental set-up for settlement assays: (A) Settlement assay petri dish with a 2 x 2 cm glass chip with microelectrodes in interdigitated comb structure. Electrode measurements and material as stated in the text. (B) Overview of devices used for a cyprid settlement assay.

Continuous AC-field settlement assay

35 to 50 cyprid larvae were placed in petri dishes with mounted electrode chips and 10 ml of sterile culture medium. Approx. 10 % of the immersed surface of the petri dish was covered by electrodes facing approximately 90 % of unfielded surface. The electric field was generated by a rectangle AC-voltage of 4 V_{rms} and a frequency of 100 kHz. Of the 48 electrodes (Figure 4a), 6 neighbouring ones were grouped and switched to the generator by a computer controlled time-relay every 7 seconds for 1 second (Figure 4b). Living larvae (a) settled on electrodes, (b) next to electrodes and (c) swimming, and

non-settled dead larvae were counted and related to the number of settled larvae in three control dishes. The assay was terminated after 44 h.

***Ulva* settlement assay**

A swarmer suspension was prepared from laboratory cultures of *U. lactuca* thalli (de Nys *et al.*, 1995, modified after Fletcher, 1989). 1 ml of the swarmer suspension was added to 9 ml of culture medium in the assay petri dish. The electrode chip used was an interdigitated comb structure with Ti/Pt-electrodes of 20 μm width and with an electrode distance of 180 μm . The electric field was generated by an AC-voltage of 6 V_{rms} and 1 MHz with a time regime of 1:1 s (voltage/break).

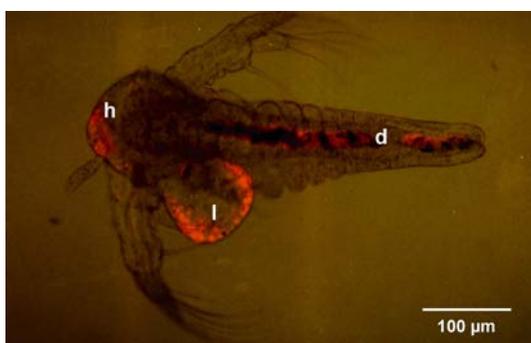


Figure 5 Ventral view of *Artemia salina* pulsed with five single field pulses of 150 V for 100 μs each and stained with propidium iodide (l = labrum, h = head, d = digestive tract) (digital overlay of bright field and fluorescence picture).

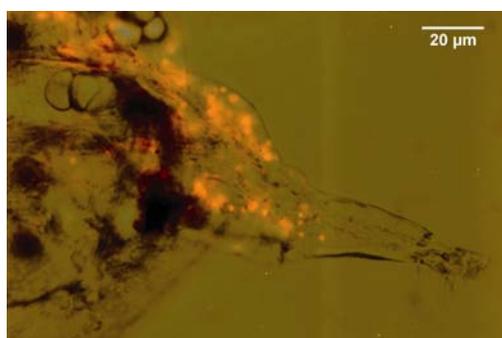


Figure 6 View of first antenna (antennula) of a cyprid larva of *Balanus amphitrite* pulsed with one single field pulse of 150 V for 40 μs and stained with propidium iodide (digital overlay of bright field and fluorescence picture).

RESULTS

Single field pulse experiments

Artemia salina larvae were exposed to single field pulses with PI added to the distilled water. Figure 5 shows an overlay of two pictures of *Artemia* pulsed 5 times with 150 V for 100 μs . Red fluorescence indicates cell damage around the rim of the labrum (l) and on one side of the head (h) due to membrane poration. Fluorescence in the digestive tract (d) is not field induced but due to free nucleic acids in digested food.

Cyprid larvae of *Balanus amphitrite* when exposed to one single field pulse of 150 V for 40 μ s, persistently retracted their first antennae and distinct cell damage was evident along the first and second segment of the first antenna and along the thorax towards the head (Figure 6). No damage was visible at the attachment organ on the third nor the fourth segment.

In the controls, stain in unpulsed animals of both taxa did not fluoresce, apart from stain in the digestive duct in *Artemia*. Scanning electron microscopy showed no evidence of mechanical damage to the larvae due to these field pulses.

Settlement assays

In single field pulse settlement assays with barnacle cyprids, the settling rate of pulsed larvae was comparable to that for unpulsed larvae (88 % settling rate) (Figure 7a). Single pulses of 50 V (73 % settling rate) and 100 V (89 % settling rate) for 40 μ s did not inhibit attachment of cyprid larvae of *Balanus amphitrite* and suggest reversible cell damage. Larvae were observed microscopically to react by temporarily retracting their first antennae during pulse application.

In a continuous AC-field settlement assay only 1 out of 50 larvae settled on an electrode (= 2 %). Approx. 40 % of the larvae attached to the unfielded petri dish surface, and 60 % of the larvae were either dead or still swimming at the end of the assay. Mortality of larvae indicates irreversible cell damage, while the free-swimming larvae at the end of the assay suggest a repellent effect.

In controls for both assays, the settlement rate was 83 % and 77 % for unpulsed larvae and unfielded larvae respectively (Figures 7a, b).

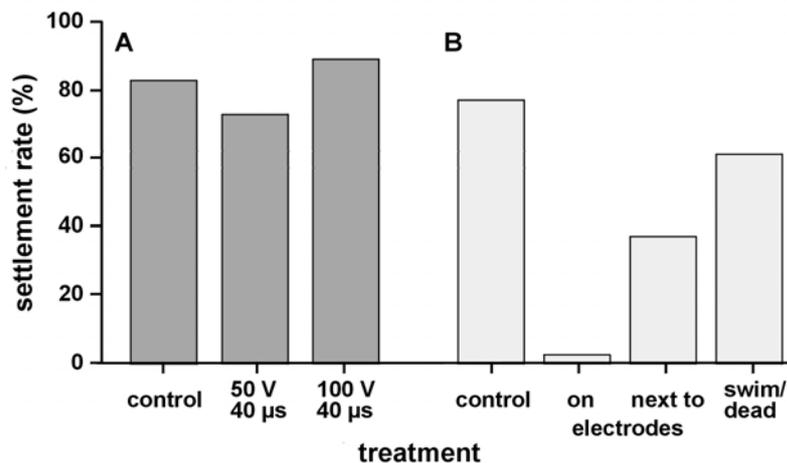


Figure 7 Settlement rates of cyprid larvae of *Balanus amphitrite* exposed to (A) single field pulses of 50 V and 100 V respectively and 40 μ s duration and (B) a periodic electric field generated by an AC-voltage of 4 V_{rms} and 100 kHz.

The *Ulva* settlement assay demonstrated the effects of negative dielectrophoresis (Figure 2b). The electric field did not repel the algal swarms from the glass chip. However, when compared to the control, swarms settled, germinated and grew preferentially between the two electrodes but not on them, while plaques of bacteria were also observed on and between the electrodes. No attempt was made to quantify these effects.

DISCUSSION

Some studies have been undertaken into the possibilities of using electrode systems as a method to control fouling on submersed panels, pipe systems or ship hulls (Smith & Kretschmer, 1984; Fears & Mackie, 1997; Ryan, 1998; Smythe et al., 1998; Bioplan, 1999). These have primarily investigated electrolysis and electrode processes that cause changes in the pH at the boundary layer of the electrode. No systems have been developed for the long-term protection of glass substrates on the small scales required for optical sensors. The effect of dielectric breakdown pulses on fouling organisms has also not previously been examined.

We have attempted to prevent the fouling of glass substrates by applying AC-fields and intense field pulses (Figure 1). Both field types may be used to influence an organism's settling behavior by increasing membrane potential to a level which causes electrical breakdown of the membrane. Unidirectional field pulses, with pulse durations longer than 100 μ s and AC-fields in the lower kHz range, may lead to electrophoresis, significant changes in pH of the medium, and the formation of toxic electrolytic products. These effects may all interfere with the settling behavior of fouling organisms. However, they have disadvantages when using microelectrodes, especially the latter two which can accelerate corrosion of the electrode material. We have therefore used uni- or bidirectional field pulses with pulse durations less than 100 μ s because Friedrich *et al.* (1998) showed that such short pulses minimize changes in pH and generation of byproducts.

By fluorescence staining of nucleic acids, we showed that single field pulses of 100 μ s (*Artemia*) or 40 μ s (cyprids) at 150 V cause membrane poration in single cells and also membrane breakdowns (Figures 5, 6). However, no damage was observed at the attachment organ on the third segment as described by Glenner & Høeg (1995), nor was the fourth segment visibly affected. For cyprid larvae, cell damage caused by short field pulses with voltages of 50 V and 100 V was reversible since, in a settlement assay, settling rates of pulsed and unpulsed larvae were similar (Figure 7a). However, microscopic observation showed that larvae do react to pulses by quickly retracting their first antennae. This is considered to be an explicit repellent reaction from the electric field. We consider that field pulses of 150 V and 40 μ s will cause irreversible damage and inhibit settlement of cyprid larvae.

While relatively long field pulses (40...100 μ s) cause membrane breakdown, very short AC-pulses at frequencies in the higher kHz and MHz range temporarily induce an

additional membrane potential. By this technique, an antifouling effect is evoked by negative dielectrophoresis without damage to the electrodes.

In settlement assays, an AC-field of $4 V_{\text{rms}}$ was found to deter cyprid larvae from settlement on the glass substrate (Figure 7b). A model for the estimation of dielectrophoretic forces and induced transmembrane potential acting on a cyprid larva (Figure 3) showed that with our AC-field technique no membrane damage is caused. The additionally induced transmembrane potential does not exceed 0.2 V and consequently the critical threshold for a total membrane potential of approximately 1 V is not reached (Figure 3, top). However, negative dielectrophoresis can be observed at all frequencies between 1 kHz and 100 MHz (Figure 3, bottom). We suggest that short, but intense, field pulses will have lasting antifouling effects. Furthermore they require less energy than continuous or even periodic AC-fields. The settlement behavior of cyprids is also an advantage for our antifouling technique as the delicate first antennae, which the larvae use to detect the optimal location for settlement, are a preferred place of entry for the electric field.

The settlement of algae and bacteria may not be prevented by a pulse regime effective against invertebrate cyprid larvae. Equation (2) shows that at constant voltages smaller organisms experience a weaker electric field. This is one reason for a non-inhibitory effect, on the other hand also the organism type may be decisive, as with more complex cellular processes and a nervous system one may expect the organism to be more responsive and susceptible to damage. However, a computer controlled pulse regime of AC-fields alternating with intense field pulses may be variable enough to create unfavorable conditions for different types of fouling organisms. Markx & Kell (1990) successfully used electrolytic cleaning pulses to remove bacterial biofilms.

In conclusion, we consider that electrical fields generated by short but very intense field pulses and periodic AC-pulses will provide antifouling protection for small scale substrates, especially optical sensors. Our studies show that field pulses cause damage at the cellular level and that AC-fields deter the settlement of fouling organisms.

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Properties of a Titanium Nitride Electrode and its Application for Electrochemical Prevention of Marine Biofouling

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ABSTRACT

Properties of a titanium nitride (TiN) electrode formed by reactive sputtering were studied and the electrode was used for electrochemical prevention of marine biofouling. Fourier transform infrared and Raman spectra indicated that an amorphous titanium oxide (TiO₂) film containing titanium hydrate was formed on the TiN surface by applying alternating potentials of 1.0 V and -0.6 V versus a silver-silver chloride (Ag/AgCl) electrode in seawater. This TiO₂ film had little photochemical activity, because *Vibrio alginolyticus* cells attached to the TiN electrode modified with the amorphous film were not killed when irradiated by black-light. However, the cells were killed by applying a constant potential of 1.0 V vs. Ag/AgCl. The amorphous TiO₂ film did not impede electrochemical deactivation of bacteria cells. In a field experiment, attachment of organisms to the TiN electrode was inhibited by applying alternating potentials of 1.0 and -0.6 V vs. Ag/AgCl for 279 days. The TiN electrode can be used as a practical electrode material for electrochemical antifouling.

INTRODUCTION

Attachment of microbial cells to the substratum, subsequent formation of a biofilm and consequent attraction of other macroorganisms is a biofouling mechanism in marine structures (Anwar *et al.*, 1990; Carson & Allsopp, 1983; Dempsey, 1981). The formation of biofilms is important for the subsequent attachment and development of

invertebrate larvae (Brancato & Woollacott, 1982; Henschel & Cook, 1990). Microbial films are known to affect the settlement and metamorphosis of oyster larvae and mussels (Satuito *et al.*, 1995; Weiner *et al.*, 1989). Marine biofouling has so far been prevented by chemical methods using copper and organotin coatings (Austin, 1988), and by injection of chlorine at suitable concentrations. However, these methods are becoming increasingly unsuitable due to the residual toxicity that pollutes the marine environment (Jackson & Jones, 1988). Therefore, deactivation of microbial cells attached to the substratum in the initial stage might be an effective method of preventing biofouling.

Previously, we have reported an deactivation method based upon the electrochemical oxidation of microbial cells (Matsunaga & Namba, 1984; Matsunaga *et al.*, 1984). In this method, it was possible to deactivate microbial cells without generation of toxic substances. This deactivation method has been applied to electrochemical prevention of marine biofouling. Electro-conductive polymer, carbon-chloroprene sheet and conductive paint have all been used as the electrode for electrochemical antifouling (Matsunaga *et al.*, 1998; Nakasono *et al.*, 1992, 1993; Okochi *et al.*, 1995). We have recently developed a practical method for electrochemical prevention of marine biofouling using titanium nitride (TiN). It is a biocompatible material (Behrndt & Lunk, 1991; Buchanan *et al.*, 1987; Rostlund *et al.*, 1990), with very low resistance (Ahn *et al.*, 1983; Igarashi *et al.*, 1978), good electrochemical stability (Endo *et al.*, 1994) and does not release toxic substances (Tateishi *et al.*, 1990). The electrochemical deactivation of marine bacterium, *V. alginolyticus* cells was studied using a TiN electrode (Nakayama *et al.*, 1998a). Furthermore, the method for formation of TiN film on a plastic plate by radio-frequency arc spraying was also investigated and used for the prevention of marine biofouling (Nakayama *et al.*, 1998b).

In this paper, we have investigated the surface properties of the TiN electrode formed by reactive sputtering when alternating potentials of 1.0 V and -0.6 V were applied. The effect of electrochemical antifouling using this TiN electrode was also studied in field.

MATERIALS AND METHODS

Materials

Titanium plate was obtained from Tokyo Titanium Co., Ltd, Tokyo, Japan. Marine broth 2216 was purchased from Difco Laboratories (Detroit, MI). Fluorescent dyes, propidium iodide (PI) and 4',6-diamidino-2-phenylindole (DAPI) were obtained from Molecular Probes, Inc. (Pitchford, OR) and Merck (Darmstadt, Germany), respectively. Other reagents were commercially available analytical reagents or laboratory grade materials. Seawater from Miura Peninsula (Japan) was treated by autoclaving for 1 min and filtering with a membrane filter (pore size: 0.2 μm).

Preparation of the TiN electrode

The TiN layers were formed on the titanium plate by reactive sputtering of the elemental titanium target in a mixture of argon and nitrogen. Pure titanium (99.999 %) and very pure nitrogen gas (99.9999 %) were used for the TiN deposition. After loading titanium on a glass plate into the sputtering device (model: SPF-312H, Nichiden ANELVA Co., Tokyo, Japan), the system was evacuated to 3×10^{-4} Pa prior to backfilling with argon. Argon gas was introduced into the system to a pressure of 3×10^{-3} Pa and the titanium surface was presputtered with the argon for 10 min. Next, nitrogen gas was introduced into the system to a pressure of 0.8×10^{-3} Pa. TiN was then deposited onto titanium plates by sputtering for 60 min and this material was used as the working electrode. A freshly prepared TiN electrode was used for each experiment.

Properties of the TiN electrode

The surface of the TiN electrode was analyzed by a Fourier transform infrared spectrophotometer (FT-IR, model FT/IR-610: Jasco Co., Tokyo Japan) and a Raman spectrophotometer (model NRS-2100: Jasco Co., Tokyo Japan). Exchange current density was measured from a polarogram obtained at a sweep rate of 10 mV/s in seawater.

Electrochemical deactivation of *Vibrio alginolyticus*

V. alginolyticus ATCC 17749 was cultured aerobically at 27°C for 12 h in 10 ml of marine broth, followed by preculture at 22°C for 12 h in 15 ml of marine broth. The cells were centrifuged at 1600g at room temperature for 10 min, washed then suspended in sterilized seawater. The cell concentration was determined using a hemacytometer. The TiN electrode was immersed in 200 ml of *V. alginolyticus* cell suspension (10^8 cells/ml) and incubated for 90 min at room temperature with continuous magnetic agitation (350 rpm). The electrode was then rinsed to remove weakly attached cells and subsequently immersed in fresh sterile seawater. A constant potential was applied with a potentiostat (model HA-151: Hokuto Denko Co., Tokyo, Japan). The reference electrode was silver-silver chloride and counter electrode a platinum wire.

Photochemical deactivation of *V. alginolyticus* cells attached to the TiN electrode

A TiN electrode modified with amorphous TiO₂ film formed by applying alternating potentials of 1.0 V and -0.6 V vs. Ag/AgCl and crystalline TiO₂ film formed by applying a constant potential of 80 V were used. The electrode was immersed in 200 ml

of *V. alginolyticus* cell suspension (10^7 cells/ml) and incubated for 90 min at room temperature with continuous magnetic agitation (350 rpm). This allowed *V. alginolyticus* cells to attach to the surface of the TiN electrode. The treated electrode was then rinsed by immersion in fresh sterile seawater to remove weakly attached cells. The electrode was subsequently immersed in 10 ml of fresh sterile seawater and irradiated by black-light (wavelength; 300-400 nm, 15W, model FL15 BL-B, Panasonic Co., Osaka, Japan).

Determination of viable cell number

The number of viable cells attached to the electrode surface was determined by plating suitably diluted samples after the cells had been recovered by pipetting and then counting the colonies, which appeared on marine broth agar plates after 24 h incubation at 25°C. The survival ratio was defined as follows: Survival ratio = (viable cell number after UV illumination or applied potential / viable cell number when no UV was illuminated or no potential was applied) 100.

Field experiments

Field experiments on antifouling using the TiN electrode were performed at a depth of about 30 cm in seawater on a floating pier located in Tateyama Bay, Chiba, Japan. A TiN film was deposited onto a Ti plate (10 x 10 cm) and used as the working electrode. The counter electrode was an iron bar (5 mm diameter, 50 cm length) and the Ag/AgCl electrode was used as a reference. The potential applied to the TiN electrode was controlled using a pulse generator (model HB-211: Hokuto Denko Co.) and a potentiostat (model HA-151: Hokuto Denko Co.). Alternating potentials of 1.0 V vs. Ag/AgCl for 60 min and -0.6 V vs. Ag/AgCl for 10 min were applied to the electrode. The experimental period was 279 days, from 9 July 1997 until 24 April 1998. A separate control experiment was carried out with no applied potential. The wet weight of biofouling organisms on the TiN was measured.

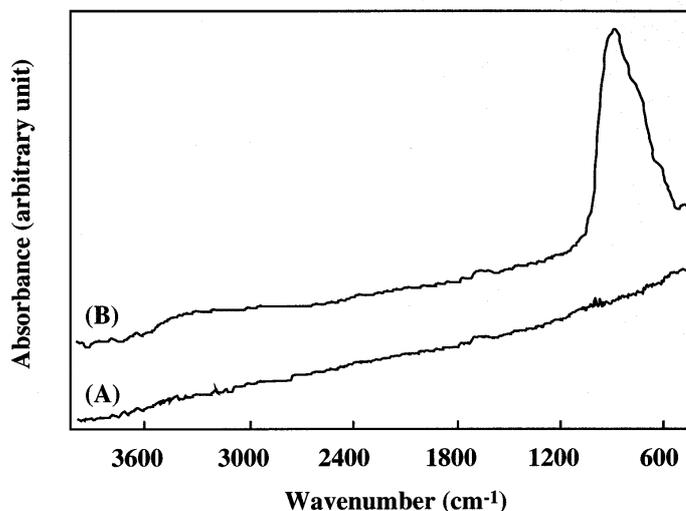


Figure 1 IR spectra of the TiN electrode surface with (B) and without (A) applied alternating potentials of 1.0 V vs. Ag/AgCl for 60 min and -0.6 V vs. Ag/AgCl for 10 min in seawater.

RESULTS

Properties of the TiN electrode

The TiN electrode surface after applying alternating potentials of 1.0 V vs. Ag/AgCl (60 min) and -0.6 V vs. Ag/AgCl (10 min) for 48 cycles in seawater was analyzed by FT-IR (Figure 1). Absorbance ascribed to TiO₂ did not appear in IR spectrum obtained from the TiN electrode surface before applying an alternating potential. IR spectrum of the TiN electrode surface after applying alternating potentials indicated the existence of TiO₂ on the basis of absorbance around 900 cm⁻¹.

Figure 2 shows Raman spectra obtained from the TiN electrode surface. As shown in Figure 2, the Raman band (A) obtained from the TiN electrode surface after applying alternating potentials was broad and the sharp Raman band ascribed to crystalline TiO₂ such as rutile or anatase did not appear. These results suggest that amorphous TiO₂ film formed on the electrode surface by application of alternating potentials in seawater. Figure 2 also shows Raman spectra (B) obtained from the TiN electrode surface after applying a constant potential of 80 V for 10 min in aqueous solution containing sodium carbonate (200 g/l) and sodium fluoride (10 g/l). Gray oxide film was formed on the TiN electrode surface. Raman bands are sharp and indicated that gray oxide film consists of crystalline TiO₂ such as rutile and anatase (Arsov *et al.*, 1991; Ohtsuka *et al.*, 1986).

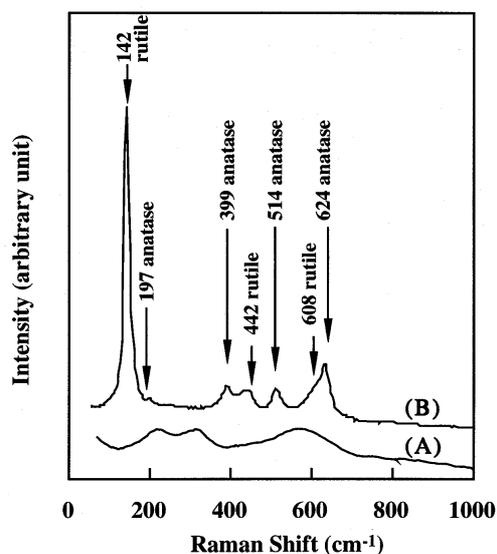


Figure 2 Raman spectra obtained from the TiN electrode surface after application of potential. (A) Alternating potentials of 1.0 V vs. Ag/AgCl (60 min) and -0.6 V vs. Ag/AgCl (10 min) for 48 cycles in seawater. (B) A constant potential of 80 V was applied for 10 min in aqueous solution containing sodium carbonate (200 g/l) and sodium fluoride (10 g/l).

Exchange current density of the TiN electrode

Electronic conductivity of the TiN electrode surface was investigated by measuring exchange current density. Figure 3 shows the effect of time on the exchange current density of the TiN electrode in seawater with applied alternating potentials of 1.0 V vs. Ag/AgCl and -0.6 V vs. Ag/AgCl. When alternating potentials applied for 10 min and 7 h were compared, the exchange current density markedly decreased from 20.5 $\mu\text{A}/\text{cm}^2$ to 6.3 $\mu\text{A}/\text{cm}^2$. After 14 h of potential application, the exchange current density was almost constant.

Electrochemical deactivation of *V. alginolyticus*

Electrochemical deactivation of *V. alginolyticus* cells attached to the TiN electrode modified with amorphous TiO_2 was examined. When a constant potential of 1.0 V vs. Ag/AgCl was applied to the electrode for 10 min in sterile seawater, survival ratio of *V. alginolyticus* decreased to 2.2%. With the modified TiN electrode, the survival ratio was 0.5%. The electrochemical deactivation effect was maintained in spite of amorphous TiO_2 formation.

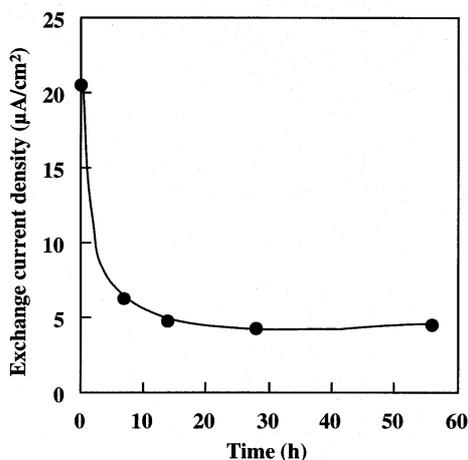


Figure 3 Changes with time in exchange current density of the TiN electrode in seawater with applied an alternating potential of 1.0 V vs. Ag/AgCl for 10 min and -0.6 V vs. Ag/AgCl in seawater.

Photochemical deactivation effect of TiO₂ film formed on TiN electrode

The photochemical deactivation effect of TiO₂ film formed on the TiN electrode surface was investigated. Figure 4 shows the viable cell number of *V. alginolyticus* cells attached to various electrode surfaces before and after irradiation using black light. The number of viable *V. alginolyticus* cells attached to the TiN electrode modified with amorphous TiO₂ film did not decrease with irradiation for 1 h. In contrast, the number of viable cells attached to the TiN electrode modified with crystalline TiO₂ film decreased and survival ratio was 2.2%. The amorphous TiO₂ film formed by applying alternating potentials in seawater had little photochemical deactivation effect.

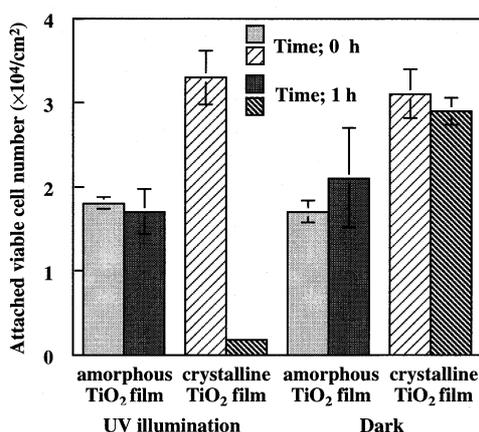


Figure 4 Attached viable cell number of *V. alginolyticus* cells on the TiN electrode modified with TiO₂ film after UV was illuminated. Amorphous TiO₂ film was formed on the TiN electrode by application of alternating potentials of 1.0 V vs. Ag/AgCl for 60 min and -0.6 V vs. Ag/AgCl for 10 min in seawater. Crystalline TiO₂ film was formed on the TiN electrode by application of a constant potential of 80 V for 10 min in aqueous solution containing sodium carbonate (200 g/l) and sodium fluoride (10 g/l).

Field experiments

Figure 5 shows TiN electrodes after immersion for 279 days in the sea, with and without applied alternating potentials. When alternating potentials were applied, attachment of organisms was prevented (Figure 5A). When no potential was applied, organisms attached and grew on the TiN electrode (Figure 5B). These organisms were mainly *Crassostrea gigas*, *Hydroides ezoensis* and barnacles. The wet weight of organisms attached to the TiN electrode after 279 days was 217.0 g/plates. When alternating potentials were applied, the corresponding wet weight was 10.3 g/plates. Upon application of alternating potentials, attachment of organisms to the TiN electrode did not increase during the experimental period.

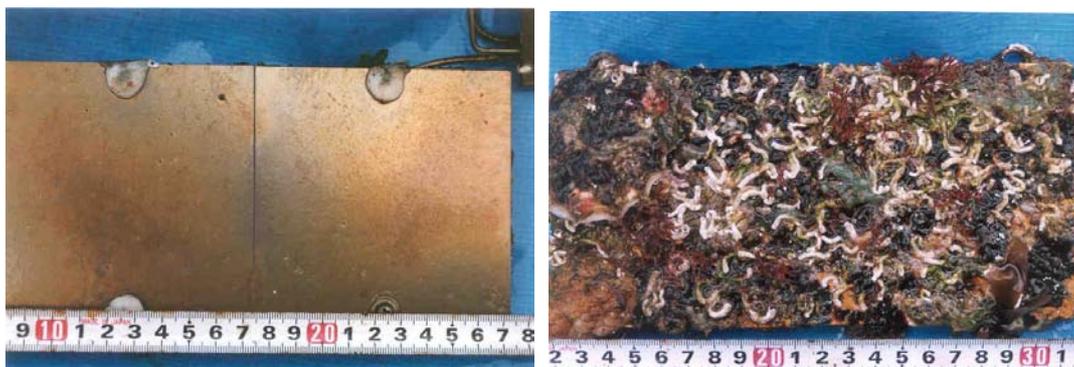


Figure 5 TiN electrode after 279 days with (left) and without (right) application of alternating potentials of 1.0 V vs. Ag/AgCl for 60 min and -0.6 V for 10 min. The experimental period was 9 July 1997 to 24 April 1998.

DISCUSSION

TiN has low electrical conductivity and is not degraded by an applied potential. It is a good material for electrochemical prevention of biofouling. Use of TiN prevented the attachment of biofouling organisms at a lower potential compared with carbon-chloroprene sheet electrodes or conductive-paint electrodes described previously (Matsunaga *et al.*, 1998; Nakasono *et al.*, 1993; Okochi *et al.*, 1995). The effectiveness of alternating potentials for prevention of biofilm and consequent attachment of other macroorganisms has been previously reported (Matsunaga *et al.*, 1998; Okochi *et al.*, 1995). Investigation of changes in TiN electrode surface properties by application of alternating potentials is important. If a TiO₂ film grows with applied alternating potentials and electronic conductivity of the TiN electrode surface decreases, electron transfer between the microbial cell and the electrode will be impeded, and long-term electrochemical antifouling cannot be guaranteed. Recently, the formation of titanium oxide (TiO₂) film on a TiN electrode has been reported by application of a constant potential (Azumi *et al.*, 1998; Nakayama *et al.*, 1998a). However, formation of the TiO₂ film by application of alternating potentials and its properties were not reported.

IR and Raman spectra indicated that amorphous TiO₂ film was formed on the TiN electrode surface by application of alternating potentials (Figures 1, 2). The appearance of broad absorbance around 3300 cm⁻¹ in IR spectrum suggests that the hydroxyl-

group exists in a TiO₂ film (Figure 2B). It had been reported that IR spectra obtained from oxide film on an aluminum surface by application of positive potential indicated that broad absorbance ascribed to alumina hydrate appeared around 3300 cm⁻¹ (Wada *et al.*, 1986; Wefers & Misra, 1987). Therefore, these results suggest that amorphous TiO₂ film containing titanium hydrate was formed. The formation of amorphous TiO₂ film on a TiN electrode reduced the exchange current density to 4.5 μA/cm². However, it did not further at longer electrolysis times. This indicates that the amorphous TiO₂ film formed on the TiN electrode was not a significant barrier to electron transfer and allows electrochemical deactivation of *V. alginolyticus* cells. These results suggest that long-term electrochemical antifouling with the TiN electrode can be achieved.

The photochemical inactivation effect of an n-type semiconductor such as crystalline TiO₂ was investigated (Matsunaga *et al.*, 1985, 1988; Matsunaga & Okochi, 1995). TiO₂ film formed on the electrode surface by application of alternating potentials could be involved in the deactivation of attached microbial cells. The amorphous TiO₂ film formed on the electrode surface has no effect on photochemical deactivation. This result revealed that microbial cells attached to the electrode were electrochemically deactivated.

The TiN electrode formed by reactive sputtering effectively prevents attachment of microbial cells to the substratum and subsequent formation of biofilm. As shown in Figure 5A, when alternating potentials were applied to the TiN-coated Ti plate, microbial fouling was not observed. Furthermore, attachment of invertebrates did not occur. *Crassostrea gigas* and *Hydroides ezoensis* attached to the TiN electrode without applied alternating potential (Figure 5B). These invertebrate larvae are known to attach on biofilm (Miura & Kajihara, 1984; Weiner *et al.*, 1989). Throughout our field studies, formation of cracks and reduction of electrical conductivity did not occur. These facts suggest that electrochemical antifouling with the TiN electrode formed by reactive sputtering can be maintained in the long-term. Thus, TiN formed by reactive sputtering is a practical electrode for electrochemical prevention of marine biofouling.

CONCLUSION

An electrochemical prevention of biofouling was investigated using a titanium nitride (TiN) electrode. As the results, following concluding remarks can be drawn:

- Amorphous TiO₂ film containing titanium hydrate was formed on the TiN electrode surface by application of alternating potentials. The film formation did not affect on photochemical deactivation of bacteria cells.
- *V. alginolyticus* cells on the TiN electrode forming amorphous TiO₂ film were deactivated electrochemically. Formation of the film did not impede the electrochemical deactivation of bacteria cells.
- Without generation of any toxic substances, attachment of organisms was inhibited by application of alternating potentials to the TiN electrode. This electrochemical prevention system against biofouling is a practical method without environmental problems.

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Electrochemical Prevention of Diatom Adhesion and Direct Estimation of Diatom Viability using TO-PRO-1 Iodide

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ABSTRACT

The adhesion of the marine diatom, *Nitzschia closterium* was controlled electrochemically and its viability was estimated by staining with fluorescent dye, TO-PRO-1 iodide. When cyclic voltammetry of *N. closterium* cells was carried out using a basal-plane pyrolytic graphite electrode at a scan rate of 20 mV·s⁻¹ in seawater, an irreversible anodic peak was observed at around 0.8 V versus saturated calomel electrode (SCE). *N. closterium* cells attached to the graphite electrode were killed by applying a potential of 1.0 V for 1 h in seawater. The efficiency of the electrochemical treatment was estimated using TO-PRO-1 iodide as a vital stain. Under blue excitation, viable and dead cells were visible as red and yellow colored cells, respectively. By applying -0.4 V, more than 80 % of the *N. closterium* cells attached to the electrode were removed. Therefore, by applying an alternating potential of 1.0 V and -0.4 V, the adhesion of marine diatoms, one of the main components of primary microbial films, was controlled.

INTRODUCTION

Marine diatoms and bacteria are the main microorganisms in primary microbial films, which can be precursors to the attachment and development of invertebrate larvae (Henschel & Cook, 1990; Weiner *et al.*, 1985). Fouling attachment and growth on surfaces can reduce the flow of seawater in cooling pipes, decrease heat transfer rate in heat exchanges and increase frictional resistance of ship hulls. Prevention of biofouling involves the use of biocidal chemicals such as copper (Austin, 1988) and

organotin (Jackson & Jones, 1988) and chlorine. However, these biocides cause marine environmental pollution and can adversely affect human health. Some species of diatom can also tolerate copper and organotins, which are used in antifouling paints (Daniel & Chamberlain, 1981; Callow, 1986). Environmentally acceptable methods for prevention of biofouling are therefore required.

We have previously reported on an electrochemical method for prevention of marine biofouling based on electrochemical sterilization (Nakasono *et al.*, 1993; Nakayama *et al.*, 1998). Microorganisms on the electrode surface were killed by applying a potential of about 1 V (Matsunaga *et al.*, 1984). Generation of toxic byproducts during electrolysis did not occur. Electrochemical sterilization results from oxidation of intracellular substances leading to cell membrane disruption and death (Matsunaga & Namba, 1984; Matsunaga *et al.*, 1995). Recently, we also reported that the marine diatom, *Nitzschia closterium* can be killed electrochemically and cell viability can be estimated by staining with the fluorescent dye, Quinolinium, 4-[(3-methyl-2(3H)-benzothiazolylidene)methyl]-1-[3-(trimethylammonio) propyl] - , diiodide (TO-PRO-1 iodide) (Okochi *et al.*, 1999). TO-PRO-1 iodide is a cationic cyanine-based nucleic acid stain and provides spectral alternatives for the detection of dead cells (Glazer & Rye, 1992; Hirons *et al.*, 1994; Li *et al.*, 1995).

In the present study, electrochemical prevention of algal biofilms and biofouling was investigated using the adhesive marine diatom, *N. closterium*. Potential settings were varied and the number of diatoms attached to the electrode surface and their viability estimated directly by staining with TO-PRO-1 iodide.

MATERIALS AND METHODS

Materials

Fluorescent dye, TO-PRO-1 iodide, was obtained from Molecular Probes, Inc. (Eugene, OR). Basal-plane pyrolytic graphite was obtained from Union Carbide Co. (New York, NY). Other reagents were commercially available analytical reagents. Seawater collected from Hachijojima offing (Japan) was filtered through a membrane filter (pore size; 0.45 μm) and autoclaved for 10 min.

Culture of *N. closterium*

N. closterium (IAM B-16) was used as a model marine fouling diatom. *N. closterium* was cultured in 40 ml of SW2 medium under continuous illumination with cool white fluorescent lamps (50 $\mu\text{Einstein}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 25°C with air bubbling through the media. The SW2 medium contained 72.0 mg KNO_3 , 4.5 mg KH_2PO_4 , 10.0 mg β -glycerophosphate disodium salt, 0.5 g tris(hydroxymethyl)aminomethane, and 5 ml Fe solution (as EDTA; 1:1 molar; containing 0.70 % $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2\cdot 6\text{H}_2\text{O}$ and 0.66 %

Na₂EDTA·2H₂O in distilled water) in 1.0 liter of seawater (pH 8.2). After culturing *N. closterium* for 7 days, the cells were centrifuged at 2300×g at room temperature for 10 min and resuspended in sterile seawater. Cell concentration was determined with a hemocytometer. Counting of about 20 cells in the chamber (area; 0.64 mm², depth; 0.02 mm) was repeated 10 times and mean cell numbers were calculated.

Cyclic voltammetry

The basal-plane pyrolytic graphite electrode (0.19 cm²) was polished and washed by sonication in distilled water. *N. closterium* cells (1.0×10⁷ cells) were immobilized on the membrane filter (pore size; 0.45 μm) by aspiration and the membrane filter placed in contact with the electrode surface. The reference electrode was a saturated calomel electrode (SCE) and the counter electrode was a platinum wire. The cyclic voltammogram was obtained in sterile seawater using a potentiostat with a wave generator (model HAB-151, Hokuto Denko Co., Tokyo, Japan) and x/y recorder (model F-35, Riken Denshi, Tokyo, Japan).

Electrochemical inactivation and detachment of *N. closterium*

The electrochemical system was the same as in the cyclic voltammetry experiment. A graphite electrode (1.78 cm²) was immersed in a *N. closterium* cell suspension (3.0×10⁶ cells·ml⁻¹) for 120 min at room temperature and stirred at 300 rpm. During this period, *N. closterium* cells were attached to the electrode surface. The electrode was removed from the cell suspension and immersed once in sterile seawater to remove poorly attached cells. The electrode was immersed again in sterile seawater and constant potentials were applied with a potentiostat (model HA-151, Hokuto Denko Co., Tokyo, Japan). After applying constant potentials, the electrode was removed from seawater. The number of viable and dead *N. closterium* cells attached to the electrode was determined by staining with TO-PRO-1 iodide. Detachment of diatoms was estimated by counting the cells that remained attached to the electrode surface.

Electrochemical prevention of the adhesion of *N. closterium*

Prevention of the adhesion of *N. closterium* cells was carried out by applying alternating potentials. Graphite electrodes were immersed in stirred *N. closterium* cell suspensions containing 2.0×10⁶ cells·ml⁻¹. The potential was controlled using a pulse generator (model HB-211, Hokuto Denko Co., Tokyo, Japan) and the numbers of viable and dead cells attached to the electrode determined over time.

Direct estimation of viable and dead *N. closterium*

N. closterium cells on the electrode surface were stained with TO-PRO-1 iodide and a cover slide placed on the electrode. After 30 min incubation, the electrode surface was observed with an epifluorescence microscope (Olympus Optical Co., Ltd., model IMT-2, Tokyo, Japan) under blue excitation, and four positions were photographed for each condition. Viable and dead cells were visible as red and yellow fluorescent cells respectively. Cells were counted, and the TO-PRO-1 iodide-stained cell ratio was defined as follows:

$$\text{TO-PRO-1 iodide-stained cell ratio (\%)} = (\text{yellow fluorescent cell number} / \text{red and yellow fluorescent cell number}) \times 100$$

RESULTS

Cyclic voltammetry of *N. closterium*

Figure 1 shows a cyclic voltammogram of *N. closterium*, obtained in sterile seawater. An anodic peak current appeared at 0.8 V in a first scan in the positive direction. Upon scan reversal, no responding reduction peak was obtained. An irreversible direct electron transfer between *N. closterium* and graphite electrode was therefore implicated. When cyclic voltammetry taken without *N. closterium* cells, the corresponding peak was not observed, demonstrating that this peak only appeared when cells were in contact with the electrode.

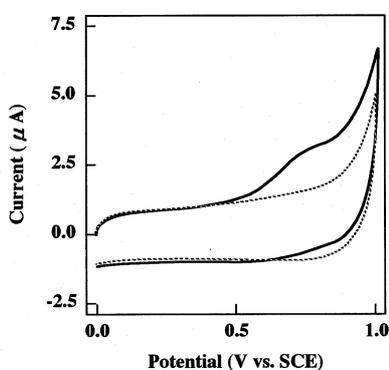


Figure 1 Cyclic voltammogram of marine diatom, *N. closterium* on basal-plane pyrolytic graphite electrode (0.19 cm²). Dashed line indicates the cyclic voltammogram without cells. Scan rate was 20 mV·s⁻¹.

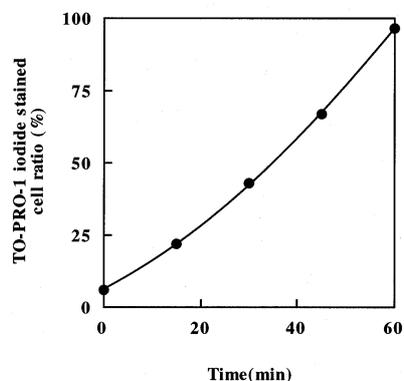


Figure 2 Time course of electrochemical killing of *N. closterium* when potential of 1.0 V vs. SCE was applied. TO-PRO-1 iodide concentration was 64.5 µg·ml⁻¹.

Electrochemical inactivation of *N. closterium*

Electrochemical inactivation of *N. closterium* was investigated by applying a constant potential of 1.0 V, at which inactivation had previously been observed (Okochi *et al.*,

1999). When cell viability was followed with time, TO-PRO-1 iodide-stained cell ratio increased linearly with time to 98 % after 60 min (Figure 2). After the electrochemical treatment, *N. closterium* cell viability was directly estimated with dead cells visible as yellow fluorescent cells.

The pH of seawater used in this experiment was 7.54 and after application of 1.0 V for 60 min was 7.51. The chlorine concentration after applying 1.0 V was below the detection limit of 0.02 ppm. However, after the potential of 1.2 V was applied, the pH of seawater decreased and chlorine concentration increased. In further experiments, application of 1.0 V for 60 min was employed for killing *N. closterium* cells.

Detachment of *N. closterium* from electrode surface

The effect of applied potential on the number of *N. closterium* cells remaining on the electrode is shown in Figure 3. Potentials of -0.6, -0.4, -0.2 V were applied for 30 min in sterile seawater after applying the potential of 1.0 V for 60 min. When no potential was applied, the number of cells on the electrode was 3.3×10^4 cells.cm⁻². In contrast, when negative potentials were applied, the cell number of attached cells decreased. At -0.4 V, cell numbers dropped to 0.6×10^4 cells.cm⁻², with more than 80% of *N. closterium* cells therefore detached. The detachment experiment was performed in duplicate and was reproducible with a standard deviation of 10%.

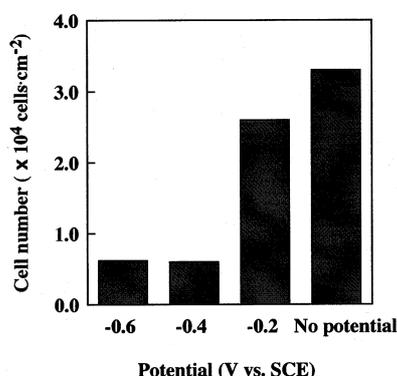


Figure 3 Detachment of *N. closterium* cells on the electrode (0.79 cm²). The potential was applied for 30 min in sterile seawater. Initial adhered cell number was 3.3×10^4 cells.cm⁻².

Prevention of the adhesion of *N. closterium*

Inactivation and detachment of *N. closterium* were achieved by applying alternating potentials of 1.0 V and -0.4 V in cell suspension of *N. closterium*. When no potential was applied, the number of *N. closterium* cells attached to the electrode increased to 1.1×10^5 cells.cm⁻² with all cells alive after 540 min (Figure 4A). On the other hand, when a constant potential of 1.0 V was applied, the number of attached cells was reduced to 5.9×10^4 cells.cm⁻² and the TO-PRO-1 iodide-stained cell ratio was 88 % after 540 min immersion (Figure 4B). In contrast, with alternating potentials of 1.0 V and -0.4 V, the

number of attached cells after 540 min was 1.0×10^4 cells.cm⁻² and the TO-PRO-1 iodide-stained cell ratio was 95 % (Figure 4C). It was shown that adhesion of *N. closterium* cells on the electrode was controlled by applying alternating potential of 1.0 V and -0.4 V.

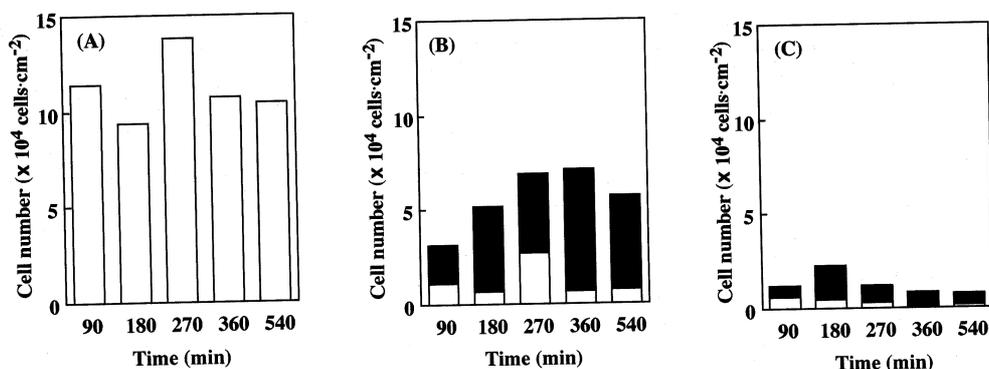


Figure 4 Time course of adhered viable (□) and dead (■) cell number of *N. closterium* on the electrode (4.0 cm²). Electrodes were immersed in cell suspension containing 2.0×10^6 cells·ml⁻¹. A, no potential; B, constant potential of 1.0 V vs. SCE; C, alternating potential of 1.0 V vs. SCE for 60 min and -0.4 vs. SCE for 30 min.

DISCUSSION

Although the adhesion of diatoms on the substratum is slow compared to bacterial cells, diatoms do secrete polysaccharides and form biofilms (Bhosle *et al.*, 1993; Daniel *et al.*, 1980). We have previously investigated the electrochemical prevention of the accumulation of bacterial cells on electrode surfaces by application of alternating potentials, which effectively killed and desorbed bacterial cells (Nakayama *et al.*, 1998; Okochi *et al.*, 1998). In this study, similar electrochemical treatment was applied to diatoms. The cyclic voltammogram of diatom *N. closterium* (Figure 1), was found to be similar to that of gram-negative and gram-positive bacteria (Matsunaga & Nakajima, 1985) or the yeast, *Saccharomyces cerevisiae* (Matsunaga & Namba, 1984). The cyclic voltammogram suggested that diatoms could also be controlled electrochemically. *N. closterium* cells attached to the surface of a graphite electrode were killed by applying a potential of 1.0 V, which is higher than the potential at which the oxidation peak was observed. Changes in pH and chlorine generation in the electrolyzed seawater occurred at potential of 1.2 V, due to the anodic conversion of H₂O to 1/2 O₂ and 2H⁺, and Cl⁻ to Cl₂. At 1.0 V, little change in pH was observed and the chlorine concentration was below the detection limit of 0.02 ppm. Bromide ions in the seawater could possibly react electrochemically at constant potential of 1.0 V but, considering the abundance (65 mg/l, that is 1/10³ compared to chlorine) and effectiveness of the bromide ion in seawater, it is not considered likely to have caused the mortality of *N. closterium* cells. Mortality of *N. closterium* cells is considered to be due to the direct

electrochemical oxidation of intracellular substance rather than from electrochemically generated toxic substances.

The detachment of diatoms by negative potentials might be due to the electrical repulsion of cells from the electrode due to the cells generally being negatively charged. At 1.0 V, diatoms were killed and the secretion of polysaccharides was prevented, but cells did not detach. Cell accumulation might therefore be observed overtime despite constant application of potentials of 1.0 V. By applying alternating potentials of 1.0 V and -0.4 V, both death and detachment of diatoms was achieved. The formation of the algal biofilm could therefore be prevented. An electrochemical method for the prevention of algal biofilm formation and biofouling is therefore demonstrated. Use of the TO-PRO-1 iodide staining technique for direct estimation of cell numbers and viability was also demonstrated. This staining method might also be useful for estimation of cell viability in other microalgae, such as cyanobacteria and green algae that contain intracellular chlorophyll (Okochi et al., 1999).

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Development of a New Antifouling Paint Based on a Novel Zinc Acrylate Copolymer

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ABSTRACT

Anti-fouling paints based on tributyl tin acrylate copolymers have been proven to provide excellent performance when applied to ocean going vessels. However, pollution of the marine environment from the release of tributyltin has been a matter for concern for some time now, such that their use has been restricted in many countries. It seems likely that these products will be phased out by the year 2003 at the very latest. We have managed to develop a zinc acrylate copolymer for use in anti-fouling, as a replacement for the tributyltin (TBT) copolymer. Zinc ions from the new copolymer exchange with sodium ions from the sea, the resulting structure will then dissolve in a controlled manner into the seawater. It is possible to control the erosion rate of this novel copolymer by altering one or more of either the molecular weight of the polymer, the concentration of the functional group or the hydrophilicity of the zinc acrylate polymer. The performance and long-term physical properties, the erosion rates and the surface profiles of the new anti-fouling paints formulated with various zinc acrylate copolymers have been evaluated by dynamic immersion. The results suggest that paints formulated using this new zinc acrylate copolymer will show similar performance to existing TBT acrylate copolymer anti-fouling paints.

INTRODUCTION

Fouling is the growth of animals and plants on the surface of submerged objects. The fouling of ships results in a reduction of speed, increased fuel consumption, and consequently losses of both time and money. (Woods Hole Oceanographic Institution, 1952). In 1984, a committee of the Japanese Ship Research Association investigated the effects of fouling on ship speed. They found that a speed reduction of 0.03 knots would occur due to increased friction resulting from 10 μ m increase in surface roughness. This

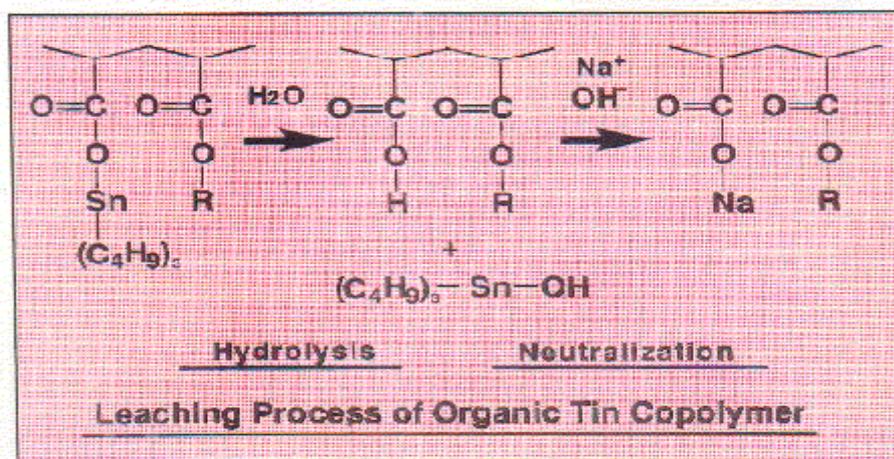


Figure 1 Leaching process of organic tin copolymer

would increase fuel consumption by 1%. They also found that a speed reduction of 1.11 knot/ year would increase fuel consumption by 52%/ year. Various types of anti-fouling paints have been used to address the fouling problem and the most effective have been tributyltin (TBT) acrylate copolymer based self-polishing anti-fouling (SPC) paints. The TBT copolymer facilitates continuous surface renewal and release of active ingredient TBT by its hydrolysis reaction with seawater (Figure 1).

The hydrolysis/erosion process confers several key properties on the TBT copolymer paint system, which includes:

- the ability to control/ regulate biocide leaching rate, and
- the erosion process generates a smoother surface (Bennett, 1996).

TBT is now known to be toxic or to have harmful effects on a range on non-target marine species (de Mora, 1996; Maguire, 1996; Hall & Bushong, 1996; Laughlin *et al.*, 1996). The use of organic tin-based anti-fouling paints has therefore been restricted or banned in many countries. The Marine Environment Protection Committee (MEPC) of the International Maritime Organization (IMO) is presently developing a legal instrument to implement a global ban on TBT anti-fouling coatings. At MEPC 42, in November 1998, the committee set dates of 1 January 2003 and 1 January 2008 for the ban on application, and total absence of TBT respectively (Anon., 1999)). The committee also urged member governments to encourage industries to continue to develop, test and use, as a high priority, anti-fouling systems which would not adversely impact non-target species or otherwise degrade the marine environment (MEPC, 1998). Paint manufacturers have responded to this call and are actively seeking to develop alternative products.

As illustrated above, after hydrolysis, the organic tin co-polymer dissolves and is subsequently neutralized by seawater (Figure 1). The resulting carboxylic sodium salt then makes the polymer soluble. Similarly, to make acrylic polymers soluble in seawater, metallic ions can be introduced which can readily exchange with sodium ions in the seawater. We have evaluated several materials and, from these, zinc was

found to be the most suitable candidate for the purpose. The structure of the zinc acrylate co-polymer is shown in Figure 2.

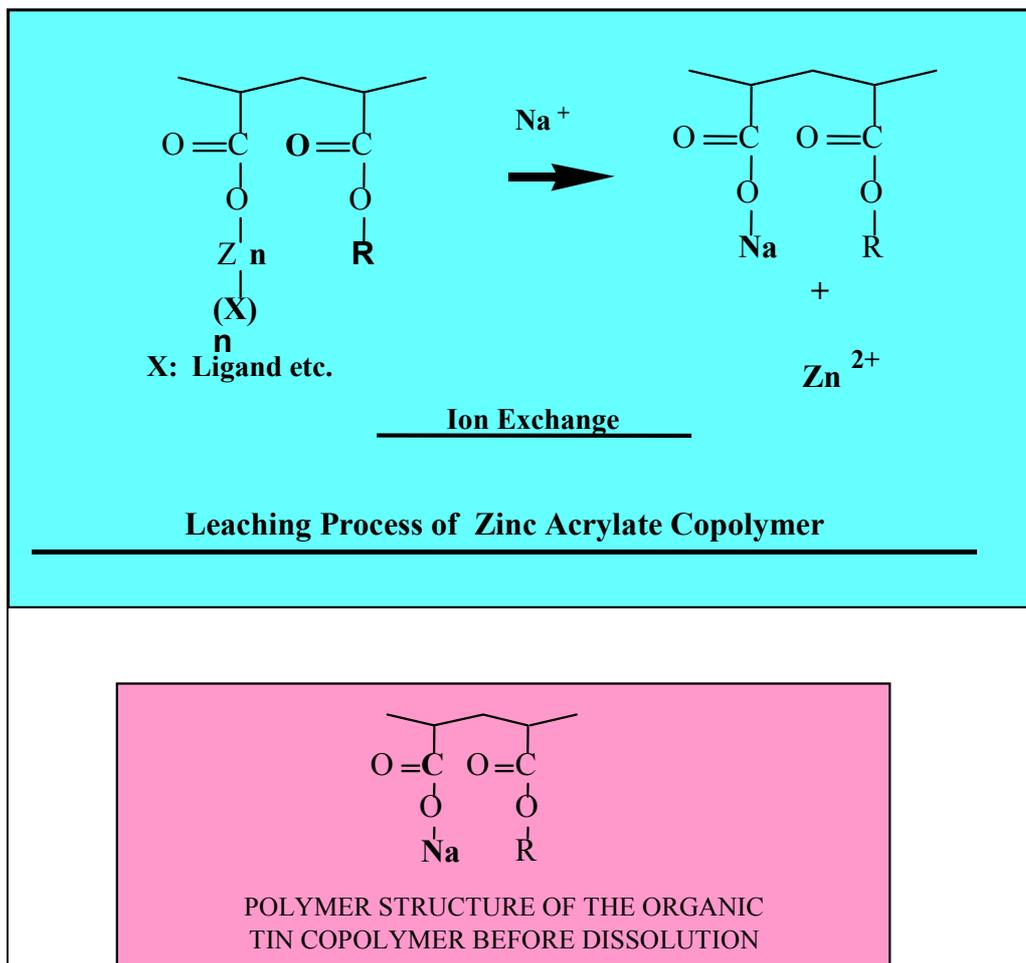


Figure 2. Leaching process of zinc acrylate copolymer

MATERIALS AND METHODS

This study examined two materials: zinc acrylate copolymer and anti-fouling paints based on the zinc acrylate copolymer. We considered various factors that may be responsible for dissolution rate of the copolymer: molecular weight, functional group concentration, and hydrophilicity of the zinc acrylate copolymers. We evaluated various copolymers by varying these parameters.

Hydrophilicity of the copolymers was measured in terms of their solubility parameter using the turbidity titration method (Suh & Corbett, 1968):

$$\delta = \frac{(V_{ml})^{1/2}\delta_{ml} + (V_{ml})^{1/2}\delta_{mh}}{(V_{ml})^{1/2} + (V_{ml})^{1/2}}$$

- δ : Solubility parameter of polymer
 V_{ml} : Volume of low SP solvent
 V_{mh} : Volume of high SP solvent
 δ_{ml} : SP of low SP solvent
 δ_{mh} : SP of high SP solvent

Evaluations of various zinc acrylate copolymers were performed by application of the clear resin film to a glass panel using 400 μ m applicator (dry film thickness 100 μ m). These glass panels were then immersed in the natural seawater for one month. Dissolution, or leaching rates were then calculated in terms of μ m / month.

Numerous anti-fouling paints formulated with various zinc acrylate co-polymers were also evaluated. Typical formulation, test items and test methods are described in Tables 1 and 2.

Table 1 Paint formulation

Ingredients	%wt
Zinc acrylate copolymer	14
Cuprous oxide	35
Iron oxide	10
Polyamide wax	1
Solvent	40
Total:	100

Table 2 Test parameters & test methods

Parameter	Test Method
Polishing rate	Rotary machine @ 15 knots
Tensile strength & elongation	Tensilon
Internal stress	Phosphor brons plate
Hardness	Tukon hardness gauge
Surface profile	Laser microscope

Self-polishing or erosion rate tests were carried out by dynamic immersion using a rotary machine immersed in the seawater (Figure 3). Test panels were prepared, and test condition were as follows:

Panel preparation:

Panel material:	Mild steel
Surface treatment:	Sanding using 240 mesh sand paper
Shop primer:	Zinc Epoxy 15 μ DFT
1 st & 2 nd coats:	Epoxy AC 2 x 100 μ DFT coats
Final coat:	AF Paint 150 μ DFT

Test conditions:

Testing Equipment:	Dynamic rotary machine
Location:	Shimizu, Japan
Seawater temperature:	Average 20°C
Speed of the rotor:	15 knots
Test duration:	6 months

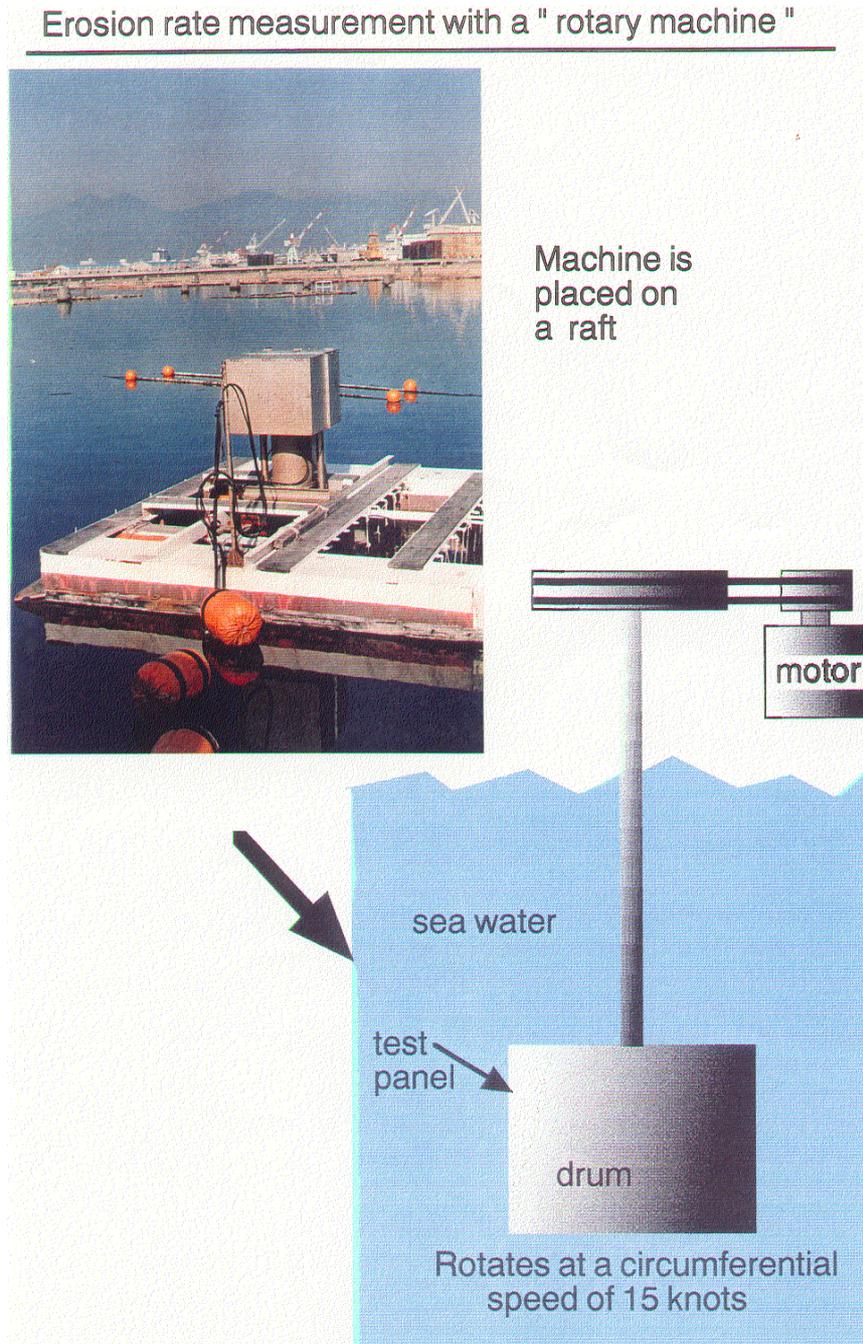


Figure 3 Dynamic immersion test using rotary machine.

RESULTS & DISCUSSION

In the clear resin film tests of the zinc acrylate copolymer, molecular weight did not greatly affect the dissolution rate. However, the dissolution rate could be controlled by functional group concentration and hydrophilicity of the copolymer. As functional group concentration increases, the dissolution rate of the copolymer also increases (Figure 4). Monomer composition determined the hydrophilicity of the polymer and the dissolution rate could therefore be controlled by the type of monomer composition. We have used the solubility parameter of the polymer to derive the relation between the hydrophilicity and dissolution rate. Figure 5 shows the relationship between the solubility parameter and dissolution rate. We have also tested dissolution of the polymer at different salt concentrations and pH levels and both affected (Figure 6).

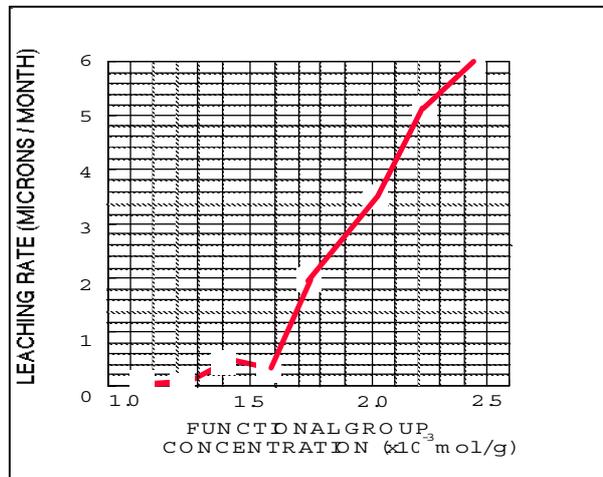


Figure 4 Functional group concentration vs dissolution rate in natural seawater

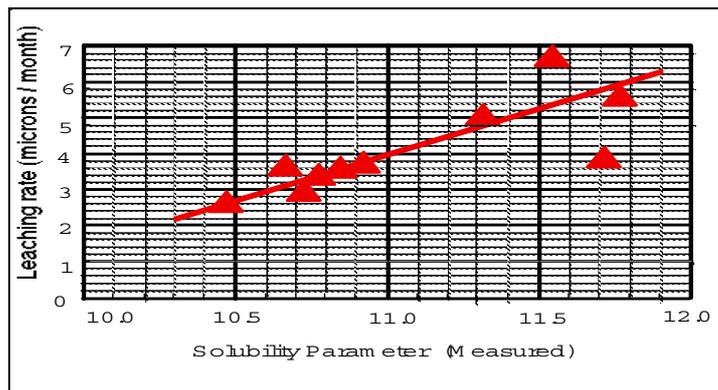


Figure 5 Solubility parameter vs dissolution rate in seawater

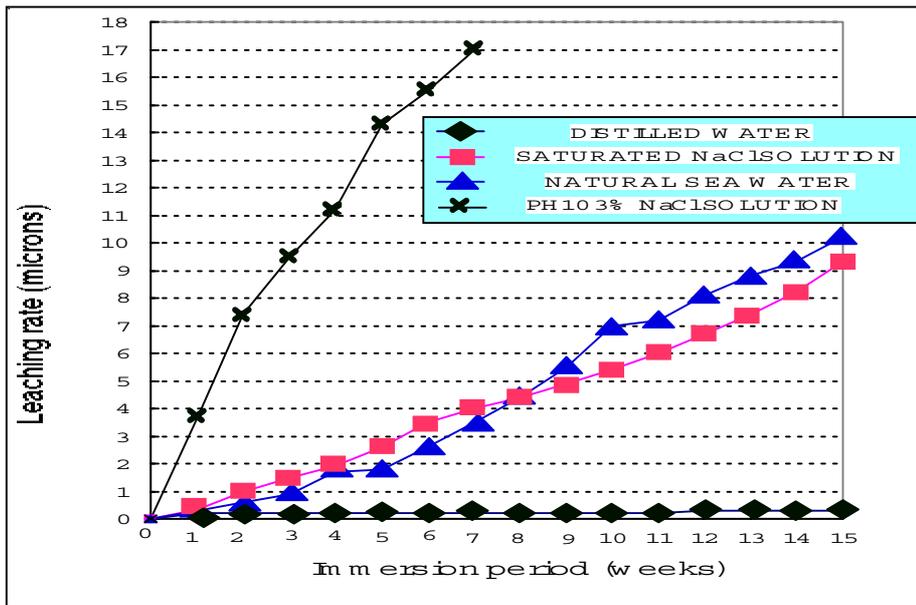


Figure 6 Dissolution rate of zinc acrylate copolymer in various solutions

The dissolution rate is also affected by water penetration. However, due to the unique structure of zinc acrylate polymer, the water resistance is still sufficient to enable effectiveness in underwater conditions, even with high levels of hydrophilic monomers in the polymer (Figure 7).

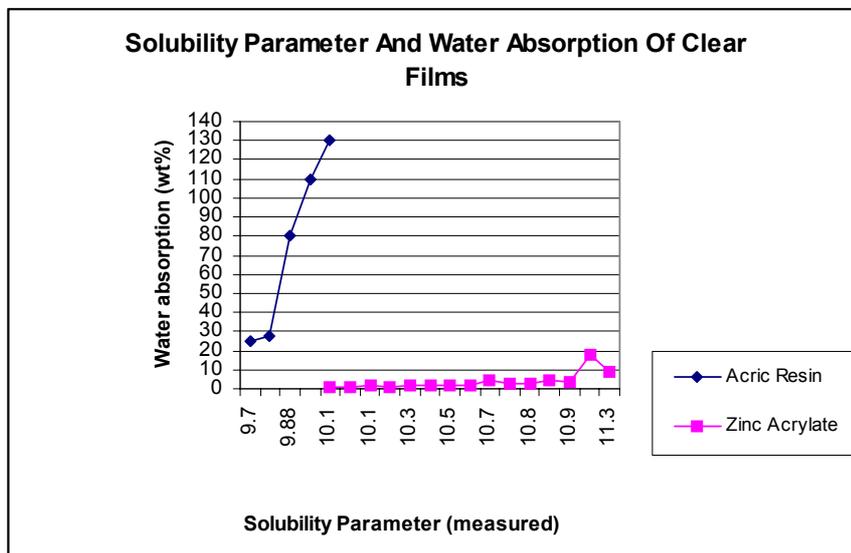
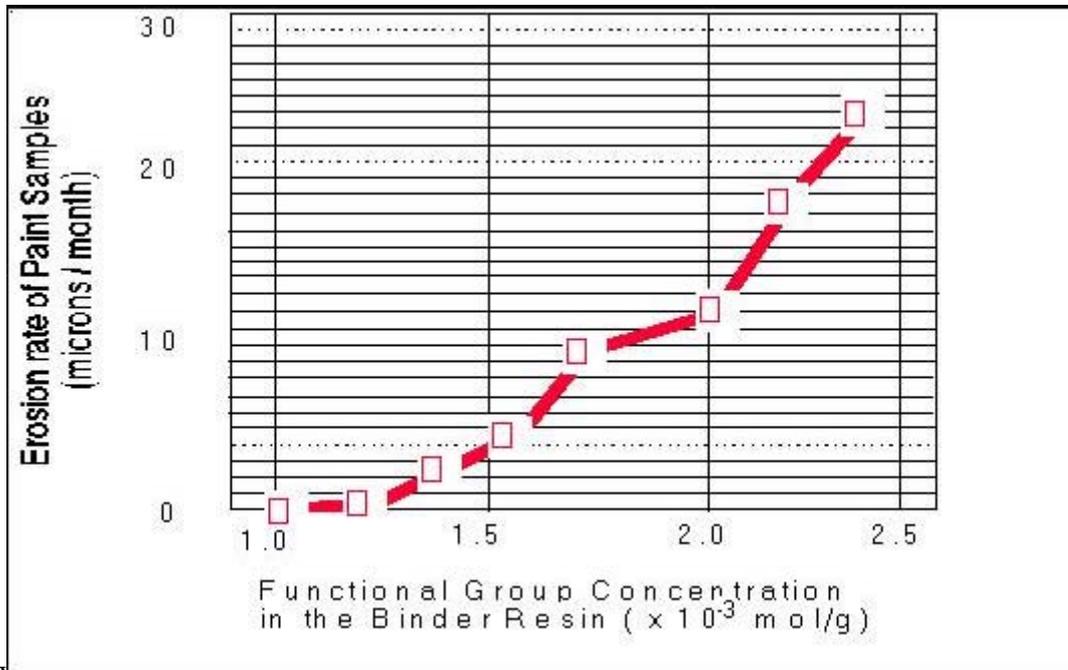


Figure 7 Water absorption of acrylic resin & zinc acrylate copolymer

As expected from the dissolution rate of the copolymer itself, anti-fouling paints formulated with various zinc acrylate copolymers were found to have erosion rates mainly dependent on function group concentration and hydrophilicity of the resin polymer (Figures 8, 9). The surface profile of the zinc acrylate anti-fouling, observed



by atomic microscopy) was also found to be similar to that of the zinc acrylate anti-fouling paints. The roughness was less than 10 microns (Figures 10, 11).

Figure 8 Erosion rate vs functional group concentration

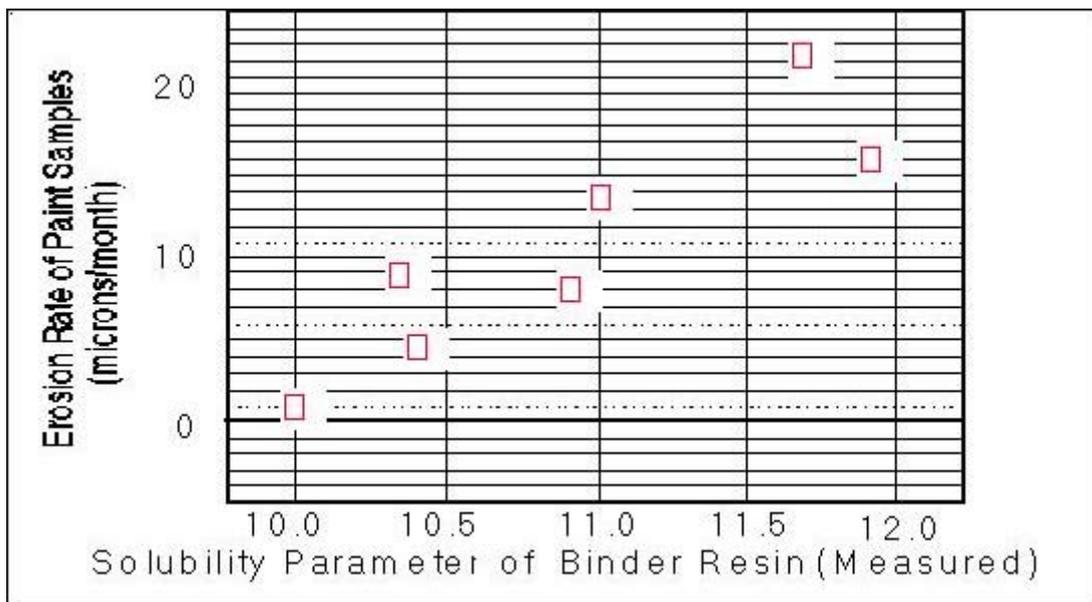
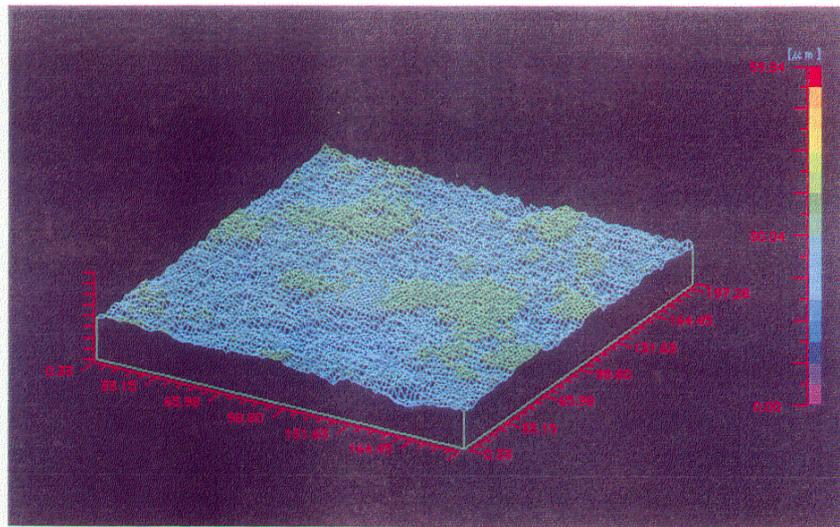
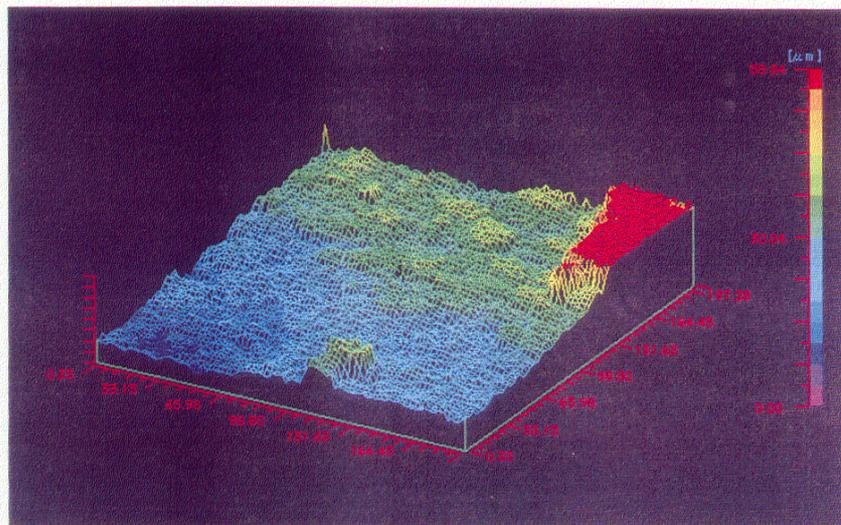


Figure 9 Erosion rate vs solubility parameter



Tin Free SPC Type

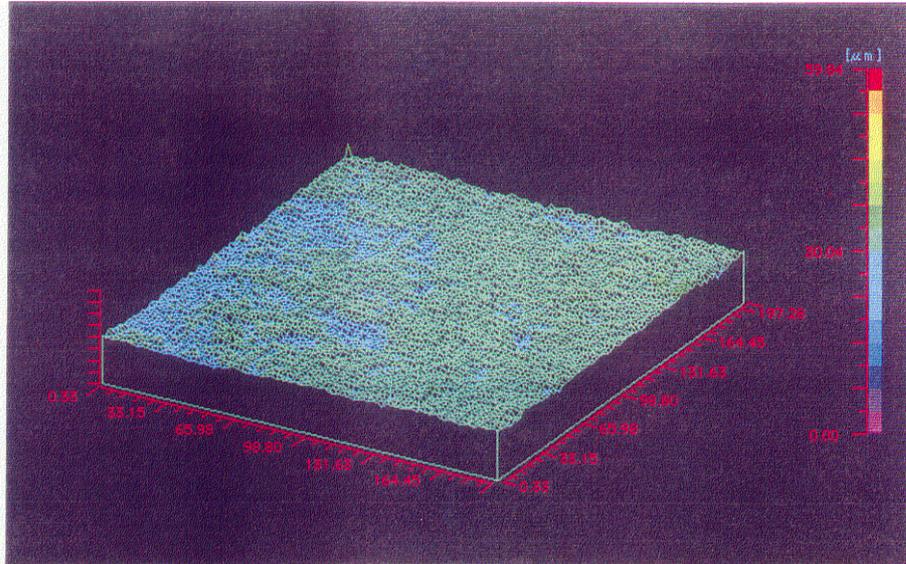


Conventional Tin Free Type

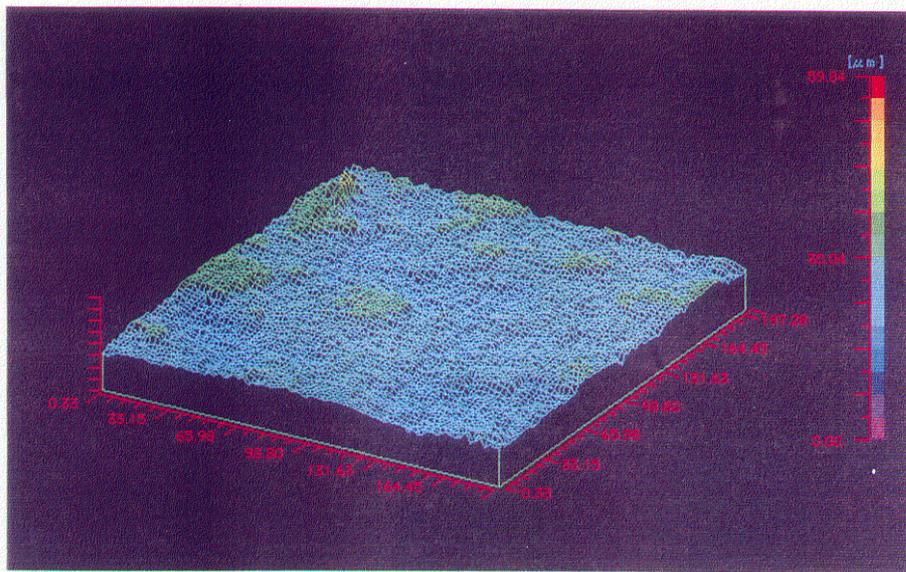
Surface Profile of the Antifouling

3D-Image by laser micro scope (magnification X 500)

Figure 10 Surface profiles of paints based on Tin-free SPC and Conventional Tin-free



A paint based on the Zinc Acrylate Copolymer



Organic tin SPC Type

Surface Profile of the Antifoulings

3D-Image by laser micro scope (magnification X 500)

Figure 11 Surface profiles of paints based on zinc acrylate copolymer and organic tin SPC

Retention of physical properties is an important aspect of anti-fouling, otherwise serious defects such as cracking, peeling etc. can occur. We have tested elongation, tensile strength, internal stress and hardness before and after immersion in seawater (Figures 12-16). Physical properties of the paint film were optimised by selecting proper ranges of the functional group concentration and the solubility parameter of binder resins. There was not much difference in the hardness of the paint film before and after seawater immersion. This is due to the stability of the polymer in seawater while dissolution of the outer layer is taking place.

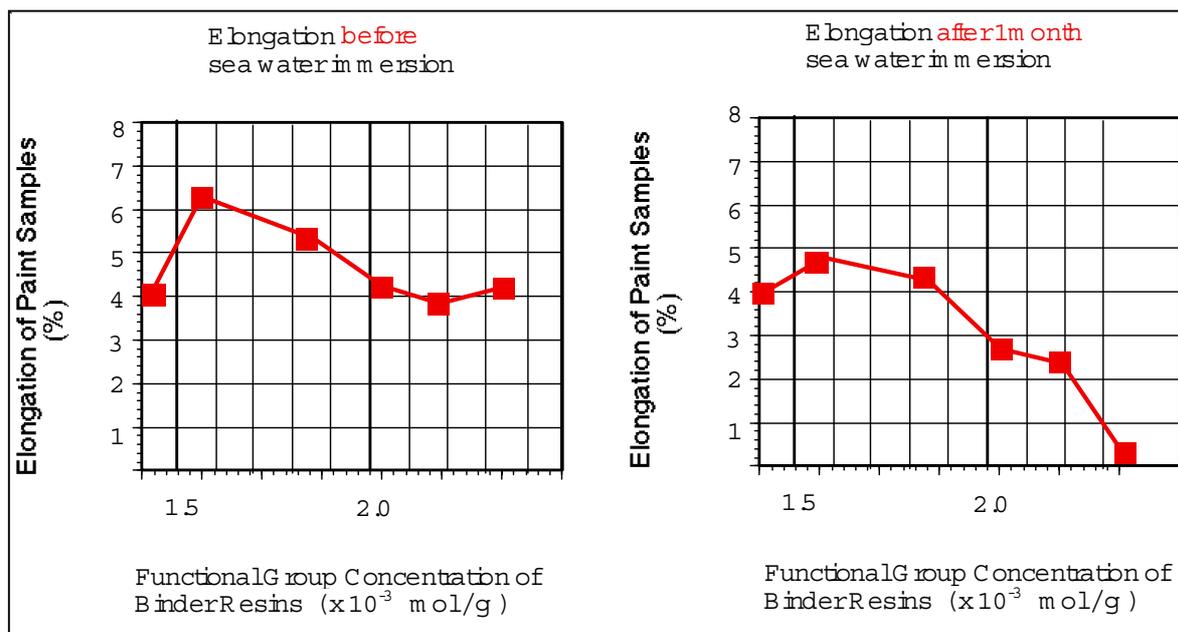


Figure 12 Elongation vs functional group concentration before & after immersion

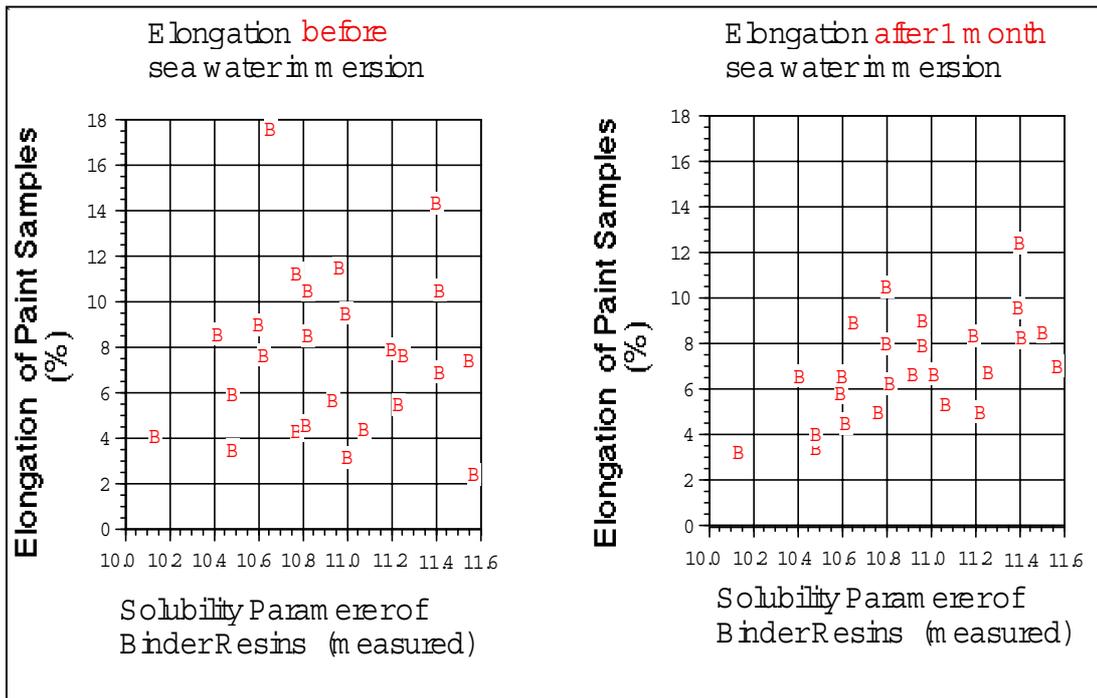


Figure 13 Elongation vs solubility parameter before & after immersion

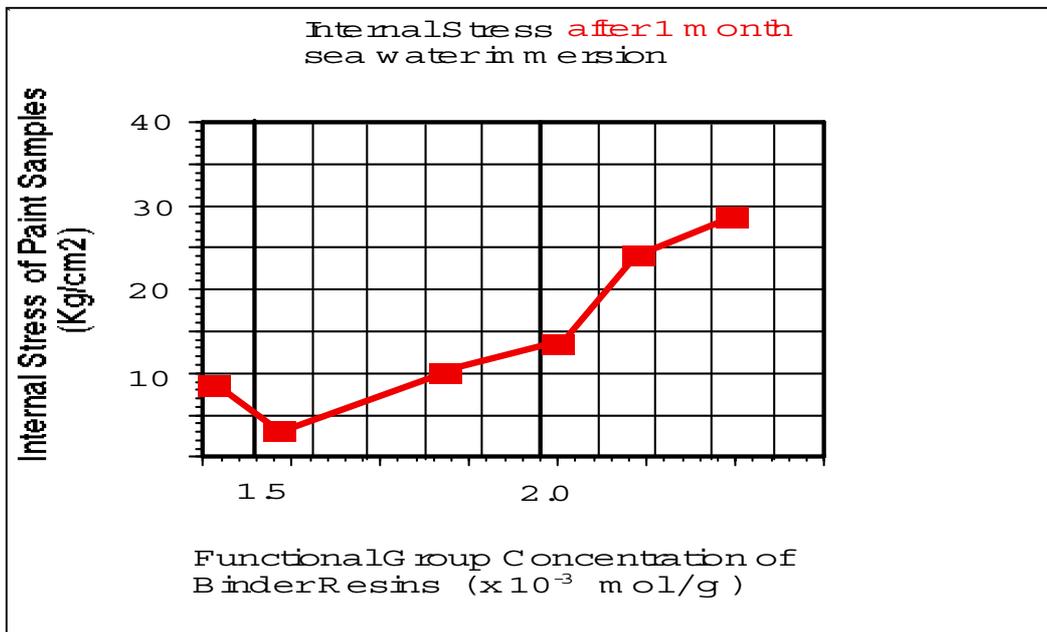


Figure 14 Internal stress vs functional group concentration after seawater immersion

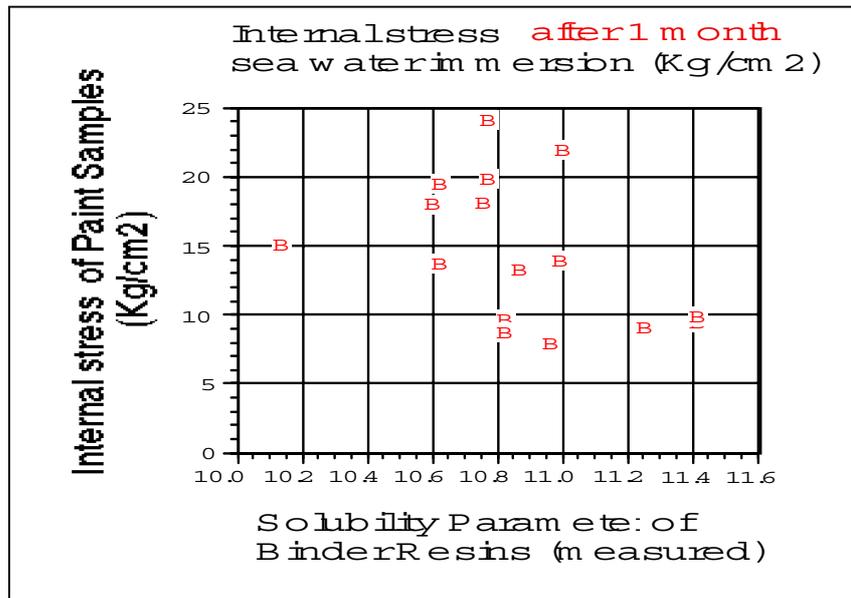


Figure 15 Internal stress vs solubility parameter after seawater immersion

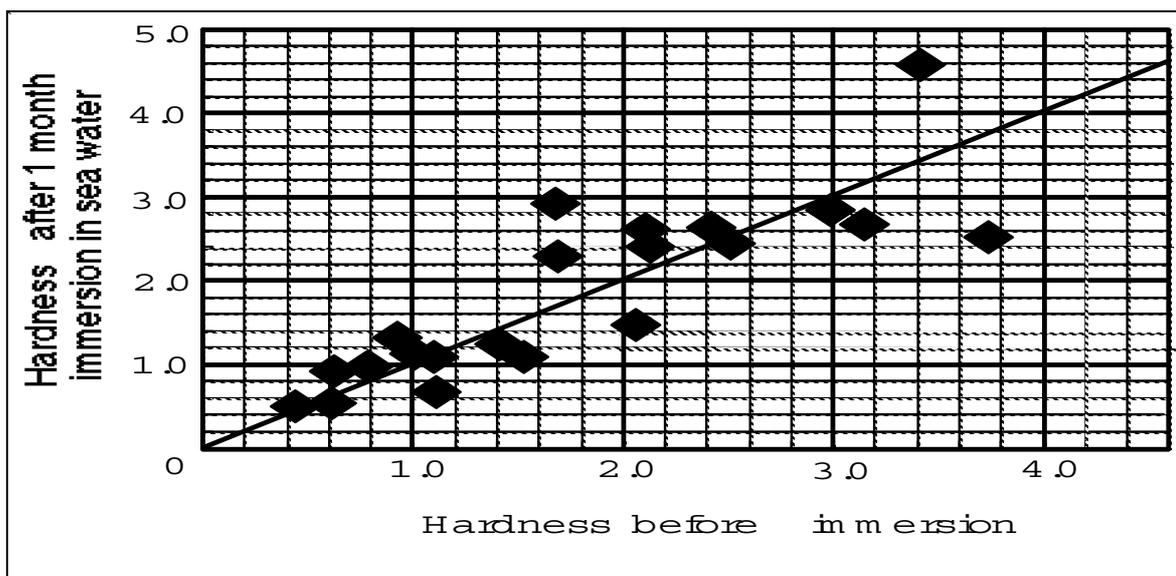


Figure 16 Paint film hardness before and after immersion

SUMMARY & CONCLUSION

In summary, the zinc acrylate copolymer has the following characteristics:

- Its dissolution rate can be controlled by varying the concentration of the functional group and the hydrophilicity of the polymer.
- The molecular weights do not greatly affect the dissolution rate.

- Its dissolution rate depends on the salt concentration and pH of the immersion medium.

In performance tests anti-fouling paints based on zinc acrylate it was found that:

- The erosion rate can be controlled easily and accurately by varying the functional group concentration and the solubility parameter of the binder resins.
- In rotor tests, the surface roughness of the zinc polymer based anti-fouling paint was similar SPC AF and smoother than other tin-free anti-fouling paints.
- To achieve and maintain optimum physical properties, correct solubility parameters and functional group concentrations are required.

We conclude that a novel zinc acrylate copolymer is a potential candidate to replace tributyltin based anti-fouling coatings. We found that it is possible to control the erosion rate of this novel copolymer by altering one or both of either the concentration of the functional group or the hydrophilicity of the zinc acrylate copolymer. By using zinc acrylate copolymers in combination with cuprous oxide and other biocides, long term anti-fouling performance and physical durability is expected to be similar to existing TBT acrylate copolymer coatings.

Acknowledgement

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5. Antifouling Biocides in the Environment

The Legacy of 110 Years of Dockyard Operations
Patricia Shaw and Barbara Hickey

The Effects of Changes in Environmental Parameters on the Release of Organic
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10th International Congress on Marine Corrosion and Fouling
University of Melbourne, February 1999: Additional Papers

The Legacy of 110 Years of Dockyard Operations

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ABSTRACT

The Devonport Naval Base, including Calliope Dock, is the only naval dockyard in New Zealand. Both naval and commercial shipping have used it for over 100 years. The seriousness of the impact of dockyard activities over the years, particularly on the marine environment, has only recently been recognised. A variety of contaminants have been detected in the sediments around the dockyard. Of particular concern are the high levels of TBT, DDT and mercury present. The RNZN has been actively working to develop ways of reducing the environmental impact and remediate existing contaminated areas adjacent to the dockyard.

INTRODUCTION

Areas around the wharves and drydock entrance of the Devonport Naval Base require periodic maintenance dredging. Dredging and disposal of marine sediments requires the approval of regulatory authorities. Since the late 1980's the authorisation process for dredging and disposal of marine sediments has included a requirement to document sediment quality.

In 1993 the sediment at the entrance to Calliope Drydock was sampled in preparation for an application to dredge. Analysis revealed the presence of a range of contaminants including tributyltin (TBT), polychlorinated biphenyls (PCBs) and heavy metals (Kingett Mitchell, 1994). In particular, DDT and mercury were present at unexpectedly high levels. Subsequent investigations were primarily concerned with DDT and mercury due to the high levels present, but the whole range of industrial pollutants was expected to be present.

As a result of the contamination, dredging at the drydock entrance did not take place as planned. Instead the RNZN has conducted investigations into the scope of the contamination around the whole dockyard, and the way forward, including remediation options and prevention of further contamination. These investigations subsequently raised concerns about possible sources of future contamination.

This paper presents an overview of the 110 year history of the dockyard and the status of the resulting contamination, including the steps taken and planned to manage the site.

HMNZ DOCKYARD

The Devonport Naval Base is located in the Waitemata Harbour in Auckland, New Zealand’s largest city and a major port. The Waitemata harbour is heavily used for recreational activities and has bulk fuel storage facilities and a number of marinas, boatyards and slipways. Large portions of the harbour are identified as conservation areas in the regional coastal plan.

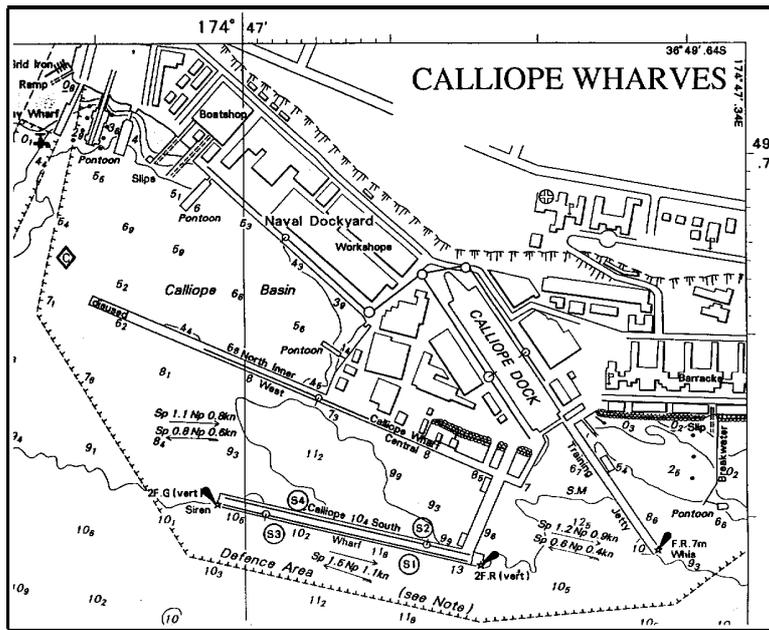


Figure 1 Devonport Naval Base
 NZ Chart 5322 Auckland Harbour, Reproduced by permission of Land Information New Zealand.
 CAUTION: NOT TO BE USED FOR NAVIGATION

HMNZ Dockyard is part of the Devonport Naval Base (see Figure 1). It is the only naval dockyard in New Zealand. It includes the Calliope Dock, the larger of the two drydocks in New Zealand, which has been used by both naval and commercial shipping since its opening in 1888. Other facilities and activities on the naval base

include a 200 tonne syncrolift, engineering workshops, berthing for naval vessels and RNZN training schools. There has been a fuel storage installation on the naval base in the past.

Key events in the development of the dockyard are summarised in Table 1.

Table 1. Key events in the development of HMNZ DOCKYARD

Year	Highlight
1888	Calliope Dock opened, owned by Auckland Harbour Board (AHB)
1890	Navy moved onto a site adjacent to dock and workshops
1926	Oil fuel installation developed
1936	Navy took over all facilities (land, workshops, wharves, jetties, seabed) except dock
1987	Navy took ownership of Calliope Dock
1989	Partial ban on the use of TBT antifoulants introduced in New Zealand
1992	Interim (rudimentary) waste treatment plant installed in dock
1993	Total ban on the use of TBT antifoulants introduced in New Zealand
1994	Management of dockyard contracted to Babcock New Zealand Ltd
1999	Full-scale waste treatment plant for dock commissioned

SEABED CONTAMINATION

Historically, dockyard stormwater and drydock wastewater was discharged to the harbour untreated, in accordance with standard practice at the time. When investigations began in 1993 it was initially thought that all of the contamination was historic. As investigations progressed, it became apparent that there was ongoing contamination, possibly due to current practices or ongoing discharges from historic contamination such as residual industrial waste in the stormwater system gradually being flushed through. A number of investigations were undertaken as the extent, both area and depth, of the contamination was progressively discovered.

In 1989, the sediment in front of the drydock entrance was dredged to rock. This area is fairly shallow and dredging is a regular maintenance activity. At this time only an application to dump the dredged waste was required. Therefore the sediment was characterised only to the extent required to obtain the dumping permit (Kingett Mitchell, 1989). However, the material from in front of the drydock was disposed of at a sanitary landfill as the material was in excess of the dumping permit received from the regional council for dredging in Calliope Basin.

The same area was again scheduled to be dredged in 1993. Legislation by this time required that material being removed be characterised prior to dredging. Contaminants detected in the sediment included heavy metals, total petroleum hydrocarbons (TPHs), polycyclic aromatic hydrocarbons (PAHs), PCBs and organochlorines (Kingett Mitchell, 1995). In particular, the Kingett Mitchell (1995)

PCBs, PAHs, mercury, copper lead and zinc (Woodward-Clyde, 1997). It was found that:

- Quality guidelines were in general exceeded, often by a large margin. Sediments near the Naval Base can be classed as heavily contaminated (Kingett Mitchell, 1995; Woodward-Clyde, 1997).
- Contamination was most severe close to the drydock outfall and entrance but extended a significant distance along the shoreline (Kingett Mitchell, 1995).
- Based on environmental guidelines, individual contaminants near the drydock can be expected to result in adverse effects on organisms (Kingett Mitchell, 1995; Woodward-Clyde, 1997).
- Toxicity tests showed the highly contaminated sediment close to the drydock was significantly more toxic to the test species (amphipod) than a control sample. The survival rates of the amphipods ranged from 28-77% (Woodward-Clyde, 1997a).

A survey of sediment quality in an area to the west of the dock was carried out recently by NIWA (1999). Again a whole range of contaminants was present, including lead, copper, DDT, mercury, nickel, PCBs and TBT. Patches of high contamination levels were identified, suggesting localised point sources with little dispersal of material occurring. Much of the contamination was found at depth in cores, indicating residual contamination from past activities. Some indications of recent or fresh inputs of TBT and copper were observed in the vicinity of the syncrolift, but the levels were low relative to historical inputs. This was encouraging as it shows measures taken to manage waste have been at least partly successful. A further aim of this survey was to assess the contribution of the contaminated sediments around the drydock entrance to contamination of adjacent sediments. The identification of hot-spots found in this survey prevented this assessment.

Some positive conclusions were noted from these investigations:

- A 1998 study (Stewart et al) carried out using transplanted mussels show that the levels of TBT and other organotins bioaccumulated are significantly lower now (up to 0.9 mg/kg) than they were in similar studies (King et al, 1989; Phillips et al, 1991) around the time the ban on organotins was introduced (up to 9 mg/kg).
- Concentrations of DDT and mercury in fish and shellfish from the area were slightly above background but did not exceed New Zealand Department of Health regulations (Woodward-Clyde, 1997).
- Elutriate quality tests showed there was little re-dissolution of heavy metal contaminants or TBT into the water column from the sediment. Therefore, with the effect of tidal action, water quality was not considered to be a significant concern (Kingett Mitchell, 1995).

The RNZN has an operational problem. Dredging at the entrance to the drydock is and will continue to be a regular maintenance procedure but dredging disturbs the

contaminated sediment. The contamination is a problem which they want to deal with once, not every time they need to dredge.

The issue of contaminated sediment is complicated by the New Zealand legislative framework and the resulting difficulty in determining liability. The New Zealand environmental legislation has changed significantly in recent years, particularly with the introduction of the Resource Management Act 1991 (RMA). The RMA amalgamated 25 major pieces of legislation, including the Clean Air Act, Town and Country Planning Act, and Water and Soil Conservation Act. The RMA is binding on the Crown.

The RMA is not retrospective so there is no liability for contamination events prior to introduction of the Act in October 1991. However, if contamination has an ongoing environmental impact, the land owner or occupier can be required to avoid, remedy or mitigate the adverse effect, regardless of when the contamination occurred.

In 1989 New Zealand underwent local government re-organisation. Fewer, larger, territorial and special function local authorities were created and regulatory and service delivery functions were generally separated. Prior to this re-organisation the Auckland Harbour Board was responsible for all aspects of managing Auckland's ports, including planning, dredging, structures, and the commercial port activities. During its ownership of the drydock, it apparently ignored a number of legislative requirements. For example, from 1966 it was required to obtain discharge consents for contaminated discharges or to confirm its existing use rights. Upon amalgamation, regulatory services of the AHB passed to the revamped Auckland Regional Council. Land (except seabed) and assets were transferred to the newly formed commercial organisation, the Ports of Auckland company, although the drydock had been sold to the navy in 1987. Some seabed was transferred (briefly) to the Regional Council and then to the Crown.

The Crown owns the seabed around the Naval Base. A small portion of the seabed is administered by Defence while the bulk is administered by the Department of Conservation. Currently, under the RMA, the land owner is liable only for contamination occurring after October 1991. While the RNZN may have liability for continuing sources of contamination (subject to separate on-shore cleanup), the majority of contamination is historic and the current liability regime is unable to address this adequately. Central government is currently considering problems of this nature and is working to determine the way forward.

Calliope Dock is scheduled as an historic item. A resource consent and Historic Places Act authority to disturb is required every time physical work is carried out on the dock. This includes works associated with controlling the discharge of contaminants, but not the discharges themselves.

ON-SHORE CONTAMINATION

The source of some contaminants, especially DDT and mercury, continued to be a puzzle. It was suggested that antifouling paint may be one source (Kingett Mitchell, 1989), but this seemed unlikely to account for the level of contamination found or the presence of DDT in the stormwater system. There is a mercury arc rectifier in a substation over Calliope Central. It has recently become apparent that the rectifier was broken at some stage. As the substation is over both land and sea, it may have contributed to the mercury contamination. The site is currently being sampled.

As investigations progressed there appeared to be ongoing inputs of contaminants. It became a priority to find the sources of ongoing contamination, and to eliminate them. Stormwater drains discharge to the harbour in the same area as the drydock outfall pipe and potentially contribute to the sediment contamination. Studies by Kingett Mitchell (1994a; 1996) found contamination in the stormwater sumps around the drydock, with DDT being the main contaminant of concern. Subsequent stormwater studies have focussed on this toxin. Cleaning the stormwater system made little difference to the levels of DDT found during subsequent monitoring.

In 1998 the investigation of the stormwater pipes around the drydock was repeated to determine if DDT levels were reduced and to identify a potential source for the DDT (Woodward-Clyde, 1998). The results suggested a recent or ongoing release of fresh DDT, source as yet unknown. A range of samples were subsequently taken, including dust, paint, pipeline scrapings and sump sediment. DDT dust was found in the ladders shop. The building was cleaned by dry vacuum to remove the chemical.

DRYDOCK

Since taking ownership of the drydock in 1987, the RNZN has taken steps to improve the management of liquid waste streams from the dockyard and drydock. In particular, the partial ban on the use of TBT antifouling, introduced in 1989, prompted an awareness of the need to control effluent discharges from the drydock.

Ships entering Calliope Dock are required to provide information on the type of antifouling used on their hull so that appropriate measures can be taken. A rudimentary treatment plant was installed in 1992, designed primarily to separate the solids from the pressure washing wastewater prior to discharge. From 1995 paint from ships' hulls below the waterline was sampled. If analysis showed the presence of TBT, the solid waste from pressure washing activity was treated and disposed of to an authorised off-site facility by specialised waste management contractors, at the ship owner's expense. Solid waste is stored until it can be treated in bulk (50-70 tonne) by stabilisation, tested for leachability (TCLP), and disposed of to landfill.

In 1999 the RNZN commissioned a permanent treatment plant for the drydock. The new plant will also be used to treat waste from the syncrolift, as the waste is similar. The new plant will discharge treated wastewater to the foul water sewer under a trade waste consent.

Management of the waste streams in the drydock has been improved to minimise the volume of water passing through the treatment plant and to eliminate sea water and oil which would reduce the efficiency of the plant. A weir at the dock gate prevents leaking sea water from combining with the fresh water waste stream. Facilities have been established to ensure shipboard waste streams are not discharged into the dock.

The dock itself is washed down with a high-pressure hose immediately after pumping down to remove sediments which may have washed in. The dock is again washed down immediately after a ship is waterblasted and again immediately before flooding up if spray painting has been undertaken. All of the washdown water is passed through the treatment facility. These steps are important as the ship owners are required to pay the costs of waste disposal (sludges, sweepings etc), either as general waste or as hazardous waste if TBT is present. Proper dock cleaning also enables the contamination to be attributed to a particular ship.

CONCLUSION

The seabed around the Devonport Naval Base is seriously contaminated, particularly with high levels of TBT, DDT and mercury. These, together with a range of other contaminants also present, have the potential to cause ongoing adverse effects on the environment. The risk to human health is considered low. The evidence indicates that the drydock outfall is the primary source of the contaminants. It is unlikely that the observed contamination is due to movement of contaminated sediments from other areas of the harbour.

The way forward is complicated by a number of factors:

- New Zealand's complex environmental legislation.
- The changing ownership and management of the drydock, dockyard and seabed.
- The use of the drydock by both naval and commercial ships.
- It is clear, however, that 'no action' is not an option.

Levels of TBT observed in mussels are dropping, indicating that the management of TBT use and TBT waste treatment has been effective.

On-shore contamination is also present and requires further investigation. While the source remains uncertain, DDT has been detected in stormwater system adjacent to the drydock. The extent of the contamination by DDT, and other contaminants, must be determined so a management plan can be developed.

Acknowledgements

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The Effects of Changes in Environmental Parameters on the Release of Organic Booster Biocides from Antifouling Coatings

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ABSTRACT

Understanding the effects of environmental variables on the rate of booster biocide release from antifouling formulations is necessary so that the measurements obtained by laboratory-based tests are correctly interpreted in environmental risk assessment. To allow the effects of anti-fouling actives on aquatic systems to be predicted effectively, we evaluated alterations in release rate as environmental variables change during tests simulating normal use. A standardised laboratory based release rate determination method (ISO/DIS 15181-1) was used to obtain release rate data for a number of booster biocides; Irgarol 1051, diuron, dichlofluanid, zinc pyrithione, Kathon 5287, TCMS pyridine and TCMTB. These data were then compared with those determined using a flume system designed to simulate environmental conditions. The effects of short-term changes (4 days) in environmental parameters such as temperature, pH, salinity, suspended particulate matter and vessel speed on TBT, copper, Irgarol 1051, diuron, dichlofluanid, zinc pyrithione, Kathon 5287, TCMTB and TCMS pyridine release rates was also evaluated. Release rates determined using the flume system were consistently lower than those obtained using the ISO method for all biocides, excluding dichlofluanid and TCMS pyridine. It was observed that salinity, temperature, pH and 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 rates were investigated. Using the same test systems it was observed that increased temperature (15-25°C) significantly increased the release of Irgarol 1051 from 0.4 to 2.0 $\mu\text{g cm}^{-2} \text{ day}^{-1}$. Comparisons between the data obtained from both laboratory and environmentally relevant test systems, along with possible implications on the environmental risk assessment of antifouling paint biocides, are discussed.

INTRODUCTION

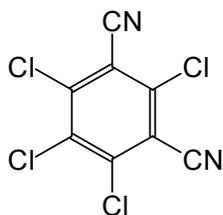
Antifouling coatings are used on the hulls of most ships to prevent the growth of fouling plants and animals. Until recently, the most effective and widely used antifouling paints released tributyltin (TBT) as the primary antifouling biocide. Widespread bans on the use of TBT on vessels less than 25m in length has now led to the use of a range of alternative organic booster biocides in combination with copper. There are eight booster biocides currently approved for use in the UK (Figure 1) which are currently being assessed for continued use by the UK Health and Safety Executive (HSE). An important factor in the risk assessment of biocidal paints, is the rate of active ingredient released from the vessel hull. Often this parameter is an important variable in environmental risk assessment models, such as the US Environmental Protection Agency (EPA) Exposure Assessment Modelling System (EXAMS). Small variations in release rate can lead to large margins of error in predicting environmental concentrations (PEC values).

Laboratory and field-based tests are used to determine antifouling paint biocide release rates and standardised methods are available (*e.g.* ASTM, 1991 and ISO, 1999). Recently, we described the use of a flume system to determine more environmentally representative TBT and Cu release rates and investigate the effects of environmental variables on their release (Thomas *et al.*, 1999). In this paper, we report the use of the same flume system to determine the rate of booster biocide release from seven antifouling formulations (each containing different booster biocides), assess the effect of environmental variables on their release, and compare these values with those determined using the standard ISO laboratory based method (ISO, 1999).

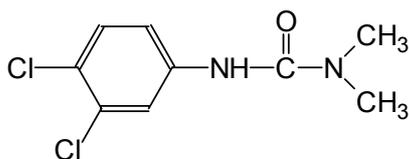
MATERIALS AND METHODS

Booster biocides and paint formulations

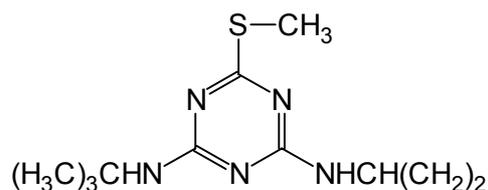
Irgarol 1051 (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine) was obtained from Ciba Geigy, Switzerland, Kathon 5287 (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one) from Rohm and Haas, USA, diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and zinc pyrithione (ZPT) from Sigma-Aldrich, UK, TCMTB ((2-thiocyanomethylthio) benzothiazole) and dichlofluanid (N'-dimethyl-N-phenylsulphamide) from Promochem, UK, and TCMS pyridine ((2,3,5,6-tetrachloro-4-methylsulfonyl)pyridine) from Zeneca, UK. These biocides were used as authentic reference compounds for the analytical methods employed. A summary of the paints used in this study is presented in Table 1. All the paints used, except those containing Kathon 5287 and dichlofluanid were commercially available in the UK. The paints containing Kathon 5287 and dichlofluanid were obtained from Hempels Marine Paints, Spain.



chlorothalonil
(2,4,5,6-tetrachloro-isophthalonitrile)

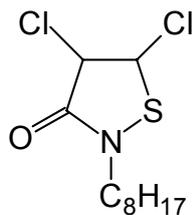


dichlofluanid
(N'-dimethyl-N-phenylsulphamide)

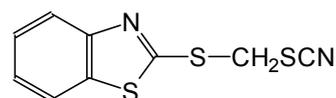


diuron
(3-(3,4-dichlorophenyl)-1,1-dimethylurea)

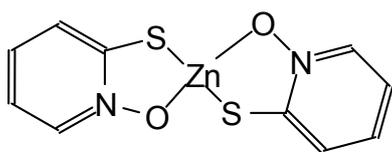
Irgarol 1051
(2-methylthio-4-tertiary-butylamino-6-cyclopropylamino-s-triazine)



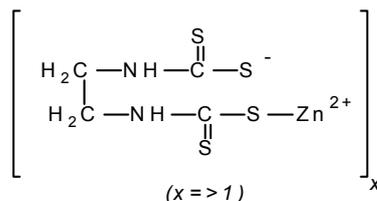
Kathon 5287
(4,5-dichloro-2-n-octyl-4-isothiazolin-3-one)



TCMTB
(2-thiocyanomethylthio benzothiazole)



zinc pyrithione
(bis(1hydroxy-2(1H)-pyridethionato-o,s)-(T-4) zinc)



zineb
(zinc ethylenebis-(dithiocarbamate))

Figure 1 Actives approved for use in antifouling coatings by the UK HSE

Table 1 Paint formulations used

Biocide	Paint	Paint type
Irgarol 1051	Blakes Titan True Blue	Copper based ablative
	Nautix SA A2 Antiflouling	Metal-free self-polishing
Diuron	Cruiser Premium (International Paints)	Copper based ablative
Zinc pyrithione	Micron Optima (International Paints)	Copper based ablative
Kathon 5287	Test formulation (Hempel)	Copper based ablative
Dichlofluanid	Test formulation (Hempel)	Copper based ablative
TCMTB	Nautix SA A2 Antiflouling	Metal-free self-polishing
TCMS pyridine	Leighs Grassline M396	Copper based ablative

Booster biocide analysis

All the analytical methods used in the study were scaled down from previously published techniques (Thomas, 1998; Thomas, 1999; Fileman *et al.*, In preparation). Irgarol 1051 and dichlofluanid were extracted by octadecylsilane solid phase extraction (C18 SPE) using atrazine ethyl amine-d₅ as an internal standard and analysed by gas chromatography-mass spectrometry (GC-MS) operated in the single ion monitoring (SIM) mode (Fileman *et al.*, In preparation). Diuron, Kathon 5287, TCMTB and TCMS pyridine were extracted by C18 SPE using chlorotoluron (dimethyl-d₆) as an internal standard and analysed by high performance liquid chromatography coupled to atmospheric pressure chemical ionisation mass spectrometry (HPLC-APCIMS) operated in the SIM mode (Thomas, 1998). Zinc pyrithione (ZPT) was determined by formation of the Cu²⁺ chelate, extraction into dichloromethane and analysis by HPLC-APCIMS (Thomas, 1999).

Table 2 Set points of environmental parameters

Parameter	Set points for test runs			
pH	8.2	6.5	7.0	8.5
Salinity (‰)	Linear decrease with time (35-0)			
Temperature (°C)	25	1	15	30
Vessel speed (knots)	3	7	10	1
Suspended particulate matter (mg/L)	0	50	500	1000

Release rate determination

Two methods were used for release rate determination; ISO method ISO/DIS 15181-1,2 (ISO, 1999) and a custom designed flume system previously described by Thomas *et al.*, (1999). The ISO test consists of a polycarbonate cylinder painted with the candidate paint. The cylinder is rotated at 60 rpm in a baffled beaker containing synthetic seawater and concentrations of the biocide are measured periodically to calculate the release in terms of micrograms of active ingredient released per square centimetre per day ($\mu\text{g cm}^{-2} \text{ day}^{-1}$). This is done using the following equation;

$$R = \frac{(C_{Cu} \times V \times D)}{(T \times A)}$$

where

R= release rate ($\mu\text{g cm}^{-2} \text{ day}^{-1}$)

D= hours/day = 24

V= volume (l) of seawater in measuring beaker= 1.5

T= time (h) cylinder is immersed in measuring container

A= surface area (cm^2) of paint film = 100.

The flume system consisted of a rectangular polyethylene tank (450L) fitted with a dividing plate and a pump that forced water around the tank. The candidate paint was applied to a Perspex panel and the water flow passed across the painted surface. Seawater flowed through the tank at a rate sufficient to maintain a constant concentration of biocide. The painted panel was initially immersed for ~28 days to allow for the biocide release to reach steady state. After this period the effects of short-term changes in environmental conditions were assessed by maintaining the set conditions for 4 days and then sequentially changing the conditions as described in Table 2. A longer-term experiment was also performed whereby the conditions were maintained for a 90 day period. Following 90 days the release rate was calculated as a mean release over the following 5 days. Both methods allow the close control of pH, temperature, salinity, speed and suspended particulate matter.

Table 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±6.5
TBT		1.5-4.0 ^a	1.6
Irgarol 1051		5.0	2.6 ^b
Diuron		3.3	0.8
Dichlofluanid	Euparen	0.6	1.7
Zinc pyrithione	Zinc omadine	3.3	- ^c
Kathon 5287	Sea-Nine 211	2.9	3.0
TCMTB	Busan	- ^c	0.9
TCMS pyridine	Densil S	0.6	3.8

^a Obtained from Thomas *et al.*, 1999 for comparison.

^b Mean of two data points for the same paint.

^c No data available.

[†] $\mu\text{g Cu cm}^{-2} \text{ day}^{-1}$ for copper, $\mu\text{g TBT cm}^{-2} \text{ day}^{-1}$ for TBT, and $\mu\text{g biocide cm}^{-2} \text{ day}^{-1}$ for booster biocides

Calculation of dry-film thickness

The painted panels were removed from the test system and any slime removed. After allowing the panels to dry (~5 days), the difference in dry-film thickness before and after the exposure period was calculated by weighing;

$$t_d = \frac{M_d - M_p}{A \cdot l_d}$$

where

M_d = mass of dried coated test piece (g)

M_p = mass of test panel (g)

A = area of painted surface (m^2)

l_d = density of dry paint (g/cm^3).

For the purpose of this study a density of 1g/cm^3 was used.

RESULTS

Biocide release rates

Biocide release rates, determined using both the ISO and flume methods, are presented in Table 3 and the ISO release rate profiles in Figure 2. The rate of biocide release varied from 0.6 to $5 \mu\text{g cm}^{-2} \text{ day}^{-1}$, with Irgarol 1051 having the fastest rate of release.

All booster biocides were found to be released at a much slower rate than Cu from a copper containing TBT-methacrylate based formulation and were comparable with the rate of TBT release from the same paint (Thomas *et al.*, 1999). The rate of Irgarol 1051 and diuron release was higher when tested using the ISO method than the flume, whilst the opposite was true for dichlofluanid and TCMS pyridine. The rate of Kathon 5287 release was similar from both methods.

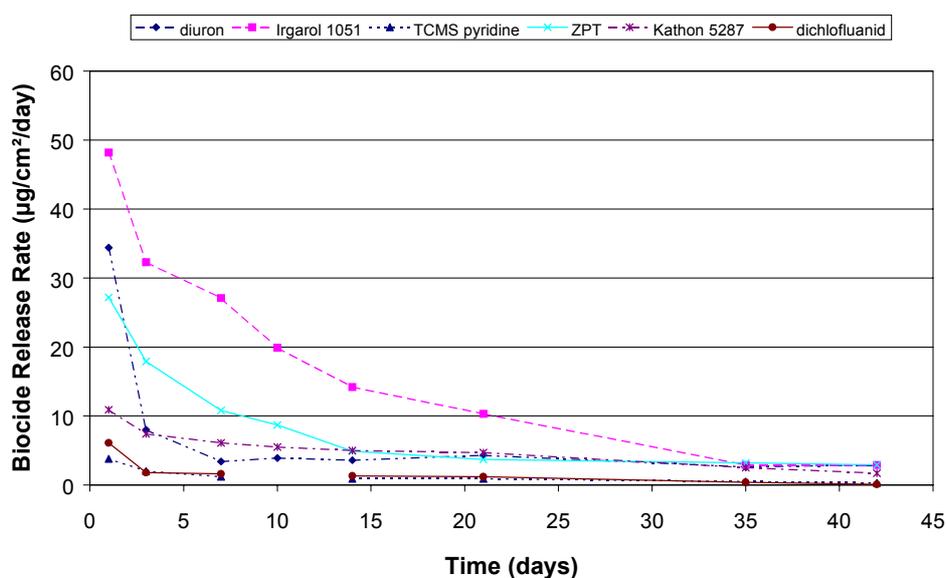


Figure 2 Biocide release versus days of immersion determined using ISO 15181-1

Effects of changes in environmental parameters

Irgarol 1051

The release rate data presented in Table 3 are within the range previously published (2.5 to 16 $\mu\text{g cm}^{-2} \text{day}^{-1}$, Ciba-Geigy, 1995). The effect of both short and long-term changes in environmental parameters on this release rate was assessed. A graphical representation of the effect of changes in pH is presented in Figure 3 and shows a slow decline in Irgarol 1051 concentration throughout the experiment. Since the flow of seawater through the test system is set to maintain a constant concentration of biocide within the flume system, this decrease represents a slow decline in Irgarol 1051 release. The same effect was observed when testing the effect of short-term changes in salinity (0-35‰), temperature (1, 15, 25, 30°C), vessel speed (1, 3, 7, 10 knots, 0.52, 1.6, 3.6, 5.2 ms^{-1}) and suspended particulate material (0, 50, 500, 1000 mg/L). It therefore appears that short-term changes in pH, salinity, vessel speed, temperature and suspended particulate matter have minimal effects on Irgarol 1051 release.

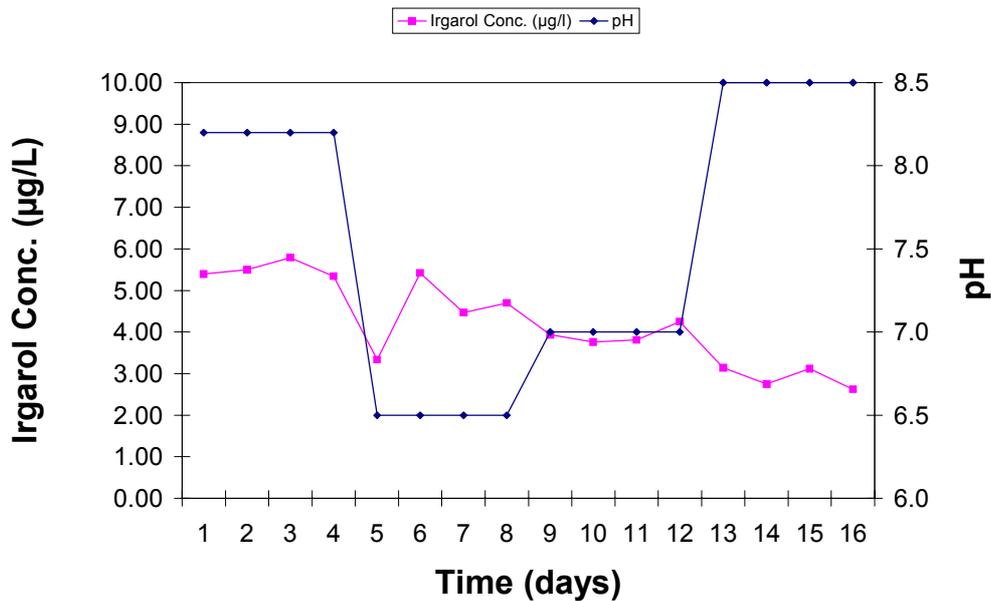


Figure 3 Effect of changes in pH on Irgarol 1051 release at 25°C, 35‰ and 3 knots

Experiments were then conducted over a 90 day period to assess the effects of temperature and salinity over a longer period. For these experiments changes in film thickness was also calculated.

The first experiment in this series was conducted to provide a baseline release rate of Irgarol 1051 from the two Irgarol 1051 containing formulations. The test parameters used were; temperature 15°C, salinity 33‰, pH 8.2 and a constant flow of water across the painted surface of 3 knots. The rate of Irgarol 1051 release following 90 days was calculated to be $0.4 \mu\text{g cm}^{-2} \text{ day}^{-1}$ for an ablative paint and $0.5 \mu\text{g cm}^{-2} \text{ day}^{-1}$ from the self-polishing coating whilst determination of the film thickness following the experiment showed varying results (Table 4). The effect of long-term differences in salinity and temperature were assessed by comparing the Irgarol 1051 release rates determined at 0‰ and 25°C. The release rates for the two formulations tested are summarised, along with change in film thickness, in Table 4. The release rates determined during the baseline and salinity experiments are similar for both the self-polishing and ablative coatings, although there is a major difference in dry film thickness of the self-polishing paint between the two experiments. It is evident that paint removal has been occurring from the ablative formulation. However, there appears to have been little or no decrease in film thickness from the self-polishing formulation that does not correlate with the measured release rates. Release rate data for the temperature experiment are only available for the ablative paint. However, these data suggest a significant increase in Irgarol 1051 release at the higher temperature. It is therefore apparent that where short-term (4 days) changes in salinity and temperature had no effect on Irgarol 1051 release, significant changes in release rate may occur over the longer-term (90 days) at increased temperatures.

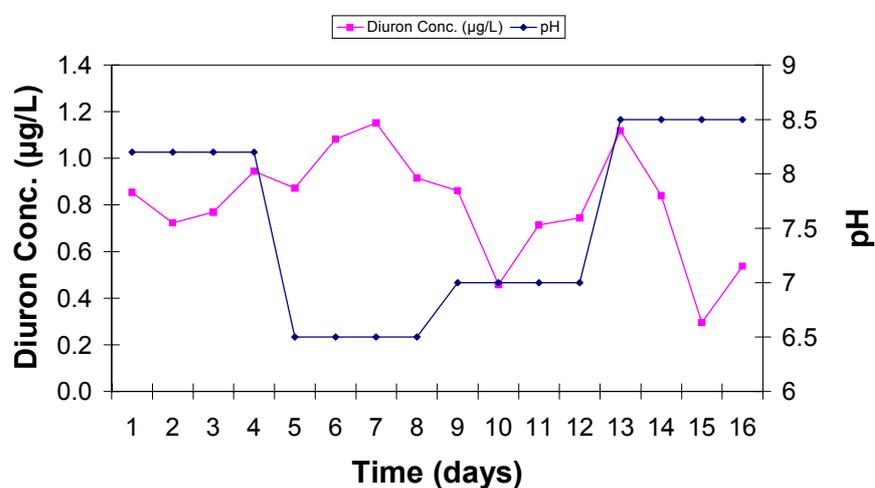
Table 4 Dry film-thickness of painted surface and release rate for long-term Irgarol 1051 release experiments

Experiment (Conditions)	Determinand	Paint type	
		Self-polishing	Ablative
<i>Baseline</i> (15°C, 33‰, pH 8.2)	Irgarol 1051 release rate ($\mu\text{g cm}^{-2} \text{ day}^{-1}$)	0.5	0.4
	Loss in dry film-thickness (μm)	0	26
Salinity (15°C, 0‰, pH 8.2)	Irgarol 1051 release rate ($\mu\text{g cm}^{-2} \text{ day}^{-1}$)	0.7	0.5
	Loss in dry film-thickness (μm)	+184	38
Temperature (25°C, 33‰, pH 8.2)	Irgarol 1051 release rate ($\mu\text{g cm}^{-2} \text{ day}^{-1}$)	-	2.0
	Loss in dry film-thickness (μm)	92*	174

*Loss through paint flaking. - no data

Diuron

Similarly to copper, TBT and Irgarol 1051, the release rate obtained from the flume system was significantly lower than that obtained using the ISO protocol ($0.8 \mu\text{g cm}^{-2} \text{ day}^{-1}$ compared to $3.3 \mu\text{g cm}^{-2} \text{ day}^{-1}$). Short-term changes in pH appear to have very little effect on the release of diuron from this particular antifouling formulation. However, when the pH or temperature of the test system was changed there appeared to be an increase in the variability of diuron release when compared to the negligible effects observed when in salinity and speed were changed (Figure 4, 5).

**Figure 4** Effect of short-term changes in pH on diuron release at 25°C, 35‰ and 3 knots

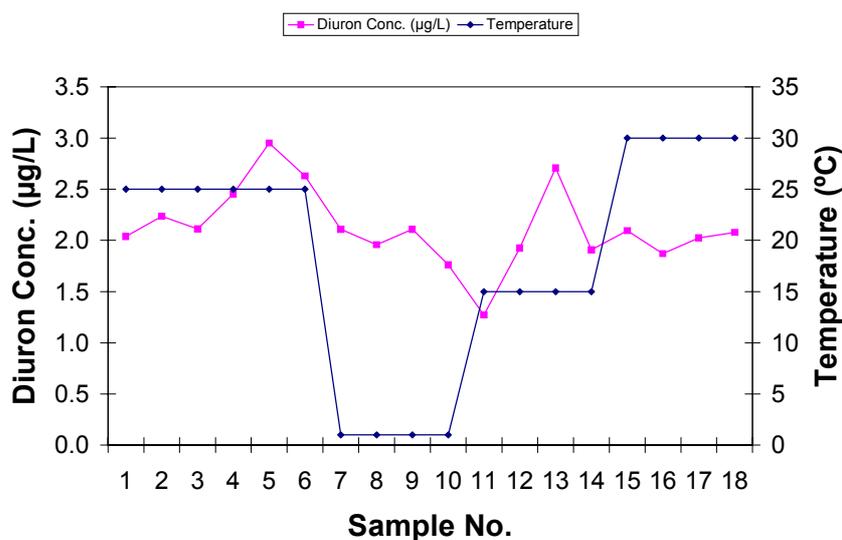


Figure 5 Effect of short-term changes in temperature on diuron release at 3 knots, 35‰ and pH 8.2

Dichlofluanid

Experiments conducted to assess the effect of short-term changes in environmental parameters on dichlofluanid release suggest very little direct effect. A variable release rate was observed throughout the experiment that showed no correlation with the changes in environmental parameters.

Zinc pyrithione

Using the ISO method a zinc pyrithione (ZPT) release rate of $3.3 \mu\text{g cm}^{-2} \text{day}^{-1}$ was obtained from a commercially available ZPT containing formulation. This falls within the broad range of literature values available for ZPT ($1\text{-}18 \mu\text{g cm}^{-2} \text{day}^{-1}$) (CEFIC, 1998). An assessment of the effect of environmental variables on ZPT release was not possible due to the rapid degradation of ZPT in the test system. This was confirmed by calculating ZPT degradation rate of $t_{1/2}=4\text{h}$ within the test system (Thomas, 1998).

Kathon 5287

A Kathon 5287 release rate of $2.9 \mu\text{g cm}^{-2} \text{day}^{-1}$ was determined using the flume system. This compares with a release rate of $3.0 \mu\text{g cm}^{-2} \text{day}^{-1}$ determined using the ISO method (Table 3) and a range of between 0.1 to $5 \mu\text{g cm}^{-2} \text{day}^{-1}$ reported in the literature (Rohm and Haas, 1992). The similarity of these two results is somewhat surprising since it has been reported that when tested using the ASTM method, Kathon 5287

seems to be rapidly released from the coating in the initial stages of the test (A Jacobson, Personal Communication).

Experiments examining the effect of pH, temperature, vessel speed and salinity over short-times scales showed very little direct effect on Kathon 5287 release. It was observed that pH and temperature changes caused an increase in the variability of Kathon 5287 release from the paint formulation under investigation (Figure 6).

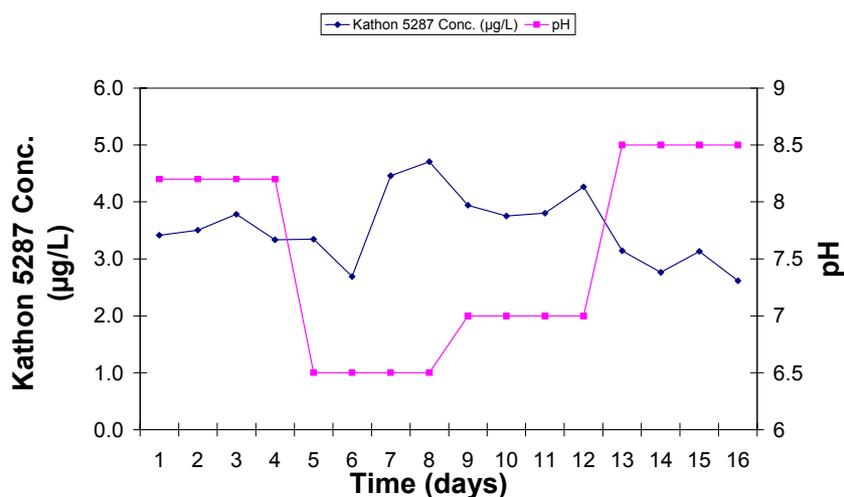


Figure 6 Effect of short-term changes in pH on Kathon 5287 release at 25°C, 35‰ and 3 knots

TCMTB

A TCMTB release rate from this particular formulation could not be determined using the ISO protocol. Although all the reference data was acceptable, concentrations of TCMTB released during the test period were insufficient to allow release rates to be calculated. A release rate was determined using the flume system ($0.9 \mu\text{g cm}^{-2} \text{day}^{-1}$; Table 3), however, short-term changes in the parameters under investigation showed that its release can be highly variable.

TCMS pyridine

Unlike many of the other booster biocides analysed, the ISO method produced a TCMS pyridine release rate lower than that produced using the environmental systems ($0.6 \mu\text{g cm}^{-2} \text{day}^{-1}$ compared to $3.8 \mu\text{g cm}^{-2} \text{day}^{-1}$). Little effect was observed from short-term changes in environmental parameters suggesting that over short periods of time, changes in environmental variables have little effect on TCMS pyridine release.

DISCUSSION

These data provide a comparison release rates derived from standard laboratory procedure and a system that more closely simulates the field for all organic booster biocides registered for use in the UK. The data showed that the release rates for each biocide cover a wide range ($0.6\text{--}5\ \mu\text{g cm}^{-2}\text{day}^{-1}$) when tested using ISO method ISO/DIS 15181-1. This is likely to be due to a number of factors, including the paint type, surface erosion rate, amount of biocide present in formulation and rate of biocide removal through degradation processes. When compared with data generated using the flume system, diuron, Irgarol 1051 and ZPT measured release rates were faster using the ISO method. Slower release rates were determined using the ISO method for dichlofluanid, TCMTB and TCMS pyridine whist Kathon 5287 release was similar for both methods.

These data also provide an indication of the potential effect of short-term changes in environmental parameters on biocide release in the environment. For all the biocides tested very little direct effect on release rate was observed over a 4 day period. For a number of the compounds tested the rate of biocide release became more variable with changes in pH. This may be due to pH having an effect on the dissolution processes that allow the biocides to be released from the paint and/or having an effect on the stability of the biocide once released into the water. However, this may be due to the variable release characteristic of ablative paints and requires further more detailed study.

When Irgarol 1051 containing paints were exposed over a longer time period to different environmental conditions the effects on release rate and film thickness were more pronounced. From these tests it was apparent that exposing an ablative paint formulation to increased temperatures over periods of 90 days caused a significant increase in the rate of release. This is probably due to increased rates of dissolution at higher temperatures and suggests that the same paint formulation would release Irgarol 1051 faster when used in warmer waters for prolonged periods of time than colder waters. However, exposure to waters of different salinity over this extended period was observed to have little effect on Irgarol 1051 release. Environmental parameters which show very little direct effect on Irgarol 1051 release over short periods of time (4 days) may still have a major influence on release rates when a coating is exposed over a longer period and does raise concern about extrapolating results from short-term tests.

A ZPT release rate could not be determined using the flume system since it was not possible to raise the ZPT concentration to $\sim 5\ \mu\text{g L}^{-1}$, required to begin the test. This is likely to be due to the rapid photolytic degradation of ZPT (half-life, $t_{1/2}=15\text{min}$ under laboratory conditions and $t_{1/2}=4\text{days}$ in natural estuarine conditions at a depth of 1m) (Turley *et al.*, 2000). This was confirmed by the determination of a degradation rate of $t_{1/2}=4\text{h}$ within the flume system (Thomas, 1999). It was also not possible to generate a TCMTB release rate using the ISO method. It is unlikely that the degradation of TCMTB within the test system was responsible for failing to determine a release rate using the ISO method. The compound possesses a $t_{1/2}$ of 740h that would have had little

effect at the start of the experiment. It is likely that the problem lay with the formulation used; either this had a low concentration of TCMTB or the co-polymer based paint did not release TCMTB during the early stages of paint exposure.

The release rates determined for the compounds investigated are in the same range as data available in the literature. The study allowed a comparison to be made between antifouling paint booster biocide release rates determined using the laboratory based ISO test and an environmentally representative flume system. The methods employed also allowed the effect of exposing booster biocide containing antifouling coatings to different environmental conditions, over both short and long-term time periods to be assessed. Booster biocide release rates varied little when exposed to short-term changes in environmental parameters, whilst temperature was observed to have a significant effect on Irgarol 1051 release from a copper based ablative formulation during a longer experiment. Results suggest that the biocide release from an antifouling coating on a vessel rapidly passing through waters with different environmental properties will vary little. However, the biocide release from a coating continually exposed to increased temperatures could have an increased rate of release to one operating in colder waters.

Acknowledgements

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6. Biodeterioration of Wood

Recent Marine Wood Preservation Research in Australia

L. J. Cookson and D. K. Scown

Copper-Chromium-Arsenic Levels in Barnacles Growing on Timber Marine Piles

D. K. Scown and L. J. Cookson

Recent Marine Wood Preservation Research in Australia

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ABSTRACT

Marine wood preservation research over the last 15 years in Australia has tackled two main problems for the industry. The first was to demonstrate that existing commercial treatments could give reliable performance in the sea, despite earlier erratic performance and inadequate specifications. The key solution was to study the biology of marine borers. Collating and identifying marine borers from different areas around Australia, and examining their preservative tolerances, enabled the Australian coastline to be divided into seven major hazard zones. Piling options can now be specified that will give more than 20 years service life in each zone. More recent difficulties for the industry are questions concerning the fate of copper-chromium-arsenic (CCA) and creosote, preservatives commonly used to protect wood in the sea. Current research focuses on the CCA levels found in barnacles growing on treated piles. Alternative chemicals and methods of protection that seem intrinsically more appealing environmentally are also being examined. These include new generation wood preservatives that are arsenic and chromium free, alternative oil and silica treatments, and potentially active agents extracted from marine organisms.

INTRODUCTION

Timber is a material naturally abundant in the sea, as at least 130 described species of marine borer have evolved to make it home. Those marine borers are a diverse group derived from several families that vary in their modes of attack and timber substrate preferences. Without an understanding of these differences, unexpected failures of timber structures can occur. Some timbers such as *Syncarpia glomulifera* (Sm.) Niedenzu (turpentine) are naturally resistant to marine borers (Barnacle, 1976), but most are susceptible and can be completely riddled within several years. Despite this, the qualities of wood; its strength, resilience, corrosion resistance, ease of handling and

association with sustainable forest management practices, make it an ideal building material for the sea.

Engineers and researchers in Australia have long searched for the most reliable timber for use as piles. The most important early works were by Iredale *et al.* (1932) for the Maritime Services Board of Sydney Harbour, and Watson *et al.* (1936) for the Port of Brisbane, both of whom combined the expertise of wood technology, engineering and taxonomy. These and other studies mainly examined the natural durability of timber in the sea (Maiden & De Coque, 1895; Armstrong, 1916; Mackenzie, 1927; Wilson, 1941; Shillinglaw & Moore, 1945; Johnson & Moore, 1950; Moore, 1961). The application of toxins by surface coating and the floating collar technique were also practised but gave temporary protection. Long term protection of non-durable timbers was achieved with physical barriers such as Muntz metal (Anon., 1907), pipes filled with sand, and concrete jacketing (Watson *et al.*, 1936). Modern physical barriers are generally plastic wraps (Steiger & Horeczko, 1982; Eaton, 1996), although concrete jacketing continues to be used.

The first commercial pressure treatment plants in Australia were built in the late 1950s. The first marine borer trial of pressure treated wood was installed by CSIRO in 1960 (Barnacle, 1960), but the marine wood preservation industry got off to a difficult start. In 1969 and the early 1970s, there were several disasters in the sea with the CCA (copper chromium arsenic) preservative that had proved so successful on land. CCA treated *Corymbia maculata* (Hook.) K.D. Hill & L.A.S. Johnson (spotted gum) mooring piles failed after three years during a storm in Bowen Queensland, taking several boats with them (Cokley, 1969). CCA-treated *Pinus radiata* D. Don (radiata pine) oyster posts in Port Stephens NSW broke, dumping valuable crops in the mud. Creosote-treated *P. radiata* in marine trials was being heavily attacked by *Limnoria* (Beesley, 1969). Even the double treatment being promoted in the USA (CCA followed by creosote) was found in 1970 to be severely attacked by *Limnoria* in our small specimen test (Barnacle & Cookson, 1995), although this was concluded to be due to the use of low temperature rather than high temperature creosote (Cookson & Barnacle, 1987a). These early failures spurred the first comprehensive survey of marine borers around Australia in 1970-1972 to generate more fundamental knowledge. Beesley (1971) distributed panels to 42 ports in Australia and PNG, and invited Dr Ruth Turner, a mollusc specialist, and students Jeanette (Marshall) Ibrahim and Suzanne Rayner to collect and identify the molluscs found during the survey. Unfortunately, the details of much of this work remained unpublished (Marshall, 1973), but a checklist of known Australian teredinid species is provided here (Table 1). Later work by Harrison & Holdich (1984) and Cookson (1991; 1994; 1996a) concentrated on the crustacean borers.

MARINE BORERS AND SUBSTRATE TOLERANCES

Limnoriidae

The Limnoriidae contains three genera, the *Paralimnoria* (wood borers), *Limnoria* (wood, algal and seagrass borers) and *Lynseia* (seagrass borers). *Paralimnoria* has the most plesiomorphic features in the family (Cookson, 1990), whereas *Lynseia* are apomorphic (Cookson & Poore, 1994). The group probably evolved in the tropics from species boring into dead wood, later moving into live plant material (Cookson, 1990). There are two known species of *Paralimnoria* and 28 species of *Limnoria* found in wood (Menzies, 1957; Cookson, 1990), of which seven of the latter genus have been found in commercial timber piling in Australia. Some of the substrate preferences have been noted (Menzies, 1951; Barnacle *et al.*, 1983; Cookson & Barnacle, 1987b; Cookson, 1990; Karande *et al.*, 1993). The most important species in Australia are *L. tripunctata* Menzies (found often in softwoods, less so hardwoods, treated with creosote, process oil, chlorothalonil etc.), *L. quadripunctata* Holthuis (found often in dense hardwoods, including those treated with CCA), and both *L. saseboensis* Menzies and *L. indica* Becker and Kampf (often in dense hardwoods and turpentine). Minor species are *L. unicornis* Menzies (found often in CCA-treated hardwoods), *L. platycauda* Menzies and *L. insulae* Menzies.

When designing structures it is important to attach bracing above the tidal zone, as *Limnoria* have a tendency to settle and bore within crevices. This behaviour is due mainly to their negative reaction to light and to their tendency to seek contact with surfaces (thigmotactic behaviour) (Eltringham, 1971). This behaviour is also noticed in aquaria bioassays where *Limnoria* generally align on test blocks beside rubber bands used to hold the test blocks in place (Cookson & Woods, 1995). The effect can be quite pronounced over time. In a recent inspection of 70 year old turpentine piles at Station Pier, Melbourne, most piles were in excellent condition, with just 5-30 mm radius lost from the heartwood (sapwood entirely lost). However, those piles which at various times had bearers fixed for work platforms in the tidal zone had mostly failed or were in poor condition due to erosion by *L. quadripunctata* (unpublished data).

While this review is about Australian research, much has been written recently on the biology and mode of attack of *Limnoria* by researchers from the Buckinghamshire College and Portsmouth University, UK (Pitman *et al.*, 1995; Henderson *et al.*, 1995; Wykes *et al.*, 1997; Praël *et al.*, 1999).

Table 1 Checklist of Teredinidae found in Australia and Papua New Guinea. Compiled from Watson et al. (1936), Marshall (1973), Turner & McKoy (1979), Ibrahim (1981), and Rayner (1983).

Teredinid species	Distribution in Australia (Fig. 1) and PNG
<i>Bactronophorus thoracites</i> (Gould)	5, PNG
<i>Dicyathifer manni</i> (Wright)	5, PNG. 4 (=Ballina, NSW)
<i>Teredothyra dominicensis</i> (Bartsch)	PNG
<i>Teredothyra matocotana</i> (Bartsch)	5, PNG
<i>Teredothyra excaovata</i> (Jeffreys)	5 (=Townsville), PNG
<i>Teredothyra smithi</i> (Bartsch)	PNG
<i>Teredora princessae</i> (Sivickis)	5, PNG
<i>Lyrodus bipartita</i> (Jeffreys)	2-5, PNG
<i>Lyrodus massa</i> (Lamy)	5, PNG
<i>Lyrodus medilobata</i> (Edmonson)	3-5, PNG
<i>Lyrodus tristi</i> (Iredale)	4,5 (east coast), PNG
<i>Lyrodus pedicellatus</i> (Quatrefages)	1-5, PNG
<i>Teredo fragilis</i> Tate	1-5 (northern limit = Townsville)
<i>Teredo clappi</i> Bartsch	1, 3-5, PNG
<i>Teredo furcifera</i> von Martens	1-5, PNG
<i>Teredo fulleri</i> Clapp	PNG
<i>Teredo johnsoni</i> Clapp	PNG
<i>Teredo poculifer</i> Iredale	4-5 (southern limit = Brisbane), PNG
<i>Teredo navalis</i> Linnaeus	1-4
<i>Teredo mindanensis</i> Bartsch	5, PNG
<i>Teredo somersi</i> Clapp	PNG
<i>Teredo triangularis</i> Edmonson	5 (Wyndham)
<i>Spathoteredo obtusa</i> (Sivickis)	PNG
<i>Nototeredo edax</i> (Hedley)	3-5, PNG
<i>Nausitora dunlopei</i> Wright	4 (east coast), 5, PNG
<i>Nausitora globosa</i> Sivickis	5 (east coast), PNG
<i>Nausitora hedleyi</i> Schepman	PNG
<i>Bankia barthelowi</i> Bartsch	PNG
<i>Bankia australis</i> (Calman)	1-5, PNG
<i>Bankia bipalmulata</i> (Lamarck)	5 (east coast), PNG
<i>Bankia bipennata</i> (Turton)	5 (Cairns), PNG
<i>Bankia campanellata</i> Moll & Roch	1,5, PNG
<i>Bankia carinata</i> (Gray)	1-5, PNG
<i>Bankia gracilis</i> Moll	5, PNG
<i>Bankia johnsoni</i> Bartsch	PNG
<i>Bankia neztalia</i> Turner & McKoy	1,2,4 (east coast)
<i>Bankia nordi</i> Moll	PNG
<i>Bankia philippinensis</i> Bartsch	PNG
<i>Bankia rochi</i> Moll	3,5, PNG
<i>Uperotus rehderi</i> (Nair)	PNG
<i>Uperotus clavus</i> (Gmelin)	5 (Green Island, Qld), PNG

Sphaeromatidae

The two important wood boring species of *Sphaeroma* in Australia are *S. quoyanum* Milne Edwards and *S. terebrans* Bate (Harrison & Holdich, 1984). *S. triste* Heller is sometimes found in untreated turpentine piles in Queensland, and *Ptyosphaera alata* (Baker) can be found at low salinity locations such as the Yarra River in Victoria and the Brisbane River in Queensland. *P. alata* causes shallow etches or small holes in decaying wood. In New South Wales (NSW) and Queensland, *Sphaeroma* generally causes more damage than other marine borers to the commercially available piling options listed in Table 2. Cragg (1988) provides a list of piling timbers that are susceptible to *Sphaeroma*. On the coast of West Australia the extent of the *Sphaeroma* hazard to marine timbers is less clear. *S. quoyanum* occurs in the Swan River (Serventy, 1955) and *S. terebrans* occurs at Exmouth Gulf (Harrison & Holdich, 1984). Generally, the presence of *Sphaeroma* limits the use of CCA-treated softwoods, and they are usually the first marine borers to attack older double treated eucalypt piles. Some of this tolerance derives from the ability to immobilise and store copper in the hepatopancreatic caeca (Cragg & Icely, 1982). Also, *Sphaeroma* is a filter feeder rather than a wood feeder (Rotramel, 1975), and so can avoid some of the toxic effects of wood preservatives.

In marine localities *Sphaeroma* damage is virtually restricted to the tidal zone (see, for example, Barnacle *et al.*, 1986). However, in estuarine water a lower percentage of the *S. terebrans* and *S. quoyanum* population will attack timber below the low water mark (Cheriyian, 1973; Cookson, 1994). Because *Sphaeroma* activity is mostly concentrated in the tidal zone, remedial treatments and protection can often be applied economically. The floating collar technique was once a standard maintenance procedure in Sydney Harbour (Iredale *et al.*, 1932), but is no longer applied because of creosote loss to the environment. In Townsville Port, PVC tape is often applied to untreated turpentine piles at the predicted tidal zone depth before being driven; this prevents attack unless boats damage the tape. In NSW and Queensland, merely placing and maintaining physical barriers on piles in the tidal zone would double the life of double treated piles, CCA-treated softwood piles and untreated turpentine piles. The average life of turpentine piles in Sydney Harbour was 34 years, whereas those protected by the floating collar method had average service lives of 70 years (Moore, 1961).

There are many questions to answer about the environmental cues that cause *Sphaeroma* to bore. For example, *S. quoyanum* is very destructive and able to bore holes as readily as *S. terebrans* in central and northern NSW, but in Victoria, while still common, *S. quoyanum* only occasionally bores shallow etches into softened timber. Presumably the difference in hazard intensity is related to temperature, or a differing geographical population, but this remains to be demonstrated. In Victoria, *S. quoyanum* seems most responsible (with other wood grazers) for an unusual pattern of attack found in old hardwood piles. *S. quoyanum* can excavate longicorn beetle emergence holes, common in some untreated hardwood piles, to give scalloped indentations throughout the tidal zone region of the pile. Neither is it known what drives *Sphaeroma* to live mainly in the tidal zone, be it predation, planktonic food levels, substrate weathering that makes wood easier to bore, salinity, temperature, or competition from

Martesia. At Townsville, *S. terebrans* has a minor preference to bore on the shady side of fender piles (Cookson, 1994). In estuaries it has an increased tendency to bore upwards, perhaps to reduce the amount of silt that might clog the burrow (Cookson, 1994).

Table 2. List of marine borer hazards and appropriate piling options that should provide service lives of at least 20 years (modified from Cookson, 1987).

	Hazard Zone								
	1	2	3	4	5	6	7		
Salinity ppt	30-36	30-36	30-36	30-36	30-36	10-30	1-10		
Approximate location	Tas	Vic	SA	Sth WA NSW	Nth WA Qld, NT	Port Stephens	Brisbane River		
Marine borer:									
<i>Limnoria tripunctata</i>	-	low	mod	ext	mod	high	-		
<i>L. quadripunctata</i>	mod	mod	mod	high	-	-	-		
<i>L. indica/saseboensis</i>	-	-	-	high	high	-	-		
<i>L. unicornis</i>	-	-	-	-	mod	-	-		
<i>L. insulae</i>	-	-	-	-	low	-	-		
<i>L. platycauda</i>	-	-	-	-	low	-	-		
<i>Sphaeroma terebrans</i> ^A	-	-	-	high	ext	ext	mod		
<i>S. quoyanum</i> ^A	low	low	mod	high	low	ext	-		
<i>S. triste</i>	-	-	-	-	low	-	-		
<i>Ptyosphaera alata</i>	-	-	-	-	-	low	low		
High salinity teredinids	mod	mod	mod	high	ext	high	-		
<i>Nausitora</i> spp.	low	low	low	low	low	mod	ext		
<i>Martesia striata</i>	-	-	-	mod	ext ^B	low	-		
mod = moderate, ext = extreme									
Marine Pile	Min. retention kg/m ³		Piles should last at least 20 years						
	CCA	HTC							
<i>S. glomulifera</i>	none	None	✓	✓	✓	✓ ^C	✓ ^C	✓ ^C	x
<i>E. marginata</i>	none	None	✓	✓	✓	x	x	x	x
<i>E. camaldulensis</i>	none	None	✓	✓	✓	x	x	x	x
<i>E. muellerana</i>	none	None	✓	✓	x	x	x	x	x
Hardwood	32	None	✓	✓	✓	x	x	x	x
Softwood	32	None	✓	✓	✓	✓ ^D	✓ ^D	x	? ^E
Hardwood	none	220	✓	✓	✓	✓	✓	✓	?
Softwood	none	220	✓	✓	✓	x	?	x	?
Hardwood	32	150	✓	✓	✓	✓	✓	✓	✓
Softwood	32	220	✓	✓	✓	✓ ^C	✓ ^C	✓ ^C	? ^E
Any listed & full length barrier			✓	✓	✓	✓	✓	✓	✓

A = *Sphaeroma* hazard in WA to be determined.

B = *Martesia* hazard extreme, especially in estuaries with variable salinity (often below 30 parts per thousand).

C = May require a barrier in the tidal zone.

D = Will require a barrier in the tidal zone. May also require knot protection.

E = *P. radiata* given this treatment were in good condition after ten years in the Brisbane River, but were then washed away by floods.

Teredinidae

Turner (1966) made a major contribution to the taxonomy of this group, and produced a useful key to the species (Turner, 1971a). In Australia and Papua New Guinea (Table 1), 33 species were collected in the 1970-1972 survey, with the major species found listed by Turner (1971b). *Uperotus clavus* (syn *Glumebra elegans*) can also be found in Australia in drifting coconuts and other seed pods (Watson *et al.*, 1936). Ibrahim (1981) examined the settlement of 17 species, while Rayner (1983) examined teredinids in Papua New Guinea. Rimmer *et al.* (1983) examined the distribution of *Bankia australis* in a mangrove creek. Brackish water species of *Nausitora* appear to be particularly destructive, as demonstrated in the Brisbane River (Watson *et al.*, 1936). Some taxonomic difficulties have arisen in that differences in breeding habits and rate of larval release suggest the division of some species. Calloway and Turner (1983), working with material from Florida, separated *L. pedicellatus* and *L. floridanus* (Bartsch), but did not determine the wider species distribution. Turner & Calloway (1987) indicated that some other species that were identical by external morphology could be separated by life cycle and histology, but unfortunately did not give details.

Preservative tolerances within the teredinid species are not as well known as for the crustacean borers. Johnson & Lebow (1996) showed that adult *Bankia gouldi* are much more tolerant of CCA in softwoods than larvae, so that if larvae can establish in untreated or poorly treated heartwood sections of timber, treated timber is more at risk. This may partly explain the susceptibility of CCA-treated eucalypt sapwood to teredinids, as this tissue has less uniform preservative distribution on a microscopic scale by comparison with pine sapwood (Greaves, 1974). A number of other species are also able to bore into CCA-treated timbers. CCA-treated *C. maculata* at Bowen failed in three years (Cookson, 1987), mainly because of *Dicyathifer manni*, a borer normally more common in nearby mangroves. *L. bipartitus*, *B. australis* and *T. fragilis* were found in CCA- and CCB (copper chromium boron)-treated eucalypts (Cookson and Barnacle, 1985). *L. pedicellatus* is another species common in failing treated timber (unpublished data). While teredinids can destroy timber faster than other marine borers, they are generally more sensitive to oil-borne preservatives such as creosote and chlorothalonil.

Pholadidae

A number of *Martesia*, *Lignopholas*, *Pholas*, *Barnea*, and *Xylophaga* species within the molluscan family Pholadidae are able to bore into soft timber (Turner, 1971a). The wood boring Pholadidae are well represented by deep water species (Turner, 1972; Santhakumaran, 1982). Of most concern to coastal structures are the *Martesia*, with four known species, *M. fragilis* Verrill and Bush, *M. nairi* Turner and Santhakumaran, *M. cuneiformis* (Say), and *M. striata* (Linnaeus). *M. fragilis* occurs in tropical and warm temperate locations, mainly in driftwood and pelagic plant material (Turner, 1971a). *M. nairi* was found in the Northern Territory and on the east coast at several locations from Cairns (Queensland) to Wallis Lake (NSW) (Turner & Santhakumaran, 1989). It was collected mainly from mangroves, although at Cairns it was found in wooden test panels. *M. cuneiformis* occurs in the western Atlantic ocean (Turner, 1971a).

M. striata is the dominant wood-boring species in Australia, and is found widely along the tropical coastline of Australia. This range may extend occasionally as far south as Sydney, where it was reported attacking creosote-treated wood and untreated turpentine (Moore, 1947). *M. striata* is common in tropical estuaries. On untreated turpentine piles in rivers in Cairns, Port Douglas and Townsville, most severe attack occurs near low tide, below but overlapping with the heaviest band of *Sphaeroma* attack at mid tide. It also produces a more scattered distribution of holes below the tidal zone through to mud line. In the Burnett River at Bundaberg, however, most severe attack was recorded by under-water video just above mud line in turpentine piles in three metres of water (presumably by *M. striata*, although specimens were not collected). Attack in the tidal zone by *M. striata* was sparse. For Townsville the *Martesia* hazard is severe in the Ross River estuary, but virtually absent about four kilometres away in the full salinity location of Townsville Harbour. *Martesia* juveniles actively bore into wood or soft substrates, but cease boring upon the formation of the callum in the adult (Boyle & Turner, 1976). CCA inhibits the settlement of *M. striata* more than creosote (Barnacle & Ampong, 1983). The deterrent effect of CCA was noticed in experimental piles at Port Douglas, where untreated turpentine piles had severe *M. striata* attack near low tide and similar CCA-treated piles had very limited attack (Cookson *et al.*, 1989). *S. terebrans*, however, could severely attack either pile.

MARINE BORER HAZARD MAP

A better understanding of marine borer species, in particular their distribution and tolerances, has led to the division of the Australian coastline into seven major hazard zones (Figure 1). Table 2 provides a list of piling options that should give a minimum service life of 20 years in each zone. Cookson (1987) provides an in-depth explanation for this table. This work was incorporated into the Australian Standard (AS 1604-1997), where in order to ensure treatment compliance the hazard was simplified into just two zones, southern and northern. Today in Australia the problem for timber marine piling is no longer the risk of premature failure. There are enough timber piling options available to ensure that a pile correctly specified and placed anywhere along the Australian coastline will have a service life of at least 20-30 years (Cookson, 1987; AS 1604-1997), a time sufficient to satisfy many coastal development needs. Longer service lives can be achieved using some of the treatments listed or when used in conjunction with physical barriers. The main task now is to address concerns that the wood preservative treatments being used can cause problems to non-target organisms and the environment. Our approach is to both quantify the risk (Scown & Cookson, 2001) and search for preservatives or systems that might be intrinsically safer.

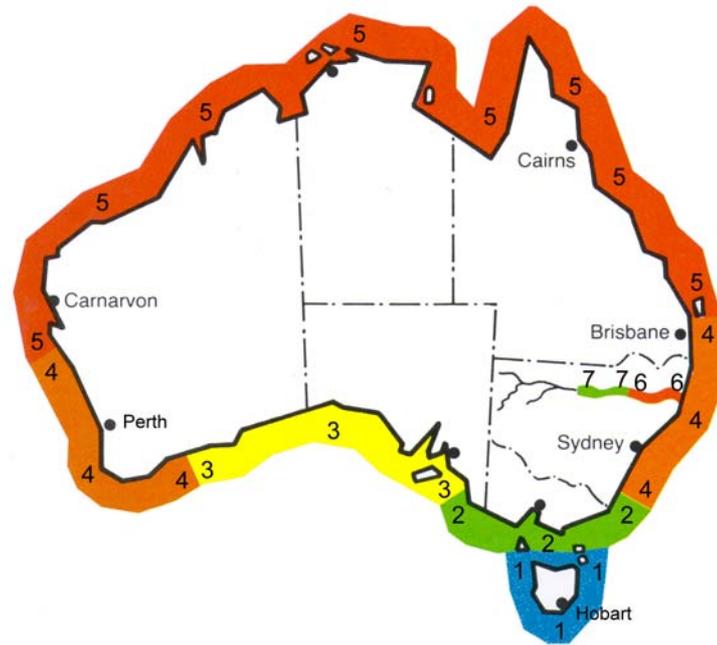


Figure 1 Marine borer hazard zones of Australia. Generalised estuarine river (zones 6 & 7) not to scale (after Cookson, 1987).

ALTERNATIVE MARINE WOOD PRESERVATIVES

Marine wood preservatives such as CCA and creosote that are used on their own as single treatments will often be successful in cool temperate waters. However, for most applications in warm temperate or tropical waters, a double treatment or preservative combination will be needed to protect timber from the variety of marine borers found. Often a wood preservative will be effective against a particular marine borer but less efficient against others. Alternative marine wood preservatives will probably need to be used in conjunction with other preservatives, either as a double treatment where the preservatives are applied in separate stages, or as a mixed formulation within one treatment (fortified creosote for example). Another factor to consider is that the marine wood preservation industry in most countries is relatively small, so it is unlikely that a treatment plant will be developed for preservatives that solely target marine borers. To be successful commercially, a marine preservative should also be effective against either termites or decay fungi and as such, will become an adjunct to more routine treatments. The other possibility is preservative fortification, where specific marine borer biocides are mixed with standard preservatives currently in use at a treatment plant when a call for marine timbers is made. A variation sometimes attempted is protection of timbers by brush or dip treatment, which could allow specific marine borer biocides to be applied over a standard treatment. However, such applications do

not normally allow deep preservative penetration, and service life might only be extended by 1-3 years due to leaching of the surface chemical.

The process found most successful to date for double treatment is to combine a fixed water-borne preservative such as CCA (mainly for *Limnoria* and *Martesia*) with an oil-borne preservative such as creosote (mainly for teredinids and *Sphaeroma*). Whereas the components of a CCA solution react chemically with wood to become fixed, the creosote remains an insoluble liquid trapped by surface tension within the wood's cellular structure. The following discussion will be divided into water-borne and oil-borne alternatives.

ALTERNATIVE WATER-BORNE PRESERVATIVES

Copper chromate

Copper chromate is not being sold commercially in Australia. CCA is the preferred water-borne preservative because the inclusion of arsenic is said to improve performance against termites and marine borers (Richardson, 1993). The arsenic also improves performance against some copper-tolerant fungi in laboratory bioassays (Da Costa & Kerruish, 1964). However, our results suggest that arsenic contributes little towards the control of marine borers and is an unnecessary inclusion for that purpose. Copper chromate performed as well or better than CCA over 25 years in marine trials near both Sydney and Perth (Barnacle & Cookson, 1995). Good performance of copper chromate was also shown by Preston & Chittenden (1980) after 2.5 years in New Zealand waters, and by Bergman & Lundberg (1990) after 16 years in Sweden. In-ground field tests in Australia showed that copper chromate performed as well as CCA preservatives against decay fungi and termites after 25 years (Johnson & Thornton, 1991). It should be noted that the copper chromate in the Australian trials included 5% chromium acetate in the formulation.

Other evidence suggesting no need for arsenic in marine exposure was derived from an international marine trial of CCA and copper chromium boron (CCB). There was no apparent difference in performance against marine borers between the two preservatives after up to eight years (Eaton, 1989), even though most boron had leached from the CCB-treated samples within a short time (Leightley, 1987). When these stakes were examined again after 17 years at the Australian test site in Sydney Harbour (Table 3), CCA and CCB were still performing similarly in the timber species remaining in test. An interesting observation was that the CCB-treated specimens were more eroded than those with the corresponding CCA treatment (Table 3), suggesting that the treated wood was softer due to adverse preservative effects or microbial action. Such a slight difference might not be noticeable in structural-sized timbers. Similarly, the performance of creosote did not improve significantly with added arsenic after ten years (Cookson & Barnacle, 1987a) and twenty years (unpublished data).

Arsenic is often naturally high in some marine organisms, which have adapted so they can metabolise and excrete any excess (Ünlü & Fowler, 1979). Chromium leaching should be of more concern than arsenic, although chromium is the component in CCA with greatest leach resistance (Leightley, 1987).

Table 3 Final results of marine trial comparing CCA and CCB treated stakes (150 x 20 x 20 mm) in Sydney Harbour after 17 years. Mean (standard deviation) of three replicates. Marine borer rating from 4 to 0, where 4 = sound and 0 = destroyed. Volume (cm³) determined by water-displacement on air dried blocks returned to laboratory. Original volume was 59.7 cm³.

Treatment solution	Factor	<i>Alstonia scholaris</i>	<i>Fagus sylvatica</i>	<i>Pinus sylvestris</i>
6% CCA	Rating	3.8 (0.3)	0.0 (missing)	3.5 (0.0)
	Volume	39.3 (1.0)		37.6 (1.8)
6% CCB	Rating	3.8 (0.3)	0.0 (missing)	3.5 (0.5)
	Volume	28.9 (4.4)		23.4 (0.2)
10% CCA	Rating	4.0 (0.0)	3.5 (0.0)	3.5 (0.0)
	Volume	50.1 (1.4)	30.6 (0.6)	50.3 (1.7)
10% CCB	Rating	3.8 (0.3)	3.8 (0.3)	3.7 (0.3)
	Volume	40.7 (0.8)	26.3 (0.8)	31.3 (1.0)

Copper dimethyldithiocarbamate

There has been a great deal of research on copper-based preservatives, as alternatives to CCA, that are chromium and arsenic free. Some of these have been examined in Australia for marine borer control. Copper dimethyldithiocarbamate (CDDC) is being developed as a CCA alternative. The treated timber has a chocolate brown appearance. It was compared with CCA in a 1.5 year laboratory aquaria trial for the sapwood of *P. radiata*, southern yellow pine (a mixture of probably three species) and *E. regnans* F. Muell. (Cookson *et al.*, 1998a). CDDC performed similarly to CCA against *Limnoria*, but was much more effective against teredinids than CCA as it prevented larval settlement. This preservative contains a higher proportion of copper than CCA, but in a limited five day trial leached less copper than CCA. Therefore, the reason for its improved performance in inhibiting teredinid settlement is still unclear (suspected copper leaching or the presence of dimethyldithiocarbamate). However, CDDC requires a two-step application procedure, making it a more expensive treatment than CCA. Commercialisation of this product is currently focusing upon the home decking market in North Carolina.

Ammoniacal copper quaternary

Ammoniacal copper quaternary compound (ACQ) is being evaluated by the Timber Research Division of the Queensland Department of Primary Industries at a marine test site in Mourilyan Harbour, North Queensland. After three years exposure, ACQ is performing as well as CCA (K. Archer, personal communication, 1996).

Table 4 Six year inspection results for a marine trial of small specimens of *P. radiata* (250 x 50 x 40 mm) and *E. obliqua* (natural rounds 40-80 mm diameter and 250 mm long) exposed below low tide at Townsville. Mean rating (standard deviation) of six replicates. Marine borer rating from 4 to 0, where 4 = sound and 0 = destroyed.

Treatment	<i>P. radiata</i>			<i>E. obliqua</i>		
	Mean retention kg/m ³	Teredinid	Limnoria	Mean retention kg/m ³	Teredinid	Limnoria
Water	427	0 (0.0)	0 (0.0)	506	0 (0.0)	0 (0.0)
P9 Oil	429	4 (0.0)	1.4 (1.2)	346	3.3 (0.5)	3.6 (0.3)
CCA	32 (salt)	2.4 (1.3)	3.9 (0.2)	32 (salt)	0.4 (0.7)	4 (0.0)
PROCCA	32 (salt)	3.9 (0.2)	4 (0.0)	33 (salt)	1.4 (0.7)	4 (0.0)
Basic zinc chloride	59 el. Zn	4 (0.0)	3.8 (0.3)	Not tested		
HTC	304	4 (0.0)	3.5 (0.0)	210	3.9 (0.2)	3.7 (0.3)
PEC	274 (as HTC)	4 (0.0)	3.9 (0.2)	205 (as HTC)	3.9 (0.2)	4 (0.0)
Chlorothalonil in oil	29	4 (0.0)	3.4 (0.2)	23	4 (0.0)	3.9 (0.2)
CCA + PEC	32 + 279	4 (0.0)	4 (0.0)	33 + 191	4 (0.0)	3.9 (0.2)
CCA + chlorothalonil	33 + 29	4 (0.0)	3.9 (0.2)	32 + 22	4 (0.0)	3.9 (0.2)

Zinc hydroxide

Pendleton & O'Neill (1986) reported that Douglas fir piles treated with basic zinc sulphate were still in excellent condition after 19 years in Hawaii. The treatment is based on the formation of insoluble zinc hydroxide within wood. We investigated basic zinc chloride, where six *P. radiata* blocks were treated with zinc chloride to a mean retention of 58 kg/m³ zinc, and then re-treated with a solution of ammonia and sodium hydroxide to precipitate the zinc. After six years exposure below the tidal zone in Townsville, those specimens are still in excellent condition, as four blocks are without attack and two blocks have only superficial attack by *Limnoria* (Table 4). However, the zinc-treated timber was softer than comparative CCA-treated specimens due to the acidic effects of unreacted zinc chloride. A marine preservative based on the formation of zinc hydroxide could lead to a relatively safer environmental option for water-borne preservatives.

ALTERNATIVE OIL-BORNE PRESERVATIVES

Pigment emulsified creosote

A major improvement in the environmental credentials of creosote was the development of pigment emulsified creosote (PEC) (Greaves *et al.*, 1986). This preservative combines 65% high temperature creosote (HTC) as an emulsion with 35% water containing pigment, emulsifiers and stabilisers. Virtually all hardwood marine piles in Australia are now treated with PEC rather than HTC. The benefits are cleaner wood surfaces, reduction of crud and vapour emissions, and improved performance. This creosote is particularly suited to eucalypts, in which the pigment blocks vessels and pathways to reduce crud formation. Laboratory work with an earlier formulation of PEC showed less leaching of fungitoxic components than from HTC (Cookson & Greaves, 1986). More recent comparison in a marine trial at Townsville showed that

PEC is performing better than HTC, either because of reduced leaching or (less likely) the presence of additives such as pigment (Table 4).

Creosote in the marine environment is biodegradable. There are a number of marine microorganisms such as *Pseudomonas creosotensis* O'Neill, Drisko & Hochman that are capable of degrading creosote (Drisko & O'Neill, 1966). These bacteria grow on pile surfaces and possibly adjust to the slow release of creosote from the pile. Any long term leaching must be slow. In a marine trial of low temperature creosotes (Barnacle & Cookson, 1990), which contain more water soluble fractions than modern creosotes, 70% of the creosotes remained in the wood after 25 years (Cookson *et al.*, 1996). Other studies in the USA found up to 75% creosote remaining in piles after 40 years service (Bramhall & Cooper, 1972). The main environmental problem for creosote-treated piles occurs shortly after installation when a combination of hitting the pile with a driving hammer and the swelling of wood with water forces some creosote from the pile. Leaking sometimes endures for several weeks after installation until the pile 'equilibrates'. There is also some volatilisation of creosote from surface fibres above the tidal zone. With age, brown-grey coloured double treated eucalypt piles usually turn light green above the tidal zone when CCA dominates surface colour after creosote loss. Creosote contains a number of compounds that are suspected carcinogens (Bestari *et al.*, 1998), so appropriate safety equipment should be worn during pile installation.

Chlorothalonil

Chlorothalonil is a fungicide widely used in agriculture for the control of various plant diseases (Davis *et al.*, 1997). Although chlorothalonil is an organochlorine it is readily metabolised by mammals and so has a very low oral toxicity (Woods & Bell, 1990). It is nearly insoluble in water, but highly toxic to fish. However, any chlorothalonil that leaches from treated wood should rapidly degrade as its half life is only about 48 h in estuarine water containing unsterile sediment (Walker *et al.*, 1988). Chlorothalonil is also effective against decay fungi and termites (Preston, 1986; Creffield & Chew, 1993). In an aquaria trial (Cookson & Woods, 1995) and a six year old sea trial in Townsville (Table 4), oil-borne formulations of chlorothalonil provided timber with good resistance to teredinids, but in softwoods was attacked by *L. tripunctata*. Chlorothalonil therefore follows a similar pattern of performance to creosote. Performance against *L. tripunctata* was improved by fortification with chlorpyrifos (Cookson & Woods, 1995). Chlorothalonil is also proving to be effective as a double treatment with CCA (Table 4).

Oil

The oil-type preservatives have both toxic (or repellent) and physical (bulking) effects on teredinids. The relative importance of the bulking effect where wood cells are filled with oil is not yet determined. However, simply treating wood with process oil such as P9 oil from the USA will protect *P. radiata* from teredinids for more than six years, although the specimens will rapidly decline due to *L. tripunctata* (Table 4). The treatment is also useful in *E. obliqua* L'Herit. (Table 4). A similar effect was noticed in a

comparison of CCA with a CCA-oil emulsion called PROCCA (see Cookson *et al.*, 1998b). The PROCCA formulation contains just 5% oil, but still slightly increased *E. obliqua* service life at our severe Townsville test site by one year by comparison with CCA. PROCCA has also improved performance in *P. radiata* over that achieved by CCA (Table 4). A double treatment of CCA and 100% oil is not likely to provide the protection gained by CCA and creosote; however, treatment with oil should markedly improve the performance of eucalypt piles treated with CCA alone. Just which oils would be best is not known.

Table 5 Performance of supplementary treatments of biocides dissolved in creosote against marine borers after 21.3 years in Sydney Harbour. Mean (standard deviation) rating for 4-6 specimens. Rating scale of 4-0 where 4 = sound and 0 = destroyed.

Conventional timber/treatment	% Biocide dissolved in a suppl. creosote treatment	Main biocidal action	Mean rating (sd)
160 kg creosote/m ³ <i>P. radiata</i>	3.0% Lindane	insects	2.9 (1.6)
	2.8% Tributyl lead acetate	fungi	0.6 (1.3)
	2.8% Captan	fungi	0.0 (0.0)
	3.0% Fenitrothion	insects	0.0 (0.0)
	1.1% Endrin	insects	3.7 (0.3)
12 kg CCA/m ³ <i>P. radiata</i>	1.7% Aminocarb	insects, molluscs	0.0 (0.0)
	3.0% Tributyl tin oxide	fungi	1.5 (1.6)
	1.5% Azinphos-ethyl	insects	3.5 (0.6)
	1.5% Thiram	fungi	0.0 (0.0)
	1.9% Toxaphene	insects	0.7 (1.1)
120 kg creosote/m ³ <i>E. obliqua</i>	3.0% Malathion	insects	2.3 (1.3)
	3.0% Tributyl tin oxide	fungi	2.1 (1.3)
	0.3% Methiocarb	insects, molluscs	1.8 (1.4)
	1.9% Niclosamide	molluscs	0.7 (0.8)
16 kg CCA/m ³ <i>E. obliqua</i>	3.0% Aldrin	insects	2.3 (1.6)
	3.0% Tributyl lead acetate	fungi	0.0 (0.0)
	0.5% Tetrachlorvinphos	insects	0.0 (0.0)
	2.9% Fenthion	insects	2.6 (1.8)

Fortification of creosote or oil

The performance of creosote (and probably oil) can be improved by fortification with other biocides that dissolve in the oil-based preservatives. The aim of such an approach could be several: to improve creosote performance, to lower the level of creosote needed in a treatment, or to avoid the need for double treatment with CCA.

This approach was explored in a study where 16 different organic biocides were added to creosote (Table 5). After 13.5 years the results showed that insecticides were generally more effective additives than fungicides or molluscicides (Cookson *et al.* 1991). This test was continued in Sydney, where after 21 years the best supplementary treatments were the insecticides lindane, endrin, azinphos-ethyl, and fenthion (Table 5). As the organochlorines lindane and endrin have no real future in Australian wood preservation, the best biocide worthy of further study is azinphos-ethyl. Chlorpyrifos is another insecticide that improves the performance of oil-type preservatives such as creosote (Johnson & Gutzmer, 1990) and chlorothalonil (Cookson & Woods, 1995).

A more recently installed trial of supplementary treatments in Australia was described by Eaton & Cragg (1995) and Cragg & Eaton (1997). They examined the performance of synthetic pyrethroids (permethrin, cypermethrin and deltamethrin) as single or supplementary treatments for *Pinus sylvestris* L. Specimens were exposed for 59 months at Mourilyan Harbour, Queensland. Those treated with the pyrethroids dissolved in white spirit were severely attacked, whereas all creosote containing samples were only slightly or moderately attacked. Fortification of creosote with deltamethrin generally gave better results than the other two pyrethroids (Eaton & Cragg, 1995).

UTILISING THE PROTECTIVE MECHANISMS FOUND IN PLANTS

The natural durability of a range of timber species against decay fungi and termites has been examined extensively (Johnson *et al.*, 1996), but performance against marine borers is poorly known. Barnacle (1976) noted that natural durabilities on land do not necessarily apply in the sea. Some comparative marine studies were undertaken by Watson *et al.* (1936) for eight timber species in Brisbane waters, and Johnson & Moore (1950) compared seven West Australian timbers in Sydney Harbour. Cookson (1996b) conducted a one-year aquaria trial to compare the relative durability of 22 timber species available in Australia. Results from a parallel sea trial will be reported at a later date.

The chemicals causing marine borer resistance in some plants could be extracted and used to treat other less durable timbers. For example, an obtusaquinone isolated from the central American timber *Dalbergia retusa* Hemsley (cocobolo) was found to be effective against teredinids, although not against *Limnoria* (Bultman & Parrish, 1979).

It has long been recognised that many of the timbers that are naturally durable in the marine environment have high silica content (Amos, 1952). The theory proposed was that hard siliceous granules within the timber blunt the boring organs of marine borers, thereby preventing attack (van Iterson, 1933). Additional support for this theory came from the fact that saws tend to blunt more quickly when used on siliceous timbers. However, attempts to replicate this natural means of preservation by impregnation with silicon-containing compounds have been generally unsuccessful (Edmondson, 1953; Roe *et al.*, 1957; Serpa, 1980). This inability to duplicate marine natural durability by silicon impregnation has led some researchers to doubt the role of silica in natural durability (de Silva & Hillis, 1980).

Recent work, however, has shown that timber can be impregnated with certain ethyl silicate formulations in a way that will mimic the pattern of resistance found in some naturally durable and siliceous timbers such as turpentine. This silica treatment, described in a provisional patent (Cookson *et al.*, 1999), has protected *P. radiata* blocks from teredinids for 2.5 years in the extreme hazard found at Townsville, though the treatment fails for *Limnoria*. The silica could therefore be used as an alternative to

creosote in a double treatment with CCA or one of the water-borne alternatives mentioned previously. Action by silica may occur in the gut of borers rather than by affecting wood boring organs such as the mandibles or shell. Teredinids must swallow all that they chew, while *Limnoria* may be able to avoid consuming silica granules.

In searching for pesticides that are environmentally benign to non-target organisms in the sea, another useful approach might be to obtain active ingredients that are already produced naturally by certain marine organisms (Holmström & Kjelleberg, 1994). The presumption is that those chemicals will also be biodegradable in the sea. Possible candidates are derivatives of secondary metabolites obtained from marine red algae that are being examined for antifouling activity (de Nys *et al.*, 1995). One of these compounds looked promising in a laboratory bioassay against *L. quadripunctata* (Scown *et al.*, 1999). It also provided moderate protection to treated *P. radiata* blocks exposed for 13 months at Williamstown (Scown *et al.*, 1999). While analogues of this product might not provide the long term protection normally required in wood preservation, it might be useful in aquaculture where timbers can be recoated on a more regular basis.

CONCLUSIONS

Marine wood preservation research in Australia has reached the stage where treated timber marine piles can now be specified to provide long service life. Knowledge of marine borer species, distributions and substrate preferences has led to the development of a marine borer hazard map that can be used to aid in the selection of the correct timber pile. Further research is driven by the need to evaluate the environmental impact of using treated timber in the sea, and an interest in the development of alternative preservatives. New formulations are likely to be preservative combinations due to the need to protect timber from the wide range of marine borers found, especially in warmer waters.

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Copper-Chromium-Arsenic Levels in Barnacles Growing on Timber Marine Piles

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ABSTRACT

Barnacles collected from experimental marine piles that had been in service for 5.7 years at Townsville were analysed for copper, chromium and arsenic (CCA) content in both their tissue and shell. Compared to barnacles on untreated piles, copper levels in tissue and shell were significantly elevated in barnacles growing on CCA-treated *Pinus elliottii* Engelm. piles. Similarly, chromium levels in shell were higher in barnacles collected from CCA-treated *Corymbia maculata* (Hook.) K.D. Hill & L.A.S. Johnson piles. Although elevated, the copper and chromium concentrations in the tissue and shell of barnacles growing on CCA plus creosote-treated eucalypt piles were not significantly different from background levels. No elevation was evident in tissue arsenic concentrations. Comparison with CCA levels in barnacles collected from piles after two and four years service revealed that the rate of leaching of copper and chromium continues to decline. Barnacles growing 20-120 mm away from the CCA-treated *P. elliottii* piles did not have increased levels of copper or chromium.

INTRODUCTION

Wood used in a marine environment is prone to attack by a variety of marine borers. In some regions, particularly tropical waters, this hazard can be extreme. All but the most durable of timbers, for example, turpentine (*Syncarpia glomulifera* (Sm.) Niedenzu), require preservative treatment in order to provide useful service lives.

Much of the treated timber used in the sea around the world is treated with copper-chromium-arsenic preservative (CCA). Although predominantly fixed in the timber, it is widely recognised that some copper, chromium and arsenic will leach from CCA-treated timbers submerged in water (Brooks, 1996). The environmental impact of this leaching has been investigated in a number of studies, often with conflicting

conclusions. Weis & Weis (1992) and Weis *et al.* (1993a, 1993b) reported that metal levels in green algae (*Ulva lactuca* and *Enteromorpha intestinalis*), barnacles (*Balanus eburneus*) and oysters (*Crassostrea virginica*) found growing on CCA-treated timber structures were higher than those found in similar organisms gathered from other areas. On the other hand, Brown & Eaton (1997), Baldwin *et al.* (1996) and Albuquerque & Cragg (1995) suggest that, while leaching from treated timbers does occur, if the timber is properly seasoned and the preservative fixed, levels of metals leached are not great enough to have an adverse effect on the environment.

The majority of studies investigating the issue of metals leaching from CCA-treated timber in aquatic environments have concentrated on the introduction of freshly treated wood (Weis *et al.*, 1991; Hegarty & Curran, 1986). It is well documented that the degree of leaching from CCA-treated timber is greatest immediately after installation and decreases with time (Weis & Weis, 1996; Albuquerque & Cragg, 1995; Archer *et al.*, 1994). Little information is available regarding the leaching of copper, chromium and arsenic from treated wood that has been in service for an extended period of time. Such information would be more indicative of the long-term effects of the use of CCA-treated wood in the marine environment.

The ideal organism to monitor for evidence of contamination might be one actually growing on the pile itself. Barnacles are common fouling organisms in the tropical waters of northern Australia. Barnacles accumulate heavy metals from the surrounding environment, and their apparent inability to regulate metal levels make them useful as a biomonitor (Powell & White, 1990; Philips & Rainbow, 1988; Rainbow, 1987). A marine trial of commercially available timber mooring piles was established in three ports in Queensland in 1991 through the collaboration of CSIRO Forestry and Forest Products, Queensland harbour authorities, Queensland Department of Primary Industries (Forestry) and Koppers Timber Preservation. Our studies investigate the levels of copper, chromium and arsenic present in the tissue and shells of barnacles inhabiting the surface of these marine piles at the Townsville test site. Results after two and four years exposure were provided by Cookson *et al.* (1996), whereas the present article concentrates on the 5.7 year results.

MATERIALS AND METHODS

Timber piles

The Townsville test site contains 36 piles encompassing a range of different timber species and preservative treatments. Timber species and treatment combinations (number of replicates) include CCA-treated *Corymbia maculata* (Hook.) K.D. Hill & L.A.S. Johnson (spotted gum) [syn *Eucalyptus maculata* Hook.] (6), pigment emulsified creosote (PEC)-treated *C. maculata* (4), double treated (CCA + PEC) *C. maculata* (6), double treated *Eucalyptus pilularis* Sm. (blackbutt) (6), CCA-treated *Pinus elliottii* Engelm. (slash pine) (5), double treated *P. elliottii* (6) and untreated *S. glomulifera* (3).

Chemical analyses were performed on four samples from all piles to determine actual chemical loadings of both CCA and creosote wherever relevant. Samples were taken from both the outer five mm and a cross section of the full sapwood depth in the hardwood species, and from the outer five mm in treated *P. elliotii*. Timber species, treatment type and chemical loadings derived from analysis of samples collected are presented in Table 1.

Piles were installed at Townsville in September 1991 in the Ross River estuary. Piles were arranged randomly in a grid pattern covering an area 90 x 126 metres, the longer dimension being in the direction of water flow.

The piles were inspected after two, four and 5.7 years' exposure. The original purpose of the trial was to assess the efficacy of the various piles against marine borers. In order that an accurate appraisal of the performance of the piles could be made, inspections were conducted at low tide and all barnacles growing on the piles removed to expose timber surface. It follows that, since the four year inspection of the piles was performed in October 1995, the barnacles collected for this study would have been growing for no longer than 19 months.

Table 1. Mean preservative retentions in experimental mooring piles exposed at Townsville

Timber species	Treatment	CCA retention % m/m	Creosote retention % m/m	CCA retention kg/m ³	Creosote retention kg/m ³
<i>C. maculata</i>	Double (CCA/PEC)	1.4	10.7	13.3	101.9
<i>E. pilularis</i>	Double (CCA/PEC)	2.0	18.1	17.6	154.9
<i>P. elliotii</i>	Double (CCA/PEC)	8.3	26.2	63.7	201.0
<i>P. elliotii</i>	CCA	4.8	-	36.8	-
<i>C. maculata</i>	CCA	2.7	-	25.5	-
<i>C. maculata</i>	PEC	-	15.3	-	145.7
<i>S. glomulifera</i>	-	-	-	-	-

Barnacle analysis

Barnacles were collected from all experimental marine piles in April 1997. A metal scraper was used to dislodge barnacles from the piles, and care taken to ensure that all barnacles collected had the shell intact. As larval settlement occurs a number of times a year, the size distribution within barnacle populations was quite varied. No preference was given to barnacles of a particular size; rather, each sample consisted of upwards of 50 individuals encompassing the entire range of shell sizes present. Sizes ranged from about three to 15 mm diameter. Generally, the smallest individuals collected were adhering to the shell of larger individuals. No preference was given to particular barnacle species. Samples were packed in plastic bags and frozen on the day of collection.

Barnacles were stored in the laboratory at -5°C until required for analysis. Upon thawing, whole soft parts were removed using stainless steel instruments. Tissue from all barnacles removed from the same pile was combined, weighed and freeze-dried overnight. Pooling of tissue from such a large number of individuals was done in order to compensate for any influence of body size on metal loading, a phenomenon observed in a number of barnacle species (Powell & White, 1990; Philips & Rainbow, 1988). Dried tissue was digested in 250 ml conical flasks by initially soaking in 2.5 ml concentrated H_2SO_4 for 15 minutes before adding one ml aliquots of 30% w/v H_2O_2 . Hydrogen peroxide was initially added to the digest at room temperature, and subsequent additions were made once the reaction from that previous addition had subsided. Generally, after the addition of the third ml of H_2O_2 reactions diminished, at which point the flasks were transferred to a hot plate; heat was gradually increased and, as the solution darkened in colour, further one ml H_2O_2 aliquots added. Digestion was complete when the solution remained clear and acid began to reflux ten mm up the sides of the flasks. Digests were made up to 100 ml with deionised water. Copper, chromium and arsenic concentrations were determined using a GBC Integra XM sequential Inductively Coupled Plasma spectrophotometer (ICP). Standards were run every six samples to confirm the absence of any significant drift.

Technique integrity was assessed through the use of standard reference material, TORT-2 (lobster hepatopancreas) (National Research Council, Canada). While chromium levels were below limits of detection, recoveries of copper and arsenic averaged 96.8% and 102.6 %.

Barnacle shells were brushed thoroughly to remove dried algae and sediment. Since whole barnacles were removed from the marine pile, timber fibres remained attached to the basal plate. Inclusion of these fibres would obviously influence results. Thus shells were carefully removed from the basal plate using insulated pliers and the basal plate discarded. Shell fragments were placed in 125 ml conical flasks with 50 ml 1 N HCl at room temperature overnight, and heated to 60°C for three hours. Solutions were filtered and analysed using the ICP spectrophotometer. In addition to containing a known quantity of copper, chromium and arsenic, the standard solution used to calibrate the ICP also contained a quantity of CaCO_3 equivalent to that in the shell solutions.

Data were treated statistically by initially deriving mean metal concentrations for samples of barnacle populations collected from the same pile type. These means for each element were consequently compared using a Model I analysis of variance with each pile treatment considered as a fixed factor. When significant differences ($p < 0.05$) were identified, comparisons between pile types employed Tukey's test for multiple comparisons.

RESULTS

Data for copper, chromium and arsenic concentrations in dry tissue and shell from barnacle populations growing on various marine piles are presented in Figures 1-5 along with comparisons by displaying honestly significant difference (HSD) ($\alpha < 0.05$) as described by Andrews *et al.* (1980).

Copper

The mean copper concentration in the tissue of barnacles growing on CCA-treated *P. elliotii* was statistically higher ($\alpha < 0.001$) than in barnacles growing on all other pile types except double treated *P. elliotii* (Figure 1). The copper concentration in tissues from barnacles growing on the double treated *P. elliotii* piles was significantly greater than in barnacles from *S. glomulifera*, PEC-treated *C. maculata* and double treated *E. pilularis* piles.

There was no significant difference between mean copper concentrations in tissue from barnacles growing on the remaining marine pile types. However, there was an apparent trend suggesting that barnacles growing on piles with at least part CCA treatment had slightly higher tissue copper concentrations than those growing on non-CCA-treated piles. Indeed, barnacle populations growing on CCA-treated and double treated *C. maculata* piles had mean copper levels 1.3 times greater than those from barnacles growing on untreated piles. So too, metal levels in barnacles collected from double treated *E. pilularis* piles were elevated by comparison with those observed in barnacles from non-CCA-treated piles.

Copper concentrations in the shell of barnacles growing on both CCA-treated and double treated *P. elliotii* piles were significantly higher ($\alpha < 0.001$ and $\alpha < 0.01$ respectively) than those in barnacles growing on any of the other experimental marine piles (Figure 2). The only other significant difference ($\alpha < 0.05$) identified was that between the mean copper level in shells from barnacles growing on double treated *C. maculata* piles and those on the non-CCA-treated piles; *S. glomulifera* and PEC-treated *C. maculata*.

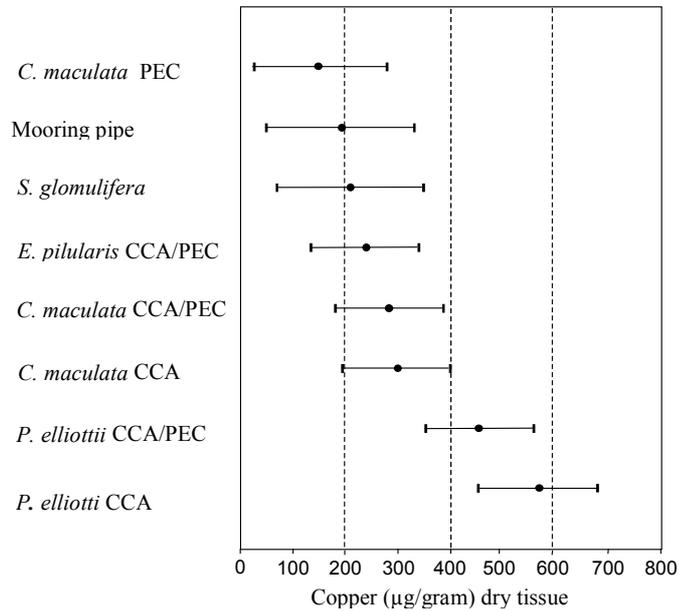


Figure 1 Mean concentration, showing honestly significant difference, of copper in dry tissue of barnacles inhabiting 5.7 year old experimental marine piles.

Barnacles removed from the metal mooring pipe attached to the CCA-treated *P. elliotii* pile had mean tissue and shell copper concentrations similar to those in barnacle populations from the non-CCA-treated piles (Figures 1 and 2).

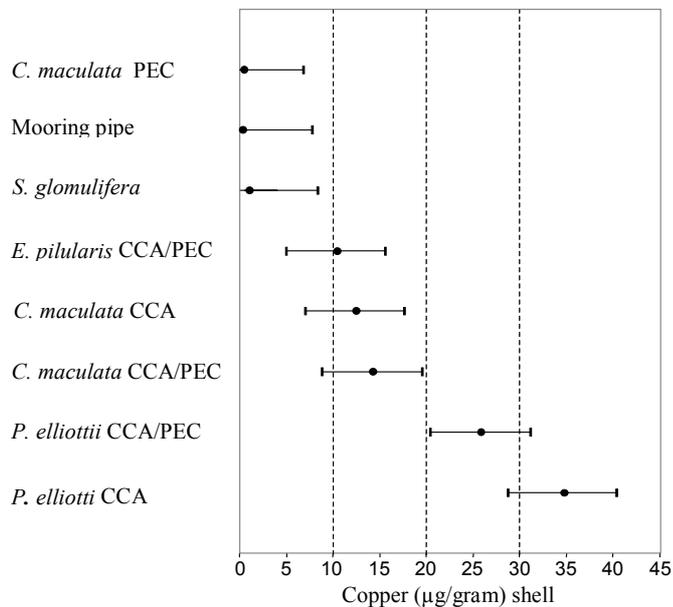


Figure 2 Mean concentration, showing honestly significant difference, of copper in shell of barnacles inhabiting 5.7 year old experimental marine piles.

Chromium

There was no significant difference between chromium concentrations in the tissues of barnacles collected from any of the experimental pile types (Figure 3). However, the ranked order of mean concentrations suggests that there is some minor correlation between chromium levels in barnacle tissue and the type of pile from which they were collected. Tissue from barnacles growing on CCA-treated *P. elliotii* piles had a mean concentration of chromium roughly 3.5 times that found in barnacles collected from non-CCA-treated piles. Barnacles growing on CCA-treated *C. maculata*, double treated *C. maculata* and double treated *P. elliotii* contained similar levels of chromium, about 2.5 times that in barnacles from non-CCA-treated piles. Chromium concentrations in the tissues of barnacles growing on double treated *E. pilularis* piles were moderately lower at 1.5 times the mean concentrations for non-CCA-treated piles.

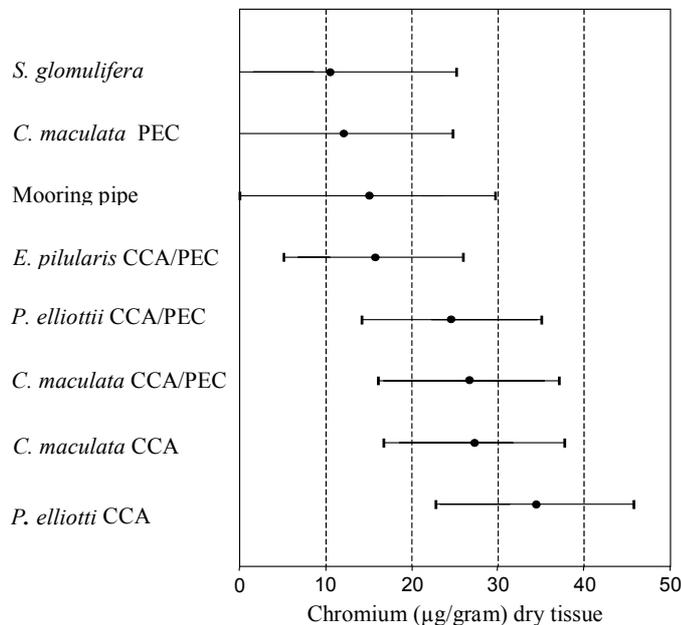


Figure 3 Mean concentration, showing honestly significant difference, of chromium in dry tissue of barnacles inhabiting 5.7 year old experimental marine piles

Mean chromium concentrations in shells from barnacles growing on both *S. glomulifera* and PEC-treated *C. maculata* piles were minimal (Figure 4). Shells of barnacles growing on CCA-treated *C. maculata* piles had a mean chromium content of 20.6 µg/g, which is significantly higher ($\alpha < 0.01$) than the mean chromium level in barnacle shells collected from any other pile type except CCA-treated *P. elliotii*. Barnacles growing on CCA-treated *P. elliotii* piles had a mean chromium concentration in their shells of 15.0 µg/g, which is significantly higher than corresponding values in barnacles collected from both non-CCA-treated pile types ($\alpha < 0.01$). Although mean chromium concentrations in barnacle shells collected from double treated *P. elliotii*, *C. maculata* and *E. pilularis* were

slightly elevated, they were not significantly different from those in barnacles from the non-CCA-treated piles.

Mean chromium levels in the tissue and shells of barnacles gathered from the metal mooring pipe were negligible, as they were in barnacles collected from non-CCA-treated piles.

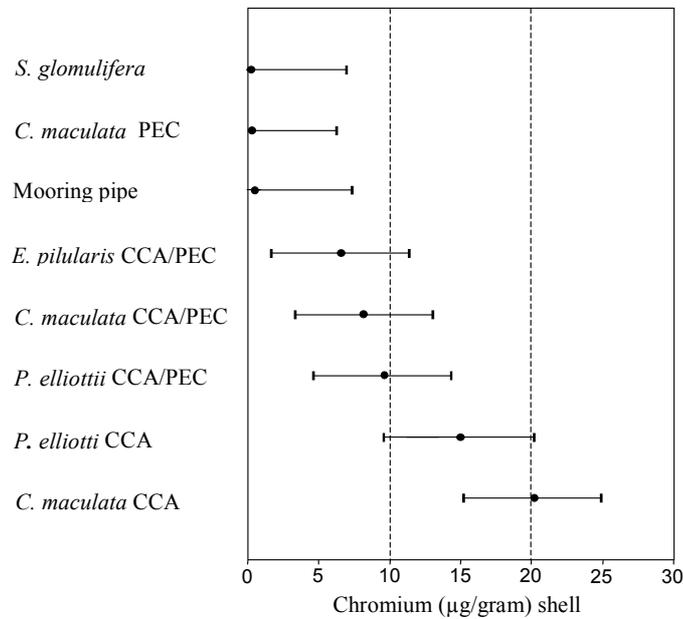


Figure 4 Mean concentration, showing honestly significant difference, of chromium in shell of barnacles inhabiting 5.7 year old experimental marine piles.

Arsenic

Our arsenic analyses using the ICP were associated with a high-percentage relative standard deviation. This was primarily due to alterations made to the ICP since earlier analyses, which were more accurate (Cookson *et al.*, 1996). While arsenic levels in barnacle tissue digests are presented, concentrations in shell solutions were below the limits of detection using the current system.

Statistical analysis of arsenic levels within the tissue of barnacles growing on experimental piles failed to reveal any significant differences (Figure 5). Barnacles collected from double treated *C. maculata* piles had the highest mean concentration, 36.7 µg/g, which is similar to that of barnacles growing on CCA-treated *C. maculata* (34.0 µg/g). Tissue from barnacles growing on *S. glomulifera* piles had a mean arsenic level of 28.4 µg/g. Barnacles collected from all other experimental pile types; CCA-treated *P. elliotii*, double treated *P. elliotii*, PEC-treated *C. maculata* and double treated *E. pilularis*, all had very similar mean tissue arsenic concentrations at 23.8, 23.5, 23.4 and 23.2 µg/g respectively. The mean arsenic concentration in the tissue of barnacles collected from the mooring pipes was 24.2 µg/g, similar to this group.

Comparison of copper and chromium levels in tissue and shell

Figure 6 compares the copper and chromium levels identified in tissue and shell. The majority of copper accumulated in the bodies of barnacles is retained in the tissue. Chromium levels in shell were up to 70% of the content measured in oven dry tissue.

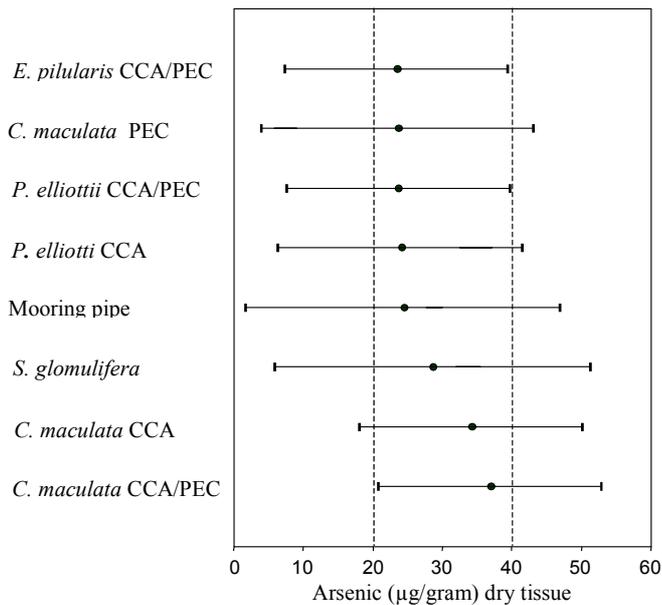


Figure 5 Mean concentration, showing honestly significant difference, of arsenic in dry tissue of barnacles inhabiting 5.7 year old experimental marine piles.

CCA losses over time

Metal levels found in barnacle tissue in this study were compared to levels identified in a previous study by Cookson *et al.* (1996) of the same piles after two and four years service. This earlier study employed a different method of extraction. The procedure was compared using the same standard reference material (lobster hepatopancreas). Copper and arsenic recoveries of 94% and 101.5% respectively were obtained. These values were similar to those achieved using the present method of tissue digestion (96.8% and 102.6% respectively). Therefore, comparison between the studies seems warranted.

Figure 7 presents the levels of CCA in the tissue of barnacles collected from the experimental piles after two and four years service (Cookson *et al.*, 1996) and from the present study (5.7 years service). There is a distinct trend of decreasing copper loadings

in those barnacles growing on CCA-treated piles over time. The trend was similar for chromium though less pronounced. No trend was apparent for arsenic, as elevated levels were not detected in either study.

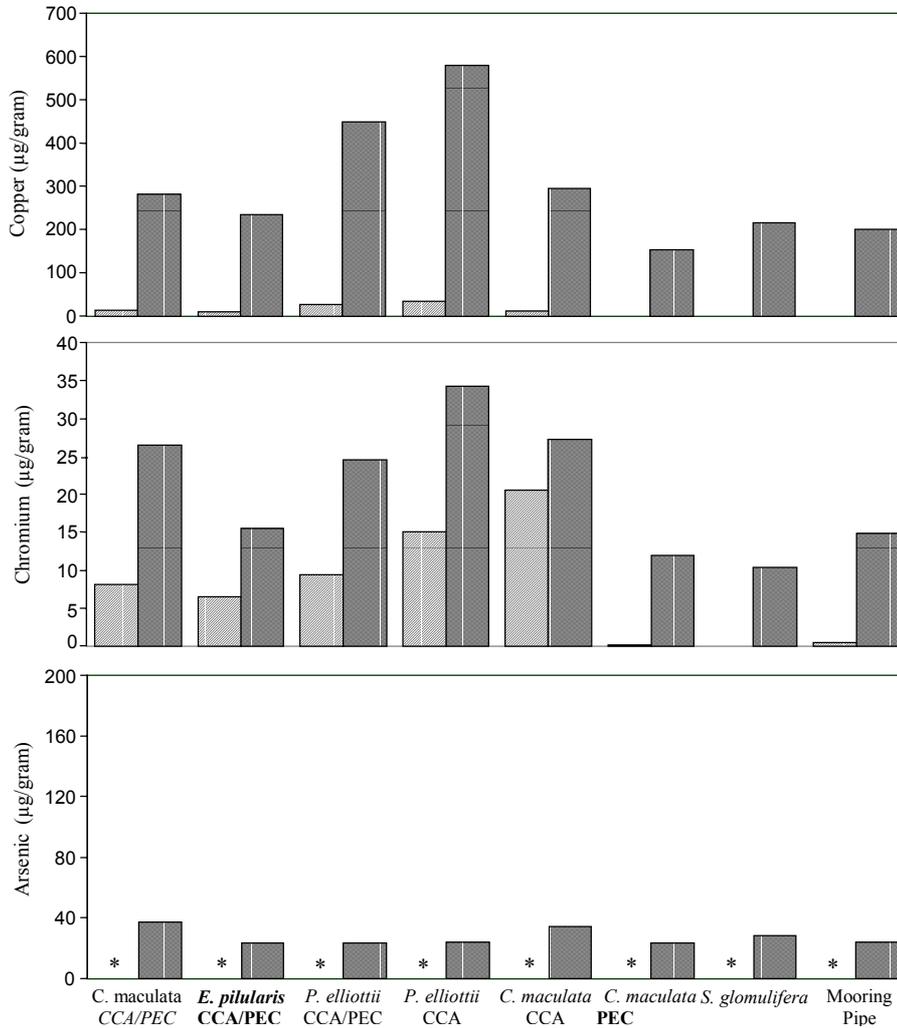


Figure 6 Comparison of the mean concentration of metals in shell and dry tissue, of barnacle populations growing on 5.7 year old experimental marine piles.

▨ shell ■ dry tissue * indicates levels at or below detection limits.

DISCUSSION

Relatively few significant differences were found in the copper, chromium and arsenic contents of barnacles growing on various treated and untreated piles that had been in service for 5.7 years. No significant difference in tissue arsenic content was found, which

is consistent with the results obtained from the two and four year inspections (Cookson *et al.*, 1996). When differences were found for copper and chromium, elevated levels were more common in barnacles on treated softwood than hardwood. The contamination profiles of copper and chromium in barnacle tissue were similar. Highest levels of accumulation for both elements were in barnacles collected from CCA-treated *P. elliotii*. These levels were both around three times that found in barnacles growing on non-CCA-treated piles. Treatment of hardwood with CCA generally results in much lower quantities of total preservative retained, as compared to softwood, because of thinner sapwood bands. Accordingly, levels of copper and chromium in tissues of barnacles growing on *C. maculata* piles treated at least in part with CCA were much lower than those from *P. elliotii* piles, and roughly 1.3 times those in barnacles from non-CCA-treated piles. Likewise, levels of copper and chromium in the tissue of barnacles taken from double treated *E. pilularis* piles were lower than from *C. maculata*. Once again, *E. pilularis* generally has a narrower sapwood band than does *C. maculata*. Therefore, while retentions of preservative within the sapwood region of both pile types are comparable (indeed that in *E. pilularis* piles was greater), overall there is substantially more CCA retained by *C. maculata* piles upon treatment.

Analysis for traces of copper and chromium in shell has revealed, in accordance with conclusions from the tissue analysis, that a distinct relationship occurs between accumulated levels in barnacles and the amount of CCA preservative retained in the timber substrate. Copper and chromium concentrations were almost undetectable in barnacles growing on non-CCA-treated marine piles, whereas populations taken from piles treated at least in part with CCA had elevated levels of both metals. Although the highest levels of chromium were recorded in barnacles from double treated *C. maculata* piles, in general the degree of copper and chromium contamination in shell reflected the initial retention of CCA in the corresponding pile type.

It is interesting to note that although the double treated *P. elliotii* piles had a CCA retention almost twice that of the CCA-treated *P. elliotii* piles, the mean levels of copper and chromium in the tissues were lower in barnacles from the double treated piles. While further studies are required to confirm this, it would seem likely that the second treatment with PEC slows the leaching of CCA from *P. elliotii* piles. The same does not appear to be true for the double treatment of *C. maculata*, as mean tissue levels of copper and chromium were similar whether the barnacles were growing on CCA-treated piles or double treated piles, even though the mean CCA retention was higher in the former. There was, however, substantially more chromium in the shell of barnacles collected from CCA-treated *C. maculata* piles than from similar double treated piles.

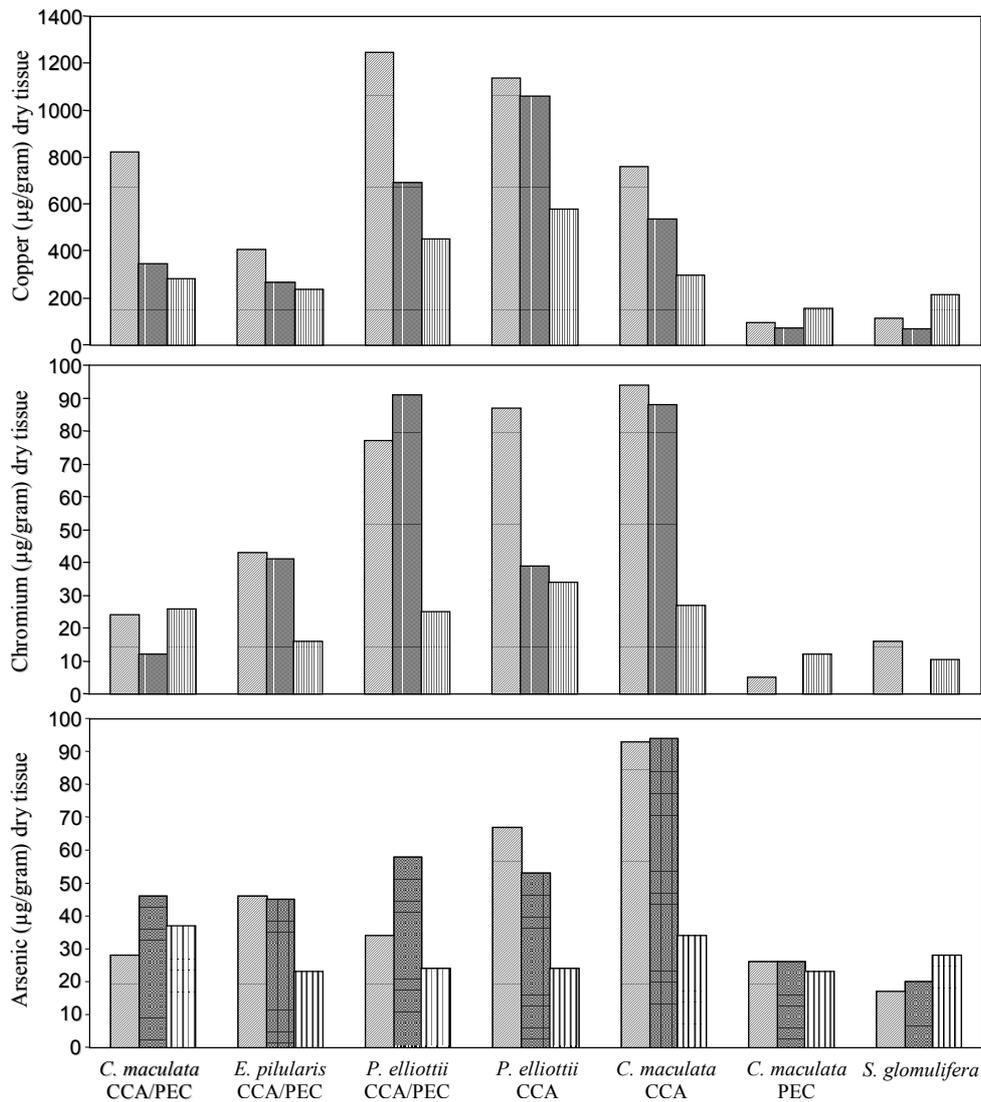


Figure 7 Mean concentration of copper, chromium and arsenic in barnacle tissue samples taken from experimental marine piles after 2, 4 and 5.7 years service life.

▨ 2 yrs ▩ 4 yrs ▮ 5.7 yrs

A number of studies have investigated the reliability of barnacle shells as a chemical record of trace elements in the environment (Watson *et al.*, 1995; Bourget, 1974), mostly with inconclusive results. Such studies found that, while barnacles had higher shell metal-concentrations from environments with higher metal loadings, there were a large number of variables that could also influence shell metal concentration such as growth rate (Bourget, 1974), seasonal change and shell size (Watson *et al.*, 1995). These studies have focussed on trace elements in the environment being the primary source of contamination, while the possible source in this study is far more direct. Mean levels of copper identified in this study, up to 35 ppm, were much greater than those

described in other reports, at up to around 4 ppm (Watson *et al.*, 1995). Further work is desirable in order to determine the process that contaminates barnacle shells to such an extent, e.g. by filter feeding or direct diffusion from the timber surface. Monitoring levels of chromium in the shell of barnacles could prove particularly useful, as the concentrations identified in the shell are similar to those in dry tissue and would exceed that in wet tissue. That is to say, the majority of the chromium burden in an individual organism may be present in the shell.

Comparison of copper and chromium levels identified in this study with those from previous work reveals an apparent trend suggesting a decline in the level of leachates being accumulated. From this it can be proposed that the degree of copper and chromium leaching from CCA-treated marine piles continues to decline during service.

The mean levels of copper and chromium detected in the tissue and shell of barnacles collected from the metal mooring pipes strongly suggests that contamination by leachates from CCA-treated timber occurs only in individuals within very close proximity of the timber/water interface. These barnacle populations were between 20-120 mm from the surface of CCA-treated *P. elliotii* piles and yet had metal levels very similar to those found in barnacles collected from untreated piles. Indeed, this can be taken further to suggest that the nature of a particular organism can make it more susceptible to acquiring leachates from treated timber. Barnacles grow with a basal plate of substantial surface area in intimate contact with the timber surface. Alder-Ivanbrook & Breslin (1999) found that the blue mussel, *Mytilus edulis*, accumulated little copper and chromium when exposed to CCA type C treated timber. A fouling organism such as *M. edulis* is secured by use of byssal threads, which provide far less intimate contact with the substrate and consequently are less likely to accumulate leachates from treated timber.

Our results from the analysis of barnacles suggest that there is no significant long-term contamination of the water column with CCA arising from the use of treated piles. However, barnacles growing directly on the surface of some pile types may have significantly elevated copper and chromium levels. Highest contamination was in barnacles growing on the surface of CCA-treated *P. elliotii* piles. A second treatment with creosote appeared to reduce the loss of CCA from treated *P. elliotii* piles. Of those pile types containing CCA preservative, least and generally insignificant contamination was found in barnacles growing on double treated hardwood piles of *E. pilularis* and *C. maculata*.

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7. Marine Corrosion & Corrosion Control

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Probabilistic Modelling of Marine Immersion Corrosion of Steels

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ABSTRACT

Marine environments can be very corrosive for mild and low alloy steels. Even though the various factors which can influence marine corrosion are generally accepted as known, quantitative relations are not well-developed. Consequently, the prediction of the likely degree of corrosion is still rather simplistic, and mathematical formulation largely has been neglected. This paper briefly reviews the factors of importance in marine corrosion, outlines previous models and describes on-going work aimed at developing a probabilistic phenomenological model for time-dependent material loss of mild and low alloy steels in immersion conditions.

INTRODUCTION

Immersion corrosion of mild and low alloy steels is an important factor for industrial coastal structures, ships and offshore systems. Such steels are still the preferred materials in marine environments, with mild steels used predominantly for ships and off-shore and harbour-side structures. Paint coatings and cathodic protection can be employed to offer some degree of protection against corrosion. However, such protection is not always sufficient, such as in sheet piling and in ships holds. For this reason there is continued interest in being able to predict, with some degree of accuracy, the likely amount of corrosion to be expected in given circumstances. Such prediction is not well developed. This is illustrated by the wide range in the typical values quoted in corrosion handbooks for differing situations. While approximate allowances usually are sufficient for new design, for the assessment of existing structures better information is preferred.

Estimates of point-in-time (i.e. short-term) corrosion rates can be obtained in-situ for existing structures using impedance, polarization and other electrochemical methods. However, for assessment of continued structural performance, longer term corrosion losses need to be estimated. This is the focus of the present research.

Only marine immersion corrosion (such as experienced by off-shore platforms at sea) is considered in this paper. Typically, such corrosion is 'uniform', that is, the loss of material is relatively uniform over the surface and pitting is not a significant factor. It is the most common form of corrosion for the mild and low alloy steels. Apart from structures which extend some way into the splash zone, the results obtained from small-scale specimens generally are directly translatable to full-scale structures.

Marine corrosion environmental influences

Factors which can influence marine immersion corrosion are shown in Table 1 (adapted from Schumacher, 1979). Not all factors are well understood. Others have become clearer only through recent research, such as the influence of marine growth (e.g. Hamilton, 1994). Seawater is known to contain a considerable amount of plant and animal life, and this can have an influence on immersion corrosion. Field observations show that micro-algal communities develop on steel (and other metals) almost immediately upon their being placed in sea water. Importantly, the biomass contains both aerobic and anaerobic bacteria, the latter being particularly important in governing longer term corrosion. Anaerobic conditions prevail when the oxygen supply to the corroding surface has been reduced due to the build-up of corrosion products, calcium and magnesium carbonates and microbiological material. Sulphate reducing bacteria then control the corrosion mechanism (Southwell *et al.*, 1979).

Table 1 Environmental factors in marine corrosion

Type	Factor	Effect on Initial Corrosion	Effect on Steady State Corrosion	Influenced by
<u>Biological</u>	• bacterial and biomass/plant life	little effect	controls rate.	<ul style="list-style-type: none"> • seawater temperature • NaCl concentration • pollutant type and level • seawater temperature
<u>Chemical</u>	<ul style="list-style-type: none"> • O₂ • CO₂ • NaCl • pH • carbonate solubility • pollutants 	<ul style="list-style-type: none"> directly proportional little effect inversely proportional little effect ? little effect varies 	<ul style="list-style-type: none"> none little effect uncertain uncertain little effect varies 	<ul style="list-style-type: none"> • unimportant at sea • little effect at sea • little known about
<u>Physical</u>	<ul style="list-style-type: none"> • temperature • pressure • water velocity • suspended solids 	<ul style="list-style-type: none"> proportional > 10°C uncertain little effect uncertain 	<ul style="list-style-type: none"> proportional ? uncertain uncertain 	<ul style="list-style-type: none"> • geographical location • oxygen effect • geographical location • geographical location

Probably the most important single parameter governing corrosion is water temperature, although field observations are often considered to be so influenced by other factors that a clear temperature effect often is not considered to be discernible (e.g. Phull *et al.*, 1997). A recent critical and detailed review of such data suggests the contrary (Melchers, 1997a). It is clear that during the initial part of the corrosion process, i.e. during the first few hours after the steel is exposed, water temperature tends to have two effects: as the temperature is increased (i) the rate of the corrosion process increases and (ii) the dissolved oxygen content in the surrounding seawater decreases, at least for seawater temperatures greater than about 10°C. Short term experimental observations show that the first is more important and that in the range 10 - 80°C higher temperatures lead to greater corrosion (e.g. Mercer & Lombard, 1995). Various longer term exposure data suggests that for temperatures $\theta > 10^\circ\text{C}$ a linear relation can be used to correct for temperature effects (Melchers, 1995a), at least for fully aerated, unpolluted 'at-sea' conditions and for up to 5 years of exposure (Melchers, 1997a):

$$d_2 = \frac{(\theta_2 - \theta_0)}{(\theta_1 - \theta_0)} d_1 \quad (1)$$

where d_2 is the depth of corrosion penetration (due to weight loss) at annual mean temperature θ_2 , d_1 is the corresponding corrosion depth at θ_1 and θ_0 is a constant obtained from fitting to the data: $\theta_0 = -10^\circ\text{C}$ appears to be consistent with data, see Figure 1.

Figure 1 shows data for mild steel specimens (to ASTM K01051) recorded in the ASTM sponsored world-wide corrosion study. This had observations at 14 different sites around the world (Phull *et al.*, 1997). The trend line shown was obtained subjectively from interpretation of the available and supplemented environmental conditions recorded at the various sites and through comparison to the corrosion behaviour of specimens of 90/10 copper-nickel (to UNS C70600) and aluminium (to UNS A95086). These materials respond differently to various environmental conditions, see Table 2.

Table 2 Influences on corrosion

Metal	Aluminium	Copper-nickel	Steel
Factors	Temperature DO	Temperature DO Salinity pH Sulphides Velocity	Temperature DO Salinity pH Calcium carbonate Velocity

Other factors

Although temperature influences corrosion kinetics, diffusion and dissolved oxygen, it is clear, as noted in Table 1, that there are a number of other factors which influence corrosion, including the concentration of dissolved oxygen able to reach the corroding surface. As noted, it is conventional to assume that for 'at-sea' conditions the waters are fully aerated (i.e. saturated). There are known also to be effects due to marine growth and due to calcite deposition, but the precise influences of these is not yet fully understood. While these matters are being investigated as part of the present research program it appears reasonable to assume that they influence corrosion 'at-sea' in a manner best modelled probabilistically. This applies also for other factors at variance with the 'at-sea' assumption, such as when steel is corroding in harbours, channels and where salinity, pH, water velocity, (different) marine growth, etc. might become influences (Melchers, 1997a). Thus, it should be clear that because corrosion is a function of many variables, not all of which are well described or can be modelled at present, a probabilistic model to describe expected corrosion is appropriate.

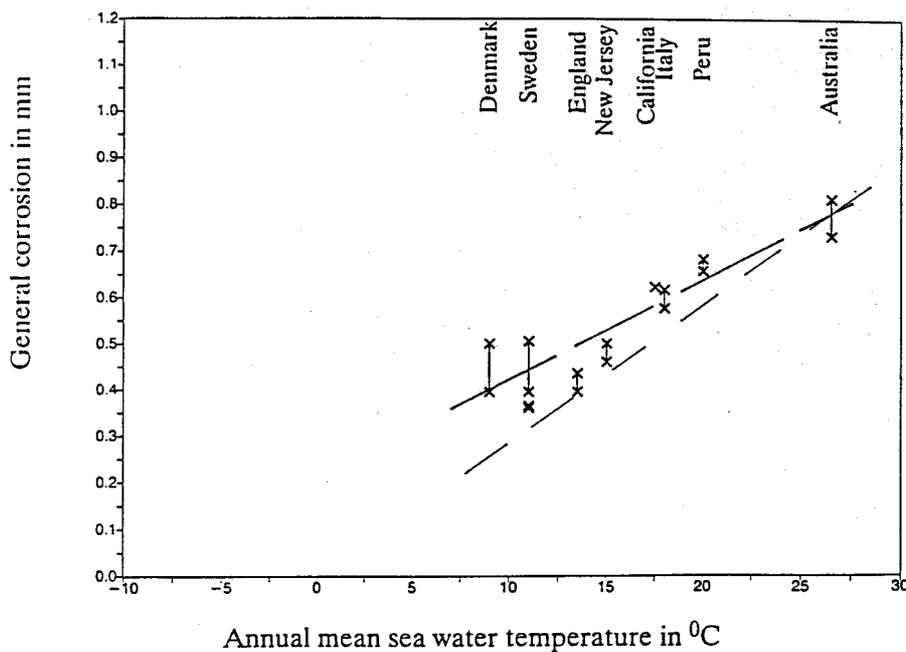


Figure 1 Five year corrosion as a function of annual mean water temperature (Melchers, 1997a).

PROBABILISTIC CORROSION MODELLING

Previous phenomenological models

Rather simple empirical models have been proposed for immersion corrosion conditions, mainly for longer term exposure conditions. Figure 2 shows longer term

corrosion data as at 1995 (Melchers, 1995a), reduced to 10°C for comparison purposes, using the temperature correction (1) together with some models. These include the linear models of Reinhart and Jenkins (1972), Southwell et al. (1979) and Melchers (1995b) and the empirical non-linear model:

$$d_w = 0.084t^{0.823} \quad (2)$$

where d_w is the nominal uniform corrosion depth and t is time since immersion. It is clear from Figure 2 that none of the models fit the data very well. The data itself shows a high degree of scatter.

A further model based on electrochemical theory and on the assumption that corrosion is at all times governed entirely by oxygen diffusion conditions and therefore by the limiting discharge current (or 'corrosion current') i has been proposed by Chernov and Ponomarenko (1991). However, difficulties were found in fitting the model to limited two-year corrosion data.

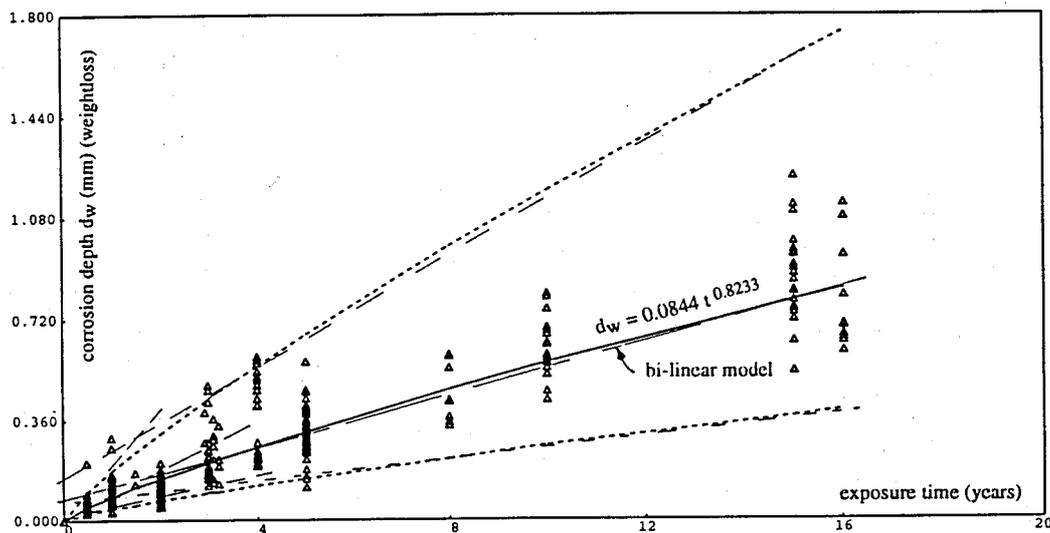


Figure 2 Corrosion data points and models for field exposed specimens.

Proposed phenomenological model

A more comprehensive model based on separately modelling the various corrosion regimes shown in Figure 3 has been proposed (Melchers, 1995a; 1997b) and is currently under development (Melchers, 1997a). To allow for the uncertain nature of the information available, the model is also probabilistic and of the following general form:

$$c(t, \mathbf{E}) = fn(t, \mathbf{E}) + \varepsilon(t, \mathbf{E}) \quad (3)$$

where $c(t, \mathbf{E})$ is the weight loss of material, $fn(t, \mathbf{E})$ is a mean valued function, $\varepsilon(t, \mathbf{E})$ is a zero mean error function, t is time and \mathbf{E} is a vector of environmental conditions.

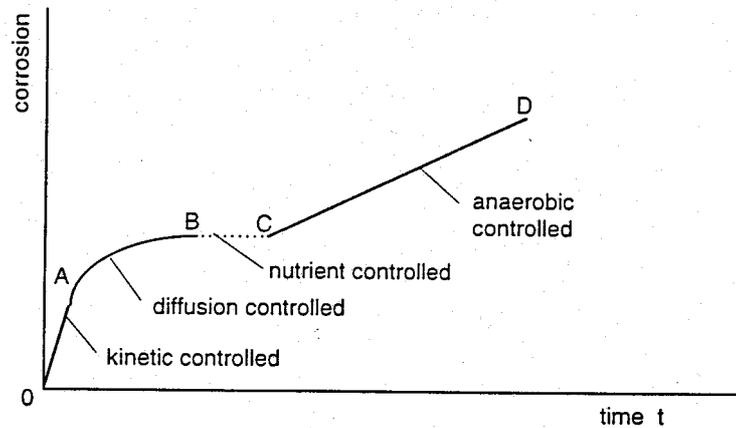


Figure 3 Proposed conceptual model for marine corrosion

In the early part (OA) of the model corrosion is initially under anodic control and then becomes controlled by cathodic ion transport or oxygen concentration control. When the thickness of the corrosion products, calcium carbonate deposits and the biomass becomes sufficient, the rate of oxygen supply to the corroding surface, will become the limiting factor. This is cathodic oxygen diffusion control which is described by AB in Figure 3 or by (Evans, 1960; Melchers, 1995a):

$$\frac{dO_2}{dt} = \text{oxygen mass flow} = \frac{k_1(C_L - C_i)}{R} \quad (4)$$

where O_2 represents the mass of oxygen transfer, C_L and C_i are the concentrations of dissolved oxygen in the seawater (assumed fully aerated) and at the corrosion interface respectively, R is the resistance to diffusion and k_1 is a constant. If it is assumed that the dissolved oxygen concentration at the corroding surface is close to zero (due to it being immediately consumed by the corrosion process), the difference in oxygen concentration across the corrosion product layer can be taken as a constant for a given temperature (as saturation depends on temperature). With a number of other assumptions it can be shown that the weightloss x as a function of time t is given by:

$$x = \sqrt{kt + c} \quad (5)$$

where k and c are constants.

It should be clear that the theoretical relationship (5) can not fit experimental data over the full range of observations (as has been attempted, for example, in the atmospheric corrosion literature by modifying the constants).

Eventually the corrosion products and other matters will restrict the oxygen supply to such an extent that anaerobic conditions begin to take over (CD in Figure 3). The activation of the anaerobic bacteria (see above) requires not only anaerobic conditions but also an adequate food supply, thought to be made available through the aerobic bacteria (BC). When this has occurred to a sufficient extent, sulphate reducing bacteria (SRB) control the rate of corrosion (Melchers, 1997a).

COMPARISON WITH AVAILABLE DATA

Work on calibrating the model described above is still in progress but some preliminary results can be given, at least for the range (AB). The ASTM data for mild steel (Phull *et al.*, 1997) was considered and corrected to a common base of 10°C, using expression (1) with $\theta_0 = -10^\circ\text{C}$. Examination of the temperature corrected data indicated that where the specimens were exposed in harbour or channel conditions, the scatter in data points was greater than elsewhere. Accordingly, six of the sites were considered incompatible with the 'at-sea' assumption. The corrosion loss versus time observations for each of the remaining sites are shown in Figure 4. It is seen that there is a considerable amount of scatter. The best fit curve based on function (5) has been plotted through the data and the 5 and 95 percentile confidence bounds shown. The means, standard deviations and variances were then calculated for each observation time (see Table 3) (Melchers, 1995c).

The standard deviations are rather smaller than those obtained from the earlier models. This is due to the elimination of test results for waters which are not under 'at-sea' conditions. Thus the present results are an improvement over the earlier models in that the influence both of temperature and of aeration has been recognized specifically.

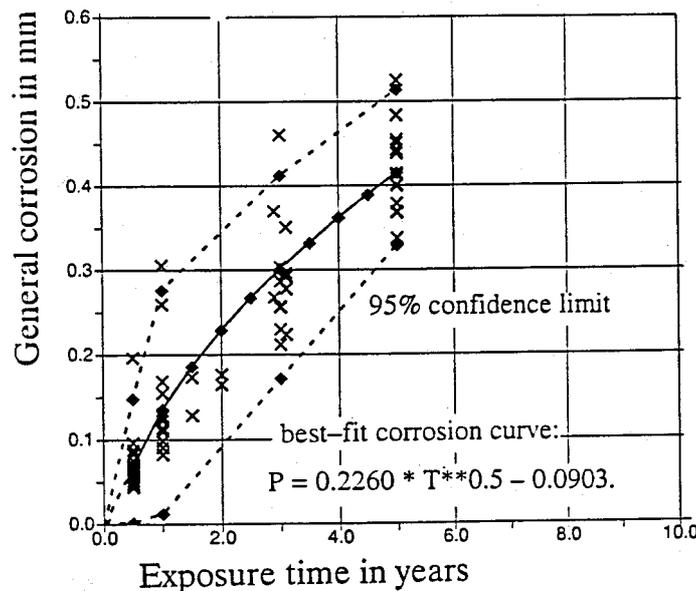


Figure 4 Corrosion data for 'at-sea' sites and fitted model and confidence limits.

Table 3 Statistics from observations

Observation Time (years)	Mean corrosion (mm)	Standard Deviation (mm)	Variance
0.5	0.075	0.0373	0.50
1.0	0.144	0.0674	0.47
3.0	0.292	0.0614	0.21
5.0	0.421	0.0470	0.12

DISCUSSION

The above discussion has been based on 'mild' steel without defining it too closely. In practice a variety of steels are in common use and it might be expected that metal composition plays some role in corrosion behaviour. However, it is necessary to distinguish between short and the long term effects.

Under initial anodic control, when the dissolution of the metal controls the reaction, the corrosion rate is likely to depend very much on the composition of the steel. Indeed, this has been noted in short-term experiments, including laboratory tests. However, when oxygen diffusion controls the rate of corrosion (cathodic control), which is usually the case after a relatively short period of exposure, relatively small differences in the composition of the steel and its heat treatment theoretically should have little bearing on its corrosion properties, at least for pH between 4 to 10 (Schumacher, 1979) and for relatively small amounts of alloying materials.

However, judging by the limited experimental observations available to date, in practice the situation is rather more complex, with possible interplay between marine growth and the effect of alloys on corrosion product properties (Melchers, 1999). At present agreement on these matters and hence on models for the effect of alloys is not available.

Further work

Comparison of steel immersion corrosion data reported by others has not been easy owing to the lack of data about environmental conditions. Nevertheless, what is available or can be deduced suggests that such earlier data are not inconsistent with the observations reported above (Melchers, 1997a).

For the further development of the model described above investigations are need at least in the following areas:

- 1 corrosion behaviour below 10°C
- 2 mechanics of water aeration and oxygen supply to the corroding surface.
- 3 influence of marine growth.
- 4 effect of water velocity.

- 5 effect of orientation of immersion specimens.
- 6 area effects (only small specimens considered so far).

This work also requires quantification of the influence of these factors along the lines described for the effect of temperature. Some of this work is currently under way, with an extensive field test program currently addressing items 2 - 5. Preparations are being made to investigate items 1 and 6.

CONCLUSION

The marine immersion corrosion corrosion-time models for mild and low alloy steels developed to date are based largely on curve-fitting, without much cognizance of basic corrosion phenomena. To improve mathematical modelling, a multi-stage phenomenological model for corrosion weight-loss is being developed. This has raised questions about the specific influences of various environmental and material property parameters, largely not considered in detail in the corrosion literature. An important parameter is seawater temperature. A linear temperature correction previously proposed is consistent with reported field and laboratory data for fully oxygenated conditions. Nevertheless, considerable scatter remains, reinforcing the notion that the model should be probabilistic. Further research required to develop the model is indicated.

Acknowledgments

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Physico-Chemical Modelling for the Prediction of Seawater Metal Corrosion

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ABSTRACT

The paper discusses the processes of corrosion products' film growth on the metallic surfaces and corrosion reagents film transport in relation to metal corrosion behaviour prediction in natural seawater. The authors' proposed combined physical-chemical approach to these processes enables correlation of the thickness of metallic corrosion K with exposure time t as follows:

$$K - \frac{P}{V_p} \left(1 - \frac{V_p}{V_o} \right) \left(1 - \exp \left(- \frac{V_p K}{P} \right) \right) = V_p t,$$

where coefficient P characterizes the protection properties of corrosion products, V_o and V_p - initial and steady corrosion rates. Data base application of metal corrosion in different ocean regions has enabled an estimation of the coefficients depending on physical-chemical seawater parameters: temperature, dissolved oxygen concentration, hydrostatic pressure, flow rate of seawater etc. The approach described seems to have been applied for the first time to numerically define the effect of seawater environment variations on metallic corrosion. The data obtained has been used to chart the corrosion activity of seawater for mild steel, copper, aluminium and their alloys.

INTRODUCTION

Considerable data exists on corrosion tests of various metals and alloys at controlled physico-chemical parameters of seawater. In most cases metals and alloys corrode with great variability under different seawater environments. However, evaluation of the influence of physico-chemical parameters on metal corrosion has not been carried out. The reason for this is the absence of a reliable physico-chemical model for the corrosion

process, which would take into consideration the corrosion rate decrease as the corrosion product films form and the physico-chemical parameters of seawater associated with this process.

MODELS OF METAL CORROSION IN SEAWATER

The first model for an estimation of the maximum corrosion rate of metals with oxygen depolarization can be determined from a limiting current of the discharge of oxygen:

$$i = \frac{nFDC_{O_2}}{\delta} \quad (1)$$

where δ is thickness of diffusion layer, $n = 4$ is the number electrons participating in discharge of oxygen $O_2 + 2H_2O + 4e \rightarrow 4OH^-$, F is the Faraday constant, D and C_{O_2} are the coefficients of diffusion and concentration of oxygen in seawater.

The equation (1) links three parameters determining the corrosion rate in seawater: oxygen concentration, temperature and velocity of the currents. At present, the distribution of these parameters in seawater is well known enough for any area of the World Ocean, because of the long history of oceanological research.

Seawater temperature influences the value of a limiting current for oxygen discharge through the diffusion coefficient and thickness of the diffusion layer because of its effect on seawater viscosity. Increasing the seawater velocity changes the diffusion processes from natural convection to an enforced mechanism that is reflected in (1) through the decrease of diffusion layer thickness.

The temperature dependence of the diffusion coefficient of oxygen in seawater can be described by the equation:

$$D = D_o \exp(-Q/RT) \quad (2)$$

where $D_o = 5.289 \times 10^{-6} \text{ m}^2/\text{s}$, $Q = 19.5 \text{ kJ/M}$ is the energy of activation similar to the energy of activation diffusion of water.

The analytical dependence of the diffusion layer thickness on current velocity is complicated and can be verified by experimental results (Rosenfeld *et al.*, 1965):

$$\delta = \frac{126 \cdot 10^{-2}}{25 + 718.3\sqrt{u}} \quad (3)$$

where u is seawater velocity in m/s.

The influence of seawater temperature and depth on diffusion layer thickness can be neglected, as their contribution is seen only in kinematic viscosity of which only 1/6 is included in the analytical equation with its range varying by less than a factor of two.

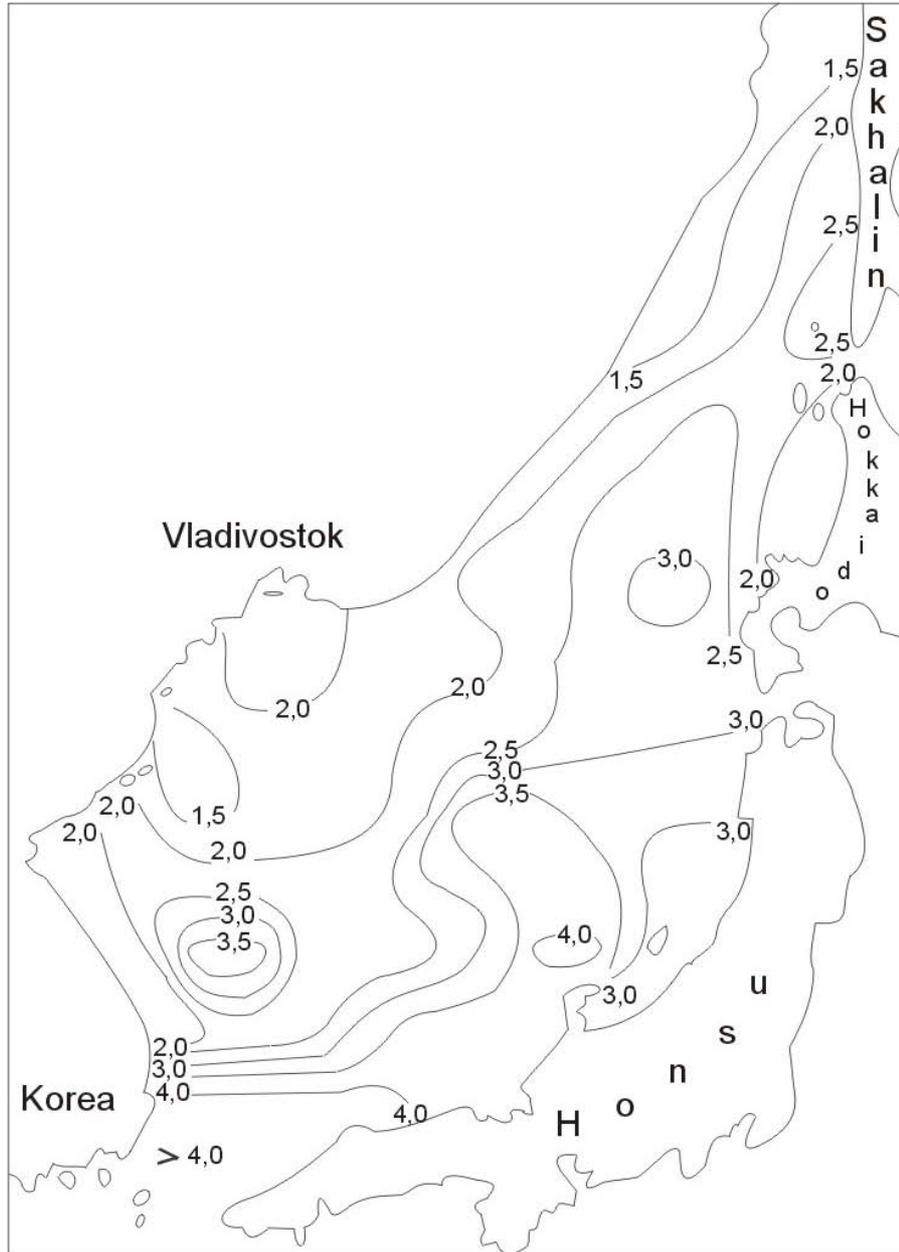


Figure 1 Distribution of the maximum corrosion rate of metals (mm/yr) in surface waters of the Japanese sea

Figure 1 illustrates the distribution of the maximum steel corrosion rate in the surface waters of the Japanese sea, in which the hydrochemical mode is essentially different from the ocean. Corrosion rates are minimal in the northwest region and increase in the southeast part of the sea, where the warm Tsushima Current enters through the Korean strait. It is possible to explain insignificant variation in the corrosion rate with oxygen

concentration close to saturation and the large influence of velocity in zones with lower oxygen concentration.

Decrease of corrosion rate with time depends on the dissolution laws on the surface of a corroding metal and is determined by the transport rate of oxygen from the seawater through the formed protective deposit. The study of such laws opens an opportunity of forecasting the corrosion of metal in seawater due to its physico-chemical characteristics.

The second model attempts to propose the calculation for the prediction of seawater metal corrosion on the basis of the corrosion product film growth laws. It is assumed that the corrosion rate at any time can be described by the equation:

$$V = \frac{\partial K}{\partial \tau} = \frac{A}{h}, \quad (4)$$

where K is thickness of metal corrosion failure for exposure time τ , A is the coefficient characterizing the penetration properties of corrosion products, h is thickness of corrosion product film. The growth rate of corrosion product film is described by the equation:

$$\frac{\partial h}{\partial \tau} = B \left(\frac{\partial K}{\partial \tau} - V_p \right), \quad (5)$$

where B is the coefficient characterizing the protection properties of corrosion products and V_p is the steady corrosion rate. Integrating Eq. (4) and (5) results in

$$K - \frac{P}{V_p} \left(1 - \frac{V_p}{V_0} \right) \left(1 - \exp \left(- \frac{V_p K}{P} \right) \right) = V_p \tau \quad (6)$$

$$\frac{\partial K}{\partial \tau} = \frac{V_p}{1 - \left(1 - \frac{V_p}{V_0} \right) \cdot \exp \left(- \frac{V_p K}{P} \right)}, \quad (7)$$

where $P = \frac{A}{B}$, $V_0 = \frac{A}{h_0}$ is initial corrosion rate, h_0 is thickness of corrosion product film at $\tau = 0$.

As $h \rightarrow 0$ or $V_0 \gg V_p$ (6) and (7) can be written as

$$K - \frac{P}{V_p} \left(1 - \exp \left(- \frac{V_p K}{P} \right) \right) = V_p \tau \quad (8)$$

$$\frac{\partial K}{\partial \tau} = \frac{V_p}{1 - \exp\left(-\frac{V_p K}{P}\right)}. \quad (9)$$

The analysis of these equations shows that the corrosion rate decreases as corrosion proceeds and in due course, asymptotically approaching the established velocity V_p . Thus the decrease of corrosion rate is determined by the value P/V_p , which is the parameter inclined asymptotes for dependence on time for having corroded metal thickness $K=P/V_p+V_p\tau$ (Figure 2). Hence, the knowledge of two parameters P and V_p completely determines corrosion losses from the equation (8).

The formulas above contain two parameters V_p and P characterizing the processes of metal corrosion in seawater. The dissolved oxygen concentration, temperature and hydrostatic pressure are the major influence on coefficient P and flow rate of seawater, pH and hydrostatic pressure influence V_p .

The results of corrosion tests of steels and copper in the Pacific and Atlantic Oceans at various depths (LaQue, 1975; Korovin, 1984; Peterson, 1984; Schumacher, 1979; Ulanovsky, 1976, 1978, 1979) have enabled estimates of the coefficients depending on physico-chemical parameters of seawater (Table 1).

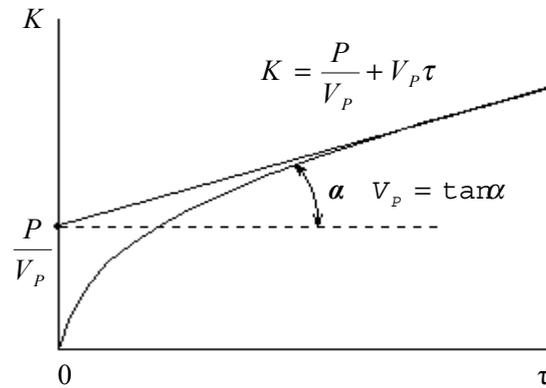


Figure 2 Change of corrosion losses with time (Equation 8)

Statistical analysis has shown, that $V_p = (3.67 \pm 2.77) \mu\text{m}/\text{yr}$ and $P=(1504.04 \pm 97.79) C_{O_2} [1+(0.033 \pm 0.006)t^{\theta}] \mu\text{m}^2/\text{yr}$ for steels, at 95% confidence. These results indicate that diffusion transport of oxygen through the surface film is the rate-controlling step of the steel corrosion. With regard to Eq. (5), after neglecting terms of higher order, it is significantly simplified and leads to the following equation

$$K = (2P\tau)^{0.5}, \quad (10)$$

where $P=(1504.23 \pm 121.35) C_{O_2} [1+(0.045 \pm 0.008)t^{\theta}] \mu\text{m}^2/\text{yr}$. It is easy to trace from (8) and (10) that uniform corrosion rates of steels vary widely and slowly decrease with time

of exposure. In particular, a corrosion rate of 20 $\mu\text{m}/\text{yr}$ at oxygen concentration 5 ml/l and temperature 20⁰ C may be reached after 17.5 years with corrosion failures of 680 μm .

The corrosion of copper is well represented by the simple equation: $K = V_p (1 + \alpha t^0 C) \tau$, where $V_p = (16.78 \pm 1.82) \mu\text{m}/\text{yr}$, $a = (1.38 \pm 1.0) \times 10^{-2} 1/\text{degree}$.

Figure 3 is indicative of an actual seawater corrosion activity chart

Obviously, the general approach above can be used for predicting the metal corrosion in different ocean regions and charting the corrosion activity of seawater.

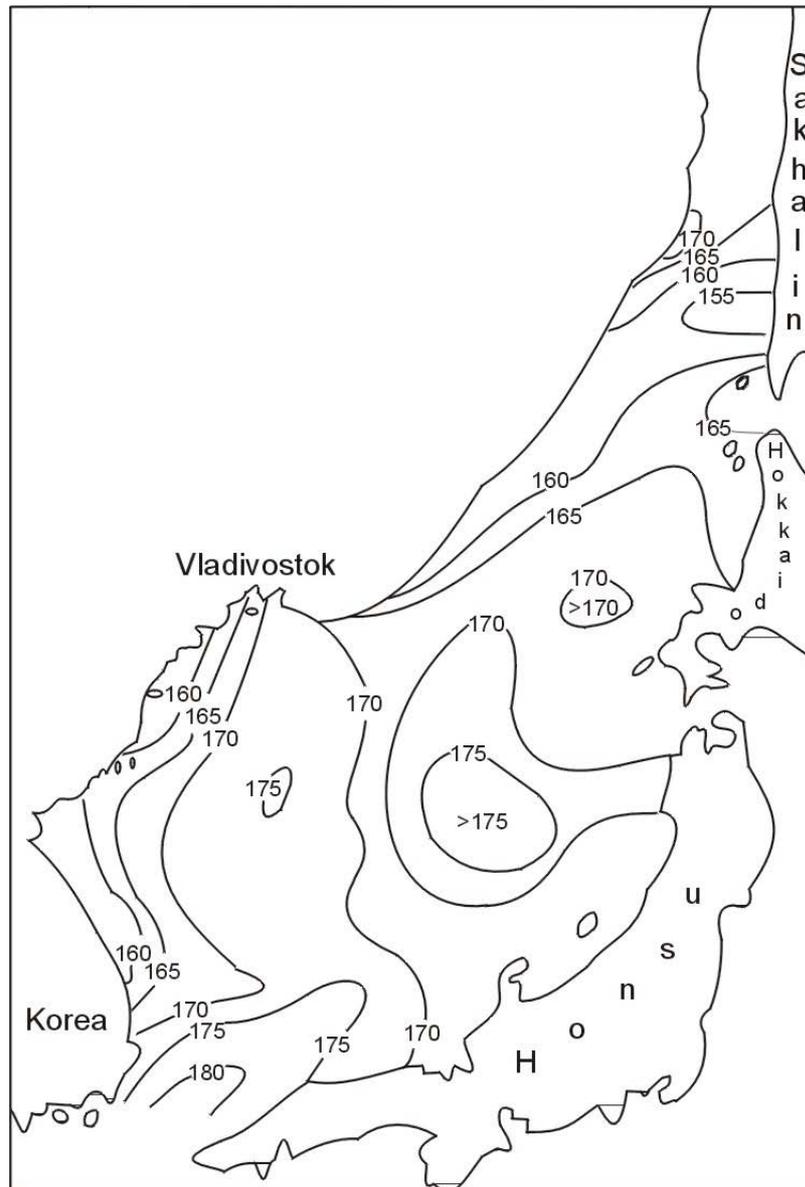


Figure 3 Distribution of corrosion rate for steels ($\mu\text{m}/\text{yr}$.) in surface waters of the Japanese sea in one year

Table 1 Data of corrosion tests in seawater (numerator-carbon and low-alloy steels, denominator - copper)

Parameters	Exposure t , year	Depth of corrosion K , μm	Concentration of oxygen C_{O_2} , ml/l	Temperature, $t^\circ\text{C}$	Depth, m
maximum	<u>2.000</u>	<u>200.00</u>	<u>8.32</u>	<u>28.31</u>	<u>5500</u>
	2.915	51.44	8.60	28.31	5500
minimum	<u>0.027</u>	<u>6.47</u>	<u>0.28</u>	<u>1.25</u>	<u>0</u>
	0.027	0.19	0.28	1.25	1
middle	<u>0.157</u>	<u>44.56</u>	<u>4.31</u>	<u>9.39</u>	<u>1433.5</u>
	0.347	8.14	4.08	9.97	1411.2

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Stress Corrosion Cracking of Duplex Stainless Steels and their Weldments in Marine Environments: An Overview

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ABSTRACT

This paper presents an overview of the phenomenon of stress corrosion cracking (SCC) of duplex stainless steels and their weldments in marine environments and a potential role of microbial activity in altering SCC susceptibility. As a precursor to the topic the paper also reviews performance of the traditional corrosion resistant alloys and their weldments, and the necessity of using duplex stainless steels (DSS) in order to alleviate corrosion problems in marine environments. Given that the performance of weldments of such steels is often unsatisfactory, this review also assesses the research needs in this area.

CORROSION RESISTANT ALLOYS FOR MARINE APPLICATIONS

Varieties of stainless steels (SS), *viz.*, austenitic SS (e.g., AISI types 304, 316 and 316L), ferritic SS (e.g., AISI type 430) and martensitic SS (e.g., AISI type 403 and 431) are the commonly used corrosion resistant alloys. Of these, austenitic stainless steels (SS) form the most popular class of materials because of their unique combination of fracture toughness, formability, fabricability and corrosion resistance. However, the austenitic SS are less commonly used in marine environments because of their susceptibility, in chloride-containing environments to stress corrosion cracking (SCC). SCC was defined by Gnanamoorthy *et al.* (1996) as *premature cracking in materials under the conjoint action of a tensile stress and corrosive medium, neither of which when acting alone would cause failure*. The ferritic stainless steels have inferior fracture toughness to the austenitic variety; however, they possess better strength and resistance to SCC.

Various Fe-Cr-Ni alloys, with alloying additions of Mo, Ti, Cu, Al, N, have been developed with a view to addressing the SCC problems in marine environments, in addition to improving the strength of the material (Table 1). However, the high cost of most of these alloys generally restricts their application as commonly used construction materials.

Table 1 Stress corrosion cracking (SCC) resistant alloys for marine applications

<i>Alloys</i>	<i>Nominal Composition (%)</i>	<i>Properties</i>
JS-777	Fe-20Cr-25Ni-5Mo-3Cu	pitting resistant
Alloy 600	Ni-15Cr-8Fe	good Cl-SCC resistance
Alloy 800	Fe-21Cr-32Ni	
Alloy B	Ni-28Mo-5Fe	high resistance to SCC
E-Brite Alloys	Fe-26Cr-1Mo Fe-29Cr-4Mo (2Ni)	good Cl-SCC resistance
Alloy 18-8-2(2Si)	Fe-18Cr-8Ni-2Mo (2Si)	good Cl-SCC resistance

SCC RESISTANCE OF DUPLEX STAINLESS STEELS (DSS) IN MARINE ENVIRONMENTS

Duplex stainless steels (DSS), which generally have a ferrite/austenite volume ratio of 1:1, show considerable resistance to SCC (Shimodaira *et al.*, 1977). DSS are used in aggressive marine environments where austenitic SS fails due to chloride-SCC. DSS derive benefits of SCC resistance and strength due to the ferritic, and toughness due to the austenitic phases. Fontana *et al.* (1984) have suggested that the pools of ferrite in the austenitic matrix tend to block the progress of cracks. In fact, Shimodaira *et al.* (1977) have reported, both yield strength and SCC resistance of duplex SS to be considerably greater than those for ferritic or austenitic SS. Hence, a variety of duplex and super duplex stainless alloys have been developed for different marine applications (Table 2).

Table 2 Duplex stainless steels for marine applications

<i>Duplex / Super-duplex SS</i>	<i>Nominal Composition</i>
SAF 2205	Fe-22Cr,6Ni,3Mo, 0.15N,0.03C
SAF2304	Fe-23Cr-4Ni-0.1N-0.03C
3RE60	Fe-18Cr-5Ni-2.7Mo-0.03C
Alloy 329	Fe-25Cr-5Ni-1.5Mo
Ferralium	Fe-25Cr-6Ni-3Mo-2.5Cu-0.17N-0.06C
Super-Duplex Zeron 100 (~S32760)	Fe-25Cr-7Ni-3.5Mo-0.8W-0.75Cu-0.25N-0.03C

In chloride media, the superior SCC resistance of the duplex SS to both austenitic and ferritic SS arises from the electrochemical behaviour (galvanic corrosion) of the two phases - austenite, which is more susceptible to SCC, is cathodically protected by the ferrite (Shimodaira *et al.*, 1977). In fact, the improvement in the chloride SCC resistance due to the presence of ferrite with austenite has been known in the case of weld metal region of austenitic SS weldments. Figure 1 shows the presence and variation in ferrite

content in the weld metal of austenitic stainless steel, caused by the variation in Cr content (which is a strong ferrite-stabilising element) in the weld metal. In moving from weld metal (fusion zone) to the unaffected base metal, different thermal treatment is experienced during welding, at different distances, resulting in the variation in ferrite contents. Gooch (1983) has suggested that the presence of retained ferrite (5-10%) in the weld metal and its absence in the base metal of the austenitic SS weldment account for the superior SCC resistance of the former. However, Baeslack et al. (1979) has reported that at higher ferrite contents in the austenitic SS weld metal, crack will propagate along the ferrite/austenite interphase interface due to a dissolution at the interface.

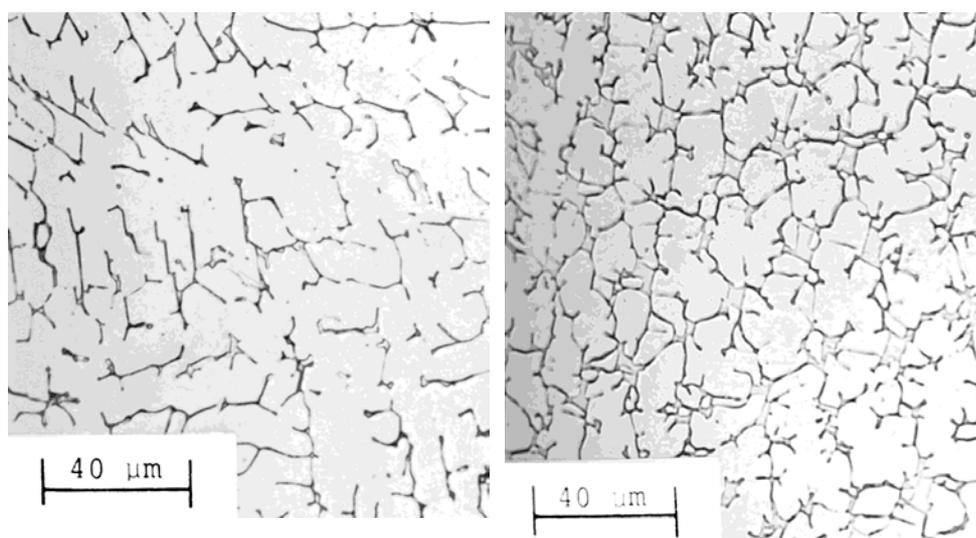


Figure 1 Presence of ferrite (which is the dark phase) and variation in ferrite content in the weld metal of a typical austenitic stainless steel with different Cr-content, resulting in ferrite volume pct : (a) 6, and (b) 16 (nearly continuous ferrite network).

SCC RESISTANCE OF DUPLEX STAINLESS STEEL (DSS) WELDMENTS

As discussed earlier, the amount of ferrite phase has a profound influence on the SCC resistance of the stainless steels. The influence of the ferrite content on SCC resistance is reasonably well understood in the case of austenitic SS welds (Gooch, 1983; Baeslack *et al.*, 1979). However, there is clearly a lack of similar understanding on the role of microstructural aspects of ferritic phase in the SCC resistance of duplex SS, particularly their welds. A ferrite/austenite volume ratio of about 1:1 has been suggested to be the optimum for intergranular corrosion resistance of duplex SS (Ward *et al.*, 1984). However, in the case of duplex SS welds and the adjacent heat affected zone (HAZ), the ferrite content will generally deviate from the optimum value due to the presence of some retained δ -ferrite. Singh Raman *et al.* (1998) have proposed that for safer operation of the welded components of duplex SS, it is necessary to know the role of the additional ferrite contents of the weld metal and HAZ in the SCC resistance of duplex SS. If the additional ferrite in the weld and HAZ is found to reduce the SCC resistance, the obvious query will be whether the weld metal and HAZ microstructure

can be suitably engineered (by suitable welding) to improve SCC-resistance of this region. For example, the ferrite content in the HAZ can be minimised if welding is carried out using low-energy input processes, such as laser or electron beam welding.

MICROBIOLOGICALLY INFLUENCED CORROSION (MIC) OF STAINLESS STEELS AND THEIR WELDMENTS

Videla (1986) has defined microbiologically influenced corrosion (MIC), microbial corrosion, or biocorrosion as an electrochemical process where the participation of the microorganisms is able to initiate, facilitate or accelerate the corrosion reaction without changing its electrochemical nature. Microorganisms can influence a corrosion process by one or a combination of the following phenomena:

- patchy microbial deposits/colonies/tubercles/biocorrosion products can form discontinuous biofilms which enable formation of new galvanic cells and/or alter the conditions in the existing galvanic cells, as shown schematically in Figure 2 (Videla, 1996; Borenstein, 1988).
- metabolic processes of the microorganisms can destroy the existing protective films on the corrosion resistant alloys.

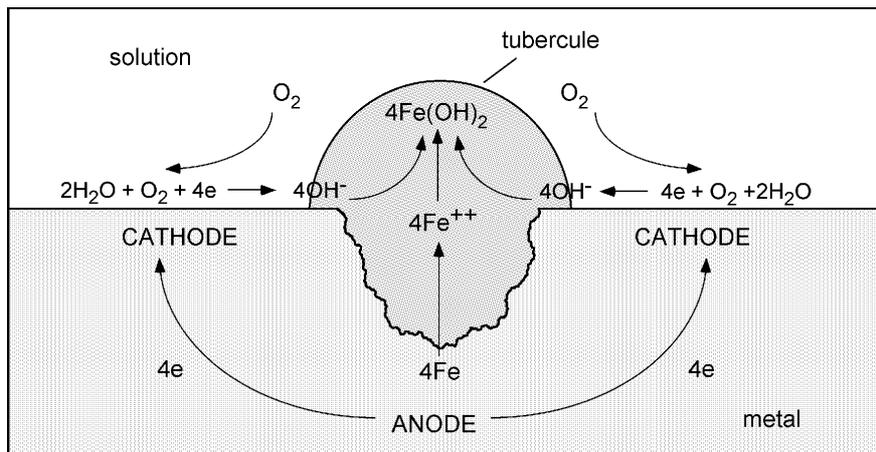


Figure 2 Schematic of a tubercle-assisted oxygen concentration cell pitting.

Newman *et al.* (1986) have suggested that, as a result of its ability for creating and/or altering the galvanic cell conditions, MIC can have a profound influence on localised corrosion, *viz.*, pitting. This influence is particularly relevant to this overview since pitting is believed to be one of the critical factors in stress corrosion crack initiation. Among the microorganisms, for most of the bacteria the chlorine/chloride (and hence the marine environment) is toxic. However, some of them (*viz.*, *Bacillus spp.*) not only survive in the marine environments, they can, in fact, live on chloride ions (Stoecker, 1995).

Stainless steels derive corrosion resistance from the formation of passive oxide films, which are stable in oxidising environments. An oxidising environment is necessary for sustaining a passive film, as well as for its repair in the event of a local mechanical or chemical damage. Localised damage in the passive films can result in the formation of pits and crevices. However, presence of aerobic bacteria can deplete oxygen within such pits and thus cause acidification which will accelerate the pitting rate. For example, Stoecker *et al.* (1995) have attributed the pitting of stainless steel in a water of very low chloride-content (20ppm, which should not normally cause pitting) to the presence of microorganisms in the environment.

Presence of microorganisms can be directly involved in increasing the corrosiveness of chloride-containing environment. For instance, iron-oxidising bacteria in chloride-containing, water can, when in contact with steel, produce ferric chloride which is known to accelerate pitting processes (Borenstein, 1988).

Videla (1986) has identified that the role of MIC involves influence of the three factors, *viz.*, metal, solution and microorganisms. To that extent, the role of metal and its microstructure are appreciated by biocorrosion scientists, such as Borenstein (1991). Borenstein (1991) has also reported that metallurgical features, such as deformation of the grain structure or presence of inclusions, act as sites of decreased MIC resistance.

Borenstein (1988, 1991) and Stoecker (1995) have found the influence of metal microstructure on MIC to be most marked in the case of weldments of stainless steels. Borenstein (1988, 1991) also emphasized that the duplex microstructure (ferrite and austenite, as shown in Figure 1), which is generally present in the weld metal and neighbouring HAZ of the weldments of austenitic stainless steels, is preferentially attacked by MIC. The fully austenetic structure in the base metal region is less often attacked. Borenstein (1988) also found that MIC (by iron-oxidising bacteria, *Gallionella*) showed no preference for one or other phase in the duplex (austenitic+ferritic) structure. However, Videla (1996) has reported that in a preferential attack on the duplex weld structure, *Gallionella* selectively attacks the austenitic phase. In this regard, as suggested by Singh Raman *et al.* (1998), it may be necessary to know the influence of the additional ferrite contents on MIC of the weld metal and HAZ in the weldments of duplex SS.

Welding of austenitic stainless steels can also result in another microstructural heterogeneity (particularly, in HAZ microstructure), *i.e.*, sensitisation, which is susceptibility to intergranular corrosion because of depletion of chromium from the area neighbouring grain boundaries as a result of chromium carbide precipitation. Borenstein (1988) has suggested that the sensitised structures are more susceptible to MIC, but lack of sensitisation did not guarantee protection from MIC.

ROLE OF MIC IN STRESS CORROSION CRACKING (SCC) OF STAINLESS STEELS AND THEIR WELDMENTS

Crack-initiation is a crucial mechanistic step in stress corrosion cracking (SCC), and pit-formation is one of the widely accepted modes of crack-initiation for SCC of stainless steels. Hence, those factors which can cause surface electrochemical heterogeneity leading to pit formation, or can alter the existing pit chemistry, can also influence SCC. In this respect, the role of MIC in increasing pitting susceptibility of stainless steels can be presumed to be of a great significance. Callejas et al. (1992) have reported initiation of pitting in presence of MIC to be responsible for facilitating notching, which led to SCC failures of stainless steel (viz., martensitic SS) pumps in the presence of vibrational stresses. Stoecker (1995) has also reported an iron-bacteria (*Gallionella*) - assisted in-service SCC failure of austenitic SS components in chloride environments.

Extensive failures of SS components (particularly, in the weldments) due to SCC have been a major concern affecting the marine application of SS. One of the difficulties in addressing the SCC failures has been the great deal of inconsistency in SCC data and the lack of consensus over the SCC mechanisms. In this regard, investigations into the role of MIC on SCC will be particularly important, since the nature of microbial content of marine environments (and hence its influence on localised corrosion and SCC) at different locations could be different. This could account for the inconsistencies in SCC data.

The possible role of microbials on SCC has the potential for triggering a relatively unexplored vista of research, *i.e.*, understanding the complex role interplay of the composition and severity of microbial activity in the marine environment and microstructural variations in SS weldments leading to SCC failures. Borenstein (1991) has documented observations on MIC and SCC of industrial weldments of stainless steels. Borenstein (1991) comments: 'for reasons not known, MIC often occurs in combination with either SCC or sensitised structure at weldments in austenitic SS'. More importantly, the conditions which are resistant to SCC may be susceptible to MIC, which in turn may alter the conditions to those conducive to SCC.

CONCLUSIONS

This overview suggests that, in order to address the inconsistencies in SCC data, there is a need for investigation of:

- the role of microbiologically-induced corrosion (MIC) in pitting and SCC of duplex stainless steels in marine environments,
- the combined role of MIC and microstructural variation in weldments of austenitic and duplex stainless steels in their SCC resistance,

Besides addressing the uncertainties in a common materials degradation issue in marine applications (i.e. failures of SS components) due to SCC, the investigations would also constitute a novel area of research.

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Rapid Assessment of the Crevice Corrosion Resistance of Stainless Steel Alloys in Seawater

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ABSTRACT

A rapid test method was developed to identify corrosion-resistant stainless steel alloys for Australian naval applications such as periscope housings, holding tanks and fasteners. The crevice corrosion resistance of a select range of new generation stainless steels was compared with the commonly used 316L austenitic stainless steel. Two standard ASTM immersion test methods, G48 (ferric chloride) and G78 (seawater) were compared with a modified version of ASTM G61 (cyclic polarization) to measure the performance of the test alloys. The cyclic polarization technique was shown to be a reliable method of assessing corrosion susceptibility, particularly for the more highly alloyed stainless steels. Duplex 2507 and austenitic 254SMO were rated the best alloys, marginally superior to another duplex alloy, Ferralium 255. The lower alloyed duplex 2205 also performed well while of the two relatively low-alloy test materials, duplex 2304 appeared to be marginally more resistant than austenitic 316L.

INTRODUCTION

The DSTO, on behalf of the Australian Defence Forces (ADF) has a particular interest in the corrosion-resistant properties of new generation stainless steel alloys (Sedricks, 1989) for a broad range of marine applications, including seawater tanks, periscope housings, pump shafts, valve spindles and fasteners. To date, the austenitic grade 316L has been the most commonly used material because of its ready availability and relatively low cost. However, its pitting and crevice corrosion resistance has often proved to be inadequate in the highly corrosive environments present on naval vessels.

AMRL undertook this study with two main aims: to compare the crevice corrosion resistance of a range of alternative stainless steel alloys with the most common currently used alloy and, in the process, develop both a reliable and rapid assessment test method for new alloys. Two corrosion test methods G48 and G78 (both ASTM, 1992) and a modified version of a third method G61 (ASTM, 1993) were used in the study.

TEST MATERIALS

Six alloys were tested, including both austenitic and duplex stainless steels. The duplex alloys were chosen because of their markedly greater yield strength (double that of austenitic stainless steel). This characteristic offered the possibility of cost and weight reductions through use of materials of thinner gauge. A nickel/chromium alloy (Inconel 625) was also included as a standard because of its high crevice corrosion resistance to seawater. The compositions of the test alloys are shown in Table 1.

Table 1. Test Alloy Compositions

Alloy	UNS No.	Cr	Ni	Mo	C	Cu	N	Other
316L	S31603	17	12	2	0.03 max.			
254SMO	S31254	20	17.8	6.1	0.02	0.8	0.2	
2304	S32304	21.5 min.	3 min.	0.05 min.	0.03 max.		0.2 max.	
2205	S31803	22.2	5.4	3.1	0.02		0.15	
Ferrallium 255	S32550	25	5.8	3.1	0.01	1.8	0.17	
2507	S32750	25	7	4	0.03		0.3	
Inconel 625	N06625	20	62	9				4.5 Fe 3.4 Nb

TEST METHODS

Five different tests (one cyclic polarization and four direct immersion) were used in this study. The field trial was used as the standard for all comparisons as it most closely represented in-service crevice corrosion conditions.

Field Trial: Seawater Immersion

A raft located at a DSTO tropical exposure site near Innisfail, Northern Queensland, was used for the field trial. The exposure was conducted in accordance with ASTM G78. Rectangular test specimens 150 mm x 100 mm x 1 mm were cut from sheet stock of each alloy. A 12.7 mm diameter hole was drilled in the middle of each panel to accommodate the crevice assembly. This assembly included a threaded *Delrin* (acetal resin) rod and two *Delrin* castellated nuts (16 crevice sites on each nut). The specimens were then suspended vertically from the raft; one panel of each alloy was immersed for 18 months and duplicates of each alloy for 3 years.

Laboratory Trial: Seawater Immersion (ambient temperature)

Test panels and *Delrin* crevice assemblies, identical to those for the field trial, were also used in this trial. Single specimens of each alloy were immersed in glass tanks containing static ambient temperature seawater for 18 months and 3 years. Seasonal temperatures in the laboratory varied between approximately 15C and 25 C.

Laboratory Trial: Seawater Immersion (30 C)

The same test procedure that was used for the ambient temperature seawater trial was again applied for this test, although the glass tank containing the static seawater was immersed in a water bath to maintain the solution at 30 C. A single specimen of each alloy was immersed for 18 months.

Ferric Chloride Immersion

Test method G48 (ASTM, 1992) was used as the basis for this test, although the test panels and crevice formers were identical to those used in the seawater immersion trials. Specimens were immersed in 6% ferric chloride solution at ambient temperature for 72 hours.

Cyclic Polarization

This test utilised G61 (ASTM, 1993), a rapid (2-4 hours) cyclic polarization technique, which facilitates assessment of localized corrosion susceptibility of iron-, nickel- or cobalt-based alloys in a chloride environment. An electrochemical cell similar to that described in G5 (ASTM, 1994) and modified (Turnbull, 1987) to enable the specific study of crevice corrosion susceptibility was used. However, the auxiliary electrode designed to help form the crevice was relocated to the side of the working electrode so that the cathodic reaction products would form outside the crevice. A blank epoxy

cylinder was used to hold the crevice former (four layers of filter paper) in place against the test specimen. This design change was thought to provide much more realistic crevice corrosion conditions. Button test specimens, 12.7 mm diameter and 1 mm thick were mounted in M epoxy resin and then immersed in seawater for 1 hour prior to testing. Identical test parameters (Turnbull, 1989) were used for each cyclic polarization. The two values pertinent to crevice corrosion susceptibility derived from these plots were:

(i) crevice breakthrough potential (E_c): the indicator of an alloy's resistance to crevice corrosion initiation and defined as the first potential (versus a saturated calomel electrode [SCE]) where a current density of $10 \mu\text{A}\cdot\text{cm}^{-2}$ occurs, and

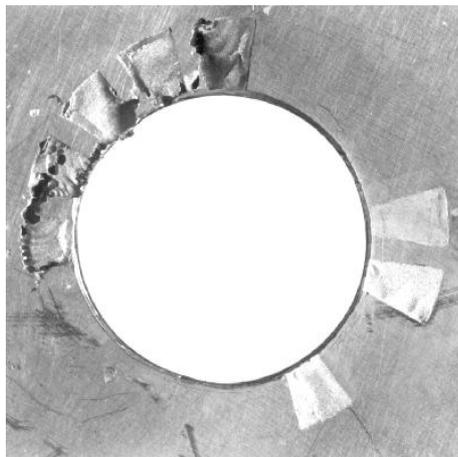
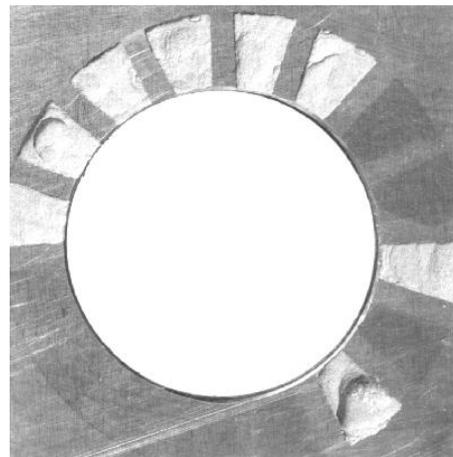
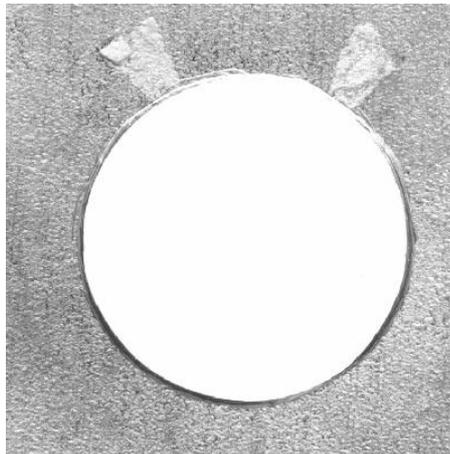
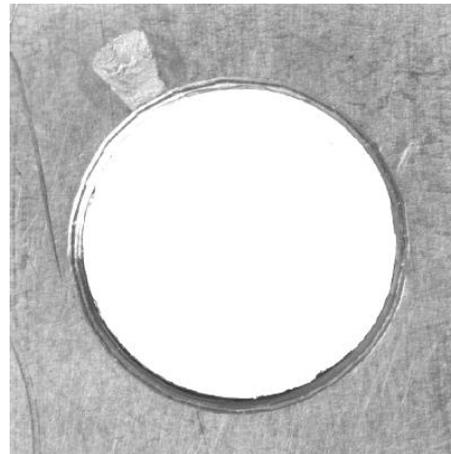
(ii) crevice propagation resistance (CPR): the area enclosed by the hysteresis loop. Values are generally negative i.e. the reverse scan potentials are lower than on the forward half of the scan. Larger negative areas indicate greater susceptibility to crevice corrosion propagation.

RESULTS

Field Trial: Seawater Immersion

Eighteen month specimens

The condition under the crevice-formers of affected alloys after 18 months immersion are shown in Figure 1. Both 316L (austenitic) and 2304 (duplex) had suffered moderate-heavy crevice attack at one-third of the crevice-former sites and some additional corrosion around the securing boltholes. The Ferralium 255 and 2205 duplexes were only lightly etched under a few crevice-former sites but had suffered moderate and heavy corrosion respectively around the securing boltholes. Inconel 625, 254SMO (austenitic) and 2507 (duplex) were all still in pristine condition.

**316L (austenitic)****2304 (duplex)****2205 (duplex)****Ferralium 255 (duplex)****Figure 1** Field Trial (18 months immersion).

Three-year specimens

Inspection of the three-year specimens generally substantiated the relative crevice corrosion susceptibility of the seven alloys determined from the 18 months panels, although the location of corrosion was quite different. The four 18 month immersion specimens that had suffered corrosion were primarily attacked at sites around the central holes under the castellated nut crevice-formers. Only the super-duplex alloys 2205 and Ferralium 255 exhibited significant corrosion around the securing boltholes at the top of the panels. In contrast, the three-year specimens were predominantly attacked around these boltholes although corrosion was relatively light on most of the

affected test alloys. Most notably, for the first time during the field trial, the super-austenitic 254SMO stainless steel exhibited mild crevice attack around the securing boltholes on both panels.

Laboratory Trial: Seawater Immersion (ambient temperature)

This trial produced only very mild etching under the crevice-formers, and then only on the lower grade alloys; weight losses were insignificant.

Laboratory Trial: Seawater Immersion (30 C)

Elevation of the static seawater temperature did not markedly increase the intensity of corrosion, although the etching under the crevice-formers was quite distinct on the 316L (austenitic) and 2304 (duplex) alloys. In addition, both 254SMO (austenitic) and Ferralium 255 (duplex) exhibited mild etching during this trial.

Ferric Chloride Immersion

The post-trial crevice-site condition of a representative sample for each alloy is shown in Figure 2.

316L (austenitic) suffered severe corrosion, including multiple perforations, whereas 254SMO (austenitic) exhibited relatively mild etching on two-thirds of the crevice sites. 2304 (duplex) was severely corroded at the crevice sites including a few perforations. In contrast, the middle grade 2205 (duplex) alloy was heavily corroded but not perforated and the two most highly alloyed duplex materials, Ferralium 255 and 2507, experienced only very mild etching at one or two sites.

Inconel 625 suffered some mild corrosion at all 32 crevice sites.

Cyclic Polarization

Cyclic polarization measurements were recorded for duplicate samples of each alloy. The crevice breakthrough potentials (E_c) and crevice propagation resistance (CPR) values determined from these plots are listed in Table 2 (weight losses included for comparison) and some typical polarization curves are shown in Figure 3.



316L (austenitic)



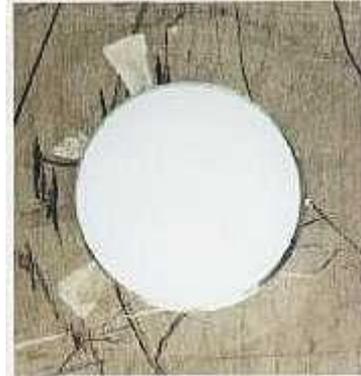
254 SMO (austenitic)



2304 (duplex)



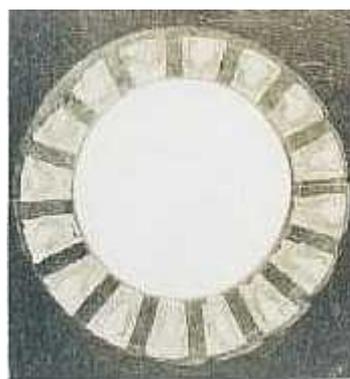
2205 (duplex)



Ferralium 255 (duplex)



2507 (duplex)



Inconel 625

Figure 2 Ferric Chloride Immersion Trial (72 Hours).

Generally, the E_c and CPR values for the middle- and high-grade alloys were closely grouped and showed good repeatability. The only noticeable variation occurred in the duplicate samples for duplex 2507, although the data still indicated a high resistance to crevice corrosion. In contrast, E_c and CPR values for the two low-grade materials were significantly different from the other five alloys; with the exception of the CPR figures for 316L, the duplicate values also varied markedly.

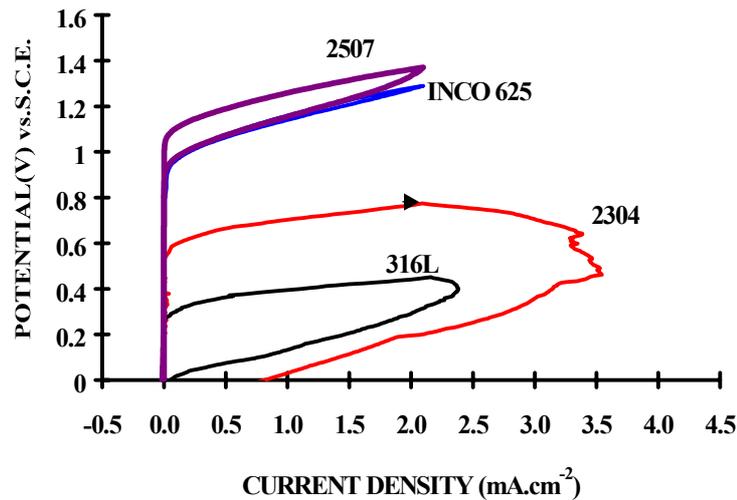


Figure 3 Representative cyclic polarization plots of four test alloys

N.B. All four cyclic polarization curves are clockwise as indicated by arrow on 2304 plot.

Table 2. Cyclic Polarization Trial - E_c and CPR Values and weight losses for 3 year field and ferric chloride trials

Alloy	E_c [Volts vs. SCE] (Crevice Breakthrough Potential)	CPR (Crevice Propagation Resistance)	Weight loss (g) 3 year field trial	Weight loss (g) Ferric Chloride trial
316L	0.341	-0.56	0.62	3.447
	0.255	-0.53	0.43	5.118
254SMO	0.972	-0.10	0.02	0.002
	0.956	-0.08	0.01	0.007
2304	0.692	-4.83	0.66	1.950
	0.578	-1.54	0.84	4.023
2205	0.960	-0.23	0.01	0.859
	0.988	-0.20	0.01	0.729
Ferralium 255	0.930	-0.08	0	0.003
	0.964	-0.09	0.02	0.004
2507	1.018	-0.16	0	0.003
	0.940	-0.09	0	0.003
Inconel 625	0.792	-0.03	0	0.006
	0.799	0	0	0.016

DISCUSSION

The five crevice corrosion trials used in this investigation did not provide consistent ratings for the crevice corrosion resistance of the seven test alloys.

Field Trial: Seawater Immersion

Examination of the field trial specimens enabled the following observations to be made:

Duplex 2507 has a very high resistance to crevice corrosion after 3 years in tropical seawater, similar to that shown by the Inconel 625 reference.

The crevice sites were in similarly good condition for austenitic 254SMO, although the light/moderate corrosion observed around the securing boltholes lowered its resistance rating slightly. This corrosion was not given the same weighting in assessing the panels because over-tightening of the bolts used to fix the panels to the exposure rack could have initiated the crevice attack. Nonetheless, it did show that the alloy has some degree of susceptibility to crevice corrosion.

The other high-grade duplex alloy (Ferralium 255) was also rated lower because of moderate attack around the securing boltholes and some light etching under the crevice formers.

Duplex alloy 2205 generally exhibited moderate crevice corrosion resistance except for moderate/heavy attack around the securing bolthole on the 18 months specimen. This attack was responsible for a relatively high weight-loss figure that was not seen in the two three-year specimens. However, the alloy was rated as significantly inferior to the more highly alloyed metals.

The two lowest grade materials, austenitic 316L and duplex 2304 were consistently the poorest performers, suffering significant crevice corrosion under both the crevice-formers and around the securing boltholes. The 2304 duplex was assessed as a slightly more resistant material because of the lower rate of attack under the crevice-formers.

Laboratory Trial: Seawater Immersion (ambient temperature)

The ambient laboratory seawater immersion trials provided relatively mild corrosive conditions that produced very little corrosion.

Laboratory Trial: Seawater Immersion (30 C)

The most significant result noted at the elevated temperature was a slight susceptibility to crevice corrosion on the high-grade alloys 254SMO (austenitic) and Ferralium 255 (duplex).

Ferric Chloride Immersion

The ferric chloride trial ratings differed significantly from the field trial for the higher-grade alloys. Inconel 625 suffered mild attack at all crevice sites while the duplexes Ferralium 255 and 2507 displayed exceptional resistance, just slightly greater than austenitic 254 SMO.

Cyclic Polarization

The cyclic polarization work indicated that neither the E_c nor CPR value for an alloy should be considered separately, when using this technique to assess crevice corrosion resistance. In particular, the average E_c value (-796 mV) for Inconel 625 showed that the alloy has a relatively low resistance to crevice corrosion initiation compared with the other high grade alloys. By contrast, its average CPR value (-0.02) was markedly superior to all other alloys tested and it appears that this characteristic was responsible for the lack of corrosion propagation in the ferric chloride test and its exceptional field trial rating. Conversely, the average E_c value (-635 mV) for the duplex alloy 2304 indicated that it is considerably more resistant to crevice initiation than the austenitic alloy 316L (average E_c -298mV), whereas its average CPR value (-3.19) showed it to be clearly the least resistant to crevice corrosion propagation. Therefore, both parameters needed to be considered when rating the crevice corrosion susceptibility of lower grade alloys by this test method.

CONCLUSIONS

This study showed that high-grade new generation stainless steels such as the duplexes 2507 and Ferralium 255 and austenitic 254SMO have excellent long-term crevice corrosion resistance to seawater immersion. The latter two alloys did show slight susceptibility to crevice attack in the field trials but not at sites under the castellated nut crevice-formers. However, the occurrence of crevice corrosion around the securing boltholes did indicate that these two alloys were marginally inferior to the 2507 duplex stainless steel alloy.

CPR data derived from the cyclic polarization trial proved to be very useful in providing a rapid method of rating the relative crevice corrosion susceptibility in seawater of more highly alloyed stainless steel alloys. Assessment of lower grade alloys

appears to be more complex and involves consideration of both the CPR and E_c values. However, the field trial still appeared to be the most reliable method for rating all alloy grades.

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Electrochemical Control of Fouling and Corrosion in a Mooring System for Use in Ecologically-Sensitive Sea Areas

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ABSTRACT

An alternative mooring system to the traditional anchorage has been developed for use in ecologically-sensitive sea areas. For this, an electrochemical approach has been applied to control of both fouling and corrosion. Biofouling growth on the floating body is prevented by means of anodic polarization, using the bottom body, made of a metallic cage filled with autochthonous stones, as a cathodic electrode. Continuous anodic polarization at +0.8 V SCE can prevent fouling formation and anodic polarization at +1.4 V SCE can remove attached fouling. Cathodic polarization of stainless steel with a Mg anode for 1 year can effectively protect metallic parts and promote the growth of calcareous deposits, which fasten the buoy system to the sea floor. Corrosion protection is provided for at least 4 years, even after consumption of the Mg anode. The whole buoy and bottom body system are designed as an integrated module, able to supply both mooring and facilities for visitors.

INTRODUCTION

Because of environmental laws, introduced in Italy to regulate access to, and protect ecologically-sensitive sea areas, a new type of boat mooring has been developed as an alternative to traditional anchorage. The seagrass *Posidonia oceanica* (Linnaeus) Delile grows in wide submerged prairies, along almost all Mediterranean sea coasts, and is a highly significant marine ecosystem. Moreover, due to its photosynthetic generation of oxygen, *P. oceanica* is considered the "green lung" of the Mediterranean Sea (Boudouresque *et al.*, 1982). *P. oceanica* beds also resist water motion and consequently act

as a natural barrier against coastal erosion (Mazzella *et al.*, 1986). In recent decades the abundance of *P. oceanica* has declined due to several causes. One damaging effect is scraping by boats at anchor (Porcher, 1984). Another important, even if not yet clearly defined role is competition with the invasive *Caulerpa taxifolia* (Vahl) C. Agardh. The spread of *C. taxifolia* has probably been favored by various factors, possibly including entrapment of algal thallus fragments in anchors, anchor chains and fishing nets (Meinesz, 1992).

Avoiding anchorage would help to prevent damage to *P. oceanica* beds and the spread of *C. taxifolia*, especially in ecologically-sensitive areas. The issue is also important in areas such as Portofino (Ligurian Sea) where tourism is important to the economy, but is opposed by those who consider it a significant cause of environmental damage. This conflict remains unresolved.

In this paper an innovative buoy system is described which aims to decrease the environmental impact of mooring. Figure 1 shows a schematic diagram of the mooring system. An electrochemical approach to prevent fouling growth was developed. The submerged part of the plastic floating body was coated with conductive concrete which was then anodically polarized. The floating body was hooked to a bottom body: a metallic cage filled with stones collected from the sea floor in the immediate vicinity of the mooring system. The bottom body functions as a cathode to anodically polarize the floating body. In situ construction of bottom bodies using autochthonous materials and protection of the metallic cage by cathodic polarization would solve a number of economic and environmental problems, related to the production, transport and installation of materials. Cathodic protection would prevent corrosion and promote calcareous growth, and the calcareous deposits would facilitate attachment of the bottom body to the sea floor, creating greater stability and mechanical resistance. Moreover, the cage would rapidly merge with the sediment, reducing its visual impact.

EXPERIMENTAL

Tests were carried out at the marine research center Mareco of TEMPE-CNR, at Bonassola on the Ligurian coast of Mediterranean sea.

The submerged part of the floating body was subjected to potentiodynamic, potentiostatic and galvanostatic tests. Potentiodynamic tests, with scanning rate 1 V/h, of anodic polarization were carried out on coupons with 1 cm² of exposed area, in 1 liter cells fed with natural seawater and with one replacement per hour. Different materials such as gold (Au), platinum (Pt), graphite, high corrosion resistance stainless steels UNS S31254 and UNS S31803 coupons were used. Potentiostatic tests at potentials of +0.5, +0.8, +1.1, +1.4 V SCE, and galvanostatic tests at current densities of 0.5, 2, 5, 20 $\mu\text{A}/\text{cm}^2$, were performed on larger, 500 mm long coupons of 200 mm diameter iron tube. These were painted with epoxy glass flake and coated externally with commercial conductive concrete. Tests were performed in open 200 l tanks, in the

open air, with natural seawater pumped through the tanks at a rate of 50 l/h. Each test continued for at least one year.

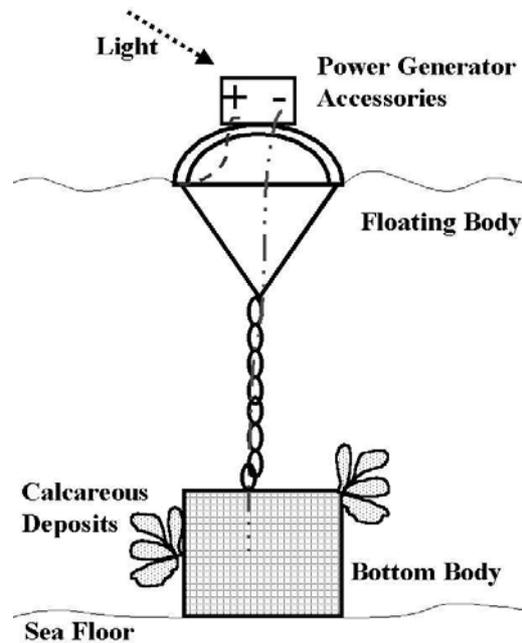


Figure 1 Schematic diagram of the mooring system.

In relation to the bottom body, cathodic polarization tests were carried out on 500 mm long carbon steel, galvanized, stainless steel UNS S30400 and UNS S31254 wires of 2, 3 and 6 mm in diameter. Coupons were exposed to seawater in 200 liters open tanks, with 50 l/h flow rate, inside the laboratory. Some coupons were coupled to a zinc anode, at about -0.99 V SCE coupling potential, or to a magnesium anode at about -1.46 V SCE. Potentiodynamic tests, with a scanning rate 1 V/h of cathodic polarization, were carried out on some wires when initially immersed in seawater and after different exposure times, both before and after galvanic coupling. X-ray, SEM, EDS analysis were performed on coupons at the end of tests.

RESULTS AND DISCUSSION

Floating body

Anodic polarization curves obtained in potentiodynamic tests are shown in fig. 2. Curves reflect not only the electrode material, but also electrode history, length of seawater exposure before the test, stirring conditions, season and environmental conditions. All tests demonstrate the presence of anodic processes, characterized by substantial current density and not exclusively related to electroodic material dissolution, which were also below the thermodynamic potential of chlorine evolution. The abrupt increase of the

current densities near the potential of 1 V SCE can be related to establishing processes of chlorine and/or oxygen evolution; the overvoltages for such processes depend on the electrodic material. In the case of UNS S31803, the anodic current densities at lower potentials can be justified considering the passive current densities of the material. Also in the case of platinum, passivation phenomena can contribute to the observed current densities; for graphite, substantially inert at such potentials, and for gold, thermodynamically inert at such potentials, the current densities observed at potentials near 0.5 V SCE, below chlorine and oxygen discharge, can not be justified through the anodic dissolution of the electrodic material.

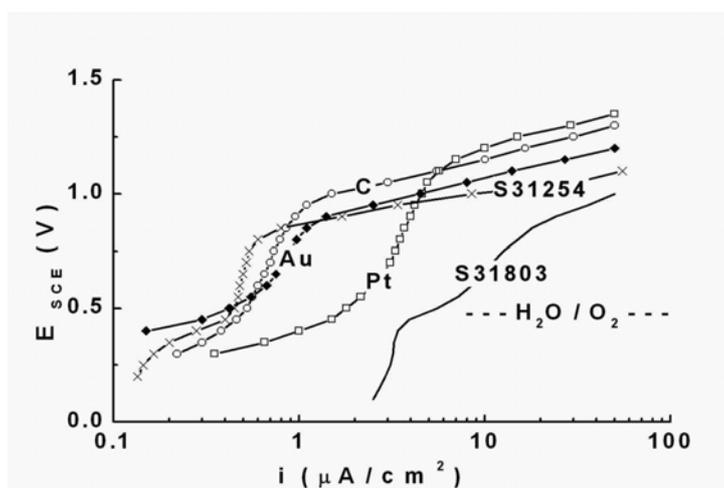


Figure 2 Potentiodynamic anodic polarization curves in natural seawater for different materials.

Tests carried out on conductive concrete coated tubes have shown that unpolarized samples, exposed to seawater in spring/summer were rapidly colonized by algae, serpulid tubeworms and barnacles. No significant macrofouling growth was observed on polarized tubes after exposure to + 0.8, +1.1, +1.4 V SCE or to 2, 5, 20 $\mu A/cm^2$, with only light fouling found after exposure to +0.5 V SCE or at 0.5 $\mu A/cm^2$. Potentiostatic, +1.4 V SCE, and galvanostatic, 20 $\mu A/cm^2$, polarizations, applied in September to samples immersed in February without polarization caused the established calcareous fouling to disappear within two months.

The antifouling effect could be the result of biocidal compounds generated by oxidation of chloride, hydrogen peroxide generation via cathodic reduction of oxygen (Dhar *et al.*, 1981; Dhar *et al.*, 1982; Christensen *et al.*, 1990), effects of current at the cellular level (Nekasono *et al.*, 1992; Bockris *et al.*, 1994) or generation of H^+ ions leading to electrode surface acidification.

Table 1 Processes which could generate current in seawater at potentials below that for chlorine evolution.

$\text{Fe}^{++} + 3 \text{H}_2\text{O} = \text{Fe}(\text{OH})_3 + \text{e}^-$	+ 3 H ⁺	$\text{NH}_4^+ + 2 \text{H}_2\text{O} = \text{NO}_2^- + 6 \text{e}^-$	+ 8 H ⁺
$\text{Mn}^{++} + 2 \text{H}_2\text{O} = \text{MnO}_2 + 2 \text{e}^-$	+ 4 H ⁺	$\text{NO}_2^- + \text{H}_2\text{O} = \text{NO}_3^- + 2 \text{e}^-$	+ 2 H ⁺
$\text{HS}^- + 4 \text{H}_2\text{O} = \text{SO}_4^{--} + 8 \text{e}^-$	+ 9 H ⁺	$\text{CH}_2\text{O} + 2 \text{H}_2\text{O} = \text{HCO}_3^- + 4 \text{e}^-$	+ 5 H ⁺

Chloride oxidation is unlikely because the observed potential of +0.8 V SCE is below that for chloride oxidation. Similarly, hydrogen peroxide is unstable and would only be formed at potentials similar to that for chloride oxidation (Pourbaix, 1974). Cellular effects are possible, but would not explain the disappearance of calcareous shells of serpulids and barnacles. The most likely cause is acidification, which would explain the dissolution of animal shells. As shown in Table 1, oxidation of seawater components such as iron, manganese, sulfides, ammonia, nitrogen oxides and organic compounds could generate the necessary H⁺ ions and, in addition to dissolving animal shells, the pH gradient could hinder biofilm formation. As a consequence, the process of biofilm development may be halted. Although some bacteria can tolerate acidic environments, a high differential in pH between the surface and its environment is possibly inhibitory, particularly when the short contact time between the bacteria and the surface is considered. If bacteria have no opportunity to acclimatize, electrodic surfaces would not be affected by microorganism presence. The antifouling effect of anodic polarization at low potentials could be similar to that described for cooling water in steam condensers (Salvago *et al.*, 1993; 1995; 1996). Dissolution of calcareous serpulids and barnacle shells, at high potential or at high current density, could also be due to acidification resulting from the chloride oxidation process rather than to biocide activity of chloride oxidation products.

In July 1995, a small PVC buoy (50 l volume) was placed in the sea in front of the laboratory. The submerged part of the buoy was coated with the same conductive concrete used for the tube, and anodically polarized by solar cells installed on the buoy. The buoy was connected to a bottom body of scrap iron which acted as the cathode. In maximum sunlight, current density was around 10 $\mu\text{A}/\text{cm}^2$ over the submerged surface of the buoy. No macrofouling was observed on the buoy when last inspected in November 1996. In February 1997, a storm damaged the research station and prevented further observation of the buoy.



Figure 3 Wire electrodes of different materials galvanically coupled to a Mg anode in natural seawater.

Bottom body

In all galvanic coupling tests, calcareous deposition was observed on the cathode. Deposits were < 1 mm thick with a zinc anode coupling and > 3 mm if a magnesium anode coupling was used. Calcareous deposits obtained with the zinc anode were poorly adherent. They were essentially composed of aragonite, rich in silica, diatoms and bacteria, and poor in brucite, with iron and manganese also present. In comparison, deposits derived from magnesium were stratified and generally well adherent. The layer adherent to the metal was made of brucite, with other layers of nearly pure aragonite without bacteria and diatoms and, on stainless steel, without iron and manganese contamination. At the end of the magnesium anode coupling test, significant calcareous deposits, 3 to 20 mm thick, formed on wires of 3-6 mm diameter (Figure 3). The thickness of these deposits varied both with season and with wire material. At the end of experiment, 2 mm diameter wires of UNS S30400 steel had calcareous deposits less than 0.1 mm thick. In this case in situ observations showed that calcareous particles were lifted away by hydrogen bubbles instead of adhering to the electrode and a calcareous deposit could be observed on non-metallic components, not coupled to the electrode.

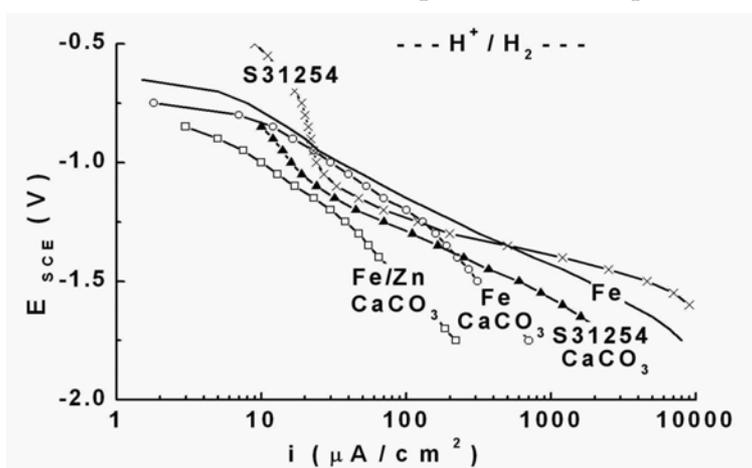


Figure 4 Potentiodynamic cathodic polarization curves in natural seawater on 3 mm diameter wires of different materials, before and after calcareous deposit formation.

Figures 4-5 show cathodic polarization curves obtained in potentiodynamic tests on different materials before and after the formation of calcareous deposits, which was found to hinder the cathodic process at the same potential. In figure 5 cathodic polarization curves are for wires of the same stainless steel but with different diameters. The cathodic process was found to be inhibited in small diameter wires. The high density of microorganisms in deposits obtained after zinc galvanic coupling and their total absence in those obtained after magnesium galvanic coupling is consistent with observations elsewhere on the effect of cathodic polarization potential and hydrogen evolution on bacterial activity (Nekoxsa *et al.*, 1991). Thickness differences in calcareous deposits are consistent with the different cathodic current densities at the polarization potential; the greater the current density, the thicker the deposit. However, wire diameter can greatly influence the cathodic process, as shown in figure 5.

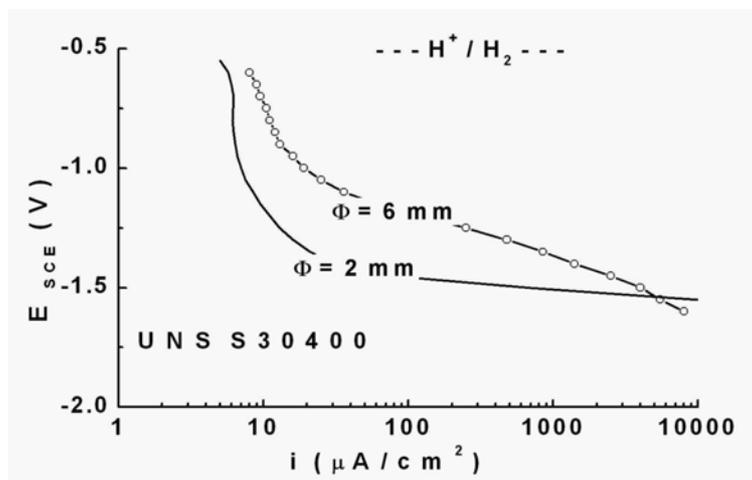


Figure 5 Potentiodynamic cathodic polarization curves in natural seawater for wires of different diameter wires.

Because of the cylindrical shape adopted of the wires, the electric potential gradient increases sharply near the wire and the potential gradient at the electrodic surface increases with decreasing wire diameter. H_2 evolution, irrespective of mechanism, requires H^\bullet formation; H^\bullet concentration in seawater is such that reaction



is controlled by a limit diffusion current that is very low at low H^+ concentrations; hydrogen evolution directly from water through reaction



is characterized by high polarization and significant current densities are produced only at potentials below -1.2 V SCE (Ives *et al.*, 1991); and more over if we consider that bicarbonate anion concentration in seawater is in the order of millimoles/l, that is at least five orders of magnitude over hydrogen ion concentration at pH = 8.3 of natural seawater, we can suppose that, in agreement with arguments already discussed

(Salvago *et al.*, 1992), during cathodic polarization at potential higher than -1.2 V SCE, most of hydrogen evolves from bicarbonate mediate process more than from directly H^+ . Anion HCO_3^- diffusion to cathode would be promoted by concentration gradient and hindered by the electric field. Therefore, according to the experimental result shown in figure 5, this process would be strongly hindered on small diameter wire electrodes, where electric field is higher.

Calcareous deposits generated on stainless steels in seawater by galvanic coupling with magnesium anode appear to hinder corrosion processes when coupling is ended, and extend the effectiveness of the initial cathodic protection (Salvago *et al.*, 1998).

In July 1993, a 2 m³ metallic cage was placed on the sea floor in front of the laboratory at 10 m depth. The cage net was made of UNS S30400 stainless steel wire of 2 mm diameter and reinforced with UNS S31254 stainless steel wire of 6 mm diameter. The cage was filled with stones collected from the surrounding sea floor. Local experience with stainless steel nets has indicated a high incidence of crevice corrosion of the wire interlacing, limiting the structure to no more than one year if unprotected. For our test, protection was afforded by a magnesium anode. By August 1993, a calcareous deposit was clearly visible on the 6 mm wire and the 2 mm wire had lost its former brightness and by March 1994, the calcareous deposit was evident on all metallic parts. In July 1994, the magnesium anode was completely gone, but the calcareous deposit was still present. In the summer of 1995, the calcareous deposit was no longer clearly visible, having been colonized by algae. However, no corrosion damage was evident, nor has it become apparent up until the most recent observations in May 1999. The bottom body had merged into the surrounding habitat and has minimal visual impact.

Project design system

A mooring buoy system integrating a number of devices has been designed. Two electric generators, a photovoltaic system and a dynamic system producing electric energy from the wave motion, are under consideration. This system would energize antifouling anodic control for the plastic floating body and cathodic protection for the metallic part of the bottom body. The bottom body acts as cathode for the anodic polarization of the submerged part of the floating body, which is coated with titanium net and conductive concrete (Salvago *et al.*, 1991). The same floating body is also used as the anode for cathodic polarization of the bottom body.

The mooring system is also proposed to have visitor information, rule for boat access, parking meter, to cover the buoy maintenance charges, and a SOS interphone on the mooring platform. A satellite detection system would act as an anti-theft device and a telematic medical service and the system would also incorporate waste disposal tanks.

CONCLUSIONS

An alternative mooring system to the traditional anchorage has been developed which consists of a permanently anchored mooring platform. Antifouling control of the floating body would be by anodic polarization of the floating body and corrosion protection of the bottom body by cathodic polarization. Continuous anodic polarization at +0.8 V SCE was found to prevent fouling formation and intermittent anodic polarization at +1.4 V SCE would remove existing calcareous fouling. Cathodic polarization of stainless steel using a Mg anode for 1 year was found to effectively protect metallic parts and promote growth of calcareous deposits, which would facilitate attachment of the buoy system to the sea floor. The system was also found to provide corrosion protection for at least 4 years.

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