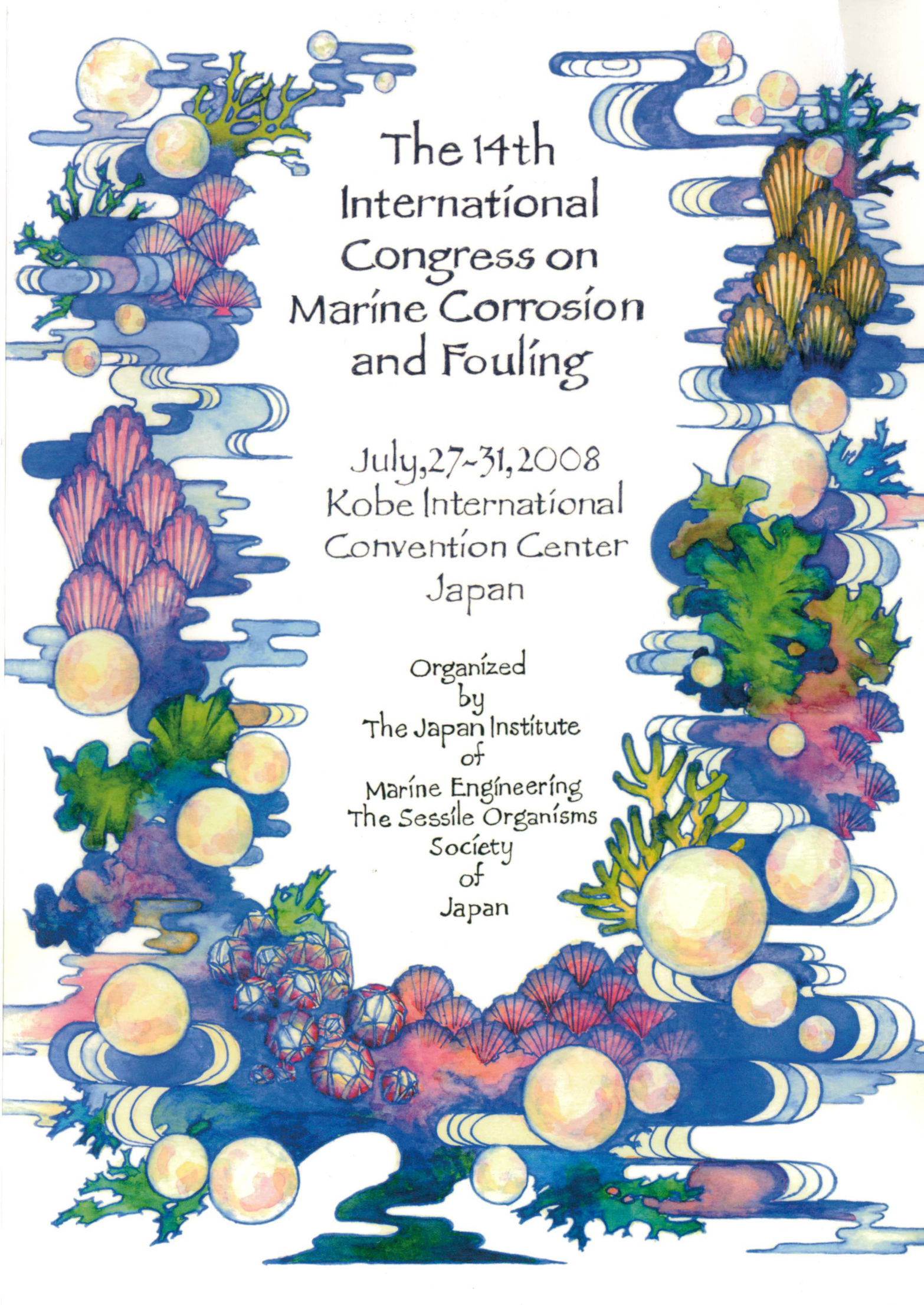

The 14th International Congress on Marine Corrosion and Fouling

July, 27~31, 2008
Kobe International Convention Center
Japan

Organized
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The Japan Institute
of
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The Sessile Organisms
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Organized by **the Japan Institute of Marine Engineering**
Co-organized by **the Sessile Organisms Society of Japan**

This work relates to Department of the Navy Grant N00014-08-1-1072 issued by Office of Naval Research Global.

Preface

Marine corrosion and fouling provide serious problems with naval architecture including ships and offshore structures. Corrosion may compromise the integrity of submerged structures resulting in premature failure and unanticipated repairs or replacement. Ships fouled with marine organisms suffer from increased hydrodynamic drag, which leads to increased fuel consumption as well as air pollution. In addition, fouled vessels may act as vectors for invasion of unwanted species.

To solve these problems we need to understand the environmental issues associated with chemistries and methods used for control. Scientists continue to make advances in such diverse areas as materials science, surface physics, analytical chemistry, physiology, biochemistry and ecology of aquatic organisms. The International Congress of Marine Corrosion and Fouling (ICMCF), the first of which was held in Paris, 1964, has been the foremost international scientific conference on these issues. This interdisciplinary conference brings together scientists from academia, industry and government organizations to present and discuss recent developments in relevant areas.

The Congress has been organized under the authorization of the COIPM (Comité International Permanent pour la Recherche sur al Préservation des Matériaux en Milieu Marin) and the 14th Congress is organized by Japan Institute of Marine Engineering (JIME) in cooperation with the Sessile Organisms Society of Japan (SOSJ). It is convened from 27th to 31st July 2008 at the Kobe International Conference Center in Kobe, Japan. This is the first Congress to be held in Asia.

The scientific program contains 4 plenary lectures, 4 organized sessions with 21 speakers, general sessions including 6 keynote lectures, 79 oral presentations and 69 poster sessions. The session topics include "analytical methods, environmental fate and risk assessment of biocides", "biofouling by macro sessile organisms and mechanisms of larval settlement", "marine corrosion and anti-corrosion", "release rate of biocides from antifouling paint", "antifouling systems using natural products", "Non-toxic antifouling systems and new strategies for fouling control", "invasive species", "biofouling and antifouling systems at power plants and fish nets", "flow effects and surface interactions" and "regulation of antifoulants and environmental issue".

Finally, on behalf of the Organizing Committee, I would like to express our sincere gratitude to all of the participants as well as to the companies and organizations who generously support 14th ICMCF.

Nobuhiro Fusetani
Chair of the 14th ICMCF
Professor, Hokkaido University

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28A-1: PLENARY LECTURE 1

28A1-1 (Plenary)

ALGAL FOULING: A STICKY PROBLEM

Maureen E Callow, m.e.callow@bham.ac.uk, The University of Birmingham

Fouling of man-made structures by algae is a major problem throughout the world. Biofilms dominated by diatoms (single algal cells encased in a silica cell wall) form slimes on even the most effective biocidal antifouling paints, as well as on non-toxic, foul-release coatings to which they adhere tenaciously. The green alga *Ulva* (previously called *Enteromorpha*) is the most widespread macroalga on ships' hulls, especially on paints containing copper. Settlement and adhesion is central to biofouling and all successful fouling organisms secrete adhesives that 'cure' underwater and are sufficiently strong to withstand the stresses of a turbulent environment.

Stringent environmental restrictions on the use of biocides has resulted in interest in the development of environmentally benign coating technologies that either invoke 'deterrence' to prevent the settling stages (spores and larvae) of fouling organisms from sticking in the first place, or the 'non-stick' or 'foul-release' principle to facilitate the detachment of permanently adhered organisms under moderate shear stress. The search for 'environmentally-friendly' solutions has stimulated basic research efforts in an attempt to understand which interfacial properties (e.g. roughness, wettability, charge, friction) are important in influencing the settlement and adhesion of fouling organisms. This has been facilitated by the advent of novel technologies, such as various forms of lithography and self-assembly, which enable the production of test surfaces with systematic variations in structure and properties at the micro- and nano-scales. Motile marine organisms such as zoospores of *Ulva*, are highly selective in their choice of a suitable surface for settlement. Therefore, one approach to developing practical coatings based upon 'deterrence' is to try to destabilize the surface cue-sensing mechanisms of the organism. The aim of this presentation is to illustrate how spores of the green alga *Ulva*, and the diatom, *Navicula,* respond to surfaces presenting a range of well-characterised interfacial properties. Examples will be taken from collaborations developed within the ONR Marine Coatings programme and the EC Framework 6 Integrated Project 'AMBIO' (Advanced Nanostructured Surfaces for the Control of Biofouling).

Callow J A, Callow M E (2006) The *Ulva* spore adhesive system. In Biological Adhesives. Smith A M, Callow J A (eds). Springer. pp 63-78

28A-2: ANTIFOULING RESEARCH EFFORT IN ASIA: STATUS AND PERSPECTIVES (ORGANIZED BY PEI-YUAN QIAN)

28A-2-1

NON-TOXIC ANTIFOULANTS FROM JAPANESE ALGAE

Tatsufumi Okino, okino@ees.hokudai.ac.jp, Hokkaido University

Natural products are possible sources and potential leads of environmentally safe antifoulants. In fact, we found that isocyano compounds isolated from nudibranchs and sponges inhibited barnacle larval settlement. Synthetic studies of their analogues revealed a simple linear alkyl isocyanide retained anti-barnacle activity in the field. After the success of isocyano compounds research project, we continued screening for anti-barnacle compounds from Japanese algae. Especially we focused on red algae *Laurencia* spp. which were known to be rich sources of halogenated compounds including the well known antifoulant, elatol. As a result of our screening for over 100 algal metabolites, several halogenated compounds showed antifouling activities against barnacle larvae at concentrations of 0.1-1.0 μg/mL. Known compounds, thyrsiferol and laurencin are examples of potent active compounds. A new compound, omaezallene, which contains bromoallene moiety, also inhibited barnacle larval at a low concentration. Toxicity of laurencin and omaezallene were estimated by using fish and a crustacean. Both compounds showed much lower toxicity than current commercial antifoulants. Laurencin has been searched by lots of organic synthetic chemists and natural products chemists for a long time. However, any biological activity of laurencin has not been reported. This fact strongly supports laurencin is non-toxic antifoulant.

28A-2-2

BIOFOULING AND ANTIFOULING RESEARCH PROGRAMS IN CHINA: AN OVERVIEW

Pei-Yuan Qian, boqianpy@ust.hk, Coastal Marine Laboratory and Department of Biology, Hong Kong University of Science and Technology

In this review, I will provide a general review of biofouling and antifouling research activities in China (including Hong Kong). Although biofouling and antifouling research can be traced back to early 30's of the last central, systematic survey of biofouling along the Chinese coast started only in early 50's, leading to a comprehensive documentation of biofouling problems in harbors, pipelines, navigation buoys, ship hulls and so on in the country. Overall, there are drastic shifts of dominant foulers and associated biofouling problems among different biogeographic regions and among different seasons, making biofouling control a very challenging task since it was very difficult to find antifouling paint that work well in different coastal provinces over different seasons. With rapid development of mariculture industries since early 80's, biofouling issues of marine farming gears (nets, cages, rafts, and shellfish surfaces) attracted substantial attention of both industry and government. Over 50 institutes have developed their biofouling and antifouling research programs with financial supports from various funding agents. However, involvement of industries and private sectors in financing research projects has remains invisible. Up to now, over 50 Chinese patents related to biofouling have been filed but most revealed new materials, polymers, surface properties for biofouling control. Only few recent patents covered the natural products as antifouling compounds. Overall, although China has become one of major ship-builder and marine coating producers in the world, antifouling program has lagged behind, in terms of fundamental research of biofouling and antifouling research, antifouling compound screening, bioassay system, antifouling mechanisms of bioactive compounds, funding supporting, and strategic planning, which need to be improved in order to support future needs of maritime industries in China.

28A-2-3

ISOLATION OF THERMOPHILIC MICROORGANISMS FROM THE HYDROTHERMAL VENTS NEAR KUEISHAN ISLAND AND DEVELOPMENT OF THEIR FUNCTIONAL GENOME

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Fu-Pang Lin, Institute of Bioscience and biotechnology, National Taiwan Ocean University

Enzymes and proteins that mediate metabolic processes and specific biological functions of microorganisms that live under extreme environments usually work under extreme conditions. Consequently, constant increasing scientific interest has been aroused not only because these microorganisms grow in some of the harshest environments on Earth but also because they provide a valuable source of unique enzymes.

A cluster of over 30 hydrothermal vents were found at a water depth of about 10-20 m off the eastern tip of the Kueishan Island (121°55' E, 24°51'N). The temperature and pH of the vents could be as high as 116 °C and as low as pH 1.52. Isolation of thermophilic microorganisms with special functions from these vents had been attempted.

Recently an anaerobic, thermophilic, and saccharolytic bacterium strain NTOU2 was enriched and isolated from the vents. This strain is of particular interest for its ability to ferment both hexose and pentose at 70 °C, and high concentration of ethanol production during fermentation of these polymers and sugars. Physiological characteristics suggesting that this strain may possess unusual fermentation pathways. Based on the thought that genomic information will be invaluable in the development of many practical applications for this strain, such as cloning and protein engineering of thermostable glycolic enzymes for industrial applications, and bioconversion of cellulose-containing municipal wastes and agricultural products into fuels such as ethanol and hydrogen, the whole genome of this strain has been sequenced by the Genomic Center of National YanMing University and predicted by BlastX. Cloning of glycolytic enzymes such as amylopulllulanase, xylose isomerase, α-galactosidase, chitinase, trehalase, maltose hydrolase, and trehalose synthase from this strain using its annotated genome sequences and expressed them in *Escherichia coil* or *Pichia* are under going.

Using this study methods for strain NTOU2 as a model, in the near future we are planning to isolate thermophilic microorganisms which produce marine natural products such as antifouling compounds.

28A-2-4

ANTIFOULING PERFORMANCE IN SINGAPORE SEAS

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Tropical Southeast Asian coastal seas are characterized by warm sea temperatures with monsoon-driven seasonality patterns, high nutrient levels and productivity, and a very rich organism biodiversity. The biotic factors coupled with warm year-round temperatures, accelerate fouling pressure and biocorrosion. Shipping and maritime industries form a significant part of the Singapore economy, and on average, there are at least 800 vessels in Port every day. As most fouling occurs when vessels are in Port, understanding of the fouling pressure and efficacy of antifouling protection is an integral part of fouling management. We will present our findings for copper ablative and silicon fouling release coatings in field static immersion over a 2 year period in Singapore waters. Strong seasonal patterns in fouling were observed, resulting in different levels of fouling pressure challenging the different antifouling technologies. The results will be discussed in context of antifouling performance and further development of marine coatings for tropical waters.

28A-2-5

MARINE BIOFOULING RESEARCH IN INDIA

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Distribution of fouling organisms and its quantitative spatial and temporal variations was the starting point of biofouling research in India. This was carried out by several R&D Organizations and Universities in the country. Research related to barnacle taxonomy and biology formed the core research area in this discipline. Expansion of maritime activities in the recent years has lead to different R&D Organizations investing substantially in biofouling research. Many organisms with a sessile or sedimentary mode of life have the dual distinction of being important in hard substratum ecology and biofouling. Their colonization on man made structures aids in their dispersion into alien environments often with devastating consequences. Ship's ballast water is also considered as an important vector in such dispersion. Owing to this consideration, India choose to be one of the pilot countries in the Global Ballast Water Management program executed by the International Maritime Organization (IMO). As the issue is transboundary in nature, countries of south Asia and south east Asia are in the process of developing a network program. The presentation will provide an overview of the initiatives underway.

28A-2-6

BIOFOULING RESEARCH IN AUSTRALIA : AN OVERVIEW

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Biofouling research in Australia is diverse and topical with biosecurity being a major environmental and economic concern. Given the scale and diversity of marine industries ranging from aquaculture and shipping, to oil and gas exploration, across a broad latitudinal range there is a large research effort aimed at preventing the spread of potential, and recently introduced, non-indigenous fouling species. There is also a focus on the biology and ecology of well-established fouling species, and their ability to dominate benthic communities through differential tolerance to copper. Given concerns relating to the sub-lethal effects of copper there is a corresponding research effort to develop new non-biocidal antifouling technologies. In the case of Australia many of these have the unifying theme of biomimetic discovery. Signalling molecules for biofilm control based on algal natural products (furanones), and physical surfaces for the control of macrofouling based on surface microtopographies modelled from marine organisms, remain the focus for developing new generation antifouling technologies. However, recent programs on novel materials including nano-fibres, photoactive materials, novel fibres and fabrics, and micro and nano-structured surfaces, have significantly broadened the scope of the field. Research on the mechanisms of adhesion of problem diatom species on low surface energy coatings is also contributing to the improvement of existing foul-release technologies, emphasising the overall focus on effective broad-spectrum surface based antifouling technologies. In this presentation the scope and scale of biofouling research in Australia is illustrated with examples from the spectrum of groups working in the field, and a compendium of recent research papers.

28A-3: ANALYTICAL METHODS, ENVIRONMENTAL FATE AND RISK ASSESSMENT OF BIOCIDES 1

28A-3-1

ANTIFOULING BIOCIDES IN MARINE ENVIRONMENT FROM JAPAN AND SOUTHEAST ASIA

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Takaomi Arai, The University of Tokyo
Madoka Ohji, Tokyo University of Agriculture and Technology
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In this presentation, the current status of antifouling biocides in marine environment from Japan (the Port of Osaka, Otsuchi Bay, Tanabe Bay and Maizuru Bay), Malaysia (Peninsular Malaysia), Indonesia (Jakarta Bay), Philippine (Manila Bay), Thailand (coastal area), and Vietnam (northern and central parts) are discussed. Among the coastal area of Japan, highest concentrations of tributyiltin (TBT) and triphenyltin (TPT) were found in Tanabe Bay which is a small fishing area and the higher percentages of TBT for total butyltin (BT) and TPT for total phenyltin (PT) in this area were also observed, suggesting that a lot of organotin (OT) compounds was used in the past. Concentrations of Irgarol 1051 and Diuron were high in the port of Osaka which is international trading port. This means that the antifouling paint is applied for ships which sail and are moored in the Port of Osaka have changed from OTs to alternative biocides. Among Southeast Asian countries, the higher concentrations of TBT were observed in the coastal area of Thailand and Malaysia. Especially, the concentrations of TBT were high in the high density area of ship and around the shipyard with poor flushing of water. Concentrations of TPT in Southeast Asia were lower than those in Japan. The lower concentrations of alternative biocides in Southeast Asia were also observed, however pyrithion was detected in sediment from Vietnam. Diuron, Irgarol 1051 and M1 were detected in green mussel (*Perna viridis*) in Thailand, in spite of lower detection frequency. Judging from these results, it was found that OT contamination is serious problem than alternative biocides in Southeast Asia.

28A-3-2

CHRONIC TOXICITY OF AN ANTIFOULING BIOCIDE, COPPER PYRITHIONE TO A MARINE FISH, THE MUMMICHOG (*FUNDULUS HETEROCLITUS*)

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Early-life stage toxicity test for copper pyrithione (CuPT) using a teleost fish, the mummichog (*Fundulus heteroclitus*), was conducted. Fertilized mummichog eggs were exposed to CuPT at various concentrations for 50 d under continuous flow-through conditions. Hatchability, survival, growth, and morphologic abnormalities were measured. Hatchability did not differ significantly between any experimental group and control groups. Survival and growth were significantly reduced at 50 d in the groups exposed to 2 or 4 μg/L CuPT. During the test, morphologic abnormalities, such as vertebral deformity and formation of inflammatory masses in the lateral muscles, occurred in fish exposed to 2 or 4 μg/L CuPT. Light and electron microscopic studies indicated that muscle dysfunction played a role in the vertebral deformity and revealed that the inflammatory mass was composed mainly of macrophages and necrotic myocytes. It is considered that macrophages infiltrated and phagocytized necrotic cells, thus forming the inflammatory mass. In addition, acetylcholinesterase activity was markedly decreased in the 2- and 4-μg/L exposure groups, suggesting the skeletal deformity was due to mechanisms similar to those proposed for organophosphorous pesticide exposure. Furthermore, in vitro study for a measurement of acetylcholinesterase inhibiting activity of CuPT and its photodegradation products revealed that two of the photodegradation products, 2,2´-dipyridyl disulfide and 2,2´-dithiobis pyridine-N-oxide, but not CuPT exhibited prominent inhibiting activity for acetylcholinesterase. The result indicates the possibility that the morphologic abnormalities was caused by the photodegradation products occurred in the interior of the body of experimental fish or in exposure tank during the toxicity test.

28A-3-3

ACUTE TOXICITY OF METAL PYRITHIONES AND PHOTODEGRADATION PRODUCTS OF THE PYRITHIONES TO THREE MARINE ORGANISMS

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Acute toxicity of zinc pyrithione (ZnPT), copper pyrithione (CuPT), and main photodegradation products of the metal pyrithiones (2-mercaptopyridine-N-oxide, HPT; 2,2´-dithio-bis-pyridine-N-oxide, (PT)2; 2,2´-dithio-bis-pyridine, (PS)2; 2-mercaptopyridine, HPS; pyridine-N-oxide, PO; pyridine-2-sulfonic-acid, PSA) to an alga (*Skeletonema costatum*), a crustacean (*Tigriopus japonicus*), and a fish, red sea bream (*Pagrus major*) representing three trophic levels were investigated. Furthermore, we investigated whether ZnPT or the photodegradation products of the metal pyrithiones were converted to CuPT in test medium for *S. costatum*. The acute toxicity values of ZnPT, CuPT, HPT, (PT)2, (PS)2, HPS, PO, and PSA for *S. costatum* were 1.6, 1.5,1.1, 3.4, 65, 730, >100,000, and >100,000 μg/L, respectively, and those for *T. japonicus* were 280, 23, >12,500, >1,250, 550, 76,000, >100,000, and >100,000 μg/L, respectively. The values of ZnPT, CuPT, HPT, (PT)2, (PS)2, HPS, PO, and PSA for red sea bream were 98.2, 9.3, 4,500, 910, 510, 45,000, >100,000, and >100,000 μg/L, respectively. Copper pyrithione was the most toxic to all three organisms among the test substances. *Skeletonema costatum* was the most sensitive of the three organisms. 2-mercaptopyridine-N-oxide and (PT)2 had similar toxicity to CuPT to *S. costatum*. Copper pyrithione was detected in the test medium containing HPT or (PT)2. The CuPT concentrations formed in the medium of HPT or (PT)2 were correspond to their toxic impact to *S. costatum*.

28A-3-4

MONITORING OF PYRITHIONE IN THE MARINE ENVIRONMENT

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As a result of the IMO-AFS treaty to ban the use of tributyltin in antifouling paints, zinc pyrithione and copper pyrithione have gained broad acceptance as effective co-biocides in copper-based, as well as copper-free, paints. Arch has conducted extensive environmental fate and aquatic toxicity studies to evaluate the safety of copper pyrithione and zinc pyrithione as antifouling biocides. However, a comparison of the predicted environmental concentration (PEC) and predicted no effect concentrations (PNEC) must be determined for a complete risk assessment. Through the extensive testing programme on the pyrithiones, we can confidently predict the PNEC. We have also calculated PEC values for both copper pyrithione and zinc pyrithione under various scenarios using a computer program originally developed to model environmental copper concentrations. In order to give more confidence to the calculations, we have initiated a program to measure the actual environmental concentrations of pyrithione in a marina during the course of a boating season. In this paper, a sensitive and reliable analytical method for the analysis of pyrithione in marina water at parts-per-trillion levels will be discussed. The results of a preliminary study conducted at a local marina to validate the sampling and analytical method will also be presented.

28A-4: ANALYTICAL METHODS, ENVIRONMENTAL FATE AND RISK ASSESSMENT OF BIOCIDES 2

28A-4-1

TIO2 PHOTOCATALYTIC DECOMPOSITION OF THE BIOCIDES CHLOROTHALONIL AND DICHLOFLUANID

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Vasiliis A. Sakkas, University of Ioannina

The photocatalyzed degradation of the biocides chlorothalonil and dichlofluanid has been investigated in aqueous suspensions of titanium dioxide under simulated solar irradiation. The primary degradation of the micropollutants follows a pseudo-first-order kinetics following the Langmuir-Hinshelwood model. In our conditions total disappearance of chlorothalonil and dichlofluanid was achieved in 90 and 20 min respectively whereas the mineralization of organic carbon to carbon dioxide after 240 min of irradiation was found to be 100% for chlorothalonil and 78% for dichlofluanid. The evolution of heteroatoms (Cl, N, S, F) followed by ion chromatography showed a mineralization into chloride, nitrate, sulfate and fluoride anions respectively. In addition Microtox bioassay (Vibrio fischeri) was employed in evaluating the ecotoxicity of solutions treated by photocatalysis. Photocatalytic intermediates detected during the degradation of biocides were identified by GC-MS techniques. Based on this byproduct identification a simple degradation scheme was proposed for both biocides including dechlorination, hydroxylation, dealkylation and decarboxylation leading to the mineralization of the starting molecules.

28A-4-2

DEVELOPMENT OF PEC MODEL FOR ANTIFOULING AGENT AND UNCERTAINTY IN THE PREDICTED CONCENTRATION

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Minoru Akiyama, Mizuho Information and Research Institute, Inc.
Yoshimi Muramatsu, Mizuho Information and Research Institute, Inc.

A model to predict environmental concentration of antifouling agent released from ship hull paint has been developed. The model includes transportation along water flow, diffusion derived by the concentration gradient, leaching from the paint, photolysis by the sun light, hydrolysis, biolysis and adsorption onto the suspended matter, as the fate processes. Special care has been taken on the photolysis among them, because the photolysis is major decomposition process of some antifouling agents in the aquatic environment. The model deals with the effect of sun light intensity change with time and its penetration into the water column. It is able to predict the concentration gradient along the water depth. The model has been designed for two typical geometric conditions. One is a pier for a large merchant vessel with uniform water flow, and the other is a rectangle shape marina which has opening to the outside environment and exchanges the water periodically by tidal. Water flow in both area has been calculated using a three dimensional fluid dynamic code. Model calculation results show concentration profile in three dimensional directions and their periodical changes with time, but some of the results are very sensitive to certain parameters. It implies uncertainty in the environment concentration prediction. The values for the parameters could be changed by time, season, weather and geographical conditions. It suggests that it is essential to evaluate adequately the environmental parameters for the place of interest using this kind of model, and that take proper sample representing the environment.

28A-4-3

EFFECTS OF MEDETOMIDINE ON MICROALGAL AND BACTERIAL MARINE COMMUNITIES

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Medetomidine is as a new antifouling substance with prevention of barnacle fouling as main antifouling property. The mode of action for medetomidine on cyprid larvae is hypothesized to be through the invertebrate-specific octopamine receptor and even though algae and bacteria lack these receptors cross-reactions with receptors in the same family could occur. Studies on algal and bacterial communities are therefore needed to avoid unforeseen environmental consequences. Static exposure, 3h, and semi-static exposure, 96h, of medetomidine to microalgae and bacteria from epipsammon, phytoplankton and periphyton communities were performed to evaluate how medetomidine affects photosynthetic activity, bacterial protein synthesis, species composition and pigment profiles. As a final investigation of medetomidine's effects on microalgae and bacteria a 4 weeks flow-through experiment was made using periphyton with physiological, structural and functional end points. The results from the short–term and the semi–static experiments show that medetomidine has limited effects on bacteria and microalgae. A significant decrease in photosynthetic activity with 16 percent was observed in the highest concentration tested, 10μM, after 3 hours of exposure. However no significant effects on bacterial protein synthesis, species composition and pigment profiles were observed from the short –term or the semi–static experiments. Results from the flow–through experiment are under evaluation. The risk from medetomidine on algae and bacteria seem negligible. However, from an efficacy point of view, it suggests that medetomidine needs to be combined with a booster biocide to prevent microfouling. This study is a part of the Marine Paint research program funded by MISTRA, the Foundation for Strategic Environmental Research.

28A-4-4

TOXICITY AND BIOACCUMULATION OF MEDETOMIDINE IN MARINE FAUNA

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Since medetomidine is a new antifouling candidate, it is important that its effects on marine organisms are investigated. Medetomidine is a commonly used sedative and analgesic in veterinary medicine and has also been shown to effectively prevent the settlement of barnacles. A variety of species, mainly non-target organisms, were chosen for sublethal effect and bioaccumulation studies.

The most obvious effect caused by medetomidine is on the pigmentation of fish. Medetomidine causes pigment aggregation in melanophores which makes the fish appear pale. This was observed in all tested fish species but was most pronounced in turbot '*Psetta maxima*'.

As a sedative, medetomidine affects the metabolism in mammals. Oxygen consumption was therefore investigated in fish, as a surrogate for metabolic rate. We observed decreased oxygen consumption with increasing concentrations of medetomidine in fish larvae, most obvious in lumpfish '*Cyclopterus lumpus*'. In addition, respiration frequency was affected in turbot.

In turbot, EROD–activity in liver microsomes increased after injection, while a non–significant decrease was observed after water exposure. No effects on EROD activities were observed in Atlantic cod '*Gadus morhua*'.

Medetomidine was shown to have a maximal bioconcentration factor 'BCF' of 1195 l/kg FW 'fresh weight) in periphyton communities at steady state. The BCF in blue mussel '*Mytilus edulis*' was 133 l/kg FW and 2.8 l/kg FW in brown shrimp '*Crangon crangon*' demonstrating that bioaccumulation is species dependent. A rapid and almost complete elimination of medetomidine was also observed.

In conclusion, the observed effects caused by medetomidine may decrease the organisms' ability to survive in nature. However, all observed effects were reversible, at least to some extent and the effects occurred only at concentrations at least 50 times higher than the predicted environmental concentrations. This study is part of the Marine Paint research programme funded by MISTRA.

28A-4-5

CONTAMINATION OF ORGANOTIN ALTERNATIVE ANTIFOULANTS IN COASTAL SEAWATER AND SEDIMENT OF HIROSHIMA BAY

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From 2002, Hiroshima City Institute of Public Health investigated the remnants of alternative compounds in the fishery harbour, dock and the environmental standard points of the north part of Hiroshima Bay and Ota River.
Over the study period, Sea-nine211, Diuron, Irgarol 1051 and the latter's degradation product M1 were detected in seawater from Hiroshima Bay. But KH101 was not detected in the seawater samples. So, it was thought that the resolution speed of KH101 was higher than the other materials and KH101 became a degradation product.
The concentration levels at the ports and marinas were high while at the environmental standard points they were low. So it was shown that the sources of the chemicals were the ports and marinas.
We detected Sea-nine211, Diuron, Irgarol 1051 and the latter's degradation product M1 in sediment from Hiroshima Bay.
Concentrations of Diuron, Sea-nine211, Irgarol 1051 and M1 in sediment samples ranged from ND-73ng/g, ND-40ng/g, ND-28ng/g and ND-9ng/g respectively. In comparison with the results of the British and Greek studies, these results showed almost the same concentrations of M1, Irgarol was much lower and Diuron was higher.

28B-1: BIOFOULING BY MACRO SESSILE ORGANISMS AND MECHANISMS OF LARVAL SETTLEMENT 1

28B-1-1

VARIATION AMONG FAMILIES IN LARVAL RESPONSE TO SURFACE WETTABILITY FOR THE BARNACLE *BALANUS AMPHITRITE*

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The attachment response of cypris larvae to surfaces of differing wettabilities varies among and within species of barnacles. I investigated whether variation in the wettability response within a population of the barnacle *Balanus amphitrite* had a genetic basis. Five surfaces spanning a range of wettabilities were generated in borosilicate glass vials using silanes. Cyprids from 20 maternal families were assayed for attachment and metamorphosis in response to the surfaces, and to a positive control (settlement factor). The proportion of larvae permanently attaching to the surface served as the character of interest. Attachment in response to all the treatments varied significantly among the families. Family means of the proportion of larvae attaching to the treated surfaces were positively correlated within surface type (hydrophilic vs. hydrophobic), but uncorrelated across surface types. Attachment in response to the positive control was uncorrelated to response to any of the glass treatments, suggesting that variation in the response to surface wettability among families was not due to variation in larval vigor or timing of acquisition of metamorphic competence. An additional experiment indicated that the observed variation may not have been due to differences among families in age-related changes in attachment behavior. These results suggest that response of barnacle cyprids to these surfaces is a product of genetic or maternal effects, and that these effects differ between hydrophilic and hydrophobic surfaces. This research was supported by funding from the Office of Naval Research.

28B-1-2

RECEPTOR CHARACTERIZATION GOVERNING CEMENT RELEASE FROM THE BALANUS IMPROVISUS CYPRID LARVAE

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Within the research program Marine Paint, located at University of Gothenburg and Chalmers University of Technology, Sweden, we investigate how substances that induce or inhibit specific biological responses may be used for antifouling purposes. The main target proteins are G-protein coupled receptors and especially the amine receptors such as dopaminergic, octapaminergic or serotonergic receptors.
Secretion of the cement proteins that attach the barnacle, Balanus improvisus, cyprid larva to the substratum is the first event in a series leading to metamorphosis and a sessile juvenile barnacle. We have investigated the dopaminergic regulation of the cement protein release from the cement gland in the cyprid larva to understand the regulatory mechanisms behind the secretion. We have used vacuole formation within the cement gland as part of the secretion process as a quantitative tool when measuring the biological response. Comparisons were made with different dopaminergic substances to present a pharmacological profile of the dopaminergic receptor that controls cement protein secretion. We then compared the different dopaminergic agonist and antagonist in the settling assay evaluating the secretion results and correlate those with the settling mechanism. Results indicate that the receptor subtype involved in cement protein release pharmacologically resembles D1-like receptors. A D1 agonist, A68930, was more effective than dopamine in inducing cement secretion and did also enhance the settlement rate of cyprid larvae. The D1-antagonist SCH 23390 were effective in inhibiting cement secretion as well as reducing settlement of when compared with other antagonists. The investigation indicate that substances with a known biological target can be used as antifoulants, with the advantage regarding a known Mode of Action, that can be used for both scientific and regulatory purposes.

28B-1-3

TEMPORAL PERSISTENCE OF THE EFFECTS OF TEXTURE AND ORIENTATION ON THE SUCCESSION OF A MARINE FOULING ASSEMBLAGE

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Studies of fouling processes are often short-term, typically only considering the time taken for surface colonisation, and rarely measure long term effects. Also, measurements made by ecologists and by technologists may be very different; the former preferring quantification of individual species or measures of assemblage diversity, the latter describing useful categories, such as hard, soft, or total percent cover. In this study we analysed the temporal persistence of strong initial colonization effects of texture and orientation of test panels through 2.5 yrs of assemblage succession. Each month, a range of response measures were also taken, including species, functional group and assemblage diversity. Orientation had strong effects at almost all levels of response during colonisation, while texture exerted most effects at group level, yet very few effects persisted through assemblage succession. We show that effects are often dependent on the choice of measurement, and that most effects disappear over time. This has fundamental consequences for both fundamental and applied studies: effects of surface properties should be tested for temporal persistence, and researchers should be aware of the potential influence of their choice of measurement.

28B-1-4

BIO-ORGANIC STUDIES FOR UNDERSTANDING THE MECHANISM OF THE ACTION OF ANTIFOULING ACTIVE COMPOUNDS WITH FLUORESCENCE-LABELED PROBES

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Development of environmentally-friendly antifouling agents is one of the most important researches. As well as the production of new antifouling active compounds, understanding the mechanism of the action of the compounds is also necessary for development of the antifouling agents. Recently, we considered the detailed structure-activity relation studies and produced various antifouling active isocyano compounds. In addition, bio-organic studies for understanding the mechanism of the action of isocyano compounds were carried out with fluorescence-compounds containing dancyl group. These studies showed that an isocyano compound would affect the oil droplet area of the cypris larvae of the barnacle. However, it was not clear whether the presence of isocyano group is important in this behavior. We disclose here further studies for understanding the mechanism of the action of antifouling active compounds with fluorescence-labeled probes.

An isocyano probe compound which has NBD group instead of dancyl group as a fluorescence moiety was synthesized, and the interaction between the probe and the cyprids was monitored under a fluorescence microscope. As well as the case of the dansyl probe, strong fluorescence was observed in the oil droplet area of the barnacle cyprid. The fluorescence signal in the oil droplet area decreased when the bioassay was carried out with fluorescence-labeled compounds in the presence of another kind of antifouling active isocyano compound. The decrease of the signal varied according to the concentration gradient of the non-fluorescence compound. This study showed that isocyano group would affect the oil droplet area of the cypris larvae of the barnacle.

28B-1-5

BARNACLE ADHESION TO A FOUL-RELEASE COATING VARIES WITH TEMPERATURE

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Accumulation of marine biofouling on manmade surfaces such as ship hulls, water intake systems, and oceanographic equipment has tremendous economic impacts. Biofouling limits the functionality and life span of such structures and increases maintenance intervals and costs. Using toxic marine coatings such as organotin and copper-based paints prevents the settlement and growth of marine organisms, however, these paints are nondiscriminatory and pollutants often build up in sediments of bays and harbors. More recently, non-toxic foul-release coatings have been used, although the efficacy of such coatings is not fully understood. In particular, we do not understand how environmental factors such as different sea temperatures will influence the functionality of coatings, and to our knowledge no laboratory testing of coatings to date has investigated such factors. In this study we examined the effect of temperature on the performance of non-toxic foul-release coatings by conducting assays using the barnacle *Balanus amphitrite*. The animals were raised on Dow Corning T-2 silastic (a polydimethylsiloxane elastomer) at 15, 25, and 30 degrees Celsius, a temperature range that spans the species' natural distribution and encompasses the service requirements of many ships. Removal forces were measured using an automated force gauge and critical removal stress (CRS) was calculated by dividing the force of removal by the basal plate area for each barnacle. A significant inverse relationship was found between temperature and CRS, demonstrating that the animals are more strongly attached to surfaces at lower temperatures. The mechanism accounting for this difference among temperatures is unknown presently and we are investigating 1) potential physical changes in barnacle cement structure or 2) possible differences in the composition of the barnacles' adhesive.

28B-2: BIOFOULING BY MACRO SESSILE ORGANISMS AND MECHANISMS OF LARVAL SETTLEMENT 2

28B-2-1

FORMATION AND INFLUENCE OF BIOFILMS: A DIATOM PERSPECTIVE

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Natural biofilms constitute a complex network of microorganisms. The development of the biofilm is regulated by many factors including nature of the substratum, environmental conditions and interactions among the components of the biofilm community. Colonization of diatoms generally follows the formation of a conditioning film and attachment of bacteria. Diatoms in marine biofilms are dominated by pennate forms which are equally important in the benthic environment, as microphytobenthos. *Navicula* and *Amphora* are among the most common pennate forms in the benthic environment. Observations in India also show *Navicula* spp. to be the dominant component of the marine diatom biofilm community.

Culture experiments with *Navicula sp.* and *Amphora coffeaeformis* showed the latter to be the superior competitor. It was also observed through incubation of natural marine diatom biofilms that excess nutrients favoured those species with comparatively higher growth rates, thereby suppressing the growth of other co-existing species. This competitive trait was found to be effective at an appropriate cell density ratio of the competitive and target species.

Diatoms and bacteria have intrinsic association. Elucidation of diatom-bacterial interactions revealed that the growth rate and extracellular polymeric substance (EPS) production of non-axenic diatoms (*Navicula* spp.) was faster/higher compared to near-axenic cultures. Incubation of benthic diatom communities with antibiotics (penicillin, streptomycin and chloramphenicol) resulted in changes in diatom abundance and community structure. The changes in the diatom community varied with the type and concentration of antibiotics used, leading to an inference that diatoms are influenced by the bacterial community. Experiments with near axenic diatom films also facilitated metamorphosis of barnacle *B. amphitrite* larva and the influence varied with diatom species and cell density. In light of the earlier observations that barnacle larvae respond to both contact and olfactory chemoreception, it is pertinent to evaluate the role of such cues in benthic and fouling community dynamics.

28B-2-2

THE DYNAMICS OF TWO SPECIES OF *MEGABALANUS* (CRUSTACEA: CIRRIPEDIA: BALANIDAE) BY A CELLULAR AUTOMATA MODEL

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The Cellular Automata model was used in a computational simulation between an introduced species in Brazilian,Rio de Janeiro State waters *Megabalanus coccopoma* and a cryptogenic species *Megabalanus tintinnabulum* (Crustacea, Cirripedia,Balanidae), obtaining a time series where *M. tintinnabulum* firstly occurs alone and then it interacts with the entrance of *M. coccopoma* in the system

The simulation also gets data about spatial distribution of both species and column formation, representing the specimens'assessment one above the other,as it occurs at the natural environment

The results show that total recruitment of each species within the maximum height of the columns is important for the predominance of the introduced species in comparison with the cryptogenic one

The comparison between CA model and natural distribution of both species shows that CA represents significantly the interaction between both species of barnacles in the studied area

28B-2-3

CURRENT STATUS OF MACRO FOULING IN PUDHUMADAM COAST OF THE GULF OF MANNAR, SOUTHEAST COAST OF INDIA

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An Underwater Biofouling Panel (UWBFP) system was erected for the qualitative and quantitative estimation of marine macro fouling organisms of Pudhumadam coast in the Gulf of Mannar. Four types of test panels, Plain wood (*Artocarpus hirsutus*), Tar coated wood, Fibre coated wood and Concrete were used for the observation of macro fouling organisms as well as to find out the durability of these materials in the marine environment. Fifty one biofoulers were identified from these test panels used in the UWBFP system. Among these, *Balanus amphitrite* was the dominant fouler. The concrete panel encouraged the highest barnacle density (2.56±0.06 no./cm^2) compared to the other panels. The barnacle density was the highest during the post-monsoon period followed by monsoon and pre-monsoon periods in all the test panels. All the test panels showed that the barnacle density was more on the topside compared to the bottom. Three series (A,B and C) of tests were done to check the seasonal difference of barnacle density. The overall variation in barnacle count in the seaward and shoreward sides of all these three series were tested using ANOVA and were found to be significantly different from each other (A-series - Seaward $P<0.001$; $F_{3,44}$ =2.81, 160.99 and Shoreward $P<0.001$; $F_{3,44}$ =2.81, 141.99; B-series - Seaward-$P<0.05$; $F_{3,44}$ =2.81, 3.85 and Shoreward $P<0.01$; $F_{3,44}$ =2.81, 4.60 and C-series - Seaward $P<0.05$; $F_{3,44}$ =2.81, 4.86 and Shoreward $P<0.05$; $F_{3,44}$ =2.81, 5.63). The Shannon-Wiener species diversity index was also calculated for identifying the substratum which supports good diversity and accumulation of biofoulers. Of all the three series, wood substratum showed larger diversity index with greater accumulation of different types of fouling organisms.

28B-2-4

RECRUITMENT OF BARNACLES ON WOODEN BLOCKS IN A TROPICAL COASTAL ENVIRONMENT; INFLUENCE OF SEASON, LARVAL ABUNDANCE AND ENVIRONMENTAL FACTORS

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Recruitment of barnacles on wooden blocks in a tropical coastal environment; Influence of season, larval abundance and environmental factors. S. Satheesh* and S. Godwin Wesley Department of Zoology, Scott Christian College (Autonomous), Nagercoil-629003, Tamil Nadu, India *presenting author, e-mail:satheesh_s2005@yahoo.co.in Abstract Barnacles are the prominent fouling organisms encountered in many waters of the world. These sessile organisms are of greater significance due to their productivity, gregarious habit and recruitment pattern. In the present study, recruitment pattern of barnacles was monitored by suspending wooden test panels (10x10x3cm) for a period of one year from January to December 2005 at Kudankulam in the East coast of India, where a new mega Nuclear Power Plant is under construction. The abundance of cirripede nauplii and the hydrological conditions of the coastal waters such as temperature, salinity, pH, dissolved oxygen content, nitrates, nitrites and phosphates were monitored throughout the study period. Results showed that barnacle recruitment was continuous with seasonal variation. Balanus variegatus, Balanus amphitrite amphitrite and Balanus tintinnabulam tintinnabulam were the common barnacles encountered on the test panels. A maximum of 1084 individuals dm-^{2} was observed during July 2005. The pre-monsoon and monsoon months recorded higher recruitment and the density of cirripede nauplii in the coastal waters was marginally high during the post-monsoon season. Correlation analysis failed to show significant relationship between larval abundance and recruitment pattern. Most of the environmental factors also did not show any significant relationship with the barnacle recruitment. The observed results may be useful for taking better antifouling strategy with reference to the Nuclear Power station.

28B-2-5

WATCHING AND FEELING PROTEIN FOOTPRINTS OF BARNACLES AT BIOFOULING SURFACES: AN ATOMIC FORCE MICROSCOPY STUDY

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The barnacle (Balanus amphitrite) is a major cause of biofouling on manmade underwater structures, with severe environmental and economic impacts. Cypris larvae of this species, prior to settling at the surface, use a rapid, temporary attachment while exploring settlement locations. We investigate the temporary adhesive footprints under its native environment by using atomic force microscopy (AFM). The interfacial morphology of the footprints was visualized by AFM imaging, while the nanoscale mechanical properties were studied in pull-off force experiments. AFM imaging revealed a fibrillar nature of the secretion, suggesting that the deposits are composed of single proteinaceous nanofibrils or their bundles. Saw-tooth characteristics were observed in the force-extension curves showed repeatable progressive peaks; characteristic of unfolding glycoproteinaceous nanofibrils. Following relaxation of bundles stretched to high strains, force –distance curves in reverse stretching experiments could be described by entropic elasticity (the worm-like polymer chains model (WLC)). The persistence length (Lp) was obtained by fitting a classical worm-like chain model to the force-extension curves. When subjected to relaxation with a time delay exceeding 500 ms, the extended glycoproteins from the footprints apparently refolded and showed characteristic saw-tooth-like unfolding peaks in subsequent force cycles. Observed rupture and hysteresis behavior was explained by a sacrificial bond model as introduced by Hansma et. al.. Longer duration of relaxation (>5 s) allowed more sacrificial bond reformation, which contributed to enhanced energy dissipation (higher toughness). At high elongation, following repeated stretching to upper strain limits, a conspicuous step in the force curve was observed, resembling a conformational transition observed during pulling of polysaccharides. We attribute this to the presence of sugar units in the footprint glycoprotein.

28-P: POSTER SESSION 1

28P-1

THE EFFECT OF BIOFOULING ON LOCALIZED CORROSION OF HIGH ALLOYED STAINLESS STEELS (AISI 904L AND ZERON 100)

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Metallic materials exposed to marine environment are susceptible to biofouling growth on the immersed surfaces. In this study, stainless steels used in marine systems (austenitic 904L and duplex Zeron 100 (120x120x2mm)) with electric contacts were hanged on rafts anchored 40m away from Farol beach, Arraial do Cabo, Rio de Janeiro, Brazil. The coupons were separated in two groups: the 'Control' treatment, covered with nylon meshes to prevent attachment of macrofouling larvae and the 'Biofouling' treatment, exposed to biofoulers development without any interference. The open circuit potential was estimated with a multimeter and an Ag/AgCl electrode, acquired on days 1, 3, 5, 7, 10 and 15, and fortnightly after this, during 285 days, together with biofoulers quantification. After the experiment, coupons were removed from the rafts, carefully cleaned and observed at SEM microscope to identify pits or crevices. Biofouling was composed by diatoms in the biofilm in the beginning of succession process. The later stages included barnacles, seaweeds, encrusting bryozoans and mussels. A sharp increase of OCP was registered. This ennoblement of potential values can be usually observed in these situations. Nevertheless, in this study its behavior was different between treatments: the OCP reached higher values and remained stable in this range along the study in 'Control' treatment. In 'Biofouling' treatment the OCP values showed a drop after ennoblement, with high dispersion around replicate average and also oscillations along the study. The later indicates a probable localized corrosion initiation caused by macrofouling. Crevices around barnacle calcareous bases were the main type of localized corrosion observed on the coupons. However, crevices initiation and establishment were not clearly detected with OCP measures. The results indicated that austenitic 904L was more sensitive than the duplex, although both steels were very resistant in the conditions provided by this experiment.

28P-2

EVALUATION OF HIGH ALLOYED STAINLESS STEELS (AISI 904L AND ZERON 100): ELECTROCHEMICAL BEHAVIOR IN THE PRESENCE OF THE BARNACLE *AMPHIBALANUS AMPHITRITE* DARWIN, 1854

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Barnacles are the dominant organisms in macrofouling and they are pointed out as one of the main cause of biocorrosion in marine environments. The species *Amphibalanus amphitrite* was used to evaluate the effect of the presence of balanids on electrochemical behavior of two stainless steels (904L and ZERON 100). The larvae were maintained in laboratory until they reached Cypris stage. Thereafter they were stored and remained 7 days under refrigeration. One hundred cypris were offered for settlement in four replicate of the steels coupons (70x50x2mm). After settlement, metamorphosis and attachment, we reared the juveniles for 3 weeks with *Skeletonema costatum* (Greville) Cleve. Open circuit potential (OCP) was registered every other day during 3 weeks. Breakdown potential was obtained weekly at potentiostatic mode. At the end of the experiment, potentiodynamic mode and electrochemical impedance experiments were performed using Autolab PG30. Additional measures were registered in two control groups of four replicates (without barnacles attached): coupons immersed in sterile seawater and immersed in seawater with *Skeletonema* solution. The experiment was performed twice. In the first experiment the number of barnacles attached varied between 13 and 56 and the basal diameter reached 2mm. In the second experiment, the number of barnacles varied between 12 and 64 and the maximum basal diameter was 7 mm. OCP measurement did not show differences between experiments, barnacle and control replicate groups or along time. However, breakdown potential showed differences between experiments and groups - the lower values were registered in the coupons with larger barnacles in both polarization modes for the two steels, indicating that organisms size seems to be important for the process. Electrochemical impedance showed high values of polarization resistance, demonstrating steels integrity. Nevertheless, the results suggest that 904L steel was more susceptible to the presence of juveniles.

28P-3

INHIBITION OF BIOCORROSION IN SEAWATER VIA SURFACE FUNCTIONALIZATION OF METALS AND ALLOYS

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As a significant portion of the corrosion damage in seawater is due to anaerobic corrosion influenced by sulfate-reducing bacteria (SRB), attempts are being made to develop novel and environmentally-friendly surface functionalization techniques to endow the metal and alloy substrates with desirable anticorrosion and antibacterial properties. Multilayer coatings of Ti oxide/butoxide were built up on the surface of stainless steel via a layer-by-layer sol-gel deposition process. Not only does the passivity of the Ti oxide/butoxide multilayer coatings remain stable under the harsh environment of D. desulfuricans inoculated seawater, the passivity is slightly enhanced due to the deposition of calcium and phosphorous compounds associated with apatite. Antibacterial and anticorrosion properties were conferred on the Cu-Ni alloy substrate via surface-initiated graft polymerization of 4-vinylpyridine (4VP) in the presence of a trichlorosilane coupling agent and benzoyl peroxide, followed by N-alkylation of pyridinium nitrogen of the poly(4VP)-grafted surface to produce a high concentration of quaternary ammonium groups. The so-functionalized alloy surface is effective in inhibiting bacterial adhesion and biofilm formation, and thus in minimizing biocorrosion by D. desulfuricans. Functional polymer brushes were also graft-polymerized from stainless steel substrates via surface-initiated atom transfer radical polymerization (ATRP) of (2-dimethylamino)-ethyl methacrylate (DMAEMA). The tertiary amino groups of covalently-bound poly(DMAEMA) brushes were further quaternized with benzyl halide to produce the bactericidal functionality. Alternatively, covalent coupling of viologen moieties to the tertiary amino groups of poly(DMAEMA) brushes on the substrate surface resulted in an increase in surface concentration of quaternary ammonium groups. The bactericidal polymer brushers were found to substantially enhance the corrosion resistance of stainless steel coupons to biocorrosion by D. desulfuricans in seawater.

28P-4

A COMPARATIVE STUDY OF CAPSAICINOID'S ENCAPSULATION TECHNOLOGY ADDRESSED TO IMPROVE THE SPOT'S OPERATIONAL HYGIENE AND TO BETTER THE CONTROLLED RELEASE OF CAPSAICINOID DURING THE ANTIFOULING

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To apply artificial capsaicinoids as expellers in antifouling paint has displayed its potential as one of the promising candidates. However one of the disadvantages of the capsaicinoid expellers is their irritant property toward the paints' spraying operators, such as sneeze, fear etc.

28P-5

A STUDY OF CO-POLYMERS TYPES IN RELATION TO THEIR ANTIFOULING PERFORMANCE

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It is generally acknowledged that the co-polymers occupy an important place in antifouling paints used for ships located at and navigated along different marine spaces.
Copolymers function as dispersing agent, membrane–forming agent and refreshing/polishing component as well. A series of factors, such as chemical nature of monomer, pluralist component combination, molecular weight distribution, the depth of metal ion crafting in case of monomer of acrylic acid etc on the antifouling performance have been investigated and summarized.

28P-6

A COMPARATIVE STUDY OF THE ANTIFOULING PERFORMANCE OF FOUR DIFFERENT CAPSAICINOIDS AS EXPELLERS IN ANTIFOULING PAINTS

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A Study of exploitation in an effort to seek the more environmentally friendly, more cost–effective and highly antifouling expellers has been the focus of attention of scientists and technicians working in field of marine chemical technology.

28P-7

IMPACT OF FEATURE SIZE, GEOMETRY, AND ROUGHNESS OF ENGINEERED SURFACE TOPOGRAPHIES ON COLONIZATION AND BIOFILM FORMATION OF MARINE BACTERIA

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Biofouling is the undesired accumulation of living organisms on a surface. Primary colonizers in the marine environment including bacteria and other microorganisms begin to settle upon submersion of the surface into seawater. The attached cells along with the extracellular material that they produce condition the surface and have been shown to enhance the settlement of secondary macro organisms such as diatoms, algae spores, and larvae of barnacles and tubeworms. Our group has designed non-toxic antifouling technologies that focus on the manipulation of surface topography in polydimethylsiloxane elastomer to deter the settlement of fouling organisms. A correlation has been made between an engineered roughness index (ERI) and the settlement of zoospores of the *Ulva linza* alga. ERI is a dimensionless ratio based on Wenzel's roughness factor, the depressed surface area fraction, and the number of geometrically distinct features in the pattern. We hypothesize that the settlement of marine bacteria will also correlate to the ERI. *Cobetia marina* will be used as a model organism for bacterial colonization and biofilm formation. Preliminary results show that the Sharklet AF surface with an ERI value of 17.1 inhibited biofilm formation of *C. marina* after exposure for up to 72 hours. Correlating bacterial settlement to the ERI will further support the use of the ERI algorithm for designing antifouling surfaces.

28P-8

ANTIFOULING ACTIVITY OF BROMINATED DIPHENYL ETHERS

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In laboratory experiments the antifouling activity of 2-hydroxy-6-(6-hydroxy-4-methylphenoxy)-4-methylbenosoic acid methylester (1), 3,5-dibromo-2-(2,4-dibromophenoxy)phenol (2); 3,4,5-tribromo-2-(2,4-dibromophenoxy)phenol (3), 3,4,5-tribromo-2-(2-bromophenoxy)phenol (4), 4,5-dibromo-2-(2,4-dibromophenoxy)phenol (5), 3,4,5,6-tetrabromo-2-(2-bromophenoxy)phenol) (6); 4-phenoxyphenol (7), 4,4-dibromobiphenyl (8), 4-phenoxyaniline (9), 1-chloro-4-phenoxybenzene (10); 1-flouro-4-(4-flourophenoxy)benzene (11), 5-chloro-2-phenoxyaniline (12), 1-bromo-4-phenoxybenzene (13), as well as 4,4-dihydroxydiphenyl ether (14) were investigated against bacteria, the diatom *Amphora coffeaeformis* and the mussel *Mytilus edulis* juveniles. The naturally occurring compound 2 from the sponge *Callyspongia* sp. showed the strongest antifouling activity in all three bioassays with no toxicity. Among the synthetic derivatives, the compound 7 showed the strongest antifouling activity. Overall, the naturally occurring diphenyl ethers showed stronger activity than the commercially available analogues and could be possible future non-toxic antifouling candidates.

28P-9

THE POTENTIAL OF AN IMMOBILISED PROTEOLYTIC ENZYME, SUBTILISIN A, AS AN ANTI-FOULING AGENT: EFFICACY AGAINST SPORES OF *ULVA* AND CELLS OF THE DIATOM *NAVICULA*

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The use of enzymes in an anti-fouling context has been the subject of numerous patent applications over the past 20+ years. Incorporating soluble enzymes into anti-fouling products however, presents a number of technical difficulties in terms of enzyme mobility, stability and impact on the physical characteristics of the formulations. One approach to overcoming these issues would be to covalently attach the enzyme to the reactive groups of the coating. Serine-proteases (Alcalase, Savinase) have emerged from laboratory-based screens as the enzymes with most promise for broad-spectrum efficacy when in solution. This communication presents results which show that Subtilisin A, the purified form of the serine-protease contained in Alcalase and Savinase, can be successfully immobilised onto maleic anhydride copolymer films. The chosen polymer coating allows for the precise adjustment of surface physicochemical properties, with the reactivity of the anhydride toward amino moieties being used for the enzyme immobilization. We demonstrate that in the immobilised form Subtilisin A is still active, retaining the ability to reduce the adhesion strength of spores of the macroalga, *Ulva* and cells of a microalga, the diatom *Navicula*. Moreover, the immobilised form of Subtilisin is revealed as being more effective than the dispersed enzyme at reducing the adhesion strength of these species, with efficacy being influenced by the physicochemical properties of the polymer used for the immobilization.

28P-10

ANTIMICROBIAL ACTIVITY OF SOFT CORAL EXTRACTS FROM BANDAR AL KHIRAN IN SULTANATE OF OMAN

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Several species of soft corals from Bandar Al-khiran, Sultanate of Oman were investigated for bioactive marine natural product. The extracts were screened against marine biofilm and various strains of pathogenic bacteria. Several extracts were shown to have bioactivity against pathogenic bacteria such as *Micrococcus* sp., *Staphylococcus* sp., *Bacillus* sp., *Salmonella* sp., *E. coli* , and *Pseudomonas* sp. The ethyl acetate extract from *Cladiella* sp. was found as a source of potential antifouling compounds as it inhibited the growth of marine biofilm bacteria *Bacillus* sp. Bioassay guided fractionation was persuaded using silica gel, poly amide, reverse-phase and thin layer chromatographys. Several bioactive compounds were isolated, and their characterization using advanced 1D, 2 D NMR and LC MS-MS techniques are on the way.

28P-11

LOCALIZED GENERATION OF REACTIVE OXYGEN SPECIES BY PULSED ELECTRIC SIGNALS AND THEIR EFFECT ON BIOLOGICAL ADHESION

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The development of antifouling alternatives based on electric signal delivery has gained attention in the last years but, to our knowledge, has been tested only from a phenomenological approach. Our work presents evidence that the generation of Reactive Oxygen Species (ROS) by electrochemical means can effectively alter adhesion patterns of barnacle larvae of the species *Amphibalanus amphitrite*. We conducted lab-scale experiments intended to first demonstrate the feasibility of the use of electric signals to inhibit barnacle settlement, and then to show that electrochemical ROS production is the driving mechanism of settlement inhibition. Biological experiments consisted in exposures of barnacle larvae to electrified and non-electrified (i.e. control) planar electrodes, which were made of titanium sputtered onto glass slides. Seawater drops containing ~30 barnacle larvae were deposited on slides and left for three days until ~35% of larvae placed on control electrodes had settled. Pulsed electric signals of 10 Volts of amplitude were applied to treated electrodes. The application of these pulses produced settlement ratios below 4%. However, the addition of reactive oxygen scavengers, such as glutathione and catalase, to electrodes treated with electric signals, increased barnacle settlement to levels comparable to the ones observed on control slides, thus demonstrating the involvement of electrolytic ROS on settlement inhibition. Additional measurements confirmed the presence of significant concentrations of H_2O_2 and hydroxyl radicals in treated slides. Thus, our further development of electrochemical coatings will optimize materials, geometry and electric signal delivery based on the optimization of ROS generation rates.

28P-12

ANTIFOULING GELS AGAINST CYPRIDS OF THE BARNACLE (*AMPHIBALANUS AMPHITRITE*)

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Marine fouling organisms (algae, barnacles, shells, etc.) cause serious problems in the world. In particular, barnacle is the most popular marine fouling organism. Tributyltin (TBT) was the most popular antifouling agent and exhibits high antifouling performance. However, TBT will completely be baned to use in the world by 2008, due to its high endocrine disruption effect. So looking for new alternative antifouling material is an urgent task. The available antifouling technologies have been applied on solid state surfaces. It is reported that a hydrogel is able to inhibit germination of algae's zoospores (Y. Katsuyama et al., 2002) and some hydrogels have been found to exhibit inhibition to barnacle' s cyprid settlement (K. Rasmussen et al., 2002). However, only limited gels have been tested. Needless to say that the antifouling mechanism of gels against barnacles is poorly understood. In this study, barnacle was used as the model marine fouling organism, and settlement tests were performed on several synthetic polymer gels with different charges. Polystyrene is used as the solid state reference. Its a results, cyprids clearly avoid to settle on hydrogel surfaces compared to polystyrene. The electric nature and chemical species of hydrogels are less important, but the elastic modulus of hydrogels seems to make a difference for inhibition effect against the cyprid settlement.

28P-13

NON-FLUORINATED, NON-PEGYLATED AMPHIPHILIC BLOCK COPOLYMERS AND THEIR CROSSLINKED FILMS AS POTENTIAL ANTI-BIOFOULING COATINGS

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Several elements are required of modern anti-biofouling polymer materials: Film modulus, chemical composition, and contact angle all play key roles, as do morphological and topographical complexities of the polymer components. In addition, the coating should perform for a long time in a marine environment while also not affecting the surroundings adversely. Recent work by our lab has demonstrated that amphiphilic polymer networks that exhibit nanoscale phase segregation can lower the incidence of biological organisms that attach to these surfaces, and provide a non-toxic, anti-biofouling coating. These advanced anti-biofouling surfaces rely on low surface-energy fluorinated polymer segments coupled with anti-protein adhesion hydrophilic segments, such as poly(ethylene glycol). Due to the cost, typically poor modulus, and uncertain biocompatibility of fluorinated polymers, as well as the recent reports about the oxidative susceptibility of poly(ethylene glycol) polymers, it would be advantageous to provide commercially available, mechanically tough and environmentally benign amphiphilic block copolymers with good anti-biofouling characteristics and long shelf life. Our lab is currently investigating amphiphilic, crosslinkable multiblock copolymers that are developed via reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization of N-vinylpyrrolidinone and isoprene. The resulting copolymers contain a hydrophilic poly(N-vinylpyrrolidinone) region for use as an anti-protein adhesion agent that can withstand harsh conditions better than commonly used poly(ethylene glycol) systems. The system also contains a hydrophobic, high surface energy polyisoprene region, which present residual pendant double bonds for crosslinking and functionalization, in effect mimicking fluorinated polymers. The study of the synthesis of the polymers, their interesting nanoscale assembly and character, subsequent crosslinking, and biological assays will be presented. Reports about similar amphiphilic polymer systems, such as poly((ethylene glycol)-b-isoprene), will be presented as well.

28P-14

ENZYMES IN ANTIFOULING: THE CHALLENGE OF PAINT-INCORPORATION

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Different proteases are recognized as efficient antifouling agents. Their mode of action is believed to be via a degradation of proteinaceous adhesive polymers or settlement cues of various organisms. As proteases are quickly and completely degraded in the marine environment they constitute an attractive alternative to the toxic and recalcitrant biocides used in antifouling paints today. However, because of their easy degradability the successful application of direct enzymatic antifouling has been limited to paints with short-term performance. Therefore, in order to improve performance, the major task is to develop ways to inhibit the degradation of the enzymes while at the same time conserving their antifouling activity.

We are working on novel ways of stabilizing and dispersing enzymes in solvent-based paints. A high initial release of enzyme to the surrounding water is often observed from paints. This is not a problem using our new technology. The methods developed ensure that the enzymes are retained in their active forms and an almost constant enzyme activity is observed at the paint surface as a function of time and wear.

To verify the antifouling effect in situ, exposure studies have been conducted both in temperate and tropical waters. Solvent-based paints with different enzyme concentrations were tested in Denmark (Baltic Sea) and Oman (Arabian Sea). The antifouling effect can be linked to both enzyme concentration and distribution in the paint at both testing sites.

28P-15

TOXIC AND NON-TOXIC MECHANISM OF SETTLEMENT INHIBITION OF NATURAL ANTIFOULANTS

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The environmental problems caused by metal-based antifouling coatings (in particular organotins and booster biocides) led to the increased interest in the search for non-toxic alternatives. Marine organisms protect the surface of their bodies with antifouling substances without causing serious environmental problems. They have a broad spectrum of efficacy and could also affect nontarget species, but it is very likely to be degraded by microorganisms. The marine natural products that inhibit settlement of potential epibionts on the host organisms could be incorporated into antifouling paints that inhibit biofouling. The ideal candidates as non-toxic natural antifouling products would be the compounds showing similar potent antifouling activity of currently used booster biocides (EC50 CuSO4=0.30 μg/mL, EC50 Copper pyrihtione <0.01μg/mL, EC50 Zinc pyrithione 0.02 μg/mL), but without the high toxicity of these compounds (LC50 *Balanus amphitrite* as model organism. Our results show generally good antifouling activity for most of the tested samples, but the mechanism of settlement inhibition (toxic or non-toxic) was variable. The most important results were obtained from a polymeric alkylpyridinium salt isolated from the Antarctic sponge *Haliclona* sp. and crude extract of *Renilla* sp. that exhibited a good antifouling activity (EC50 of 0.06 μg/mL and 0.3 μg/mL respectively) and a low toxicity (LC50>100 μg/mL). On the other side, crude extracts of *Reniera mucosa* and *Ircinia variabilis* showed good antifouling activity (EC50 of 0.48 μg/mL and 3.40 μg/mL), but high toxicities (LC50=4.49 μg/mL and 1.75 μg/mL). Our research encourages further studies for a better selection of non-toxic marine natural compounds to stimulate the industrial development of new environmental friendly antifoulants.

28P-16

INHIBITION OF MARINE BIOFOULING BY POTASSIUM SORBATE LAB-TESTS AND FIELD TRIALS APPROACHES

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Traditionally, copper or organotin based coatings were commonly used to inhibit biofouling on underwater structures. Although these paints are highly effective, they are dangerous to the marine environment due to they affect a wide spectrum of non-target organisms. New trends in antifouling technology are focused to the use of environmental friendly-compounds.

As yet, the development of antifouling coatings based on natural products has not been obtained at a commercial scale. In addition to its antifouling characteristics, products must have properties that make them suitable, i.e. low toxicity, a broad spectrum of activity against algae and invertebrates, low cost, etc. For this reason, we search for another possibility for biofouling control. The aim of this study is to evaluate the activity of potassium sorbate, a synthetic compound widely used as food preservative, on larval survival of *Balanus amphitrite* through laboratory antifouling bioassays and field trials.

In the lab potassium sorbate solutions ranged between 0.1 and 0.001M were prepared. Toxicity and settlement tests were conducted using nauplii II and cyprids respectively, and results were recorded after 24 h incubation. Significant differences between treatment and controls were observed in solutions higher than 0.0125M, above this concentration, solutions were effective in inhibiting both nauplii swimming movements and cyprids attachment. When larvae were transferred to fresh artificial seawater they could recover and follow their development, this observation confirms that the effect of potassium sorbate is not permanent.

For field trials, potassium sorbate was entrapped into a non-toxic varnish. Acrylic panels coated with the varnish were exposed in Mar del Plata harbour, Argentina. Biofouling percentage cover was estimated after 15 day. A great decrease in micro and macrofouling density and diversity in relation to controls was detected ($p<0.05$).

Because potassium sorbate is not lethal, it has an excellent potential as an antifouling agent.

28P-17

GENISTEIN AND ITS DERIVATIVES AGAINST LARVAL SETTLEMENTS OF THE BARNACLE *BALANUS AMPHITRITE*

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Genistien (4',5,7-trihydroxyisoflavon) was identified by bioassay guided isolations from the metabolites from a deep sea bacterium *Streptomyces fungicidicus*. The results of anti-larval settlement bioassays indicated it possessing remarkable antifouling potential. The EC_{50}, LC_{50}, and LC_{50}/EC_{50} ratio were detected as 3.0 $\mu g\ ml^{-1}$, >50 $\mu g\ ml^{-1}$, and >16.7 against the larval settlements of the barnacle *Balanus amphitrite*, respectively. We also detected the antifouling activities of genistein against *Hydroides elgans* and *Bugula neritina*, results of which showed that genistein also can inhibited the larval settlement both species with EC_{50} less than 20 $\mu g\ ml^{-1}$. To study the structure-function relationship, we collected 17 flavone or isoflavone derivatives and examined them in larval settlement bioassays using *Balanus amphitrite* cypris larvae. Eight compounds among them showed strong anti-larval settlement activities with their EC_{50} lower than 10 $\mu g\ ml^{-1}$. Through analysis of structure and activity relationship of these compounds, we demonstrated that 1) the structure difference between flavones and isoflavoens did not affect their antifouling activities; 2) 5-hydroxyl on the skeletons played a key role in antifouling activities; and 3) the presence of hydroxyl or bulky groups on C3, which disturbs the formation of O:H–O hydrogen bond between 5-hydroxyl and 4-carbonyl, significantly reduced the antifouling activities.

28P-18

FORMULATION AND TESTING OF NOVEL NON-BIOCIDAL ANTIFOULING NANOTECHNOLOGIES

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Project AMBIO (Advanced Nanostructured Surfaces for the control of Biofouling) is a European Union initiated integrated programme to study and develop new nano-structured surfaces for the control of biofouling in aquatic environments. The role of International Paint within the project has been to formulate and carry out field trials of candidate nanotechnologies. The challenges associated with formulation of novel non-biocidal antifouling coating technologies will be described, and the methods for field evaluation of technologies for a variety of end-uses, including ships hulls and pleasure crafts, aquaculture equipment, and water in-lets will be illustrated. Preliminary results from field trials will demonstrate the potential of nano-structured surfaces for antifouling end-uses and will provide an insight into the nature of materials that will be taken 'real world' trials later within the Project.

28P-19

MARINE PAINT OPTIMISATION: SETTLING ASSAYS WITH MARINE PERIPHYTON COMMUNITIES AND SEA LETTUCE (*ULVA LACTUCA*)

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In the Marine Paint Optimisation project the major groups of fouling organisms will be studied. From each group of organisms one model organism was selected and used in settling assays to assess the efficacy of a number of individual antifoulants. A tentative list of existing and promising compounds include medetomidine, tolylfluanide, copper pyrithione, Sea-Nine, Irgarol, copper, Borocide, and Econea. Based on the produced data we will predict the joint effects of almost all possible combinations in terms of efficacy. The optimisation will be based both on high efficacy and low environmental risk. We will present and summarise the results from two settling assays with marine biofilms (microfouling) and the zoospores of the macroalga sea lettuce (*Ulva lactuca*). The microfoulers are important parts of the fouling community with a high number of different species and individuals which form the basis for all subsequent settlers. In this periphyton assay a multispecies microbial biofilm established in the field on Plexi glas™ (PMMA) are used to prepare an inoculum using a scrape, shake and sieve technique. Using this approach we include a large set of the potential species in the microbial fouling community. Ulva lactuca belongs to the most common family of fouling macroalgae. Thalli of *Ulva lactuca* were sampled in the field in September 2007, cultivated in the laboratory until further testing of the zoospores. Both assays are semistatic and run over three days and include both settling and growth. Efficacy data for a number of antifouling agents will be presented. This study is part of the Marine Paint research programme funded by MISTRA.

28P-20

MARINORD - NANOPOROUS PARTICULATE CARRIERS FOR BIOACTIVE SUBSTANCES IN MARINE ANTIFOULING COATINGS

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The objective of this project is to develop ecofriendly, effective and economical marine antifouling paints with controlled release of active substances by nano-porous particulate carriers. Traditionally, marine fouling has been prevented by the addition of heavy-metal compounds as antifouling agents in marine paints. These heavy metals have however shown to have undesired effect on many organisms in the sea and have therefore been banned to larger extent. The current technology for keeping ship hulls and other marine constructions free from marine antifouling is to large extent based on the release of copper and organic biocides. Since these coatings are suspected to have negative impact on the marine environment, new innovative approaches are sought for. There is definitely a need for new ecofriendly effective and economical solutions.

Enzymes are biological molecules that can act as antifouling agents in marine paints. The aim of this project is to develop methods to stabilize and maintain the activity of enzymes as active antifouling agents in paints for a period of three years.

Avermectin is a new very potent anti-barnacle substance evaluated at Goteborg University. It has shown good antifouling properties in field tests and further, it shows also the very interesting contact-active principle. Avermectin will be used as a model substance that represents the contact active principle of any active substance.

By using nano-porous particles as resevoirs for the active substances in the paint, a relatively stable release rate can be achieved. Different binders' technologies exist for antifouling paints. Some are present in waterborne systems and some are present in solvent-borne systems. In this project a waterborne, acrylate-based, preferably self-polishing, binder will be developed. Solvent-borne systems will also be included in the project to cover larger parts of the potential market.

28P-21

ANTIFOULING ACTIVITY OF NOVEL TRIALKYLSILYLMETHACRYLATE-BASED PAINTS THROUGH BIOASSAYS AND FIELD TESTS

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This work focuses on the assessment of the AF performance of several binders based on novel silylated-block copolymers [1]. Varnishes and complete AF paints were prepared with notified biocides including Seanine® 211, Preventol® A4S, zinc pyrithione, and zinc oxide. Multi-well plate screening method and field tests were utilized to assess the influence of fouling species on the coating performance. Relevant fouling species have been chosen to assess the performance of paints. Five marine Bacteria (*S. baltica, P. irgensii, H. marina, P. elyakovii, P. citrea*) and five marine benthic diatoms (*H. coronata, R. marinus, P. roscoffensis, P. carterae, Sacrinochrysis sp.*) were used to evaluate the coatings. Coatings were tested for inhibitory activity against the settlement of cypris larvae of *Balanus amphitrite*, as well as for toxicity [2]. Factors including salinity, pH, temperature of natural sea water, and type of macrofouling communities were recorded during 18-month immersion test (2004-2006) in Toulon (France). Results from bioassays demonstrated that the varnishes were essentially non-toxic to *nauplii* and reduced the settlement of barnacle in comparison with a TBT based-binder. The effectiveness of the corresponding paints against the marine microorganisms was reported to be affected by, both the molar content of the silylated block of the binder and the activity spectrum of biocides. Nevertheless, those paints were demonstrated to be toxic against the barnacle larvae. The AF performance on raft were corroborated with the bioassays, showing no macrofouling organisms on the coating surface during 18 months.

[1] C. Bressy, A. Margaillan, F. Fay, I. Linossier, K. Vallée-Réhel, Advances in antifouling coatings and technologies, Chapter 12: "Tin-free self-polishing binders for chemically-active paints" Eds. C. Hellio and D. M. Yebra, to be published.
[2] Hellio *et al.*, Biofouling 20 (3) (2004) 139-145.

28P-22

THE EFFECT OF NANOCOMPOSITES ON THE ADHESION OF *MYTILUS EDULIS* TO PDMS SURFACES

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Mussel fouling is a major issue for the aquaculture industry, especially for fish cages. Recent work within the EC AMBIO project has shown that the addition of small amounts of carbon nanotubes to PDMS improves fouling-release performance with respect to barnacles, bacteria and algae. The addition of natural sepiolite clays showed variable effects, with barnacles adhering more strongly with the addition of natural sepiolite while *Ulva* sporelings showed reduced adhesive strength (Beigbeder et al in press). This study aimed to examine whether the nanofiller-modified PDMS coatings similarly affected blue mussel, *Mytilus edulis*, adhesion. Specifically, the number of byssi deposited and byssal plaque area were determined for 30 small mussels (10 to 12mm) on 8 surfaces - acid washed glass, unfilled PDMS (sylgard 184), PDMS filled with 0.01, 0.1 and 1% carbon nanotubes and PDMS filled with 0.1, 1 and 10% natural sepiolite. Average byssal number laid down by each mussel was not affected by either increasing loadings of carbon nanotubes, or natural sepiolite. The average byssal area of a mussel increased with increasing carbon nanotube loading, while the opposite was true for natural sepiolite.

28P-23

DEFENSIVE POTENTIAL OF *PALYTHOA CARIBAEORUM* (DUCHASSAING & MICHELOTTI 1860) IN ARRAIAL DO CABO REGION, RIO DE JANEIRO - BRAZIL

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Palythoa caribaeorum has a great competitive ability being the dominant organism in the rocky shores of Arraial do Cabo, Rio de Janeiro. Cnidarians of *Palythoa* genus have their tissues colonized by symbionts dinoflagelates (zooxanthellae) and are known to produce palytoxin (PTX) like chemical defense. PTX is the most potent and structurally complex marine toxin discovered. In this study, we assess the differences of defensive potential of *P. caribaeorum* considering spatial and temporal scales; test antifouling activity of polar and non-polar crude extracts of tree different treatments of *Palythoa* (*Palythoa* with mucus, *Palythoa* without mucus and mucus of *Palythoa*); and evaluate if active defense against fouling is hydrophilic and/or lipophilic. It was not found any difference of defensive potential in spatial scale, probably due to proximity of sampled sites, because the high genic influx results in high genetic differences of defensive potential in temporal scale, what can be a great indicator that production of chemical defenses by *P. caribaeorum* in Arraial do Cabo is genetically regulated in temporal and spatial scale. We conclude that the production of lipophilic chemical defenses with antifouling activity is null or very low in *P. caribaeorum* from Arraial do Cabo. The absence of antifouling activity in polar and non-polar mucus treatment suggests that mucus confer to *Palythoa* only physical protection against fouling. Only polar *Palythoa* with mucus treatment presented antifouling activity, it indicates that active metabolite is probably hydrophilic being not only in qualitative aspect, but also in quantitative because polar crude extracts of both *Palythoa* without mucus and mucus of *Palythoa* treatments did not show activity against fouling. The presence of antifouling potential in one polar extract also can be an indicative that *P. caribaeorum* from Arraial do Cabo produce PTX, because this toxin is the unique polar chemical defense described to *Palythoa* genus.

28P-24

ELECTROCHEMICAL SENSING FOR MARINE BACTERIAL BIOFILMS

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The ability to detect bacterial biofilm formation on marine platforms, heat exchangers and pipelines in-service is often limited in many cases by a lack of suitable techniques capable of sensing for the presence of these biofilm species. This investigation aims to detect for the formation and growth of marine bacterial biofilms using gold microelectrodes and electrochemical impedance spectroscopy (EIS). The presence of a bacterial biofilm modifies the electrochemical properties of the interface and the mass transport near the interface, which ultimately alters the interfacial impedance response.

EIS has been performed in 3.5 % NaCl solution, natural seawater, filtered natural seawater, artificial seawater and artificial seawater in the presence of *NCIMB 2021 Pseudoalteromonas sp. strain*, under continuously aerated conditions, in order to evaluate changes in the rate of the oxygen reduction reaction and the extent of the enhancement by enzymatic processes present within the biofilm. The EIS response has shown that the oxygen reduction reaction in seawater was significantly enhanced (by 1 and 2 orders of magnitude change in impedance) and the charge transfer resistance has been identified as a possible parameter to quantify the biofilm thickness in real-time.

Overall, using sampled natural seawater and artificial seawater in the presence of microorganisms, the microelectrodes are capable of monitoring for the initial biofilm development over a duration of 3 days under both static conditions and controlled hydrodynamic (laminar flow) conditions.

28P-25

ANTIFOULING ACTIVITY OF A SERIES OF NEW 9-METHYLENE SIDE-CHAIN MODIFIED RETINOIDS AGAINST CYPRIDS OF *BALANUS AMPHITRITE* AND SEVERAL MARINE BACTERIA

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Key words
Adhesion, *Balanus amphitrite*, fouling, *Halomonas marina*, modified side-chain retinoids. *Polaribacter irgensii, Pseudoaltermonas citrea, Pseudoalteromonas haloplanktis,* toxicity, *Vibrio aestuarianus, Vibrio pomeroyi,*

In the course of our program on new antifouling compounds, a series of new side-chain modified retinoids, relative to vitamin A, were synthesized and assayed for antifouling efficacy in laboratory assays towards bacteria and barnacles. Three of the compounds – the 9 methylene analog of vitamin A ((4E,8E)-3-methyl-9-(2,6,6-trimethylcyclohex-1-enyl)-7-methylenenona-2,4,8-trien-1-ol), the 9 methylene 13 demethyl analog of retinoic acid ((2E,4E,8E)-9-(é,-,--trimethylcyclohex-1-enyl)-7-methylenenona-2,4,8-trienoic acid) and the aromatic analog of vitamin A ((4E,8E)-3-methyl-7-methylene-9-phenylnona-2,4,8-trien-1-ol) – were active at inhibiting the growth of 5 strains of marine bacteria and the settlement of barnacle (*Balanus amphitrite*) cypris larvae at non-toxic concentrations. These compounds have the advantage of being synthesised and so easily accessible to paints manufacturers.

28P-26

NOVEL MERODITERPENOIDS AND DERIVATIVES FROM A MOROCCAN BROWN ALGA *CYSTOSEIRA BACCATA*

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As a part of our project aimed on searching for new antifouling compounds from seaweeds, we have investigated specimens of *Cystoseira baccata* collected off the Atlantic coast of Morocco. This species is commonly found along the NE atlantic shores from Ireland to Mauritania and to date only few studies have been published on the chemical composition of *C. baccata*. Specimens were harvested along the atlantic coasts of Morocco and yielded seven new meroditerpenoids (1-4) and derivatives (**5-7**), whose structures were elucidated mainly by 2D NMR and mass spectrometry. Surprisingly, for all these compounds, which possess a bicyclo[4.3.0]nonane ring system, a *trans* fusion of the bicyclic system was deduced by stereochemical studies (Noe, Mosher,...) even though such compounds isolated from *Cystoseira* species are known to have a typical *cis* orientation for the bridgehead methyls. The antifouling and antibacterial activities of compounds **1-5** and **7** were evaluated, as well as their toxicity towards non-target species. Compounds **4**, **5** and **7** showed some antifouling activities against microalgae, macroalgae settlement and mussel's phenoloxidase activity, while being non-toxic on larvae of sea urchins and oysters.

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28P-27

PHOTODEGRADATION STUDY OF THE ANTIFOULING AGENT DIURON IN AQUEOUS MEDIA UNDER SIMULATED SOLAR IRRADIATION

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Organic booster biocides were recently introduced as alternatives to organotin compounds in antifouling products, after restrictions imposed on the use of tributyltin (TBT). Replacement products are generally based on copper metal oxides and organic biocides. This ban introduced by the International Maritime Organization (IMO), Marine Environment Protection Committee (MEPC), has led to an increase in alternative "TBT free" coating products containing the biocides, Irgarol 1051, diuron, Sea-nine 211, dichlofluanid, chlorothalonil, zinc pyrithione, TCMS (2,3,3,6-tetrachloro-4-methylsulfonyl) pyridine, TCMTB [2-(thiocyanomethylthio) benzothiazole], and zineb. As a result important coastal concentrations of these biocides have been found in areas of high yachting activity, particularly in marinas and sportive harbors.

Among the different transformation processes (biotic and abiotic), photodegradation is an important factor influencing the fate of organic micropollutants in the field. The photochemical behavior of diuron was examined in aqueous media of different composition such as sea, river, lake and distilled water under simulated solar irradiation. In addition the effect of important constituents of natural water such as dissolved organic matter (DOM, isolated from Pamvotis Lake) and nitrate ions was also examined. It was found that photodegradation proceeds via a pseudo first-order reaction in all cases. Moreover, the toxicity of the water solution was also performed using the marine luminescent bacterium Vibrio fisheri. Our results indicated a toxicity increase of the irradiated solution showing that photoproducts of higher acute toxic effects were formed.

28P-28

DETERMINATION OF PYRIDINE-TRIPHENYLBORANE (PTPB) IN ANTIFOULING PAINTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Analytical method for the determination of pyridine-triphenylborane (PTPB) content in antifouling paints by high-performance liquid chromatography (HPLC) has been developed. PTPB in sample was dissolved with dimethyl formamide (DMF) by supersonic wave instrument. HPLC was carried out on a L-column C8 column (150 mm * 4.6 mm I.D.) using acetonitrile/water/IPC-TBA-P (0.5M tetra-n-butylammonium phosphate) (65:34:1) as mobile phase with UV detection at 220 nm. The recoveries of PTPB from copper-free antifouling paints were 90.0 - 100.2 % with high accuracy. As the results, the proposed method is suitable for the routine analysis of PTPB content in antifouling paints.
Reference: K.Takahashi, E.Yoshikawa, M.Akiyama, K.Kitaori and S.Masuoka: J.Jpn.Soc.Colour Mater.,78(2),pp50-57(2005).

28P-29

ANALYTICAL METHOD OF PYRIDINE-TRIPHENYLBORANE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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In order to establish the analytical method of pyridine-triphenylborane (PTPB) released from antifouling paint, analytical condition of a high performance liquid chromatography (HPLC) was investigated using a reversed-phase column and mobile phase, containing 0.5 mol/l tetra-n-butyl ammonium phosphate (TBA-P) as ion-pair reagent. Five resources of TBA-P were tested and sharp peak in chromatogram was obtained by one of the resources, but, not by other four resources. Eight kinds of reversed-phase column for HPLC were tested and clear and sharp peak was obtained by seven columns except one column. As a result, the analysis method showed a strong selectivity to the resources of ion-pair reagent TBA-P, even if the cause was not clear.

28P-30

TRIPHENYLBORANE-PYRIDINE ANTIFOULING AGENT DEGRADES BY METAL IONS?

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Triphenylborane-pyridine (TPBP) is one of the antifouling compounds used in Japan, but there is little information available on its fate and ecotoxicity in aquatic environments. The purpose of this study was to evaluate whether TPBP can degrade by ubiquitous metal ions in aquatic environments. The concentrations of TPBP and boric acid which should be produced as a final degradation product were determined by HPLC. TPBP solution was prepared at the concentration of 1mg/L in 20 mM sodium acetate buffer at pH 8 and was placed in a screw capped vial at 25°C in the dark. TPBP was completely degraded after 24 hours in the presence of 50 mg/L of copper ion and was not degraded at all by other metal ions such as zinc, manganese and iron. Copper chloride, nitrate or sulfate as copper salts was added to the TPBP solution at different copper ion concentrations (5, 10 and 50 mg/L). No TPBP was detected after 24 hours even at 5 mg/L of any copper salts and this indicated complete degradation of TPBP. The concentrations of boric acid produced were dependent on the copper ion concentrations added and copper salt species. The results suggested a production of unknown organic boron compounds. TPBP degradation was examined in the presence of low copper ion concentration at an additional concentration of 2 μg/L as copper chloride. The TPBP concentration in the control and the copper treatment decreased to 65% and 48% of the initial concentration after two months, respectively. The difference in TPBP concentration in triplicate experiments was statistically significant. It was suggested that TPBP could degrade by environmentally relevant concentration of copper to produce unknown organic boron compounds. There is a need to identify these metabolites and establish their ecotoxicity in a future study.

28P-31

SIMULTANEOUS DETERMINATION OF PYRIDINE-TRIPHENYLBORON (TPBP) ANTI-FOULING AGENT AND ITS ESTIMATED DEGRADATION PRODUCTS BY CAPILLARY ZONE ELECTROPHORESIS

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A commercial organoborane compound, pyridine-triphenylboron (TPBP), is often applied to ship hulls as an anti-fouling agent. We have developed a procedure using capillary zone electrophoresis (CZE) with direct UV detection for the simultaneous determination of TPBP and its estimated degradation products such as diphenylborinic acid (DPB) and phenylboronic acid (MPB). The stock solutions (1000 mg/L) were prepared in acetonitrile and 1% (v/v) pyridine was added to the stock solutions to stabilize TPBP. The following optimum conditions were established: a background electrolyte (BGE), 20 mmol/L sodium tetraborate solution adjusted to pH 10 with 1 mol/L sodium hydroxide; detection wavelength, 200 nm; vacuum (5 in. Hg) injection period of sample, 4 s (*ca.* 84 nL); applied voltage, 15 kV with the sample inlet side as the anode. By using the proposed procedure, limits of detection (LODs) for TPBP, DPB, and MPB were 25, 30, and 50 μg/L, respectively, at a signal-to-noise ratio of three. At concentrations of 0.5 mg/L, values of the relative standard deviation (RSD, n=6) of peak area: 4.1, 4.1, 4.7%, peak height: 3.6, 3.2, 1.7%, and migration time: 1.1, 1.1, 1.0%, for TPBP, DPB, and MPB, respectively, were obtained. The analytes were detected within 14 min. Simple photodegradation experiments are in progress to verify the usefulness of the proposed method for further TPBP degradation investigations.

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28P-32

SIMULTANEOUS DETERMINATION OF ALTERNATIVE COMPOUNDS IN ENVIRONMENTAL SAMPLES

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A rapid and simple high-performance liquid chromatographic method using solid phase extraction was developed for the determination of antifouling alternative biocides (Sea-Nine 211, Diuron, Irgarol 1051) and its degradation product M1. The analytes were extracted with Excelpak SPE-GLF solid phase extraction from water samples. After drying, the analytes were eluted with methanol and then were measured by LC/MS-MS. the analytes in sediment were extracted by acetonitrile. After the removal of residue by centrifugation, the supernatant was diluted with distilled water. In the biological samples, lipid component was removed by addition of zinc acetate and celite. The analytes were then extracted with dichloromethane, were concentrated and were measured by LC/MS-MS. The recoveries of 3 antifouling biocides and M1 in water sample were the range 68 – 97 % and the detection limits of water samples corresponding to S/N=3 were in the range of 0.3 – 1.9 ng L^{-1} in water samples. The recoveries of these compounds in sediment and biological samples were in the range of 65 – 103 % and 60 – 99 %, respectively. Detection limit of these antifouling biocides from sediment and biological samples were in the range of 0.04 - 0.18 ug kg^{-1}dry and 0.24 - 1.1 ug kg^{-1} respectively. In this presentation, analytical methods of pyrithiones and pyridine triphenyl boran are also reported.

28P-33

ANALYSIS OF ZINC PYRITHIONE AND ITS DEGRADATION COMPOUNDS RESULTED FROM PHOTOLYSIS AND HYDROLYSIS PROCESS

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Zinc pyrithione ($Zn(PT)_2$) is one of the representative booster biocides among recent antifouling biocides. Hydrolysis and photolysis of $Zn(PT)_2$ in aqueous solutions were investigated under controlled environmental conditions as a laboratory fate study. Under dark conditions, 2,2'-dithio-bis-pyridine-N-oxide ($(PT)_2$) was detected by high performance liquid chromatography (HPLC) as a hydrolysis product from $Zn(PT)_2$. Under dark conditions, $(PT)_2$ produced by hydrolysis. The half life of $Zn(PT)_2$ was found to be above 20 days. Photolysis experiments of $Zn(PT)_2$ were carried out by air-mass-filtered 500 W xenon lamp simulating natural sunlight. Photo degradation process was exhibited by 4-step, and $Zn(PT)_2$ and its degradation compounds were measured by HPLC at each stage of irradiation. At the first step,$(PT)_2$ was formed. Reduction then occurred to form pyridine/pyrithione mixed disulfide 2,2'-dithiobispyridine mono-N-oxide (PT-PS) followed by production of 2,2'-dithio-bis-pyridine ($(PS)_2$). At the third step, $(PS)_2$ changed to 2-pyridine sulfuric acid (PSA). At the final step, further transformation of PSA was observed, however its degradation compound could not be identified.

28P-34

COST-BENEFIT ANALYSIS FOR UNDERWATER HULL COATINGS AND MAINTENANCE

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Various approaches, or combination of approaches, can be used to mitigate underwater hull fouling. Minimizing fouling reduces ship operating costs by decreasing power demand. Common mitigation approaches include antifouling coatings, fouling-release coatings, in-water hull cleaning and dry-dock maintenance. Each approach is associated with particular capital, operational and maintenance costs. The U.S. Navy continually researches emerging underwater hull coating and maintenance technologies for greater cost savings and mission benefits. A new cost-benefit analysis (CBA) tool evaluates potential benefits to strategically guide research investments. Key quantifiable CBA inputs incorporate data on ship performance (e.g., powering, coating life), in-water maintenance (e.g., frequency, type) and dry-dock maintenance (e.g., frequency, coating removal, coating application). As time and data availability allow, less easily quantifiable parameters (e.g., environmental impacts, mechanical wear-and-tear, development costs) will be included. Results for several hypothetical approaches will be discussed. This work is funded by the Office of Naval Research.

28P-35

ECOTOXICITY ASSESSMENT FOR ZINC- & COPPER PYRITHIONE

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Zinc- and copper pyrithione (ZPT and CuPT, respectively) are co-biocides used in antifouling paints as replacement for TBT. TBT was banned under the International Maritime Organization's (IMO) Anti-fouling Substances treaty (AFS) because of its adverse environmental effects. It is thus necessary to investigate the environmental effects of substitute substances rigorously. This paper will discuss the acute and chronic aquatic toxicity data for the pyrithiones, the acute aquatic toxicity data for the major degradation products of the pyrithiones and the special environmental conditions required to carryout standard laboratory testing of the pryithiones. ZPT and CuPT chronic toxicity has been evaluated in a wide variety of fish, invertebrate, unicellular algae (fresh and saltwater species), aquatic plants, terrestrial plants e.g. rice, and estuary-marsh plants species. The results indicate an acute to chronic ratio consistently at 1.0 indicating low risk from chronic exposure. The bioaccumulation potential for the pyrithiones was evaluated in mollusk and fish. The BCF values observed in oysters were less than 11.0 for ZPT and less than 100 for CuPT. The principle degradant PSA was considered practically non-toxic to fish and practically non-toxic to freshwater algae in a multiple generation study. The paper goes on to establish the Predicted no effect concentration (PNEC) to be used in robust risk assessment methodologies used to demonstrate acceptable risk quotients thus complying with the intent of the IMO AFS treaty.

28P-36

REGISTRATION STRATEGY FOR A NEW ANTIFOULING SUBSTANCE; MEDETOMIDINE AS AN EXAMPLE

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There is a strong focus in finding new antifouling substances. However, the big obstacle is not to find new substances but how to proceed those towards a possible commercial success. For many years, the ideal has been to find a new substance that technically functions as well as tributyltin did, but without the adverse toxicological effects. It might be that such a substance is impossible to find. Instead a long developmental work has to be performed to include, optimize and combine new and old substances with paint formulations.
The Biocide Product Directive (BPD) is the European registration directive. It is based on two risk assessments regarding ecotoxicology and human health. A new candidate substance needs to be assessed by the BPD framework.
There are different conceptual routes. Biological specificity making it possible to use low amounts of a substance or fast degradation which will allow for a substantial release though it will degrade before causing harm. Medetomidine is an example of the former.
Medetomidine is a substance that has a specific mode of action. The effective concentration is by far less than the lethal. This means that medetomidine has a favorable starting point regarding the ecotoxicological assessment. Its high efficacy also implies that only a small amount needs to be incorporated into a paint and the leakage rate can be kept low. When using the MAMPEC model, medetomidine get favorable predicted values even though it does not degrade as quickly as some other antifoulant substances.
The main difficulties and costs are related towards mammalian toxicology testing. Medetomidine had the advantage that it has been registred before as a pharmaceutical and most of the mammalian data already exist conclusing no mutagenecity or severe toxicological side effects. Also, its pharmacokinetic and mammalian metabolism is well studied.
We will present how different parameters affect the overall risk assessments using medetomidine as an example. We will point towards a rational route in how to pick among possible new substances with a reasonable financial effort.

28P-37

KINETIC MODEL FOR THE LEACHING OF ANTIFOULING SUBSTANCE FROM SELF POLISHING PAINTS

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A simple mathematical model has been developed to simulate the leaching behavior of self-polishing anti-fouling paint, taking formation of skeleton layer, in which the antifouling substance is leached out and only paint resin structure remains, into account. The model deals with four phenomena relating to the leaching. They are reaction between water and the antifouling substance which is dispersed in the paint film, diffusion of antifouling substance in boundary layer in the water phase, that in the skeleton layer in paint film, and polishing of the paint resin. The leaching rate measurement data, which are obtained by the rotating cylinder test under the condition similar to ASTM/ISO protocol, show that the rate is very fast at the beginning and gradually decreases to a steady state. This phenomenon can be explained as follows, based on the model. At the very beginning of the leaching, the rate is controlled either dissolution reaction rate of antifouling agent or mass transfer in the boundary layer in water phase. Once the polishing rate becomes slower than above two processes, the skeleton layer is developed. At that case the diffusion of leached antifouling substance in the skeleton layer controls the overall leaching rate. Finally, the leaching rate reached a steady state, when the diffusion rate in the skeleton layer and the polishing rate are balanced. Numerical calculation based on the model with the best fit parameters, has successfully simulated long term leaching rate of cuprous oxide from self-polishing paints. It indicates that the model can predict long term leaching rate of self-polishing anti-fouling paint from the limited experimental data.

28P-38

RAPID LABORATORY SCREENING ASSAYS FOR CHARACTERIZING THE ANTIFOULING/FOULING-RELEASE PERFORMANCE OF MARINE COATINGS

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A suite of laboratory screening assays have been developed and implemented at NDSU to rapidly characterize the antifouling and fouling-release performance of marine coatings developed using a combinatorial approach. The screening workflow employs marine bacteria, microalgae and adult barnacles to efficiently and effectively evaluate coating libraries prepared in array formats. The workflow has been automated by using a variety of commercially available and custom-built robotic platforms to enhance the accuracy, reproducibility and overall throughput of the screening workflow. Several correlations have been established between the rapid laboratory screening assays and ocean immersion testing, enabling down-selection and identification of promising coating compositions year-round at NDSU. Details of the rapid laboratory screening assays, examples of coating analysis, and correlations to ocean immersion testing will be presented.

28P-39

AN ASSESSMENT ON THE PERFORMANCE OF THE CAPSAICINOIDS' EXPELLERS IN ANTIFOULING PAINTS FROM THE VIEW-POINT OF ENVIRONMENTAL ECONOMIC

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A noval expeller used in antifouling paints coated on ship hull should simultaneously meet the generally acknowledged criteria as follows;
(1) excellent antifouling performance with relatively longer serving period (lifetime),
(2) cost effective in synthesis (preparation) of the expeller as well as the formulated paints
(3) environmental friendly, Neither the expeller itself, nor its degraded products causes the harmful impact toward the ocean environment as well as ecology.

To combine and consider the technological and environmental economics as a whole, the advantages and superiority of capsaicinoids expellers over the other biocides commonly used in the antifouling paints, such as insecticide and pesticides were presented and discussed.

28P-40

DEVELOPMENT OF PHOTOGRAPHIC STANDARDS FOR THE SURFACE PREPARATION FOR THE IMPLEMENTATION OF PSPC

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In 2006, the International Maritime Organization (IMO) developed a mandatory "Performance Standard for Protective Coatings for Dedicated Seawater Ballast Tanks in All Types of Ships and Double-sided Skin Spaces of Bulk Carriers" (the PSPC). It requires surface preparation for protective coating to comply with grade "Sa2$^1/_2$" or "St3" as defined in ISO8501-1, which provides photographic examples of these grades. These examples are rusted flat steel surfaces without shop primer and do not represent many of the actual conditions in the shipbuilding process.
In response to requests by the industries concerned, JSTRA has conducted a research and compiled a new photographic collection of surface preparation examples that covers typical shipbuilding coating process for reference when implementing the PSPC. Where the PSPC provides a specific preparation grade, the collection provides samples showing conditions: i) prior to surface treatment; ii) after surface treatment that would comply with the requirement in the PSPC; and iii) after surface treatment of a higher grade than that of the PSPC. For the development and selection of the photographs, JSTRA has established an Expert Group composed of members from various fields: coating manufacturers, shipyards, shipowners, institutes, universities, and classification societies, including coating inspectors certified at NACE Coating Inspector Level 2 and FROSIO Inspector Level III. The selected photographs have also been verified by the "Research Committee on the Relationship between Coating Quality and Hull Manufacturing Quality" of the Japan Society of Naval Architects and Ocean Engineers.

29A-1: PLENARY LECTURE 2

29A-1-1 (Plenary)

CORROSION PROTECTION AND OTHER COATING RELATED ASPECTS FACING SHIPOWNERS TODAY

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Chemical engineer by education fresh out of school in Sweden in 1973 I started as a young man working for a coating supplier; Carboline Co.'s joint venture with Alcro. I was soon drawn out from the office into the shipyard environments. First involved with cargo tank lining repairs, then same task on new buildings in the Gothenburg region in Sweden – a major ship building areas in the mid 1970ies. I was also involved with coating of nuclear power plants in Sweden as well in the 1970ies.

In 1975 the work brought me to England and further, for Carboline's more worldwide network of organizations. First, to supervise tank-lining repairs on two ships owned by Stolt-Nielsen, dry docked in Cardiff in Wales, UK. Next year I was asked to take over as tank lining supervisor for Carboline Co. on several new buildings for Stolt-Nielsen in France, and the after that in Korea also for Stolt-Nielsen.

In the years following that I was working in Yugoslavia (now Croatia), Germany, Korea (several times), Singapore, Portugal, Norway, Finland, Sweden and Denmark, etc., mainly related to marine corrosion protection. I was assisting on some 46 new buildings world wide during that period, and in fact working also for shipyard and coating contractor during a period in a dual role. Hence, I had by then gained experience from three of the four sides in the marine industry; the paint maker, the yard and the contractor, and had developed a good overall understanding from these various positions.

In 1989 a ship owner in Sweden approach me for a job on their new construction site in Korea, and I accepted – thus getting experience also from the fourth party to the marine industry as it pertains to corrosion, coatings and fouling issues.

Then in 1991 Stolt-Nielsen approached wanting help with ballast tank coating management, and also to find the cause for some cargo tank coating problems they had.

I have progressed within Stolt-Nielsen to the position of Manager of Materials Protection. Basically functioning as an in-house consultant for the new building department, the ships, the ship managers, and for operations and chartering with regards to what can be loaded from a resistance point of view, in different lining materials, including stainless steels. I also assist the container and shore storage tank divisions at Stolt-Nielsen.

I have given a number of presentations, and written several articles, on marine corrosion and corrosion control over the years.

During the past 10 years I have also been involved with Tanker Structure Cooperative Forum, assisting in development of guidelines, and Intertanko a Non Governmental Organization (NGO) of independent tanker owners at the International Maritime Organization (IMO) supporting them on coating and corrosion related issues.

I recent years I have been deeply involved with energy conservation at Stolt-Nielsen, which is in fact directly related to CO_2 emissions, something very important these days.

29A-2: ACCELERATED LOW WATER CORROSION: CAUSES, CONSEQUENCES AND REMEDIAL ACTION (ORGANIZED BY IWONA BEECH AND SHEELAGH CAMPBELL)

29A-2-1

WHAT IS ACCELERATED LOW WATER CORROSION?

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There has been much concern in the maritime sector over the last few years about a recently recognised and particularly aggressive form of localised corrosion, referred to as Accelerated Low Water Corrosion (ALWC). It is believed to be a form of microbiologically induced corrosion (MIC) and can occur on unprotected, steel, maritime structures, typically at or around the low water level. This complex and high profile problem has been identified in many countries throughout the world.

If ignored, the consequences of ALWC can be serious, requiring extensive, unbudgeted and costly repair or replacement works at an unexpectedly early stage in the life of a structure, in addition to having serious implications for its safe operation. Consequently, ALWC is of significant interest to infrastructure owners, operators and their advisors, maritime engineers and asset & maintenance managers in ports, harbours and other marine locations.

This presentation will provide an understanding of (1) the background to the emergence of the ALWC phenomenon, (2) key features associated with its identification, (3) proposed mechanisms by which it acts and (4) methods currently available to deal with it both on new build and existing ALWC affected structures. In addition, it will address the uncertainties and confusion often associated with the problem and will cover ongoing technical research and what we need to know more about to more fully understand this problem and its management.

29A-2-2

INHIBITION OF BIOCORROSION IN SEAWATER VIA SURFACE FUNCTIONALIZATION OF METALS AND ALLOYS

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As a significant portion of the corrosion damage in seawater is due to anaerobic corrosion influenced by sulfate-reducing bacteria (SRB), attempts are being made to develop novel and environmentally-friendly surface functionalization techniques to endow the metal and alloy substrates with desirable anticorrosion and antibacterial properties. Multilayer coatings of Ti oxide/butoxide were built up on the surface of stainless steel via a layer-by-layer sol-gel deposition process. Not only does the passivity of the Ti oxide/butoxide multilayer coatings remain stable under the harsh environment of *D. desulfuricans* inoculated seawater, the passivity is slightly enhanced due to the deposition of calcium and phosphorous compounds associated with apatite. Antibacterial and anticorrosion properties were conferred on the Cu-Ni alloy substrate via surface-initiated graft polymerization of 4-vinylpyridine (4VP) in the presence of a trichlorosilane coupling agent and benzoyl peroxide, followed by *N*-alkylation of pyridinium nitrogen of the poly(4VP)-grafted surface to produce a high concentration of quaternary ammonium groups. The so-functionalized alloy surface is effective in inhibiting bacterial adhesion and biofilm formation, and thus in minimizing biocorrosion by *D. desulfuricans*. Functional polymer brushes were also graft-polymerized from stainless steel substrates via surface-initiated atom transfer radical polymerization (ATRP) of (2-dimethylamino)-ethyl methacrylate (DMAEMA). The tertiary amino groups of covalently-bound poly(DMAEMA) brushes were further quaternized with benzyl halide to produce the bactericidal functionality. Alternatively, covalent coupling of viologen moieties to the tertiary amino groups of poly(DMAEMA) brushes on the substrate surface resulted in an increase in surface concentration of quaternary ammonium groups. The bactericidal polymer brushers were found to substantially enhance the corrosion resistance of stainless steel coupons to biocorrosion by *D. desulfuricans* in seawater.

29A-2-3

ALLOY PLATING AND BIOFILM FORMATION

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Microbial growth and biofilm formation on practical materials can be one of the main causes for microbial corrosion. The authors have investigated some kinds of alloy films produced on some materials surfaces through the combination of heat treatment and coating processes and have been interested in their inhibitory effect against the formation of biofilm on the surfaces. In this study, the process of the alloy film formation and the surface structures investigated by XRD and SEM were introduced and the correlation between the surface structures, phases etc. and the antimicrobial effect was discussed in terms of anticorrosion in estuarine and low water environment.

29A-2-4

THE STUDY OF RELATIONSHIP BETWEEN DIVERSITY OF BACTERIAL BIOFILMS AND MARINE CORROSION OF CARBON STEEL IN HARBOUR ENVIRONMENTS

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Here is reported investigation aimed to elucidate relationship between accelerated marine corrosion of carbon steel and community structure of bacterial biofilms associated with corrosion products. The study was conducted in three different French coastal regions (English Channel, Atlantic Ocean and Mediterranean Sea). SEM-EDX, ICP-AES, XRD and micro-Raman spectrometry revealed stratification of corrosion products based on iron oxidation state, regardless of geographical location. Molecular biology techniques and standard cultivation methods demonstrated that bacteria in corrosion products were abundant and highly diverse. Moreover, corrosion products contained many species of diatoms and multicellular organisms. Characterisation of cultivable bacteria and culture-independent molecular fingerprinting method (CE-SSCP) revealed more than 20 bacterial ribotypes in the deposits. Molecular detection of sulphate-reducing bacteria (SRB) was carried out using dissimilatory sulfite-reductase gene (dsrAB) and 16S rDNA probes specific for six SRB sub-groups. The results of microbiological analysis demonstrated that the composition and diversity of bacterial populations associated with corrosion products are determined by the chemistry of different regions, i.e. steel surface, corrosion product surface and green rust / oxy-hydroxide interface, without clear stratification. Moreover, the study confirmed that, irrespective of the site investigated, bacterial species detected in corrosion products differed from the ones present in bulk water, thus emphasising the importance of biofilm sampling when investigating corrosion failures. In view of the obtained results, the role of green rust as sulphate reservoir, the localised acidification induced by biogenically produced sulphur, the plausible role of extracellular polymeric substances in connecting bacterial cells and mineral corrosion products and the impact of multicellular organisms on the corrosion product stability are discussed.

29A-2-5

EARLY TEST RESULTS AND INTERPRETATIONS FOR CORROSION OF VERTICAL STEEL STRIPS EXPOSED IN THE MARINE TIDAL ZONE

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This paper will present the results of exposures up to 2 years of the corrosion of vertical mild steel strips exposed at various tidal exposure locations on the Australian Eastern seaboard. It is shown that Accelerated Low Water Corrosion occurs at some locations already within this time frame but not at other locations. For these, high water corrosion is much more significant, consistent with the classical results reported by Larrabee, LaQue and others both for steel piles and for coupons electrically connected vertically through the tidal zone. The Australian results for the strips are interpreted using (i) data for water quality measured at the various test sites, (ii) an extension of the model for longer term corrosion proposed previously by the authors and (iii) specific observations of orange blooms also found at one Australian site for isolated mild steel coupons. The latter indicates that the mechanisms driving the ALWC phenomenon are not confined just to steel sheet piling. Although longer-term corrosion observations are still to come, it is proposed that the early ALWC phenomenon observed in the Australian tests is consistent with the influence of microbiological influences, such as SRB, as has been proposed previously by various investigators. However, such influences must be governed by the availability of nutrients and the local environmental conditions. This is consistent also with microbiological influences having been implicated in the very early (0-3 month) corrosion of steels in seawater, as shown in a number of studies. Importantly, the mere presence of SRB at the corroding surface is not sufficient to claim them as the causative agent.

29A-2-6

ACCELERATED LOW WATER CORROSION AND ITS EFFECTS ON COASTAL AND HARBOUR DEFENCES

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Accelerated low water corrosion (ALWC) is an aggressive form of localised corrosion found on steel structures, at or around the low water level, in marine tidal systems. The typical corrosion rate of steel in marine environments is ca. 0.05 mm y-1, but in the presence of ALWC unusually high rates of metal wastage of between 0.3 and 1 mm y-1 can be exhibited. Normally, only a small percentage of the surface area of the unprotected steel in the low water zone is affected. Whilst ALWC attack is random both within and between installations, the resultant damage conforms to a specific pattern, similar for particular pile geometries, irrespective of the geographic location of the installation. ALWC is considered to be a form of microbially-influenced corrosion associated with the presence of sulphate-reducing and sulphur-oxidising bacteria in biofilms present on the steel. Extensive remedial work at an early stage in the life of an affected structure is often required when ALWC is present.
This paper describes investigations to elucidate the causes of ALWC on steel piling in English harbours. Inspection of metal surfaces revealed features characteristic of ALWC e.g. the presence of poorly adherent, thick corrosion products of varying morphology randomly located on sections of structures at the low water level. Removal of these layers exposed bright grey pitted surfaces. Representative samples of corrosion products from surfaces identified as experiencing ALWC attack during site inspections, and water/sludge samples from different locations within the harbours were subjected to microbiological, chemical and microscopy analysis. Linear polarisation resistance measurements confirmed that corrosion rates similar to those reported for ALWC cases in the field could be obtained under laboratory conditions: enhanced pitting was demonstrated under biofilms. The necessity of using biofilm bacteria recovered from surfaces of corroding metallic materials and the need to carry out electrochemical testing of corrosion using conditions as close to the field environments as possible are shown to be crucial to identifying MIC as the cause of material degradation.

29A-3: MARINE CORROSION AND ANTI-CORROSION

29A-3-1

TUBERCLES AND RUST LAYERS OBSERVED ON MILD STEEL EXPOSED IN SEAWATER

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This paper deals with the corrosion product and oxides that have been formed and analysed when steel corrodes in tidal waters. The observations have been made at marine immersion sites along the eastern Australian seaboard. The oxides have been noted to morph into a number of different forms and this is known to depend on how long and where in the tidal zone the steel is immersed. At the top of the tide, pyramidal nodules develop. In the upper tidal regions thick layers of corrosion products form that enclose both horizontal voids and vertical cracks. Analysis of the voids and cracks shows them to be lined with calcium and magnesium products. Mid tide regions are usually covered with biomass that limits oxygen diffusion, and to a degree thick corrosion layers. At the low water level orange nodules or tubercles are observed. Tubercles have been reported previously in relation to fresh water piping and are known to harbour bacterial consortia that impact on corrosion of metals. Accelerated low water corrosion (ALWC) is a worldwide phenomenon in ports with its hallmark being areas of orange growths near and below the low water mark. It is suggested that the orange growths observed at various immersion sites and those reported in fresh water piping are a result the similar bacterial activity. As well as general observations, this paper details the metamorphosis of horizontal inter-layer cracks that expand into voids and nodules in the upper tidal zone and into tubercles in the lower tidal zone. The changes are documented by a sequence of cross-sectional images taken using a scanning electron microscope as well as X-ray diffraction analyses of the various corrosion product components.

29A-3-2

CORROSION CONTROL TECHNIQUE FOR MARINE AND OFFSHORE STRUCTURES USING RADIATION INDUCED SURFACE ACTIVATION

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This study examined a corrosion control technique for corrosion-resistant material or stainless steel that employs an effect of Radiation Induced Surface Activation (RISA). The experimental results revealed that; (1) the mechanism behind the corrosion control was clarified through tests that measured the amount of dissolved oxygen and iron in the solution; (2) the corrosion control technique was confirmed to be useful even in actual sea water, which is a more severe environment for corrosion control than simple salt water due to biological effects. The corrosion control technique for corrosion -resistant material using RISA in sea water has therefore significant potential for practical applications in naval architecture and marine structures.

29A-3-3

THE INFLUENCE OF MARINE BIOFILMS ON CORROSION

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Ennoblement of open circuit potential (OCP) and sulfide production due to sulfate-reducing bacteria (SRB) are both observed in the presence of marine biofilms. In general, alloys that undergo ennoblement are not vulnerable to sulfide derivitization and conversely, alloys that are subject to sulfide derivitization do not become ennobled. A universally defined mechanism of potential ennoblement in marine environments has not been established. Ennoblement in marine waters has been ascribed to depolarization of the oxygen reduction reaction due to organometallic catalysis, acidification of the electrode surface, the combined effects of elevated hydrogen peroxide and decreased pH and the production of passivating siderophores. Theoretically, potential ennoblement should increase the probability for pitting and crevice corrosion initiation and propagation, especially for alloys with pitting potentials within 300 mV of the OCP. However, attempts to relate ennoblement to increased localized corrosion have been inconsistent. Extent of ennoblement varies among locations and the extent of ennoblement for a particular material cannot be used to predict an increased likelihood of localized corrosion. Thermodynamic models can be used to predict the susceptibility of carbon steel and copper alloys to sulfide derivitization. However, there is some controversy as to the susceptibility of low and medium grade stainless steels. Laboratory experiments designed to provide data on susceptibility to sulfide derivitization have produced conflicting results because of the following: 1) laboratory media can contain anions that inhibit localized corrosion, 2) laboratory media can contain yeast extract that interferes with electrochemical measurements, and 3) deaeration procedures can produce environments that are not conducive for the growth of SRB. The aim of this paper is to review ennoblement and sulfate reduction in the marine environment and present reasons for the persistent confusion as to whether either phenomenon is important in the corrosion of specific materials.

29A-3-4

PHYSICOCHEMICAL SURFACE PROPERTIES OF SRB AND POLARIZATION INFLUENCE ON ITS BIOFILM FORMATION

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SRB is one kind of important corrosive bacteria. Its physicochemical properties were investigated in this paper. Microbial adhesive to solvents (MATS) experiment showed that the SRB cell used in this workr was hydrophilic and electron donor. Zeta potential indicated that SRB was highly negative charge. Bacteria attachment influenced by cathodic polarization was studied with epifluoresence microscopy, using 907A as working electrode. Results showed that when potential was in the range of -800 to -1000 mV (SCE), the adhering cell number on working electrode increased with the polarization potential decrease. When -1100mv was applied, the cell number reached to greatest. After this, the cell number on working electrode decreased sharply.
Key words: 907A steel; biofilm; sulfate reducing bacteria (SRB); bacterial surface properties;cathodic polarization

29A-3-5

MICROBIAL INFLUENCED CORROSION (MIC): WHY IS BIOLOGY IMPORTANT AND HOW CAN ENM HELP TO BETTER UNDERSTAND AND DETECT THE UNDERLYING BIOLOGICAL AND ELECTROCHEMICAL PROCESSES?

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MIC is known to be a dangerous process in enclosed and inaccessible areas such as ballast tanks, settling tanks and cargo tanks. Its rapid and unpredictable occurrence often leads to extremely fast local corrosion. MIC has already been studied for decades but detailed mechanisms of biocorrosion are still poorly understood. Central point in our biocorrosion research is to obtain fundamental understanding of interactions between biological and electrochemical processes at material/water/oil interfaces and identify critical steps that offer best potential to detect and monitor MIC and improve MIC control.
In this paper we provide an overview of commercially available test kits and assays for the rapid identification of MIC and we discuss other more advanced concepts for MIC detection. Based on this, we are now focusing our biocorrosion research on using electrochemical noise measurements (ENM) for detecting and monitoring MIC. ENM involves the measurement of self-generated potential and current signals in natural corrosion processes, relating the outcomes to the characteristics of specific corrosion mechanisms. Initial laboratory experiments with a range of MIC species will be presented, showing substantial differences in noise signal between MIC and non-MIC corrosion. This has promoted further work to reveal detection and discrimination of different types of MIC caused by aerobic and anaerobic species.

29A-3-6

CORROSION OF STEELS INFLUENCED BY AEROBIC AND ANAEROBIC BIOFILMS IN SEAWATER ENVIRONMENT

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Biofilm are microbial community of forerunner prior to biofouling on marine steel structures. It has been found that marine biofilm formed on the steel structures can accelerate corrosion. Marine biofilm can be divided into aerobic and anaerobic biofilms, which affect the corrosion of steel by different processes. In the current presentation, we introduced our work on marine aerobic and anaerobic biofilms on the influences of stainless steel and carbon steel. We studied the electrochemical activity of aerobic and anaerobic biofilm on the steels. Aerobic biofilm can accelerate corrosion by promoting oxygen reduction rate. A kind of Anaerobic biofilm, sulfate-reducing bacteria strain was cultured from the anaerobic biofilm on carbon-steel immersed in seawater. The corrosion behavior of anaerobic biofilm was also carried out. The important role of marine biofilm on corrosion was discussed.
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29A-3-7

ADVANCED MASS SPECTROMETRIC METHODS AS TOOLS TO INVESTIGATE BIOCORROSION

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Surface-associated microbial growth, i.e. a biofilm, on metal surfaces is known to instigate biocorrosion, or microbially-induced corrosion (MIC). The mechanism of MIC, including its dependence on microbial metabolic activities, species composition and environmental parameters, is poorly understood. For this reason, it has been extremely difficult to design reliable MIC detection and treatment strategies. While a direct interaction between microbes and metal surface may be involved in some cases, there is strong evidence that MIC is essentially chemical and/or biochemical in nature and highly dependent upon the chemical composition of extracellular polymeric substances (EPS) and their interactions with the metal surface. Furthermore, the distribution of biofilm over the surface, and bacterial activity within the biofilm, strongly influence the morphology of localized pitting corrosion. Mass spectrometry (MS) is the most powerful technique available to characterize complex chemical systems, such as biofilms. Both inorganic and organic compounds can be detected with high sensitivity and identified. With modern ionization methods, large biochemical molecules, such as proteins, can also be studied. MS methods for chemical mapping of lipids, peptides and proteins in biological materials such as tissue have recently been developed and, increasingly, MS mapping is possible in the ambient environment. For these reasons, MS is ready to deliver detailed chemical characterizations of biofilms, with high spatial resolution. Here, we will discuss recent MS developments emphasizing their potential application to the study of biocorrosion phenomena and present examples from our own investigations on characterization of organic and inorganic components of EPS recovered from biofilms of the sulfate-reducing bacterium *Desulfovibrio alaskensis*.

29A-3-8

BIOFILM-SPECIFIC MARINE CORROSION OF 70/30 CU-NI ALLOY

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In marine environments, microbial biofilms form on all surfaces exposed to environmental conditions conducive for microbial colonisation and growth. When present on metallic surfaces, biofilms can cause deterioration or protection of the underlying substratum. A number of mechanisms have been identified reflecting the variety of physiological activities carried out by biofilm micro-organisms that promote the establishment of localised chemical gradients at the metal surface. It is recognised that crystalline and amorphous minerals can be formed as a result of interactions between biofilm matrix macromolecules with e.g. inorganic cations. The accumulation of metal ions in different oxidation states within biofilm matrix can, in turn, promote electron transfer processes, which would influence anodic and/or cathodic reactions; the ultimate consequence being loss of metal from discrete locations on the surface. The laboratory investigation of biocorrosion of 70/30 Cu-Ni alloy conditioned in fresh water to encourage the formation of a protective oxide layer, was carried out using complex biofilm communities grown for 5 months under stagnant culture system in natural seawater at 24°C. The aim and the challenging aspect of the study were to reproduce the morphology of the pitting attack observed in the field. The data revealed that the level of pitting corrosion was significantly increased in the presence of biofilms obtained by exposing Cu-Ni specimens to sessile population removed from the surface of Cu-Ni failure compared with biofilms obtained by exposing Cu-Ni specimens to planktonic population recovered from the site. Both the natural populations used comprised sulphide-generating bacteria, including sulphate-reducing bacteria, as well as acid- and slime-producing bacteria. In contrast to the planktonic population, bacteria present in the sessile population harboured Cu-resistant species. The results are discussed with emphasis placed on the importance of appreciating habitat-specific microbial metabolism when attempting to mimic, in the laboratory, the morphology and the rate of corrosion attack experienced in the field.

29A-3-9

ENHANCING THE PROTECTION OF MARINE STRUCTURES FROM ALWC BY UNDERSTANDING THE COMPONENTS OF CALCAREOUS DEPOSIT IN CATHODIC PROTECTION

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Accelerated Low Water Corrosion (ALWC) is a particularly severe form of microbiologically-induced aggressive localised corrosion, which is most commonly found close to the level of the Lowest Astronomical Tide (LAT). To mitigate, cathodic protection (CP) and/or coatings have been used in the combination with surface sterilisation in most cases for about 30 years. However, it is expensive and difficult to apply conventional coatings at sea for existing corroded structures to prevent further corrosion. In those cases, the calcareous film, a deposit of $CaCO_3$ and $Mg(OH)_2$ produced on steel structures that are cathodically protected, may be effective as an inexpensive corrosion control coating. This film has two functions: *reducing the current demand from the CP system* and *acting as a protective coating*. When deposition conditions are appropriately controlled, it could provide long-term corrosion protection for steel in seawater. In this work, calcareous deposits formed under impressed current cathodic protection (ICCP) in artificial seawater were analyzed and modeled to show the dependence of the film structure (notably the relative proportions of brucite ($Mg(OH)_2$), calcite and aragonite ($CaCO_3$)) on the applied potential. Subsequently the endurance of this film will be studied both in the laboratory and the field.
Key words: ALWC, low water corrosion, calcareous deposit, brucite, calcite, aragonite, endurance, cost effectiveness.

29A-3-10

CORROSION IN THE DEFENCE INDUSTRY

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A review of corrosion costs in the military shows an unacceptable staggering figure in billions of dollars annually. It identifies three distinctly separate and at the same time interdependent categories that challenge materials science and laws of thermodynamics. These are: (i) the intrinsic material cost of weapon systems and their fabrication/ assembly projected over a design lifetime, (ii) the cost of scheduled maintenance and repair such as labor and materials used over the lifespan to keep the system operationally ready and effective, and lastly (iii) the refurbishment cost due incidental repairs from mission affected (severe environment) operations not considered in OEM design considerations for corrosion and corrosion assisted mechanical damage. For the reduction of life-cycle costs, it is essential that corrosion prevention and control management strategies must be instituted to examine all the three areas that make a difference. On legacy platforms although not all three areas could be easily addressed, but certainly on new acquisitions changes can be instituted by enforcing policies and practices that are in compliance with corrosion science and engineering principles and standards. Additionally, tools and processes that provide assessment of system health regarding structural and functional integrity must be integrated along with the others such as on board communication and navigation systems. Most of all corrosion management must not be looked at as a cosmetic action rather than an intrinsic requirement on how materials behave in an assembly and perform in the operating environment and what affects their life limiting factors.

29A-4: RELEASE RATE OF BIOCIDES FROM ANTIFOULING PAINT

29A-4-1

COPPER AND CO-BIOCIDE RELEASE FROM ANTIFOULING PAINTS AND IMPLICATIONS FOR ENVIRONMENTAL RISK ASSESSMENT

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International standards based on the rotating cylinder method for quantifying biocide release rates continue to be developed for a range of biocides. A universal mass-balance calculation model is also being developed as an international standard method. In a new study, published ASTM and ISO standard rotating cylinder methods are used to concurrently determine copper and co-biocide release rates for a series of tin-free antifouling paints containing copper oxide and two commonly used co-biocides. Copper and co-biocide release is highly synchronous and, beyond day 21, the ratio of copper:co-biocide release rates remains near-constant, even when the test period is extended well beyond the minimum period specified by the standards (45 days). The ratio of biocide release rates is consistent with the relative amounts of copper and co-biocide present in the dry paint film.
It has previously been shown that the ASTM/ISO method and (to a smaller extent) the mass-balance calculation method significantly overestimate the in-service environmental copper release rate under harbour and marina scenarios. The synchronous copper and co-biocide release rates observed in this study indicate that the determined co-biocide release rates for these paints are similarly unrealistic and that the ASTM/ISO and mass-balance calculation methods will overestimate the environmental in-service release of co-biocide by the same extent as for copper. In order to provide a more realistic estimate of in-service release rates, a correction factor approach should be adopted whenever ASTM/ISO and mass-balance calculation release rate data for these co-biocides is used for environmental risk assessment purposes. In countries where a correction factor approach has already been adopted for copper release rate data, these results support the use of the same factor for these co-biocides whenever environmental risk assessments are conducted for such paints.
As biocide release is seen to be controlled by the properties of the paint and not by the properties of the biocide *per se*, then these results suggest that this factor may also be applicable to other co-biocides.

29A-4-2

ON-SITE SAMPLER FOR MEASURING RELEASE RATE OF ANTIFOULANT FROM SHIP'S BOTTOM

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In order to measure the release rate of antifoulant from actual vessels, on-site sampler is developed.
The handy-sized sampler is designed for measuring release rate just after the landing when a vessel comes into the dry docking. To measure the release rate by this sampler, acrylic container is filled with sea water and the elute is stirred by a propeller which is equipped inside of the sampler.
Propeller speed and sampling time is discussed and performed with measuring test panels.
Release rate of copper from ship's bottom is compared with the laboratory's method of ISO 15181-1/2 and the other calculation methods.

29A-4-3

STUDY OF THE COPPER RELEASE OF ANTIFOULING PAINTS AND ITS DEPENDENCE ON DYNAMIC REGIME OF EXPOSURE. DETERMINED WERE THE COPPER RELEASE RATE, THE EROSION RATE AND LEACHED LAYER DEVELOPMENT DURING 2 YEARS

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The results of a unique, by ONR sponsored, project will be presented. It is probably a first time attempt to follow the copper release of antifouling paints by all possible means over a period of more than two years. Standard paint systems and extended ageing in natural seawater had been used to give a better insight of what antifouling paints do release in practice. The newest insights of international working groups on antifouling biocide release had been applied on the seawater conditioning. Integral part of the project was also to register the effect of dynamic and static ageing during more than two years of the service life of an antifouling. The study combined a modern analytical approach to determine the released copper in seawater accurately with methods which determined the film thickness loss and leached layer development without disturbance of the ageing by drying the film. At the end of the study the effect of a brushing of the aged antifouling paint film had been determined too.

29B-1: COPPER IN ANTIFOULING AND THE ENVIRONMENT (ORGANIZED BY NEAL BLOSSOM)

29B-1-1

COPPER IN ANTIFOULING - THE EU RISK ASSESSMENT PROCESS

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Over the past four years, the use of copper has been assessed for review by the EU Technical Committee for New and Existing Substances (TCNES) under the EU Existing Substances Regulations. The marine effects chapter has recently been completed and approved by TCNES, and is now under review by the Scientific Committee on Health and Environmental Risks (SCHER). The marine effects assessment has involved a thorough review of all available data on copper in the marine environment, and presents a methodology for the extrapolation of laboratory tests to derive meaningful Predicted No Effect Concentrations, incorporating the latest statistical techniques to derive environmentally safe levels based on a simple assessment of bioavailability.

29B-1-2

THE RELATIONSHIP BETWEEN COPPER AND MARINE INVASIVE SPECIES

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Copper has a long history of use as a biocide in shipping and maritime industries, primarily as a means to prevent the establishment of fouling communities on vessel hulls and other submerged surfaces. Following the IMO decision to phase out the use of tributyltin (TBT) paints by 2008, copper has become the number one antifouling biocide in use for most vessels worldwide. While copper is considered less harmful to the environment than TBT, it is also less effective against a broad range of organisms, which has implications for the transfer and establishment of marine species. Many invasive marine species arrive to new locations as fouling organisms attached to the hulls of ships, often despite the fact that these surfaces are treated with anti-fouling biocides such as copper-based paints. Several well documented cosmopolitan hull-fouling species are known to have considerable resistance to copper, a factor that may have contribute to their spread. Through their strong tolerance to copper, and ability to overgrow newly anti-fouled surfaces, some hull-fouling species also have the ability to act as surrogate settlement surfaces, facilitating the further establishment and spread of less copper-tolerant species. In addition to the direct relationship between an organism's tolerance to copper and its potential spread via hull-fouling, copper pollution in donor and recipient ports and harbours can indirectly assist in the successful invasion by organisms transferred by vessel traffic. The same copper tolerance that allows for the transfer of invasive species via hull-fouling, has been shown to provide them with a competitive advantage over often less-resilient native communities in metal polluted environments, providing a foothold for their establishment and spread.

29B-1-3

EFFECTS OF COPPER ON SURVIVAL AND GROWTH OF MARINE FINFISH

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Copper is used as a main component of tin-free antifouling paints for ship bottoms and water intake facilities. Although there have been general concerns about an impact of released copper on coastal ecosystems recently, toxicological information on marine organisms is limited. This study was designed to clarify acute and chronic effects of copper for two of the most popular marine finfish species along the coast of Japan, red sea bream and Japanese flounder.
Preliminary study showed that maximum dissolved concentration of copper supplied as CuSO4 in natural seawater of pH 8.1 was 1 mg-Cu/L and acute toxicity data could not be obtained based on dissolved copper concentration in natural seawater. Therefore, modified Lyman-Fleming's artificial seawater of pH 5.4 to 6.7 was used for acute toxicity tests after checking the normal growth of Japanese flounder in the seawater, and natural seawater for chronic tests. Red sea bream of 0.5 to 13 g (7 sizes) and Japanese flounder of 0.3 to 17 g (5 sizes) were exposed to the copper of 0.04 to 41 mg-Cu/L for 96 h at 20 C. Effects of water temperature of 10 to 25 C on the acute toxicity were examined for red sea bream of 0.5 and 1.0 g, and Japanese flounder of 0.3 and 0.4 g at 0.04 to 21 mg-Cu/L. Fifty-six days rearing experiments were conducted to examine the chronic effect of ambient copper for red sea bream and Japanese flounder of 0.5 g each, with 0 (control), 0.1, 0.3, and 0.9 mg-Cu/L and 0, 0.04, 0.12, and 0.36 mg-Cu/L, respectively. Median lethal concentration (LC50) and the lowest observed effect concentration (LOEC) for mortality and growth were determined.
The LC50 value of Japanese flounder after 96 h was independent of fish size, the value of red sea bream decreased with increasing fish size significantly. Water temperature has little effects on the acute toxicity for both fish species. Based on the result of rearing experiments, the 56-day LC50 for red sea bream and Japanese flounder were estimated to be 0.12 and 0.53 mg-Cu/L, and the LOEC for mortality were 0.12 and 0.9 mg-Cu/L, respectively. The LOEC for the growth was similar to the mortality for the flounder, however, no value was obtained for the sea bream because the growth of fish did not depend on the copper concentration.
Thus, physiological response of marine finfish to ambient copper differs by fish species, and sensitivity of red sea bream to copper seems to be higher than that of Japanese flounder.

29B-1-4

A REGULATOR'S EXPERIENCE WITH COPPER ANTIFOULING ISSUES

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In the late 1990's, a regional water quality control agency in California (CA) determined that the use of antifouling paints (AFPs) on the hulls of recreational boats significantly contribute to elevated water concentrations of copper in a large recreational boat basin in San Diego, CA. Copper concentrations there regularly exceeded water quality standards established for the protection of aquatic life. As a result, water quality regulations were developed for the control of copper discharge from boat hulls. Over the past few years, a number of other Southern California marinas and boat basins have subsequently been added to a federal impaired water-bodies list under similar circumstances, signaling the likelihood of more stringent regional regulations.
Since AFPs are considered a pesticide in CA, their sales and use are under the purview of the Department of Pesticide Regulation (DPR). In response primarily to the aforementioned developments, DPR initiated a comprehensive investigation of AFP pollution. DPR's investigation included an exploration of the scientific literature, a statewide assessment study of marinas, and consultation with a number agencies. DPR concluded that 1) during dry periods, AFPs are a significant source of copper in many CA marinas, and 2) AFP use leads to the widespread exceedance of copper water quality standards in many salt and brackish water marinas.
Based on these findings, DPR is preparing to engage AFP manufacturers in exploring various solutions to mitigate high levels of copper in CA marinas. DPR is also preparing to initiate a partnership with manufacturers to further explore the ecological impacts of booster biocides. At this point, DPR believes that a balanced solution to the AFP pollution concern may lie in better understanding the biological relevance of current regulatory thresholds and also in exploring less-traditional mitigation approaches to arrive at a more sustainable long-term outcome.

29B-1-5

DEVELOPMENT AND TESTING OF A MARINE BIOTIC LIGAND MODEL FOR COPPER

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The U.S. EPA has recently adopted the Biotic Ligand Model (BLM) as the basis for the latest revision to the ambient water quality criteria for copper in freshwater. However, at the time the copper criteria were finalized, a marine model for copper had not been fully developed and tested. Development of a marine copper model has progressed, and the marine copper model has recently been validated at numerous sites in North America. The model has successfully been able to predict copper toxicity to a number of sensitive marine invertebrates including *Mytilus galloprovincialis*, *Mytilus edulis*, *Strongylocentrotus purpuratus*, *Dendraster excentricus*, and *Crassostrea virginica,* and *Crassostrea gigas*. Results from this work suggest that natural organic matter is among the most important factors that affect copper bioavailability in marine environments. Application of the model to waters in San Diego Bay and Pearl Harbor suggest that appropriate site-specific criteria for copper would be higher than the national ambient marine copper criterion. In this presentation, we will discuss the development and testing of the model, application to several marine sites, and implications for criteria development and risk assessments in marine waters.

29B-2: ANTIFOULING SYSTEMS USING NATURAL PRODUCTS 1

29B-2-2

SUBSTRATUM STRATAGIES ON MICROFOULING AND ANTIFOULING POTENTIALS OF MANGROVES *AVICENNIA OFFICINALIS* AND *RHIYZOPHORA MUCRONATA*

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Four naval and fouling pattern substrata such as wood of *Artocarpus* sp, fiber glass reinforced plastic(FRP), stainless steel (316L) and carbon steel were evolved for vulnerability towards biofilm bacteria. Generally the biofilm bacterial load was increased with increase in experimental duration. Among the tested substrata, the wood was highly susceptible to biofilm bacteria and the Total Viable Count (TVC) was 221±11.86 x 10^3 $CFUml^{-1}$ at 72h and carbon steel showed the least biofilm bacterial load of 89±5.35 x 10^3 $CFUml^{-1}$at 72h. Totally ten bacterial strains were identified and among this *Pseudomonas aeruginosa* was found as a dominant and ranged from 19±1.63 to 24±2.49%, where as *Serratia liquefaciens* recorded meager prominence and found only in wooden (6±1.24%) and carbon steel panel(6±0.62%). Mangroves, *Avicennia officinalis* and *Rhizophora mucronata* were screened to explore antifouling potentials in which *A. officinalis* exerted better bactericidal activity of 7.0±0.40 to 15.1±0.62 mm than the bioactivity of 5.8±0.62 to 12.0± 0.40 mm of *R. mucronata* against the isolated biofilm bacteria. Similarly during mussel bioassay (anti macrofouling), *A. officinalis* recorded the EC_{50} of 69.17±5.72 μg/ml with LC_{50} of 357.33±2.49 μg/ml and *R. mucronata* documented the EC_{50} of 121.54±4.77 μg/ml with LC_{50} of 414.19±13.14 μg/ml. The further researches are being directed to predict the structure of antifouling compounds from *A. officinalis* and *R. mucronata*.

29B-2-3

BIOINSPIRATION - THE SOLUTION FOR BIOFOULING CONTROL?

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All surfaces in the marine environment, both biotic and abiotic, are subject to biofouling. This has significant consequences to the safe and efficient conduct of marine activities. There is a pressing need to develop environmentally and economically acceptable methods to control the problem. In nature most plants and animals have evolved techniques that prevent or limit the process of fouling. These include chemical, physical, mechanical and behavioral responses. This paper reviews the knowledge with respect to natural antifouling methods, discusses similarities between natural mechanisms and existing antifouling technology and identifies potential future bioinspired approaches for the prevention of hull fouling specifically as they apply to US Navy requirements.

29B-2-4

TRENDS IN ANTIFOULING ACTIVITY OF NATURAL PRODUCTS FROM BRAZILIAN SEAWEEDS: BIOGEOGRAPHY OR PHYLOGENETICS?

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Antifouling chemical defense is likely to be an evolutionary response to the ecological disadvantages of epibiosis, particularly on macroalgae. Natural product antifoulants can provide effective, environmentally friendly alternatives to currently used antifouling paint booster biocides. The aim of this work was to assess the antifouling potential of natural products from Brazilian seaweeds, southwestern Atlantic. For that purpose, the extracts from 51 populations comprising 42 species of macroalgae from eight locations along the Brazilian coast were tested against a relevant fouling organism in laboratory bioassays, the mussel *Perna perna*. Antifouling activity of some purified compounds was also tested. Nine macroalgae were also cultured and tested for the presence of inducible defenses against fouling. Ecologically relevant field tests were performed in 11 cases in order to confirm laboratory results. Despite the unbalanced number of macroalgae tested between different localities, there seems to be no latitudinal trend of increased antifouling activity towards lower latitudes, where fouling pressure is presumed to be higher. However, there was a clear phylogenetic pattern in antifouling activity, with red macroalgae showing the highest proportion of active species, followed by brown macroalgae. Our results appear to support known trends of secondary metabolite production among seaweeds, and suggest that research efforts should be focused on tropical red macroalgae in the quest for new antifoulants. On the other hand, it seems clear that macroalgal groups such as green algae must have mechanisms of defense against fouling other than chemical.

29B-2-5

NATURAL PRODUCTS AS ENVIRONMETALLY FRIENDLY ANTIFOULANTS

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Many marine lives prevent the surface of their bodies with various antifouling substances without causing serious environmental problems. These substances may be expected to be utilized as new environmentally friendly antifouling agents, especially as those having high anesthetic, repellent, settlement deterrent, settlement inhibitory properties, etc. without having biocidal properties. These natural products are terpenoids, nitrogen-containing compounds, phenols, steroids and others [1]. Many compounds such as sesquiterpenes, diterpenes, trigonelline, primidines, piperazines, macrocarpals and higher fatty acids, showed the same level of antifouling properties as that of CuSO4, and some compounds such as gramines, shogaols, alkylphenols, kaempferols, bufalin and furanones, showed the similar high antifouling properties as those of the organotin antifoulants.

[1]. I. Omae, Chemical. Reviews. 2003, 103, 3431. I. Omae, The Handbook of Environmental Chemistry, Vol. 5 Water Pollution, Part O, p. 227, 2006. Springer-Verlag, Berlin.

29B-3: ANTIFOULING SYSTEMS USING NATURAL PRODUCTS 2

29B-3-2

ANTIFOULING COMPOUNDS FROM *STREPTOMYCES* ISOLATED FROM DEEP-SEA SEDIMENTS

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The unexplored deep-sea Streptomyces is a potential good source for uncharacterized bioactive compounds. In this study, 11 Streptomyces strains isolated from the sediments collected in the West Pacific Ocean at depth of over 5000 m were screened for their antifouling activities and all of them were active. Strain 291 (please give the species name even it is tentative) showed remarkable inhibitive effect on both bacteria and larval settlement of several major fouling invertebrates. The crude extracts of 100 l of spent culture medium of this strain were fractionated by column chromatography and the active fractions were purified through a bioassay-guided procedure. A group of compounds belonging to the family of butenolides with strong inhibitive activity against fouling invertebrate larvae, including Balanus amphitrite, Hydroides elegans and Bugula neritina were purified and identified. The structure-activity-relation of those compounds was also investigated in a step-wise manner.
Although these compounds are highly active, they are produced by bacteria at very low concentration, which poses difficulties for further investigation or application. Response surface methodology was used to search for a good culture medium and to optimize the culture conditions for maximizing the production of these bioactive butenolides.

29B-3-3

CHEMICAL ANTIFOULING DEFENCES OF TROPICAL SEA STARS

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The role of natural products in keeping the exposed surfaces of the tropical sea stars *Linckia laevigata*, *Fromia indica*, *Cryptasterina pentagona* and *Archaster typicus* free of fouling organisms was investigated. Conditioned seawater of these sea stars did not have any effects on the settlement of the ecologically relevant diatoms *Amphora* sp. and *Nitzschia closterium* and the bryozoan *Bugula neritina*. However, dichloromethane, methanol and aqueous extracts of whole sea stars at 100, 10, 1 and 0.1 ug cm^{-2} had concentration-dependant effects on the settlement of these fouling species and the polychaete *Hydroides elegans*. Based on bioassay-guided fractionation and analysis with nuclear magnetic resonance (NMR) and gas chromatography – mass spectrometry (GC-MS), the most bioactive fractions were shown to contain several fatty acids and sterols. To determine whether the compounds responsible for the observed antifouling effects were present on the surface of all four sea star species, surface-associated compounds were absorbed onto filter paper and tested against *Amphora* sp., *N. closterium*, *B. neritina* and *H. elegans* in settlement assays. These surface-associated compounds had species-specific effects, reducing the settlement of at least two of the four fouling species for each sea star species. Using surface extractions and GC-MS analysis, the most abundant surface-associated fatty acids and sterols of each sea star species were identified as hexadecanoic acid, cholesterol, lathosterol and sitosterol, and quantified. These four compounds were tested at ecologically relevant concentrations, ranging from 1000 to 1 ng cm^{-2}, in settlement assays. Of these compounds, hexadecanoic acid, cholesterol and lathosterol significantly reduced the settlement of *Amphora* sp. and *N. closterium*. The settlement of *B. neritina* was not affected by any of the compounds. This study demonstrated that hexadecanoic acid, cholesterol and lathosterol play a role in deterring the settlement of fouling diatoms.

29B-3-4

GREEN CHEMISTRY FOR ANTIFOULING

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As a result of awareness of the environmental impacts of antifouling agents, the coatings industry has withdrawn the use of toxic organotins from commercial marine coatings. However, biocides continue to be the mainstay in the industry since conventional marine coatings based on self-polishing release of active substances continue to provide the best performance and most practical and economic practice for shipping. We argue that the way forward lies in intelligent chemical design that takes into account the biological and chemical fate of the molecules during and after application. In this paper we will present the current directions, as well as results of our work with biologically active pharmaceutical-based compounds in the development of new generation green chemicals for antifouling.

29B-3-5

PRACTICAL ASPECTS OF DEVELOPING A NATURAL PRODUCT BASED MARINE ANTIFOULING COATING SYSTEM

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This paper aims to further the study of natural product (NP) based antifoulant systems from an applied perspective. A multidisciplinary approach was used to examine the functionality of a NP antifouling coating system for large ship hulls. After careful review the red seaweed *Chondrus crispus* (Stackhouse) was selected as the NP source. Crude extracts were bioassayed then incorporated into a proprietary marine antifouling control depletion polymer (CDP) to better understand their effects. The antifouling efficacies of two differently sourced algae were tested, a 'commercial off the shelf' dried algae (extract A) and coastal algae from Calshot UK (extract B). Bioassays of the two ethanol extracts indicated good antifouling activity for extract A at concentrations of 1 μg mL^{-1} for three out of five bacterial and microalgae species. Due to its potential, extract A was directly compared to a booster biocide (chlorothalonil) and both were incorporated into the CDP using a high shear mixer at 1 % wet w/w. Assessment of the NP on the integrity (ion diffusion, water uptake) of the coating system, was made using non-destructive electrochemical techniques. Laboratory experiments of artificially thin coatings at 30 μm dry film thickness were applied to mild steel and immersed in 3.5 % NaCl solution. Electrochemical impedance spectroscopy (EIS) was used to calculate the water volume fraction in the coating, an increase due to the NP was seen. 3 month field trials were conducted using these two coatings as well as varying algae concentration on primed mild steel. In the first 6 weeks of a trial, the algal extract out-performed the biocide based system. SEM with EDX analysis was used to quantify the final coating integrity. The direct incorporation into a coating platform was successful and provided a useful means of developing techniques to screen the performance of future compounds.

29B-3-6

DYNAMIC ASPECTS OF STORAGE, TRANSPORT, EXOCYTOSIS OF SECONDARY METABOLITES IN THE SEAWEED *LAURENCIA OBTUSA*

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The production and ecological roles of secondary metabolites in seaweed have been related to a capability to partition compounds into cellular specialized storage structures and the possible transport to its surface. However, the possible mechanisms that transport these compounds to the thallus surface remain poorly understood. In addittion, to demonstrate that seaweeds use antifouling chemicals, the species should be naturally devoid of fouling; the natural concentration of chemicals at the macroalgal surface should inhibit fouling, and the bioassays to verify antifouling property should use relevant fouling organisms. It is also useful to know what compounds occur on or close to the thallus surface, what if any interactions occur between or among these compounds, and by what mechanism are these compounds transported to macroalgal surface. Recent studies using tropical specimens of *Laurencia obtusa* as a model revealed that low concentration of the compound elatol on its surface did not inhibit fouling. In addittion, confirmed that the *corps em cerise* (CC) storage halogenated compounds, and reveals the dynamics aspect of these CC in the intra-cellular transport of halogenated compounds to algal surface throughout membranous tubular connections. It was also verified the cell death events related to these mechanisms of halogenated compounds exudation to the thallus surface and consequently with defensive role against fouling or other ecological role. These studies indicate that storage cell structures and transport of compounds to thallus surfaces can provide essential and dynamic cues about surface-mediated chemical defenses. In addition, also reveal that the concentrations of the compounds on macroalgal surfaces are probably not absolute characteristics of the species, but may vary according to environmental conditions. Advances in this *surface ecology* will be fundamental to understanding and evaluating the importance of surface chemicals in the interactions between macroalgal species as they relate to benthic community structure.

29P: POSTER SESSION 2

29P-1

STUDY BARNACLE CYPRID ADHESION WITH ATOMIC FORCE MICROSCOPY

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Atomic force microscope (AFM) based force spectroscopy, a nanoscale testing and characterization technique, has allowed the scaling down of traditional mechanical materials testing by several orders of magnitude. The main advantages of using AFM to study barnacle cyprid adhesion system is its ability to measure nano-scale properties of natural bioadhesive materials and adhesive interfaces in native conditions. We used AFM to image footprints at high resolution and to acquire measurements of interaction forces. Alkyl (CH_3-) and amino (NH_2)-terminated glass surfaces were used for comparison of footprint morphology. It was noted that, footprints deposited on NH_2- comprised three times the volume than those footprints deposited on CH_3. High-resolution images of the morphology cyprid footprints revealed that a well correlated feature correspond to the ultrastructure of the attachment structures. The response of barnacle cyprid adhesives to the Alcalase was studied. Alcalase removed cyprid footprint deposits from glass surfaces within 26 mins, but cyprid permanent cement became resistant to Alcalase within 15 h of expression, acquiring a crystalline appearance in its cured state. The nanomechanical properties of barnacle (Balanus amphitrite) cyprid permanent cement were recorded from the cement disc continually over the course of its curing. Results showed a narrowing of the pull-off force distribution with time, as well as a reduction in molecular stretch length over time. There was a strong correlation between maximum pull-off force and molecular stretch length for the cement, suggesting curing of the adhesive; some force curves also contained a fingerprint of modular protein unfolding. This study provides the first direct experimental evidence in support of a putative tanning mechanism in barnacle cyprid cement.

29P-2

THE SUSCEPTIBILITY OF BARNACLE CYPRID ADHESIVES TO A SERINE PROTEASE, ALCALASE

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Barnacles are a persistent fouling problem in the marine environment, however their effects, such as reduced fuel efficiency and increased corrosion, can be reduced through the application of antifouling or fouling-release coatings to marine structures. It is necessary to develop future fouling resistant coatings that are effective, yet economical and that are not deleterious to the marine environment. The incorporation of proteolytic enzymes into coatings has been suggested as one potential option. The effects of such enzymes on the settling larvae of fouling organisms have not been studied in great detail. Here, we assess the efficacy of a commercially available serine endopeptidase, Alcalase, as an antifoulant and investigate its mode of action on barnacle cypris larvae. In situ atomic force microscopy (AFM) of barnacle cyprid adhesives during exposure to Alcalase supported the hypothesis that Alcalase reduces the effectiveness of the cyprid adhesives, thus impeding settlement of the organism. Quantitative behavioural tracking of cyprids, using Ethovision 3.1, further supported this observation - concentrations of Alcalase that reduced cyprid settlement did not significantly affect the behaviour of the larvae prior to settlement. Alcalase removed cyprid 'footprint' deposits from glass surfaces within 26 min, but cyprid permanent cement became resistant to attack by Alcalase within 15 hrs of expression, acquiring a crystalline appearance in its cured state. It is concluded, on the basis of the enzyme's effects on cyprid footprints, uncured permanent cement and its non-toxic action, that Alcalase has antifouling efficacy. Engineering of coatings that afford long-term activity of the enzyme at the surface is now required for field testing of the technology.

29P-3

ANALYTICAL MODELLING OF THE INTERACTIONS BETWEEN SETTLEMENT CUES FOR *SEMIBALANUS BALANOIDES* CYPRIDS

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Settlement of *Semibalanus balanoides* cyprids in response to texture, orientation, and the presence and density of incumbent settlers was analysed on artificial settlement tiles. Tiles were photographed and replaced every tide for nine days in one deployment, and settlement was simultaneously allowed to accumulate in another. Over 1.3 million larvae were counted using image analysis. With increasing density of settlement net loss of settlers became more likely, thus suggesting that density regulation was occurring. Smooth textures tended to be settled the least, and down-facing surfaces were settled more during daylight. However, cue effects were highly variable on each tide, and less likely in the cumulative deployment, suggesting that the presence of incumbent settlers negated other cues. There was no evidence for larval desperation. An attempt to rank the cues in terms of size, variability and frequency of effect revealed a non-linear hierarchy, so a path analysis model was employed to quantify the relative effects of each factor. The model showed that the accumulation of incumbent settlers outweighed the influence of texture and orientation by an order of magnitude, and that unmeasured variables accounted for five times more than the sum of all factors measured here. These results highlight the complex responses to multiple interacting cues in the field for just one species of barnacle, and show the difficulties that will be encountered in attempting to control settlement through manipulation of settlement cues.

29P-4

IMPACTS OF SHORE HEIGHT WITH CONSPECIFIC AND ALLOSPECIFIC CHEMICAL AND TOPOGRAPHIC CUES ON THE ZONED SETTLEMENT OF *SEMIBALANUS BALANOIDES* CYPRIDS

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Post settlement mortality of *Semibalanus balanoides* (SB) on the upper shore due to environmental extremes has long been thought of as the dominant driver of zonation of two species of barnacles. The present study was carried out at the same location as Connell's seminal work (Great Cumbrae, Scotland) and found that zonation in SB was strongly driven by choices made during cyprid settlement. Settled cyprids were counted under seven treatments at two shore heights, the mid-shore SB zone and the upper-shore *Chthamalus montagui* Southward (CM) zone. Treatments were clean bare rock, settled bare rock, live adult barnacles, and resin replica colonies of high and low densities of SB and CM. After accounting for differences in immersion period, cyprids preferentially settled in the SB zone over the CM zone, irrespective of the treatment. There were no differences among treatments within the CM zone. Within the SB zone, the live adult colonies attracted significantly more settlement than replica colonies, confirming the chemical aspect of gregarious attraction; however the replica colonies did not attract more settlement than the rocks, as would have been expected if their topography contributes mechanically to gregarious attraction; it may in fact repel settlement. As there was no topographic density effect within the replica colonies, cues conveying density information may be entirely chemically mediated.

29P-5

SEASONAL SUCCESSION OF BIOFOULING COMMUNITIES IN COASTAL WATERS OF KALPAKKAM, EAST COAST OF INDIA

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Coastal water of Kalpakkam, east coast of India, is used for condenser cooling of Madras Atomic Power Station (MAPS), where biofouling is an operational problem. A Prototype Fast Breeder Reactor of 500 MW (e) capacity presently under construction would use 30 cubic m/s of seawater for condenser cooling. Studies on biofouling was carried out for one year (May, 06- April, 07) to assess a) settlement pattern, b) seasonal succession, c) dominant species and d) impact of Tsunami on biofouling.

Teak wood panels (12 x 9 x 0.3 cm), categorized into three series viz: weekly, monthly and cumulative (30 d interval), were suspended in the coastal waters at 1 m below the lowest low water mark. The major fouling organisms observed were barnacles, mussels, hydroids, polychaete worms, flat worms, sea anemones and oysters. Considerable faunastic and biomass variations were observed with time. On weekly panels, the maximum biomass (g /100 sq cm) was 11, whereas on monthly and cumulative (150 d) panels, it was 116 and 685 respectively. The lowest and the highest number of organisms observed on weekly panels were 1 and 136 per sq cm respectively indicating two orders of magnitude variations. The present biomass build-up was relatively high as compared to the values reported from elsewhere. Barnacles (99 %- weekly, 85%- monthly) and other crustaceans (62%-weekly, 64.1%- monthly) dominated the weekly and monthly panels. However, in the cumulative observation, mussels (Perna viridis) dominated from July and gradually succeeded the barnacle population. With time, the 150d old panel was totally covered with mussels (95%). The unique features of this study is that fouling study at 7 d interval is available. The fouling density here has increased during post-Tsunami period. A shift in peak settlement period of mussels (July-September) has taken place as compared to earlier study (April-June). The influence of coastal water characteristics on settlement pattern is also discussed.

29P-6

SEASONAL VARIATION IN PHYTOPLANKTON COMMUNITY STRUCTURE IN THE COASTAL WATERS OF KALPAKKAM, EAST COAST OF INDIA

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Diatoms the earliest autotrophic colonizers on any surface immersed in marine environment, are also responsible for major energy input to the surface during the slime formation, the most vital stage in marine fouling cycle. A study was carried out from April, 2006 – March, 2007 to evaluate the variability of phytoplankton community structure at different locations viz. coastal waters, forebay & outfall of Madras Atomic Power Station (MAPS), Kalpakkam, where the problem of biofouling is persistent.

Samples collected fortnightly from intake, forebay and outfall of MAPS, Kalpakkam were analyzed for phytoplankton abundance, community organization and diversity. These locations represent different environments (intake - natural, forebay- influenced by chlorination, flow and fouling communities and outfall – influence of forebay and temperature). The population density exhibited wide variations at all location (order of magnitude). The lowest and the highest density were found in November and August, respectively for intake and forebay. For outfall, the minimum was obtained in November and the maximum in July. The minimum coincided with the lowest biofouling density. The pennate diatoms dominated over centric from April to September and reverse during October to January. Onset of northeast monsoon, which changes the coastal water quality significantly, could be the causative factor. Mostly, pennate dominated in forebay and centric in intake indicating their relative resistance to adverse environment. The pinnate to centric ratio followed in the order, coastal water <forebay< outfall indicating an increase in benthic forms from intake to outfall. Asterionellopsis glacialis, was the most dominant species (22.18 % - intake, 18.32 % - forebay, 43.65 % - outfall) indicating its hardiness to temperature and chlorine. Reduction in phytoplankton density and increase in species composition (three fold) was observed at intake during post-Tsunami period. The paper also discusses role of coastal water characteristics and phytoplankton assemblage on Biofouling.

29P-7

CONTACT ANGLE ANALYSIS FOR ADHESIVE OF THE BARNACLE *BALANUS AMPHITRITE*

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The key step in the accumulation of biofouling on immersed surfaces is the permanent attachment of fouling organisms. Patterns of adhesion of biofouling, in response to surface properties, vary both among and within species. This variation in adhesion may be mediated by interactions between surfaces and biological adhesives. We have been studying this interaction for the barnacle, *Balanus amphitrite*. Our current approach addresses the propensity of barnacle adhesive to wet commercially-available and experimental silicone fouling-release coatings. Wetting was quantified by measuring cement contact angle and spreading. Reference solutions include HPLC-grade water and bovine serum albumin (BSA) at a protein concentration similar to that of barnacle adhesive. For the nine materials tested (one commercially-available coating, eight experimental formulations), contact angles for barnacle adhesive did not track with those for water or BSA. Instead, adhesive contact angles reached a plateau between 100 and 105 degrees, while angles for the reference solutions continued to increase. Contact angles for barnacle adhesives were also more variable than those for the reference solutions. Adhesive contact angle was not correlated ($p = 0.17$) with barnacle removal stress. However, the spreading of barnacle cement did appear to show a relationship to adhesion strength ($r = 0.9$, $p < 0.001$). This research was funded by the NSWC Carderock Division In-House Laboratory Independent Research Program.

29P-8

INVESTIGATING THE WALKING BEHAVIOUR OF CYPRIS LARVAE ON SIMPLE MODEL SURFACES

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The colonization of a surface by barnacles begins with the exploration by the walking cypris larvae ‹cyprids›. In this study, we observe the walking behaviour of cyprids from Balanus amphitrite on simple model surfaces. We analyze and quantify parameters that correlate with their affinity for settling a specific surface.
Three surface chemistries were chosen: hydrophobic ‹CH3-treated glass›, hydrophilic negatively charged ‹clean, bare glass›, and hydrophilic positively charged ‹NH2-treated glass›. Two parameters were chosen: the walking distance between successive attachment points and the time spent before moving onto a new attachment point ‹probing time›. This behaviour was examined both with and without flow in a micro-fluidic device.Under no-flow conditions, cyprids take larger steps on the hydrophilic surfaces than on hydrophobic glass. Cyprids also exhibit a shorter probing time on hydrophilic surfaces than on hydrophobic glass. In the presence of flow, the latter trend is reversed. Under flow conditions, we observe no significant difference in their walking distance between the three surfaces.
These differences in the cyprids' behaviour indicate the cyprids´ response to different surfaces and flow conditions. This may have implications for studies performed only in static water. An understanding of these behavioural parameters will also provide insight into their preference for settlement surfaces, potentially leading to a simple assay to assess the performance of antifouling surfaces.

29P-9

HOME SWEET HOME ? REDUCING THE SETTLEMENT OF THE BARNACLE, *BALANUS AMPHITRITE*, THOUGH THE CHARACTERISATION OF GLYCANS INVOLVED IN GREGARIOUSNESS

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Prior to settlement barnacle cypris larvae, through surface exploratory behaviour, assess the suitability of a substratum for settlement and in so doing deposit proteinaceous 'footprints'. These footprints, termed the cyprid temporary adhesive, appear to contain as yet uncharacterised settlement inducing chemical cues. In addition to the cyprid temporary adhesive, the cuticular tissue of adult barnacles contains a glycoprotein-based contact cue referred to as settlement-inducing protein complex (SIPC). These cues are key to barnacle gregarious settlement and represent an attractive target for custom synthesis of antagonistic surfaces. Despite decades of research into marine fouling and the development of anti-fouling/fouling-release systems, detailed knowledge of the biochemical and structural composition of marine bioadhesives remains poor. Using the tropical acorn barnacle *Balanus amphitrite*, this project will seek to fully characterise the carbohydrate moiety of SIPC and cyprid temporary adhesive, illustrating possible similarities between the two and the importance of glycan based structures in conspecific identification. SIPC will be purified by bioassay-guided isolation, using ammonium precipitation, ion exchange chromatography and gel filtration. The active fraction will then be run on an SDS PAGE gel and the SIPC bands detected through immunoblotting with antibodies. Bands will be eluted from the gels for carbohydrate characterisation. The carbohydrate structure will be characterised using a combination of high performance liquid chromatography (HPLC), exoglycosidase digestion, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS). A greater understanding of the contribution of glycans to gregarious mechanisms and the success of fouling organisms, will be achieved through this full characterisation of the SIPC and cyprid temporary adhesive. With further research it may be possible to develop carbohydrate-functionalised polymers that are antagonistic to the barnacle settlement cues.

29P-11

THE EFFECT OF SURFACE COLOUR ON SETTLEMENT AND ADHESION STRENGTH OF BARNACLES

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The aim of this study was to test the effect of varying the colour of a commercial foul release coating on adhesion strength of the barnacle *Elminius modestus*. Strength of adhesion varied between and within sites for colour. The complex effect of colour on barnacle adhesion may be due to different physico-chemical surface characteristics caused by different pigment compositions and their interactions with local environmental conditions, as well as interactions with the settling organism.

29P-12

MARINE PAINT OPTIMISATION: SETTLING ASSAYS WITH SEA SQUIRT (*CIONA INTESTINALIS*) AND BARNACLES

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In the Marine Paint Optimisation project the major groups of fouling organisms will be studied. From each group of organisms one model organism was selected and used in settling assays to assess the efficacy of a number of individual antifoulants. A tentative list of existing and promising compounds include medetomidine, tolylfluanide, copper pyrithione, Sea-Nine, Irgarol, copper, Borocide and Econea. Based on the produced data we will predict the joint effects of almost all possible combinations in terms of efficacy. The optimisation will be based both on high efficacy and low environmental risk. We will present and summarise the results from two settling assays with larvae of the sea squirt *Ciona intestinalis* and the barnacles *Amphibalanus improvisus* and *Balanus amphitrite*. Adult sea squirt were sampled in the field and dissected to collect eggs and sperms to control fertilisation. Newly hatched larvae were incubated in 6-well microplates and exposed to individual antifoulants. After 48 hours of settling, water solutions and non-settled larvae were removed and fresh toxic solutions were added and growth continued for another 48 hours. Cultivated barnacle cyprids were allowed to settle for 48 hours in 6-well microplates. The number of settled and metamorphosed sea squirts and barnacles were counted and compared to control treatments. Efficacy data for a number of antifouling agents will be presented. This study is part of the Marine Paint research programme funded by MISTRA.

29P-13

THE mRNA EXPRESSION OF THE OCTOPAMINE RECEPTOR IN *BALANUS IMPROVISUS*

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Medetomidine have been found to strongly inhibit settlement of *Balanus improvisus* both in the laboratory and in field studies. Our hypothesis is that medetomidine binds to the octopamine receptor, belonging to the family of G-protein coupled receptors. The motive of the study is to investigate if medetomidine may cause tolerance development in barnacles, this due to down-regulation in the mRNA expression of the octopamine receptor. To investigate this possible mechanism for tolerance development, we have developed a protocol for a real-time PCR assay measuring mRNA expression of the octopamine receptor in *B. improvisus*. Total RNA was isolated both from cyprids and adult animals and RNA from each sample was converted to cDNA using the enzyme reverse transcriptase. To use the real-time PCR, we designed primers to the octopamine receptor sequence to give a PCR product of 69 base pairs. We used the DNA-binding dye SYBR Green, which binds nonspecifically to double-stranded DNA. The fluorescent signal from a reaction is proportional to the amount of double stranded DNA present, and will increase as the target is amplified. The primer concentration was optimized to 200 nM at a temperature of 59°C. The PCR product was sequenced and confirmed to be a part of the octopamine receptor sequence. Primers to a reference gene (actin) have also been designed and tested and we now have an assay for relative quantification with the reference gene as the normalizer. Our aim is to compare the mRNA expression of the octopamine receptor in *B. improvisus* during different life stages and medetomidine exposures.

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29P-14

CLONING AND EXPRESSION OF OCTOPAMINE RECEPTORS FROM *BALANUS IMPROVISUS*

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The octopaminergic system in invertebrates has been suggested to be the correlate of the adrenergic system in vertebrates and octopamine has been shown to be a major neuromodulator with neurotransmitter and neurohormone functions.
Settling of the *Balanus improvisus* cyprid larvae has been shown to be inhibited by the synthetic α_2-adrenergic receptor agonist medetomidine. The action of medetomidine is believed to be mediated by its binding to octopamine receptors, which belong to the G-protein coupled receptor family.
To characterize octopamine receptors from *B. improvisus*, we have cloned and expressed putative octopamine receptors in *Saccharomyces cerevisiae* and in mammalian cells. The first receptor was cloned from cDNA and genomic DNA prepared from a pool of cyprids, using PCR primers based on the published sequence of the *Balanus amphitrite* octopamine receptor gene. A region including transmembrane helix 1 to transmembrane helix 7 were amplified and the 5' and 3' ends of the gene were thereafter amplified using Rapid Amplification of cDNA Ends. Interestingly, sequencing of several receptor clones revealed relatively high sequence variability at the nucleotide level, whereas the amino acid sequence was much more conserved. One explanation for the high nucleotide sequence variability could be that there are sequence differences between individuals in the pool of cyprids used as template for cloning. To enable investigation of individual differences, genomic DNA was prepared from single *B. improvisus* adults and the receptor was cloned and sequenced.
In another approach, using degenerate PCR primers based on sequences of biogenic amine receptors, we identified two other putatitive octopamine receptors. These receptors had the highest sequence similarity to the family of β-adrenergic-like octopamine receptors, which quite recently was identified in *Drosophila melanogaster*.
Financially supported by The Foundation for Strategic Environmental Research (MISTRA), Sweden, through the research program Marine Paint

29P-15

METHANOL EXTRACTS OF SILICONE COATINGS ALTER ACTIVITY OF ENZYMES INVOLVED IN BARNACLE GLUE POLYMERIZATION

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Polymerization of barnacle, *Amphibalanus amphitrite*, glue involves trypsin-like serine protease(s) and transglutaminase(s) (Dickinson et al., 2008). When barnacles are reared on silicone surfaces they have different glue phenotypes, which have a heritable component (Holm et al., 2005). Here we tested the hypothesis that molecules associated with silicone coatings alter the activity of enzymes involved in glue polymerization. One to 6 ul droplets of unpolymerized glue were harvested from individual barnacles and used in colorimetric assays for activity of transglutaminase (using a Sigma-Aldrich CS1070 transglutaminase kit) and serine protease (using a BAPNA substrate). Soluble molecules from silicone coatings were extracted by 30 second washes of the surface of the coating with 60 to 540 ul of 100% methanol. The methanol extracts, including controls, were dried under nitrogen, re-suspended in 30 ul of methanol, transferred to assay containers and the methanol removed. For each assay 10 individual barnacles were tested for each condition. Methanol extracts yielded visible amounts of material from most silicone polymer surfaces. Results of assays were dependent upon individual barnacles. Enzymes from some individuals were not significantly impacted by methanol extracts while others were strongly impacted. Results of enzyme assays complement observations of individual barnacles with differing glue phenotypes.

29P-16

AN INVESTIGATION OF NOVEL ANTI-FOULING/FOULING-RELEASE COATINGS CONTAINING QUATERNARY AMMONIUM SALT GROUPS

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Polysiloxane coatings containing "tethered" quaternary ammonium salt (QAS) moieties were investigated for potential application as environmentally-friendly coatings to control marine biofouling. A combinatorial/high-throughput approach was applied to the investigation to enable multiple variables to be probed simultaneously and efficiently. The variables investigated for the moisture-curable coatings included QAS composition and concentration as well as silanol-terminated polysiloxane molecular weight. A total of 75 compositionally unique coatings were prepared and characterized using surface characterization techniques and biological assays. Biological assays were based on two different marine microorganisms, a bacterium, *Cellulophaga lytica* and a diatom, *Navicula incerta*, as well as a macrofouling alga, *Ulva*. The results of the study showed that all three variables influenced coating surface properties as well as antifouling and fouling-release characteristics. Characterization of coating surface morphology revealed a heterogeneous, two-phase morphology for many of the coatings investigated. A correlation was found between water contact angle and coating surface roughness with the contact angle increasing with increasing surface roughness. With regard to antifouling and fouling-release properties, coatings based on the 18 carbon QAS moieties were very effective at inhibiting *C. lytica* biofilm formation and enabling easy removal of *Ulva* sporelings (young plants) while coatings based on the 14 carbon QAS moieties were very effective at inhibiting biofilm growth of *N. incerta*.

29P-17

INCREASING SETTLING ACTIVITY; THE ROLE OF DIFFERENT DETERGENTS

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It is by far easier to disturb settling in the common settling assay than initiate or enhance the settling ratio. Almost all substances will in high concentrations inhibit settling whereas there are few to have proven to evoke settling. Why so may be due to the inherent biology of the cyprid larvae or that the settling assay may not being optimal in investigating barnacle settlement.

Our approach to study the settling behavior is to understand the importance of surfaces. Medetomidine, a new antifoulant, is a surface active molecule that exerts its effect, probably mostly at the surface. In that sense, surfaces are not only the substrate but also the delivering system for the antifoulant substance itself. With that in mind, surface activity and behaviour of the cyprid larvae are of outmost importance.

Detergents are surface active molecules and have the ability change surface properties. We have investigated a number of different detergents and noticed that the TWEEN molecules have an effect on the settling rate. In low concentrations it induces settlement while in high, decreases. Other detergents such as Brij or SDS, do not have this effect and it seems that the TWEEN molecule has some unique properties. We are now investigating how the length of the different side chains may influence the settling rate. Also interesting to notice is that, together with medetomidine, the TWEEN molecule masks the antisettling effect of medetomidine This points towards the conclusion that it is medetomidine surface concentration and affinity are of higher importance than the bulk concentration.

29P-18

DAMAGES OF EPOXY ANTI-RUST COATING ON MARINE STRUCTURES OBSERVED UNDER EXISTENCE OF SEA URCHINS AND ITS PROTECTION

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A survey was performed on the survival of sea urchins on marine structures and the damage of these on the surface coatings. Four species of sea urchins were found and damaged coatings were confirmed in locations with 3 species including the so-called "tawashi-uni"(Echinostrephus molaris). Pieces of the coatings as well as other organisms were found in their digestive canals. The "tawashi-uni" moves around on the surface of marine structures feeding on seaweeds and barnacles and during feeding their hard teeth may have damaged the coatings.
In order to protect the coating layer of marine structures from sea urchin, we investigated the relationship of coating hardness to the damage on the anti-rust paint coating using the "tawashi-uni" urchin as a test organism. We prepared test plates with different coating hardness and let the sea urchins forage through these plates for three months. During this period, observations were made on the damage condition of the anti-rust coating on the plates.
From the results, it was considered that the damage, which reached the base steel plate, done by the "tawashi-uni" urchin on the coating layer could be protected with epoxy-glass-flake paint. Moreover, based on the relationship of coating hardness and the rate of coating damage occurrence, it was thought that the damage afflicted by the sea urchins on the coating layer could have been prevented if MVH was about 40.
Key words: marine structures, anti-rust coating, sea urchins, coating damage, coating hardness

29P-19

SPATIAL DISTRIBUTION OF THE GOLDEN MUSSEL, LIMNOPERNA FORTUNEI (DUNKER, 1857) IN THE TONE RIVER SYSTEM, CENTRAL JAPAN

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Limnoperna fortunei (the golden mussel) is an invasive freshwater bivalve species originally native to China and southeastern Asia. It was first recorded in Japan in Lake Biwa, in western Japan, in 1992. Since *L. fortunei* has both economic and environmental effects, Japan's Ministry of the Environment has officially designated it as an "Invasive Alien Species" and prohibits its raising or transportation in Japan without permission from the relevant ministers.
In 2005, the mussel was newly reported in central Japan, but its spatial distribution in this area was not known. We examined the spatial distribution of the mussel in the Tone River System from 2006 to 2008. The Tone River, the second longest river in Japan, is an important water source for domestic, agricultural and industrial purposes. The mussels were attached to hard substrata, such as concrete walls and stones. We found the mussel up to 116 km from the river mouth. The mussel was found also in several other rivers, including the Kokai, Hitachitone, and Edo Rivers; ponds and lakes (Lake Kasumigaura, Lake Teganuma, Lake Ushikunuma, etc.); and canals. It was found along half of the shore of Lake Kasumigaura, the largest lake in central Japan. Size distribution data suggest that the mussel invaded Lake Kasumigaura no later than 2004. Our results show that the mussel has become widely distributed in central Japan. This species may have spread via water supply facilities such as water conveyance pipes and canals.

29P-20

ELECTROLYSIS OF BALLAST SEAWATER TO CONTROL POLLUTION WITH NONNATIVE MICROORGANISMS

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Ballast seawater can be a major cause of pollution by non-native organisms, especially toxin-producing planktons that contaminate shellfish. Also disinfection of ballast seawater containing food-poisoning bacteria or fish pathogens is needed to reduce risk to human and fish health. In the present study, bactericidal and virucidal effects of three methods, i.e., UV irradiation, ozonation and electrolyzation, widely used in the fisheries field, were compared using human and fish pathogens as well as bacteria habituated to seawater. Furthermore, a trial experiment using a set-net fishing boat equipped with a seawater electrolyzer was done. Effectiveness for disinfection of seawater for the fish reservation tank and for cleaning fisheries equipment was studied.
The number of viable bacteria in seawater decreased from 10^{4-5} to 10^{0-1} CFU/mL after UV irradiation (1.0×10^5 μWsec/cm^2), ozonation (Total Residual Oxidants 0.5mg/L for 1 min) and electrolyzation (chlorine concentration 0.5 mg/L for 1 min). In the case of electrolysis, similar levels of disinfection of coliforms or *Vibrio parahaemolyticus* were observed after exposure to chlorine at the concentration of 0.5 mg/L for 1 min. Also Feline Calicivirus, Norovirus surrogate, and fish pathogenic viruses were inactivated at a chlorine concentration of 0.4 to 1.3 mg/L for 1 min. On the fishing boat, viable bacterial counts of seawater in the tank were remained below 100 CFU/mL. Although the fishing boat was a small scale trial, electrolysis can be easily scaled up by increasing amperage. From these results, electrolysis of seawater is one of the suitable methods to disinfect ballast seawater.

29P-21

TOXICITY OF ANTIFOULING BOOSTER BIOCIDES TO FRESHWATER AND SALTWATER CRUSTACEAN

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The AFS Convention decided to ban of using organotin, have caused to serious environmental problems, toward antifouling coatings. The quantity of alternative antifouling coatings including booster biocides are increasing worldwide. However, limited information on the toxicity of booster biocides to aquatic organisms has been reported. In this study, we tested toxicity of five booster biocides, Irgarol 1051, Sea Nine 211, PK, CuPT, and ZnPT, toward freshwater and saltwater crustacean. Water flea *Daphnia magna neonates*, one of recommended freshwater crustacean in OECD guidelines, harpacticoid copepod *Tigriopus japonicus*, and barnacle *Balanus amphitrite*, distributing widely in Japanese tidal pools and intertidal area, respectively, were used as experimental species. Acute toxicity test to *D. magna* was conducted according to OECD guideline 202 at 20°C. To *T. japonicus*, five mates of copepod were exposed each test compounds with 10ml seawater volume for 48h at 25°C. Toxicity and antifouling activity to *B. amphitrite* cypris larvae were carried out according to Nogata *et al.* (2004). For *D. magna* and *T. japonicus* tests, swimming behavior after 48h exposure was observed and EC_{50} value for immobilization was decided based on the nominal concentration. For cypris larvae of barnacle, the EC_{50} value expressed as settlement inhibition activity. Sea Nine, PK, CuPT, and ZnPT affected to *D. magna* at low concentrations with the 48h-EC_{50} values of 0.039, 0.0008, 0.016 and 0.018 mg/L, respectively. On the other hand, the EC_{50} to *T. japonicus* were generally higher (twofold ~ 30 times) than those of to *D. magna*. Irgarol, Sea Nine, PK, CuPT and ZnPT were inhibited larval settlement barnacle with 48h-EC_{50} values of 0.95, 0.1, 0.01, 0.05 and 0.54 mg/L, respectively. These results suggest that booster biocides affect to non-settlement crustaceans at lower concentration compared with concentrations of inhibition of barnacle settlements .

29P-22

WATERBORNE SETTLEMENT-INDUCING PHEROMONE-LIKE PROTEIN IN THE BARNACLE-CONDITIONED SEAWATER OF *BALANUS AMPHITRITE*

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Gregarious barnacles sometimes cause serious damage to cooling water systems at electric power stations. In order to develop effective antifouling techniques, understanding of settlement mechanism is necessary. Settlement process of intertidal barnacle larvae is affected by several environmental factors including chemical factors such as settlement-inducing pheromones. Several studies have been shown involvement of two types of pheromones, substratum-bound and waterborne, in inducing larval settlement. Recently, substratum-bound type was purified from adult extracts of *Balanus amphitrite* and primary structure was determined. The pheromone is a glycoprotein consisting of 76, 88, and 98 kDa subunits, and reported as SIPC. On the other hand, little information has been obtained for the waterborne type. We tried to identify the waterborne types in adult extract of *B. amphitrite*, and distinct protein from SIPC was purified. Results of the larval settlement assay using 24-well polystyrene plate showed that almost 90% of cypris larvae settled in the presence of the purified protein at 10 μg/mL. The molecular mass of the protein in reduced and denatured form was 32 kDa, and partial amino acid sequences showed no similarity with previously reported proteins. Therefore, we may find a novel pheromone-like protein inducing larval settlement of barnacles. However, it is uncertain whether this protein is actually released into seawater. To clarify this, following experiments were executed. Adult *B. amphitrite* were reared in filtered seawater at inanition. After rearing of two days, barnacle-conditioned seawater was collected and centrifuged. Then the supernatant was concentrated using the ultrafiltration membrane, and was applied to gel-filtration column chromatography. The column eluate was monitored for larval settlement-inducing activity, and the fractions showing activity were offered to SDS-PAGE for detection of settlement-inducing pheromone-like protein. The molecular characterization and function of this protein compared with SIPC and/or 32 kDa pheromone-like protein will be discussed in the presentation.

29P-23

SPECIES-SPECIFIC DETECTION AND QUANTIFICATION OF FOULING BARNACLE LARVAE BY REAL-TIME PCR

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Abstract
Barnacles are major fouling organisms at several electric power stations in Japan. It is useful to forecast larval settlement period of the fouling barnacle species for effective antifouling measures. In order to forecast it, precise larval detection method must be developed. We showed that species of barnacle larvae can be identified by DNA analysis of 12S rRNA gene, and developed easy detection method for the major fouling species, *Megabalanus rosa*, by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the restriction enzyme *Spe I*.
In the present study, we aimed for establishment of a quantitative identification method for the fouling species of barnacle larvae based on the previous study. Recently "real-time PCR" has been well developed in the field of molecular biology as quantitative methods for specific DNA, expression of specific gene and so on. The "real-time PCR" was adopted for the larval quantification of the fouling barnacle, *M. rosa*. First of all, we selected *M. rosa* specific sequence in 12S rRNA gene and designed a pair of specific primers, RTMr12S-F and RTMr12S-R for the real-time PCR. When template DNA extracted from a series of numbers of *M. rosa* nauplius larvae were used for the real-time PCR, their threshold cycles (Ct) were well correlated with the number of larvae. The correlation was not affected by the presence of other barnacle larvae or mixed planktons collected at natural sea. The differences in quantitative results of real-time PCR between in early nauplius stage and in late cyprid stage were observed but the differences were less than 5 times. These results indicate that the species specific detection and quantification by "real-time PCR" is useful for the fouling barnacle larvae.

29P-24

BIOFOULING ON AQUACULTURE NETS IN NORWAY: CURRENT STATUS AND FUTURE DIRECTIONS

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The control of biofouling on aquaculture infrastructure is an essential operational feature of fish farming throughout the world. Along the Norwegian coast, over 10 million m^2 of nets are in use at any one time across approximately 1500 fish farms. These nets can become fouled with up to 10 kg m^{-2} wet weight at the end of autumn. At present, the main antifouling strategies are: (1) copper-based coatings on nets combined with regular washing, (2) copper-based coatings on nets combined with regular drying, and (3) uncoated nets with frequent cleaning. The current trend to increase the size of net pens and hold the large nets in the sea for the whole fish production cycle will make the traditional techniques for net handling, changing and washing less effective.
The hydroid *Tubularia* sp. has become an increasing problem in the Norwegian aquaculture industry, because it is difficult to remove through the current cleaning procedures. Rapid re-colonisation and growth occurs, leading to more or less monocultures of *Tubularia* sp. on nets. The attachment and growth patterns of established *Tubularia* sp. colonies on nets were observed under a dissecting microscope. The hydrorhiza and hydrocaulus of *Tubularia* sp. wind around the threads and may grow between the threads and loose filaments, which increases their attachment strength. There is an urgent need to develop novel antifouling strategies and technologies to reduce, control and remove hydroids on aquaculture infrastructure in an efficient and environmentally sustainable manner. Further research to be conducted at the Centre for Research-based Innovation in Aquaculture Technology will investigate the reproduction and feeding biology of *Tubularia* sp., determine the settlement preferences and growth rates of these hydroids with regards to surface nano- and micro-topographies, temperature and light, and develop strategies to remove them more efficiently.

29P-25

MEXEL® PROCESS: AN ALTERNATIVE FOR COOLING WATER CIRCUIT TREATMENT

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In 1990, Mexel Industries SAS. (France) developed an innovative process, using the surface-active properties of Mexel® 432 to inhibit inorganic fouling (mud or scale deposits), microfouling (biofilm), and macrofouling (mollusks, crustaceans, hydroids) in fresh, brackish and sea water cooling circuits. This product is a patented emulsion of alkylamines in water. It is registered for use world-wide, including EC (BAT's), the USA (EPA), Australia etc. Furthermore, it is included in the European Integrated Pollution Prevention and Control reference document on the application of best available technology (BAT's) for industrial cooling systems. This product is injected daily for 30 minutes at a residual concentration of approximately 3 mg/L. It is use at full scale since 1996 in power plant cooling water circuits with flow rates up to 22 m^3/s. This alternative to oxidizing treatments has resulted in (*i*) the elimination of discharges of chlorinated products into the environment, (*ii*) the improvement of plant efficiency in the range of 1% (3% heat rate reduction) by offering continuously a cleanliness factor (HEI) closed to 100%, (*iii*) extension of the service life of the plant by reducing the corrosion rates (especially of copper alloys) and provides a solution to CO_2 emissions reduction. At the same time, supported by numerous toxicological, ecotoxicological and impacts studies, it has proven its safety (human and environmental) and its cost-effectiveness. This product does not contain any known carcinogenic, mutagenic or reprotoxic substances and is biodegradable due to its composition. Moreover, biodiversity studies performed after 10 years of use by a power plant demonstrated the absence of any specific effects on fauna at the discharge of this plant and the total absence of plume impact (600 m) downstream from the discharge point.

29P-26

DOMINANT ATTACHED ORGANISMS ON THE PLANE NETTING OF TRAP NETS IMMERSED IN FISHING GROUNDS OFF HIRADO CITY, NAGASAKI, JAPAN

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The taxonomic groups of organisms that attach on nettings of trap nets were analyzed in a study conducted on two different types of sea areas in Nagasaki, Japan using nettings of two mesh types immersed for varying periods of time from fall to spring of 2001. Species from families Ischyroceridae and Ampilochidea (sub-order Gammaridea) and *Caprella acutifron* (sub-order Caprellidea) were dominant on the plane nettings, with the 2 sub-orders accounting for >90% of the biomass of the attached organisms. By contrast, barnacles were the dominant organisms on PVC plates (control) that were immersed simultaneously with the test nettings for comparison. The density of attached organisms reached a maximum of 13,000 individuals/100cm^2 during the spring season on the smaller mesh size nettings immersed at the inner part of the bay. Thus, nettings of trap nets are fouled mainly by amphipods and not by the common macro-fouling organisms, i.e. barnacles and mussels.

29P-27

RESIDUE ANALYSIS OF FISH EXPOSED TO FISHNETS COATED WITH ANTIFOULING PAINT CONTAINING ZINC PYRITHIONE OR COPPER PYRITHIONE

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Zinc pyrithione and copper pyrithione are very effective in the protection of netting used in aquaculture from marine fouling. Although the release of pyrithione into the aquatic environment is not predicted to cause adverse effects on the marine ecosystem, we believe that it is imperative that the pyrithione residues in the farmed fish are determined to assess the risk associated with the human consumption of these farmed fish. In order to accomplish this, a sensitive method has been developed for the analysis of pyrithione in salmon meat at low part-per-billion levels. A risk assessment based on the method detection limit and the consumption pattern of fish in South East Asia will be presented.

29P-28

DEVELOPMENT OF MONOCLONAL ANTIBODIES AND IMMUNOCHROMATOGRAPHIC ASSAYS FOR RAPID AND SPECIFIC DETECTION OF THE SETTLING STAGE LARVAE OF THE MUSSEL AND THE BARNACLE

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Massive settlement of the mussel *Mytilus galloprovincialis* and the barnacle *Megabalanus rosa* often causes serious problems in coastal industries: electric power plants, fisheries, marine services. The objective of this study is to develop the rapid and sensitive methods for on-site monitoring the larval settlement of the fouling species to make efficient the antifouling systems. Monoclonal antibodies (MAbs) were prepared against pediveliger larvae of the mussel and against cypris larvae of the barnacle. As the results of ELISA and immunofluorescence testing, MAbs against the mussel larvae showed the specific reactivity with the surfaces of the larval velum and foot, and MAbs against the barnacle larvae did with the basal tissues of the larval thoractic appendages. Using these MAbs, immunochromatographic assay systems were developed for specific detection the settling stages of the mussel and the barnacle larvae. The immunochromatographic assay systems (based on the sandwich immunoassay) were applied to the crude extracts of various samples. As the results, the immunochromatographic assay systems were useful to detect specifically the one individual larva of the fouling species within 20 minutes from the field plankton samples. This is the first application of the immunochromatographic assay system for reproductive ecology of the marine sessile invertebrates and for maintenance of the power plants.

29P-29

CHARACTERIZATION OF MANGANESE RICH DEPOSIT

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Three different kinds of deposit, such as biofilms, particulate deposits and corrosion products, are known to form on the surface of aluminum brass condenser tubes in the cooling seawater. The particulate deposits can be classified into a protective ferrous(Fe)deposit and a manganese(Mn)rich deposit. The author reported the protective Fe deposit elsewhere and it was found to be a biofilm. The present paper deals with the Mn rich deposit. The Mn rich deposit was collected from the surface of aluminum brass condenser tubes by scraping off with granular sponge balls at a power station. At the power station conventional treatments of cooling systems have been conducted for the protection of aluminum brass condenser tubes from corrosion by adding ferrous ions in the cooling seawater, and cleaning with sponge balls for removing deposits formed on the inner surface of the tubes, but chlorination of the seawater has not been conducted. The bacterium species in the Mn rich deposit was identified. The water content and its chemical composition in the Mn rich deposit were also determined. Characterization of the Mn rich deposit was made by using an electron microscope, an electron micro probe analyzer(EPMA) and X-ray diffraction analysis. The Mn rich deposit was also found to be a biofilm similar to the protective Fe deposit. The water content in biofilms was 96.4% by weight, but those in the Mn rich deposit and in the protective Fe deposit were 85.4% and 95.5% by weight, respectively. It is noted that the water content in the two deposits containing either metal was less than other biofilms. Manganese oxidatioin bacteria in the Mn rich deposit was only 0.4% by weight. The Manganese-related substance in the Mn rich deposit is thought to be oxidized from Mn^{2+} in seawater by Mn oxidation bacteria in the deposit and would possibly be a manganese complex rather than MnO_2.

29P-30

THE EFFECTS OF SPONGEBALL CLEANING ON BIOFILM AND PROPTECTIVE IRON FILM

Atsushi Kawabe, Kansai Techno Co.

Seawater is divided into two classes, clean and polluted. For this experiment, supposedly clean seawater was used, however its turbidity and the amount of suspended solid were much more than what would normally be classified as clean. This seawater was obtained from an intake located at a depth of approximately 2 m at the Aioi power plant and was introduced to model condenser. Test tubes were installed beyond the model condenser. Test tubes were titanium condenser type with an outer diameter of 25.4mm and a wall thickness of 0.7mm. Seawater velocity was 2.2m/s. Chlorine was not added. In the 1st.series of tests, neither spongeballs nor ferrous ion injection were used, resulting in the formation of a biofilm. In the 2nd. series, ferrous ion injection were used however spongeballs were not, resulting in a protective iron film formation. In the 3rd. series, spongeballs were used however ferrous ion injection were not. In the 4th. series, both ferrous ion injection and spongeballs were used. The ferrous ion injection rate was 0.3mg/l×5h/d..Spongeball cleaning occurred 3times/week,2 balls at a time without ferrous ion injection and 3 balls per time with ferrous ion injection. Each test was repeated three times over 42~47 days.
The results indicated that the protective iron film`s structure is the same as biofilm. With spongeball cleanings, both the deposit thickness and dry weight decrease substantially. After spongeball cleanings, residual deposit structure remains the same as that of biofilm. FeOOH particles(less than 1μm diameter) are concentrated in the residual deposits while mineral particles(more than sereral μm diameter) and potozoans(more than 20μm diameter) are diluted. The number of sessile bacteria in the residual deposits is more than biofilm. The main composition of biofilm is water(over 90%), the same is true residual deposits. Therefore, heat conductivity of residual deposits is the same and that of biofilm.

29P-31

CHARACTERIZATION OF PROTECTIVE IRON FILM

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Seawater is divided into two classes, clean and polluted. For this experiment, supposedly clean seawater was used, however its turbidity and the amount of suspended solid were much more than what would normally be classified as clean. This seawater was obtained from an intake located at a depth of approximately 2 m at the Aioi power plant and was introduced to model condenser. Test tubes were installed beyond the model condenser. Test tubes were titanium condenser type with an outer diameter of 25.4mm and a wall thickness of 0.7mm. Seawater velocity was 2.2m/s. Chlorine was not added. In the 1st.series of tests, neither spongeballs nor ferrous ion injection were used, resulting in the formation of a biofilm. In the 2nd. series, ferrous ion injection were used however spongeballs were not, resulting in a protective iron film formation. The ferrous ion injection rate was 0.3mg/l×5h/d. Each test was repeated three times over 42~47 days.
The results indicated that the protective iron film's structure is the same as biofilm. Iron compounds on the surface of tube are accompanied with microorganisms and organic particles. It suggests that they are crystallization nucleus of iron compounds.

30A-1: PLENARY LECTURE 3

30A-1-1 (Plenary)

CAN THERE BE AN ENVIRONMENTALLY 'SAFE' ANTIFOULING PAINT BIOCIDE?

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Over twenty years on from the first reports of detrimental environmental effects associated with the use of TBT in antifouling paints, are we any nearer reaching the goal of an environmentally friendly antifouling biocide? Or is a 'biocide-free' paint the only option since intrinsically a biocide must be toxic? Post-TBT there has been a large amount of research focused at better understanding the factors that inform the assessment of the environmental risks associated with antifouling biocide use. These data can effectively be split into two; on one hand we have regulatory tests performed by the biocide manufacturers in support of the registration of their product and on the other, the data produced by independent researchers and published following peer review. More often than not data produced for registration purposes are not available in the public domain; however the increasing quantities of peer-reviewed material make an initial assessment possible. The type of data required are those that allow us to understand the environmental behaviour and potential effects of the biocide; typically, occurrence, persistence, partitioning and toxicity. In the context of environmental risk assessment these data are used to generate predicted environmental concentrations (PEC) which are evaluated against predicted no-effect concentrations (PNEC). When a PEC exceeds a PNEC it is considered that the environment may be at risk. PECs are often determined using models specific to antifouling paint exposure scenarios, whilst PNECs are generated from laboratory ecotoxicology tests. Where a biocide is already used in paint formulations occurrence data can be used to measure the quantities of a biocide present in the environment and therefore validate PECs, whilst behaviour and fate studies can assist in better establishing the persistence of a particular biocide. From a review of the data currently available it is apparent that certain biocides persist in the aquatic environment and can have detrimental effects (e.g. Irgarol 1051 and diuron). For other biocides few data are available for their occurrence, due to the absence of survey data, low usage and low persistence. Typically these compounds are poorly stable and sometimes reports are contradictory. What is clear is that there are compounds with good efficacy and that are relatively labile when assessed under laboratory conditions. Extrapolating from the laboratory is challenging and can have a major influence on PECs. This key-note presentation will offer some insight into the data that currently informs the assessment of the environmental risk posed by antifouling biocides and will hopefully demonstrate that although no biocide can be guaranteed to be environmentally 'safe', it is possible to select those biocides that offer the least risk to the environment when used in a responsible manner.

30A-2: NON-TOXIC ANTIFOULING SYSTEMS AND NEW STRATEGIES FOR FOULING CONTROL 1

30A-2-1

WHY DO DIATOMS STICK TO FOULING-RELEASE COATINGS?

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Diatoms are the most frequent and successful microalgal foulers of illuminated submerged structures, forming a brown, slimy biofilm. Paradoxically, unlike most other fouling organisms, raphid diatoms stick more strongly to (and therefore foul) low-energy surfaces, such as silicone-based or fluorinated fouling-release coatings, than they do to more wettable, high-energy surfaces. It is therefore of interest to know how diatoms detect the wettability properties of a substratum as this may provide insight into the improved design of antifouling surfaces.
Adhesion of diatoms to surfaces is mediated by the secretion of extracellular polymeric adhesives, which also play a role in regulating motility. Coordinated adhesion and motility underpin diatom strategies for growth and survival in the natural environment in response to external environmental cues. For example, diatoms may detach from a surface if the microhabitat is unfavourable. Light and salinity are examples of behaviour-modifying environmental cues for diatoms, and we postulate that substratum wettability is another. We hypothesise that a hydrophilic surface that is unfavourable to diatoms, i.e. a surface to which they do not adhere well, could be regarded as stressful, signalling a stress response in the cells that changes their adhesion processes in such a way that they stick less strongly. Conversely, a hydrophobic surface, which is more conducive to adhesion, could be regarded as a less stressful surface.
To test this hypothesis we have explored one aspect of stress-signalling pathways used by other organisms, namely the intracellular production of nitric oxide (NO). We will present data from studies on *Seminavis* and transgenic, NO-overexpressing cell lines of *Phaeodactylum*, which support the underlying hypothesis and which demonstrate that NO acts as a signal of surface wettability in diatoms.

30A-2-2

TOWARD TOUGH FOULING-RELEASE COATINGS: SELF-STRATIFIED SILOXANE-POLYURETHANE COATINGS

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Self-stratified siloxane-polyurethane coatings have been found to be a promising concept for fouling-release coatings. The fouling-release coating concept is a promising approach to non-toxic underwater marine coatings for fouling control. The greatest success--including several commercial coating systems--has been achieved using silicone elastomers. These coatings have the low surface energy and modulus needed for the easy removal of most fouling organisms. However, silicone elastomers are mechanically weak and they are difficult to adhere to most substrates. To overcome these issues the concept of a self-stratified crosslinked hybrid coating system has been explored. The siloxane-polyurethane system consists of an organofunctional siloxane, a polyol and a polyisocyanate. Dueing film formation, the siloxane component predominates on the surface, yielding a coating with the surface properties of siloxanes and the bulk properties of polyurethanes. High throughput screening was used to synthesize libraries of siloxanes and polyols to determine the effect on coating surface properties and interaction with biological organisms in laboratory screening tests. It was found that organofunctional siloxanes with aminopropyl end groups and an acrylic polyol with a low glass transition temperature yielded the best performance. Lower amounts of siloxane modification of the polyurethane system seem to be preferred. Field testing of selected coatings at several different test sites has indicated that the coatings having good release properties in laboratory assays are demonstrating similar release performance. Additional characterization using XPS and TEM, have been carried out to understand the morphology of the coatings that leads to good release properties.

30A-2-3

FABRICATION OF LARGE AREA MICRO-PATTERNED POLYOLEFIN BLEND COATINGS HAVING FOUL RELEASE PROPERTIES

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Surface roughness, surface free energy, elastic modulus properties are the most important characteristics to affect the antifouling and foul-release properties. The interaction between the coating and the marine microorganisms can be reduced by decreasing the contact area of the surface with the microorganism. For this purpose, a surface roughness of below 5 micron in size on the substrate is needed. Such coatings having a definite degree of geometric patterning could be manufactured mostly from expensive materials with time consuming and expensive physical processes. However, the chemical and marine paint industries need these surfaces to be manufactured from inexpensive polymers with cheap procedures. In this study, large area micro-patterned surfaces were prepared by dip coating using mixed polyolefin solutions. Polypropylene, high-density polyethylene, cyclic olefin copolymer and ethylene vinyl acetate polymers were used to prepare these polymeric blend coatings. Micro-patterning with polymer protrusions having 2-4 micron diameter could be achieved by using different variations of polyolefin blend compositions. The change in the surface morphology of these thin coatings with the change in the composition and concentration of polymer solutions, and also dipping conditions were investigated. Wettability and the surface characteristics were examined by measuring contact angles and calculating surface free energy values of the sample surfaces. These coatings can be kept in seawater for long time without decomposition or release and displayed good fouling release results as determined by partners within the European Commission-AMBIO (Advanced Nanostructured Surfaces for the Control of Biofouling) project.

30A-2-4

MARINE PAINT: FROM MULTIDISCIPLINARY RESEARCH TO ENVIRONMENTALLY SOUND ANTIFOULING PAINTS

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Marine Paint is a multidisciplinary and highly integrated research programme where all competences needed to develop new concepts for environmentally sustainable antifouling coatings are represented. The research is funded by The Foundation for Strategic Environmental Research, (MISTRA) in Sweden. Twenty researchers from six different departments are active in the programme.

The first substance from the programme – medetomidine – has been proven highly effective against barnacles and with an acceptable ecotoxicological risk profile. The mode of action of medetomidine, an adrenoceptor agonist, has been elucidated in the barnacles. Deeper understanding of the barnacle on a genomic, physiological and behavioural level has been established. Concepts for paint formulation with controlled release of medetomidine based on nanotechnology have been developed.

The programme has now taken the next step - developing recipes for biocide combinations aimed at hindering all major settling organisms in an effective and sustainable way.

The programme collaborates closely with I-Tech AB, holder of the patent rights to medetomidine and adjoining technologies and largely as a result of this collaboration - medetomidine is now being filed for the approval process with regulators in Europe and in the USA.

30A-2-5

A PREDICTIVE MODEL FOR ANTIFOULING USING ENGINEERED TOPOGRAPHIES

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The biocomplexity of the marine environment is a significant challenge for both antifouling and foul-release coating designs. Our research has focused on the investigation of the interactions between physical and chemical properties of anti-fouling surfaces. We have designed topographies that provide super hydrophobicity or super hydrophilicity properties. We have extended our designs to include the more fundamental chemical surface modifications that allow the study of surface energies from a low of ca. 18 mN/m to a high of ca. 72 mN/m. These combinations of high fidelity, engineered topographies and chemistry have been subjected to fouling assays that include the Ulva algae zoospore, Balanus Amphitrite cyprids, and two different bacteria, e.g., Cobetia marina, and Cytophaga lytica. The results of the assays were used to develop a number of algorithms that describe the inhibition of settlement as a function of the topography. The factors included in the algorithm are the Wenzel roughness factor, the fractional depressed surface and the number of unique features of each pattern. The preliminary indication is that surface energy is not the predominant term. Data will be presented that supports the inclusion of geometric factors in combination with surface energy as a mechanism of inhibiting settlement by marine organisms.

30A-2-6

ANTI-FOULING PROPERTIES OF POLY(METHYL METHACRYLATE) FILMS GRAFTED WITH POLYETHYLENE GLYCOL MONOACRYLATE IMMERSED IN SEAWATER

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Bio-fouling[1] of all structures immersed in seawater constitutes an important problem and many strategies are currently developed to tackle it. In this context, our previous work shows that Polyethylene glycol mono-acrylate (PEGA) macro-monomer grafted on pre-oxidized[2] poly (methyl methacrylate) (PMMAox) films exhibit an excellent repellence against the bovine serum albumin (BSA) used as a model protein[3]. This study aims to evaluate firstly, the prevention of marine protein (MP) adsorption by the modified surfaces. Then, the antifouling property of the PEGA-g-PMMAox substrates when immersed in natural seawater at two seasons: Season 1: end of April-beginning of May 2007 and, Season 2: end of October-beginning of November 2007). The anti-biofouling performances of the PEGA-g-PMMAox films are investigated for different PEG chain lengths and macromonomer concentrations into the PEGA-based coatings. These two parameters are followed as a function of the immersion time, which evolves up to 14 days. The influence of the PEGA layer on marine species (proteins and phospholipids) adsorption is evidenced by Time-of Flight Secondary Ion Mass Spectrometry[4] (ToF-SIMS) and X-ray Photoelectron Spectroscopy (XPS). It was found that the anti-biofouling efficiency of the PEGA grafted surfaces increases with both PEGA concentration and PEG chain length.
Keywords: PEG, biofilm, seawater, protein, adsorption, ToF-SIMS, XPS, principal component analysis
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30A-2-7

CONTROL OF *BALANUS RETICULATUS* SETTLEMENT USING PETROLEUM JELLY: RESULTS OF LABORATORY AND FIELD EXPERIMENTS

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Oyster culture in Konagai-machi, Nagasaki, Japan suffers yearly damages from fouling mainly by the barnacle *Balanus reticulatus*. The effect of petroleum jelly (PJ) on the inhibition of settlement of *B. reticulatus* was investigated in the laboratory and in the field.
In the laboratory, two types of bioassays were conducted in flow-through overflow-type tanks with plankton net cages (mesh size: 100μm, cage size: 3L). The first experiment investigated settlement of 3-d-old cyprids on plates (26mm x 76mm ceramics plates pre-treated with extracts of adult *B. reticulatus* to promote settlement, CP), when these were coated with PJ (PJ-CP) and compared with those that were not. The second was a choice assay between CP and PJ-CP placed simultaneously in a single cage. Cyprid settlement on spatted *Patinopecten yessoensis* shells coated with PJ and survival of the spats were also investigated in 1-ton tanks. A field experiment was also conducted on an oyster farm in Konagai-machi, Nagasaki from July to September in 2007.
B. reticulatus settlement on CP was 45% while that on PJ-CP was <1%. In choice bioassays, settlements were 23% and 0% for CP and PJ-CP, respectively. In the 1-ton tank experiment, the number of cyprid settlements were 45 and 2 individuals per *P. yessoensis* shell for the control and the PJ coated groups, respectively. Survival of spats was the same in the two groups. In the field, PJ effectively reduced barnacle fouling on oyster cultches and no difference in survival and growth of oysters were observed between coated and control groups.

30A-2-8

MARINE PESTS, NICHE AREAS AND VARIABLE SPEEDS: BIOFOULING ISSUES FOR DEFENCE

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Biofouling is a complex problem with many issues in addition to hull fouling. This talk will examine the problems with current antifouling systems from a Royal Australian Navy (RAN) perspective. Military platforms have a very different operational tempo than commercial vessels and therefore require different antifouling strategies. Problems with copper-based antifouling coatings and the limitations of foul-release coatings will be highlighted. RAN platforms often operate in warm waters and the difficulty in maintaining fouling resistance under such conditions, particularly when stationary for extended periods is addressed. The importance of protecting niche areas on a vessel will be discussed along with the potential they present to translocate marine pests. The difficulty with inspecting niche areas and methods to assist diver inspections are outlined. Lastly some complimentary non-toxic strategies to reduce fouling that are under investigation by DSTO are presented.

30A-3: NON-TOXIC ANTIFOULING SYSTEMS AND NEW STRATEGIES FOR FOULING CONTROL 2

30A-3-1

INFLUENCE OF SILICONE OIL ADDITION ON PROPERTIES OF SILICONE FOULING RELEASE COATINGS

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Many researchers have investigated and developed nontoxic antifouling coatings because of the marine environmental damage by the use of traditional antifouling coatings, fouling release coatings are the most possible successors to toxic antifouling coatings. This paper investigated the influence of silicone oil addition on the surface properties and antifouling properties of silicone fouling release coatings. The results showed that the surface energy became lower by addition of PDMS oil which could not diffuse to the coating surface. The coating surface energy increased with the increase of PDMDPS oil with low phenyl content which could not diffuse to the coating surface, too. The PDMDPS oil with high phenyl content exhibited poor compatibility with the silicone resin, it could easily penetrate the interior coating to the surface and change the surface morphology. The structure surface energy caused by the PDMDPS oil diffusion was much higher than the intrinsic surface energy of the coating with no silicone oil. The silicone fouling release coatings possessed excellent stability during the seawater exposure experiments, and the antifouling properties were improved by the addition of PDMDPS oil with high phenyl content.

30A-3-2

EFFECT OF SUBSTRATE ROUGHNESS AND FOULING CONTROL COATING TYPE ON SHIP DRAG RESISTANCE, FUEL CONSUMPTION AND GREENHOUSE GAS EMISSIONS

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A 240 meter long, 5.5 meter deep towing tank fitted with a specially-made test bench was used to characterize the hydrodynamic performance of two different generic types of fouling control coatings. Variables studied include not only coating chemistry and its intrinsic surface morphology, but also the underlying substrate roughness reproducing several real-life hull scenarios. Skin friction coefficient values measured under highly-reproducible, low-wave and wake conditions are extrapolated to power efficiency improvements by means of a commercial software including realistic full-ship wave, wake and air resistance. Estimations point at an increase in up to 10.6% in power efficiency for large container ships when a fouling release topcoat is compared to a commercial, copper-based, self-polishing coating. Such increased power efficiency translates into important reductions in fuel consumption and, subsequently, greenhouse gas emissions. Some key assumptions behind such extrapolation are critically discussed and compared to e.g. reported 8% real-life savings on an ultra-large crude carrier coated with an epoxy siloxane/amino siloxane hybrid tie-coat and the poly(dimethyl siloxane) topcoat studied in this paper. Open questions resulting from the use of this experimental methodology will be addressed in further tests so as to fully characterize real-life fuel savings from current and upcoming generations of FR coatings.

30A-3-3

RECENT FOCUS OF U.S. NAVY UNDERWATER HULL COATING SYSTEMS PROGRAMS

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The U.S. Navy already eliminated the use of organotin coatings over a decade ago and continues to maintain an ongoing commitment to reducing the adverse environmental impact of underwater-hull coatings by actively evaluating heavy metal-free, reduced copper, and foul-release underwater hull coating systems. The recent International Maritime Organization's (IMO) adoption of the International Convention on the Control of Harmful Antifouling Systems on Ships (AFS Convention) that bans the use of organotin-based marine coatings demonstrates that reducing the adverse environmental impact of underwater-hull coatings is an issue of international concern. At the same time, local, regional, and international concerns over high levels of copper in harbors and sediments continue to grow. This paper will describe the U.S. Navy's recently completed qualification testing of its first copper-free, 3-year service life underwater hull coating system. Approval of a more desirable 7-year and 12-year service life copper-free coating system will require additional advancements in available technology. Efforts to develop copper-free coating systems are of particular interest to the U.S. Navy since cuprous-oxide-based coatings are incompatible with the US Navy's growing fleet of aluminum-hulled vessels such as the Littoral Combat Ship, Joint Maritime Assault Connector, and Joint High Speed Vessel. In addition to the copper-free, biocidal coatings, the US Navy is also reevaluating their commitment to non-toxic fouling release coating technology. We will outline how recent panel tests, small boat tests, and MSC and Navy surface ship experience has provided the basis for current and future plans for larger-scale evaluation of this technology and the possibilities for implementation.

30A-3-4

DEVELOPMENT OF ENVIRONMENTALLY BENIGN, DURABLE AND EFFECTIVE ULTRA LOW FOULING MARINE COATINGS

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Biofouling on ship hulls and other marine surfaces has become a global environmental and economic issue. Antifouling paints are of increased environmental concern while non-toxic silicone and fluorinated fouling-release coatings are under development. In this work, we introduce a new type of ultra low coatings, to which marine microorganisms can not attach. These nonfouling coatings are as effective as anti-fouling coatings, but do not contain or leach any biocides while they are much more effectively in both performance and cost than environmentally benign fouling-release coatings, particularly for low ship moving velocities.
This talk will cover two topics - (a) design of ultra low fouling materials beyond PEG to resist nonspecific protein adsorption, bacterial adhesion/biofilm formation, and marine microorganism attachment, and (b) development of non-toxic, durable, effective, and low-cost ultra low fouling coatings with excellent mechanical strengths for marine applications. We have demonstrated for the first time that several zwitterionic-based materials and coatings are ultra low fouling (less than 0.3 ng/cm2 protein adsorption). While many hydrophilic surfaces are low fouling as compared to hydrophobic surfaces, very few materials can achieve ultra low fouling, which is essential to effectively defer the settlement of microorganisms. Under support from the Office of Naval Research (ONR), we have developed several nontoxic and stable ultra low fouling coatings and have used several approaches to apply these materials onto surfaces. Marine laboratory tests have confirmed the outstanding performance of our coatings against marine microorganisms (Ulva spores and barnacle cypris larvae) and marine bacterial adhesion/biofilm formation. We have developed paints and spray-coated them onto surfaces covered with an epoxy primer, which is the normal base coat on ship hulls. Field tests of these panels clearly demonstrated that our coatings are very effective to defer the settlement of biofoulants.

30A-4: NON-TOXIC ANTIFOULING SYSTEMS AND NEW STRATEGIES FOR FOULING CONTROL 3

30A-4-1

DEVELOPING AN ENZYME-BASED ANTIFOULING COATING, PART I: THE ENZYME SYSTEM

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Advanced biotechnology has been applied to the development and analysis of a novel antifouling system based on *in-situ* generation of hydrogen peroxide through enzymatic catalysis. Results and experiences are presented from the development of an enzyme system, which is inherently free of bioaccumulating active agents. Insight is given to the extensive research in the activity of this compound and, most notably, the viability of the enzyme system as well as its chemical and physical characteristics.
Two leading companies in their respective fields have joined the bioscience of Genencor and the paint technology of Hempel in an effort to fight biofouling without the hazard of having damaging substances accumulate in the sea and harbours. This collaboration is manifested in two parallel industrial PhD projects in affiliation with the University of Aarhus and the Technical University of Denmark, respectively, and outcomes of the projects are presented in two connected parts under the shared headline "Developing an enzyme-based antifouling coating".

30A-4-2

DEVELOPING AN ENZYM-BASED ANTIFOULING COATING, PART II: THE COATING SYSTEM

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Antifouling coatings based on hydrogen peroxide as the active antifoulant provide an environmentally friendly solution to the fouling challenge. In seawater hydrogen peroxiode rapidly degrades to water and oxygen. However, its instability makes in-situ production of hydrogen peroxide in the coating necessary. The enzymes used here, glucoamylase and hexose oxidase, together with starch will provide hydrogen peroxide when water and oxygen are present. In order to use this for antifouling purposes, enzymes must be active in the surface of the coating. However, addition of enzymes and starch to a coating is challenging. Not only enzyme activity, but coating parameters such as mechanical integrity and leaching rate are also affected. As the coating parameters of interest are interdependent, detailed knowledge of the impact of the constituents on the coating parameters is essential. This paper presents the engineering initiatives taken to overcome the obstacles that emerged when biochemical compounds were used as ingredients in antifouling coatings. Background:Antifouling coatings based on enzymes present biochemical challenges to coating engineers, and/or paint obstacles to biotecnichians. The bioscience of Genencor and the paint technology of Hempel have joined in an effort to fight biofouling using enzymes. The collaboration is manifested in two parallel industrial Ph.D. projects in affiliation with the University of Aarhus and the Technical University of Denmark, respectively, and outcomes of the projects are presented in two connected parts under the shared headline "Developing an enzyme-based antifouling coating".

30A-4-3

ADVANCED MARINE COATINGS MAKING USE OF NANO-CARBON TUBES

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The authors have investigated the advantages of using Nano Carbon Tubes (NCT) and supplementary nano-technology particles in epoxy based marine coatings. Experiments conducted by the authors show that adding a small volume of NCT and other nano-particles into such epoxy based coatings reduces the surface roughness, improves the foul release properties and abrasion resistance significantly. These properties provide benefits in a variety of marine coating applications. The authors experiments show that hull friction resistance is reduced, cleaning is easier and abrasion resistance is 2-3 times better than standard epoxy coatings. Utilizing NCT addresses current environmental concerns as it is uses little or no solvents, has a longer time between reapplication and is easier to clean. Advanced Marine Coatings AS has developed hull of different marine coatings for different applications to take advantage of the benefits provided by NCT and other nanotechnology.

30A-4-4

NOVEL TRIALKYLSILYLMETHACRYLATE BLOCK COPOLYMERS AS SELF-POLISHING BINDERS FOR CHEMICALLY ACTIVE ANTIFOULING PAINTS

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The most successful antifouling paints in terms of long time efficiency in service life (up to five years) were self-polishing copolymer based-paints (SPC paint). These well-known chemically active paints contain statistical methacrylic copolymers bearing tributyltin ester moieties which could undergo a hydrolysis reaction in basic environment like seawater. The success of these paints was clearly related to their mechanism of action defined by a constant release rate of TBT-based products and co-biocides and a control of the polishing rate in seawater under stationary and dynamic conditions. Soon after the recognition of the detrimental side effects of TBT-based compounds on non-target species at the early 80's, tin-free alternatives have been developed.
In our laboratory, research works are focused on the development of erodible (meth)acrylic binders bearing silylated, titanate and ammonium salt moieties.[1] Here will be presented results of polishing rates obtained from novel trialkylsilyl-methacrylate block copolymers as self-polishing binders. Random and block methacrylic copolymers were investigated to show the effect of the microstructure on the erosion rate of the corresponding coatings. Diblock copolymers poly(MMA-b-tert-butyldimethylsilyl methacrylate) showed a better control of the erosion with a constant erosion rate over a long-time service in artificial seawater [1]. Moreover, experiments showed that the erosion rate could be modulated by varying the molar proportion of hydrolysable side groups onto the block copolymer and the weight amount of copolymers mixed with PMMA in solution.
[1] C. Bressy, A. Margaillan, F. Faÿ, I. Linossier, K. Vallée-Réhel, Advances in antifouling coatings and technologies, Chapter 12: Tin-free self-polishing binders for chemically-active paints, Eds. C. Hellio and D. M. Yebra, to be published.

30A-4-5

APPLICATION OF ANTIBACTERIAL ABILITY OF METALS FOR BACTERIAL CONTROL -A VIABLE STRATEGY TO CONTROL BACTERIAL ATTACHMENT AND MICROBIOLOGICALLY INFLUENCED CORROSION-

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Microbiologically Influenced Corrosion (MIC) is reported to occur in various types of construct materials. Thus MIC has been one of major concerns in material engineering. When a metal surface comes in contact with a non-sterile fluid, a conditioning film forms, and biofilm formation starts. This might result in the initiation of MIC. Thus bacterial attachment, biofilm formation, and their roles in MIC have become the focus of scientific attention.

Bacterial attachment on the metal surface is considered as the precursor of MIC. Some closer observation of the MIC failure case analyses showed the interaction of some metallurgical factors and the preferential bacterial colonization on welds. Naturally, the effect of the surface roughness or ruggedness shapes is considered as one of main reasons. However, some reports showed the influence of substratum microstructure or the elemental segregation at the grain boundaries on bacterial attachment.

In current researches, authors have investigated that the alloying elements such as sulfur and nitrogen, which have possibility to become essence for bacterial growth, enhance bacterial attachment. From these results, it was considered that that if the elements are profit for bacterial growth, bacterial attachment should be enhanced and if the elements are harmful, bacterial attachment should be deterred. And this hypothesis led us to the study for antibacterial ability of metals to control bacterial growth.

In this reports, two kind of experiment were performed. In the first experiment, Silver and copper, the well-known toxic elements were tried as alloying elements in stainless steel and the efficiencies of these antibacterial stainless steels were tested in the laboratory and in a freshwater environment as well. In the other experiment, In order to collect a base line data on the antibacterial efficacies of different candidate alloying elements, various metals were tested for their antibacterial efficacy using a standard method, known as film contact method.

30A-5: NON-TOXIC ANTIFOULING SYSTEMS AND NEW STRATEGIES FOR FOULING CONTROL 4

30A-5-1

CHEMICALLY FUNCTIONAL NANOSTRUCTURED ANTI-FOULING COATINGS

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We recently demonstrated that nanostructured polymer films of poly-(*p*-xylylene) (PPX) can be fabricated using oblique-angle polymerization[1,2]. The production technique does not require any template, lithography or a surfactant for deposition. The structure is composed of approximately 40,000,000 aligned columns (50-150 nm in diameter) per square millimeter. These films offer the possibility of fabricating coatings exhibiting tunable antifouling behaviors by systematically varying and controlling the surface chemistry and topology. Nanostructured PPX films also show superhydrophobic and low adhesive properties[3]. The PPX surfaces are used to study fundamental aspects relating to the settlement and attachment of fouling organisms. Our results showed that *Ulva* zoospores settle onto the nanostructured PPX surface with a density of 697/mm^2 as compared to 879/mm^2 for a standard poly(dimethylsiloxane) elastomer coating. In addition, spores showed a lower strength of attachment for the modified PPX film with a 38.5% removal of the attached spores under a simulated water shear stress of 52 Pa.

References

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30A-5-2

NEW STRATEGY FOR DESIGN OF OPTIMISED COMBINATIONS OF ANTIFOULANTS: MIXTURE EFFICACY PREDICTIONS, RISK WEIGHTING AND MICROCAPSULE TECHNOLOGY

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Organisms differ in their sensitivity to toxicants, and each biocide will have its own efficacy profile. Whenever an antifoulant is used to affect organisms beyond its high-efficacy profile, an excess of biocides will be emitted to the environment. We propose an unprejudiced and rational design of efficacy-optimised combinations with minimum environmental risk. To control release of several antifoulants independently we use microcapsules bound to a polymer coating. The approach is based on three initial steps:
Mixture toxicity concepts are used to predict efficacy of >100 000 combinations of 2-8 antifoulants. Predictions are based on full concentration-efficacy relationships with regard to prevention of settling of fouling model organisms (e.g. periphyton, sea lettuce, barnacles, sea squirt, blue mussel). Based on the predictions we will identify a set of > 100 000 combinations that are efficacious to all model organisms, and define their mixture concentrations and mixture ratios.
Risk ratios (e.g. PEC/ PNEC) for the individual antifoulants are then used as weighing factors to rank the combinations according to their estimated relative risk to the environment. This results in a set of promising antifoulant combinations defined by their constituents, mixture ratios and total concentrations in water.
The release rate from each of the individual mixture components will be regulated by microcapsule numbers, chemical and physical properties, to deliver the expected combination at the surface of the ship hull. The flexibility of the paint formulation - with one antifoulant only in each capsule - suggests that the coating can easily be reformulated to adjust to more demanding conditions.
This study is a part of the Marine Paint Research Programme funded by MISTRA, the Foundation for Strategic Environmental Research, Sweden.

30A-5-3

SHIP HULL CLEANING: AN EVOLVING METHOD FOR BIOFOULING CONTROL

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The careening of boat hulls to remove unwanted marine growth is the oldest method for biofouling control. Today, underwater hull cleaning is still practiced; however, it is typically used as a reactive measure to remove excessive fouling. This means that the ship may already be operating at a significant penalty. Advances in underwater robotics and the development of more sophisticated ship hull coatings now enable methods to be developed that allow ship hull cleaning to become proactive. This should guarantee that a ship is always operating in a hydrodynamically smooth condition and is not a vector for invasive species.
This paper will provide a short history of hull cleaning and then present a novel method for the proactive maintenance of ship hulls, the HullBUG (Bioinspired Underwater Grooming). This research, funded by the United States Office of Naval Research, has been directed towards the development of fully autonomous underwater vehicles that groom the hull at frequencies that maintain the hull in a smooth and fouling free condition. The method raises challenges with respect to vehicle design, navigation, grooming tools and interactions with the biofouling and coating surface. Preliminary data are presented with respect to the effectiveness of grooming on copper, silicone and epoxy based surfaces.

30A-5-4

BARNACLE SETTLEMENT BEHAVIOR ON CONTROLED MICRO-TEXTURED SURFACES

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In order to keep clean marine environment, one important thing is to avoid settlement of larval barnacles. There are two factors which affects settlement of barnacles, such as mechanical and chemical factors. Cyprid larvae swim around and explore favorite surface with their first antennas. When they detect the surface, they test it and settle on. This study focused on the relationship between the micro-texture of substrate surface and settlement behavior. Two types of disc shaped substrate were prepared; one was made of SUS316L stainless steel, the other was made of acrylic resin. Rough SUS316L steel disc surfaces were prepared by an FPP (Fine Particle Peening) treatment. FPP is a surface treatment method which shoots out particles of several ten micrometers in high speed of about 200 m/sec. Acrylic resin surfaces with regular channel, width of 30, 50 and 100 μm were made by injection molding. The number of settlement of barnacles *Amphibalunus amphitrite* on controlled micro-textured surfaces and polished surface were measured with a specially developed laboratory testing cell.

30A-5-5

INTEGRATING FIBRE FILTERS, ULTRASOUND AND UV-C INTO A CHEMICAL-FREE WATER TREATMENT SYSTEM : STUDY ON THE EFFICIENCY OF ALGAL REMOVAL IN LABORATORY AND FIELD EXPERIEMENTS

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The CHEM-FREE project develops a process instrument to integrate and optimise three well known water treatment devices: Fibre filters, ultrasound and UV-C. The devices are expected to restrain suspended solids as well as dissolved nutrients, to counteract algae growth and care for disinfection. Additionally, it must allow the application for the treatment of different water qualities without the use of chemicals.
Lab-scale experiments regarding removal of algae in a controlled environment result in a better understanding of the principal removal mechanisms to decrease the contamination for the applications using a) the individual devices and b) their combinations to determine the optimal operational parameters.
The main output of CHEM-FREE will be a prototype and technical specification as basis for patent registration for a process control instrument allowing integration and optimisation of the three devices as an integral unit. The optimal integration and control of fibre filters, ultrasound devices, and UV-C sets will result in chemical-free water treatment enabling ecological prevention of algae, in decreasing biofilm growth on walls, in pipelines, on fittings and in containers. CHEM-FREE will provide completely new solutions for sensitive water treatment systems where chemicals are an unsatisfying solution.

30B-1: BIOFOULING AS A VECTOR FOR MARINE SPECIES INVASIONS (ORGANIZED BY NAOMI PARKER)

30B-1-1

BIOFOULING INVASIONS - WHAT CAN WE DO?

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Introduced species are one of the greatest risks to marine environments world-wide. The evidence that vessel biofouling continues to represent a serious risk for transporting species from one region to another is mounting. Research world-wide and particularly in Australia and New Zealand has demonstrated that even well maintained vessels can carry potentially invasive species, especially in niche areas including sea chests, bow thrusters, propellers, bilge keels etc., while slow moving or poorly maintained vessels can become virtual floating reefs. In New Zealand biofouling is the most likely pathway for the majority of introduced species, with new species being detected every year.
Many countries are starting to look at how these risks can be mitigated and the International Maritime Organization, having completed the Ballast Water Management Convention, is now looking at what can be done to address biofouling - the other major pathway for species spread. Critical areas that will need to be considered in minimising invasions from biofouling include: selection and maintenance of the most appropriate antifouling coating systems; effective management of niche areas, in-water cleaning, containment of biological waste in cleaning facilities, and the need for appropriate documentation. All of these areas need to be addressed while at the same time recognising that one size will not fit all, different vessel types will require different solutions and all measures will need to be practical and pragmatic. The talk will provide an overview of the biofouling invasion problem and some of the initiatives being taken internationally to address it.

30B-1-2

THE RELATIVE IMPORTANCE OF SHIP HULL FOULING AS ALIEN SPECIES INTRODUCTION VECTOR INTO EUROPE

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This presentation will show the relative importance of hull fouling of commercial vessels as species introduction vector into Europe based upon results from one of the most recent hull fouling studies, carried out in Germany (Gollasch 2002, Minchin & Gollasch 2003). In total 131 ship hulls were sampled during the study, carried out 1992 to 1996 in German ports. Most of the commercial ships investigated were docked after the regular interdocking period, i.e. ca. 3 to 5 years after the application of the antifouling paint. The ship size ranged from 1,000 to 45,330 DWT. Heavily fouled hull areas were emphasised during the study. The hull fouling samples contained 81 alien species. Most species were sessile, i.e. living attached to the hull such as barnacles and oysters. However, mobile organisms, such as decapod crabs, were also frequently found. The highest number of species in a single sample was 15 and the highest fouling density recorded during the study consisted of 107 barnacles plus 64 mussels per 100 cm^2 vessel surface. In maximum the wet weight of heavily fouled vessels was ca. 12.2 kg/m^2 vessel surface. At least one alien species was documented in 97.7 % of all hull fouling samples. During the study a new species to science was also found and described. Further, an introduction vector analysis carried out for the Baltic, North and Mediterranean Seas as well as the Atlantic seaboard of Europe in the framework of the recently completed European Union funded study "Delivering Alien Invasive Species Inventories for Europe" (DAISIE) will be discussed.

Gollasch, S. (2002): The importance of ship hull fouling as a vector of species introductions into the North Sea. Biofouling 18(2), 105-121
Minchin, D & S. Gollasch (2003): Fouling and ships' hulls: how changing circumstances and spawning events may result in the spread of exotic species. Biofouling 19 (Suppl.), 111-122

30B-1-4

BARNACLES ON THE HULL OF AN INTERNATIONAL BULK CARRIER VISITING THE PORT OF KAWASAKI, AND THEIR INVASION RISK

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Barnacle fouling on the hull of a bulk carrier from Australia was surveyed at the Port of Kawasaki in 2007 to assess their risk of invasion to the port. Twenty-one barnacle species were found on the hull: Lepadidae (1 species), Chthamalidae (1 sp.), Tetraclitidae (5 spp.), and Balanidae (14 spp.). Species composition varied with location on the hull, with small Chthamalid barnacles dominant on the bulbous bow, and large Balanids, especially *Megabalanus*, on the poop. Survival and reproductive condition also varied with hull location. More living and brooding barnacles were found on the poop than on the bulbous bow. We attribute this variation to the difference in water pressure around the hull during ship voyages. We also assessed the risk of invasion of 12-recorded species not previously found in the Port of Kawasaki or its adjacent seas. For this, we followed the method proposed by Hayes et al. (2005) with a minor modification. In addition to the difference of latitude, we also considered another environmental similarity. On the basis of these two, we concluded that *Striatobalanus amaryllis* and *Austrominius modestus* (syn. *Elminius modestus*) were the species most likely to establish in the port. The most likely source ports of these species were Newcastle, Gladstone, Brisbane and Port Headland for the former species and Newcastle and Albany for the latter.

30B-1-5

IN SITU ASSESSMENT OF RECRUITMENT AND SURVIVORSHIP OF FOULING ORGANISMS AT DIFFERENT HULL LOCATIONS AND VESSELS TYPES PLYING EXTENDED VOYAGE TIMES

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Shipping is generally accepted as the principal mechanism for the transport of marine non-indigenous species (NIS) worldwide, with hull-fouling (or biofouling) one of the primary means of dispersal. However, the relationship between vessel biofouling and specific vessel types is poorly understood - particularly the influence of vessel characteristics (e.g. speed, shape, design, use) on the composition and survivorship of fouling assemblages following extended voyages. Pre-fouled settlements plates can be attached to metal-hulled vessels using specially designed magnetic plates, enabling us to undertake *in situ* studies that examine hull-fouling survivorship over a range of vessel types and hull locations. Our recent work has focused on determining levels of recruitment experienced by different classes of vessels, assessing *en route* survival of fouling taxa, identifying morphological characteristics of fouling taxa that increase survivorship over prolonged vessel voyages, and identifying 'niche' areas of a vessel that have an increased propensity for fouling. In this study, we attached both blank and pre-fouling settlement plates to a range of vessel types to assess the recruitment and/or survival of common fouling taxa over extended vessel voyage times (7 – 28 days). We also compared the recruitment and survivorship of organisms within different areas of the same vessel hull (e.g. exposed bow region versus sheltered areas around the stern). This paper presents results of these recent field trials and discusses potential future directions of our research.

30B-1-6

EXOTIC ORGANISMS IN FOULING COMMUNITIES ON BULK CARGO CARRIERS, CONTAINER AND PCC SHIPS

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We investigated fouling communities on four intercontinental bulk cargo carriers, two intercontinental container ships and two intercontinental PCC ships in Japan to identify 90 taxa of seven phyla, including nine species of barnacles, amphipod and bryozoan which have not been yet found in Japanese waters, and further six species of barnacles and mussels exotic to but already recorded in Japanese waters. The bulk cargo carriers showed higher species richness and larger biomass/abundance of fouling communities than both of the container and the PCC ships. The barnacles indicated the highest species richness and largest biomass/abundance in these fouling communities: *Balanus trigonus* in first rank followed by *Tetracilitella purpurascens* and *Amphibalanus improvisus* on smooth hulls, while *Megabalanus coccopoma* in first rank followed by *Austrobalanus imperator*, *M. rosa* and *M. volcano* on rudders, rope guards and sea-chest. The above three species (*Tetracilitella purpurascens*, *Megabalanus coccopoma* and *Austrobalanus imperator*) have not been yet found in Japanese waters. It was indicated that living/dead barnacle shells contributed to structuring habitats suitable for free-living organisms on hulls. About 200 species as native barnacles, as well as six species as exotic ones, have been found in Japanese waters. In near future, species newly exotic to Japan are possible to be introduced mainly by bulk cargo carriers.

30B-1-7

VESSEL FOULING IN NEW ZEALAND; HOW BIG THE TOOLBOX NEEDED?

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The hulls and other external surfaces of vessels are ideal pathways for the transport of invasive species between and within countries. Merchant vessels, mostly foreign-flagged, make more than 7000 visits to New Zealand ports annually and New Zealand is also a popular destination for international recreational yachts, which arrive predominantly during the Austral spring/summer period. Recreational vessels are an integral part of the outdoor lifestyle of New Zealanders with 15% of households owning at least one boat and 41% of New Zealanders going boating each year. Added to these vessels is a domestic fleet of approximately 1700 registered fishing vessels. New Zealand began quantifying the biosecurity risks associated with the biofouling on these vessels in 2000 and more recently, has been developing tools to manage them. Significant differences in risk have been identified, both among and within vessel types, so multiple tools are needed in the management toolbox. The diversity of this vector of invasive species and the approaches being taken in New Zealand to find tools to manage the risks are discussed.

30B-1-8

DOES SPECIES RICHNESS AND COMPOSITION INFLUENCE RECRUITMENT OF INVASIVE SPECIES? A CASE FOR THE GREEN MUSSEL IN TAMPA BAY, FLORIDA, USA

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Since its introduction in 1999, the green mussel, *Perna viridis*, has become one of the most abundant and prolific organisms in epibenthic coastal communities of Florida, colonizing a wide variety of artificial and natural hard substrates, and displacing resident species. To better understand the role of resident species on invasion processes, I tested the long-held view that communities of high diversity should be more resistant to invasion by new species. For this, I examined the effect of species richness and composition on mussel recruitment by gardening assemblages on acrylic settlement plates for 1 year. Gardened assemblages consisted of one, two or three common fouling species (barnacles, encrusting bryozoan, and/or colonial tunicate), which were used to produce four levels of richness (0, 1, 2, and 3). Paired combinations were used for the 2-species treatments. Field experiments showed that mussel recruitment was positively and significantly associated with species richness and final percent cover of plates and mussels avoided colonizing areas of open space, suggesting that space occupation and/or habitat heterogeneity created by resident species may be an important factor for mussel recruitment. Mussel recruitment varied with assemblage composition with mussels more frequently associated with barnacles than with the bryozoan or colonial tunicate. This suggests that resident species could play a disproportionate role on the recruitment of introduced species, and with habitat-forming foundation species such barnacles distributed throughout estuaries, could increase opportunities for mussels to colonize and invade these coastal communities.

30B-2: BIOFOULING AND ANTIFOULING SYSTEMS AT POWER PLANTS AND FISH NETS

30B-2-1

BIODETERIORATION AT THE POWER STATIONS IN JAPAN

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1.SRB induced corrosion of copper alloys(Polluted water corrosion,sulphide corrosion)
Polluted waters are divided into a)coming from outside and b)production in the station.
The former are divided into a)continuous coming(no coming at present) and b)short period of time(summer,dredging etc.)The latter are divided into a)macro production(no flowing water) and b)micro production(under attached macro sessile organisms)
Counter measures are a)Ti b)APF(by Sumitomo.More than 430thousands tubes have been delivered.)c)AP bronze by Sumitomo d)Ferrous protective film
2.Mn- oxidizing bacteria induced corrosion of copper alloys .
Mn- oxidizing bacteria in the deposit oxidizes Mn^{2+} in the water and Mn^{4+} deposits on the surface of cell. Mn-rich deposit is hard,but easily peel off by debris.
Ferrous ion injection stops it completely.
3.Erosion-corrosion by macro sessile organisms of copper alloys(3/4 of leakages)
Macro sessile organisms are divided into a)attached one and b)clogged one.
Spongeball cleaning(380units have been delivered by Taprogge) prevents attachment.Mussel filter(565units have been delivered by Taprogge) is very effective to remove debris.
Inlet attack have been preventing by Cumberland method.
4.Organism induced erosion-corrosion.
It occurs at the scraped groove by passing of macro organism,at the corrosion pit by the sulphide corrosion.
Ferrous ion injection recovers the grooves and the pits,therefore proceeding corrosion stops.
5.Heat transfer decreasing by macrofouling
6.Micro-biofouling control on the surface of tubes.
Spongeball cleaning.
7.Macro-biofouling control.
a)High flowrate of water at the full load.(about 10% of the fossil stations)b)Ultra low concentration chlorination with 0.03~0.05ppm residual chlorine is effective.(only several stations)There are many stations with no effective chlorination less than 0.03ppm. c)Copper powder and Cu2O contained paints(1/3 of the fossil stations)d)Silicone paint(44% of the fossil stations)

30B-2-2

MONITORING OF BLOCKAGES BY SHELLFISHES IN A PIPE UTILIZING AN OPTICAL FIBER ACOUSTIC EMISSION SYSTEM

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A blockage inside pipes by shellfishes is one of the serious problems for safe operation in plants. We developed the system to monitor the blockage inside a pipe utilizing acoustic emission(AE) technique. We used an optical fiber as an AE sensor. The monitoring system was based on a Michelson-type laser interferometer with phase compensation feedback circuit. The sensor is intrinsically safe, immune from corrosion and electro magnetic noise. Additionally, an optical fiber is flexible, light weight and has water proof.
We first studied the mechanism and characteristic of AE signals from the blockage. The blockage was modeled by inserting small shells into a PMMA pipe. AE signals were monitored by winding an optical fiber multiple times around the pipe. We found that AE was caused by collisions of the blockage to the wall. But, there is no clear relationship between the flow velocity and amplitude or frequency component of the detected AE signals. However, generation rate of AE signals was increasing depending on the flow velocity.
We next monitored AE from pipes which were blocked by attached shellfishes (Mytitus Edulis). We prepared three pipes with different shellfish number. AE generation rate increased corresponding to number of shellfishes in the pipe. AE waveforms were classified according as amplitude of the detected AE signals. It was estimated that strong AE was produced by the shellfish attached weakly and weak AE was produced by the shellfish attached strongly. The developed system successfully monitored AE from blockage by shellfishes.

30B-2-3

CHLORINATION FOR BIOFOULING CONTROL IN POWER PLANT COOLING WATER SYSTEM – A REVIEW

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Fresh water is becoming a scarce commodity day by day and thus power plant authorities are turning into sea to make use of the copious amount of seawater available at an economical rate for condenser cooling. Unfortunately, biofouling; the growth and colonization of marine organisms affecting the smooth functioning of power plant plague these cooling water systems. This is more so, if the plant is located in tropical climate having clean environment, which enhances the variety and density of organisms. Thus, biofouling needs to be controlled for efficient operation of the power plant. Biocide used for biofouling control is decided based on three major criteria viz: it should be economically, operationally and environmentally acceptable to the power plant authorities. Chlorine among others stands out on the top and meets all above requirements in spite of a few shortcomings. Therefore it is no wonder that chlorine still rules the roost and chlorination remains the most common method of biofouling control in power plant cooling water system all over the world. Although, it is easier said than done, a good amount of R&D work is essential before a precise chlorination regime is put into pragmatic use.

This paper discusses the problem of biofouling in each section of cooling water system such as intake, pre-condenser & condenser section and suggests appropriate remedial strategy for each of them. To delineate the above a typical case study is discussed. It also reports in details the chemistry of chlorination such as chlorine demand, chlorine decay, speciation of chlorine residual and role of ammonia on chlorination in biofouling control. In addition, the environmental connotation of chlorination and its synergistic effect with temperature is also touched upon.

30B-2-4

OPTIMISING COOLING SEAWATER ANTIFOULING STRATEGY THROUGH PULSE-CHLORINATION®: ASSOCIATED BENEFITS TO OPERATING FACILITIES FROM ADOPTING AN ENVIRONMENTALLY FRIENDLY / BAT TECHNOLOGY

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Sodium hypochlorite is the industry standard biocide for controlling biofouling, however, the application involves environmental issues surrounding the discharged residual chlorine (oxidants) and formation of chlorination by-products. Pulse-Chlorination®, a new method for chlorine dosing in cooling water developed by KEMA, is based on the principle that bivalve biofouling species like oysters, mussels and clams, in general have a recovery period after exposure to a chlorination period, before fully opening their valves and start filtering water. Pulse-Chlorination takes advantage of this recovery time by short successive pulses of chlorine, alternating with periods without chlorine. Using continuous chlorination, bivalves will close for longer periods and switch to an anaerobic metabolism. When dosing intermittently, i.e. for several hours a day, bivalves will only close during the dosing period. During Pulse-Chlorination, bivalves are forced to continuously switch their metabolic mode between aerobic and anaerobic, leading to a rather quick physiological exhaustion. This dosing procedure does not apply chlorine as biocide, but rather as a trigger to force mussels to switch between their metabolic modes, resulting in a rapid effect. Pulse-Chlorination is Best Available Technique under the European terms of the Integrated Pollution Prevention and Control (EU-IPPC) for macrofouling mitigation in once-through seawater systems and is successfully implemented through on-site field tests worldwide (Europe, Middle East, Asia and Australia). The method is universally applicable, but needs to be attuned to local conditions. The overall aim is to ensure an optimal and reliable cooling seawater system and condenser / heat exchanger performance. The backgrounds and main results of Pulse-Chlorination at different locations will be discussed in the light of improving the protection of the cooling water system from biofouling, reduce the amount and concentration of oxidants discharged to sea in line with (new) stringent regulatory requirements and lower the operational costs of related equipment (e.g., electro-chlorinators).

30B-2-5

IN SITU FEEDING OF THE HYDROID *TUBULARIA* SP. ON AQUACULTURE NETS AND THEIR LARVAL RELEASE AS A RESPONSE TO THE WASHING PROCEDURE

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Leif M. Sunde, SINTEF Fisheries and Aquaculture, CREATE

Biofouling on aquaculture nets is known as a significant problem for the global fish farm industry and is a focus area within CREATE. The Norwegian aquaculture industry faces an increasing problem due to the rapid re-growth and re-colonization of the hydroid *Tubularia* sp. on the nets after the washing procedure. To investigate nutritional aspects of *Tubularia* sp. on aquaculture nets, the total lipid content and fatty acid composition of both hydroids and fish feed were measured and compared. The results demonstrate that *Tubularia* sp. feed on particles of fish feed, which may partly explain their high growth rates on nets.

The cleaning of the nets by high pressure underwater washing is common in the Norwegian fish farm industry. To investigate the response of *Tubularia* sp. to the washing procedure, water samples were taken before, during and after the washing at 10 m and 25 m depths, respectively. The samples were categorized as swimming actinula larvae, settled actinulae, and hydranths with or without gonophores. The number of actinulae increased up to five times (mean ± SE: 26 ± 4.2 actinulae at 10 m depth and 40.3 ± 6.4 actinulae at 25 m depth) during the washing procedure, compared to the number of larvae before the washing (5 ± 1.5 actinulae at 10 m depth and 7.7 ± 0.7 actinulae at 25 m depth). After less than 2 h, the number of actinulae decreased to the values before the washing (5.7 ± 1.2 actinula larvae at 10 m depth and 7.3 ± 3.2 actinulae at 25 m depth). The numbers of settled actinulae and polyps with and without gonophores also significantly increased during the washing procedure. These results suggest that the washing procedure itself promotes the spreading of *Tubularia* sp. and the rapid re-colonization on the cleaned netting.

30B-3: FLOW EFFECTS AND SURFACE INTERACTIONS

30B-3-1

EFFECTS OF BIOFOULING AND ITS SPATIAL HETEROGENEITY ON SHIP RESISTANCE AND POWERING

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Predictions of full-scale ship resistance and powering are made for antifouling coating systems with a range of roughness and fouling conditions. The estimates are based on results from laboratory-scale drag measurements and boundary layer similarity law analysis. Predictions are made for a mid-sized naval surface ship at cruising speed and near maximum speed. The results for uniform fouling coverage indicate that slime films can lead to significant increases in resistance and powering, and heavy calcareous fouling results in powering penalties of up to 86 percent at cruising speed. These estimates show good agreement with results from full-scale ship power trials. The present work is an extension of that reported in Schultz (2007) in which the results of recent experiments are used to account for nonuniformity in fouling coverage. This spatial heterogeneity is shown to have a significant influence on the predicted resistance and powering penalties.

Schultz, MP 2007, Effects of Coating Roughness and Biofouling on Ship Resistance and Powering, *Biofouling* 23: 331-341.

30B-3-2

A ROLE OF HYDRODYNAMIC INVESTIGATIONS IN MULTIDIRECTIONAL ASSESSMENTS OF MODERN ANTIFOULING COATINGS ELABORATED IN FIELDS OF NANOTECHNOLOGIES

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Cezary Kania, Ship Design and Research Center S.A. (CTO)

The paper intends to present a role hydrodynamic tests in the assessment of improved, anti-fouling coatings, based on advanced nanostructured materials created by molecular surface engineering, elaborated within the EU sponsored Ambio Project. After the initial stage of nanomaterials technologies, the newly elaborated and formulated coatings are to be a subject of the both biological and in-field evaluations. Where the lattest depend on end-use applications, from which the maritime sector puts very high requirements in scope of coating properties and ship hull drag reduction, seen from the point of view of costly fuels savings.
In this situation, hydrodynamic investigations are a group of the complex field testing which aims to quantify a relationship between widely understood surface roughness and hydrodynamic performances so as to determine the branch usefulness of new products. It demands a respective surface characterisation from the phase of quasi-industrial coating application technology, up to multidirectional quantitative surface assessments, meeting the maritime requirements. Since, the hydrodynamic tests can be conducted in, different in size, testing facilities like big towing tanks or small cavitation tunnels, a series of samples including: flat plates, axisymmetric bodies, hydrofoils and propeller models has been selected for tests. The scheduled, dedicated experiments will include not only new coatings but presently used reference ones. The received results will be elaborated in the uniform way to be afterwards compared with existing, objective data banks contents, giving respective recommendations for end users. As an additional achievement, it will make possible to determine an optimum testing method in respect to both the testing facility potential and disposable coating quantities.

31A-1: PLENARY LECTURE 4

31A-1-1 (Plenary)

DEVELOPMENT OF MARINE PAINT AND ENVIRONMENT

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As Japan is a seafaring country, a lot of people have taken part in the development of the marine business since olden days. The first patent at Japan was claimed in 1885 which was "Hotta's anticorrosion formula and its application method" claiming anti-corrosion and also anti-shell effects. It looks marine paint was militarily important at that time and thought the high technology.

When looking back on the history of the development of the antifouling paint in Japan in early 1970's, organic arsenic and the organic mercury agents were introduced as active substances and withdrawn because of adverse effect to worker, which led to establish consortium SR-141 participated by shipbuilding and paint industries, academy and authority to investigate workers' safety. In the same decade at 1973, chemical substance control law of Japan was established to control the adverse effect of PCB.

In 1980's organic-tin compounds were introduced for antifouling paints. Although the consortium reviewed the workers effect of organic-tin compound, its increment use caused accumulation of organic-tin tin in sediment at seabed, pressed Japanese industry for the decision to introduce the self-regulation to ban the organic-tin since 1992. At the same time, consortium RR-76 started a study on risk assessment of alternative active substances including TBT, which influenced the adoption of AFS convention 2001. This self-regulation of TBT have led the development of TBT-free self polishing antifouling system in Japanese industry.

The risk management of chemical product is social requirement. Johannesburg Resolution was adopted in 2002, which targeted chemical risk reduction by the year of 2020.

Looking back to the history of marine paint, we are required to find out the adverse effect of the chemicals before introducing that product to the market. Paint industries are using wide range of chemicals and which will be reviewed by the year of 2020. Harmonization with environment is one of the targets of development for marine industries and we hope many active researches are published.

31A-2: REGULATION OF ANTIFOULANTS AND ENVIRONMENTAL ISSUE

31A-2-1

ANTIFOULING PAINTS - PAST, PRESENT AND FUTURE

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Throughout history the biofouling of ship hulls has been a major problem for mariners around the world. They had to constantly work to protect their wooden ships from the invasion of Teredo worms and to eliminate marine growth slowing the ship's speed. The development of copper sheathing was a major advance in fouling control; but as steel replaced wood, new solutions were needed to prevent unwanted marine growth. This search culminated in self-polishing paints using organotin (TBT) biocides. We will discuss how using TBT biocides in combination with cuprous oxide appeared to be the ultimate solution but how the harmful environmental effects and persistence of tributyltin outweighed the performance advantages. It took 20 years of work at the International Maritime Organization (IMO) to finally ban the use of organotins as an antifouling biocide. In that time the barrier to new antifouling systems has increased as new standards and regulations have been developed to measure the environmental impact of new technologies. People developing new antifouling systems now have to deal with the Biocidal Product Directive, the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, the Japanese proposed ISO system for measuring risk and the recognition that fouling on ships can be a major contributor to the transmigration of species. With globalization the modern world is interconnected by and dependent on the oceans for the international shipping of food and goods. These issues have become of paramount importance. As we search for environmentally friendly solutions to fouling on ships, both scientists and mariners must understand this new world in which we live and work.

31A-2-2

INDUSTRY IN IMO - A RESPONSIBILITY BEYOND BUSINESS OPPORTUNITIES

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It is 60 years since the Convention establishing IMO was adopted and 50 since it entered into force. Since the early 1970-ties, IMO has been an influential force in protecting the marine environment. Recent examples are the Anti-fouling System Convention which will enter into force in September this year and the 2004 Convention on Ballast Water Management. Shipyard waste is currently on the agenda. IMO established in February a correspondence group that will start work leading to measures for reducing the transport of invasive species on ships' hulls.

Non-governmental organisations representing industry or environmental groups play a vital role in the development of IMO instruments. The International Paint and Printing Ink Council (IPPIC) early this year received its formal observer status in IMO. However, the paint industry had a strong involvement in an environmental issue in IMO already a decade ago during the development of IMO's Anti-fouling System Convention. IPPIC's observer status in IMO commits the organisation to be active and constructive when having expertise to offer.

Classification societies play a key role, not only in the development but also in the enforcement of several IMO Conventions. Therefore, they have an extra responsibility for being attentive to the spirit and the text of these instruments. IPPIC has earlier this year submitted a letter of complaint to the International Association of Classification Societies. The type approval schemes introduced by a couple of classification societies place a costly and unnecessary burden on industry.

The paint industry appreciates IMO's new initiative for reducing the problem with invasive species transported on ships' hulls. Together with IMO's increased focus on greenhouse gas emissions from the world's merchant fleet, this represent a recognition of the huge environmental benefits of effective fouling protection. Paintmakers will, however, work even more vigilantly to avoid a similar situation with respect to classification societies as is now the case for the Anti-fouling System Convention.

31A-2-3

ENVIRONMENTAL RISK ASSESSMENT FOR ANTIFOULING PRODUCTS FOLLOWING EU GUIDELINES – SOME EXPERIENCES AND EXAMPLES

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Regulatory compliance systems are becoming increasingly more sophisticated within Europe. The implementation of the Biocidal Products Directive (BPD 98/8/EC) will fundamentally change the way that antifouling products are reviewed and authorised in the majority of European countries. The presiding central tenet of the BPD is one of risk assessment; a task which relies upon defining environmental inputs of biocides as a result of antifouling use, not only during the service of the paint but at the application and removal stages as well.

This paper gives an overview of the common approaches used to establish the risk of using biocidal antifouling paints as defined under the current BPD guidelines and an insight into the complexity of assessing the risk of biocide use. It considers the methods available for the determination of release rates and identifies other critical information required in order to provide a realistic risk assessment for all typical exposure scenarios. Specifically: Defining the exposure scenario; Establishing the release rate; Market share issues; Defining the Predicted No Effect Concentration; Calculating the Predicted Environmental Concentration.

The use of the computer modelling software MAMPEC (Marine Antifoulant Model to Predict Environmental Concentration) is also presented as an effective tool for providing estimates of environmental loading.

These critical points are considered for two theoretical biocides outlining the levels of detail required to ensure confidence in the results. It is concluded that whilst these methods are effective in predicting the potential risk of using antifouling paints, the confidence in the final result is defined by the amount of available data. The BPD and the risk assessment processes therein may restrict the development of new biocides within Europe. More pragmatic approaches to risk assessment will be needed to ensure future product development.

31A-2-4

STANDARDIZATION OF MARINE ENVIRONMENTAL RISK ASSESSMENT METHOD FOR ACTIVE SUBSTANCES IN ANTI-FOULING SYSTEMS

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Tetsuya Senda, National Maritime Research Institute

A draft standard of "Marine Environmental Risk Assessment Method on Active Substances Used for Anti-fouling Systems on ships" is prepared as a part of the marine environment protection – Risk assessment on anti-fouling systems on ships.

International Convention on the Control of Harmful Anti-fouling Systems on Ships (the Convention) was adopted at the IMO Diplomatic Conference in October 2001, and the Convention is expected to become effective in September 2008. Annexes 2 and 3 of the Convention include the list of information required to determine whether the anti-fouling system is harmful, but the marine environmental risk assessment method for evaluating the harmfulness of the anti-fouling system is not included.

The system being proposed in this draft aims to provide

- a useful method to be used for supporting the evaluation of the harmfulness of the anti-fouling system in the Convention, and
- a pragmatic approach to introduce systems in the countries with either no system, or a less-well developed system and to allow such countries to further contribute to protection of the aquatic environment.

This draft standard specifies a method of risk assessment that performs to protect marine environment from negative impacts by active substances used intentionally for the anti-fouling system on ships applied during its service time. This draft standard can also be used for risk assessment of their impacts in freshwater environment.

Drafting of this standard of International Standardization Organization (ISO) has been conducted by the Japan Ship Technology Research Association (JSTRA) since 2006, and was funded by a grant from the Nippon Foundation.

31A-2-5

PHOTODEGRADATION OF ANTIFOULANTS WITH WATER DEPTH: CORRELATION OF LIGHT INTENSITY AND CALCULATED BOND ENERGIES

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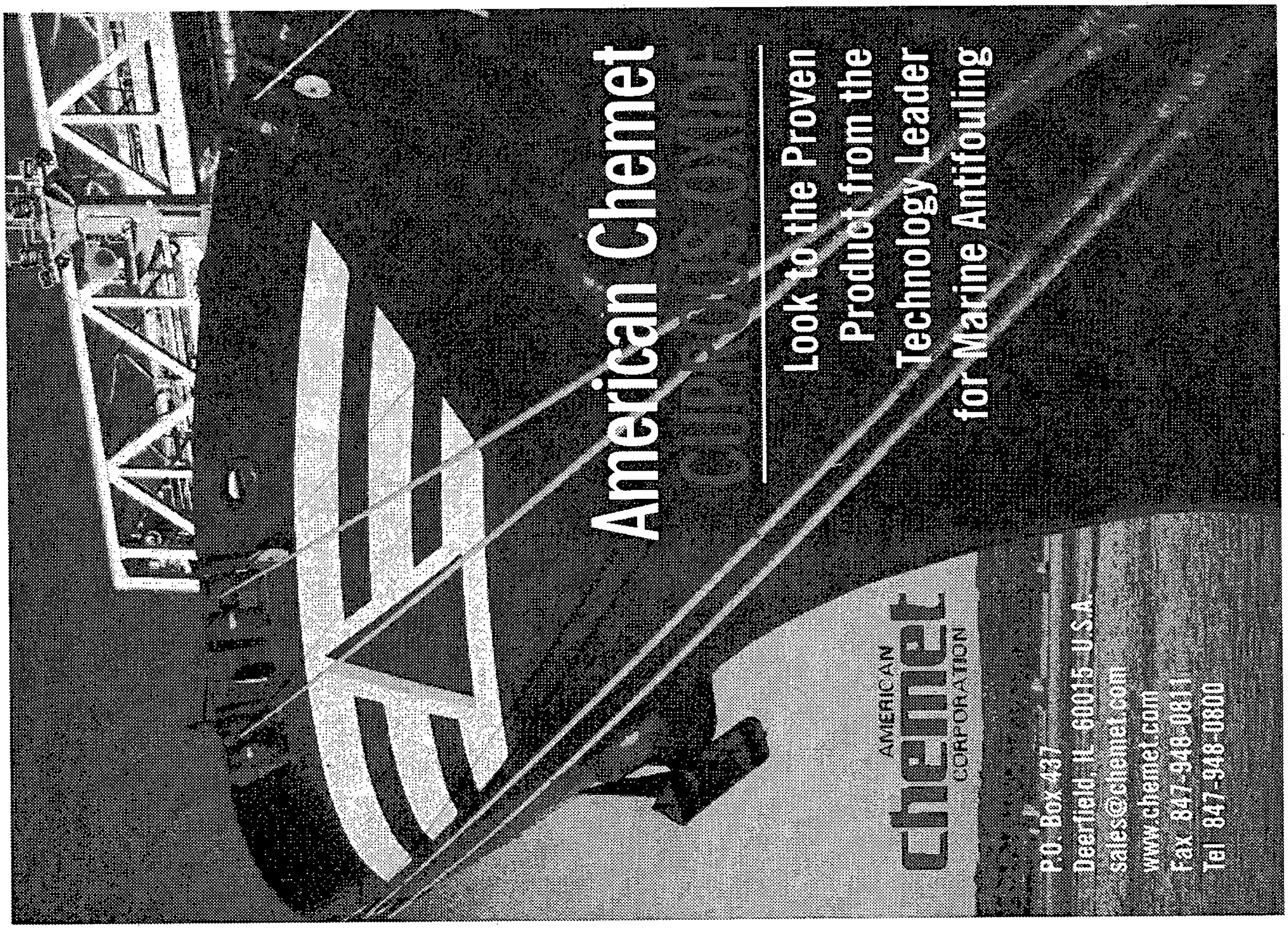
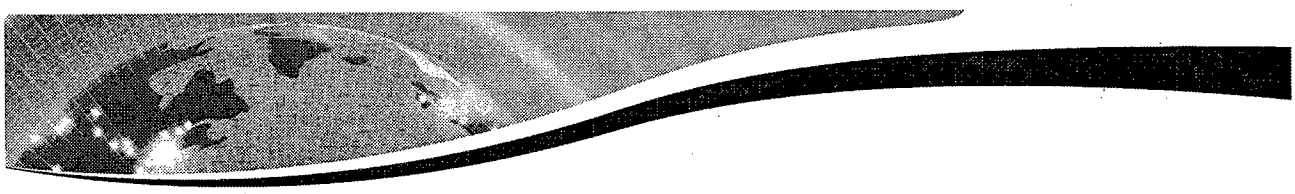
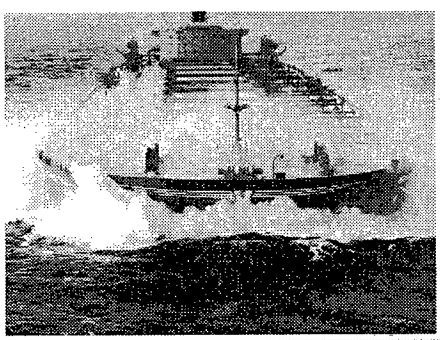
Previously we have examined the effect of water depth on the photodegradation of five marine antifoulants; DCOIT [4,5-dichloro-2(n-octyl)-isothiazolin-3-one], Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], Irgarol [2-tert-butylamino-4-cyclopropylamino-6-methylthio-1,3,5-triazine], TPBP [triphenylboron-pyridine], CuPT/ZnPT [zinc/copper bis-1-oxide-2(1H)-pyridenethionate]. Kinetic analysis was performed on these five compounds in two harbors, Oslofjord, Norway and Osaka Bay, Japan. DCOIT, Diuron, and Irgarol had essentially no photodegradation even in full sunlight at the water surface. In full sunlight at the water surface, TPBP and CuPT/ZnPT showed rapid photodegradation, however with increasing water depth photodegradation kinetics decreased. At one meter water depth, the rate of photodegradation was decreased by about 50% compared to the surface. In this current study we have expanded the scope of this study by measuring the light intensity at varying depths in a number of harbors. Additionally, using various thermodynamic models we have calculated the light energy required to break the initial chemical bond on these five molecules. By comparing the measured light intensity to the bond cleavage energy we can correlate the rate of photodegradation at varying water depth in a number of harbors and develop a general equation to determine the effect of photodegradation kinetics for marine antifoulants at varying water depths.

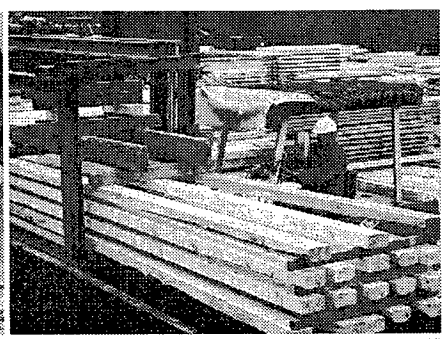
AUTHOR INDEX





Spiess-Urania is a leading source of copper chemicals for industry and agriculture. In our Hamburg plant copper-based active ingredients are manufactured for use in antifoulings, wood preservatives, and agricultural pesticides.





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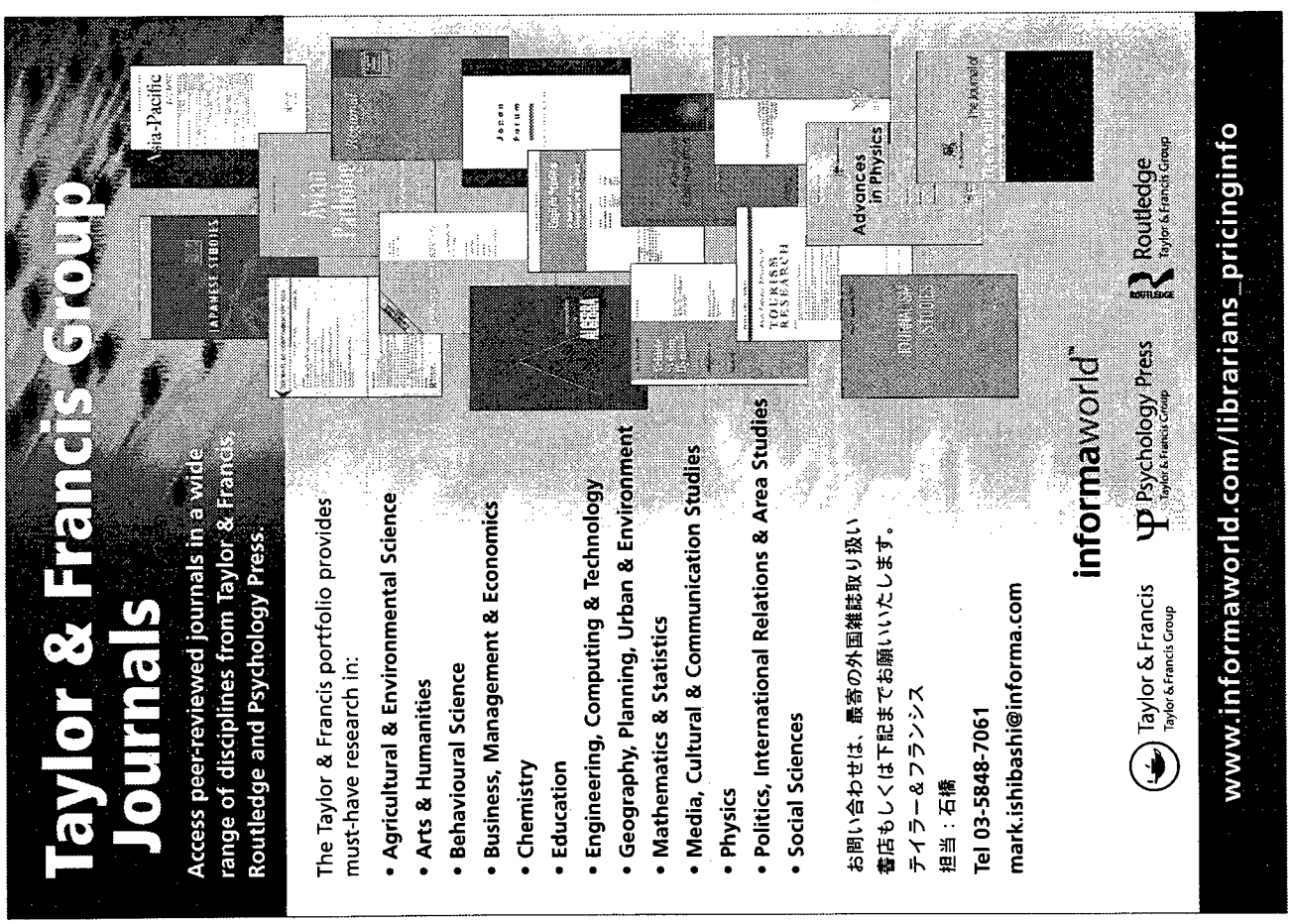

July,13, 2012

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Dear John

Enclosed are the abstract book of ICMCF14 Kobe, and the CD.

Form the top page of the CD, clicking the date, you can see the program of that day, and the numbers in red indicating that presentation file is provided.
We have made this CD, after the congress. Honestly speaking, before the congress we had no intention to make such CD, but we had some amount of money left, because of unexpected number of attendees. Therefore, we decided to s to share the benefit, sending the CD to all the attendees. Around the half of the presenters were eager to provide the presentation files.

The front page of the abstract book was designed by a friend of my daughter. She is studying Japanese painting. I hope you will find something Japanese.

Best regars,

Kiyoshi