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6th INTERNATIONAL CONGRESS ON MARINE CORROSION AND FOULING

6ème CONGRES INTERNATIONAL DE LA CORROSION MARINE ET DES SALISSURES

MARINE BIOLOGY

Athens 5-8 SEPTEMBER 1984

GREECE

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MARINE BIOLOGY
BIOLOGIE MARINE

	Pages
L.YANSHUN, Y.DEZAN, L.ZIYUAN, Y.RUIMEI: "Characteristics of Microfouling Organisms in Xiamen Harbour" (China)	9-21
Z.G.HUANG: "Ecological and Biological Studies of Fouling Organisms Along the Coast of China"(China).	23-37
A.F.A.GHOBASHY: "Polycheatous Fouling in the Egyptian Marine Waters" (Egypt)	39-51
A.F.A.GHOBASHY, S.F.HAMADA: "The Fouling Organisms in the Damietta Harbour (Egypt)" (Egypt)	53-63
I.TITTLEY, R.L.FLETCHER: "Sullom Voe Oil Terminal Shetland: Benthic marine algal fouling communities" (England)	65-79
N.GUNN, R.L.FLETCHER, E.B.G.JONES: "Observations on the Strength of Attachment of Spores and Germlings of the Marine Fouling Alga Enteromorpha" (England).	81-98
S.PYNE, R.L.FLETCHER, E.B.G.JONES: "Attachment Studies on Three Common Fouling Diatoms" (England)	99-112
I.BROWN, G.BLUNN, E.B.G.JONES: "Attachment of Marinw Fouling Protozoa" (England)	113-127
R.L.FLETCHER, R.E.BAIER, M.S.FORMALIK: "The Influence of Surface Energy on Spore Development in Some Common Marine Fouling Algae" (England)	129-144
N.BALAKRISHNAN NAIR: "Ecology of Reproduction in Tropical Shipworms" (India)	145-157
B.MEENAKUMARI, N.BALAKRISHNAN NAIR: "Ecology of Fouling in Cochin Harbour" (India)	159-171

	Pages
A.TURSI, A.MATARRESE, C.MONTANARO: "Mytilus Galloprovincialis(L.) in Fouling Research" (Italy).....	173-180
S.GERACI, V.ROMAIRONE, P.PICONE: "Balanus Larvae (Cirrop dia) in Genoa Harbour" (Italy).....	181-194
G.RELINI: "Three Years Investigation on Macrofouling of A Tyrrhenian Power Station" (Italy).....	195-210
P.A.COOK, J.R.HENSCHEL: "The Importance of a Primary Film of Microorganisms on the Subsequent Establishment of a Macrofouling Community" (South A rica).....	211-220
C.H.GREENE, A.SCHOENER: "Multivariate Analysis of Three-Dimensional Data in the Study of Succession in Marine Fouling Communities" (U.S.A.).....	221-235
J.R.DePALMA: "Fouling Production in the World Ocean" (U.S.A.).....	237-244
D.P.DeVORE, G.H.ENGEBRETSON, C.F.SCHACHTELE, J.J. SAUK: "Isolation and Characterization of Adhesive Proteins Secreted by the Sea Mussel, Mytilus edulis" (U.S.A.).....	245-258
C.DREW HARVELL, Ch.GREENE: "Foul Play: Some Consequences of Life Histories Among Temperate Bryozoans" (U.S.A.).....	261-267
ANTIFOULING COATINGS PEINTURES ANTISALISSURES	
C.A.GIUDICE, J.C.BENITEZ, V.RASCIO, O.SINDONI:"Antifouling Paints of the Soluble Matrix Type Based on WW Rosin and Chlorinated Rubber" (Argentina)...	269-281
C.A.GIUDICE, J.C.BENITEZ, B.del AMO: "Bioactivity of Chlorinated Rubber Antifouling Paints Tested in Sea Water" (Argentina).....	283-292
C.A.GIUDICE, B.del AMO, V.RASCIO: "Dissolution of Cuprous Oxide and its Influence on Antifouling Paints Effectiveness" (Argentina).....	293-305

Pages

E.B.NIELSEN: "Bio-Active Materials for Antifouling Coatings" (Denmark)..... 307-324

M.E.CALLOW: "A World-Wide Survey of Fouling on Non-Toxic and Three Anti-Fouling Paint Surfaces" (England)..... 325-346

A.A.KARANDE,R.B.SRIVASTAVA: "Pollution of Coastal Sea Water and Sulfate Reducing Bacteria" (India).. 347-354

N.UNNIKRISHNAN NAIR, A.G.GOPALAKRISHNA PILLAI, K. RAVIDRAN, R.BALASUBRAMANYAN: "Studies on Marine Biofouling and its Prevention with Special Reference to Fishing Craft" (India)..... 355-368

K.RAVIDRAN, A.G.GOPALAKRISHNA PILLAI: "Observations of the Inter-relation on Marine Corrosion and Fouling in a Tropical Environment" (India)..... 369-384

SAMIR M.SAID, N.J.PAUL, DARWISH M.GOBAISI: "Deterioration of Paints and Coatings by Marine Organism" (ABU DHABI)..... 385-398

C.M.SGHIBARTZ: "Antifouling Paints-Today and Tomorrow" (Norway)..... 399-413

MICROBIOLOGICAL CORROSION
CORROSION MICROBIOLOGIQUE

E.S.AYLLON, D.A.BISCIONE, B.M.ROSALES: "Microbiological Corrosion in Water Displaced Ship Fuel Tanks" (Argentina)..... 417-428

R.S.SALVAREZZA, H.A.VIDELA: "Corrosion of Carbon Steel Induced by Sulfate Reducing Bacteria. Effect of Chloride and Sulfide Anions" (Argentina)..... 429-441

L.RUIMU, L.DEKAI, H.XUEBAO, Z.JIANCHENG: "The Effects of Macro-Fouling Organisms on Steel Corrosion and its Electrochemical Behavior" (China).... 443-451

M.J.WHITE, D.C.SIGEE, S.J.HAWKINS, E.J.BELLINGER, S.J.HUTCHINSON, S.WILSON: "Association Between Early Marine Microfouling and Corrosion of Steel" (England)..... 453-467

	Pages
R.G.J.EDYVEAN: "Interactions Between Microfouling and the Calcareous Deposit Formed on Cathodically Protected Steel in Sea Water" (England).....	469-483
Ch.SINICKI, C.GABORIAU: "Étude électrochimique d´un acier dans des cultures de bactéries sulfato-réductrices. I-Mécanisme de corrosion" (France)..	485-496
C.P.DE: "Present State of Technology for Control of Marine Corrosion and Fouling in Tropical Indian Waters" (India).....	497-509
B.LITTLE, M.WALCH, P.WAGNER, S.M.GERCHAKOV, R. MITCHELL: "The Impact of Extreme Obligate Thermophilic Bacteria on Corrosion Processes" (U.S.A.).	511-520
D.MARTINEZ, R.BASTIDA, G.BRANKEVICH: "Ecological Aspects of Marine Fouling at the Port of Ingeniero White" (Argentina).....	521-537
A.ABU-SAFIAH: "Performance of Copper Base Alloys in Polluted Sea Water" (Jordan).....	539-555
R.J.DAVIES, R.L.FLETCHER, S.E.J.FURTADO: "The Effects of Tributyltin Compounds on Spore Development in the Green Alga Enteromorpha Intestinalis(L)Link" (Netherland).....	557-565
G.BRANKEVICH, R.BASTIDA, D.MARTINEZ: "Ecological Aspects of Marine Fouling at the Necochea Power Station (Puerto Queuqen,Argentina)" (Argentina)....	567-583
H.GUCINSKI, R.E.BAIER, A.E.MAYER, M.S.FORMALIK, R. W.KING: "Surface Microlayer Properties Affecting Drag Phenomena in Seawater" (U.S.A.).....	585-604
A.E.MEYER, M.S.FORMALIK, R.E.BAIER, R.W.KING, J.J. ZAMBON, P.S.HUBER, B.J.WATTLE, H.GUCINSKI: "Microfouling Survey of Atlantic Ocean Waters" (U.S.A.).	605-621
E.D.MOR, L.FERRETTI, P.FERRARONE: "Compositions anti-salissures pour des structures immergées fixes" (Italy).....	623-636

MARINE BIOLOGY

BIOLOGY MARINE

CHARACTERISTICS OF MICROFOULING ORGANISMS IN XIAMEN HARBOUR

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ABSTRACT

The essential kinds of microfouling organisms on non-toxic substrates exposed to seawater in Xiamen Harbour were bacteria, diatoms, fungi, micro-algae and protozoa, of which bacteria and diatoms dominated. The order of adhesion was bacteria, diatoms and fungi. The microfouling peak would be from June to August in a year. Apparent two-tier fouling layer structure occurred on plates after 12 days' exposure by observation using SEM. The stages of slime film development are discussed in this paper.

RÉSUMÉ

Les microorganismes de la salissure fixés sur les plaques non-toxiques immergées dans le port de Xiamen sont composés essentillement par des bactéries, diatomées, fungi, microalgues et protozoaires parmi lesquels les bactéries et diatomées représentent les peuplements dominants. L'ordre de la fixation est successivement: bactéries, diatomées et fungi. Après l'examen des variations annuelles, on constate que la période de fixation la plus élevée est au mois de juin-août. L'observation sur SEM indique qu'au 12^{e} jour de l'immersion de la plaque, apparaît des couches doubles sur la pellicule dont le développement est discuté dans l'article.

INTRODUCTION

The slime film on a solid surface exposed in seawater ge-

nerally consists of certain microorganisms such as bacteria, diatoms, micro-algae, fungi and protozoa. However, the species, numbers, and the successive sequence of the organisms in a fouling community vary with sea locations. It has been reported that diatoms first occured on a freshly exposed surface in some harbours of New Zealand, Australia, England[1-3], but in other areas, such as California, US, bacteria were first[4-8]. It is obvious that the formation and succession of fouling microorganisms in a community are affected by a variety of marine environmental factors such as temperature, light, organic and inorganic material levels, hydrometeorological and biological factors and components of surface substrate (including coat)[9,10]. Therefore, there are some characteristics in the formation of marine microfouling community and succession of organisms in different sea areas and so are our investigation in Xiamen Harbour [9, 11, 12, 13].

This paper is about the study on the developmental dynamics of early microfouling, the structure of the community, the succession of the population and its seasonal changes as well as some other characteristics, on the surfaces of glass and some metals exposed to natural seawater in Xiamen Harbour, a subtropic area.

MATERIALS AND METHODS

Specimen preparation and exposure

Test plates for this study were quality glass and metal, such as stainless steel, copper, copper-nickel alloy (70/30) and aluminium which had been polished with emery paper of gradient sizes and treated with xylene and ethanol. The metal plates were fixed in parallel with a certain distance on a wood cuboid frame with rubber strings and as the glass slides placed in the shape of radioactivity on a rubber block. All the test plates were hung at a depth of 60-70 cm, in the buoy (24°27' N, 118°04' E) for the test of corrosion and fouling in Xiamen Harbour. The exposed periods were from Dec. 1980 to Jan. 1981 (in winter with water temperature of 12-14 °C) and from June to July, 1982 (in summer with 27-29 °C), respectively.

Microbial analyses

Three plates for each kind of material and seawater at the exposure depth were collected as following intervals of 1, 4 and 8 hours, and 1,2,3,4, 5, 6, 7, 10, 13, 16, and 19 days. The triplicate after rinsing in sterile aged seawater was prepared for the isolation and plate-count of heterotrophic microbes, microscopic observation of the surface structure, classification, and counting of diatoms, and determination of the dry weight of the film. The seawater was only for the plate-count of heterotrophic bacteria.

Scanning electron microscope (SEM)

All test plates were fixed in 4% glutaraldehyde in seawater for 4 hr before they were dehydrated. The metal plates were immersed in xylene and dried by air while the glass ones were at critical-point dried and coated with Au in a vacuum evaporator. Then, they were observed by scanning electron microscope (Model Hu-12A, Hitachi). The working voltage was between 5-25 KV. Further details were presented in the reference[11-13].

RESULTS AND DISCUSSIONS

Bacteria

It was shown, by microscopic observation, that a species of bacterium was one of the most dominant microfouling organisms and the first species to appear on the plates (1 hr's exposure) in both winter and summer in Xiamen Harbour. Most bacteria on plates of 24 hrs' exposure were G⁻ ones, and almost entire surfaces of a plate were coverd by a thin slime film after 7 days' exposure. The film became coarse partly and thicker as the extension of exposure time. After 1-19 days' exposure, a mature micro-ecosystem was formed on the plate surface in which bacteria, diatoms, and some other microorganisms were growing. The continuous determination of the numbers of bacteria and diatoms in different time of exposure was, therefore, helpful in explaining dynamics of film formation and organism succession. Fig. 1 shows the relationship between heterotrophic microbial numbers and the

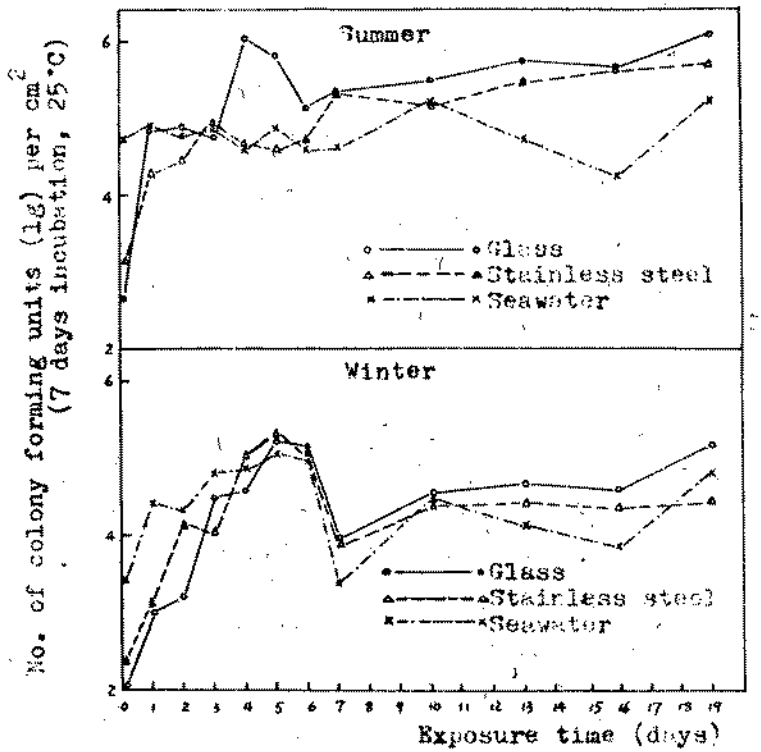

Fig. 1. The developmental dynamics of heterotrophic microbes attached to surfaces of glass and stainless steel.

exposed time during the periods of 1-19 days.

It is suggested in Fig. 1 that heterotrophic fouling microbial numbers in summer (average numbers ranging from 4.5 (lg) cell/cm^2 to 6.0 (lg) cell/cm^2) generally were greater than those in winter (average numbers ranging from 3.8 (lg) cell/cm^2 to 5.2 (lg) cell /cm^2). In both summer and winter there was a fouling increase phase during the early exposing period(1-6 days exposure)and afterwards there were relatively stable periods which extended to the end of exposure(19 days). Perhaps,the fouling increase phase resulted from colonization by bacteria on the surface before the formation of a complex physical-chemical film. Seven

genera of bacteria were isolated from the plates exposed in winter and were determined to be Pseudomonas sp., Xathomonas sp., Staphyloxcous sp., Bacillus sp., Corynebacterium sp., Flavobacterium sp and Micrococcus sp., with Pseudomonas sp. dominant. And besides those listed above, Vibrio sp. and Mycobacterium sp. were also separated from these plates in summer. It was obvious that both species and numbers of heterotrophic fouling bacteria in summer were more than those in winter.

Diatoms

After bacterial adhesion, diatoms the second group of colonist occurred on the plate. It was observed that Cocconeis scutellum and Licmophora flabellata were the first to appear on the plates each exposed for 4 hrs and 24 hrs respectively in winter. In summer it was Navicula longa and Cocconeis scutellum (24 hrs' exposure) were the earliest appearance. Generally, within 1-5 days' exposure, most diatoms would adhere individually, and from that time on diatoms would grow rapidly and cluster on some parts of plate. For instance the observation by SEM showed that Cocconeis sp. were locally gathered (Fig. 3a,b).

We[1] collected from the plates exposed in winter, 61 species of diatoms which distribute in 21 genera. Among them, marine species, benthic species, and permanent-adhesion species were most numerous, planktonic species were next, but the brackish-water species were very few. About the species of fouling diatoms occurring in summer we will report in another paper. It is shown in Fig. 2 that there was a fouling peak of diatoms in the period of 7-9 days' exposure, but their greatest density was about 3 orders of magnitude fewer than that of bacteria, and diatoms grew apparently slower than bacteria. In addition, the numbers of fouling diatoms in summer were much more numerous than those in winter.

Other microfouling organisms

In our investigation, it was observed that fungi, actinomycetes, micro-algae, protozoa, etc. adhered to the surfaces of glass and stainless steel. Fungi occurred after 24hrs and their numbers were

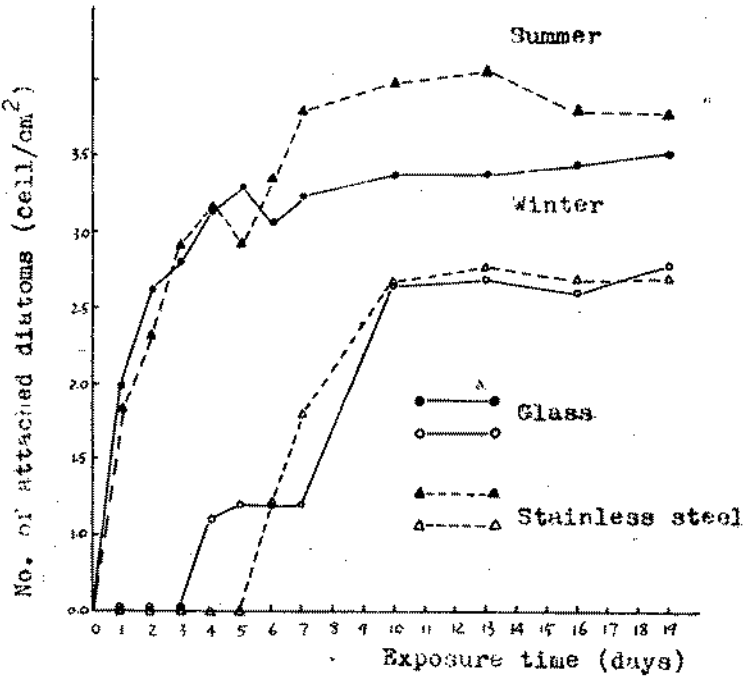

Fig. 2. The developmental dynamics of diatoms attached to surfaces of glass and stainless steel.

just fewer than those of bacteria and diatoms[11]. Seven species of fungi belonging to 4 genera, Aspergillus versicolor., A. japonicus., A. flavus., Penicillium citer-viride., P. corilophilum., Trichoderma viride and Cladosporium herdam were isolated from two kinds of plates in winter. In summer, Penicillium sp., Aspergillus sp., Dendryphion sp., Cladosporium sp., Ceratosporium sp, Paecilomyces sp., Triposporium sp., Fusarium moniliforme and Mucor racemosus. were common. The numbers and species of fungi in summer were more than those in winter.

Numbers of yeast were apparently smaller. The common species were Stergmatomyces sp., Rhodotorula sp. and Crytoccus sp. in winter, and Debaryomyces sp., Rhodotorula sp., Hansenula sp. and Trichosporon sp. in summer. Besides, actinomycetes, such as Streptomyces sp. were only few numbers on the plates in both seasons.

A few fouling micro-algae were on the plates for 3 days' exposure and they would become one kind of organism on the second layer when two-tier microfouling phenomenon occurred on the plates (Fig. 3b). The species in summer were a few more than those in winter with presence of Pseudichomosiphon., Derbesia marina., Bangia fuscopurea and Ectocarpus sp. Additionally, Difflugiidae were observed swimming on the slime film with only a few number while Micronenatode sp. — a kind of common species in summer, were also present along with Carchesiem polypinum., Hemiophrys pleurosigma and Ceratocorys hrrid as well as protozoa such as Vorticella sp.

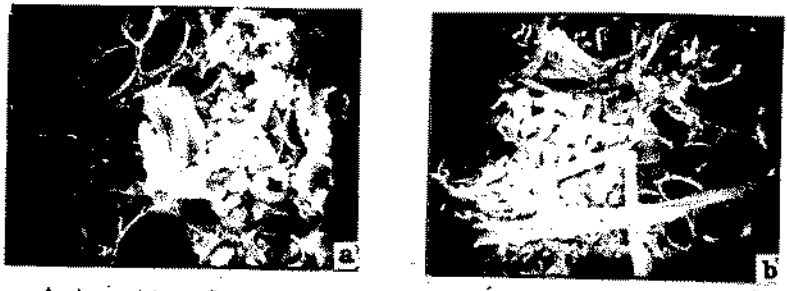

Fig. 3. A two-tier layer structure on the glass (a) and stainless steel (b) for 12 days' exposure observed by the SEM. Diatoms onthe glass (a) and filamentious algae on the stainless steel (b) were second tier layer microorganisms. (1650 x)

Macrofouling

Observation showed that macrofouling was apparent on the plates after 7 days'(in winter)or 5 days'exposure(in summer). Although their numbers and species in winter were fewer than those in summer,after 6 days'exposure it would be common in winter, fouling of Tubularia mesembryoan themem and Obelia sp..Barnacles were usually absent in this season.In summer,besides the species mentioned above,Clytia sp.,Membranipora sp.,Acanthodesia sp.,Larva and adults of Balanus sp.,Amphipoda sp.,Corophium sp.(worm tube)

Polychaeta sp. and Oysters were present. Among them, Obelia sp., Clytia sp. and Membranipora sp. considerably affected the forming of the slime film. In this test, they covered with a net , two fifths of the glass surface and one fifth of the stainless steel surface after 13 days' exposure. So they affected development of microorganisms in the early time of the exposure period.

Two-tier layer phenomenon

A structure of two-tier microfouling layer occurring during the forming and developing period of slime film, to a certain extent illustrated the complexity of community configuration in the slime film, Which has been evidenced in recent years by Cherchakov [14], Mitchell [15] and Marshall [16] using SEM. In Xiamen Harbour, the structure observed by light microscope and SEM, generally came into existence on the plate of glass after 10 days' exposure, but the more visible one on the plates of both glass and stainless steel appeared after 12 days' exposure (Fig. 3a, b and Table 1).

Table 1. Two-tier microfouling formation on submerged surface

Day of exposure	Glass	Stainless steel	Copper	Copper-nickel 70/30 alloy	Aluminium
1	-	-	-	-	-
4	-	-	-	-	-
7	+	+	-	+	-
12	+++	+++	-	+	-

Note: - = no formation, + = just forming, +++ = apparently formed

The results in Table 1 show that besides sea area and exposure time and depth, composition of substrate also played an important role in the occurrence of two-tier microfouling layer phenomenon.

Community succession

Lately, Corpe (1976)[17] and Mitchell (1978)[15] have described the developing stages of the primary slime film and the ecological succession of complex microfouling communities in the sea. We[11] would describe the major stages of developments of slime film in Xiamen Harbour in winter (Table 2).

Table 2. Developmental stages of slime film on non-toxic substrates exposed in Xiamen Harbour (in winter)

Time of immersion (hrs)	Dominant group of fouling organisms	Main feature of slime film development
1-24	Bacteria (most of which, G^-)	The surfaces were polished or transparent with organic or inorganic particulate adhesion.
48-144	Bacteria, diatoms, fungi and filament-algae	The surfaces were getting dull and untransparent, the locally roughing with something tipping, and appearing a thin, light-grey and visible slime film.
168-456	Besides above the organisms, the protozoa and macro-organisms remarkable increasing.	The film with green-brown color areas extended, numbers and two-tier after 10 days' exposure was obvious under microscope.

According to the results of observation and comparision, the essential feature of slime film development in summer was similar to that in winter, except for the apparent occurrence of macrofouling in summer; film development began at the fifth day of exposure, but it was a little earlier in winter.

In order to observe the tendency of development of slime film, dry weight of the film (including organic and inorganic compositions) was also determined (Fig. 4).

It can be seen from Fig. 4 that the weight increased gradually before 7 days' exposure, but rapidly after 7-10 days' exposure. This time when growth of the heterotrophic microbes were in a stable phase (Fig. 1), diatoms occurred in larg

numbers (Fig. 2), and the fouling of macro-organisms gradually increased. Thus, we believed that the biomass on the plates was supposed to be mainly relevant with the fouling of diatoms and macro-organisms after 7 days' exposure. Moreover, the weight in summer was much higher than in winter. This seemed to be in good agreement with the results observed above variation of fouling dynamics of varied population.

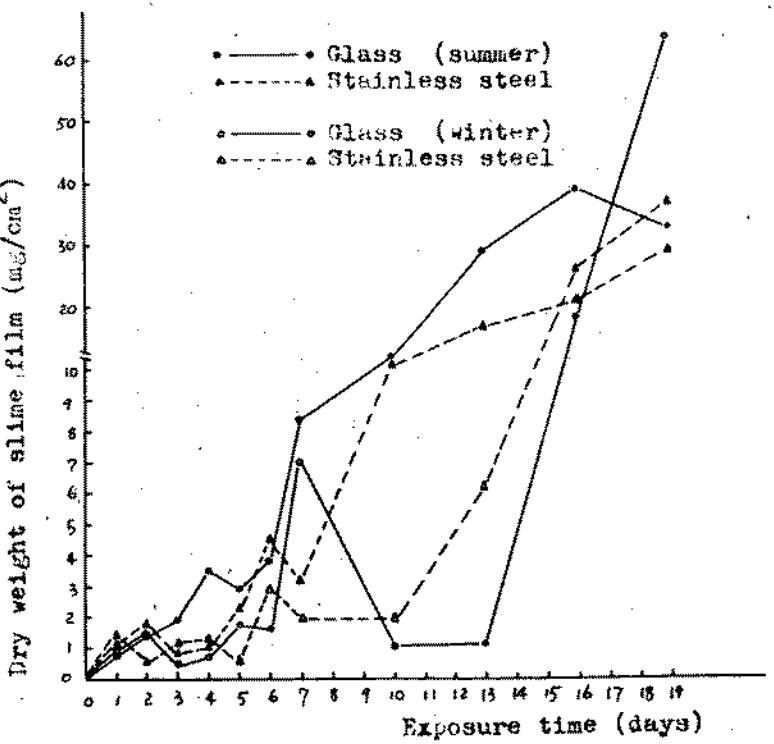

Fig. 4. The development dynamics of slime film on immersed surfaces.

Annual changes

The changing in numbers of microfouling organisms was observed

on two kinds of plates, twice a month around the year. As shown in Fig. 5, the fouling peak of aerobes (bacteria, fungi, yeast and actonomycetes) extended from June to August (in summer). Namely, it was present in the season when the temperature was at the highest of a year and macrofouling organisms bloomed[13, 18]. Moreover, the numbers of microfouling organisms on the carbon steel were much less than those both on glass and stainless steel.

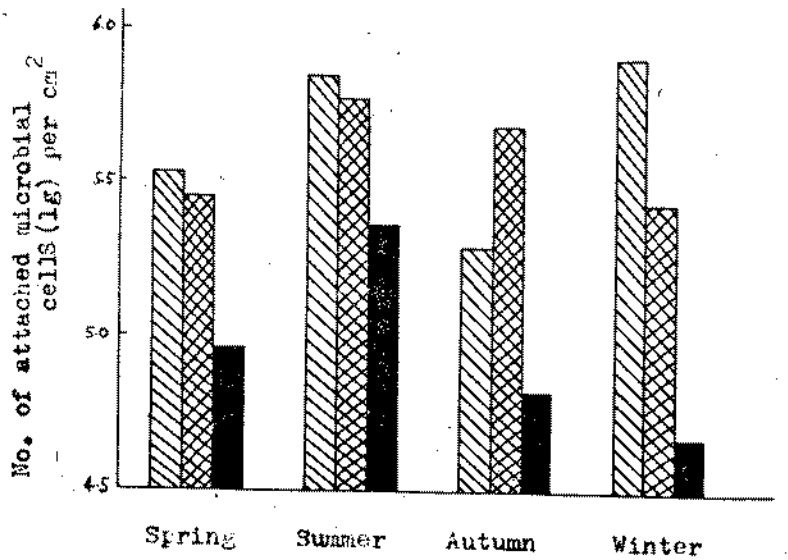

Fig. 5. Annual changes of microbial attached numbers immersed in natural seawater.

▨ = glass ▩ = stainless steel ■ = carbon steel

As indicated above, the primary species of the slime film of microfouling organisms and the characteristics of community succession in Xiamen Harbour are slightly similar to those in some other bays in China and in the world as well. The species and numbers of microfouling organisms such as bacteria and diatoms and other fouling organisms are numerous, the succession is rapid, and the structure of the community is complex.

Perhaps these are the characteristics of the relatively mature and entire micro-ecosystem in Xiamen Harbour, as well as in sub-tropical areas generally.

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ECOLOGICAL AND BIOLOGICAL STUDIES OF FOULING ORGANISMS ALONG THE COAST OF CHINA

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ABSTRACT

Reported in the paper are the history, the method and the major result of the studies of marine fouling organisms along the coast of China. Species, attaching seasons and quantities of fouling organisms are also described briefly. At the end of this paper, sixty related literatures are attached.

RÉSUMÉ

Ce rapport l'histoire, les méthodes et les résultats importants de la recherche sue la bio-salissure dans les eaux côtières chinoises, et donne un apercu des espèces, des saisons d'attachement ainsi que de la quan ntite des salissures dans les eaux côtières chinoises. Soixante documents intéressés sont donnés en détail à la fin du rapport.

INTRODUCTION

Seas along the coninental coast of China, which has more than 18,000Km of coastal line, are divided from north to south into the Bohai Sea, the Yellow Sea, the East and South China Seas, distributing in over 20 latitudes of temperate, subtropic and tropic zones. In addition, she has over 5,000 islands such as Taiwan and Hainan Island, Xisha, Dongsha and Nansha Islands in the South China Sea. Geological environments and hydrological conditions in harbours and bays along the coast vary greatly, especially in water temperature, salinity and the wideness of the harbours and bay, consequently, the fouling organisms there are very complicated and various.

Studies on fouling organisms and antifouling with excellent results have been conducted in China. Antifouling paint's based on Cu_2O and organotin

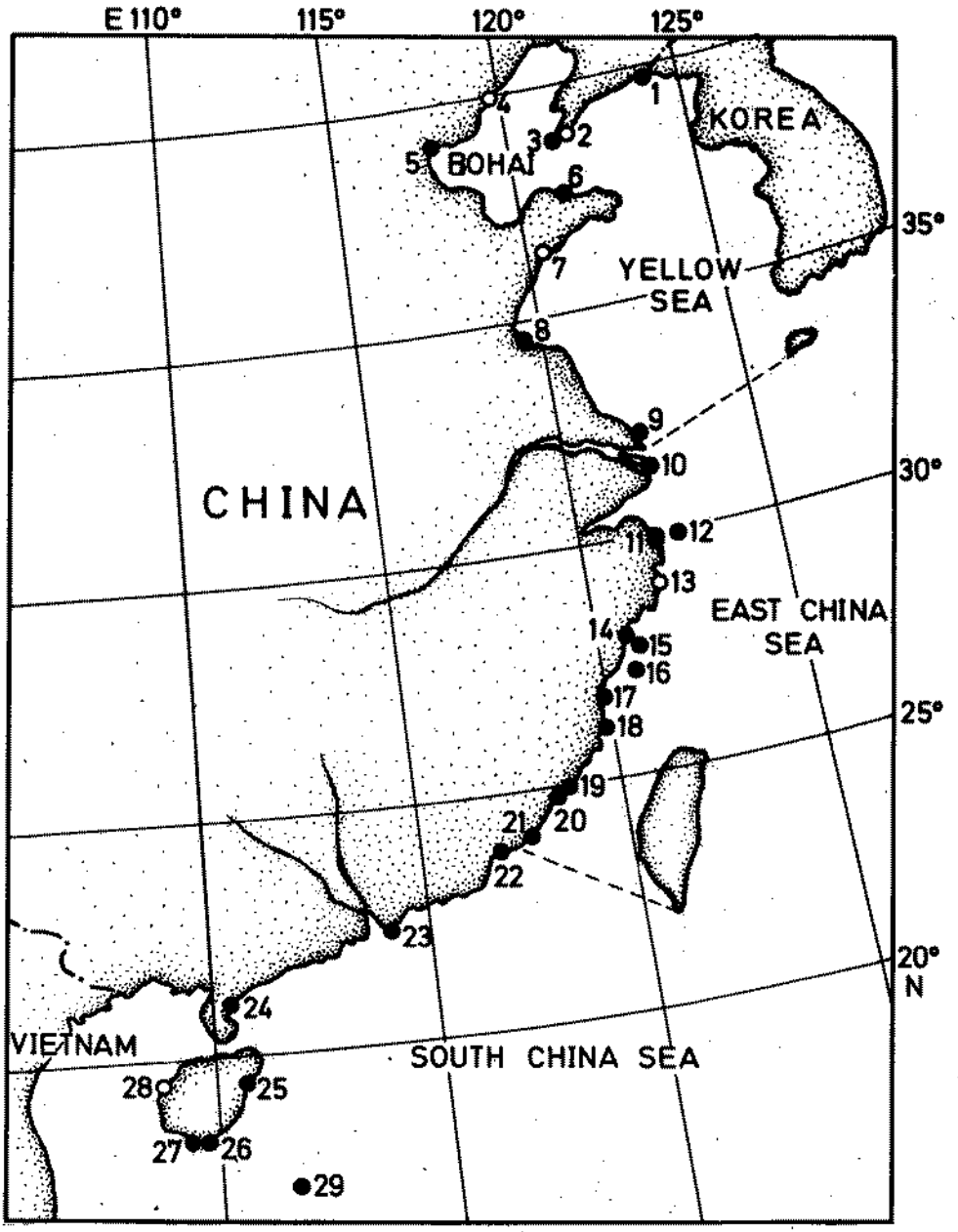

Fig. 1. Panel areas of fouling organisms along the chinese coast.

1. Dandong, 2. Dalian, 3. Lushun, 4.Qinhuangdao, 5. Tianjin , 6. Yantai, 7. Qingdao, 8. Lianyun Harbour, 9. Lusiyang, 10. Changjiang River Estuary, 11. Ningbo, 12. Zhoushan waters, 13. Shipu, 14. Wenzhou, 15. Dongtou, 16. Nanji, 17. Shanduao, 18. Pingtan, 19. Quanzhou, 20. Xiamen, 21. Dongshan, 22. Shantou, 23. Hong Kong Waters, 24. Zhanjiang, 25. Qinglan, 26. Langya Bay, 27. Yulin, 28. Yangpu, 29. Xisha.

(R_3SnX) have been developed successively and antifouling system of electrolyzed seawater has been successively applied in tubes of ships and tidal power stations. Studies on fouling organisms have also been carried out from various aspects. In this paper, a brief acount of studies on ecology and biology of fouling organisms as well as their ecological features are presented.

HISTORY AND METHODS

Institutions engaged in the study of fouling organisms in China are the National Bureau of Oceanography (3rd Institute of Oceanography), Chinese Academy of Sciences (Institute of Oceanology and The South China Sea Institute of Oceanology) and two universities (Xiamen University and Shangdong College of Oceanology).

In the fields of the ecology of macrofouling orgamism communities, professor Zheng Zhong and his colleagues in 1950 conducted panel experiments for nine months and published paper in this field for the first time in China. Li Jiemin and Huang Xiuming et al. started from 1959 panel experiments for three years in four harbours along the Chinese coast. Huang Xiuming et al. surveyed biofouling on 18 ships. Dong Yu et al. started panel experiments from 1961 in some harbours in the Soyth China. A group of biofouling study led by the author, inherited the cause of my teacher, professor Zheng Zhong, conducted systematic panel experiments in the major harbours and bays along the Chinese coast from 1960 onward, and great varieties of surveys have been made on ships, buoys, pier (floating or fixed) seabottom cables, tubes, rafts and mariculture net and cages. Panel experiments have been completed up to the present, generally annual, at most eight years, at 27 bays (34 stations) (Fig. 1. areas indicted by black dots), from which 3,300 panels have been recovered and analyzed. In the mean time, biofouling on 100 ships, 120 buoys and rafts, 33 piers and underwater constructions have been surveyed. Twenty-four papers om these studies have been published.

The above-mentioned surveys or experiments were conducted generally in uniform approaches, consequently the results are comparable. In 1975, the National Bureau of Oceanography published STANDARD OF MARINE SURVEY, in wich the chapter concerning the survey approaches of fouling organisms was based on experiences of many years, and the surveys at present just follow the standards.

From 1970's onward, University of Hong Kong and Hong Kong Chinese Univ-

ersity and have also been conducting the studies in this field, of which, professor B. S. Morton and his colleagues and postgraduates have gained outstanding results. Since 1980, the author has several times joined in their studies. Mak and Tseng rather deeply in the studies of biofouling on mariculture net and cages.

Im the fields of microfouling organisms, Wang Qiu et al. have studies primary microbial films on both the surface of antifouling paint and nontoxic surface. In their studies in the Qingdao Harbour, they discovered lots of small nematodes in the films. Lin Yanshun et al. have studies species, quantitative variations and the development and succession of communities on microfouling in the Xiamen Harbour, and reported 60 species of fouling diatoms as well as some bacteria, fungi, and yeasts. Chin Dexiang et al. reported 60 species of fouling diatoms in the Hong Kong waters. Ma Shide studied the relationship between microfouling organisms and corrosion.

In the field of individual biology, the life circle, growth and development as well as larval culturing of major fouling organisms, such as Balanus amphitrite amphitrite, B. reticulatus, Mytilus edulis, Perna viridis, Ostrea cucullata, Hydroides ezoensis, Tubularia mesembryanthemum have been conducted. The expansion of distribution limits of fouling organisms due to the carrying of ships has been noticed. The effects of salinity as well as the relationship between macrofouling organisms and corrosion have been made.

Based on the data collected in the studies of author for more than 20 years, and by making references to above-mentioned researches and the related reports in the world, the author has written a monography, MARINE BIOFOULING AND ITS PREVENTION. The first volume of which including introduction and natural ecology of fouling organisms has been published, and the coming second volume will put emphasis on the individual biology, experimental ecology and antifouling. In the first volume, species, quantity and attaching seasons of fouling organisms in the major harbours in China and the other parts of the world have been comprehensively summarized.

SOME ECOLOGICAL FEATURES OF FOULING ORGANISMS ALONG THE CHINESE COAST

Species: Up to the present 654 species of fouling organisms, rather different due to salinity, temperature and the windeness of various harbour and bays, have been recorded in the Chinese coast (TABLES 1, 2). Based on temperature, lots of them are eurytopic species in China, or even in the other parts of the world, such as Balanus amphitrite amphitrite, Bugula neritina etc.

TABLE 1. Number of Species of Fouling Organisms in Each Groups Reported from Coastal Waters of China

Groups	Numbers
Bacteria	7
Fungi	7
Yeasts	3
Actinomyces	1
Bacillariophyta	119
Chlorophyta	31
Phaeophyta	12
Rhodophyta	16
Cyanophyta	6
Protozoa	4
Porifera	28
Hydrozoa	34
Anthozoa	14
Ectoprocta	57
Platyhelminthes	2
Nemertea	2
Nemathelminthes	1
Polychaeta errantia	64
Polychaeta sedentaria	43
Sipunculida	2
Amphineura	2
Gastropoda	44
Bivalvia	71
Cirripedia	33
Copepoda	1
Ostracoda	1
Tanaidacea	1
Isopoda	12
Amphipoda	19
Stomatopoda	1
Decapoda	32
Pycnogonida	1
Insecta	2
Echinodermata	12
Tunicata	20
Pisces	9
Total	654

TABLE 2. Major Species of Macrofouling Organisms and Its Distributions Along the Coast of China

Species	Bohai	Yellow Sea	East China Sea	South China Sea
Algae				
Enteromorpha clathrata		+	+	+ +
E. compress	+	+	+	
E. intestinalis	+ +	+ + +	+ + +	+
E. tubulosa		+	+	+ +
E. prolitera	+	+	+	+
Ulva lactuca			+ +	+
Monostroma nitidum			+	+ + +
Porifera				
Mycale adhaerens				+ + +
Lissodendoryx isodictyalis			+	+ +
Halichondria panicea	+	+	+	+
Tedania ignis				+ +
Coelenterata				
Tubularia mesembryanthemum		+ + +	+ + +	+
Obelia geniculata	+	+	+ +	
O. gracilis		+	+ +	+
Anthopleura pacifica		+ +	+ + +	+
Ectoprocta				
Acanthodesia granudicella	+	+	+	+
Bugula neritina	+	+ +	+	+ + +
B. californica	+	+	+	+

Celleporina costazii		+	+ + +	+
Cryptosula pallasiana	+	+	+	
Dakaria subovoidae	+	+	+ +	+ +
Electra anomal		+	+	+
Membranipora savartii	+	+	+ +	+
Schizoporella unicornis	+	+	+	+ + +
Scrupocellaria spatulatoidea				+ +
Petraliella umbonata				+ + +
Polychaeta				
Hydroides elegans	+	+	+	+ + +
H. ezoensis	+	+ + +	+	
H. dirampha			+	+ +
H. longispinosa				+ + +
Serpula vermicularis	+	+	+	+ +
Pomatoleios kraussii			+ +	+ + +
Mollusca				
Crepidula onyx				+ +
Barbatia virescens	+	+	+ +	+ + +
Mytilopsis sallei				+ +
Mytilus edulis	+	+ + +		
Perna viridis			+	+ + +
Isognomon ephippium				+ + +
Dendostrea plicata	+	+ +	+ + +	+
Alectryonella crenulifera				+ + +
A. radix				+ +
Cirripedia				
Lepas anserifera		+	+	+ + +
Balanus improvisus	+ +	+		
B. amphitrite amphitrite	+	+ + +	+	+ + +
B. reticulatus			+ + +	+ + +
B. uliginosus	+	+	+ + +	+
B. trigonus			+ +	+ + +
Megabalanus rosa			+ + +	+
M. t. tintinnabulum			+	+ + +
Chirona amaryllis	+	+	+	+
Amphipoda				
Corophium acherusicum		+	+ +	+
Ericthonius pugnax			+	+ +
Tunicata				
Ciona intestinalis	+	+ + +	+	+
Styela clava	+ +	+ + +		
S. plicata			+	+ + +
S. canopus		+	+	+ + +
Molgula manhattensis	+ + +	+		
Ascidia sydneiensis				+ +
Symplegma schlosseri		+	+	+
Pisces				
Prionobutis koilomatodom			+ +	+
Salarias dussumieri			+ +	+ +

some of them are temperate species limited along the coast of Northern China , such as Styela clava, B. improvisus, Mytilus edulis etc.. And some of them are tropical or subtropical species limited to the coast of Southeast China, such as B. reticulatus, Styela plicata, Megabalanus t. tintinnabulum and Perna viridis etc.. And still some such as M. zebra and Hydroides longispinosa are limited in the offshore waters south of the Hainan Island in the South China Sea. Based on salinity, some are hypersaline or sub-hypersaline species, such as Mycale adhaerens, B. trigonus. Scrupocellaria spatulatoidea, Ascida sydneiensis. Some are hyposaline species, such as B. uliginosus, Carbasea carbasea. Some freshwater species, such as Cordylophora lacustris, Limnoperna fortunei were also found in the esturies and embayments along the coast of China. The great majoiity of fouling orginisms live most suitably in waters unimpeded, some of which are even limited in the offshore waters with stronger tides and currents, such as M. t. tintinnabulum.

Attaching Season: Temperatures in Chinese waters differ greatly as they cover many latitudes. The attaching periods in various waters very greatly since temperature is a major factor controlling the attaching seasons of fouling organisms, generally speaking, attaching periods are incremingly prolonged from north to south. For example, the bloom period of fouling organisms in the Bohai Sea is from June to September (monthly mean temperature 20-26 ℃), and no attachment from December to January. In the Southern Yellow Sea and the Northern East China Sea, macrofouling organisms could be found from May to October (18.5-28.4 ℃), no or less attachment from December to February. In the waters south of the Hainan Island and around the Xisha Islands, attachment takes place all the year round (monthly mean temperature 20 ℃). The attaching period of the same species differs in different harbour and bays, shorter in the north and longer in the south (Fig. 2).

There are mainly three types of attaching periods of fouling organisms along the Chinese coast.

1. Those with only one attaching period in a year: Attaching period limited only in the months with higher water temperature in a year, mainly in the harbours and bays with greater differences of temperature, such as those in the Bohai Sea, the Yellow Sea and the Northern East China Sea.

2. Those with two separated attaching periods: Especially evident for Tubularia mesembranthemum, Obelia geniculata as well as algae such as Enteromorpha spp. in some harbours in the East China Sea. These species form a peak of attachment in spring, stop or decrease in summer and then form another small peak in autumn.

3. Those attach the whole year round, with distinct seasonal variations: This category are the most harbours in the South China Sea, especially those in the areas south of the Hainan Island.

Figure 2 shows that the attaching period of the same species in different harbours may belong to differing types while different species in the same harbour may also fall into differing types.

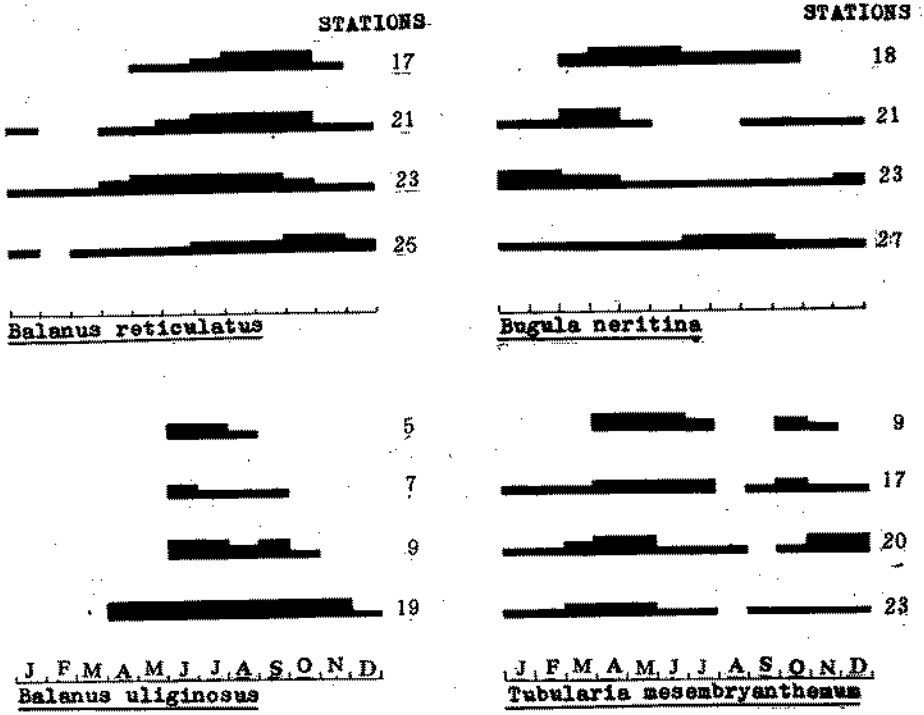

Fig. 2. The attaching periods of four fouling organisms in different harbours along the Chinese coast (station locations are shown in Fig. 1)

Quantity: We measured the quantity of organisms by their weight (dry or wet), coverage area, individual number and thickness. Panel data from 37 areas showed that the wet weight of fouling organisms varied greatly in different harbours or at different sites in a same harbour. The differences may be explained by the following points, 1). in open and not impeded waters, there are greater varieties of organisms, quicker growth, thus larger wet weight; 2). in coastal waters, farther from the coast, larger wet weight; 3). in estuar-

ine waters, salinity too low or approaching freshwater, less number of organisms smaller wet weight (TABLE 3).

TABLE 3. A Comparision of Wet Weight of Fouling Organisms from 37 Areas along the Chinese Coast (June - August)

Order	Wet Weight (Kg/m^2)	Number of Stations
1	19	4
2	10- 8	6
3	7- 3	13
4	2- 1	4
5	0.6	10

TABLE 4. The Maximum Wet Weight Records of Fouling Organisms along the Chinese Coast

Seas	Objects	Wet Weights (Kg/m^2)	Dominants
Bohai	Lock gate of dock	25.1	Ostrea rivularis, Balanus uligimosus
Yellow Sea	Ship's bottom	28.1	Hydrcides ezoensis, Styela clava, Ostrea plicata
	Platform's pile	34.4	O. rivularis, B. uligimosus
East China Sea	Floating pier	27.9	O. rivularis, Perna viridis
South China Sea	Fixed pier of H.K.	51.0	Isognomon ephippium
	Buoy	59.6	Megabalanus t. tintinnabulum

Gemerally speaking, the number of species and quantity of fouling organisms on ship-bottoms and other underwater constructions are greater than that on panels. For example, great amount of very large barnacles were found on buoys in the rapid flow area at the center of the Qiongzhou Straits, the wet weight of which was as high as $59.6 Kg/m^2$ (Table 4).

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Polychaetous Fouling In the Egyptian Marine Waters.

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Abstract.

Studies proved that polychaetes, namely the tube worms are the leading fouling organisms in most of the Egyptian marine areas. Lists of the polychaetes at Alexandria and Suez Canal, the most fouled regions in Egypt shows that Hydroides elegans dominate but in Damietta estuary Mercierella enigmatica represents the only polychaetous fouling organism there. Fouling at Sallum on the Mediterranean and in the Red Sea is practically absent. Features of the Hydroides fouling is discussed.

Les etudes out provees que les polychaetes notament les vers tubule sont les pioniera dans les organisms responsible de salissures dans eau marine en Egypte. Les listes de salissures organisms fait a Alexandria et au Canal de Suez, les regions les plus effectiues de salissures en Egypte moutre que Hydroides elegans dominee, dans L'eusuair Damietta Mercierella enigmatica, represent le seul polychaete responsible de salissures. Salissures a Sallum en Mediterranee et en Mer Rouge est practicement absent. Les caracteistique de salssures de Hydroides est egalment discutee.

Introduction .

Egypt lies on the biggest two seas in the world. Her coasts extend thousands of miles on the Mediterranean and the Red seas, in addition to the Suez Canal and Sinai coasts on the gulf of Suez and gulf of Aqaba. On these extensive coasts lie several harbours, ranging from Alexandria; the biggest to small ones on the Red Sea such as El Ghardaqa. Incidence of marine fouling in the Egyptian harbours varies from very severe in Alexandria and Ismailia(on the Suez Canal) to almost negligible at Sallum (west to Alexandria) and in the Red sea in general.

The author and his colligues during their investigations and observations on the biology of fouling gathered a clear idea about the fouling state nearly in all Egyptian regions. In all cases polychaete worms, causing permanent or temporary settlement constitute the principal fouling organisms.

The importance of the polychaetes is not only due to their marked role in fish feeding (Thorson,1956) and as marine foulers but also because some worms proved to be indicators for pollution(Reish,1957).

Unfortunately the report Of Dor Pow(1978) about the "Lessepsian migration" neglected the presence of the serpulids in the Suez canal although they are among the dominating benthic animals in the canal as well as in other Egyptian localities, and this is the aim of this paper to explain.

Material and Methods.

The area of this survey includes Sallum, Alexandria, Damietta and Port-Said on the Mediterranean, the whole Suez canal , Suez on the gulf of Suez, Sharm El Shaikh and Nwebaa on the gulf of Aqaba And El Ghardaqa on the Red sea (see the map). Test panels of different sizes and made of glass (Banoub, 1960), of different materials (Megally,1970) or of impact resistance polystyrene (Ghobashy and his colligues from 1976 to 1984) served as fouling collectors in various Egyptian marine waters. Collection has been made also from buoys, piers and other marine installations.

A- The region west to Alexandria.

The author during heading a team of researchers to study the benthos in the Egyptian western region from September 1974 to December 1975 , investigated the fouling conditions in the Sallum bey(Ghobashy,1977). no fouling attachments on the pier or on the wharf were observed. However, dredging in the whole bey which reaches about 50 m deep showed that few worms of Lanice sp. and Eunice sp. are the leading polychaetous forms there. Little number of the serpulid Protula tubularia encrusting dredged stones were found at the depths 10 and 50 m during winter.

Farag(1981) also found the region from Alexandria to Sallum generally poor in the marine benthos and the polychaetes mainly represented by Eunice and Syllis are very rare. Fouling forms in the whole area are practically absent.

B- Alexandria region.

Most of the attention has been paid to the fouling growths in the Eastern harbour of Alexandria. Work of Banoub(1960) was done in 1958 before the erection of the high dam ,i.e. at the time when the Nile flood was lowering the salinity from 39‰ in December to 31‰ in September annually and causing dramatic changes in the marine environmental conditions in the south-eastern Mediterranean (Halim et al, 1967). Banoub observed that the serpulid Hydroides norvegica Gunnerus - which identification has been corrected to H.elegans (Zibrowius,1971) - formed the principal fouling organism and its settlement reached maximally, in May, about 6.2 worms /cm^2/month and the maximum tube length reached the maximum value, 4 cm, in June.

Ten years later, after the erection of Asswan high dam , Megally(1970) revealed that the same Hydroides species persisting as the leading fouling animal in the harbour. After the cease of the Nile flood the water salinity became stable and its range lies between 37 and 39‰. This species constituted about 7.1 worms /cm^2/month during July 1968.

Ghobashy(1976) and Ghobashy and Selim(1976 a and b)observed that H.elegans is the major fouler in the harbour throughout most of the year and particularly in September. The rate of its settlement was 72.9 worms/cm^2/month in September 1972 and 57.3 worms/cm^2/month in September 1975. These authors studied the settlement behaviour of this species. Setthement is markedly favoured on surfaces already dwelled by the same species worms , treated by its extract, roughened or darkened. The tubes do not extend on the substrates as a complete one but as an arch because ventrally the tubing is lacking. The tube prolongation is highly promoted on the rough surfaces(0.59mm/day).

Using the width of the anterior opening of the tube as a function of the growth rate proved more practical than using the tube length. It was observed that a tube while continuously prolonging its anterior opening ceased to widen after having a tube length equal to 6 cm. The tube increase in length ,therefore is not necessarily led by the animal growth. Having the worm lying and protruding from the tube mouth which ceasd to widen as a result of the ultimate growth of the animal, it has been concluded that the further tube prolongation only serves to carry the animal to new places. In other words ,the tube prolongation is a sort of a very slow movement performed by this serpulid.

The wide spreading and the tremendous growth of this worm in the harbour stimulated the study of its reproduction potential therein. Ghobashy,Abdel Hamid and Mona(1981) found that the females often form over 70% of the population. Maturation occurs very early in life and a female in the greatest chapter in its life can produce eggs and manages to carry about 20000 ova in average at any time. Fertilization takes place, partially on a safe place, the operculum and the process is enhanced by the gregariousness phenomenon.

Another tube worm is largely found encrusting fronds of the green alga Caulerpa prolifera Forssk., that is Spirorbis corrugatus Montague which spreads from April to September(Ghobashy and Selim, 1976 c). Its tube does not exceed 1.4 mm in diameter after about 3 weeks of settlement.

Its responses to the different substrate conditions are similar to those of H.elegans. By choice experiments Ghobashy(1978)found that preference of this spirorbid to settle in the harbour on Caulerpa over Ulva which spreads on the sea surface seems to be due to the occurrance of the former plant on the dimly-lit sea bottom and not to chemical factors.

Ghobashy,Reda and Mona(1981)determined, histochemically, the position of the calcium secreting glands in H.elegans on both sides of the thorax, immediately posterior to the first body segment. In S.corrugatus such a distinct gland is absent seemingly because the larval two big glands secrete almost the whole adult tube which attains its maximum diameter shortly after settlement.

A litre of the annual fouling may contain the polychaetes mentioned in table 1 which numerically constitute more than half the litre. The species in the table are arranged according to their abundance and evidently H.elegans forms over 99%of the total number of the worms.

Table 1. Quantity of the polychaetes in a litre representing an annual crop of fouling collected throughout a year,as estimated by Selim(1978).

Species	Quantity	Species	Quantity
Hydroides elegans	1999260	Cirratulus cirratus	5621
Polydora caeca	2944	Trypanosyllis variegata	1556
Spirorbis corrugat#s	1181	Lumbriconereis funchalensis	401
Trypanosyllis zebra	208	Eulalia sp.	179
Psedonereis anomala	178	Dasychone lucullana	122
Trebella lapidaria	117	Nereis falsa	92
Platynereis dumerilii	81	Magalia perarmata	74
Lepidomotus clava	66	Ceratonereis costae	55
Glycera convoluta	41	Hydroides dianthus	36
Myxicola sp.	12	Nephthys hombergii	12
Diopatra neapolitana	8	Sthaurocephalus rurolphii	7
Neanthus caudata	5	Eunice vittata	4
Capitella capitata	3	Lepidonotus squamatus	3
Perinereis cultrifera	3	Hydroides dirampha	3
Nainereis laevigata	1	Vermiliopsis infundibulum	1

Statistical analysis has led Mona(1982) to conclude that the fouling polychaetes in Alexandria harbour may have the same habitat preference and reach full maturity and peaks of settlement nearly during the same period.

C-Damietta estuary.

The worksof Hamada(1980)and Mona(1982) in that estuary reveal that the estuarine serpulid Mercierella enigmatica Fauvel is eclusively the major fouling organism throughout. In March and April 1979 its maximal settlement achieved 69.4worm/cm^2/month but in September and October the settlement rate did not exceed 0.3worms/cm^2/month, Macrofouling in the estuary is poor both qualitatively and quantitatively. Its principal constituents are the serpulid (Mercierella) and the barnacle Balanus amphitrite and its heaviest growths were less than 0.5g/cm^2/month in May 1979(Hamada,1980).

The tube growth rate in Mercierella reaches 0.2mm/day and its maximum length in the estuary is about 7.5cm. Histochemical tests proved that the two calcium secreting glands occur under the collar in a form of granulated cells in addition to a single rod-like structure on each side of the thorax.

D-Suez Canal.

Ghobashy,El Komy and Ramadan(1980) through an extensive work on the fouling of 14 stations distributed along the canal, including Port Said, Ismailia , Suez and the lakes traversed by the canal have made it clear that the polychaetes and particularly Hydroides elegans are the major fouling organisms in the whole water-way. Absence of fouling assembleges in the Red sea raised the feeling that a southward current is responsible for conveying the larvae to the southern region of the canal. Although fouling in the latter region is quantitatively rare, its components are mostly similar to those at the north.

Scarcity of fouling in the southern region of the canal is most probably due to the silty conditions there(Ghobashy,and El Komy,1981). However, polychaetes led by H.elegans as well as Pomatoceros triqueter, Spirorbis sp. and Dasychone lucullana form the principal settlers on both short and long

term exposures.

Fouling generally increases in the canal northward and reaches the peak in the middle way i.e. in the Lake Timsah. The study of Ghobashy and El Komy (1981 b) shows that the tube worms ,particulrly, H.elegans constitute the dominating foulers in the lake, nearly throughout the year. Maximum settlement of this worm (about 64.1/cm^2/month)in months from May to October. As the depth increases the mean tube length decreases as shown in table 2. Salinity increase from about 37‰ at the surface to about 43‰ at six metres below the surface(Gerges,1976) may have some effect in this respect.

Table 2. The mean tube length(mm)of H.elegans at various depths in Lake Timsah.

Depths(metres)	0.5	1.5	2.5	3.5	4.5
Mean tube length	22.7	21.9	19.0	15.8	12.0

The present work , by the author, reveals that the list of the polychaetes in the lake includes : Hydroides elegans, H.dirampha, Serpula vermicularis, Pomatoceros triqueter, Spirorbis sp., Spirorbranchus sp., Dasychone lucullana ,Polydora sp.,Lepidonotus sp., Phyllodoce sp., Polycerrus sp. and Nereis sp.

Surprisingly the tube worms nearly disappear in the water-way north to Ismailia except at El Bellah by-pass, the widest region in the canal between the Lake Timsak and Port Said harbour. In this harbour the polychaetes dominate again and about 2000worms/panel (each 12.5x12.5 cm) were observed in May-June 1977. South to the Lake Timsah the water-way is also nearly free from the tube worms ,except at Devresoir; the northern entrance of the Great Bitter lake and at Kebret in between the two Bitter lakes.

E- Red Sea

Author's observations at the Gulf Of Aqaba namely at Sharm El Shaikh, Dahab and Nebaa and at El Ghardaqa showed that fouling assembleges are practically absent in all these localities. Worm tubes scattering on rocks could not be identified with certainity.

Discussion.

Wherever marine fouling assembleges occur in an Egyptian harbour the polychaetes and particularly *H.elegans* predominate. In Alexandria waters and in the Lake Timsah(Suez Canal), the two most fouled areas in this country, a panel immersed for a month, from May to September, would be hidden completely under the heavy crust of the tube worms which make the panel as a sphere mostly made up of *Hydroides* worms (figure 1).Such similarity in the polychaetous fouling in both Alexandria and the Suez Canal emphasizes the migration of these animals,which are not found in the Red sea, from the Mediterranean to the Canal,i.e.antilessepsian migration(Dov Por,1978).

Formation of huge pilings, is due to the ability of these animals to form coalescing layers without damaging those pioneers which settled first and formed the base of the crust. Barnacles fail to form such successive layers beaause their survival is limited by crowding , and the death of the first settlers leads to the subsequent repelling of further cyprid settlement. Virtually the first settlers of *Hydroides* form the most strongly attaching layer. Their heads become always directed outward as if to avoid the competition in obtaining food and oxygen(Ghobashy and Selim, 1976 b). If a growing part of the tube is damaged compensation takes place in a brief time. The newly formed layers usually leave spaces which appear inside a big crust of worms as deep trenches(figure 1) that allow the deepest layers worms to breath and feed.

Neverthless the piled growth of the tube worms on a surface does not, eventually, form a heavy burden and most of can be easily removed from the surface without causing any damage to it. Conversely the overwhelming number of the worms may have a marked contribution to increasing the potential production of the site they settle at. They are palatable to fishes(Thorson, 1956)and the tremendous number of the liberating larvae will eventually be added to the zooplankton. Therefore the polychaetous fouling can be considered a rather a beneficial one; it is light and productive.

Shalla(1984) and Ramadan(1984) observations of the persistence and high growth of H.elegans in the oil-polluted areas in the lake Timsah and Alexandria respectively point to the role of these animals as pollution indicators and studies on this role are now going on in the lake.

The ceasm of the Nile flood after the erection of the high dam apparently has created conditions favourabl to fouling growth in the harbour of Alexandria. From 6.2 worms/cm^2/month in May 1958 and 7.1 worms/cm^2/month in July 1968, the settlement of Hydroides elegans jumped to 72.9 worms/cm^2/month in September 1972. Seemingly the shability of the salinity nearly throughout encouraged the settlement of fouling organisms(Huchin,1952) and the tube worms in particular.

Being more or less confined to the widest areas of the Suez canal, i.e. harbours, lakes and by-passes where the water disturbance is minimal the tube worms evidentally prefer the calm water rather than the turbulent one.

Absence of fouling communities throughout the Red sea may be due to the oligotrophic condition of the sea and the depletion of the available plankton by the wide-spreading filter feeding corals. The biological and the environmental factors which might be causing this phenomenon need to be thoroughly studied.

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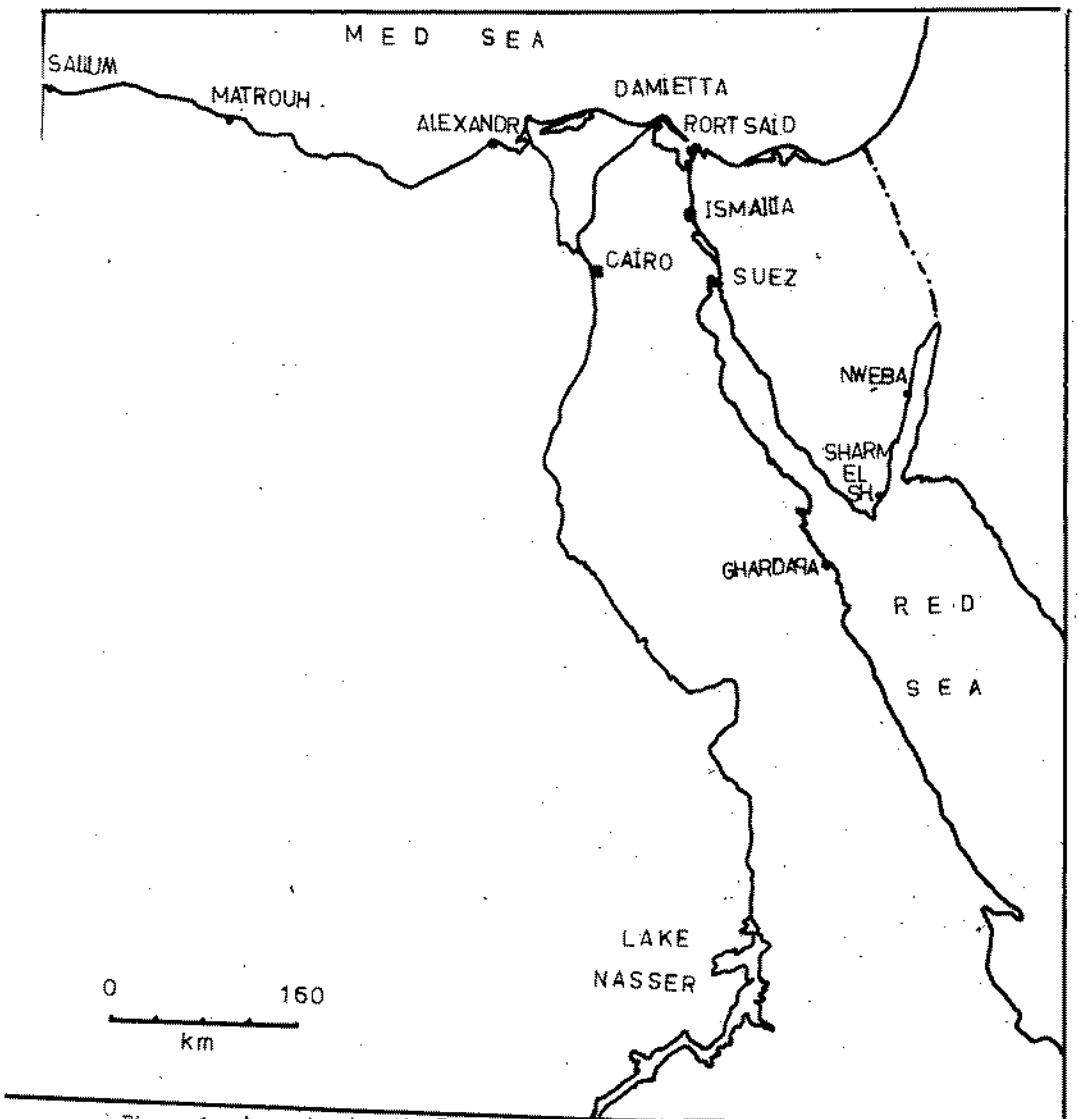

Figure 1. A map to show the Egyptian harbours and sites where the work on marine fouling has been done.

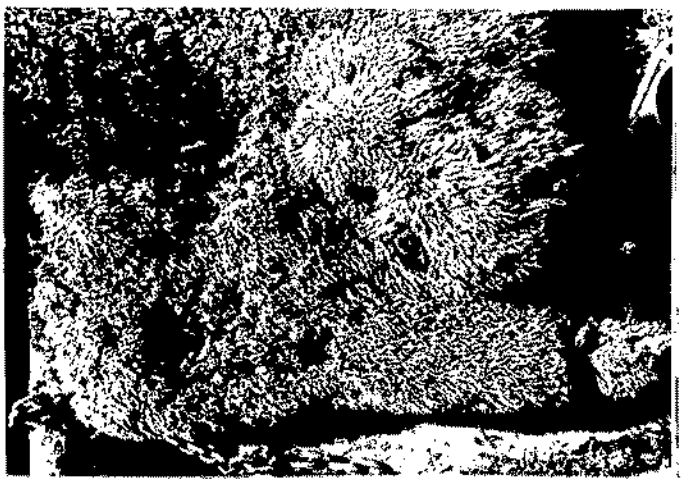

Figure 2. Panels completely hidden under heavy crusts of Hydroides and other tube worms after immersion in the lake Timsah for a month.

The fouling Organisms In The Damietta Harbour(Egypt).

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ABSTRACT

The hydrographic conditions in the harbour are estuarine; salinity varies from 12.6 to 36.1‰ and pH from 7.3 to 8.47. Settlement seasonality is inconsistent and the fouling intensity is weak and the species are few in comparison to other marine localities. The community is dominated by brackish-water polychaete worm Mercierella enigmatica and the barnacle Balanus amphitrite. Growth rate of these two forms, during three periods was persued. Persistance of the estuarine conditions in the harbour in spite of its isolation from the River Nile for more than 15 years suggests that its invasion by the sea water from the Mediterranean is not effective enough to cause changes towards the marine conditions. The study precedes the construction of the harbour which will make the whole area a part of the sea and tremendous changes in the biotic conditions are anticipated.

RESUME

Les conditions hydrographiques dans le port sont estuariennes, le salinite varie de 12,6 à 36,1‰ et pH entre 7,3 à 8,47. L'incrassement est faible et les espèces sont peu en nombre en comparaison avec les autres localites en Egypte. La communauté est dominée par les polychaetes d'eau saumatre Mercierella enigmatica et le barnacle Balanus amphitrite. La croissance de ceux deux formes était faite pendant trois périodes. La persistance des conditions estuariennes du port, malgré sa séparation du Nile dépuis 15 ans montre que l'invasion de l'eau de mer saline de la Méditerranée est faible et n'est pas efficace pour changer vers une condition typiquement marine. Cette étude précède la construction du port qui changera la région comme une partie de la mer,de façon que les conditions biologiques le suivrent.

Introduction.

Since the erection of the Asswan high dam about 16 years ago , a permaneht dam has been vuilt at Farskour, 21 km south to the Mediterranean, (see the map). This dam isolated the estuary at Damietta from the rest of of the River Nile and made it in continuety with the water of the sea only. Being undergoing invasion by the Mediterranean water for this long time, a change in the hydrographic and biotic conditions towards the marine state is anticipated.

Furthermore a harbour is planned to be constructed at the estuary to be the second in Egypt after Alexandria instead of being a fishing vessels harbour as it is now. Presumably the estuary will become a part of the sea and tremendous altrations in the ecosystem are expected to take place. This approach to study the fouling in a location about 12 km south to the sea will giva a background information about the standing condition inthe estuary area. This will clarify the eventual biotic changes after damming the Farskour Nile region and make a comparison between the conditions before and after the construction of the Damietta harbour possible.

Material and Methods.

In addition to collecting the fouling organisms attaching to the Nile installations (piers,bridges etc.), test panels made of non-impact resistant polystyrene(12.5x12.5 cm) were placed successively(five in each month) under water to collect the monthly settling organisms. The number of the settlers was counted and their sizes were measured. The mean wet weight of the panel fouling was also determined. The values of temperature, PH and salinity of the water during the exposure periods were obtained as well. The temperature ranged between 19°C in December and January and 29°C in June and July. The study lasted from March 1978 until July 1979. Further observations by the senior author made in 1982 indicated that the conditions mentioned in the present paper are still prevailing there. Figures 1 and 2 illustrate the monthly variations in pH and salinity respectively in the harbour during the study.

Results.

Although various groups of foulers prevail in the area under investigation . the crop, for the majority of exposures, was very poor. Algae,Protozoa, polychaetes, barnacles, amphipodans and ascidians formed the fouling populations procured. Figure 3 represents an estimation for the quantity of organisms collected every month throughout in the present work.

Here is an account of the constituents of the fouling groups and their settlement frequency.

Algae : Filamentous algae comprise the dominant plants. Entermorpha appeared throughout the period of the study, maximally in March. Ulva and Oscillatoria also prevail but in less density. Ulva appeared in December and the other form from September to March 1979.

Protozoa : Large number of Epistylis flavicana colonies settled in November and in December 1978, and these were the only identified protozoans.

Polychaeta : The serpulid Mercierella enigmatica is exclusively the major fouling organisms at the Nile mouth. Its settlement reached the peak in March and April1979(9815 and 10829 worms/panel/month respectively). However, only 27 and 47 worms, in average, dispersely settled on a panel during September and October 1978.

Throughout three durations in 1978;April-June, July-September and October-January the growth of Mercierella was persued. The mean length of the tubes at the begining of the examination was 0.2 cm forabout 15 worms. When the experiment ended, the mean lengths were2.2,2.2 and 1.9 cm for April, July and October groups respectively. The growth rate was generally low during the first 10 days but the respective growth rates were as a whole equal to 0.2, 0.16 and 0.05 cm/day for the groups.

Nereis diversicolor worms were also encountered occaisonally on some panels.

Barnacles : Balanus amphitrite and B.eburneus were the only representatives of the barnacles in the samples. The former is more available but the peak settlement of both coincided in July 1979 when the number of B.amphitrite

was 691 and of B.eburneus was 348 specimens/panel.

Along with Mercierella the growth rate of B.amphtrite was examined. Within the first 20 days of settlement, July individuals were five times as large as those settled in April and October. The maximum size was attained after 90 days in the case of July specimens(1.5cm in base length), but in the case of October worms the maximum size(1.4cm in base length) was reached after 120 days.The growth rate for the three groups in the first fifty days of settlement were 0.174, 0.25 and 0.13 mm/day for April, July and October groups respectively.

The maximal basal diameter attained in the field was 1.7cm for Balanus amphitrite and 1.2 cm for Balanus eburneus.

Amphipoda tubes: Corophium volutator was the only tube making amphipod found on the panels. Its tubes attached to the panels from March to July 1978 and numerically ranged between 32 and 63 tubes/panel /month.

Gammarus locusta and the isopod Sphaerome serratum are also present in the estuary waters.

Ascidians : In addition to colonies of Symplegma viride which colonies attach to the Nile piers, large number of minute specimens belonging to the genus Styela were inhabiting panels submerged in April-June 1979.

Discussion.

As far as the marine fouling communities are concerened the area surveyed is poor in comparison to the the other Egyptian marine areas. The heaviest fouling was observed during March,April ,May and July 1979 when the panels collected, in average, 58.2 g /month. This value is mush less than that had been estimated for the Eastern harbour of Alexandria(Ghobashy,1976), Port-Said(Ghobasgy, El Komy and Ramadan,1980) and Lake Timsah(Ghobashy and ElKomy, 1981). In the latter region,for example, a panel collectes about five times that collected by it in Damietta harbour.

Considering Tebble(1953),Naylor(1959) and Moroz(1977) statement, fouling at Damietta is characteristically estuarine. The salinity and pH are

fluctuating and the sewage discharge as well as the silt deposition add to the discrepencies of the composition and quantity of fouling. No definite seasonality is observed there and in the years of investigation the area was ecologically inconsistent. Figure 4 reveals that the standing crop of the panel fouling is variable and does not conform with any definite system.

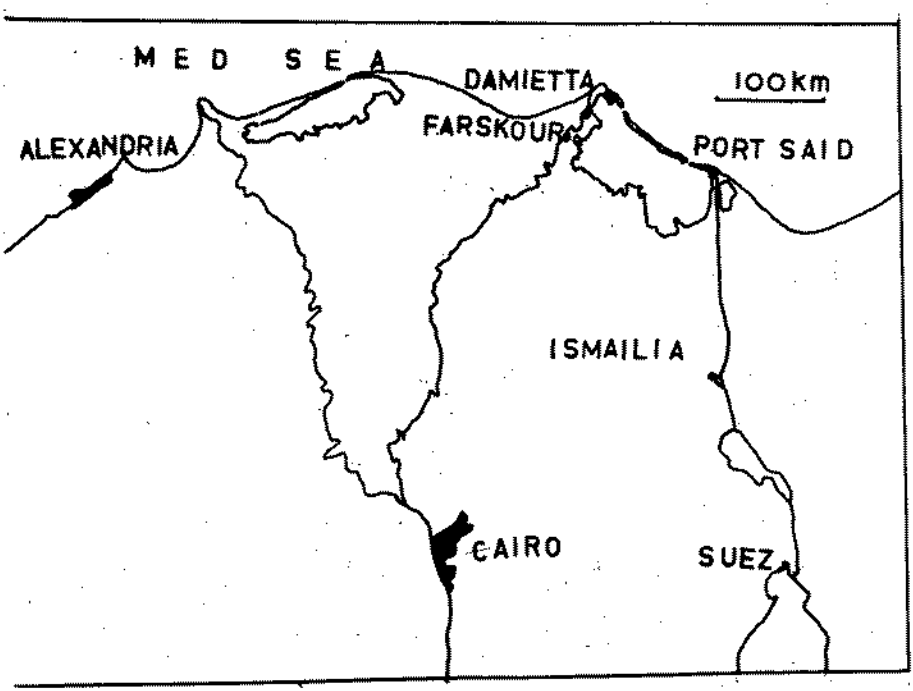
The fouling community at Alexandria,Port Said and Ismailia(Lake Timsah) are generally the same but all are quite different from that recorded at Damietta. In the three harbours the multispecis populations of hydroids, tube worms , barnacles, amphipods, bryozoans and ascidians almost settle in the majority of year. At Damietta on the other hand,Mercierella enigmatica which is an estuarine tube worm(Kuchl,1977) dominates the fouling settlers. In Egypt, this species has only been found in the brachish-water lakes by Steuer(1935). Moreover, Nereis diversicolor, Sphaeroma serratum and Coronhium volutator are typically estuarine forms(Meadows,and Campell,1978).

The foregoing suggests that the influence of the sea on the biotic conditions of the Nile at Damietta is still weak, despite of the isolation of the area from the rest of the river. Mixing with the Mediterranean waters is thus not effective enough to cause a change in the ecosystem towards the marine side. Clearly it is difficult to determine the factors which influence the settlement period and the rate of any organism in the investigated area. Temperature was nearly the same in May and July 1979 but the settlement dropped in density from68.6 g/panel in May to 16g/panel in June and increased again in July to be 42.1g/panel. In the meantime pH and salinity were steadily increasing. In 1978 salinity dropped from 29.3‰ in May to 18.8‰ in the next month, but the settlement conversely jumped from 5.1g to 18.3g/panel. The abrupt increase in pH from 7.3 to 8.47 in the same period does not encourage such organismic flourish (Al Kholy and El Wakeel,1975).

The effect of any single parameter on the growth rate is also uncertain beacause of the fluctuating conditions, but it is worthy to mention that the

growth rate of Balanus amphitrite here is more or less similar to that found by Ghobashy(1975) in the Eastern harbour of Alexandria for the same barnacle.

Presumably, construction of a lrge harbour at Damietta estuary mouth will cause profound changes in the ecological conditions and the complete mixing with the sea water will give rise to the establishment of new circumstances. These circumstances will be more or less comparable with the other Egyptian marine harbours. Serious measures will thus be required to overcome the expected severe fouling.



A map to show the position of Damietta harbour and Farskour dam.

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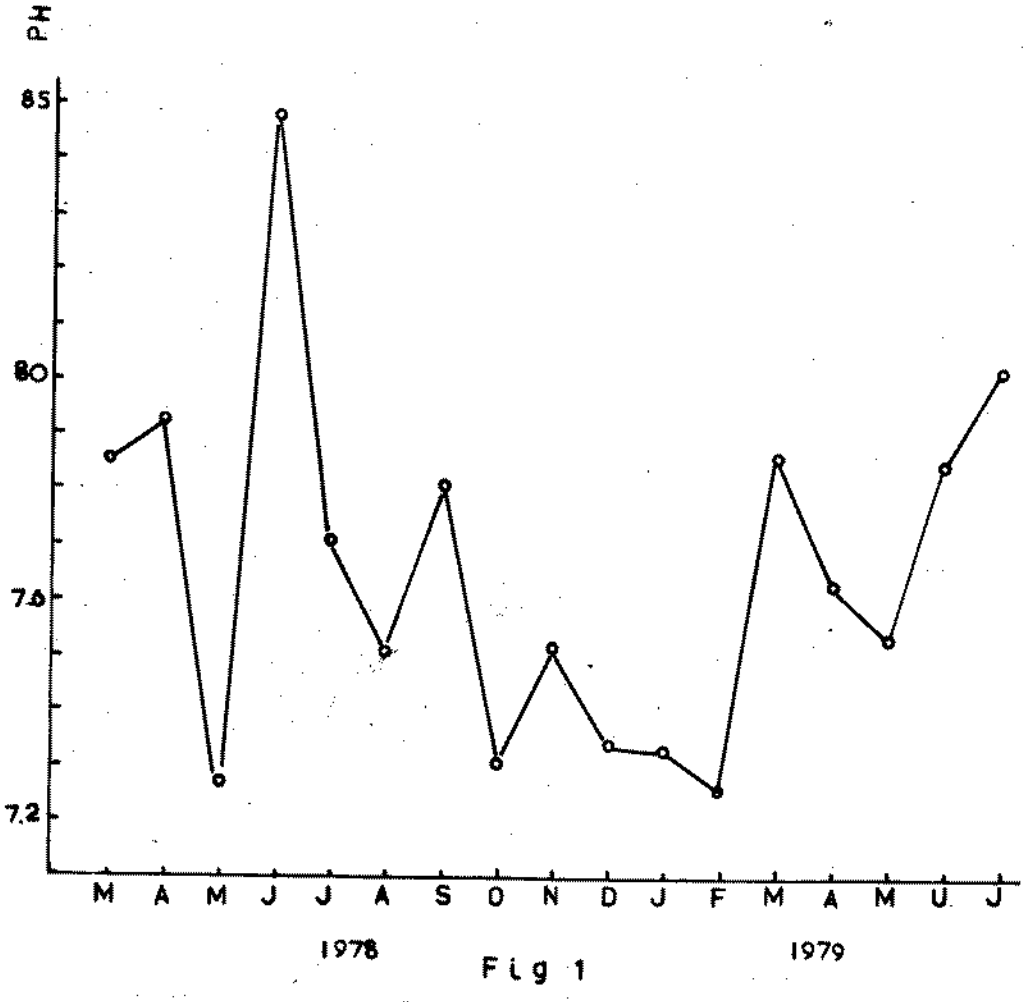

Figure 1. Values of pH during the period of investigation.

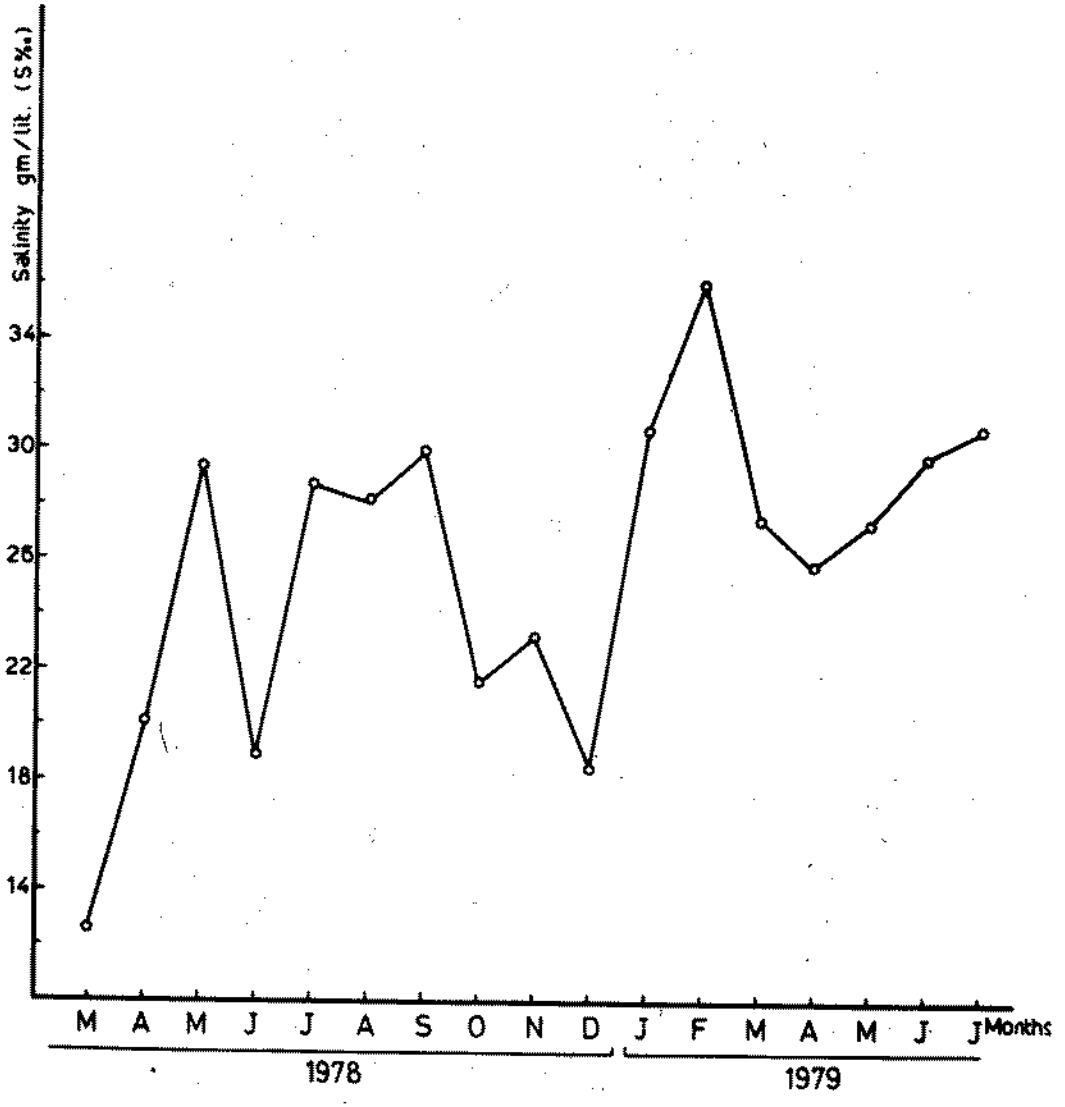

Figure 2. Values of salinity during the period of investigation.

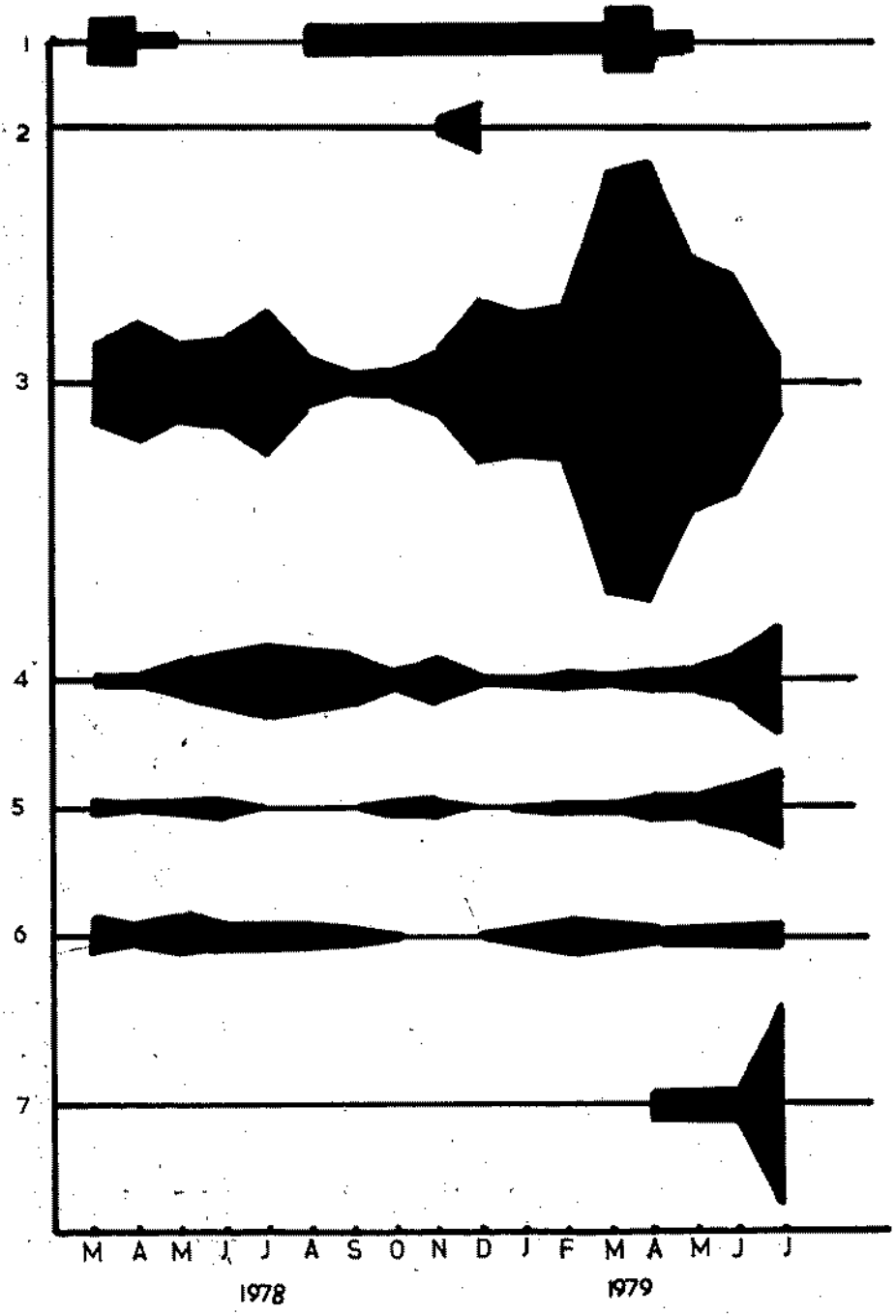

Figure 3. Monthly settlement variations of the fouling organisms in Damietta Nile estuary. Ordinates are the square roots of the monthly settlement of the following foulers:
1-Algae; 2-Protozoa; 3-Mercierella; 4-Balanus amphitrite; 5-Balanus eburneus; 6-Amphpoda tubes;7Ascidians

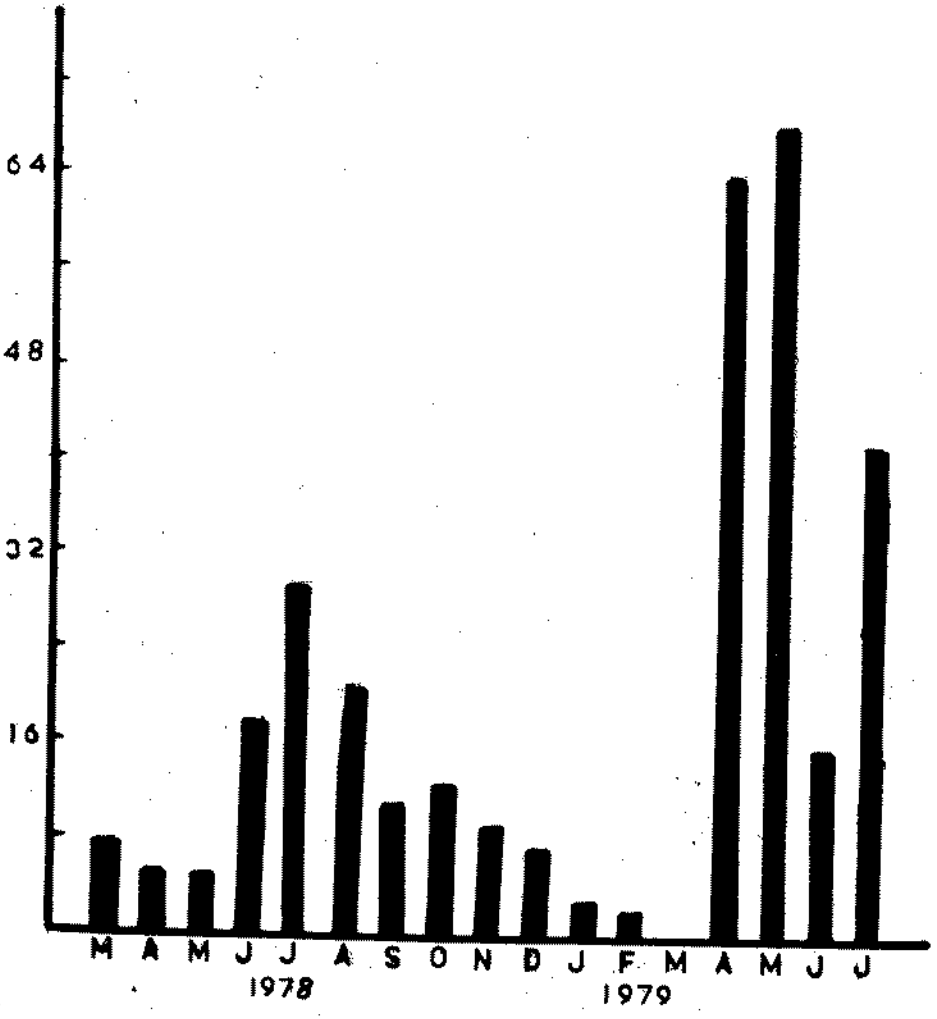

Figure 4. The variations of the wet weight of the panel fouling during the period of investigation.

Sullom Voe Oil Terminal, Shetland: benthic marine algal fouling communities.

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Fouling communities widely recorded on floating structures in Sullom Voe showed close similarities in floristic composition and zonation with those in other British and North sea locations. They were characterised by wave-washed green algae (*Ulothrix*, *Urospora*), water line brown communities (*Petalonia*, *Scytosiphon*) with kelps (*Laminaria* spp.) below water level. Communities on fixed structures were similar to those of natural rocky habitats. The principal fouling species on inshore structures are also those of offshore fixed installations in the North Sea.

Agglomerations surjalantes souvent marquées sur les constructions flottantes à Sullom Voe, avaient une ressemblance exacte en composition en floristique et en ceinture avec celles trouvées dans des localités de la Mer du Nord. Elles avaient été caracterisées par des algues verts qui reçoit des embruns (*Urospora*), des agglomerations brunes de flottaison (*Petalonia*, *Scytosiphon*) avec des *Laminaria* spp. qui se trouvent au dessous de la ligne de flottaison. Les agglomerations marquées sur les constructions fixées ressemblaient celles des habitats naturels rocheux. Les principaux espèces surjalantes sur les structures près de la côte sont les mêmes qu'on trouve sur les appareils fixés au large de la Mer du Nord.

Sullom Voe fouling

Introduction

The Shetland archipelago lies in the northern Atlantic Ocean some 200km north of mainland Scotland and west of the extensive North Sea oil fields. The close proximity of Shetland to these fields led to the construction of Europe's largest oil terminal alongside the deep, sheltered waters of the fjord-like Sullom Voe. The outer reach of the Voe is now a major port servicing world shipping. For a full account of the hydrography, chemistry, geology and biology of Sullom Voe, see Proceedings of the Royal Society of Edinburgh 80B (1981).

Prior to construction of the terminal, a comprehensive algal survey of Sullom Voe was undertaken (Tittley et al., 1976). Later (1983), when the terminal was in full operation, the algal vegetation was reassessed (Tittley et al., 1984). During this latter period of field work, algal communities on some new man-made installations, providing additional or alternative habitats, were studied.

Plant and animal fouling communities are widely recognised as a major problem. They impede efficient operation, inspection and maintenance, accelerate corrosion of shipping and offshore structures (Eikers, 1978; Freeman, 1977; Hardy, 1981), block coolant water intake pipes and screens, and interfere with mariculture equipment. As a consequence these fouling communities are being studied throughout the world; for full reviews see Fletcher (1980a,b). Most British studies have been carried out on southern coasts, particularly adjacent to major harbours and ports. Only recently has greater attention been given to fouling communities on offshore North Sea rigs (Fortreath et al., 1982; Hardy, 1981; Moss et al., 1981). The present investigation therefore contributes towards better understanding of algal fouling in the region.

Materials and Methods

Sixteen floating installations (two barges, fourteen navigation and mooring buoys; Table 1, Figure 1) were investigated in late July 1983. The moored barges had been longer in the sea than had the buoys, regularly lifted for maintenance (Table 1). Macro-algal cover was field recorded, while communities of smaller species were investigated by sampling (1) above, (2) at, and (3) below, water level.

The inner faces of two fixed structures, a

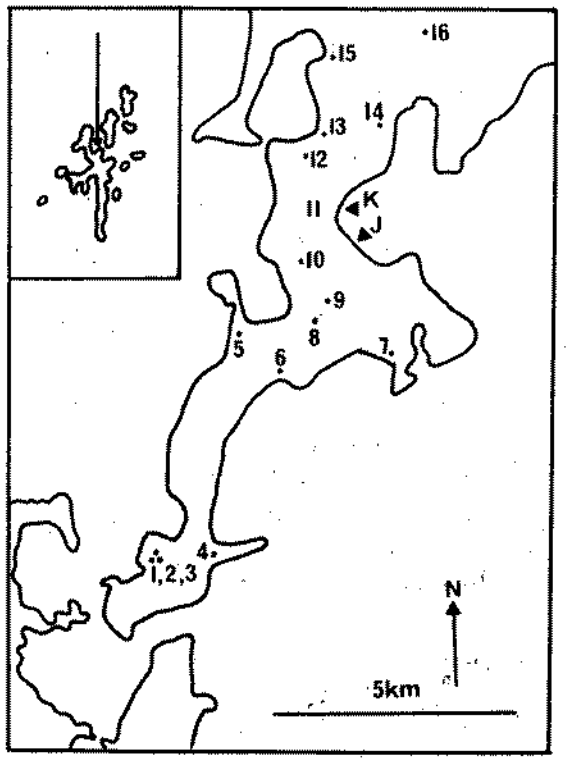

Figure 1. Distribution of study sites in Sullom Voe. Inset: Shetland Isles: For key to numbers and letters see Tables 1 and 2.

steel supporting leg of oil terminal jetty 3 and a vertical concrete wall of the Kames jetty, were investigated by recording along continuous transect lines. Macro-algal cover was field-recorded while smaller species were investigated by sampling.

Results

Seventy-nine benthic macro-algae were recorded as fouling organisms in Sullom Voe; lists of algae detected on floating and fixed structures are presented in Tables 1 and 2 respectively.

Floating structures

Fifty-five species (12 Chlorophyta, 24 Phaeophyta, 19 Rhodophyta) were detected; barges and buoys in the inner sheltered Voe were richer than on buoys in the outer Voe. Most commonly recorded were Ectocarpus siliculosus (algal nomenclature follows Parke & Dixon, 1976) and Petalonia fascia. Greens such as Urospora penicilliformis were also common. In general, the floating structures were richer in brown algae but poorer in red algae.

The vertical zonation showed three fairly distinct bands. An upper band of the finely filamentous Urospora penicilliformis and Ulothrix flacca at wave washed levels was particularly prominent (reaching 100 - 150mm above water level) on buoys in the outer more exposed parts of the Voe. In shelter the band was less well-developed, being replaced just above water level by Enteromorpha spp.

At standing water level a second band consisted of Enteromorpha spp., dense growths of the browns Petalonia fascia, P. filiformis, Scytosiphon lomentaria, and filamentous Ectocarpus siliculosus, Giffordia granulosa and Pilayella littoralis. This band was dominated on buoys 5 and 7 by dense growths of Chordaria flagelliformis.

Below water level, most structures supported dense growths of large browns; Laminaria saccharina was more frequent than L. digitata. Beneath the canopy, a low lying community of smaller algae was intermixed with barnacles (noticeably on buoys 8 and 15) and tube building amphipods.

A characteristically well-developed community at inner Voe sites included Bryopsis plumosa, Desmarestia viridis, Dictyosiphon foeniculaceus, Sauvageaugloia griffithsianus, Ceramium rubrum, Polysiphonia brodiaei and P. urceolata.

A generally noteworthy feature was the numerous

Table 1 Chlorophyta on floating structures in Sullom Voe

Species	Site	1 IS	2 IS	3 IS	4 IS	5 IS	6 IS	7 IS	8 IS	9 IS	10 IS	11 IS	12 IS	13 IS	14 IS	15 IS	16 IS	T Isp	T Ssp	T Site
Acrosiphonia aeruginosa		+	C		+	+		+										2	3	5
A. arcta	!											+			C			0	2	2
Blidingia minima	*o		++															1	1	1
Bryopsis plumosa	o		+															0	1	1
Enteromorpha clathrata	!								+									0	1	1
E. intestinalis	!*o		+	+											+			1	2	3
E. prolifera	*o	A	A+	A		+			+						+		C	5	3	7
Rhizoclonium riparium	*o	+		+	+													1	2	3
Ulothrix flacca	*o	+					A				+		C		C	+		6	0	6
Ulva lactuca	*o	+	+	+	+	+			+						C		C	1	7	8
Urospora penicilliformis	!*o	+			A+				C	A	+	+	C	+	C	C	A	11	1	11
U. wormskioldii	*															+		0	1	1
Totals		6	6	4	4	3	2	1	4	1	2	2	2	1	6	3	2			

Key: ! = Recorded by Grieve & Robertson (1864); * = recorded by Beth (1953);
o = recorded by Fletcher (1980a). I = intertidal; S = sublittoral; T = totals;
A = abundant; C = common; + = present. (E) = Epiphyte.

Table 1 (continued) Phaeophyta on floating structures in Sullom Voe

Species	Site	1 IS	2 IS	3 IS	4. IS	5 IS	6 IS	7 IS	8 IS	9 IS	10 IS	11 IS	12 IS	13 IS	14 IS	15 IS	16 IS	T Isp	T Ssp	T Sites
Asperococcus fistulosus	!		+		+	+		+										0	4	4
Chordaria flagelliformis	!*			+	+	A		A	+	+		+			C		C	0	9	9
Desmarestia viridis	!*o		+						+									0	2	2
Dictyosiphon foeniculaceus			+			+												0	2	2
Ectocarpus fasciculatus	!*o			C		+	C	C	+	C	+				+			0	9	9
E. siliculosus	!*o	+A	C+	++	C		C	C		C	A	C	C	C	C	C	A	3	14	14
Giffordia granulosa	*o	C	C+	++	C								C				C	2	6	6
G. hincksiae	! o												C					0	1	1
Hecatonema foecundum (E)	.	C	C	+		+	C	+	+	+			+				C	0	10	10
h. maculans (E)			+		+													0	2	2
Laminaria digitata	!*o		C	+	+			+						C			+	0	6	6
L. saccharina	!*o	A	A	A	C	+	A		A	+	+		A		+		A	0	12	12
Leathesia difformis						+												0	1	1
Myrionema aecidioides (E)													C					0	1	1
M. strangulans (E)	o	+	C+	+					+								+	2	4	5
Myriotrichia clavaeformis (E)		+			+													0	2	2
Petalonia fascia	*o	C+	+		C			C	C	A	+	A	A	A	A	A	A	2	13	13
P. zosterifolia	*o	+		C										A		A		2	2	4
Pilayella littoralis	!*o	+	+	+		+								C			+	2	4	6
Protectocarpus speciosus (E)									+									0	1	1
Punctaria latifolia			+		+	+			+	+	+	+					+	0	8	8
P. plantaginea	!*				+													0	1	1
Sauvageaugloia griffithsianus					C													0	1	1
Scytosiphon lomentaria	!*o	+	CC		C	C		C	A	+		A	A	C	A	A	A	2	12	13
Totals		10	14	9	13	10	4	8	10	8	5	5	8	6	6	4	11			

Table 1 (continued) Rhodophyta on floating structures in Sullom Voe

Species	Site	1 IS	2 IS	3 IS	4 IS	5 IS	6 IS	7 IS	8 IS	9 IS	10 IS	11 IS	12 IS	13 IS	14 IS	15 IS	16 IS	T Isp	T Ssp	T Sites
Antithamnion boreale												+						0	1	1
A. plumula	o		+														+	0	2	2
Audouinella daviesii(E)	!	C																0	1	1
A. purpurea	*			+														0	1	1
A. secundata(E)			C															1	0	1
A. virgatula(E)	!		+	+													+	1	2	3
Audouinella sp. (E)	o					+	+											0	2	2
Callithamnion corymbosum		+																0	1	1
Ceramium diaphanum	o		C					C										0	2	2
C. rubrum	! o		A	A	+	+	C			C			+				+	0	8	8
Chylocladia verticillata	o		+															0	1	1
Erythrotrichia carnea (E)		+				+			+									0	3	3
Polysiphonia brodiaei	! o	+				+			+						C			0	4	4
P. elongata	!*o		+										+					0	2	2
P. fibrata			+															0	1	1
P. nigra	!*		+															0	1	1
P. urceolata	!*o	+			+													0	2	2
Porphyra purpurea	!	+																1	0	1
P. umbilicalis	*o																C	1	0	1
Totals		7	9	3	2	4	1	1	3	0	0	2	1	0	1	0	3			
Total algal species		23	29	15	19	17	7	10	17	9	7	9	11	7	13	7	17			
Months immersed		12	12	12	13	..	13	12	12	11	6	4	12	9	12	3	11			

epiphytes; commonly the hosts were Laminaria spp., less frequently Petalonia fascia and Ceramium rubrum. Apices of Laminaria saccharina blades often carried Giffordia granulosa, G. hincksiae, Ectocarpus fasciculatus, Hecatonema foecundum, Myrionema aecidioides, M. strangulans, Myriotrichia clavaeformis, Protectocarpus speciosus, Asperococcus fistulosus, Antithamnion plumula and Audouinella spp.

Tables 1 shows that the number of species present and floristic composition differed with how long substrate had been floating. Some older structures (e.g. barges 2 and 3) supported more red algae, others (buoy 6) carried poor growths.

A further influence on the extent of fouling was surface roughness. Uneven surfaces (particularly abraded paintwork, cables, edges and ridges) usually supported denser growths of algae than did smooth painted surfaces. This was particularly noticeable on buoys 10, 12 and 13.

Fixed structures.

These barnacle covered sites were in moderately wave-washed situations. They bore similar patterns of littoral algal occurrence and zonation (Tables 2 and 3). The uppermost band, Blidingia minima, was 200 - 400mm in width. Below, Porphyra umbilicalis formed a band of similar width, and merging at lower levels into a wider band of Enteromorpha prolifera and E. intestinalis. Ulva lactuca descended thence to water level, with Fucus spp. also present on jetty 3. At low water level and below on jetty 3 were sequential bands of Laminaria saccharina and L. digitata ; this canopy shaded an underflora mainly of small reds (Table 2). At about 1m depth, laminarians were replaced by Desmarestia aculeata and Delesseria sanguinea; at still lower levels tunicates, sponges and starfish predominated over the associated small growths of algae. Other jetty legs revealed L. hyperborea, with abundant epiphytes.

Discussion

Only about one-half of the species recorded from Sullom Voe (Tittley et al., 1976) were detected as fouling algae, it is likely that intensive and seasonal studies especially of fixed man-made installations, would undoubtedly reveal additional species. Nevertheless this period of field work revealed species (55) on floating structures, comparable with the number (53) obtained from

Sullom Voe fouling

Table 2 Fouling species on fixed structures in Sullom Voe

Chlorophyta	Acrosiphonia arcta J-
	Blidingia minima J+ K+
	Bryopsis plumosa J-
	Codium fragile ssp atlanticum J-
	Derbesia marina J-
	Enteromorpha intestinalis J- K+
	E. prolifera J- K+
	Rhizoclonium riparium J-
	Ulothrix flacca K+
	Ulva lactuca J+- K+
	Urospora penicilliformis K+
Phaeophyta	Cutleria multifida J-
	Desmarestia aculeata J-
	Ectocarpus fasciculatus J-
	Fucus spiralis J+
	F. vesiculosus J+
	Giffordia granulosa J-
	Laminaria digitata J-
	L. hyperborea J-
	L. saccharina J-
	Litosiphon filiformis J-
	Punctaria filiformis J-
	Scytosiphon lomentaria J-
	Sphacelaria radicans J-
Rhodophyta	Antithamnion boreale J-
	A. plumula J-
	Audouinella purpurea J-
	A. secundata J+
	Bonnemaisonia hamifera J-
	Ceramium diaphanum J-
	C. rubrum J-
	Cystoclonium purpureum J-
	Delesseria sanguinea J-
	Erythrotrichia carnea J-
	Hypoglossum woodwardii J-
	Lomentaria clavellosa J-
	L. orcadensis J-
	Membranoptera alata J-
	Phycodrys rubens J-
	Plumaria elegans J-
	Polysiphonia brodiaei J-
	P. elongata J-
	P. nigrescens J-
	P. urceolata J-
	Porphyra leucosticta J-
	P. umbilicalis K+
	Ptilota plumosa J-
	Rhodomela confervoides J-

Key: J = Jetty 3; K = Kames Jetty; + = littoral; - = sublittoral

Table 3: Zonation of species on Jetty 3 leg and Kames Jetty

Depth	0 MHWS	20	40	60	80 MTL	100	120	140	160 MLW	180	200	220	240	260 MLW	280 -1m	300cm
Species																
Jetty 3																
Barnacles		+	+	+	+	+	+	+	+	+	+	+				
Blidingia minima		+	+													
Porphyra umbilicalis			+	+												
Enteromorpha spp.				+	+											
Ulva lactuca					+	+	+	+								
Fucus spp.					+	+	+	+								
Laminaria saccharina									+	+						
L. digitata										+	+	+				
Underflora spp.										+	+	+	+	+		
Desmarestia aculeata													+	+		
Delesseria sanguinea													+	+		
Sponges, tunicates														+	+	+
Kames Jetty																
Barnacles		+	+	+	+	+	+	+								
Blidingia minima		+	+													
Porphyra umbilicalis			+													
Enteromorpha spp.			+	+	+	+										
Ulva lactuca					+	+	+	+								

prolonged study of 35 buoys near Helgoland (Beth, 1953). Our results contrasted with others (Table 4) in which only eleven species were recorded on nine buoys off eastern England. Grieve & Robertson (1864) recorded substantially higher numbers on nine buoys in the Firth of Clyde (west Scotland). highest diversity (68 species) was recorded by Fletcher (1980a) on floating structures along southern English shores. That study involved mature, well-established communities and therefore differs from our time restricted investigation of structures not long immersed. Similarly restricted fouling communities on functional shipping (Evans & Chrisitie, 1973) are not directly comparable with those of stationary buoys.

Species composition on floating structures in Sullom Voe is similar to that described for structures elsewhere in the British Isles and North Atlantic (Grieve & Robertson, 1864; Beth, 1953; Lodge, 1949; Fletcher 1980a,b). Commonly recorded on all were Ulothrix flacca, Urospora penicilliformis, Enteromorpha spp., Giffordia granulosa, Petalonia fascia, Scytosiphon lomentaria, Ceramium rubrum, and Polysiphonia spp. (Table 1). We, as did others, noted more Phaeophyta than Chlorophyta and Rhodophyta (Table 5). Such similarities on diverse and widely-scattered floating structures are not surprising in view of the unique and artificial environment, an upper physically harsh wave-washed level merging almost directly into the shallow subtidal with high light intensities.

Restriction on the development of buoy fouling communities results from frequent raising for regular cleaning and repainting; many communities are therefore of fast-growing pioneer algae. Beth (1953) noted rapid colonisation of cleaned buoys by Ulothrix and Urospora, often within one month of re-immersion. These algae are also pioneers on new concrete sea-walls (Tittley & Shaw, 1980). Other frequent pioneer colonisers of floating structures are ectocarpoid browns (Beth, 1953; Fletcher, 1980a,b; Grieve & Robertson, 1864; Table 4). These algae occur at and below standing water level; in Sullom Voe, Ectocarpus siliculosus and Giffordia granulosa were widespread on buoys and barges.

The few red algae detected probably resulted from the short immersion-periods of most buoys; pontoons in the Solent (in water for much longer period) were richer in reds (Fletcher, 1980a,b). Only Ceramium and Polysiphonia (generally of widespread occurrence) were regularly recorded.

Sullom Voe fouling

Table 4 Species recorded from East coast buoys.

	No. of buoys
Enteromorpha intestinalis	2
E. linza	1
E. prolifera	7
Ulothrix flacca	1
Urospora penicilliformis	3
Ectocarpus fasciculatus	2
E. siliculosus	2
Giffordia granulosa*	9
Petalonia fascia*	6
Scytosiphon lomentaria	1
Bangia atropurpurea	3

* = dominant sp.

Table 5 Totals of species recorded

	C	P	R	To
Sullom Voe	12	23	19	54
Helgoland	16	19	18	53
E. England	5	5	1	11
Clyde	7	17	15	39
Solent	17	23	28	68

C = Chlorophyta
P = Phaeophyta
R = Rhodophyta
To = Totals

Table 6 Preliminary list of species recorded from North Sea Rigs

<u>Chlorophyta</u>
Acrosiphonia aeruginosa
Blidingia minima
Cladophora spp.*
Enteromorpha intestinalis**
E. prolifera
Enteromorpha spp*
Rhizoclonium riparium
Ulothrix pseudoflacca
Ulva lactuca* **

<u>Phaeophyta</u>
Alaria esculenta**
Desmarestia viridis* ***
Ectocarpus-like*
Giffordia granulosa
G. sandriana
G. secunda***
Laminaria digitata**
L. hyperborea**
L. saccharina+
Laminaria sp.*
Scytosiphon lomentaria (&*, -like)
Spongonema tomentosu

<u>Rhodophyta</u>
Audouinella purpurea
Audouinella spp.*
Bangia atropurpurea*
Lomentaria clavellosa
Lomentaria orcadensis***
Polysiphonia brodiaei & **
P. urceolata & **
Porphyra umbilicalis
Polysiphonia spp.*

* = Hardy (1981)
** = Fortreath <u>et al.</u> (1983)
*** = Price <u>et al.</u> (1977)
\+ = Goodman & Ralph (1979)
unmarked = unpublished sources

Ceramium rubrum, being an early coloniser, differed from Polysiphonia urceolata which did not appear before 5 months immersion (Beth, 1953). The depauperate red algal vegetation on buoys in Sullom Voe also contrasted with rich populations on natural rock and fixed structures (Tittley et al., 1976, and Table 2). Lomentaria clavellosa, a community dominant on Helgoland buoys (Beth, 1953) and on floating structures in the Clyde (Grieve & Robertson, 1864) and the Solent (Fletcher, 1980a,b) was not found on floating structures in the Voe despite abundance on adjacent natural substrates. Differing species richness on structures immersed for similar durations in the Voe is not easily explained.

The occurrence of three horizontal bands of algae on floating structures is in general agreement with reports (Beth, 1953; Milne, 1940; Fletcher, 1980a,b). The upper wave-washed green band is similar to that found at supralittoral levels on the shore. The two habitats are not dissimilar, being only intermittently wetted. Absence of Fucus spp. from buoys is probably due to severe limitations on surface area (Beth, 1953). Luxuriant growths of opportunist annual greens and browns at standing water level on buoys may have been encouraged by high insolation and temperatures; in nature such algae occur in shallow upper-shore rock pools, sharing these same conditions. The poor cover of Enteromorpha on Sullom Voe buoys contrasted with 0.5m bands on Helgoland buoys. Beth (1953) suggested that the extent of Enteromorpha cover on buoys depends both on time floating and distance from the coast.

A subtidal laminarian community has also been widely reported elsewhere (Beth, 1953; Milne, 1940; Fletcher, 1980a,b; Grieve & Robertson, 1864). As in Sullom Voe L. saccharina was most common, L. digitata being only infrequent. However compare Milne's (1940) report of L. digitata abundant on buoys in Plymouth Sound. According to Beth (1953) a laminarian community was best developed on buoys immersed for long periods. Speed of new surface colonisation by algae and the eventual floristic composition are dependant on both adult plant fertility and on abundance of spore inoculum in the water. Rapid appearance of L.saccharina on Sullom Voe buoys compared to on offshore structures (Goodman & Ralph, 1979) probably results from the greater spore abundance in inshore waters. Beth (1953) drew particular attention to decrease in algal growths with increasing distance offshore. Oil and gas rigs

at considerable distances offshore, however, support well developed fouling communities and it is not clear whether these are acquired by (a) natural spore dispersal, (b) in inshore waters while en route to site, or (c) from vectors such as shipping. What is clear from preliminary listing (Table 6) of fouling algae from North Sea rigs is that many species are also principal growths inshore floating structures. There are differences which relate to the rigs fixed situations. Well-developed bands of red algae (e.g. Polysiphonia brodiaei) cover lower littoral levels on legs (Fortreath et al., 1982), contrasting with sporadic occurrence on buoys. P. brodiaei was important at subtidal levels on jetty legs in Sullom Voe. Fortreath et al. (1982) and Hardy (1981) recorded L. digitata as the principal laminarian on oil rigs, with sporadic L. hyperborea and Alaria esculenta. Both Laminaria spp. commonly occur on fixed structures in Sullom Voe. Only Goodman & Ralph (1979) noted L. saccharina on oil rigs; its restricted occurrence on offshore installations is surprising since it is widespread on buoys and jetties in Sullom Voe and elsewhere.

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Observations on the strength of attachment of spores and germlings of the marine fouling alga Enteromorpha

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The strength of attachment of settled zoospores and young germlings of the common marine fouling green alga Enteromorpha was determined using the radial flow chamber. The attachment strength measurements obtained for different settlement times were correlated with scanning electron microscope observations. Spores settled very rapidly and secured an early attachment by the release of a small pad of adhesive material. A marked increase in attachment strength of the spores was recorded with increasing settlement time up to 60 minutes. This rise appeared to be concomitant with increased production and outward spread of the adhesive. During the subsequent stages of spore germination and sporeling growth a continued more gradual increase in attachment strength was recorded. This was attributed to the production and adhesive properties of the primary and secondary rhizoidal filaments.

Observations sur la puissance d'attachement des spores et des jeunes plantes de l'algue marine salissant Enteromorpha.

La puissance d'attachement des zoospores fixes et des jeunes plantes de l'algue verte marine salissante Enteromorpha a été déterminé en employant la chambre à l'écoulement radial. Les mesures de la puissance d'attachement obtenues, pour les périodes différentes d'établissement, ont été mises en corrélation avec des observations d'un microscope électronique. Les spores ontres vite colonisé l'endroit et s'y sont très tôt attachés par le dégagement d'un petit bourrelet d'un produit collant. Une augmentation nette de la puissance d'attachement des spores a été enregistré en prolongeant la période d'établissement d'un maximum de soixante minutes. Cette augmentation apparait concomitant de l'augmentation de la production et de la dispersion vers l'extérieur de l'adhésif. Pendant les phases suivantes de la germination des spores et la croissance des jeunes plantes, une augmentation progressive de la puissance d'attachement a été enregistré. Ceci a été attribué aux capacities de production et de pouvoir adhérent des filaments rhizoidals primaires et secondaires.

Strength of attachment of Enteromorpha spores

INTRODUCTION

The growth of marine algae on artificial structures has been well documented with special reference given to the colonisation of ships, buoys and offshore platforms and the problems this causes (Baker and Evans, 1973a,b; Evans and Christie, 1967; Fletcher and Chamberlain, 1975; Moss, 1973). The success of a marine alga in colonising a structure is initially dependent upon the settlement and attachment of the reproductive spores. After this, the spore must then develop an attachment mechanism to support the rapidly developing young erect shoot system. In recent years it has been felt that algae are at their most vulnerable during these early stages of development and that it may be possible to control algal fouling at this time. Although numerous studies have been made on the algal spore attachment process (Christie, 1973; Evans and Christie, 1970; Fletcher, 1976; Moss, 1975; Rawlence and Taylor, 1972) only a few have been concerned with measurement of the strength of adhesion and factors affecting it (Charters et al., 1973; Christie et al., 1970; Houghton et al., 1972; Jones et al., 1984; Norton, 1983).

Various techniques have been used in these studies to measure the strength of attachment of the settled spores. These include the water broom method used by Charters et al. (1973) and Norton (1983), the water jet technique of Christie et al. (1970) and the water velocity technique used by Houghton et al. (1972) and Jones et al. (1984). A more recent improved experimental approach for attachment strength measurement in organisms, which would lend itself readily to algal studies, is the Radial Flow Chamber (R.F.C.) described by Fowler and McKay (1979). To date this method has only been used to measure the strength of attachment of bacteria (Duddridge et al., 1982) and has not been involved in the study of any other group of organisms.

In the present study the R.F.C. of Fowler and McKay has been used to examine the strength of attachment of presettled zoospores and developing germlings of the cosmopolitan ship fouling green alga Enteromorpha intestinalis (L.) Link. Measurements of the attachment strength have been correlated with light microscope and scanning electron microscope observations.

MATERIALS AND METHODS

1. Preparation and treatment of algal material

Samples of fertile E. intestinalis with zoosporangia were collected locally on the northern sheltered shore of the Ferry Road Peninsula, Hayling Island, surface-cleaned in pasteurised filtered seawater (P.F.S.) and then allowed to air dry at 10°C for 20-24 hours. After this time the fertile material was excised from the remainder of the plant and flooded with P.F.S. Zoospore release was normally observed within 5 minutes but further stimulation by directing an artificial light source at the material was occasionally required. Upon release the zoospores were pipetted directly on to the surface of each test

disc (see later). The discs were then immediately placed in the dark for a maximum of two hours to encourage zoospore settlement. The discs were then removed after the following time periods: 1,2,5, 10, 20, 30, 40 and 60 mins, 7 and 28 days. They were then placed in the R.F.C. for 15 minutes at various flow rates and the attachment strength determined. For the longer time periods of 7 and 28 days the discs were removed from the dark after two hours, immersed in Von Stosch (1963) culture medium in large (11cm dia) plastic petri dishes and incubated at 20°C, 4000 lux white light intensity, 16h-8h light-dark photo-regime.

After removal from the R.F.C. the discs were examined under the light microscope and spore counts taken at 5mm intervals along 10 radii.

Percentage attachment was then calculated in relation to the number of zoospores originally present. For times greater than 30 minutes this was achieved by making a similar number of counts on the test disc before and after testing. Due to the time involved in the counting process it was not practical to follow this routine for the shorter settlement times and so the number of spores present after testing was related to untreated identically inoculated control discs. For each time interval tested replicates of both test and control discs were used and the mean percentage attachment calculated.

2. Light microscope and scanning electron microscope studies

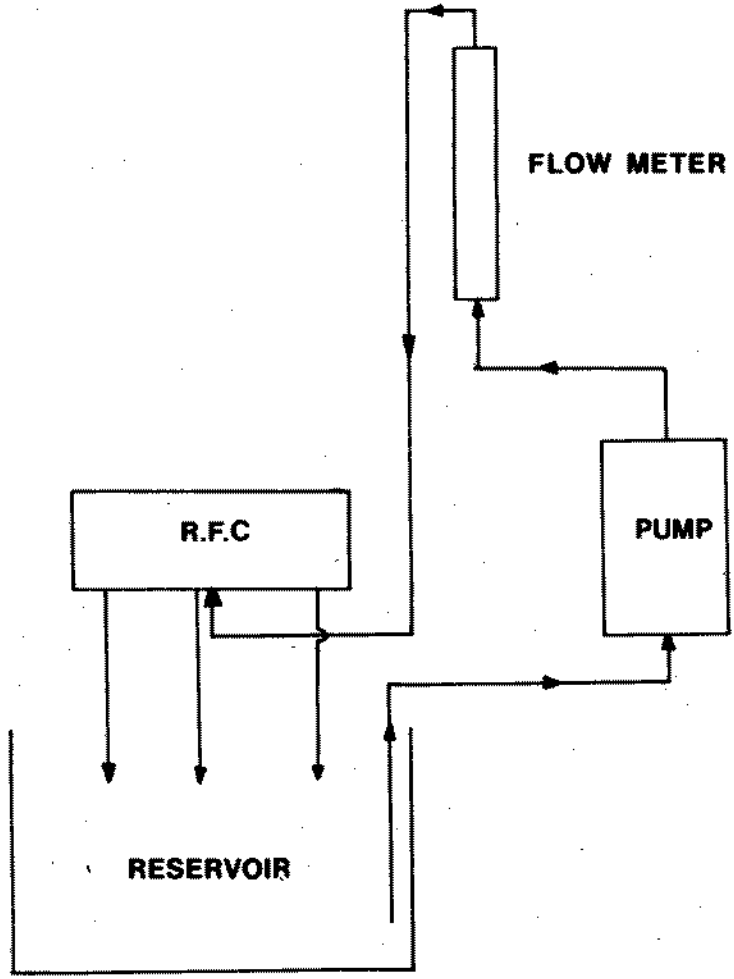
Enteromorpha zoospores were allowed to settle and develop on glass coverslips for the same time periods used in the attachment studies. The coverslips were then examined under both the light microscope and scanning electron microscope. Standard methods of fixation and preparation were used. The material was prefixed in a 4% (v:v) solution of glutaraldehyde in a 0.25M sucrose-0.1M cacodylate buffer mixture for 2 hours. Fixation was followed by three 20 minute washes in 0.1M cacodylate buffer of decreasing sucrose concentration (0.25M, 0.125M and zero). After this the samples were taken through an alcohol dehydration gradient starting at 15% with each wash lasting for 20 minutes. The samples were then given two 15 minute immersions in dry absolute alcohol (dried with $CaCl_2$) followed by dry acetone. The specimens were then critical point dried and mounted on S.E.M. stubs using double-sided adhesive tape. Once mounted the specimens were sputter coated in gold and examined in the JEOL T-20 scanning electron microscope.

3. Operation of the radial flow chamber

Details of the R.F.C. and its operation are illustrated in Figures 1 and 2. It is based on a recirculating system with water passing from a reservoir into the pump, the flow meter and then the R.F.C. (Fig. 1). The water enters the chamber through the inlet port, a (see Fig. 2), flows radially out across the base plate, A, and test surface, C, to the exit ports, b_1 and b_2 and then back to the reservoir. Leakage of water and ingress of air is prevented by the presence of a silicon rubber O ring, D. As the pumped water passes

FIGURE 1:

FLOW DIAGRAM OF RADIAL FLOW APPARATUS



Strength of attachment of Enteromorpha spores

KEY TO FIGURE 2

A = Base plate with legs d, and fastening screws e, extending from it.

B = Upper plate with central viewing port.

C = Test disc.

D = Silicon rubber "O ring".

a = Central entrance port for test fluid.

b_1 = Sectioned exit port for test fluid.

b_2 = Exit port for test fluid.

d_1 = Sectioned leg attached to base plate A.

d_2 = Leg attached to base plate A.

e_1 = Sectioned fastening screw.

e_2 = Fastening screw.

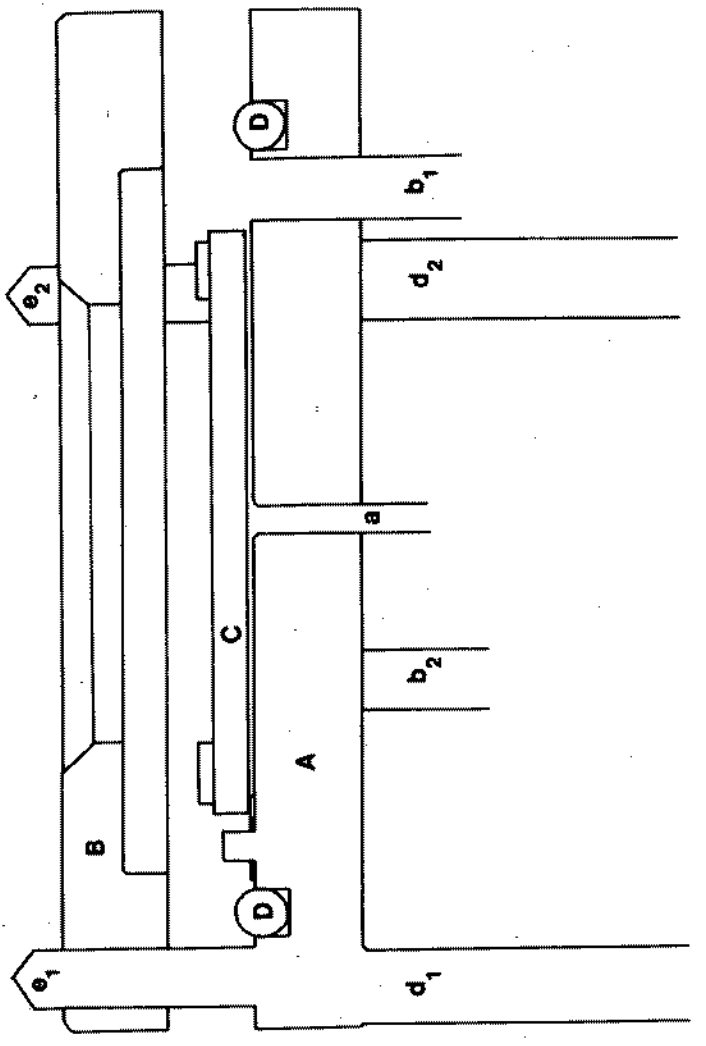


FIGURE [illegible] ROUGH RADIAL FLOW CHAMBER

across the test disc it decreases in speed and there is a resultant decrease in shear stress exerted on attached cells or spores. By regulating the flow rate it is possible to determine the shear stress operating at any point along the radius of the disc.

On the basis of preliminary trials using Enteromorpha sp. zoospores the water flow rate chosen for the present study was in the region of 6l/min for 15 minutes. Determination of the relative numbers of zoospores along the radius can then provide information on the critical shear stresses required for their detachment.

RESULTS

1. Measurements of attachment strength

Tables 1, 2 and Figures 3, 4 present data on the variation in attachment strengths of the Enteromorpha zoospores settled for the different periods of time. It can be seen that for any given shear stress value increasing the time of settlement of the spores from 1 min to 28 days gave a corresponding increase in the number of spores remaining attached to the surface. Particularly interesting was the observation that this increase in percentage attachment over the experimental time period was not uniform. For example, a marked increase in attachment strength was recorded between 1 min (1% attached cells at 3.21 $N.m^{-2}$ shear stress) and 10 min (25% attached cells at 3.21 $N.m^{-2}$ shear stress). Between 10 and 30 minutes there was little change in percentage attachment whilst a significant increase was observed between 30 and 40 minutes (25% to 46% attachment at 3.21 $N.m^{-2}$) and between 40 and 60 minutes (46% to 72% attachment at 3.21 $N.m^{-2}$ shear stress). This second increase in attachment strength was particularly evident under conditions of higher shear stress. For example at 51.8 $N.m^{-2}$ shear stress increasing the settlement time from 30 to 60 minutes resulted in a dramatic increase in the percentage attachment from 1% to 50%.

Thereafter, between 1hr and 7 days settlement time, across the range of shear stress values operating (2.0-67 $N.m^{-2}$), there appeared, in general, to be a reduction in the percentage attachment of material. After 28 days in culture however the percentage attachment once more increased (90% attachment at 67 $N.m^{-2}$).

2. Light microscope and scanning electron microscope observations

S.E.M. micrographs of the early stages of zoospore attachment in Enteromorpha are shown in Figures 5-16. It can be seen that after only one minute spores were settled on the coverslips. They were vertically orientated with the narrow anterior region in contact with the surface and the wider posterior end projected up into the water. The four flagella were usually extended away from the spore body along the substratum surface and were sharply attenuate at the tip, ending in a curled hair-like appendage. After 2 minutes a small pad of mucilage was observed on the substratum associated with the anterior end of the spore body. Between two and five minutes a number of structural changes were observed in the appearance of the

Table 1. Effect of settlement time on the percentage numbers of cells remaining attached following treatment in the R.F.C. (6.1 litres per minute flow rate).

DISTANCE FROM DISC CENTRE (m)	0.045	0.04	0.035	0.03	0.025	0.02	0.015	0.01	0.005
SHEAR STRESS ($N.m^{-2}$)	2.14	2.41	2.75	3.21	3.85	4.82	7.13	14.8	51.8
LOG S.S.	0.331	0.382	0.456	0.507	0.586	0.683	0.853	1.17	1.71
TIME	PERCENTAGE ATTACHMENT								
1 min	1.0	1.0	1.0	1.0	1.0	1.0	0	0	1.0
2 min	4.0	3.0	4.0	4.0	2.0	1.0	0	0	1.0
5 min	16.0	17.0	4.0	12.0	10.0	5.0	5.0	1.0	1.0
10 min	20.0	27.0	19.0	25.0	32.0	19.0	19.0	11.0	5.0
20 min	23.0	24.0	22.0	28.0	29.0	16.0	16.0	9.0	2.0
30 min	24.0	23.0	21.0	26.0	25.0	11.0	11.0	1.0	1.0
40 min	53.0	54.0	37.0	46.0	45.0	21.0	21.0	19.0	19.0
60 min	86.0	87.0	68.0	72.0	92.0	79.0	79.0	65.0	50.0

Table 2. Effect of settlement time on percentage attachment (7.0 litres per minute flow rate).

DISTANCE FROM DISC CENTRE (m)	0.045	0.04	0.035	0.03	0.025	0.02	0.015	0.01	0.005
SHEAR STRESS ($N.m^{-2}$)	2.47	2.78	3.18	3.71	4.45	5.57	9.24	19.2	66.7
LOG S.S.	0.393	0.444	0.502	0.569	0.648	0.745	0.945	1.28	1.82
TIME	PERCENTAGE ATTACHMENT								
7 days	71.0	83.0	66.0	67.0	66.0	55.0	45.0	68.0	53.0
28 days	86.0	87.0	100	83.0	93.0	79.0	74.0	100	89.0

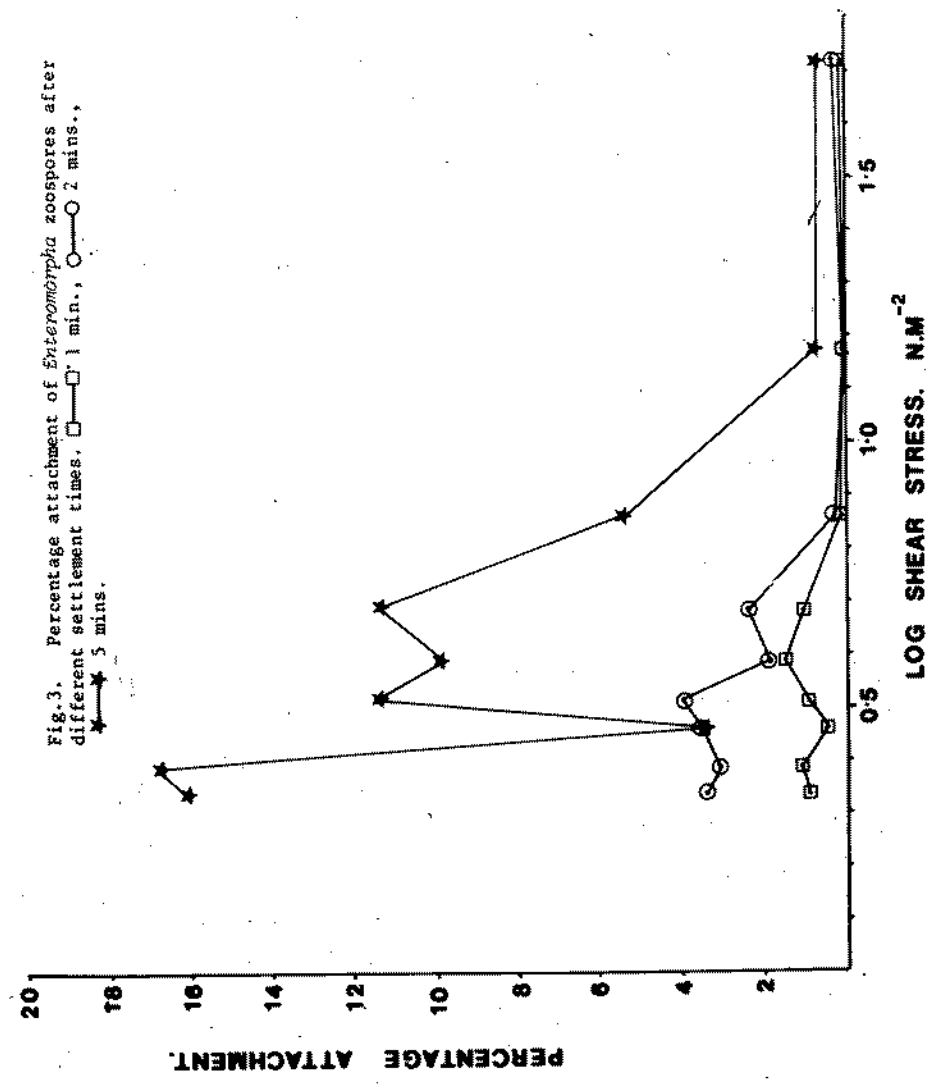


Fig.3. Percentage attachment of *Enteromorpha* zoospores after different settlement times, □—□ 1 min., ○—○ 2 mins., ✱—✱ 5 mins.

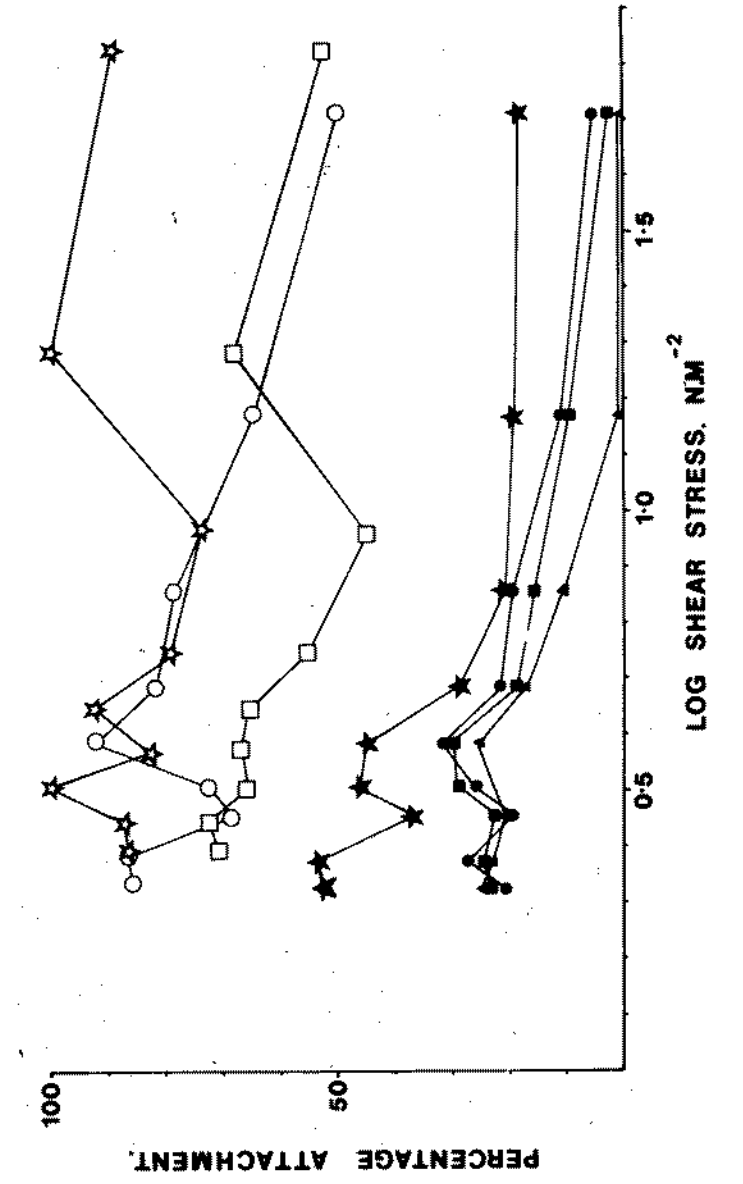


Fig.4. Percentage attachment of *Enteromorpha* zoospores and germlings after different settlement and growth times.
10 mins., 20 mins., 30 mins.,
40 mins., 60 mins., 7 days., 28 days.

spores. They were now much more closely adpressed to the substratum and were distinctly hemispherical in shape with a larger surface contact area. There was a much greater amount of mucilaginous material present which could be clearly seen extending out a short distance across the substratum around the spore periphery. The flagella were now less obvious, and on many of the spores were clearly contracted and showing signs of decay. Between 10 and 30 minutes very little change was observed with the possible exception of greater production of mucilage and further loss of flagella (Figs. 9, 10, 11). Between 30 and 60 minutes the most noticeable change was the more copious production of mucilage, which extended out a considerable distance across the substratum surface. The material was hyaline, rather than reticulate in appearance, and appeared to "flow" out from the spore forming an intimate contact with the surface (Figs. 15, 16).

Between 1-3 days the spores showed signs of germination. The spore cell elongated vertically and divided transversally to produce a short erect, multicellular filament. During the early stages of development the filament was attached by the basal rhizoid initial cell; later, after 4 to 7 days, the erect filament of 7-10 cells had commenced primary rhizoid production. These were produced as 2-4 short, lateral extensions of the rhizoid initial cell, laterally adjoined and disc-like in appearance. Continued growth and branching of these primary rhizoids, plus the additional production and outward spread of secondary rhizoids at the base of the developing parenchymatous shoot, contributed to the formation of the large attachment base.

DISCUSSION

For most marine macroalgae, the colonisation of new substrata is carried out, almost entirely, by specialized reproductive spore bodies. Experimental observations on these spores reveal them to be particularly well suited for this process. For example to assist their passage through the water many spores are actively motile (green and brown algae) or can, at least, exert some control over their sinking rate (red algae). The motile spores can also respond to external stimuli such as light, and surface topography which increases their chance of finding suitable substrates and habitats for settlement. Upon settlement most spores then attach by the production of an adhesive material. It would be considerably advantageous, during this critical phase of 'establishment' if the processes of spore settlement and attachment could be rapidly achieved. The present experimental study clearly demonstrates that this occurs in the zoospores of Enteromorpha. Settlement for large numbers of the spores took place in less than 1 min, whilst firm attachment was clearly demonstrated after 1 hour.

The R.F.C. experiments showed that, during the early stages, with increasing time of settlement the spores become more firmly attached. Two critical stages in attachment strength development were discernible, between 1-10 minutes and between 30-60 minutes. Time periods between 10-30 minutes revealed no significant change in the attachment strength of the spores. From the S.E.M. studies it can be seen that a number of distinct stages in the development of the spores

occurred. Following settlement, these included loss of flagella, increased surface contact and secretion of increasing quantities of mucilage. It is likely that some initial and weak attachment of the spores is accomplished by the spreading, closely adherant flagella (Fig. 8). However, within 2 minutes the attachment appears to be enhanced by the presence of a small pad of connecting mucilage. It is probably the presence of this mucilage, the spread of the flagella and the more closely adpressed nature of the spore which determines the increase in attachment strengths observed during the first 10 minute period of settlement. Between 10 and 30 minutes only small quantities of mucilage appeared to be produced; however between 30 and 60 minutes copious mucilage production was apparent which concurs with the rise in attachment strength at this time. The concomitant production of the mucilage and the recorded increase in the strength of attachment of the spores suggests the former has adhesive properties.

Electron microscope observations by Evans and Christie (1970) presented evidence of a similar release of adhesive material, which originated from anteriorly positioned vesicles. These authors also noted a rapid release of the material, usually within 5 minutes of settlement. The continued production of mucilage throughout the first 10 minutes of settlement, as revealed under the S.E.M., in the present study, suggest that vesicle synthesis occurs during the whole of this time. However it is possible that the apparent increase in the mucilage results from the expansion of the small quantity of originally released material, either by inbibition of water or by structural modification. Alternatively the increase in mucilage around the periphery of the spore may be associated with the process of cell wall synthesis. In this respect it is interesting to note that Evans and Christie (1970) reported the presence of a thin but distinct wall in Enteromorpha zoospores fixed only 30 minutes after settlement. Certainly there are reports of germ tubes and rhizoids of algae possessing an outer amorphous cell wall layer (Fletcher, 1981) which is often revealed under the S.E.M. to have similar outward flowing mucilaginous properties to the released spore material observed in this study (see Figs. 14, 15).

It has been speculated that the rapidity of attachment in Enteromorpha zoospores contributes to its great success as a fouling organism (Christie, 1973). It is confirmed here that settlement and attachment in Enteromorpha zoospores is extremely rapid, and after only 1 hour many can survive shear stresses well in excess of 50 $N.m^{-2}$. This can be equated with water speeds of 6 knots indicating that their removal by ships in motion is extremely unlikely. The results from the germling studies indicate that they can withstand similar, if not higher, shear stresses. It seems likely, therefore, that once the plants have established themselves on ships' hulls their continued presence will more likely depend upon their physiological tolerance to environmental extremes than the force of water movement. This characteristic is further compounded by the settlement of spores in clumps (Fig. 13). This appears to allow them to resist even higher shear stresses and so would offer an ecological advantage over any competitors.

ACKNOWLEDGEMENTS

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EXPLANATION OF FIGURES 5-10

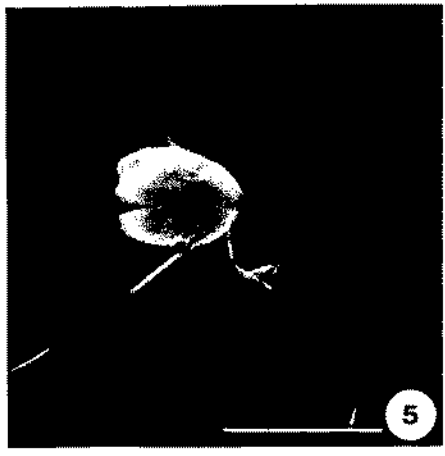
Scanning electron micrographs of Enteromorpha sp. settlement
Bar markers = 5µm

Fig. 5. 1 minute settlement: settled zoospore with intact flagella.
Fig. 6. 2 minutes settlement: settled zoospore with apical mucilage arrowed.
Fig. 7. 2 minutes settlement: extended flagella of settled zoospore showing sharply attenuated hair point tip (arrowed).
Fig. 8. 2 minutes settlement: flagella tip showing attenuation and possible presence of mucilage.
Fig. 9. 5 minutes settlement: settled zoospore showing the presence of substantial amounts of mucilage and a reduction in flagella length.
Fig. 10. 10 minutes settlement: continued mucilage production and flagella decay.

EXPLANATION OF FIGURES 11-16

Scanning electron micrographs of Enteromorpha sp. settlement
Bar markers = 5 m.

Fig. 11. 20 minutes settlement: a spreading mucilage pad is present appearing continuous around the periphery of the spore. Note, contracted flagella showing attenuated tip (arrowed).
Fig. 12. 30 minutes settlement: further mucilage production extending out and over the remaining portions of flagella.
Fig. 13. 40 minutes settlement: a clump of spores showing aggregation of mucilage.
Fig. 14. 40 minutes settlement: spore showing large amounts of adhesive material extending up the side of the spore body.
Fig. 15. 60 minutes settlement: a large mucilage pad is present extending away from the spore and covering what are possibly the remains of flagella.
Fig. 16. 60 minutes settlement: the mucilage pad (arrowed) is observed extending away from the rounded spore body.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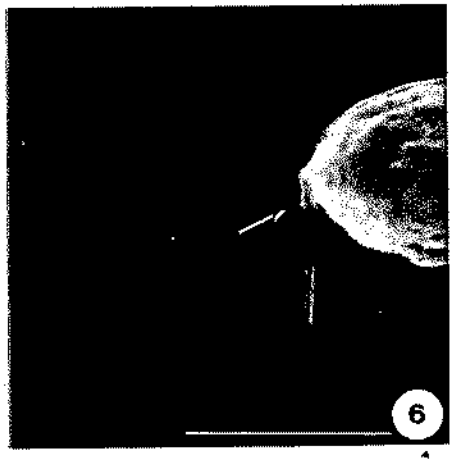
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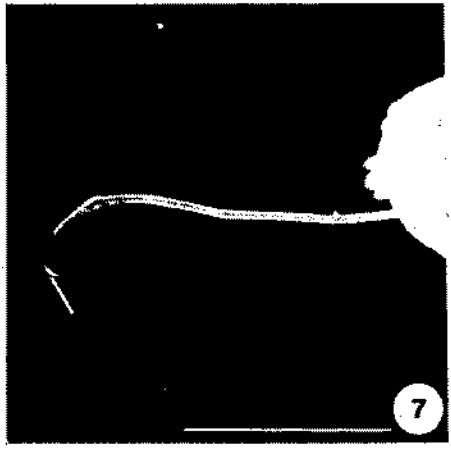
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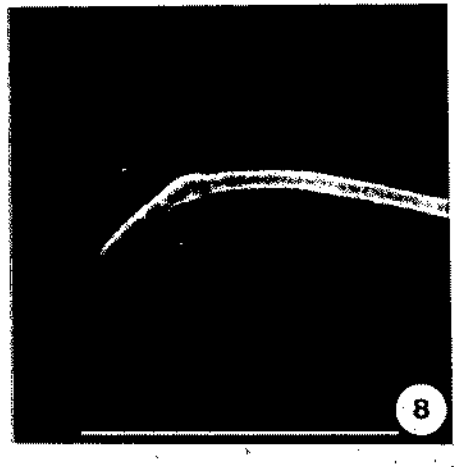
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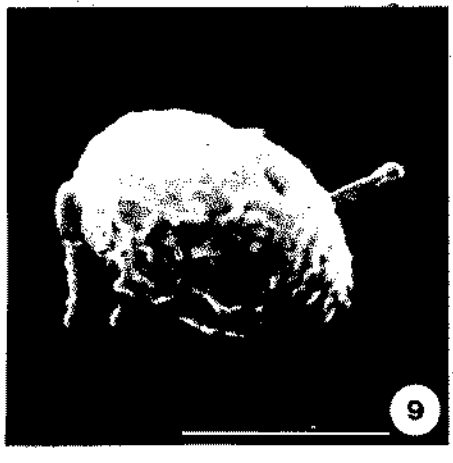
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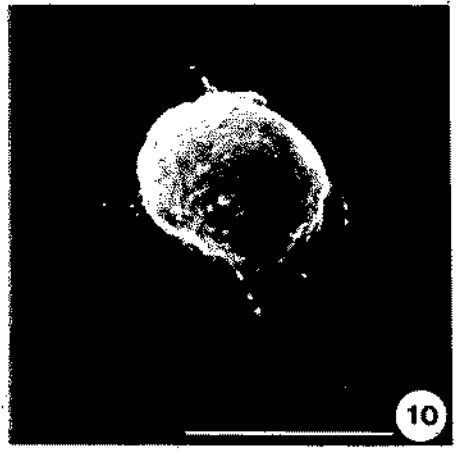
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Strength of attachment of Enteromorpha spores

EXPLANATION OF FIGURES 11-16

Scanning electron micrographs of Enteromorpha sp. settlement
Bar markers = 5μm.

Fig. 11. 20 minutes settlement: a spreading mucilage pad is present appearing continuous around the periphery of the spore. Note, contracted flagella showing attenuated tip (arrowed).

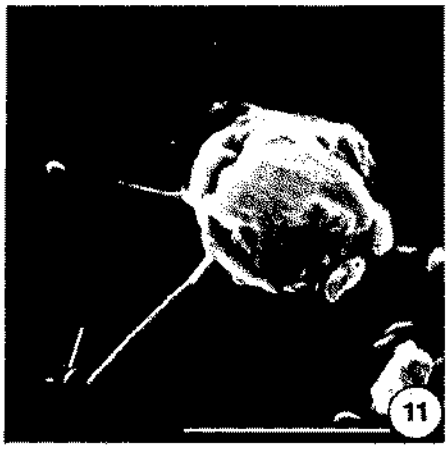
Fig. 12. 30 minutes settlement: further mucilage production extending out and over the remaining portions of flagella.

Fig. 13. 40 minutes settlement: a clump of spores showing aggregation of mucilage.

Fig. 14. 40 minutes settlement: spore showing large amounts of adhesive material extending up the side of the spore body.

Fig. 15. 60 minutes settlement: a large mucilage pad is present extending away from the spore and covering what are possibly the remains of flagella.

Fig. 16. 60 minutes settlement: the mucilage pad (arrowed) is observed extending away from the rounded spore body.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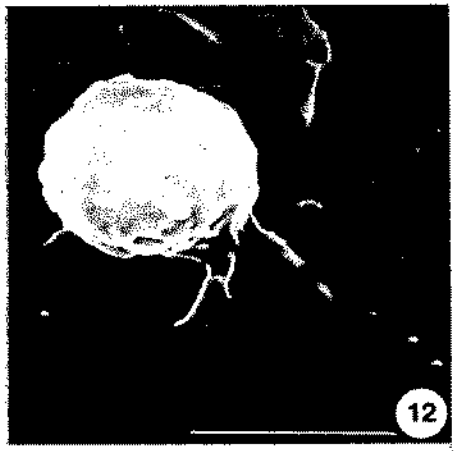
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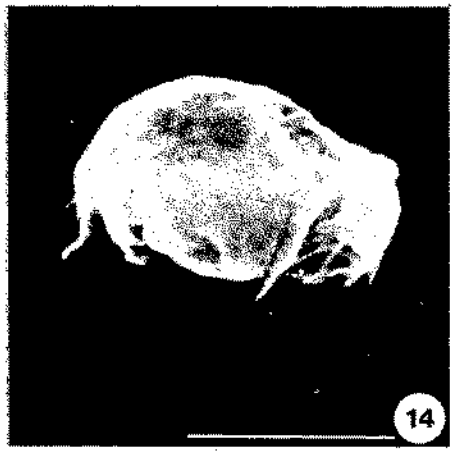
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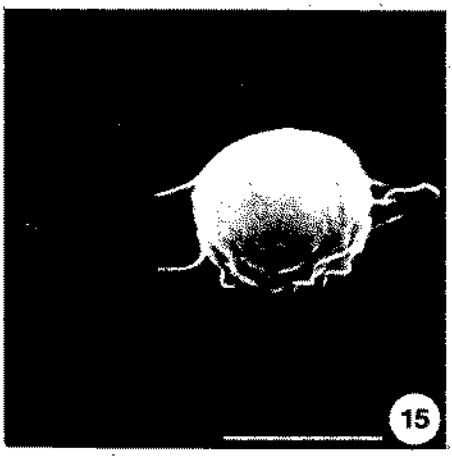
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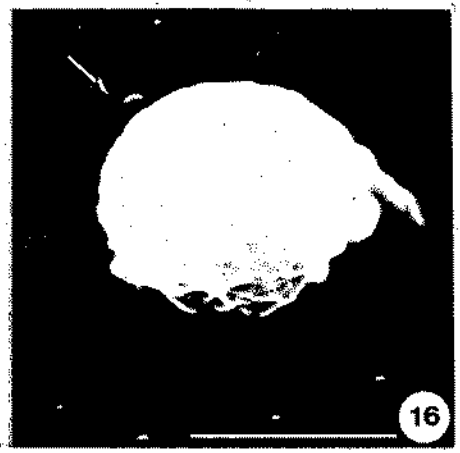
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Strength of attachment of Diatom cells

Attachment Studies on Three Common Fouling Diatoms

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The use of the radial flow growth chamber to examine the development of attachment processes in several fouling diatoms is reported. The attachment strengths of Licmophora flabellata, Achnanthes longipes and Amphora coffeaeformis to perspex test plates are shown to be dependent on the time in incubation. With areas of low shear stress (1.00 N/m^2) the maximum number of cells were attached. The highest attachment values for Licmophora flabellata and Achnanthes longipes were about 4.00 N/m^2 after 120 hours incubation. With an increase in incubation time there was a noticeable increase in the force needed to dislodge the attached cells.

Etudes sur la puissance d'attachement baseés sur trois diatomées salissantes.

L'utilisation de la chambre de croissance à l'écoulement radial pour examiner le développement des méthodes d'attachement dans plusieurs diatomées salissantes, est déscrit ci-dessous. Les puissances d'attachement de Licmophora flabellata, Achnanthes longipes et Amphora coffeaeformis aux assiettes d'essai sont démontrées dépondre de la période de leur incubation. Avec certains endroits d'effort de cisaillement faible (1.00 N/m^2) le nombre maximum de cellules a été attaché. Les valeurs d'atttachement les plus élevés pour Licmophora flabellata et Achnanthes longipes etaient 4.00 N/m^2 après 120 heures d'incubation. Aven une augmentation de la période d'incubation, il y avait une augmentation nette de la force nécessaire pour détacher les cellules attachées.

Strength of attachment of Diatom cells

INTRODUCTION

There have been many reports of the attachment of algal spores/germlings to test plates in laboratory culture (Charters et al., 1973; Norton, 1983). Previous work has examined the mode of attachment of macro-algae (Chamberlain and Evans, 1973; Boney, 1975), and the strength of attachment of spores/rhizoids (Christie et al., 1970; Coon et al., 1973; Charters et al., 1971). However, there have been few studies made on the effects of diatoms on ships' hulls or their strength of attachment (Hendey, 1951; Bishop et al., 1974; Daniel et al., 1980). With the development of more effective antifouling systems, it has been possible to control the growth of macro-algae. Consequently, the development of bacterial and diatom slimes (primary film) have become more evident and there is a requirement to study their effects on: 1. the frictional resistance of a ship, 2. reducing the efficiency of an antifouling system, and 3. their strength of attachment to a surface.

In this study, the strength of attachment of three major fouling diatoms will be studied - Licmophora flabellata E. Grun, Achnanthes longipes Agardh (stalk formers) and Amphora coffeaeformis Agardh (true slime former).

MATERIAL AND METHODS

1. Laboratory culture of diatoms

The test diatoms were isolated from a copper antifouling panel (medium loading) immersed in Langstone Harbour, by establishing a mixed population in an enriched von Stosch (1963) medium with the addition of niacin and thiamine hydrochloride (20mg/l of seawater of each). By sub-culturing, axenic cultures of the selected diatoms were established.

For the experimental work, diatom cultures were obtained by inoculating 500ml von Stosch medium in a 1 litre flask. The medium was agitated by means of a magnetic stirrer, the flasks incubated at 10°C with a 14-10 hour photoperiod and a light intensity of 2K lux. Cultures were harvested at 10 days (Achnanthes longipes) and 14 days (L. flabellata, Amphora coffeaeformis).

Strength of attachment of Diatom cells

2. Radial Flow Growth Chamber (RFGC)

A test perspex disc (10cm diameter) from the RFGC was placed into a large petri dish and flooded with the required diatom culture. These were then left to attach to the disc over a given incubation period (ranging from 1 to 120 hours). After the required settlement time the test disc was placed into the RFGC, the water flow initiated and maintained for 30 minutes. The water flow rate was dependent on diatom incubation time, e.g. after 1 hour incubation a flow rate of 3.033 litres per minute was used, while at 120 hours incubation it was 6.066 litres per minute. After 30 minutes in the RFGC, the water flow was stopped, disc removed and the number of diatoms remaining attached counted. Counts were made of the number of cells per field of view in a light microscope (x10 eye piece, x10 objective) at 5mm intervals along the radius of the disc. For each disc 4 radii were counted. For each single 5mm area counted the shear stress acting on the attached diatom can be calculated using the formulae present in Duddridge et al. (1982). Tables of critical shear stresses (that force needed to dislodge the diatom) are presented in the following results.

3. Light microscope histochemistry

To study the development of attachment systems in the diatoms used several histochemical tests were carried out. The stalk of Achnanthes longipes was stained using toluidene blue at pH = 6.8 (Kramer and Windrum, 1955; McCully, 1970), while the stalk of Licmophora flabellata shows a different polysaccharide reaction and hence was stained with Azur A at pH = 5.0 (McManus and Mowry, 1964; Pearse, 1960). Amphora coffeaeformis slime layer was stained by the alcian dye technique of Parker and Diboll (1966).

RESULTS

The attachment strengths of the three diatoms tested vary considerably from 0.741 N/m^2 to 5.635 N/m^2, and was dependent on 1. species tested and 2. time of incubation. However, during the settlement period there was a general increase in the force needed to detach the diatom cells. Tables 1, 2 and 3 represent the detachment values (critical shear stresses) for Achnanthes longipes, Licmophora flabellata and Amphora coffeaeformis respectively.

Achnanthes longipes was incubated for 1 to 120 hours and attachment strengths calculated for each incubation period (Table 1).

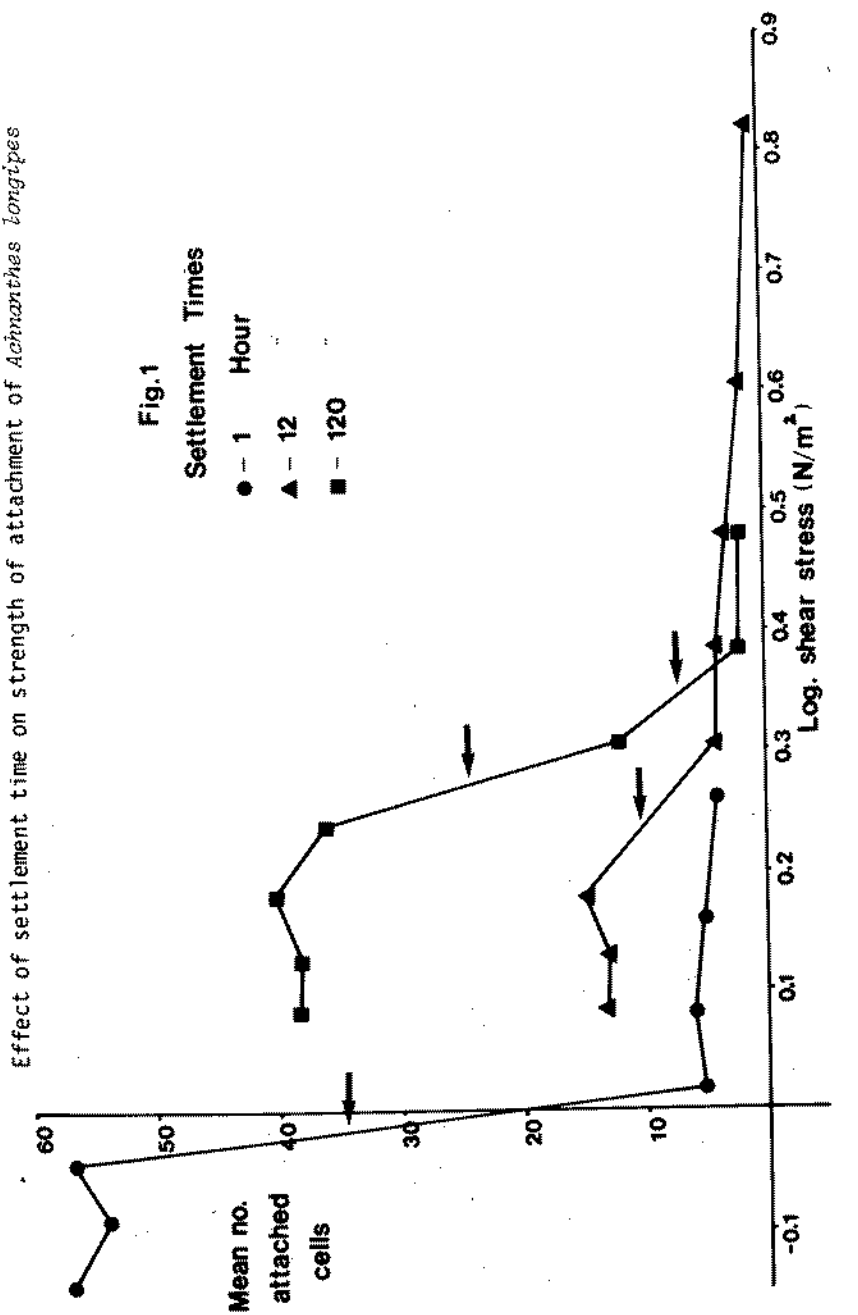
Table 1. Detachment values (Newtons/m^2) for Achnanthes longipes

Settlement times (hours)	Shear stress (N/m^2)
1.0	0.957
2.0	0.964
8.0	1.499
12.0	1.884
36.0	2.698
60.0	1.496 & 1.742
120.0	1.888 & 2.208

The results show a gradual increase in the shear stress needed to dislodge the attached cells and is time dependent. Figure 1 gives the corresponding plots for three incubation periods, an initial settlement time of one hour, an intermediate period of 12 hours and a long term settlement time of 120 hours. Detachment values are calculated as the mid-point between two consecutive log. shear stress values in which there is the greatest reduction in cell numbers (arrowed on the graphs).

The slopes for 1 and 12 hours show only a single shear stress value of 0.957 and 1.884 N/m^2 respectively. However, during the longest incubation period, 120 hours, there was a two stage detachment process on the outer part of the disc, cells were attached by a pad and stalk (an area of low shear stress) while in the inner region of the disc only cells with a stalk remained attached (an area of high shear stress). Figures 4-9 illustrate stages in the development of the stalk of A. longipes from a basal attachment pad (Fig. 4) to a long pliable stalk (Fig. 8).

Table 2 lists the critical shear stresses required to dislodge cells of Licmophora flabellata from 0.5 to 120 hours.


Effect of settlement time on strength of attachment of Achnanthes longipes
Fig.1
Settlement Times
● – 1 Hour
▲ – 12
■ – 120
Mean no. attached cells
60
50
40
30
20
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
-0.1
Log. shear stress (N/m²)

Strength of attachment of Diatom cells

Table 2. Strength of attachment for Licmophora flabellata

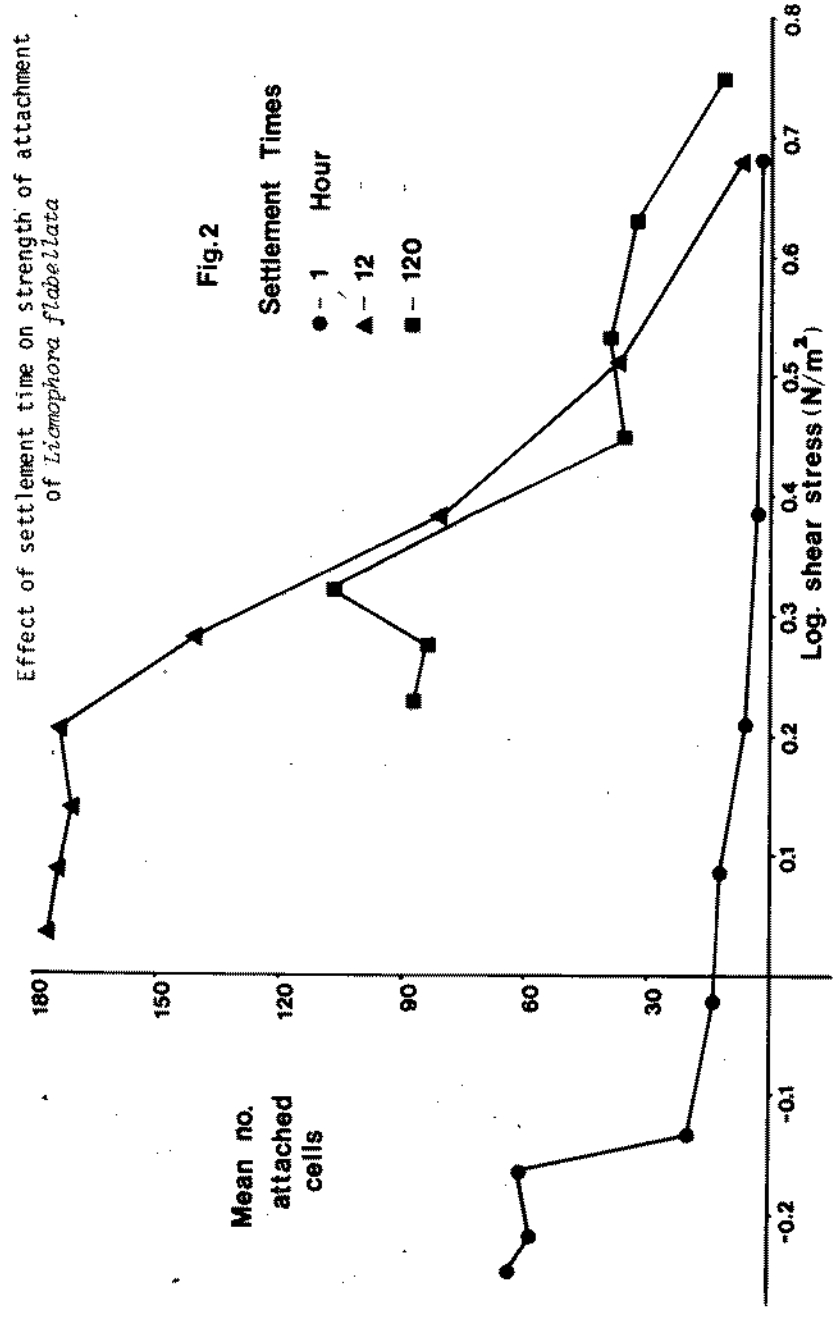
Settlement times (hours)	Shear stress (N/m^2)
0.5	1.0
1.0	0.745
5.0	1.995
12.0	2.239
24.0	5.635
72.0	2.344 & 3.162
120.0	2.512 & 4.467

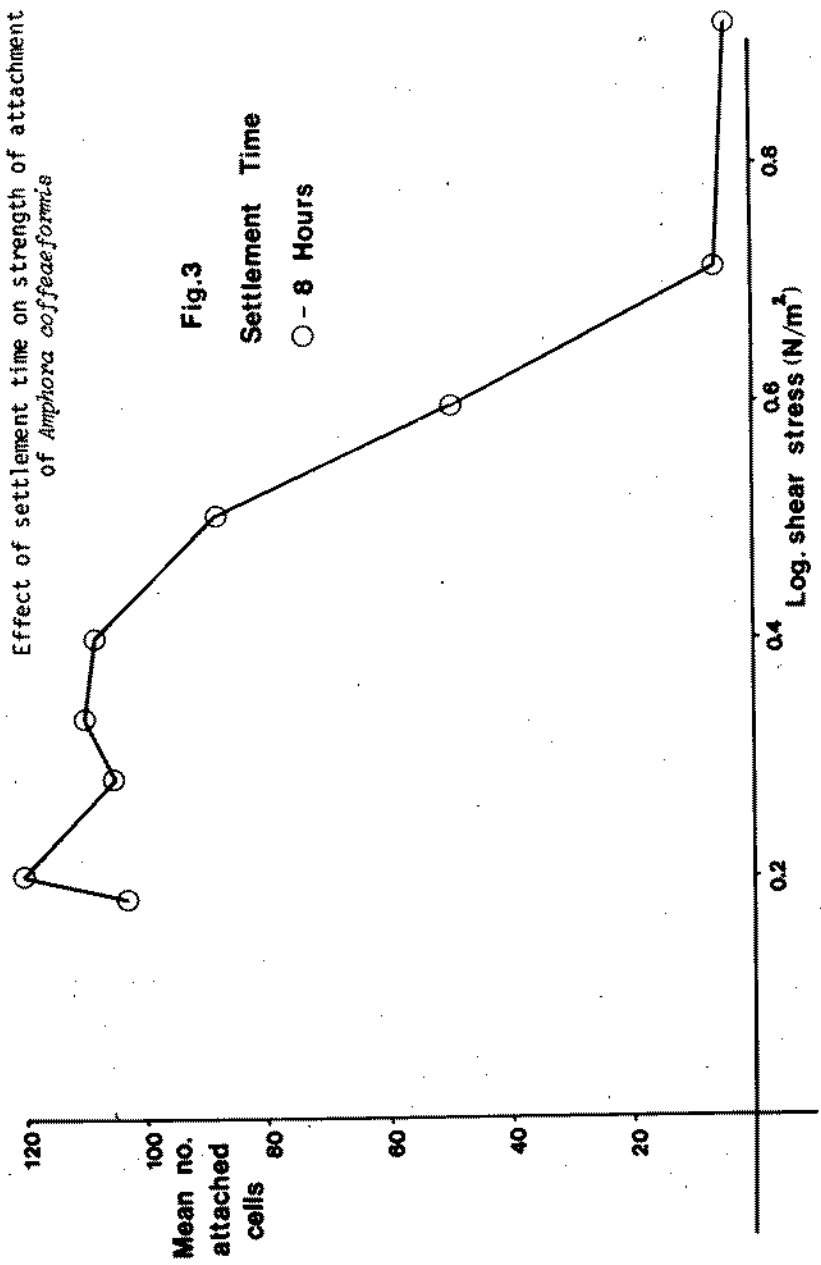
As for A. longipes, the force required to detach cells of Licmophora flabellata increases with period of settlement. Figure 2 shows the log. shear stresses at three incubation times (1, 12 and 120 hours). Results for the initial settlement periods are 1.0 and 2.239 N/m^2 respectively (1, 12 hours). However, at the higher settlement time, two stages in the detachment of cells was observed, with padded and stalked cells present at the outer area of the disc and stalked cells only in the inner region where high shear stresses were operating. In Figures 10-14 the stages in the development of the stalk in L. flabellata is illustrated, showing cells attached by pads and older cells with a well developed stalk.

The third fouling diatom tested was Amphora coffeaeformis, a true slime former, cells closely adhere to the surface producing copious amounts of mucilage which envelop the diatom and spreads onto the substratum. In Table 3 the critical shear stress required to dislodge cells are presented.

Table 3. Strength of attachment for Amphora coffeaeformis

Settlement time (hours)	Shear stress (N/m^2)
8.0	2.98


Effect of settlement time on strength of attachment of Licmophora flabellata
Fig.2
Settlement Times
● – 1 Hour
▲ – 12
■ – 120
Mean no. attached cells
180
150
120
90
60
30
-0.2
-0.1
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Log. shear stress (N/m²)


Effect of settlement time on strength of attachment of Amphora coffeaeformis
Fig.3
Settlement Time
○ - 8 Hours
Mean no. attached cells
120
100
80
60
40
20
0.2
0.4
0.6
0.8
Log. shear stress (N/m²)

Cells were incubated for similar time periods as L. flabellata, namely 0.5 to 120 hours. However, at low settlement times (0.5, 1.0 and 5.0 hours) and low flow rates the number of cells remaining attached were too low to calculate the critical shear stress. After 8.0 hours settlement the cells had divided sufficiently so that the shear stress could be calculated (Fig. 3). At incubation periods higher than this (16, 24, 72 and 120 hours) there was now a mono-layer of attached Amphora cells which merely sloughed off at low flow rates. Therefore, at these times no critical shear stresses could be calculated. Figure 15 shows an Amphora slime after 24 hours incubation.

DISCUSSION

Several methods and test surfaces have been used to measure the attachment strengths of bacteria (How et al., 1983; Duddridge et al., 1982) and algal spores (Christie et al., 1970; Charters et al., 1971, 1973; Jones et al., 1983). However there have been few studies examining the adhesive properties of fouling diatoms (Harper and Harper, 1967; Cooksey, 1981).

The development of the attachment system with time in Achnanthes longipes and Licmophora flabellata has been examined. Initially attachment was by means of a pad of mucilage/adhesive (Figs. 4, 10) produced from the raphe area. This was produced within 30 minutes of settlement. After 5 (L. flabellata: Fig. 11) and 24 hours (A. longipes: Fig. 5) a stalk is formed. However, stalk formation in these two species is laid down in different ways (Blunn and Evans, 1981; Daniel, 1983).

A direct comparison of the results presented above with other work on diatoms is not meaningful due to the fact that different techniques and ways of expressing the results have been used (Cooksey, 1981). For example, Harper and Harper (1967) estimated the attachment strength of Amphora ovalis Gregory to be approximately 410 millidynes using a physical removal of cells by a drawn out glass rod.

The results can more readily be compared with the work of Duddridge et al. (1982) on the bacterium Psuedomonas fluorescens. They gave a detachment value for cells of between 4.5 (18 hours settlement) and 7.8 N/m^2 (72 hours), using a similar method to that used in this work. These are slightly higher than the values obtained for A. longipes and L. flabellata. Results for macro-algal spores are lower than for diatoms, e.g. 2 N/m^2 (20 dynes/cm^2: Charters et al., 1971) after 24 hours settlement for Gracilariopsis sp. However, Jones et al. (1983) reported greater shear stresses were required to remove Ceramium rubrum (Huds.) C.Ag. spores after 60 hours than for the diatoms tested above for the corresponding settlement period.

From the results presented, three conclusions can be drawn. Firstly, strength of attachment is interspecific (e.g. Achnanthes longipes

1.5 N/m^2; L. flabellata 2.1 N/m^2; Amphora coffeaeformis 2.9 N/m^2 all at 8 hours). Secondly, the force required to dislodge cells increases with sett ement time (e.g. L. flabellata: 0.7 and 5.6 N/m^2 at 1 and 24 hours respectively). Thirdly, at high shear stresses (e.g. L. flabellata: 4 N/m^2 at 120 hours) cells attached by means of pads were removed while the stalked cells remained attached. This is attributed to the greater flexibility of the stalks so that the cells lay closer to the surface, thus reducing drag and orientated with the water flow.

The highest shear stress at which diatoms remained attached to the disc was 4.4 N/m^2 (approximately 0.25 knots) after 120 hours. This represents a low shear stress when compared to ships in service. However, from published literature it is known that diatoms remain attached to ships' hulls where higher shear stresses are operating (Daniel et al., 1980). Gunn (unpublished data) has shown that diatoms allowed to attach to discs for 2-3 weeks are able to withstand shear stresses of 120 N/m^2, which is equivalent to 10 knots. This confirms our observation that strength of attachment increases with settlement time.

The results presented give comparative data on the strength of attachment of diatoms, an area for which we have little information.

ACKNOWLEDGEMENTS

This project was financed by a research grant from the Research Organisation of Ships' Composition Manufacturers (ROSCM), and we are grateful for their support. We are grateful to: Dr. Peter Morris for all his help and discussion, Mr. E. Hawton for photographic assistance and Dr. J. Duddridge for all his help.

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Explanation of Figures 4-15

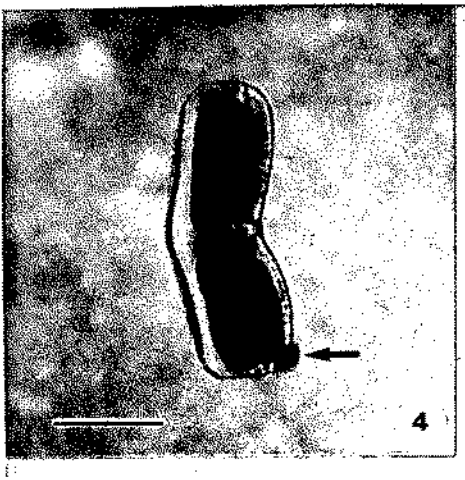
Figs 4-9. Development of attachment mechanisms in Achnanthes longipes (light micrographs).

Fig. 4. 1 hour settlement. Stained with toluidene blue. Note adhesive material (arrowed).
Fig. 5. 24 hours settlement, short stalk visible.
Fig. 6. 30 hours settlement.
Fig. 7. 36 hours settlement.
Fig. 8. 60 hours settlement. Note differential staining of stalk (arrowed).
Fig. 9. 120 hours settlement. Padded and stalked cells present. (Bar markers: 20µm).

Figs 10-14. Development of attachment mechanism in Licmophora flabellata (light micrographs).

Fig. 10. 30 minutes settlement stained with Azur A. Note adhesive pad (arrowed). (Bar: 2.5µm).
Fig. 11. 5 hours settlement. Note short stalk. (Bar: 10µm).
Fig. 12. 60 hours settlement, stalk longer. (Bar: 15µm).
Fig. 13. 72 hours settlement. Note cell division. (Bar: 10µm).
Fig. 14. 120 hours settlement. Stalk may exceed 300µm. (Bar: 20µm).

Fig. 15. 12 hours settlement of Amphora coffeaeformis forming a thick laayer on the surface of the disc. (Bar: 10µm).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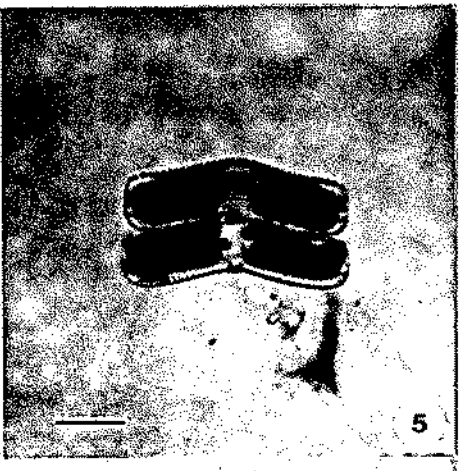
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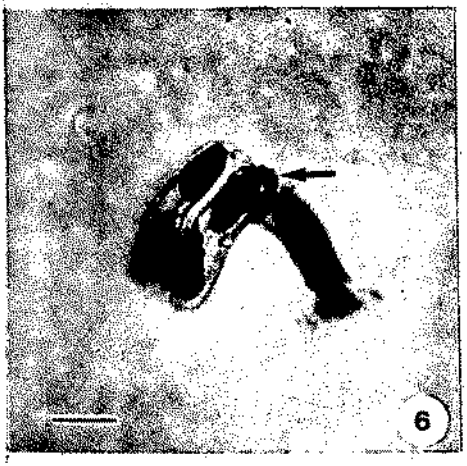
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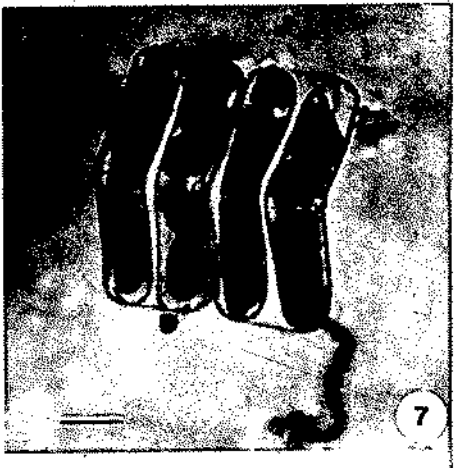
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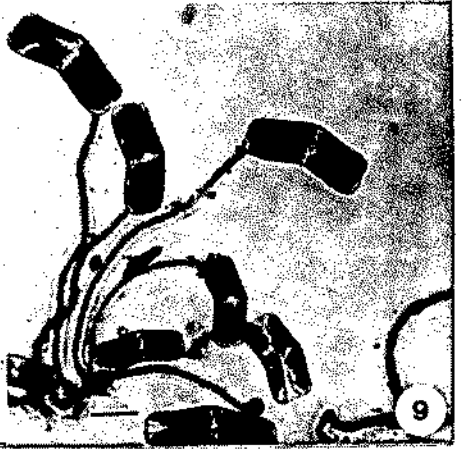
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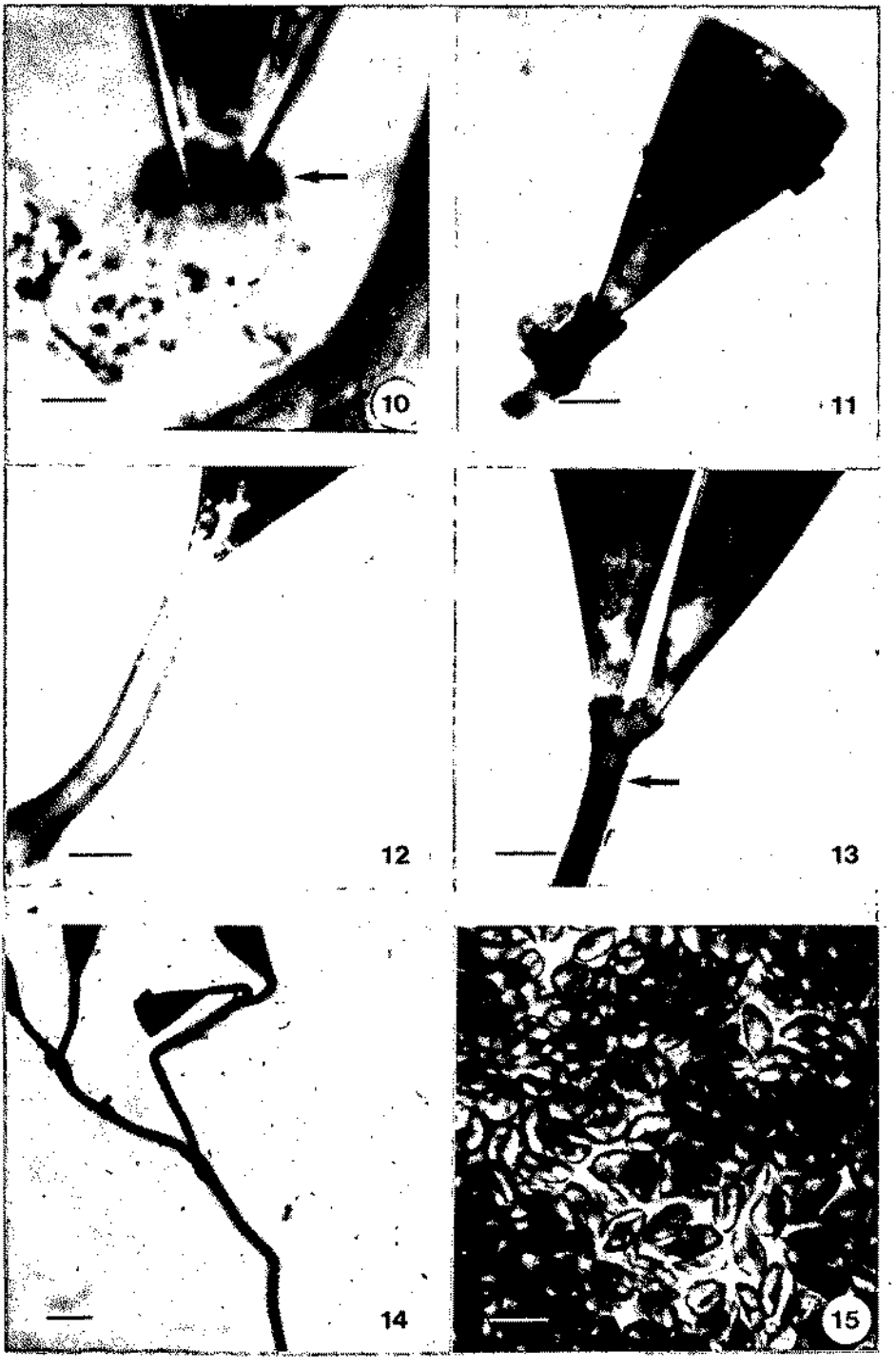
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Attachment of marine fouling protozoa

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The successional microbial colonization of titanium surfaces in estuarine water were investigated using scanning and transmission electron microscope techniques. Initial colonization by bacteria, diatoms and protozoa is described. A wide range of protozoa were found to colonize the titanium sections, and the settlement and attachment of these were investigated. Four groups were discernible based on the morphology and structure of cell attachment: a) solitary peritrichs, b) colonial peritrichs, c) loricate and d) suctorian.

L'attachement des protozaires marines salissants.

La colinisation microbienne successive des surfaces de titane dans l'eau de l'estuaire a été étudié avec l'aide d'un microscope électronique en employant les techniques de scansion et de transmission. La colonisation initiale de la bacterie, des diatomées, et des protozaires est décrit ci-dessous. Il a été constaté qu'une large gamme de protozaires colonisasient des parties en titane, et l'établissement et l'attachement de ceux-ci a été étudié. Selon la morphologie et la structure de l'attachement des cellues, quatre groupes ont été discerné; a) "solitary peritrichs" (b) "colonial petritrichs" (c) "loricate" et (d) "suctorian".

INTRODUCTION

Titanium is now widely used in a range of industrial environments, from construction material in heavy engineering plant to condenser tubes in heat exchange systems such as those found in power stations. Its advantages over other commonly used metals is that it is strong, of medium weight and its corrosion resistance is excellent in oxidising and reducing environments (Perry and Chilton, 1974). It is not usually affected by impingement attack, crevice corrosion or pitting attack by sea water and it is even superior to stainless steel in this medium. However, it has a number of disadvantages. One is its

relatively high cost and it is also difficult to form. Additionally due to its inert nature microbial colonization of its surface readily takes place when exposed in aquatic systems (Brown and Jones, 1982).

This study investigated the sequential colonization of titanium condenser tubes in an estuarine environment and paid particular attention to the role of sessile protozoa in the build up of the fouling biofilm.

MATERIALS AND METHODS

1. Preparation and exposure of titanium sections

Titanium sections were prepared by cutting 1cm wide rings from a normal 2cm diameter power station condenser tube. The sections were thoroughly cleaned with HCl followed by distilled water and acetone. Cleaned sections were stuck to a metal frame with epoxy resin and the frame then anchored at a set depth in the river so that it was always submerged, irrespective of the state of the tide. Sections were orientated so that the water flowed directly through them as in a tube. The average flow rate of the river Thames where the study took place (Grid Reference TQ 5676) was $1ms^{-1}$, which is similar to that found in power station heat exchange condenser tubes. At this site the river Thames was tidal with a salinity range of 6-17°/oo.

Sections were removed at the following time intervals: six, twenty-four and forty-eight hours as well as at 15, 30, 45 and 60 days. On removal from the frame the sections were immediately placed in sterile river water and returned to the laboratory for investigation.

2. Preparation of titanium samples for Scanning Electron Microscopy (S.E.M.)

In the laboratory, the titanium was cut into $1cm^2$ sections which were placed in a 0.16% aqueous solution of chlorbutanol for 20 minutes. The samples were then washed in distilled water and placed in a 2% aqueous osmium tetroxide solution and left for twelve hours in the dark. After thorough washing with water the samples were dehydrated in an ethanol series and then transferred through four ethanol/acetone dilutions into 100% acetone. After critical point drying they were mounted on stubs, coated with gold in a sputter coater and examined using a JEOL 35C scanning electron microscope.

3. Preparation of titanium samples for Transmission Electron Microscopy (T.E.M.)

Titanium sections were taken and fixed for 2 hours in 4% glutaraldehyde buffered at pH 7.2 with 0.1M sodium cacodylate. After washing twice in 0.1M sodium cacodylate for one hour sections were dehydrated in an acetone series and then embedded in an Epon-araldite mixture (Mollenhauer, 1964). The polymerized resin with the fouling slime layer was removed from the titanium surface by immersing in liquid nitrogen for twenty seconds and then in water at 40°C.

Silver sections were cut using a diamond knife on an LKB 3 ultramicrotome. They were stained in 50% ethanol saturated with uranyl acetate for 20 minutes and then in lead citrate (Reynolds, 1963) for 10 minutes. Stained sections were observed in a JEOL 100S transmission electron microscope.

RESULTS AND DISCUSSION

Using S.E.M. and T.E.M. the initial microbial colonization of titanium surfaces and the build up of a slime film composed of protozoa with different attachment devices was observed.

1. Initial Microbial Colonization

The initial events of microbial colonization of titanium surfaces are illustrated in Figures 1-7. Adsorption of non-living organic and inorganic material took place within the first hours of exposure (Figure 2). This film has been reported as conditioning surfaces (Baier, 1973; Dexter et al., 1975; Fletcher, 1976) providing a favourable substratum for the attachment of periphytic bacteria (Floodgate, 1966, 1971). This, coupled with the fact that the titanium has a micro-rough surface (Figure 1), allows the initial colonization by microorganisms to take place quickly. Bacteria are present on the titanium surface after 24-48 hours (Figures 2, 3).

The bacterial biofilm continues to build up (3-30 days) and entraps river detritus and fungal spores (Figure 4). As in other studies (Daniel and Chamberlain, 1981) diatoms are found embedded in this fouling layer (Figure 5), and at the same time sessile peritrich protozoa are found on the titanium sections (Figure 6). Suctorian protozoa are present at 30-60 days (Figure 7).

The sequence of colonisation outlined broadly conforms to that described by Corpe (1970), Floodgate (1971) and Marshal et al. (1971), with the exception of the presence of fouling protozoans. The remainder of this paper, therefore, concentrates on the types and mode of attachment of some of the protozoa found during this study.

2. Protozoan settlement and attachment

Initial settlement, attachment and growth of a colonial sessile peritrich is shown in Figures 8-13. The first stage is the encystment of an immature zooid from a cyst adhering to the biofilm surface (Figures 8, 9). The cyst wall breaks up and disappears revealing a small zooid with a short attachment stalk (Figure 10). At this stage the zooid has a well developed peristome region with buccal cilia. Figure 11 shows the attachment pad of the stalk which is composed of polymeric material. After stalk growth, the organism divides (Figures 12, 13), each zooid producing its own stalk (Figure 12). Examination of the basal attachment pad (Figure 13) shows that an extra layer of

adhesive has been secreted, so as to give it a double ring effect. This may be in response to the increased size of the colony, thus the larger the colony the thicker the adhering stalk and attachment pad. The zooids continue to divide to form a bush-like colony (Figure 6).

3. Attachment of different types of protozoa

In this study a range of different protozoa were found and the principal types are described below. In each case we shall describe the external morphology of the protozoan and then the structure of the stalk and pad attachment.

a. Solitary Peritrichs (Vorticellids)

Members of this group belong to the genus Vorticella (Figures 14-17). Each cell is solitary, and composed of a zooid and a contractile stalk which is attached to the substratum by an attachment pad (Figures 14, 17). Zooids are formed after cell division (Figure 15), when one daughter cell develops a posterior band of cilia, breaks away to become a motile telotroch stage (Figure 15), the other cell remains attached to the original stalk. The telotroch settles (Figure 16) and metamorphosis into a zooid, stalk and attachment pad. pad.

The stalk is composed of 1) an outer zone of fibrillar material with non-striated fibres derived from zooid cilia which extend a short distance into the stalk (Figure 27), 2) a central myoneme composed of contractile fibres which extends almost the complete length of the stalk and 3) a cell wall which surrounds the myoneme (Figure 26). The stalk is attached to the titanium surface by a pad (Figure 17) which in the T.E.M. appears to be composed of the outer fibrillar material of the stalk (Figure 28).

b. Colonial Peritrichs

Species of Carchesium and Zoothamnium are colonial protozoa composed of zooids, branched stalks which are attached by a circular pad (Figures 6, 12, 13). Colonies can be quite large and may extend over 400µm beyond the surface. Initial attachment of Zoothamnium was described above, however, in Carchesium a motile telotroch stage is produced (Zagon, 1971).

Zoothamnium differs from Carchesium in that the former has a zooid with a distinct lip-like peristome region (Figure 22). This is lacking in Carchesium (Figure 21).

In Carchesium and Zoothamnium species the stalks are composed of: a) an outer zone of fibrillar material with striated fibres (Figures 31, 23 respectively) derived from cilia (Figures 31, 23), b) a central myoneme (Figures 23, 24, 29, 30, 31) and c) the cell wall surrounding the myoneme. In Zoothamnium the myoneme extends to the

base of the stalk and all zooids are connected to this, thus all contracting simultaneously, while in Carchesium, the myonemes are not continuous and each zooid may contract independently.

Attachment pads are circular (Figures 11, 13) and composed of fibrillar material with striated fibres (Figure 25). In the S.E.M. the pad of Zoothamnium appeared to be composed of 2 zones (Figure 13), however, this could not be resolved at the T.E.M. level.

c. Loricate protozoa

The genus examined was Vaginicola and is characterized by cells enclosed by a mucilaginous lorica (Figure 20) with a short stalk (approximately 4μm) (Figures 32, 33). Although cells are able to contract into their lorica, the stalk is non-contractile and does not possess a central myoneme (Figure 34). At the interface between the cell and the stalk there are fine extensions of cytoplasm (Figure 34) bounded by the cell wall which surrounds the stalk (Figure 35).

The stalks consist of fibrillar material with longitudinal orientated, coarsely striated fibres (Figure 34) which are derived from the cilia (Figure 35). The lorica appears to be composed of material which is similar to that of the stalk matrix, however the stalk is separate from the lorica which suggests that the former is produced before loricate production.

Attachment pads in Vaginicola are poorly developed (Figure 34) and made up of fibrillar material. However, despite the weakly developed pads, the lorica are firmly attached to the substratum.

d. Suctorian protozoa

Suctorians are classified among the ciliated protozoa, however, only the free swimming larval stage possess cilia. The adult stage found on titanium surfaces does not possess cilia and feeding in this species is accomplished by capturing algae and other protozoa with tentacles (Figure 18). These protozoa are characterised by heart-shaped cells with two groups of apical, contractile, tentacles, a long thin stalk and a well developed bulbous attachment pad (Figures 18, 19).

Stalks are composed of fibrillar material and coarsely striated fibres which form a pronounced ring to the outside of the stalk (Figure 38). The ring of fibres branch to form a reticulate system within the centre of the stalk (Figure 39). Fibres extend the full length of the stalk, but their origin is unknown. The cell wall comprises 3 layers: an inner electron-dense layer; a thin electron-dense layer with an outer serrated margin from which fibrillar material may be derived and an outer thick fibrillar layer. The latter is continuous with the matrix of the stalk.

Figure 39 shows a cross section through a pad which is composed of an electron-dense amorphous layer and a sponge-like layer which is in

contact with the substratum.

Protozoa have not been regarded as major fouling organisms (Jones, Eltringham and Callame, 1971) yet they were present in significant numbers on titanium sections immersed in the Thames. They were present in far greater numbers than has been previously recorded by Cundell and Mitchell (1977) on wood and Berk et al. (1981) on metal surfaces. This study has shown that there is great variation in their structure and mode of attachment. Four types of attachment processes have been described, differing in stalk ontogeny and structure. Clearly, there is a requirement to investigate these fouling organisms in more detail.

ACKNOWLEDGEMENTS

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Explanation of figures

Figures 1-7. Sequence of colonization on titanium sections.

Fig. 1. Section of clean titanium showing the micro-roughness of the surface. x700.

Fig. 2. Surface of titanium after 6 hours exposure. Organic layer adsorbed with a few solitary bacteria attaching (arrowed). x5,500.

Fig. 3. Surface covered with well attached rod-shaped bacteria after 48 hours exposure. Note the copious mucilage produced by the cells. x4,300.

Fig. 4. Gross bacterial fouling with entrapped fungal spores (arrowed) after 30 days. The depth of the fouling layer is clearly seen. x1,800.

Fig. 5. Two diatoms firmly embedded in the biofilm after 30 days exposure. x1,900.

Fig. 6. Well established colonial sessile peritrich protozoa on the surface of titanium after 30 days exposure. x110.

Fig. 7. Protozoan biofilm after 60 days exposure. x400.


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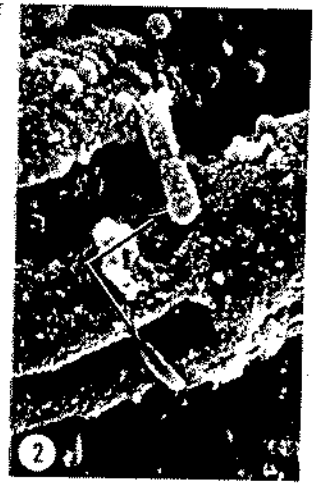
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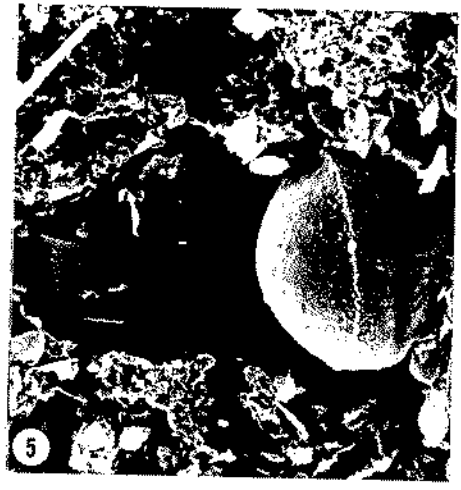
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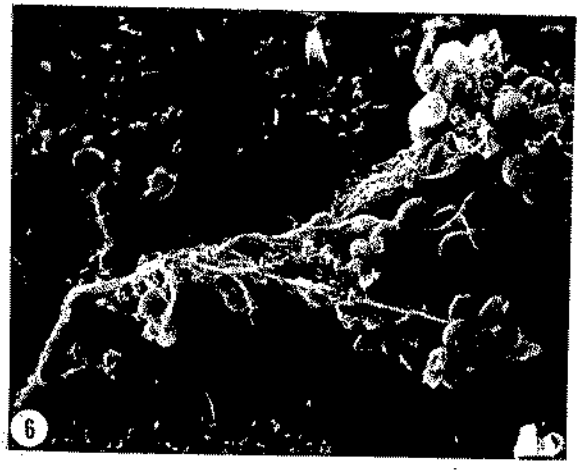
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Figures 8-13. Encystment and attachment of Zoothamnium.

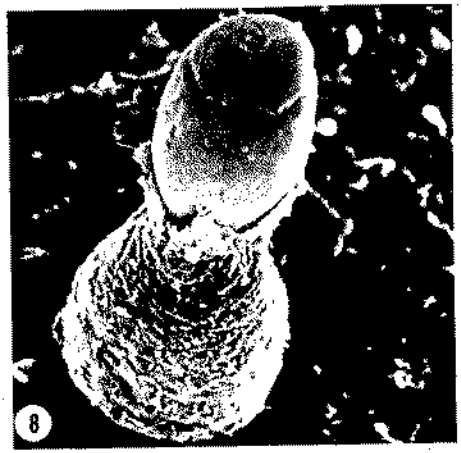
Fig. 8. A settled Zoothamnium excysting from titanium surface. x780.
Fig. 9. Detail of an excysting zooid of Zoothamnium. x1,700.
Fig. 10. Stalk formation in an immature peritrich. x570.
Fig. 11. Detail of base of immature peritrich stalk with the attachment pad. x6,000.
Fig. 12. S.E.M. of a colonial peritrich after one cell division. x1,000.
Fig. 13. Detail of basal area of the above peritrich (Fig. 12) showing two rings of adhesive material. x1,200.

Figures 14-22. Protozoa found on titanium surfaces.

Fig. 14. Zooid and stalk of Vorticella. x1,800.
Fig. 15. Two cells of Vorticella directly after cell division. One cell becomes the motile telotroch stage, the other remains attached to the stalk. x1,400.
Fig. 16. Settlement of telotroch stage. x1,300.
Fig. 17. Attachment pad and stalk of Vorticella. x2,100.
Fig. 18. Suctorian protozoa with tentacles extended and attached to a thin stalk. x6,200.
Fig. 19. Attachment pad of suctorian protozoa. x5,000.
Fig. 20. Loricate protozoa on titanium surface. x400.
Fig. 21. S.E.M. of buccal region of Carchesium. x600.
Fig. 22. S.E.M. of peristome region of Zoothamnium. x1,600.

Figures 23-28. Stalk structure of Zoothamnium and Vorticella.

Fig. 23. Section through stalk and cell region of Zoothamnium showing central myoneme (M) and short cilia (C). x6,200.
Fig. 24. T.S. of stalk of Zoothamnium showing central myoneme (M) surrounded by cell wall and periphery containing numerous striated fibres. x7,500.
Fig. 25. Attachment pad of Zoothamnium stalk. x4,400.
Fig. 26. T.S. of stalk of Vorticella showing central myoneme (M) surrounded by periphery of fibrillar material. x9,200.
Fig. 27. Section showing short cilia (C) with their kinetosomes (K) projecting into the fine fibrillar periphery of the stalk. x30,000.
Fig. 28. Section through the attachment pad of a Vorticella stalk. x5,700.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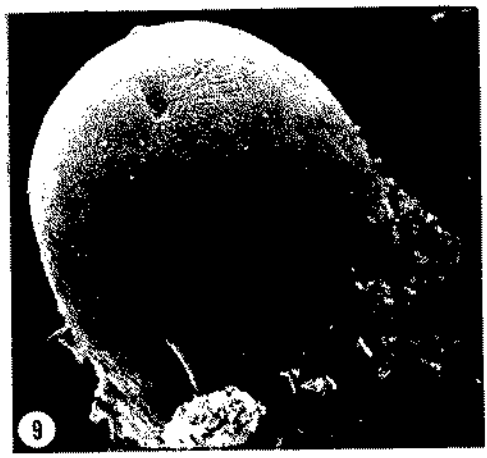
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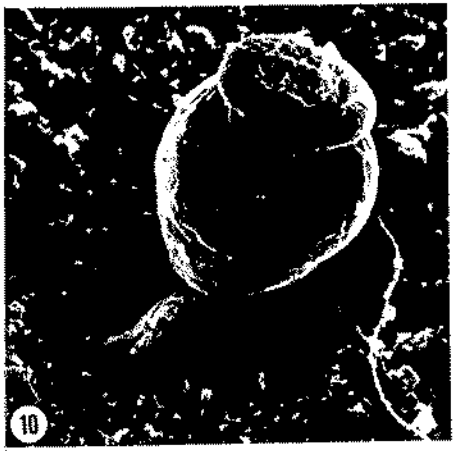
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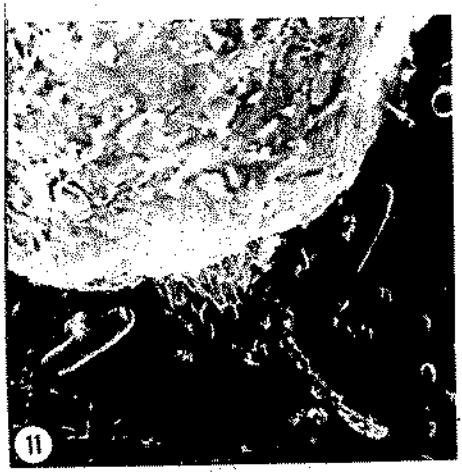
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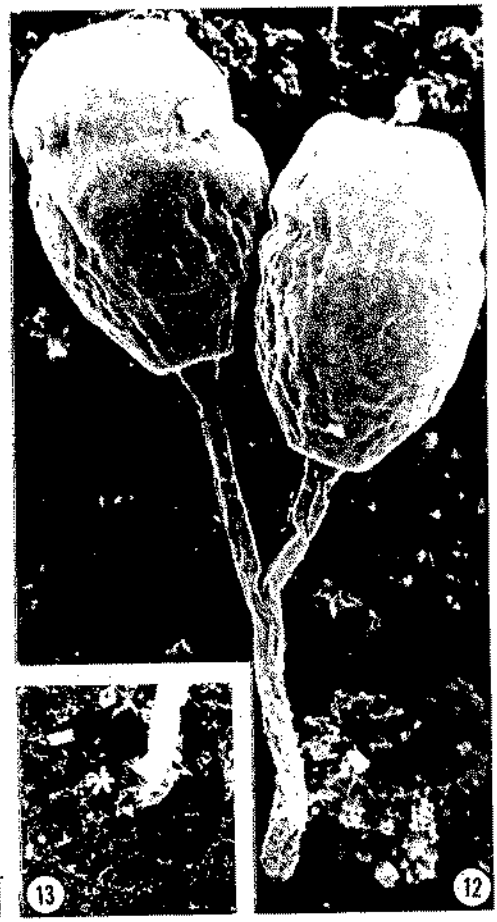
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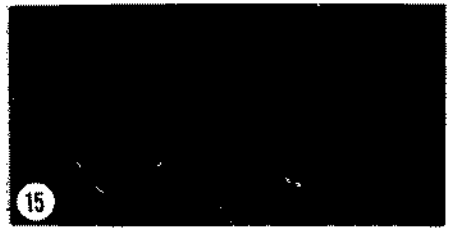
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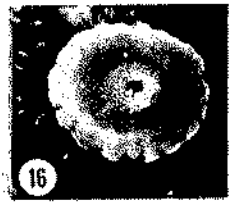
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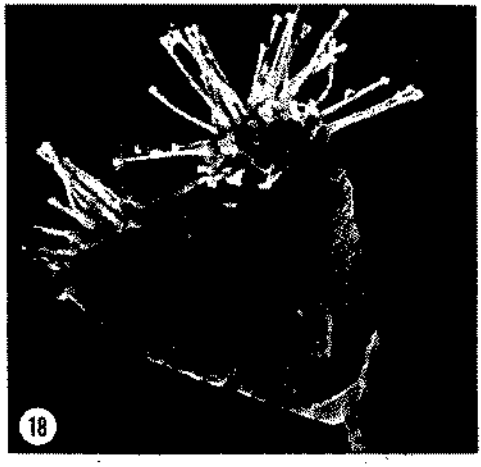
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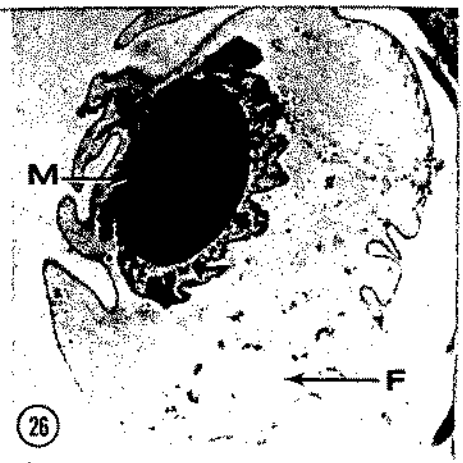

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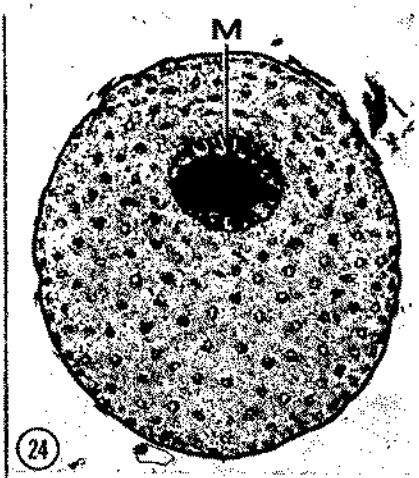

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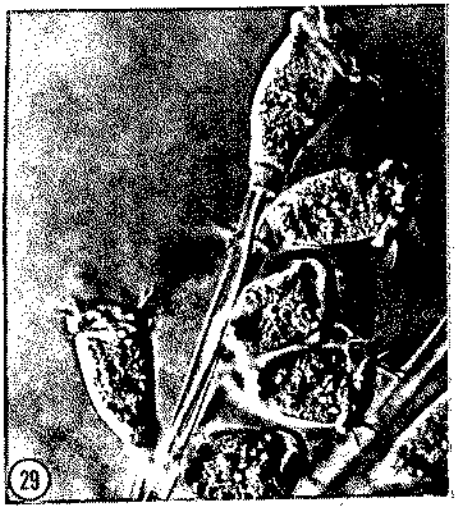
Figures 29-35. Stalk structure of Carchesium and loricate protozoa.

Fig. 29. Light micrograph of Carchesium zooids and stalks. x580.
Fig. 30. Section through Carchesium cell and stalk showing central myoneme (M). x1,600.
Fig. 31. Stalk and cell region of Carchesium showing myoneme (M) with fibres (F) in periphery of stalk derived from the cilia which penetrate from the cell into the stalk. x5,600.
Fig. 32. Loricate protozoa (Vaginicola) which has contracted into lorica (L). Note the non-contractile stalk (S). x1,000.
Fig. 33. Micrograph of fully extended loricate protozoa. x700.
Fig. 34. Section through the stalk (S) and partly through lorica (L) of a loricate protozoa. x6,100.
Fig. 35. Section taken through part of the lorica (L) and stalk (S) of Vaginicola, with an extension of cytoplasm bounding the stalk (arrowed) and ciliary derived fibres (F). x23,000.

Figures 36-39. Stalk structure of Suctorian Protozoa.

Fig. 36. Section taken through stalk and cell region of suctorian protozoa showing stalk (S) which is a continuation of cell wall (W). x6,700.
Fig. 37. Higher power micrograph of the stalk-cell interface showing cell wall (W) and origin of striated fibres (F). x2,700.
Fig. 38. T.S. of stalk showing ring of fibres (F). x18,000.
Fig. 39. Stalk (S) and attachment pad (P) of suctorian protozoa which has attached to a slime film containing bacteria (B). x10,900.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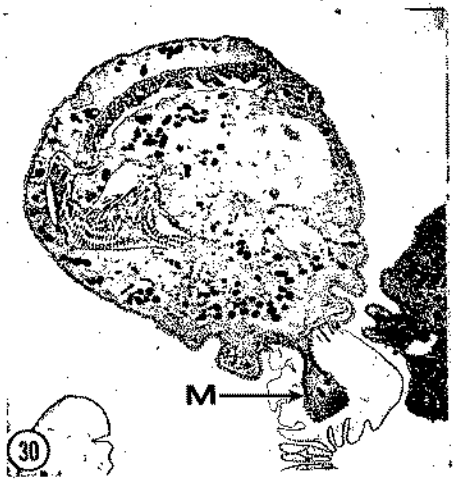
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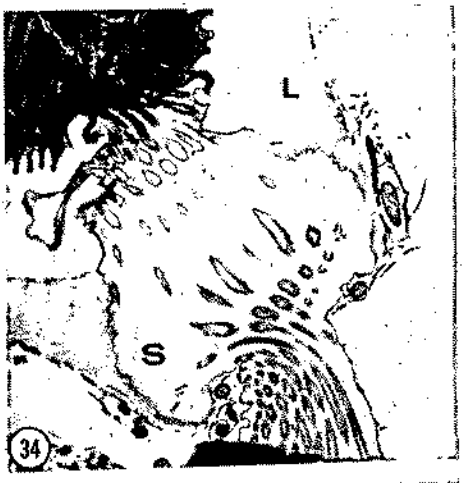
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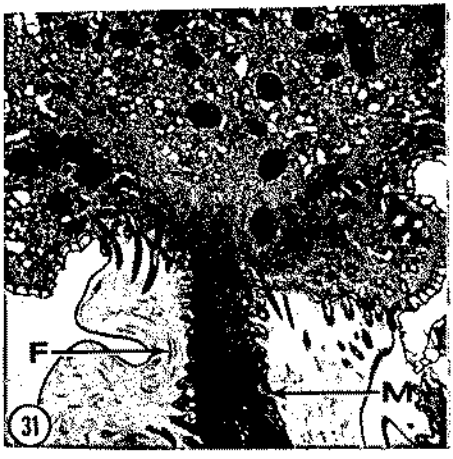
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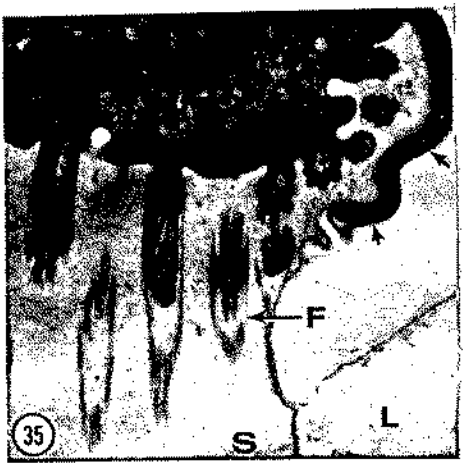
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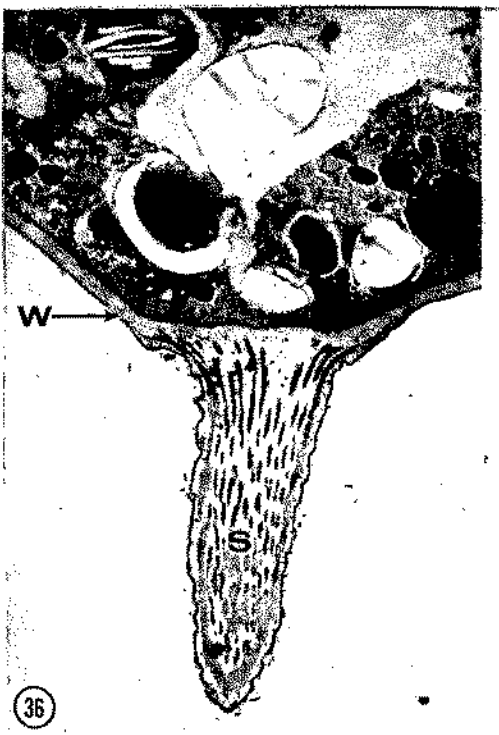
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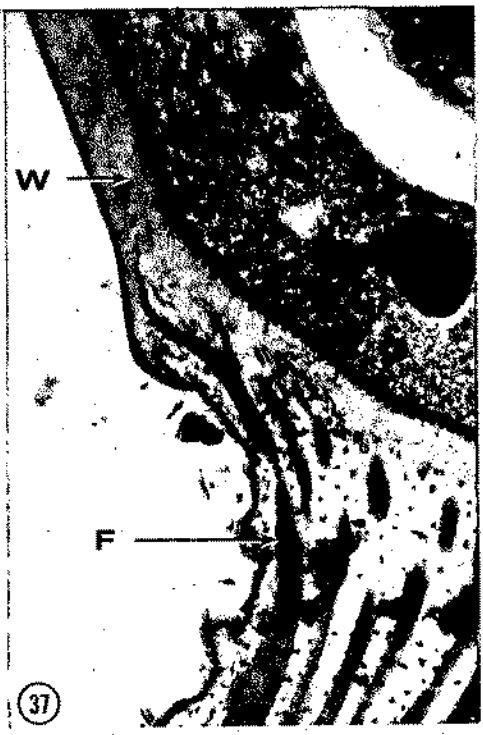
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The influence of surface energy on spore development in some common marine fouling algae

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A study was made of the influence of surface energy on the early development and attachment of sporelings of five common marine fouling macroalgae in the North Atlantic viz. Enteromorpha intestinalis, Ulva lactuca (Chlorophyta), Giffordia granulosa (Phaeophyta), Bangia atropurpurea, Polysiphonia brodiaei and P. urceolata (Rhodophyta). The sporelings were grown on five types of surface-controlled glass coverslips, exhibiting surface energies over the range from less than 20 mN/m to greater than 70 mN/m. The low surface energy ranges were prepared from different silane-based coatings. Over the range of surface types used, marked differences were observed in the extent of outward growth, morphogenetic appearance and adhesive strength of the rhizoids in each alga. These results are discussed in relation to the development of novel non-stick control mechanisms against fouling growths.

Introduction

Marine algae are major, world-wide fouling organisms reducing the efficiency of a wide range of immersed structures including ships (Christie, 1973), buoys (Anon, 1952), pilings (Haderlie, 1977) and offshore platforms (Freeman, 1977). The fouling of container ships and large tankers is a particular problem as the algae increase the frictional resistance of the hull and necessitate increased fuel consumption to maintain cruising speeds (Banfield, 1972). The more recently documented occurrence of algae as major components of the marine communities on the support legs of oil and gas platforms in the North Sea has further contributed to an appreciation of their importance as fouling organisms. Adverse effects of the fouling growths include an increase in both the structural and hydrodynamic loading (Freeman, 1977), enhanced corrosion rates (Terry & Edyvean, 1981) and the prevention of important inspection and maintenance

work (Freeman, 1977). Fouling control has so far proved both costly and generally ineffective. Fouling growths on the support legs are removed by divers which is labour intensive (Goodman & Ralph, 1979), whilst on ships' hulls some limited protection from the often dominant algal growths is given by the use of toxic antifouling paints, usually containing copper or organotin compounds (Fletcher & Chamberlain, 1975). There is, therefore, a continuing need for improved antifouling systems (Houghton, 1970; Fletcher & Chamberlain, 1975).

For this reason considerable interest has been directed towards obtaining a better understanding of the biology of some of the important fouling organisms. The biological processes which have received most attention are the mechanisms of settlement and attachment, and in the algae this has been well documented for both colonising spore stages and developing plants (Jones et al., 1983). One aspect of this work which could have important implications in the development of control methods is the role played by the surface characteristics of substrata during the period of settlement and attachment of the organisms. Influencing characteristics include the presence of macromolecular and microbiological films (Baier, 1973; Thomas & Allsopp, 1983); surface texture (Crisp & Ryland, 1960; Muller, 1964; Christie, 1973); surface charge (Marshall et al., 1971) and surface energy (Baier, 1980, Dexter et al., 1975; Young & Crisp, 1982). To date the surface energy studies have been concerned with bacterial adhesion, a notable exception being Young & Crisp's (1982) work on the bivalve Mytilus edulis. In the present paper, we report, for the first time, on the influence of surface energy on the development and attachment of some common marine macroalgae.

Materials and Methods

Species used

Table 1 lists the marine algae used in the present study along with details of habitat, date of collection and spore type. Six common fouling species on the south coast of England were investigated representing the 3 major macroalgal groups - Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). With the exception of Enteromorpha intestinalis which was removed from a concrete breakwater wall in the upper littoral at Southsea, Hampshire, all the algae were collected at the water-line, from a floating, non-toxic, steel pontoon situated in Langstone Harbour.

Preparation of test surfaces

Five types of surface-controlled glass coverslips were used for the experiments, exhibiting a range of critical surface energies from less than 20 mN/m to greater than 70 mN/m. These are outlined in Table 2. The low surface energy ranges (surface types 3-5) were prepared by covalently coupling 3 different silane-based coatings to

coverslips that were radiofrequency glow discharge cleaned in air for 3 minutes just prior to coating. The coverslips were immersed in the individual coating liquids within 30 seconds after R.F. glow discharge cleaning, obtaining optimal coating-to-substrate coupling (Baier & dePalma, 1970). Prior to spore inoculation (see later) all the coverslips were initially immersed in pasteurised filtered seawater (PFS) for 24 hours.

Inoculation of prepared coverslips

Following collection, all algal samples were brought back to the laboratory and epiphytes, diatoms and surface debris removed using cotton wool pads soaked in PFS. Small fertile portions were then excised for spore production. For the green and brown algae these small portions were placed directly into the compartments of a repli dish (10 cm square - 25 compartments) containing 3 mls of PFS. The released motile spores were then concentrated in one corner of the compartment, using their negative phototactic response to a strong unilateral light source. The spores were then pipetted in drops directly onto the surfaces of the coverslips. After spore settlement and attachment had occurred (allowing 2-3 hours) the coverslips were then placed into the culture vessels (plastic petri dishes, 90 mm diameter) containing fresh PFS.

For release of the non motile red algal spores, small portions of the fertile thalli were carefully laid out over the surfaces of the coverslips immersed in PFS in a petri dish. Following release and settlement of the spores which usually took 10 to 24 hours, the coverslips were removed, rinsed gently to remove unattached spores and placed into new petri dishes containing PFS.

For each alga investigated, replicate specimens of each of the 5 surface types were used. All cultures were grown in 15°C temperature, 4000 Lux 'white' light intensity, 16-8 hours light-dark photoperiod. Observations were then made on the initial development and growth of the germlings on the range of surface types. Particular attention was given to examining the rhizoidal 'attachment' systems produced (see Fletcher, 1976, 1977 for further information on this process) and the sizes of at least 100 germling bases recorded for each surface type.

Results

Measurements and illustrations of the attachment bases produced by all the experimental algae are presented in Table 3 and Figures 1-18 respectively.

Chlorophyta

The early stages of zoospore development were very similar for both *E. intestinalis* and *U. lactuca*. The spores germinated by elongating

vertically and dividing transversely to form a short, erect, multicellular filament attached at the base only by the slightly enlarged 'rhizoid initial' cell (Fig. 1). After 4 days in culture, the Enteromorpha germlings were up to 12 cells high on all surface types without any indication of the development of primary rhizoids. After 9 days growth the parenchymatous nature of the young erect shoot was discernible and attachment was provided at the base by a small, discoid, rosette of cells (Fig. 2). These were produced by short bifurcating rhizoidal filaments emerging from both the rhizoid initial cell (primary rhizoids) and the immediately adjoining basal shoot cell (secondary rhizoids). These disc-like rosettes of cells were produced by germlings growing on all 5 surface types; however, the constituent filaments of the discs on the low energy surfaces appeared less tightly packed and more projected than those on the high energy surfaces. The difference in appearance of the attachment base on germlings grown on the different surface types became increasingly prominent during the subsequent production and growth of the secondary rhizoids. The bases of the plants grown on the high energy surfaces remained small and disc-like in appearance, with short, tightly packed, rhizoidal filaments (Figs. 3, 7) while those on the low energy surfaces became more tufted and unilateral in their growth pattern and comprised much longer, outwardly spreading, free, rhizoidal filaments (Figs. 4-6, 8). Table 3 presents data on attachment base diameter of germlings of both E. intestinalis and U. lactuca after 2 weeks growth in culture. It can be seen that a reduction in the critical surface tension produced a corresponding increase in the size of the basal attachment systems.

Perhaps of more significance, however, was the observed differences in the attachment strength of these 2 morphological expressions. The disc-like attachment base produced on the high energy surfaces was strongly adherent and difficult to remove by gentle brushing, while the more filamentous attachment base produced on the low energy surfaces was more loosely adhered and quite easily brushed off.

Phaeophyta

The germination process of the zoospores of G. granulosa was similar to that described by Fletcher (1981). Germination was usually unipolar and the single germ tube grew out over the substratum to form a prostrate system of outwardly radiating, freely branched, multicellular, adherent primary rhizoids. During the growth of the primary rhizoids the original spore cell produced an additional, but this time upright filament which developed into the characteristic, erect branched shoot. The above described developmental pattern was observed on all surface types. However, there were distinct differences in the extent of development of the primary rhizoids on each surface (see Table 3). For example, the average diameter of bases developed on intermediate energy surface type 3 was much reduced (79 µm) compared to those developed on the other surface energy types (248 µm to 298 µm). The appearance of the rhizoids on surface energy

type 3 was also very different to those produced on the other surfaces. They were generally shorter, more pseudodichotomously branched and comprised short, closely adherent cells (Figs. 9, 10). Unlike the rhizoids produced in the other cultures (Figs. 11, 12) they were also very firmly attached and could not be removed by gentle brushing.

Rhodophyta

a) Bangia atropurpurea

The early stages of spore development in B. atropurpurea were not unlike those of E. intestinalis. The spore germinated by vertical elongation and cell division to produce an erect, multicellular filament attached at the base to the large spore glue pad by a slightly enlarged rhizoid initial cell. After 6 days in culture, with the erect filament up to 20 cells long, rhizoidal filaments were apparent, emerging from the rhizoid initial cell. The number of rhizoids produced and the extent of their development appeared to be influenced by the surface energy properties of the coverslips (see Table 3). In general 2 to 5 bifurcating rhizoids were produced on the high energy surface types 1 and 2 which were characteristically short, broad, thick walled, closely adherant and disc-like in appearance (Figs. 13, 14). On surface energy types 4 and 5 however, usually only single rhizoidal filaments were produced at the base of the germlings. These rhizoids were comparatively thin walled, long, irregularly contorted and undulating in appearance and only occasionally branched (Fig. 15). On surface energy type 3 usually a mixture of both discoid and filamentous rhizoids was recorded. In agreement with the observations on the green and brown algal cultures the more compacted rhizoidal expressions developed on surface types 1 and 2 were more closely adherent and firmly attached than the filamentous expressions developed on the other surface types.

b) Polysiphona spp.

The early stages of tetraspore development were very similar for both species of Polysiphonia investigated (see Fletcher, 1976). Vertical extension followed by transverse divisions produced an erect, cigar-shaped, 2-3 celled filament after 3 days, adhering at the base to the spore glue pad. After 4 days the erect filaments were 5-8 celled and a small bulbous germ tube (primary rhizoidal filament) could be seen emerging at the base of the rhizoid initial cell. Usually one of 2 types of attachment system resulted from the growth of this germ tube viz. filamentous or discoid. Filamentous systems consisted of rhizoidal filaments which were usually long, unicellular, colourless, unbranched and widely spreading across the substratum (Fig. 16). Discoid systems, which developed at the tip of the filamentous rhizoids, consisted of short, thick walled, much branched outwardly radiating filaments closely compacted together into a disc (Figs. 17, 18). They were occasionally multicellular with characteristically darkly tanned cell walls. The discoid attachment systems were largely

confined in both species to surface energy types 3 to 5 (93-100% disc production) and were of much rarer occurrence on surface energy types 1 and 2 (0-11%). Filamentous rhizoids, however, were the most common morphological expression on surface energy types 1 and 2. The development of discoid rather than filamentous rhizoids on surface types 3 to 5 markedly reduced the horizontal spread of the attachment system: average lengths of the primary rhizoids developed on surface types 3 to 5 ranged from 114 µm to 224 µm compared to the range 810 µm to 1049 µm recorded on surface types 1 and 2. With regard to the strength of attachment of the discoid systems, this was not very much different to that observed for the filamentous systems.

Discussion

Colonisation of new substrata is probably one of the most critical stages in the life history of a marine benthic organism. An essential feature of this process in the macroalgae is the rapid germination of the spore cell to form a prostrate attaching system for the developing erect shoot. This is accomplished initially by the growth of 'primary' rhizoids from the spore cell (Fletcher, 1976) and later by the production of secondary rhizoids from the basal cells on the erect shoot (Fletcher, 1977). Both types of rhizoids are highly suited to their role for attachment. Their outwardly radiating growth pattern and their ability to detect and respond to a range of external stimuli such as variations in surface texture, light, gravity, etc., greatly enhances their chances of finding suitable substrata and obtaining a firm foothold. Their morphogenetic flexibility also ensures colonisation of a wide variety of surface types, while the secretion of an outer mucilaginous glue-like material greatly increases their adherent properties. Perhaps not surprisingly, therefore, rhizoids have been attributed with a major role in the early more critical stages of germling establishment on substrata. The present paper demonstrates significant modification of their morphogenesis and attachment strength in response to surface tension and this will undoubtedly have considerable ecological implications.

The surface tension properties of the substrata appeared to exert an important influence on 2 main features of rhizoid development in the algae. They influenced the growth rate and consequently the outward spread of the rhizoids and they modified the degree of surface contact by affecting, either the lateral spread of individual rhizoids or the degree of branch formation. Basically this resulted in the development of 2 diverse morphological expressions in each alga on the range of surface energy types investigated: compact, sometimes disc-like structures of short, much branched, tightly adjoined and adherent rhizoidal filaments and loose, fibrous-like structures of long outwardly spreading, free rhizoidal filaments. One noteworthy aspect of this development was the variation in the response shown by individual algae, irrespective of their phylogenetic group, to each of the surface energy types. For example production of the short, much branched rhizoidal systems (disc-like) occurred on the

high surface energies in E. intestinalis, U. lactuca and B. atropurpurea, on intermediate surface energies in G. granulosa and on low surface energies in the 2 species of Polysiphonia. In addition unpublished work on another brown alga, Petalonia fascia has revealed a different response to that of G. granulosa with discs only produced on the high surface energy types. This absence of a model response in algal attachment to the surface energy properties of substrata finds support in recent work on bacteria by Fletcher and Loeb (1976). These authors reported preferential attachment of a bacterium to low energy, low negative charge surfaces in contrast to most reports on tissue cells and some reports on bacteria in which high energy, high negative charge surfaces were preferred (Dexter et al., 1975; Maroudas, 1975).

Of particular interest was the observed relationship between the morphological expression and the strength of attachment of the basal systems. In most of the algae investigated, the possible exception being Polysiphonia, the short, branched, disc-like bases were quite firmly adhered and not easily removed by gentle brushing. However, the more elongated, flexuous, rhizoidal filaments were much more weakly attached and easily brushed off. Indeed, in these cultures, in which the latter morphological expression predominated, much greater numbers of loose-lying, free floating germlings were observed, this undoubtedly being a result of culture vessel movement associated with frequent microscopic examination. However, it is also possible that the surface energy properties of the substrata may have exerted an earlier influence on the settlement and attachment process of the spores. In this respect Eiben (1976) reported surface energy to influence settlement of larvae of the bryozoan Bowerbankia gracilis.

With regard to the differences in attachment strengths of the 2 principal morphological expressions, although the densely branched disc-like bases were more compact with greater surface contact, this alone is unlikely to account for their usually greater adhesive property. Probably the surface energy properties of the substrata directly influence the adhesive quality of the rhizoids, either by reacting with the cell wall material, the rhizoid glue material, or both. It is possible, for example, that the different surface energy types exert an influence on the spread of the cell wall and/or glue material, similar to its influence on byssus pad size in the mussel Mytilus edulis (Young and Crisp, 1982). In this respect more detailed scanning electron microscope observations on rhizoids attached to the different surface energy types might make a useful contribution.

The involvement of surface energy properties of substrata in algal development might well have industrial implications. Baier (1973) suggested that the application of controlled surface energy coatings could be used to combat biological fouling. The present report, which shows that different critical surface energy coatings can be used to manipulate the basal, morphogenetic development in algae and thereby effectively control the strength of attachment of the plants,

supports this proposal. With respect to the macroalgae, low surface energy coatings appear as the least favourable to the development of efficient attachment systems, in agreement with Young and and Crisp's (1982) study on byssus pad adhesion in the mussel Mytilus edulis. Although settlement of macroalgae can take place on immersed structures coated with low critical surface energies, only relatively weak forces will be required for dislodgement.

Acknowledgements

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Explanation of Figures

Figs. 1-6. Enteromorpha intestinalis.
Fig. 1. Base of 4-day-old germling showing attaching rhizoid initial cell. S.T. 1; Scale Bar (S.B.) = 12μm.
Fig. 2. Base of 5-day-old germling with small disc-like base. S.T. 1; S.B. = 11μm.
Fig. 3. Enlarged disc-like base of 19-day-old germlings. S.T. 1; S.B. = 26μm.
Fig. 4. Base of 9-day-old germling with emerging primary and secondary rhizoids. S.T. 4; S.B. = 11μm.
Fig. 5. Base of 12-day-old germling showing outwardly spreading rhizoids. S.T. 4; S.B. = 25μm.
Fig. 6. Base of 15-day-old germling showing filamentous rhizoid base. S.T. 4; S.B. = 26μm.
Figs. 7, 8. Ulva lactuca.
Fig. 7. Disc-like attachment base of 12-day-old germling. S.T. 1; S.B. = 24μm.
Fig. 8. Filamentous attachment base of 12-day-old germling. S.T. 5; S.B. = 46μm.
Figs. 9-12. Giffordia granulosa.
Figs. 9, 10. Bases of 15-day-old germlings with short, compacted rhizoids. S.T. 3; Fig. 9, S.B. = 50μm; Fig. 10, S.B. = 26μm.
Figs. 11, 12. Bases of 15-day-old germlings with long, filamentous rhizoids. S.T. 2; Fig. 11, S.B. = 48μm; Figs. 12, S.B. = 80μm.
Figs. 13-15. Bangia atropurpurea.
Fig. 13. Base of 6-day-old germling attached by enlarged rhizoid initial cell. S.T. 1; S.B. = 9μm.
Fig. 14. Disc-like base of 10-day-old germling. S.T. 1; S.B. = 11μm.
Fig. 15. Base of 10-day-old germling showing single, elongate, branched rhizoid. S.T. 4; S.B. = 10μm.
Figs. 16-18. Polysiphonia brodiaei.
Fig. 16. Base of 6-day-old germlings showing long, filamentous rhizoids. S.T. 1; S.B. = 50μm.
Figs. 17, 18. Disc-like attachment base of 6-day-old germlings. S.T. 5; Fig. 17, S.B. = 50μm; Fig. 18, S.B. = 25μm.

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Table 1

Details of experimental algae

The nomenclature of Parke & Dixon (1976) is used

Phyla	Alga	Habitat	Date of collection	Spore Type
Chlorophyta	Enteromorpha intestinalis	Breakwater wall, Southsea	9.2.84	zoospores
	Ulva lactuca	Pontoon side, Langstone Harbour	7.2.84	zoospores
Phaeophyta	Giffordia granulosa	Pontoon side, Langstone Harbour	15.1.84	zoospores
Rhodophyta	Bangia atropurpurea	Pontoon side, Langstone Harbour	7.2.84	monospores
	Polysiphonia brodiaei	Pontoon side, Langstone Harbour	14.1.84	tetraspores
	Polysiphonia urceolata	Pontoon side, Langstone Harbour	21.1.84	tetraspores

Table 2

Experimental Test Surfaces

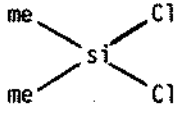
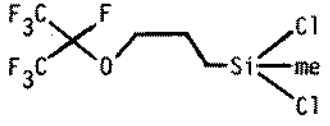
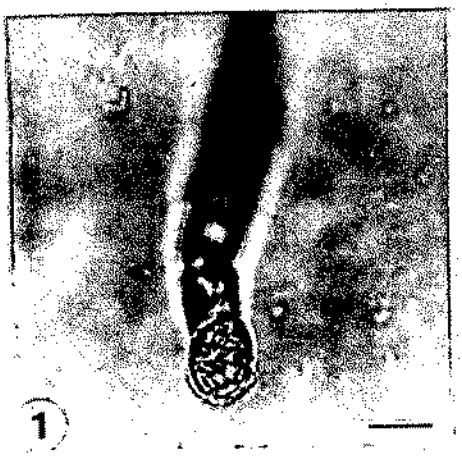
Surface Type	Treatment	Molecular Structure	Critical Surface Tension mN/m
1	Radio Frequency Glow Discharge Treated (RFGDT) and stored in boiled 3 x distilled water	-	>70
2	RFGDT followed by equilibration in a laboratory 'white' room and packaged in tissue	-	30-40
3	Chloropropyltrichlorosilane-coated	$ClCH_2CH_2CH_2SiCl_3$	30
4	Dichlorodimethylsilane-coated		20-30
5	Fluorosilane coated		<20

Table 3

Influence of surface energy on basal attachment in the experimental algae

Surface Type	Measured Critical Surface Tension (mN/m)	E.intestinalis	U.lactuca	G.granulosa	B.atropurpurea	P.brodiaei	P.urceolata
		17 days	12 days	10 days	10 days	5 days	6 days
1	>70	85	104	272	45	836(1)	1049(11)
2	30-40	104	127	296	31	810(0)	885(8)
3	30	151	163	79	64	160(94)	238(100)
4	20-30	150	192	298	102	146(95)	222(100)
5	<20	174	234	248	72	114(93)	224(100)

Open figures represent average diameters in µm of the attachment base (per 100 germlings) after the given time periods.
Figures in brackets represent% number of germlings with discoid type rhizoids.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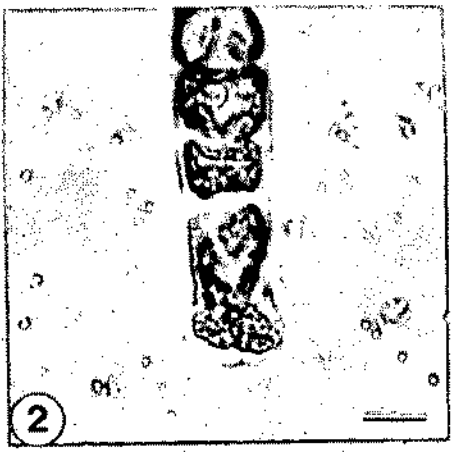
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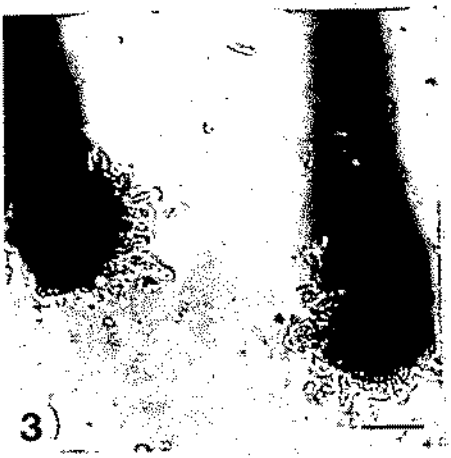
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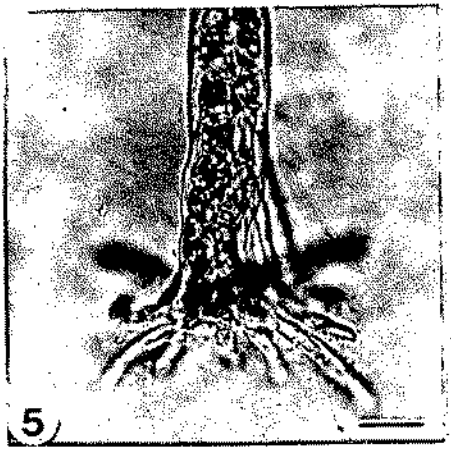
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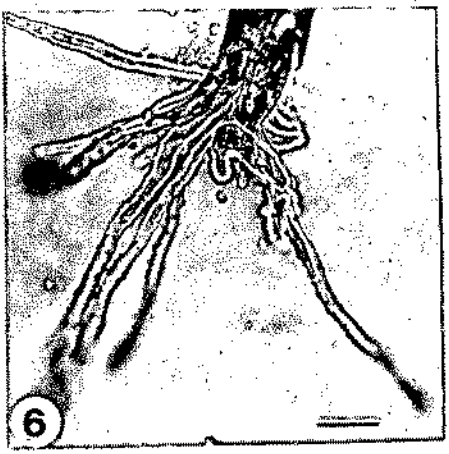
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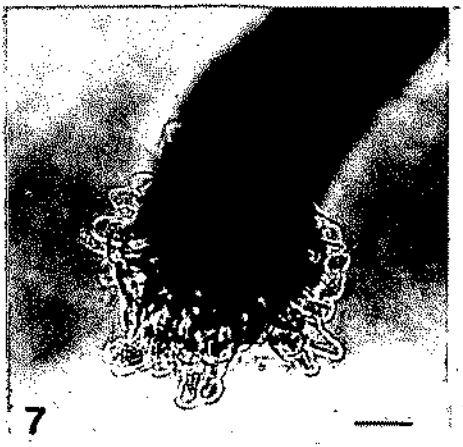
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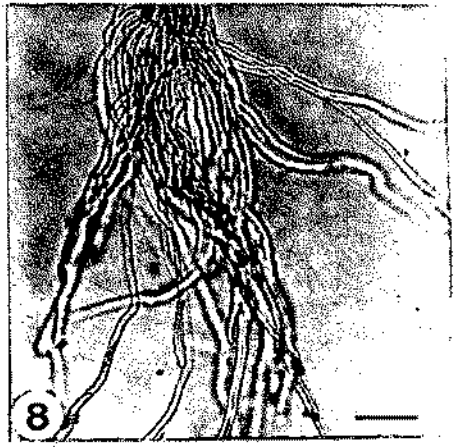
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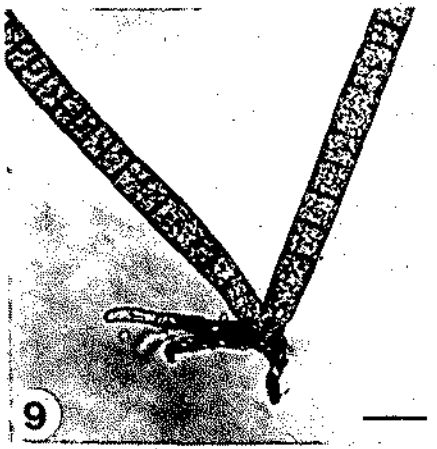
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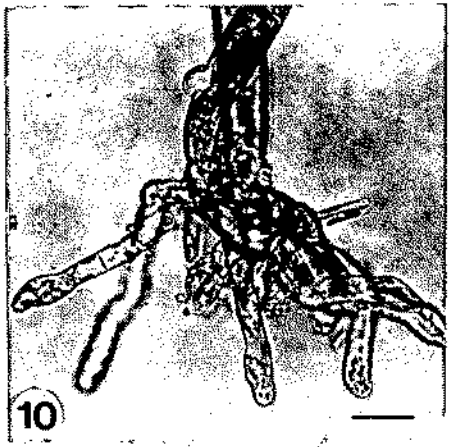
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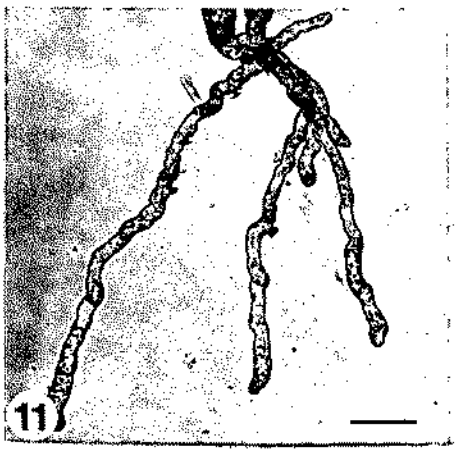
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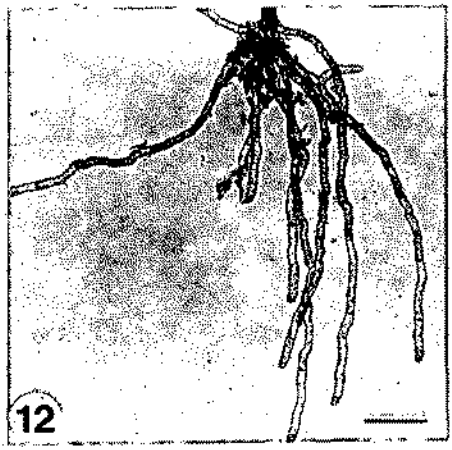
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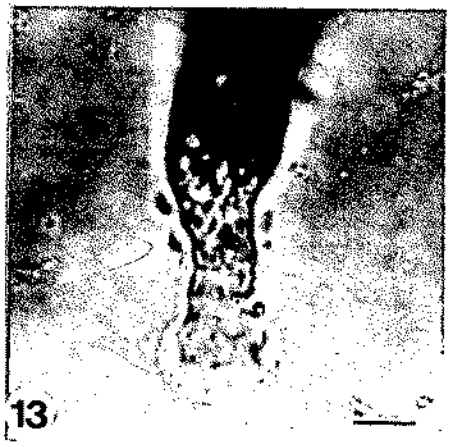
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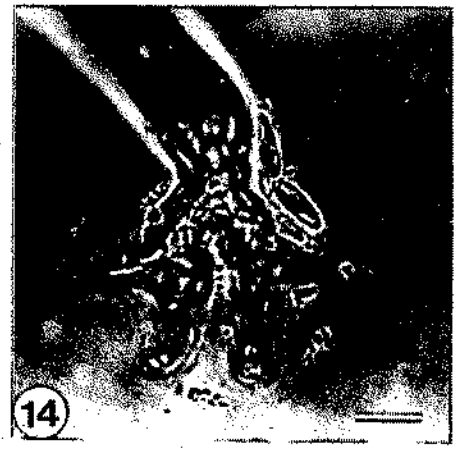
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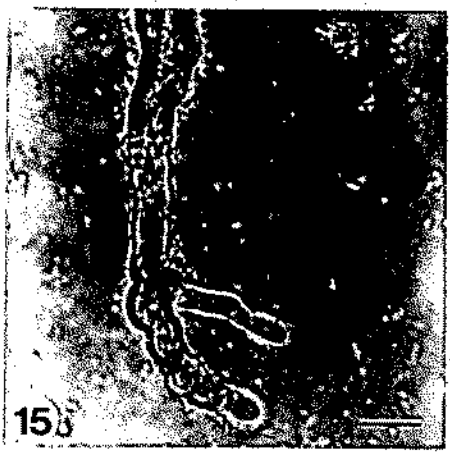
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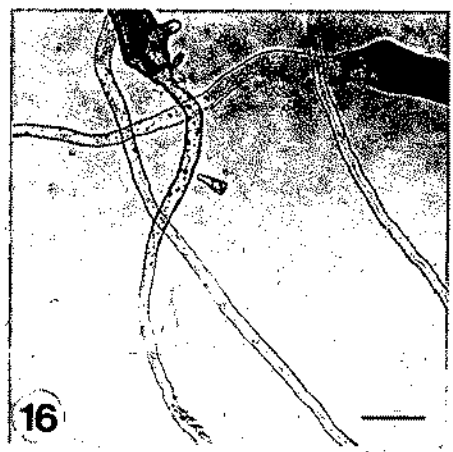
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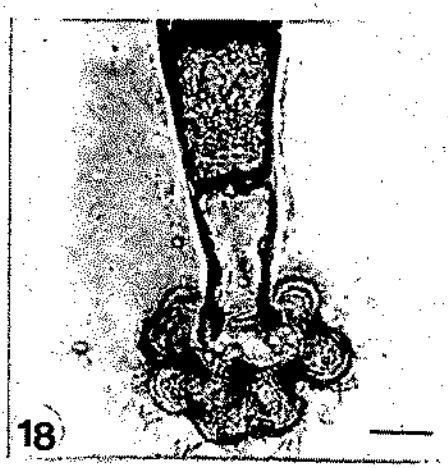
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ECOLOGY OF REPRODUCTION IN TROPICAL SHIPWORMS

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ABSTRACT

Results on the different environmental factors that influence the reproductive biology of four representative tropical shipworms namely, the oviparous Bankia carinata (Gray), and Nausitora hedleyi Schepman and the larviparous Teredo furcifera von Martens and Lyrodus pedicellatus (Quatrefages) are presented. Information from the Gulf of Cariaco is compared with that from the Indian Ocean. Significance of detailed studies on the ecology of reproduction is highlighted with a view to employing and/or suitably manipulating some of the environmental factors to check the damage by these pests.

RESUMÉ

Ici sont présentés les résultats des différents éléments écologiques qui influencent la reproduction biologique de quatre types de vers tropicaux attaquant les bateaux - les ovipares Bankia carinata (Gray) et Nausitora hedleyi Schepman et les larvipares Teredo furcifera von Martens et Lyrodus pedicellatus (Quatrefages). Les resultats du golf de Cariaco sont comparés avec ceux de l'Océan Indien. La signification des études détaillées sur l'écologie reproductive est dirigée pour un usage éventuel ou pour manipuler ces éléments écologiques dans le but d'arrêter les dommages effectués par ces animaux nuisibles.

INTRODUCTION

Reproduction in tropical shipworms exhibits a close relationship with environmental conditions to ensure maximum survival of the offspring. This aspect has great significance since these bivalves depend for their existence on a precarious and undependable substratum which is of terrestrial origin and scarce in the medium in which they live.

In the present paper an effort has been made to indicate the nature of the environmental adaptations concerning the reproductive biology of four representative species namely (1) the oviparous stenohaline Bankia carinata (Gray), (2) the oviparous euryhaline Nausitora hedleyi Schepman (3) the short-term larviparous Teredo furcifera von Martens, and (4) the long-term larviparous Lyrodus pedicellatus (Quatrefages).

MATERIAL AND METHODS

Shipworms were methodically collected by exposing a system of preconditioned wooden test panels of soft wood 10 x 10 x 10 cm identified by plastic tags and supported by a length of nylon rope threaded through a hole bored through the centre of the block. The blocks were suspended from a raft and a heavy weight at the lower end kept the blocks in position. The blocks were placed at three levels, one near the surface, a second at the middle of the water column and the third a little above the sea floor. The borer holes were counted using a binocular microscope. The seasonal settlement was studied by tabulating the species of boring organisms present on the blocks collected at monthly intervals. Their intensity of settlement was recorded on the basis of careful examination of three series of panels namely (1) Short-term panels consisting of 12 ropes each with 3 units as stated above, each put out and changed at the end of thirty days. These showed the settlement during the thirty days of immersion. (2) Long-term blocks of 12 ropes each with 3 units as in the previous series exposed simultaneously and removed one by one at intervals of thirty days. These gave an idea of the nature of settlement and growth for the respective period of immersion and showed how the monthly set was modified by the animals already present over the blocks. For assessing the condition of the gonad during the yearly cycles the method of gonad index developed by Giese and his collaborators (Giese et al., 1964) has been used with good results. The

gonad index provides accurate information on the breeding condition of individual animals and represents a measure of the reproductive condition of the population. In the calculation of the index the gonad weight is expressed in terms of the percentage of the body weight. The data thus obtained were supplemented with those drawn from the incidence of larvae in the plankton.

RESULTS AND DISCUSSION

All the species examined exhibit a sequence of sexual phases. In Table I the sexual condition of B. carinata is presented. In this species the majority of individuals are bisexual males from 10-50 mm while females predominate from 50-200 mm and longer with a few hermaphrodites occurring in the intermediate range 50-150 mm. The bisexual males contained both functional sperms and developing oocytes. A small percentage of males with functional sperm only, were found in individuals from 10-200 mm.

Table I - Sexual condition in Bankia carinata

Length in mm	True males	Ambisexual males	Hermaphrodites	Females	Total
10 - 20	9	60	-	-	60
20 - 50	9	169	2	5	185
50 - 100	16	77	7	211	311
100 - 150	1	16	1	27	35
150 - 200	1	9	-	17	27
Above 200	1	-	1	3	5

Gametogenetic activity and salinity

Nausitora hedleyi is unique in exhibiting a restricted breeding habit in the tropics closely related to the saline regime in its brackish water habitat. There is a preponderance of ambisexual males among smaller individuals (1-50 mm) and as growth continues there is a tendency towards increase in the percentage of females with hermaphrodites appearing in the intermediate size group (50-200 mm). This suggests protandry and a probable change of sex from the initial bisexual male phase to the female phase. A few of the hermaphrodites had gametes of both sexual types in the same follicle, some were changing from male to the female phase and one in the 250-300 mm was apparently changing from female to male with the germinal epithelium proliferating

spermatogonia and the lumen containing spermatozoa, a few oocytes and ova (Table II).

Thus it appears that the proportion of individuals in the two functional sexual phases changes with the advance of the breeding season. There is a preponderance of ambisexual males at the beginning of the breeding season, with an increase in the number of females in the large size groups collected during the final phases of the breeding season, probably as a result of a change in sexual phases from male to female. There is a possibility of self fertilisation during the intervening hermaphrodite period. A second sex reversal after the assumption of the definite female phase may also occur. The conclusions reported above are drawn from the data collected during the breeding season of the species that extends from June to December; the animals settled on test panels during July-August when the salinity of the ambient water is low consequent on the monsoon rains.

Table II - Nausitora hedleyi condition of the sex of specimens

Length group in mm	True males		Ambi-sexual males		Hermaph-rodites		Females		Immature		Total no. of speci-mens
	No.	%	No.	%	No.	%	No.	%	No.	%	
1-50	4	1.7	128	55.9	-	-	45	19.7	52	22.7	229
50-100	5	4.5	53	48.2	6	5.5	33	30	13	11.8	110
100-150	1	1.4	23	31.4	14	19.2	27	37	8	11	73
150-200	-	-	3	14.3	5	23.8	13	61.9	-	-	21
200-250	-	-	7	43.8	-	-	9	56.2	-	-	16
250-300	-	-	2	20.0	1	10	7	70	-	-	10
Above 300	-	-	2	18.2	-	-	9	81.8	-	-	11

From October onwards, a small percentage of the females examined histologically showed indications of inactive gonads. There was noticeable reduction in the gametogenetic activity, at lowest level in late January and in February. During the non-breeding months, from January to May specimens examined histologically revealed the presence of the following categories of gonads. Indeterminate gonads where the follicles are not clear and sex not clearly determinable. The epithelium of the gonad apparently showed no sign of activity. Common during March-April. Among specimens distinguishable as females at least three types of histological conditions could be .

recognised. (1) Ova loose in the follicles with a few ovocytes in the cortical region both undergoing a process of disintegration, and the epithelium apparently inactive: Common during February. (2) Ova free in the lumen of the lobule with the walls containing proliferating spermatogonia. The cortical region is apparently active indicating a probable transition from a female to a male condition, in March. (3) Follicles shrunk but the cortical layer containing ovocytes showing progress of oogenesis in May, June and July.

Two types of males were discernible during the non-breeding period : (1) with the cortical layer containing spermatogonia and spermatids and the germinal epithelium showing some activity without sperms in any of the follicles. Common in February; (2) with active spermatozoa free in the lumen of the lobule and the cortical layer with primary and secondary spermatids, with a few differentiated oocytes representing an ambisexual gonad, common during May and June.

Thus in N. hedleyi in the estuarine locality of Cochin backwaters during the hot, highly saline period January to May the gonads usually are in a state of apparent quiescence. The unspawned gonadial elements of the previous breeding season left in the follicles are probably resorbed and the epithelium undergone a period of rest. By May or June gametogenic activity is particularly evident in the male phase and it is probable that these may provide the male gametes for the specimens bearing the type '3' gonad of the female recorded above.

Delineation of the breeding period

The delineation of the breeding period has been done on the basis of (1) the incidence of larvae based on regular collection of a given species from a known volume of water and the assessment of the density of larval populations during different months in the plankton, and (2) the determination of the time of settlement based on a study of the relative abundance of post settlement stages in methodically operated test panels. This second method is of great practical significance since this alone provides a reliable measure of breeding success, there being the possibility of spawning without settlement. In certain special situations it is essential to employ a quantitative assessment of the condition of the gonad using the technique of gonad index to avoid the possible errors due to recruitment of larvae from marine sources in

enclosed areas of the harbour. A systematic examination of the gonad condition gives a fairly accurate picture of the breeding season. The data thus obtained have been supplemented with those drawn from the two other methods. The average values for the different months in the case of N. hedleyi are presented in Table III and Fig.2.

Table III - The seasonal settlement of shipworms on short-term panels ("A" series) at Cochin harbour

Period of immersion	Nausitora hedleyi			Teredo furcifera		
	Inter-tidal	Sub-tidal	Bottom	Inter-tidal	Sub-tidal	Bottom
Pre-monsoon						
1 Feb. - 1 Mar.	-	-	3	11	159	507
1 Mar. - 1 Apr.	-	-	-	35	217	305
1 Apr. - 1 May	-	-	-	30	145	340
1 May - 1 Jun.	-	-	-	81	113	258
Monsoon						
1 Jun. - 1 Jul.	-	-	-	2	5	43
1 Jul. - 1 Aug.	-	-	7	-	-	5
1 Aug. - 1 Sep.	9	3	21	-	-	-
1 Sep. - 1 Oct.	-	6	9	-	-	-
Post-monsoon						
1 Oct. - 1 Nov.	2	15	9	-	-	-
1 Nov. - 1 Dec.	-	43	31	-	2	-
1 Dec. - 1 Jan.	-	11	9	7	14	25
1 Jan. - 1 Feb.	-	3	1	13	12	49
Total for the year	11	81	90	179	667	1532

It is evident that the gonad index is low during the period January to June. For the rest of the period the values are above 15. The lowest value recorded is for June (4.99). The sudden decrease is probably owing to an initial spawning which took place during that month. From this low index in June the values move up steadily to a peak in October (32.45). Thereafter the values indicate a downward trend with a steep fall in November (15.81). In December also the value remains about that level followed by another major fall (6.11) in January. From January values show a slight rise through February and March and attain a secondary peak

in April. This is followed by a fall in values to the lowest recorded for the year in June.

This data, perhaps the first of its kind in the case of shipworms show that N. hedleyi is not a continuous breeder in this locality. The high values noted for females during the months following June till October suggest very high activity of the ovary during this period. High gonad activity in N. hedleyi is thus restricted in this habitat, to a period of nearly six months from July to December. The peak period of settlement will, however, be slightly different from the period of maximum activity of the gonad since there is a time lag between spawning and the time of settlement. It is interesting to note that there is reasonable correlation between the data on gonad index and the data collected from a system of test panels used to find out the time of settlement of the larvae.

Season of settlement

Biologically, the period and extent of settlement are significant since they are reflection of the breeding season and a reliable measure of the breeding success. This is due to the fact that there is the possibility of spawning without settlement. In certain instances detailed studies on the season of settlement of timber boring organisms have shown effective ecological adjustments, the different species occurring in an area showing interrelationships so that interspecific competition is reduced to a minimum through characteristic zonation in settlement (Nair, 1959). The breeding activities of closely allied species show differences and even those of the same may vary according to the hydrographic conditions prevailing in the area (Nair, 1965). The density of distribution of shipworms has fluctuated over long periods and within the same period their attacks have differed considerably in various locations along the same stretch of coastline.

This aspect of the breeding activity of shipworms has been the subject of study in 4 localities along the coasts of India. At Cochin harbour south-west coast of India Teredo furcifera settles chiefly during the hot, highly saline premonsoon period, February to June with sparce settlement during the early part of the monsoon and later part of the post monsoon period. In Visakhapatnam on the east coast of India this species settles on test panels throughout the year with a maximum attack during the summer months between March and June with a

peak in May (Nagabhushanam, 1959). The comparatively smaller attack rate during the winter months (November-January) is attributable to a biological competition with B. campanellata whose intensity was greatest during the winter months. According to Karande and Pendsey (1968) settlement of T. furcifera at Bombay occurs throughout the year in varying intensity. In the Gulf of Cariaco, Venezuela the pattern of settlement of shipworms has been examined. The incidence of T. furcifera was almost continuous with a small gap during August-September. Highest settlement was noticed in October-November. Lyrodus pedicellatus, settled in Bombay Harbour in great numbers during late January to late October. After October only a few specimens were seen on panels until January. Santhakumaran (1973) found that seasonal abundance and vertical distribution are both influenced by prevailing hydrographic conditions and also by the presence or absence of fouling organisms. In an earlier report Santhakumaran and Alikunhi (1971) reported that at Trombay attack by L. pedicellatus started in March, gradually increased in intensity up to July and reached a peak in August. After September only a few specimens were seen settling till March. These results indicate that settlement of even closely related species might show difference in time and space and even the same species may show variation according to the prevailing hydrographic conditions. In the Gulf of Cariaco the pattern of settlement of L. pedicellatus was quite different from that in India (Fig. 1). This species is the most common and therefore, the most destructive species in the Gulf. The settlement was continuous throughout the year with a slight reduction in its intensity during May-June. During October-November the settlement reached a prominent peak when more than 54 per cent of the total settlement has been recorded.

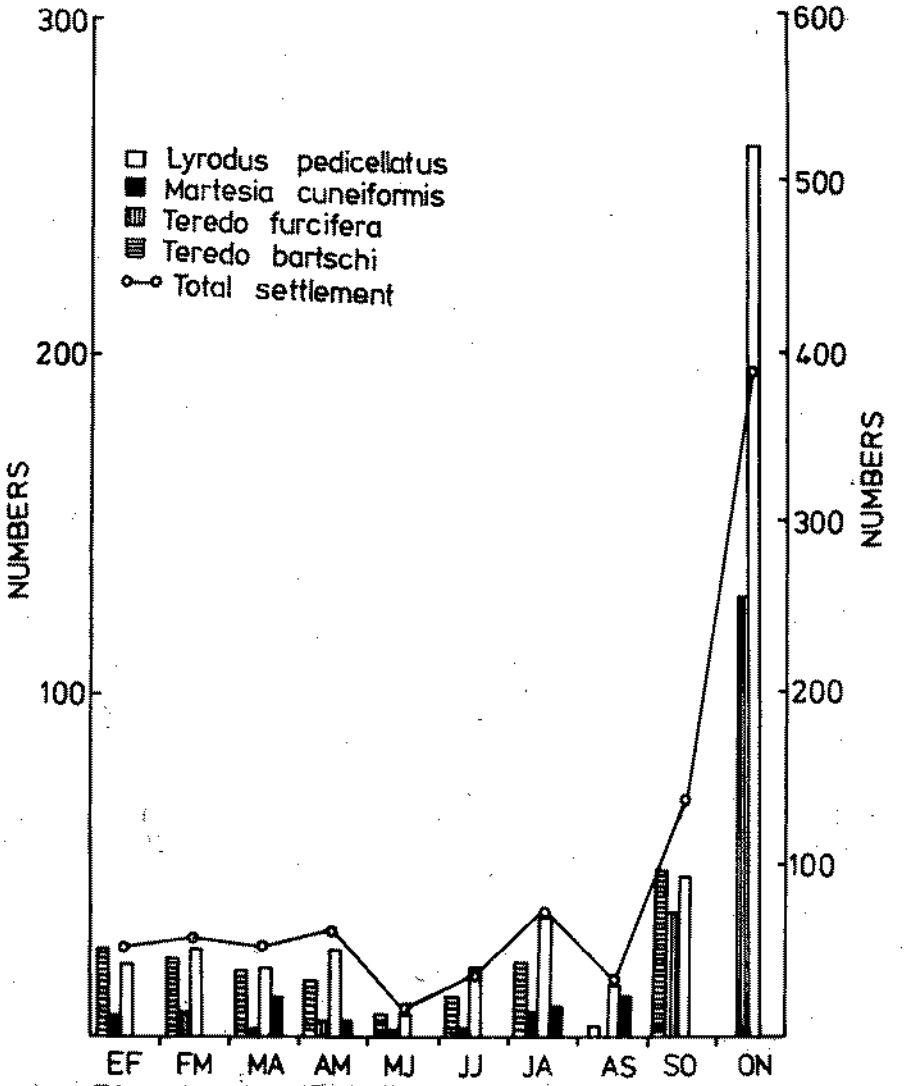

Fig.1. Histograms showing the nature of settlement of wood-boring molluscs over short-term blocks in the Gulf of Cariaco

The settlement of the oviparous Nausitora hedleyi is quite different from any of the previous cases in that at the Cochin Harbour it is strictly seasonal (Fig. 2). Beginning in July, the settlement continues uninterruptedly till about February. The data also show that

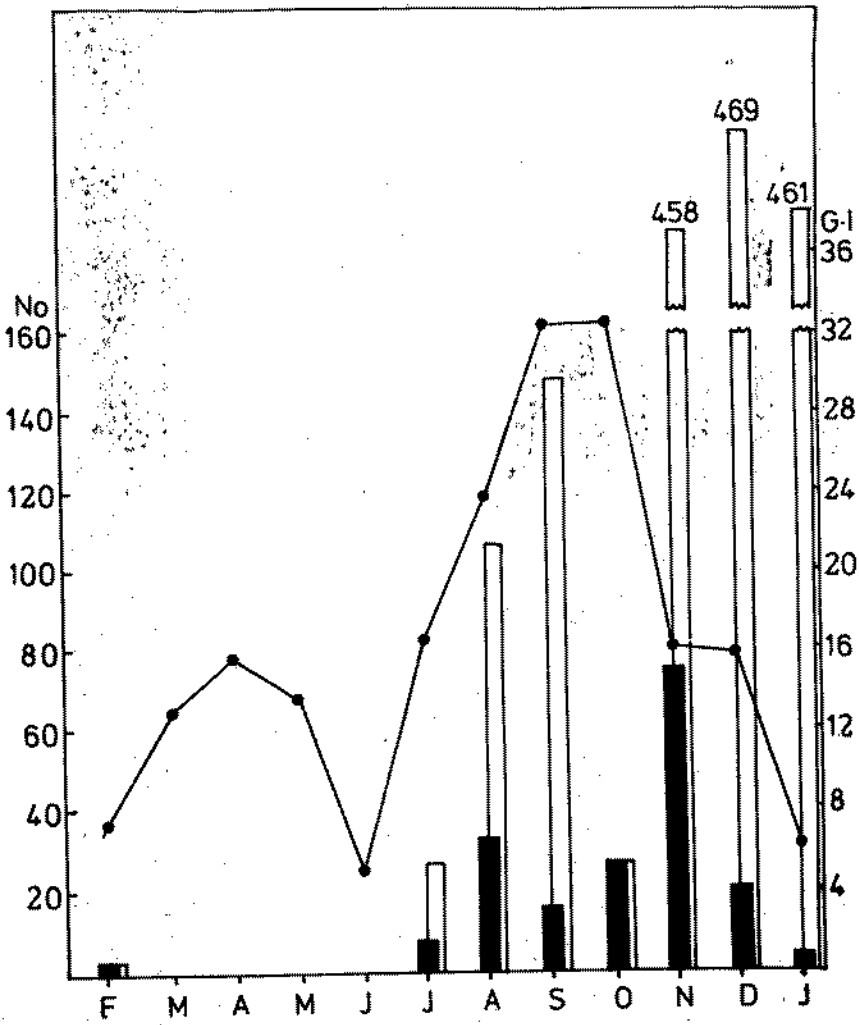

Fig. 2. Histograms representing the nature of settlement of Nausitora hedleyi on test panels. Short-term (shaded) and long-term (blank) during the different months at Cochin Harbour. The graph presents the average gonad indices for the respective months

November represents a period of intense settlement with a period of fair settlement in August. Probably the dense settlement of November may be on account of the fact that those settled from July become mature and reproduce to record intense settlement by November. The data from the long-term panels show how the monthly settlement may be modified when test panels are submerged for longer periods than 1 month and how the presence of organisms on the panels influences further settlement. The relatively greater number of specimens in panels of this set was due to longer exposure and so continuous settlement by waves of larvae during periods of immersion ranging from 1 month to 4 months.

The breeding period of the circumtropical oviparous Bankia carinata has been delineated on the basis of the frequency of occurrence of veliger larvae in the plankton, the presence of post-settled stages on test panels and the condition of the gonads of the adults. These studies indicate that the breeding is continuous in Madras waters and that there is a peak in reproductive activity during July-August when maximum settlement has been recorded (Fig. 3).

There are several environmental factors which affect the nature of populations of shipworms. These are the physicochemical variables of the sea water such as temperature, salinity, oxygen tension, turbidity and pollutants, the presence and intensity of fouling organisms, the nature of the wood, depending on the species of timber, its softness and orientation of the grain, the length of exposure of the wood sample in water, the presence or absence or the nature of the preservatives used on it, the location of the wood in relation to tidal changes, whether or not it is periodically exposed to desiccation, the orientation of the sample in relation to death; nature of the bottom, mechanical effects of currents, their velocity; conditions of illumination, the interaction of the species of wood boring animals present in the area; the availability of a suitable substrate during settlement; the effectiveness of local larval sources and the presence or absence of predators and parasites. The occurrence, abundance, and so the intensity of attack in any locality is dependent on these factors which vary widely from year to year. Probably there are several more, but these factors are the most important. The variations in the borer populations from year to year in

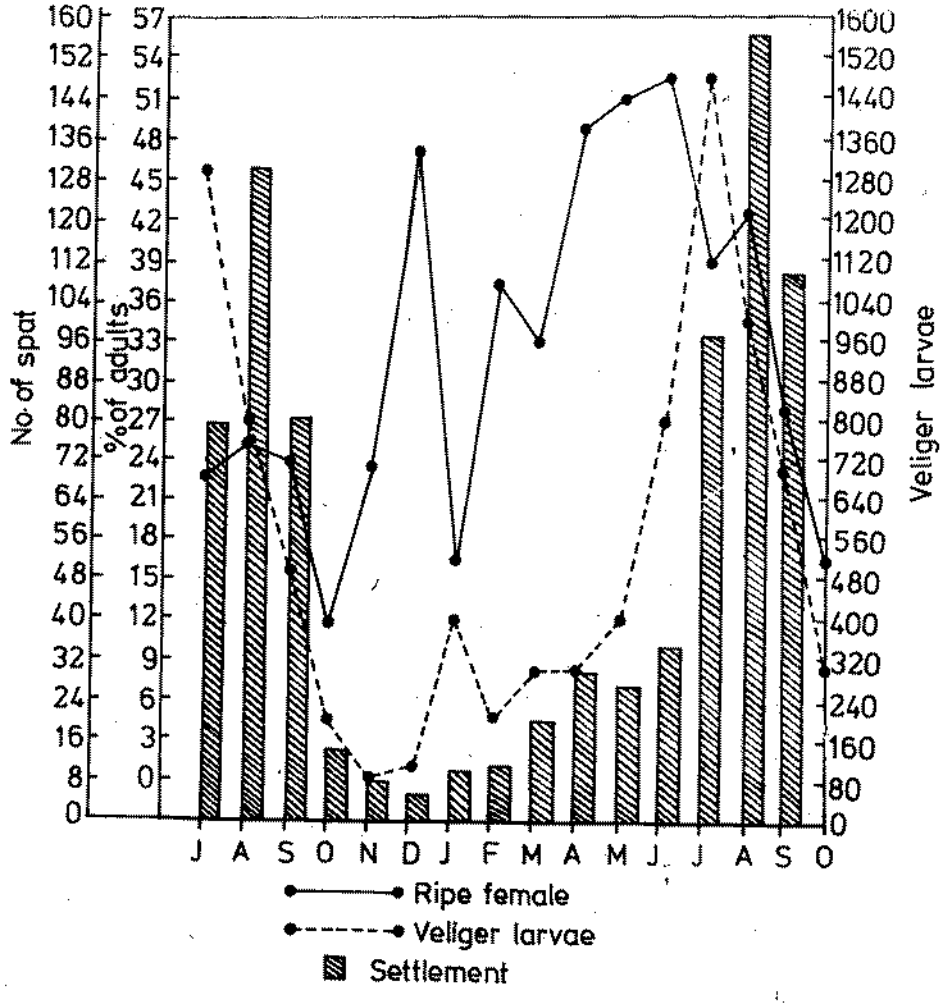

Fig. 3. Curves illustrating the occurrence of veliger larvae in plankton, settlement of spat on test panels (Histograms) and the condition of the gonad of adult females of B. carinata in respective months in Madras waters.

any locality are no doubt due to a very involved association of these factors, some of which occasionally stand out as the most responsible ones while other factors, none attaining conspicuous importance by itself, may collectively exert as much or more influence

than more prominent and easily followed factors. It is by a constant shifting of importance of these factors and new alignments in their associations owing to ever varying conditions, that account for variations in local abundance of shipworms. This would explain periodically recurring devastation separated by often lengthy intervals of comparative freedom from attack.

Reasons for increased attacks have not all been investigated but they may be different in different localities such as difference in salinity caused by reduced inflow of fresh water or reduced rainfall causing an increase in salinity high temperature and dry summer or an unusual increase in water temperatures.

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ECOLOGY OF FOULING IN COCHIN HARBOUR

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Abstract:

A comprehensive study of the fouling organisms encountered at the Cochin Harbour, India was made for a period of one year. Hydroids, bryozoans, polychaetes barnacles and bivalves are the dominant groups encountered in this area. Monthly settlement of the different fouling groups has been recorded. Number of animals present on the test panels was high during the pre-monsoon and the post-monsoon periods and low during the monsoon period. The low saline condition consequent on the south - west monsoon imposes restrictions on the otherwise continuous settlement of fouling organisms in the Cochin Harbour. The fluctuation of salinity is very much pronounced here and the correlation coefficients with salinity and number of animals settled were worked out for the most important fouling groups.

The severity of the problem of fouling and its prevention are well brought out in the monograph on Marine Fouling and its Prevention (1952) by the Woods Hole Oceanographic Institution. The problem varies from locality to locality and a thorough understanding of the biology of the various fouling animals is very much essential for the formulation of preventive measures. Several authors have studied fouling occurring in different harbours of the world. Notable among them are those

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of Coe & Allen (1937), Milne (1940), Mc Dougall (1943), Allen & Ferguson (1950), Skerman (1958), Stubbings & Houghton (1964), Kawahara (1969), Long (1972), Lee & Trott (1973), Ghobashy et al. (1980), Bastida et al. (1980), Ehrler & Lyke (1980) and Viviani & Disalvo (1980).

In India various aspects of fouling have been dealt by Paul (1942), Daniel (1954), Ganapathi et al. (1958), Antony Raja (1959), Nair (1967), Dharmaraj & Nair (1981) and Rao et al. (1982).

In spite of these studies our knowledge pertaining to this problem from various harbours of India remains still inadequate. The structure and composition of the fouling complex exhibit wide temporal and regional variations which are governed mainly by varying hydrographical conditions and geographical locations (Dharmaraj & Nair, 1981). Changes could clearly be seen in the composition of fouling communities on permanent and temporary substrata, but a correct analysis and interpretation of this complex phenomenon is possible only by a planned study with aid of experimental panels (Menon et al. 1977).

Material and Methods

The present study was carried out from the north oil tanker berth in Cochin Harbour in the Ernakulam channel. Fouling organisms were collected by exposing glass panels of size 150 mm x 100 mm, kept at 1m below the lowest low water mark arranged on a grooved teak immersion rack. The test panels were exposed from January 15, 1981 to January 15, 1982.

A - series (short-term):

Twelve panels were exposed one by one on the 15th of every month and removed on the 15th of the succeeding month. This shows the settlement occurred during the 30 days of immersion.

B - series (long-term):

Twelve glass panels were exposed together on the 15th January 1981 and removed one by one at the end of every 30 days which gave an idea of the cumulative fouling complex for one year. The fouled panels were preserved in 5% formalin after determining the weight and volume. The quantity and quality of fouling were determined by identifying the foulers and counting them wherever possible. Salinity and oxygen content of the water

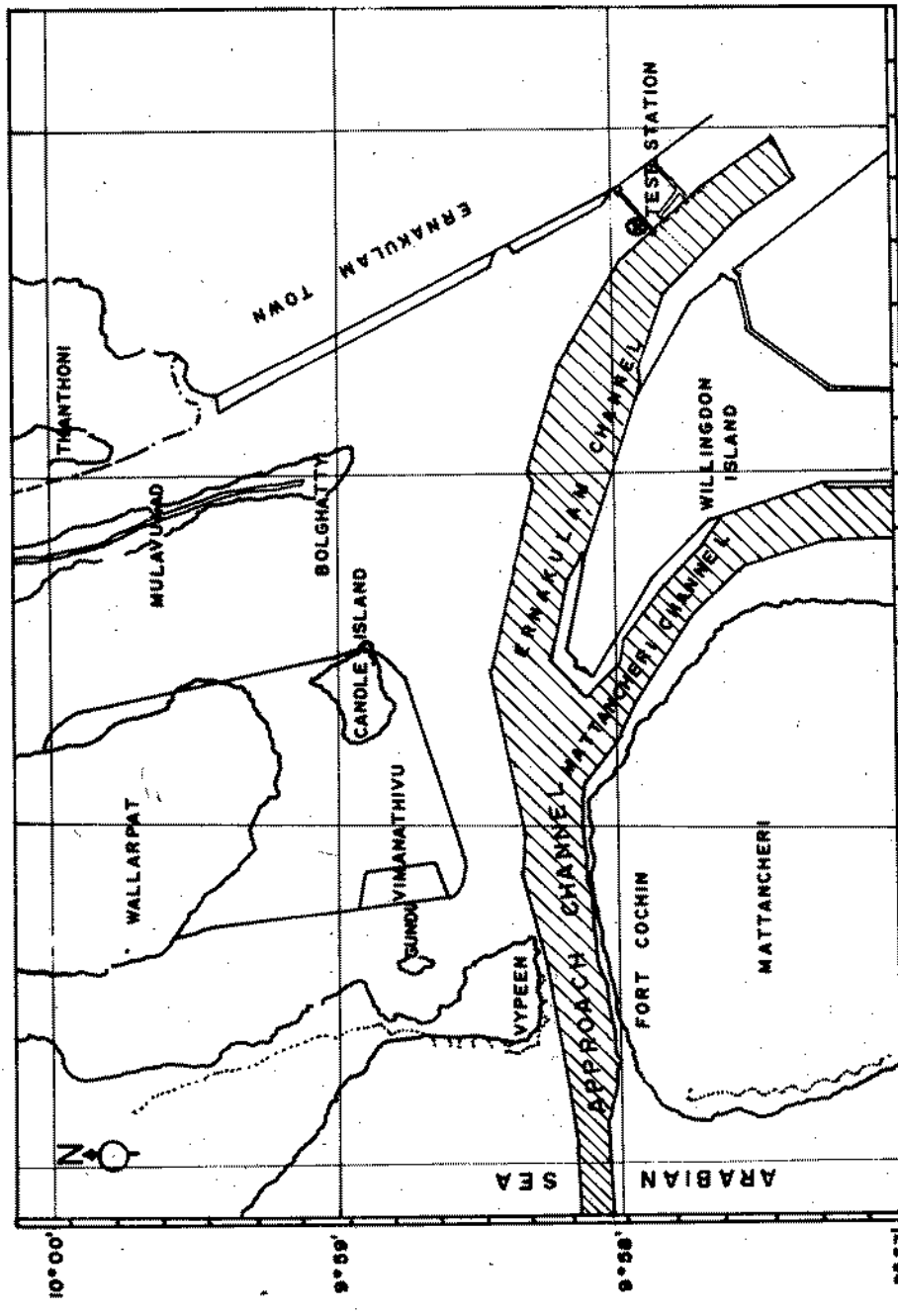

Fig.1. Cochin Harbour showing the test station

were determined following Strickland and Parsons, 1968. Air temperature and surface water temperature were measured with a mercury thermometer correct to 0.1°C.

Cochin Harbour (Lat 9°58' N Long 76°16' E) is situated in the Cochin backwaters which is the northward extension of the Cochin backwaters, maintains a permanent connection with the adjoining Arabian Sea through an opening of 450 m wide. The approach channel leading to the Cochin Harbour (Fig.1) is 12 m deep. The influence of the sea strongly felt in the backwaters, throughout the year except during monsoon, during which large quantities of freshwater are flushed into the estuary through river discharge and land drainage.

The south-west monsoon is very much active in this area, from May to August. The north-east monsoon bursts over the region during October to November. Based mainly on the quantity of precipitation during different months, the year can be divided into three seasons, pre-monsoon (February - May), monsoon (June - September) and post-monsoon (October - January) (Nair, 1967). The pre-monsoon is a period of high salinity with almost all the characteristics of coastal marine environments, the monsoon period is characterised by very low salinity and the post-monsoon period represents a transitional stage between the other two periods. During the course of the present study the South-west monsoon started in late June and was prevalent over the region till September.

Results

Salinity of the surface water fluctuated between 19.7 - 26.5‰ during the pre-monsoon period reaching the maximum in April (26.5‰) and in May it declined to 19.7‰. During the monsoon period lowest salinity was recorded in July (0.93‰). Low saline conditions continued till November, and then slowly increased reaching 33.75‰ in January 1982. Surface water temperature fluctuation between 28° - 32°C and air temperature fluctuated between 25° - 33.1°C during the period of investigation. Dissolved oxygen of the surface water varied from 2.8 to 4.2 ml/l and a definite pattern of seasonal change could not be observed. The hydrographical characteristics are shown in Fig.2.

The fouling community at the Cochin Harbour consisted of chiefly of sponges, hydroids, sea anemones, bryozoans, mud-tube dwelling polychaetes, calcareous-tube dwelling polychaetes, barnacles and bivalves. A list of various foulers commonly met with on experimental panels is presented in Table 1.

Table 1. List of fouling organisms

Diatoms

- Bacillaria sp.
- Biddulphia sinensis
- Coscinodiscus excentricus
- Coscinodiscus sp.
- Fragilaria sp.
- Licmophora sp.
- Navicula sp.
- Nitzschia longissima
- Thalassionema sp.
- Thalassiosira sp.
- Pleurosigma sp.
- Rhizosolenia sp.

Protozoa

- Zoothamnium sp.
- Vorticellids
- Folliculinids

Coelenterates

- Garveia franciscana
- Halocordyl disticha
- Obelia bicuspidata
- Obelia gracilis

Sponges

- Unidentified

Sea anemones

- Sagartia sp

Polyzoans

- Alderina arabianensis
- Bowerbankia gracilis
- Electra anomala
- Electra crustulenta
- Electra bengalensis
- Bugula neritina
- Nolella paupensis
- Schizoporella unicornis
- Watersipora cucullata

Mud-tube dwelling Polychaetes

- Polydora ciliata
- Polydora flava

Ecology of Fouling in Cochin Harbour

Calcareous-tube dwelling polychaetes

Mercierella enigmatica
Ficopomatus macrodon

Errant Polychaetes

Perinereis capensis
Ceratonereis keiskama
Nephthys oligobranchia
Dendronereis zululandica
Platynereis sp.

Crustacea

Cirripedes

Balanus amphitrite communis
Balanus amphitrite insignis

Caprellids

Metaprotella sp.

Amphipods

Corophium triaenonyx
Corophium abdictus
Tanais phileterius
Ampelisca scarbripes

Isopods

Cirolana bicarinata

Bivalves

Perna viridis
Crassostrea madrasensis
Modiolus carvalhoi
Musculista senhausia
Musculista arcuatula

Ecology of Fouling in Cochin Harbour

The seasonal settlement of the most important groups of foulers are shown in Fig.3.

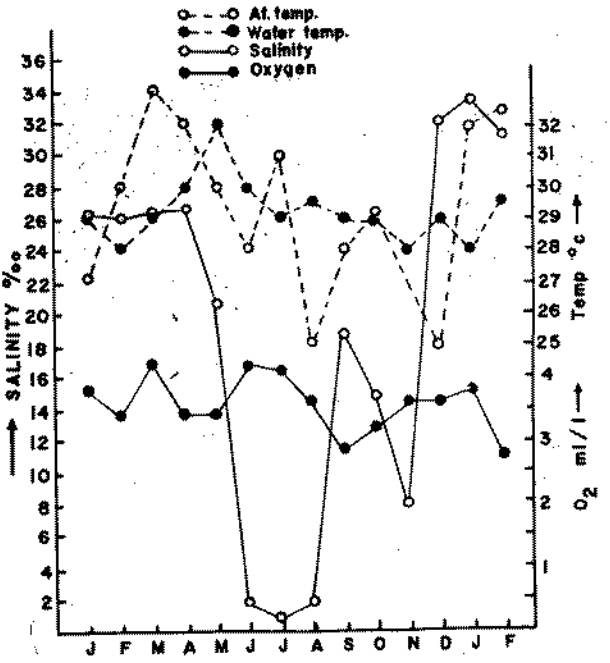

Fig.2
Figure showing the monthly recordings of salinity, oxygen content, air temperature and surface water temperature at the test site from January 15, 1981 to February 15, 1982

Diatoms, hydroids and bryozoans were present through out the year. During the high saline period, hydroids exhibited luxuriant growth. Sea anemones were absent during the low saline period of the monsoon. Bryozoans were found settling throughout the year.

Polychaete (Polydora sp.) was absent during monsoon in both the test series. Their peak settlement was from March to May. Settlement was comparatively low during the post-monsoon period. Two minor peaks were noticed during October and December. Settlement of calcareous-tube dwelling polychaetes were fewer compared to the mud-tube dwelling polychaetes. Errant polychaetes were also found in good numbers among the fouling community both in long-term and short-term panels.

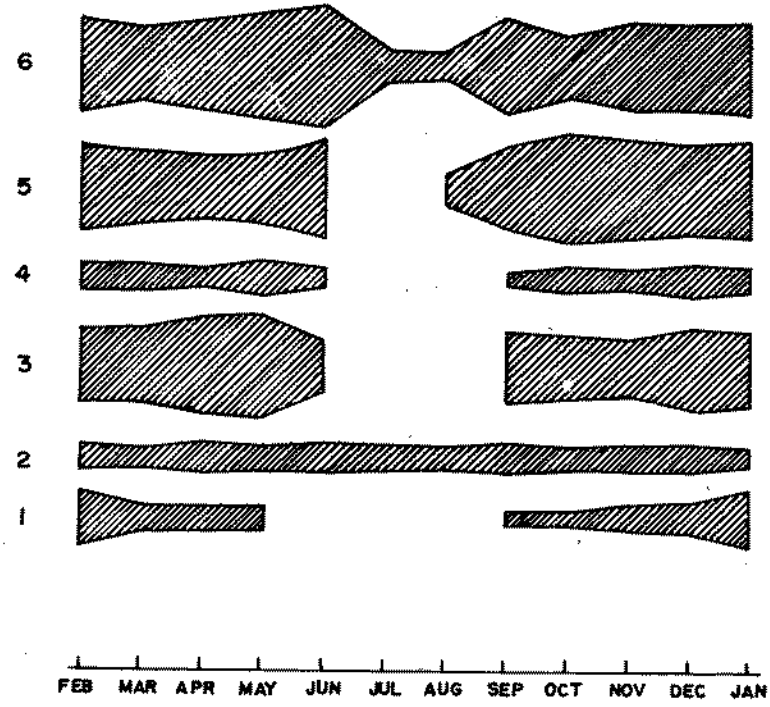

Fig.3
Seasonal variations in the settlement of the principal fouling groups in the harbour. Ordinates are proportional to the cube root of the number settled per m^2 in each month. 1. Sea anemones 2. Bryozoans 3. Mud-tube worms 4. Calcareous-tube worms. 5. Balanus sp. 6. Modiolus sp.

Ecology of Fouling in Cochin Harbour

From the settlement pattern on the short-term panels it is evident that the barnacles settled throughout the year except during July and August. The major peak in settlement of barnacles on short-term panels was in October during the post-monsoon period when the salinity was 14.6‰. There were two other minor peaks, one in January and another in June. In short-term and long-term panels barnacles settlement was very much pronounced during the post-monsoon period.

Among bivalves Modiolus sp. also exhibited a settlement pattern similar to that of barnacles. Together they contribute the major share to the total biomass of fouling. Moreover Musculista sp. accumulated considerable quantity of mud over the panels thereby increasing the weight of the panels. Bivalves were found occurring throughout the year except when the salinity is low during the monsoon period. Their peak settlement was during the pre-monsoon period (May-June) in short-term panels and June-July in long-term panels. There was no settlement during the monsoon period. Settlement of the mussel Perna viridis and Crassostrea madrasensis was observed only very rarely on test panels.

Among the free living forms, amphipods were noticed in large numbers during the pre-monsoon and post-monsoon periods. Isopods were less in number compared to amphipods. Associated with hydroids numerous caprellids were found browsing on them but were totally absent during low saline periods.

Discussion:

The collection of data based on an examination of the test panels provides a fairly reliable method for the study of the seasonal settlement of marine foulers and their rates of growth (Nair, 1967a). The present investigation clearly shows that settlement of foulers in Cochin Harbour is considerably influenced by the south-west monsoon. The low saline condition consequent on the monsoon imposes restrictions on the otherwise continuous settlement of fouling organisms in Cochin Harbour. The fluctuation of salinity is very much pronounced when compared to temperature and oxygen content of the water and plays the major role in the settlement of marine foulers. All marine organisms and most estuarine organisms can withstand full seawater, but some of them cannot withstand lowered salinities and thus the species numbers decline with the salinity gradient-decline in estuaries. Sessile or only slightly motile marine organisms have optimal salinity ranges for best growth rates and when the salinity varies away from the optima,

either upward or downward, the population becomes stunted - says Gunter (1961). Panikkar & Aiyar (1939) have observed that the lowering of salinity affects the breeding of the brackish water animals of Madras. Ganapathi et al. (1958) observed peak settlement of barnacles during April, May and August at the Visakhapatnam Harbour. No such period of intensity has been recorded at the Madras Harbour (Antony Raja, 1959). Different peaks in the settlements of barnacles have been observed by Menon et al. (1977). Nair (1967a) registered maximum settlement of barnacles at Cochin Harbour during the post-monsoon period. At Neendakara Port barnacles appeared throughout the year except during February with a peak in August and November (Dharmaraj & Nair, 1981). In the present investigation too, the settlement of barnacles was observed throughout the year except during monsoon months with peaks in May-June and September-October which is in agreement to what has been observed by Nair (1967a) and Pillai (1958). Karande (1967) noticed lack of breeding of barnacles during low saline conditions of July to September in Bombay Harbour. Crisp (1950), Hutchins (1947) and Barnes (1957) have shown that the liberation of larve in barnacles depends on factors such as temperature, salinity, oxygen tension and the availability of nutrients.

Peak settlement of the bivalve Modiolus sp. was during the pre-monsoon and post-monsoon periods similar to that of barnacles. Musculista arcuatula settled densely during the post-monsoon, while Modiolus carvolhoi and M. plumicens preferred high saline periods for their dense settlement at Neendakara Port (Dharmaraj & Nair, 1981). Polychaetes also followed a similar pattern as that of bivalves. They had a peak in the pre-monsoon period and two minor peaks in October and December. These are very sensitive to low salinity and disappeared during the monsoon period. Polychaete fouling was varied and pronounced during the post-monsoon owing to the combined settlement of both estuarine and marine forms at Neendakara Port (Dharmaraj & Nair, 1981). At Mangalore, polychaetes were absent during June-July and August at both stations investigated by Menon et al. (1977).

The correlation coefficient with salinity and number of animals settled were worked out for the groups which were absent from the fouling community in the low saline period. The total number of animals on the sets of one month panels was significantly correlated to salinity changes in the case of mud-tube dwelling polychaetes (Polydora sp.), r=0.7371 (significant at 1% level, sea anemones, r=0.884 (significant at 1% level), calcareous-tube dwelling polychaetes r=0.7513 (signifi-

cant at 1% level). In the case of barnacles and Modiolus sp. also the r-values were significant at 5% level (r=0.524 and r=0.4786 respectively).

It is clear from the foregoing account that salinity is the determining factor in the incidence of fouling animals at Cochin Harbour. So there is no true seasonal succession in the settlement of foulers in this area as seasons are not well marked here. What is happening here is only an ecological succession as has been observed by Antony Raja (1959) in the Madras Harbour. Gunter (1950), Ladd (1951) and Parker (1959) have shown that the distribution of invertebrates in estuaries and hypersaline lagoons shows definite patterns when related to salinity. Day et al. (1952) presented a bar graph showing clearly the decline of species numbers with the fall in salinity in a South African estuary. Marked lowering of salinity is known to affect the sedentary organisms leading to a decrease in their number (Edmondson & Ingram, 1939; Mc Dougall, 1943 and Weiss, 1948). During the present investigation in the Cochin Harbour the salinity fluctuated between 0.93‰ to 33.75‰. The rough salinity climate characterised estuaries physiologically as stress habitats which strongly challenge their inhabitants as well as potential new immigrants from sea or fresh water (Kinne, 1966). The most obvious basic characteristics of estuaries are the increased gradients and fluctuations of environmental factor intensities relative to the more stable situation in the neighbouring sea and freshwater areas where salinity turns out to be the 'ecological master factor'.

Acknowledgements:

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Ecologie des Salissures au port Cochin

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Une étude complète des organismes des salissures est faite en Inde, au port Cochin pendant une période d'un an. Hydroids, bryozoans, polychaetes barnacles et bivalves sont les groupes dominants rencontrés dans cette aire. L'installation mensuelle des groupes différents des salissures était relevée. Le nombre d'animaux présents dans la liste d'epreuve était assez élevé pendant la période de pré-mousson et de post-mousson et bas, pendant la période de mousson. La basse condition saline résultante du sud, le mousson de l'ouest impose des restrictions sur les autres installations continuelles des organismes des salissures au port Cochin. La fluctuation de la salinité est très marquée et la corrélation coefficie avec la salinité et le nombre d'animaux installés ont été calculés en ce qui concerne les groupes des salissures les plus importants.

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MYTILUS GALLOPROVINCIALIS (L.) IN FOULING RESEARCH.

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Résumé

La fixation de Mytilus galloprovincialis(L) sur structures artificielles immergees en mer est decrite.
Les données se rapportent à une période supérieure à dix annees de experimentations effectuées sur le fouling de la Mar Piccolo de Taranto (Italie du Sud) employant des panneaux d'asbeste de 20x30 cm immergés selon différentes méthodes. On a observé que la fixation de Mytilus galloprovincialis s'effectue uniquement sur des panneaux déjà colonises ou sur des structures filamenteuses qui peuvent fournir un support méchanique a la fixation des larves. En effet, sur des panneaux d'un seul mois d'immersion et colonisés par le seul film algal, les larves de moules étaient absentes tandis qu'elles résultaient fixées sur les panneaux immergés dans la même station mais depuis trois mois. On a observé que dans la dynamique des populations des moules présentes dans les communautés fouling, une grande importance est due, pas seulement au recrutement mais aussi à la mortalité due surtout , dans ce cas, au détachement des individus plus gros à cause des mouvements des vagues. Cela détermine, naturellement, des aspects différents dans les faciès qui se retrouvent après une année d'immersion, sur les panneaux fouling selon la saison dans laquelle on effectue l'observation. Le recrutement se vérifie, en effet, surtout dans l'hiver-printemps, la plus importante croissance des individus arrive en été et le détachement en général en automne.

Abstract

The settlement of Mytilus galloprovincialis on artificial structures is described. The data discussed refer to more than 10 years of experiments carried out on fouling in the Mar Piccolo of Taranto(Southern Italy), using 20x30 cm asbestos panels immersed following different methods.It has been observed that settlement of Mytilus galloprovincialis takes place only on panels which are already well colonized, or on filamentous structures wich can supply a mechanical support for the larvae settlement. In fact, no mussel larvae developed on panels immersed for 1 month, colonized only by an algal film, while mussel

larvae settlements on panels immersed for at least 3 months in the same place. It was observed that in the dynamics of mussel populations present in fouling communities, an important role can be played not only by recruitment, but also by the mortality rate of individuals. The latter phenomenon must be due mainly to the detachment of the larger individuals caused by hydrodynamic factors. Obviously, this, determines the different terminal facies found after a year's immersion on fouling panels in relation to the season when the observations were made.In fact, recruitment, occurs mainly in winter and spring, maximum individual growth takes place in summer, whereas detachment generally takes place in autumn.

Introduction

The mussel (Mytilus galloprovincialis) is a fairly common species of the fouling communities which can be found settled on artificial substrata and which can often cause deterioration of mechanical equipment such as the keels of boats, aspiration tubes etc. This species is present in nearly all the Italian coastal regions with rare exceptions such as the area around Palermo (RIGGIO, 1979). It can be found in great quantities, especially in some particular environments such as eutrophic areas, where, from a purely ecological point of view, it represent a species characterized by fouling communities (RELINI et al., 1976; TURSI et al., 1979; GHERARDI et al., 1974; CALLAME, 1954a, 1954b; BOMBACE, 1977; ARDIZZONE et al., 1980; BARBARO et al., 1976).

Actually, the mussel represents the cardinal species of the "climax" communities found in eutrophic environments (RELINI, 1974) here it forms mussel beds which are often exploited by man for alimentary purposes, and which are the sourse of several hygienic and sanitary problems (see, for example, the Italian law n. 192 of 1977). The mussel can also colonize some man-made structures immersed in certain environments, thus "underwater mussel-culture" may develop (BOMBACE, 1982), and settle itself even in the open sea, where hydrodynamic effects often make traditional mussel culture impossible.

The data reported in the present work were obtained during more than ten years of experimentation on fouling, carried out in the "Mar Piccolo" of Taranto (Southern Italy) where mussels represent a widely spread species both on hard natural substrata and on artificial substrata.

Materials and methods.

The data reported here refer to many experiments carried out in the Mar Piccolo of Taranto, at the station called Buffoluto (GHERARDI, 1973; GHERARDI et al., 1974; TURSI et al., 1977; TURSI et al., 1979; TURSI et al., 1982; TURSI et al., 1982).

The settlement of various fouling organisms, including Mytilus galloprovincialis, on asbestos panels (20x30 cm), immersed in different ways and for different periods of time, in relation to the set objectives, were studied. In some of the experiments, series of panels were used; some of which were immersed for only one month, others for three, others for six and still others for a whole year. The panels used were 19 (12 x 1 month; 4 x 3 months; 2 x 6 months and 1 x one year= 19) and they were positioned in particular ways (horizontally, vertically, and obliquely) in order to study the influence of the positioning factor on the settlement and development of the fouling communities (TURSI et al., 1977).

A recent study (TURSI et al., 1982) on the mussel development on artificial

structures, immersed for nine years in the same area as the Mar Piccolo, has established both biomass and density in relation to depth. Another experiment, carried out in 1980-82 (TURSI et al., 1982) analysed the settlement of mussels on panels immersed for twelve months, but whith the immersion beginning in different seasons (summer, autumn, winter and spring). In order to study the effect of depth, as well as of season on the development of Mytilus galloprovincialis for this experiment ten panels (20x30 cm) were used for each of the four seasonal series.These panels were chained together so that they formed a transect 20 x 300 cm long. In addition, at monthly periods, a chain made up of 10 panels tied together, up to a total of 120 panels,was immersed in order to study the larval settlement, month by month, on virgin panels.

Finally, in January 1983, twelve series, also of ten panels each, were immersed, and then each of them was removed at monthly periods, so as to study the dynamics of mussel settlement on artificial structures of varying age.

Results.

a - Settlement

An extremely interesting aspect characterizes the settlement of Mytilus galloprovincialis on artificial panels immersed during the various years of experimentation.The first result obtained was that although their larvae were present in the sea and they settled themselves at the same time on other types of artificial structures, the mussels never settled on panels immersed for a period of only one month. This reveals a rather curious characteristic of the mussels,which in order to settle themselves, need already colonized hard substrata, or else both natural and artificial filamentous substrata (BAYNE, 1976). In fact, young barely settled mussels were never found on panels immersed for monthly periods since their larval fixation in the seas of Taranto occurs in the winter-spring period, when fouling, after thirty days of immersion, is characterized by a simple algal film of diatoms which is unsuitable, as receptacle for the mussel veliger.

However, during the same period, the larvae settled themselves on fouling panels immersed for a longer interval (from three months onward) so that there was a benthic association of a different level of maturity already present on them. This was nearly always represented by hydroids and ascidians, therefore, the influence of a pre-existing biological association on the larvae's settlement, is obvious. In another series of experiments (not yet published and still partially under way), the settlement of mussel larvae on artificial collectors made up of vegetal fibres is being studied. These are usually used in local mussel culture to gather the larvae, and are immersed for only thirty days in the same manner as the panels immersed for monthly periods. Thus it has been observed that these ropes can play an important part in the settlement of mussel larvae, even if the latter are deprived of a biological substrata, probably due to their filamentous nature, which seems to attract the mussels (BAYNE, 1976). The density of the larval settlement differed notably in relation to time of immersion. The spring months yielded the best results, whith maximum mussel settlement.

On the basis of the results obtained from the experiment carried out from January 1983 onwards, when the development of the fouling community in the Mar Piccolo of Taranto was observed at monthly intervals, it was also noted that the first mussel larvae appeared on panels from March onwards and contin-

ued to settle themselves until the beginning of summer, after which nearly all settlement was completed.

As far as the effect of substratum inclination on the mussel settlement is concerned, it was observed that it had little effect on the initial phase of colonization but it played a significant role during the mussels' growth-period (due to the hydrodynamic effects discussed later).

The F values obtained from the ANOVA carried out on data related to settlement on panels immersed at varying angles were not significant.

As for the depth-gradient, studied from 0 - 3 m, we observed that the larvae settled themselves along the whole transect but that they were numerically more dense on the top levels due to reduced competition between species.

These results have also been confirmed in other Italian regions where Mytilus galloprovincialis has never been found on panels immersed for a month (Manfredonia, Orbetello, Ravenna, Crotone, Venezia, etc.) and has shown a predominantly spring cycle of settlement, except for the Ravenna region where the effect of the waters from the River Po can cause a second settlement-period during October and November (Relini et al., 1976).

b - Development

By means of experiments carried out in the "Mar Piccolo" of Taranto it was also possible to calculate the density and development of the mussels on fouling panels. It was found that the density of individuals on artificial substratum depends on factors such as the duration of the immersion period and the season, both of which play an important role. In fact, larvae settlement increases proportionally in relation to the increase in the period of panel-immersion. The latter factor in turn increases population density. This, however, takes place up to when they are recruited (therefore until the summer), after which the individual density remains more or less constant. Actually, on hard artificial substrata, such as fouling panels, this density can even diminish in the course of one year because of the mussel detachment brought abaut by the combined action of hydrodynamic effects and of their biomass (expressed as g/cm^2) which often as too high with respect to the larvae capacity to stay fixed onto the substratum. However, it is obvious that the seasons, as well as alternating recruitment of young specimens and detachment of large individuals, play an essential role on the numerical density of the mussels present on panels immersed for long periods of time.

In the experiment carried out in 1980 - 1982 (TURSI et al., 1982) it was noted that, beginning in spring or summer, after one year of immersion, it was possible to find high individual-density levels (more than 2 - 3 individuals per cm^2). The latter were almost always young specimens fixed in the period immediately preceding settlement. Vice-versa, on panels immersed in autumn and winter, and sampled after one year, a rather scarce mussel population was found (only a few dozen individuals on 600 cm^2) so that the individuals settled in the following spring at the beginning of the immersion period grew considerably during the summer, detaching themselves immediately after, for the above-mentioned reasons. Though at different time-intervals this phenomenon was also noted on natural beds (TURSI et al., 1983 in press) so that the individual density can vary during the seasons and during the years, in relation to natural events. Dense mussel populations develop in nearly all the afore-mentioned Italian coastal regions. In particular, high densities are to be found especially at Ravenna (RELINI et al., 1976) and Ancona (BOMBACE, 1982) due to the eutrophic conditions of the Adriatic Sea, and in the Tyrrhenian Sea, near the mouth of the Tiber

River (ARDIZZONE et al., 1980). In relation to the years these studies were carried aut a considerable alternation was noted in the individual mussel-density.

Estimates made in the Mar Piccolo of Taranto of yields of artificial metal structures immersed for nine consecutive years during the winter period (TURSI et al., 1982) showed that the density can reach 30 Kg/m^2, especially in the first few meters, where biological productivity guarantees survival and development.

The mussels' growth on fouling structures is very similar to that found on natural beds. It obviously depends on the season, wile the individuals' average dimensions depend on the period of observation.

In fact, on panels which remained immersed for twelve months from autumn onwards, the individuals' average length was about 26 mm, while those immersed from spring onwards, were notably smaller (approx. 4-5 mm). For the most part, These represented the new generation (the so-called "seed"). The size of mussels found settled on panels immersed for 12 months from summer onwards (they had an average size of 13+3 mm), was slightly smaller than those found in autumn. This average annual summer value has been confirmed in several analogous experiments carried out during several years. As far as the size of mussels found on three- and six-monthly panels is concerned an average value of 3.5+0,7 mm (April-June) and of 7,26+2,19 mm (December-June) respectively, was calculated.

It has been noted that the inclination factor of has little effect on the mussels' growth, these seem to have no preference, but appear to detach themselves more easily from the lower surface of the horizontal panels observed, and even more so from vertical surfaces, while they resist the hydrodynamic effects of the sea better if placed on oblique surfaces on which the tides have a lesser destructive effect.

As shown in Fig. 1, the average size of the mussels settled on artificial substrata, can be influenced by depth, where the variations in average length is shown graphically in relation to depth. It can be noted that mussels, on panels immersed for one year are larger, especially in the first 150 cm, after which they become progressively smaller as they reach the bottom where larger individuals can be found (which therefore raise the average value) which are, most probably, individuals fallen from above and successively fixed there, through the byssus, on hard substrata, so as to avoid death by burial in the substratum can be found. This phenomenon, observed directly on immersion, is found when one studies the structure of the mussel population and it can cause quite a lot of confusion.

The indvidual sizes can obviously vary because of particular environmental conditions in relation to locality but, on the basis of existing literature on this topic, it is possible to ascertain growth values such as those cited above, as average length values of mussels on panels immersed for one year. Furthermore the largest increase in length (and weight) of the mussel takes place in the second year rather than in the first (BOMBACE 1982).

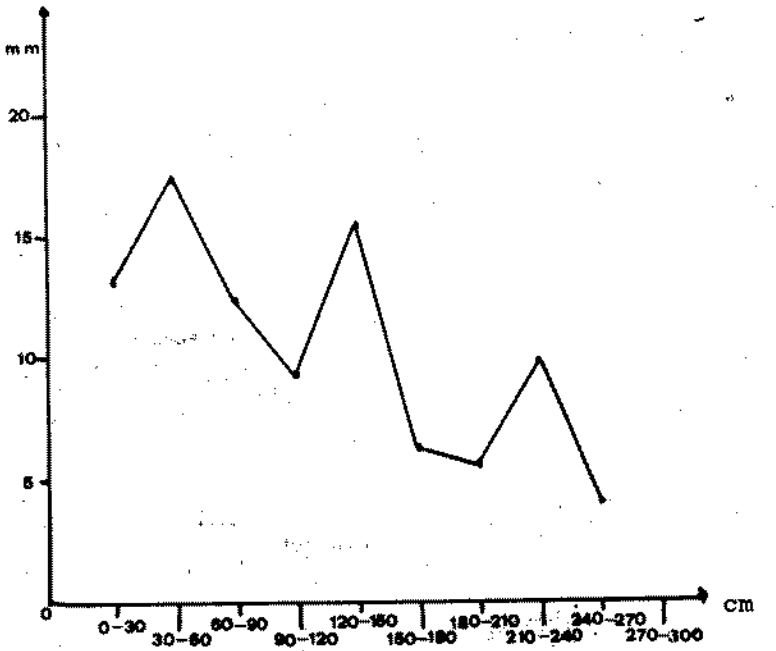

Fig. 1 - Average lengths of mussels settled on artificial substrata, in relation to depth.

Conclusion

From the data reported it is possible to draw some important conclusions regarding the mussel in fouling communities. Above all it has been observed that the mussel population, representing the final facies found on fouling panels after a prolonged immersion of one year, can vary considerably in relation to the seasons. In fact, according to the seasons, individuals can vary in density because of the new recruitment or the detachment of a part of the preceding generation. These events can determine notable variations also in average size and in the relative biomass of the mussels found fixed on the panels.

However in eutrophic environments, Mytilus galloprovincialis is the species which characterizes benthic associations present on fouling panels, not only in the Mar Piccolo of Taranto but also in other Italian areas, as can be seen in the literature cited. Another interesting fact which results from these studies is that the mussel larvae have a precise period of recruitment, operate a selective choice as to the substrata avoiding smooth surfaces and prefere filamentous and/or preconstructed biological substratum surfaces. It therefore follows that artificial structures, characterized by different structures (smooth or filamentous), immersed in the sea at the same time and for the same length of time, can present a notably different mussel density. Also, structures which present, different biological associations (e.g., only algal film or Serpulids' association)

at the time of the mussels' recruitment due to the different periods of immersion, present completely different facies concerning above all the presence of Mytilus galloprovincialis after a few months. Thus, a period of over 18 months is necessary , in order to obtain similar association structures (TURSI et al., 1982).

In conclusion, it can be observed that, although the basin of the Mar Piccolo of Taranto is highly suitable for the growth and development of mussels, the dimensions which these reach on artificial panels is still inferior to that which they reach in the same period of time in mussel cultures where a reduction in the number of individuals has been effected in order to permit optimal growth.

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BALANUS LARVAE (CIRRIPEDIA) IN GENOA HARBOUR

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ABSTRACT

This paper refers the results of the observation on fouling larvae, in particular of Balanus (Cirripedia) in a station located in the outer Harbour of Genoa between 1979 and 1982, as well as in four other stations in the same port (1981). Fouling larvae distribution shows a higher concentration in inner harbour areas where nutrients are also more concentrated ($N-NO_3$; $P-PO_4$), water movement is less and interaction with the open sea is more limited. The increasing number of Balanus larvae in june is followed by an increase of settlement, recording two peaks corresponding to the main larvae concentration rates of the last three nauplius' stages. An analysis of the Balanus larvae ratios in the port of Genoa (Balanus amphitrite, B. perforatus, B. trigonus and B. eburneus) has been made separating fourth, fifth and sixth naupliar stage. It was also found that Balanus amphitrite have the highest larval concentration with as much as 86% in average.

RESUME

Dans ce travail sont présentés les résultats des observations sur les larves de la salissure, en particulier de celles de Balanus (Cirripedia), effectués dans une station située dans l'avantport de Gênes et dans quatre autres stations du même Port (1979-1982). La distribution des larves de la salissure est plus abondante dans les stations les plus intérieures du Port, en correspondence des concentrations les plus elevées des sels nutritifs ($N-NO_3$; $P-PO_4$), d'un moindre hidrodynamisme et rapport avec la mer ouverte. L'accroissement du nombre des larves de Balanus pendant le

mois de juin est suivie par un augmentation de l'implantation, donnant lieu a deux maxima en correspondence des deux valeurs maxima principales de la concentration des larves dans les trois derniers stades des nauplius. En séparant le IV^{me}, V^{me} et le VI^{me} stade larvale, une analyse des rapports entre les larves relevées dans le Port de Gênes (Balanus amphitrite, B. perforatus, B. trigonus at B. eburneus) a eté aussi effectuée. En particulier on a trouvée que les larves de Balanus amphitrite sont les plus abondantes avec un percentage moyen de 86%.

Foreword

Various harbour environments in the Mediterranean area have been studied concerning fouling, especially along the Italian peninsular coasts where data are available on the harbours of Trieste, Venice, Ancona, Bari, Taranto, Catania Palermo, Ischia, Civitavecchia, La Spezia, Genova, Savona, Imperia and other yachting Ports and Marinas of the West. Ligurian Riviera (Relini, 1980 a). Studies on fouling and barnacle settlement on hard substrata were also made in some areas of the Po delta (Matricardi et al., 1980; Relini, 1980 b), of the Orbetello Lagoon and in coastal and open Mediterranean waters near Ravenna, Crotone, Fiumicino, Punta del Mesco, Riva Trigoso and Portofino (Relini, 1980 c).

Concerning Genoa harbour, fouling was studied continuously as far back as the 1960s by Relini and coll.

This wealth of studies on settlement is however lacking adeguate work on larval stages of fouling organisms in the Mediterranean Basin.lack already revealed by Le Reste (1965) in his paper on cirripedes larvae. So far, a few studies were made on larvae concentration in meroplankton (Picone-Zunini Sertorio, 1976; Zunini Sertorio 1980) but except for a previous note on Genoa harbour by Geraci and Romairone(1982 a, b), no work has been done to identify the various cirripedes species, in particular on Balanus, at various larval stages or to establish relationships between larvae and settlement.

This paper wants to give a contribution to this problem, while taking into account the studies already made in other countries (Bassindale,1936; Pyefinch, 1948 a, b,1949; Sandison,1951; Norris and Crisp, 1953; Costlow and Bookout, 1957,1958; Barnes and Costlow, 1961; Lang 1979, 1981; Schel tema and Williams, 1982). Furthermore, the importance of cirripedes larvae as fish nourishment should not be underestimated, so that they may prove very useful for aquaculture. This is the reason why our studies were started on cirripedes larvae, although due consideration was also given to the larvae of other fouling organisms.

Barnacle larvae in Genoa harbour

Material and methods

From 1979 to 1982, a study was made in the Genoa harbour on the existing relationship between Balanus larvae concentration and their settlement on artificial substrata (Asbestos panels 300 X 200 X 3 mm) vertically suspended at a depth of 2, 4, 6, 8 m under a raft owned by ' Istituto per la Corrosione Marina dei Metalli, C.N.R.', moored in the outer harbour of Genoa, above the 11 m deep sea floor. Every fortnight (and every week in 1982), these panels were replaced and barnacle counts were made with utilization of all barnacles having a rostro-carinal basal diameter of less than 1 mm for larvae correlation purpose. These larvae were hauled up vertically from the sea floor with a 100 µm plankton net at a speed of 0,5 m/s. Each vertical haul filtered about one cubic meter of sea water. At the same time, it was deemed advisable to determine some hydrologic data (such as $N\text{-}NO_3$, $N\text{-}NO_2$, $P\text{-}PO_4$, temperature, dissolved oxigen and water transparency).

Further plankton samples were taken in 1981 on stations located in different spots of the harbour to asses how distance from the open sea, eutrophy and sheltered waters and their isolation might affect the qualitative and quantitative selection of cirripedes larvae populations. For this reason, five equally spaced stations were established between the port entrance and the most internal harbour zones; where vertical hauls were made according to the same methodology for sampling under our raft.

The study of the weekly collected samples between may and november 1982 was not only limited to the quantitative relationship between different Balanus species in the port area, but was also extended to a comparison between later larval stages (IV, V and VI nauplius) as well as between the latter and settlement. The cyprid stage was not correlated to settlement for the very low amount of organisms at this stage due to the drastic decrease from the earlier stages.

Balanus larvae distribution

The average concentration rates of Balanus larvae collected during plankton sampling at fortnight intervals in 1981 in the five Genoa Harbour stations are reported in fig.1 together with the hydrologic parameters values taken into account and averaged over the whole sampling period. An observation of the fouling larvae and of the hydrological data shows similar situation at the more internal stations (1 and 2) where the highest nutrient values together with the lowest dissolved oxigen and highly reduced water movement were found. Larval populations, clearly differ by composition and concentration from those observed in the sta-

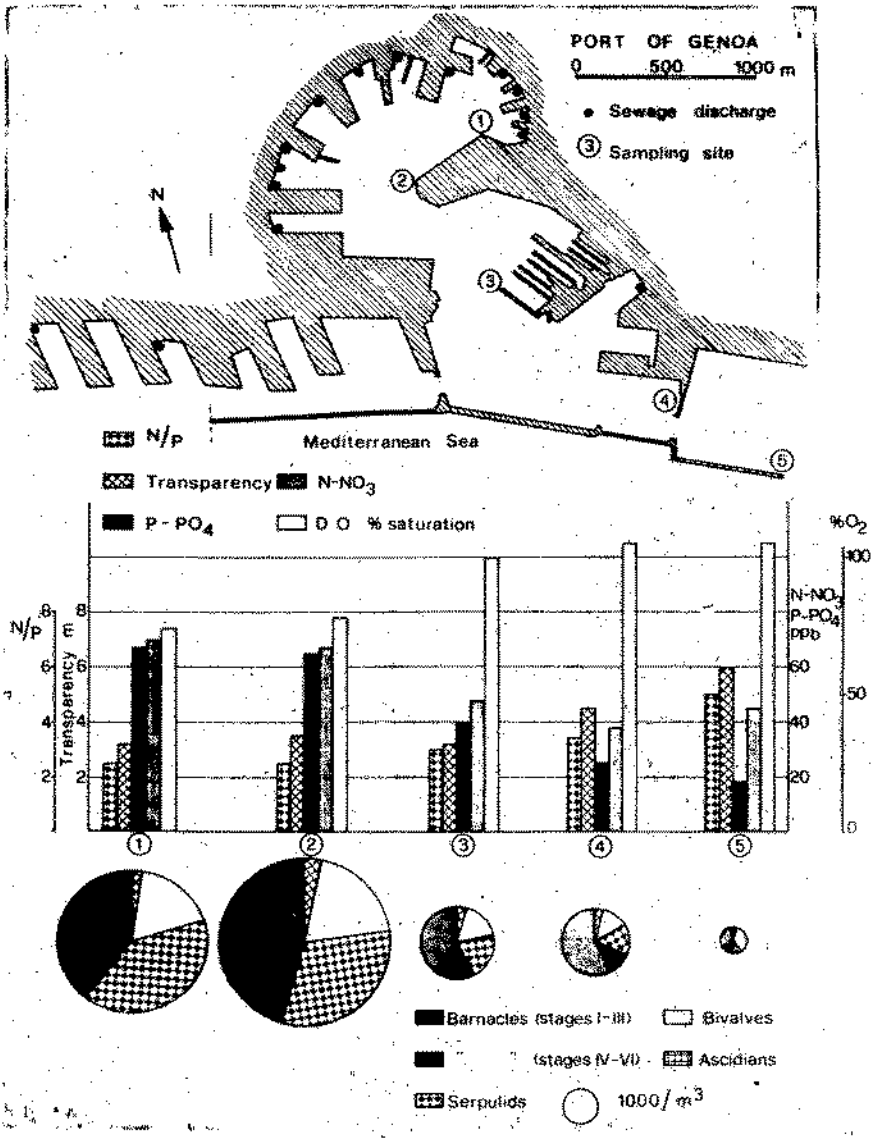

Fig. 1 - 1981: A) Location of stations and sewer headers discharges into the Genoa Port. B) Mean values of some hydrologyc data and of larval population for the single stations.

tions 3 and 4 where they were much less in number. This difference is even greater in the outer station 5, showing not only a smaller amount of larvae, but also a much lower soluble phosphate content of probable domestic origin. This in turn affects the N/P ratio which increases though still remaining below the open sea values. This is quite in agreement with the data of Picone and Zunini Sertorio (1976) regarding cirripedes larvae as well as some considerations by Fabiano et al.(1976) regarding nutrients. The data show that the water discharged through the various sewer headers into the 'Porto Vecchio' basin causes eutrophycation of these waters and the development of the larvae populations such as to affect fouling composition in this environment. Outside this basin (station 3 and 4), fouling larvae are differently influenced, either as a result of a dilution of town sewage water or due to an increased vivification of open sea waters flowing into the inner harbour through the east entrance of the port. Topographically, as well as regarding larval population, station 5 marks the boundary between the port environment and coastal waters.

Finally, it should be observed that serpulids and barnacles are much more often found in the inner zones and that the former decrease much faster when nearing the open sea.

Relationship between larvae and settlement

The data covering three years of observations regarding larvae concentrations and barnacle settlements in the outer harbour (station 4) obtained from fortnightly hauls and panels are reported in fig. 2. These data were obtained through averaging of the concentration values of all larvae belonging to stage IV, V and VI as well as of the settlement density of each sample with respect to the values of the previous and next sample, thus obtaining general trend curves. Late naupliar stages peaks were observed in all three years around june-july, when maximum settlement occurs. The first spring-summer peak is followed by a less marked second peak in september-october, thus confirming the presence of two main reproduction periods (Geraci-Romairone, 1982 a). Settlement occurs soon after the appearance of later larval stages when the average water column temperature is higher than 19°C. On the other hand, early larval stages (I,II and III) are found even earlier and a small number of these exist during the whole year (Geraci and Romairone, 1982 b).

Over the three considered years the variation in time of the density of larvae in the water column and of the settlement rate of barnacles on the panels shows a high correlation (r=0.93) only in the first settlement period (May-

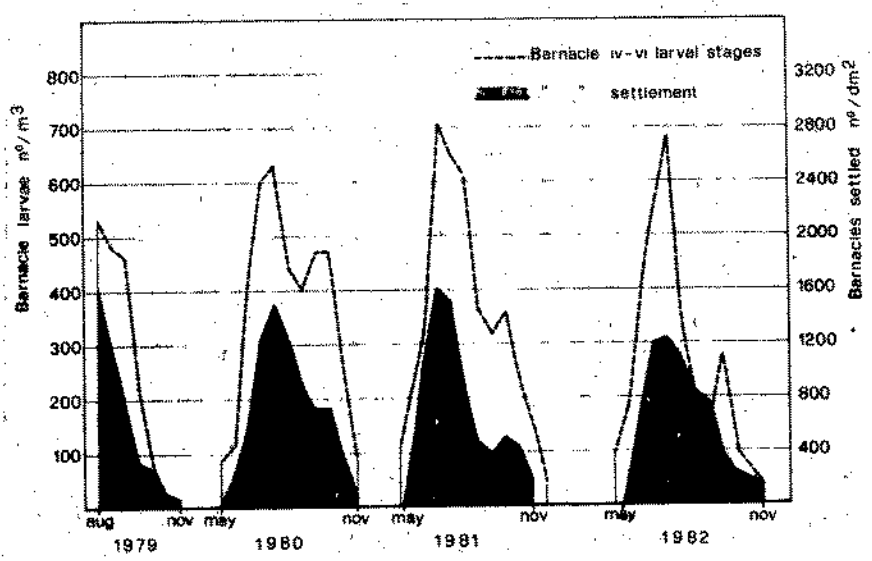

Fig. 2 – General trend curves of both concentration of last three naupliar stages and density of settled organisms (fortnightly panels) of Balanus in the outer harbour of Genoa (station 4).

june). After this period, such a high correlation between larvae and settled barnacles is no longer observed.

Cirripedes larvae of Balanus perforatus and B. trigonus reared in the laboratory, together with literature informations and the examination of the larvae collected from plankton hauls at weekly intervals from may to november 1982, made it possible to separate the larval stages IV, V and VI and to differentiate the four Balanus species that are found in the Genoa harbour at larval level, i.e. Balanus amphitrite, B. perforatus, B. trigonus, B. eburneus (table I).

When considering the percentage values reached by all larvae of the various species at stages IV, V and VI (fig. 3), the absolute predominance of Balanus amphitrite with an average 86,6% is immediately noticed. The other three species, i.e. Balanus perforatus, B. trigonus and B. eburneus are in average present at the rate of 5,6; 4,8 and 3,9% respectively, although their reciprocal ratio may vary from one period to another due to drastic cyclic lowering of the Balanus amphitrite quantities. These values are in agreement with those recorded by Relini and Giordano

	MAY		JUNE					JULY				AUGUST					SEPTEMBER				OCTOBER				NOV.
	18	24	1	8	15	22	29	6	13	20	27	3	10	17	24	31	7	14	21	28	5	12	19	26	2
Stages										*Balanus amphitrite*															
IV	56	28	76	116	92	386	78	320	276	48	60	126	30	4	34	242	116	40	6	52	48		8	24	8
V	30	8	48	90	86	272	22	192	202	52	42	64	32	6	6	142	100	36	6	4	38			8	8
VI	12	2	12	70	90	128	50	80	104	64	26	60	18	8		58	84	4			28				
Total	98	38	136	276	268	786	150	592	582	156	128	250	80	18	40	442	300	80	12	56	114	—	8	32	16
Stages										*Balanus perforatus*															
IV		2	2	22	10	6	14	60	2	2	12	16	38	18		2			2				4		
V	4		2	14	4	8		8	2	2	2	6	16	8											
VI			4	4							2	4	4												
Total	4	2	8	40	14	14	14	68	4	4	16	26	58	24	—	2	—	—	2	—	—	—	4	—	—
Stages										*Balanus trigonus*															
IV	4		8	26	4	26	44	8		4	22	8	2	8					12		2				
V			6	4		2	22	4	2		6	8	4	12											
VI			2				2				4	4													
Total	4	—	16	30	4	28	68	12	2	4	32	20	6	20	—	—	—	—	12	—	2	—	—	—	—
Stages										*Balanus eburneus*															
IV		2		4	4	6	2	36		4	2		4		2	2	8		2					4	
V		2	2	2		2		12	2		4		4	2		2									
VI							6	16	2	8		2				2	4								
Total	—	4	2	6	4	8	8	64	4	12	6	2	8	2	2	6	12	—	2	—	—	—	—	4	—

Table I – Amount of IV, V and VI larval stages of the four Balanus species for single samples.

(1969) regarding four years of Balanus amphitrite settlements amounting in the port of Genoa to 83% of all Balanus populations. They are however, not in accordance with the B. perforatus settlement ratio, only representing 0,3%. It should be pointed out that B. perforatus, like B. trigonus is a species frequently found in less eutrophic and more moved waters. It is therefore likely that this extra-harbour species suffers a further decrease of its population during settlement in this environment.

Table I shows more generally a strong reduction of larvae number from stage IV to stage VI. Not more than $4/m^3$ B. perforatus and B. trigonus stage VI larvae were found in the plankton hauls, while B. eburneus, like B. amphitrite, were recorded in a relatively higher number in the same stage, thus confirming their better adaptability in euthophic and eurialine environment.

An analysis of the total barnacles larvae population of all six nauplius stages shows weekly density values ranging from a minimum of $796/m^3$ to a maximum of $7866/m^3$; these values were respectively recorded during sampling at the end of may and early july. The average reduction over the whole period taken into consideration between the first

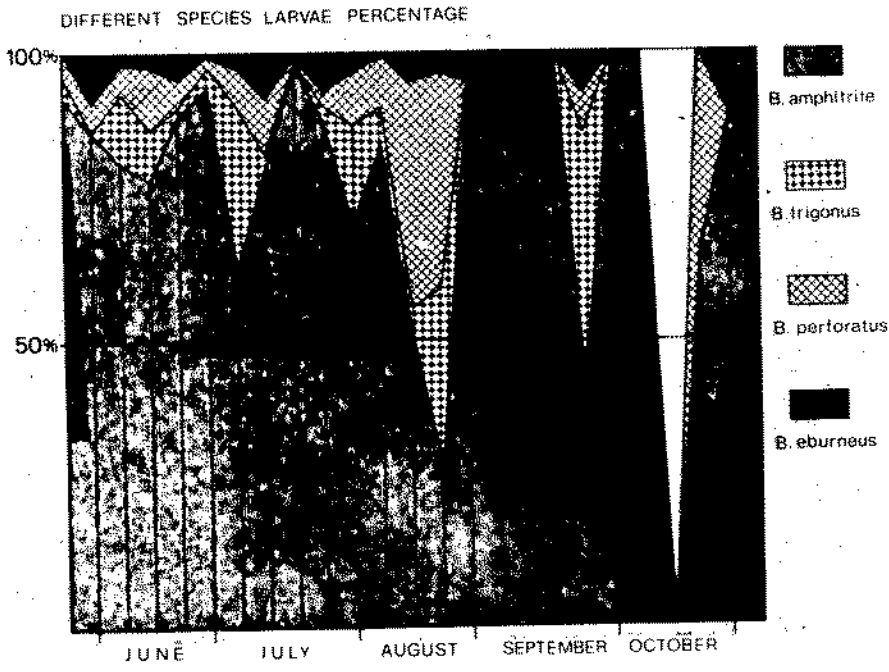

Fig. 3 - Larval (IV, V, VI stages) percent ratio of the four Balanus species in the outer harbour of Genoa (June-October 1982).

three stages (table II) and the VI stage is about 98%. When specifically considering the average percentage reduction between stage IV and V as well as between stage V and VI, we find the values 37,5% and 44,5%.

MAY	18	800	JUNE	1	910	JULY	6	7090	AUGUST	3	1005	SEPTEMBER	7	855	OCTOBER	5	695
	24	650		8	2755		13	4815		10	1520		14	2800		12	855
				15	2225		20	2350		17	2155		21	1690			
				22	3315		27	2000		24	1290		28	3460			
				29	4435					31	2695						

Tab. II - Total amount of the first three larval stages of Balanus for single sample.

The concentration curves of each single later larval stages have a similar trend, with maximum values in the june-july period (fig. 4), indicating a massive emission and optimum larval development conditions. A relative decrease, in the middle of this period, indicate global maturation of the whole population towards more advanced stages and a massive settlement (fig. 5). A partial reduction of reproductive activities and the subsequent increase seem to oc-

cur late july and early august, whereas two more larval peaks are observed at the end of august-early september and late september. It therefore seems that we can speak of successive larvae waves. These waves are difficult to notice during the initial period in which most adult barnacles expel larvae.

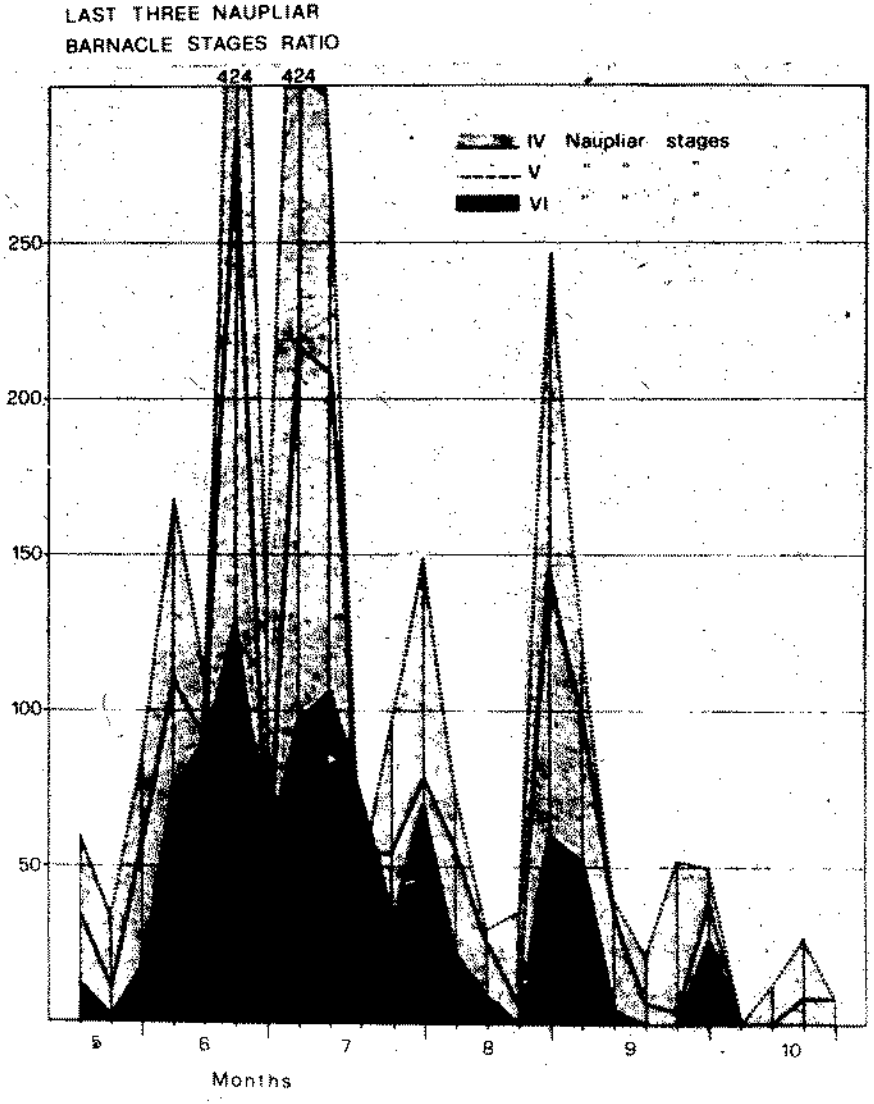

Fig. 4 - Last three naupliar stages of Balanus density from may to October 1982 (weekly samples).

Subsequently however, each wawe is interrupted by longer rest periods, as in mid-august and mid-september when low larvae concentration figures in all three later naupliar stages were found for three weeks on end. No stage VI larvae were found in october.

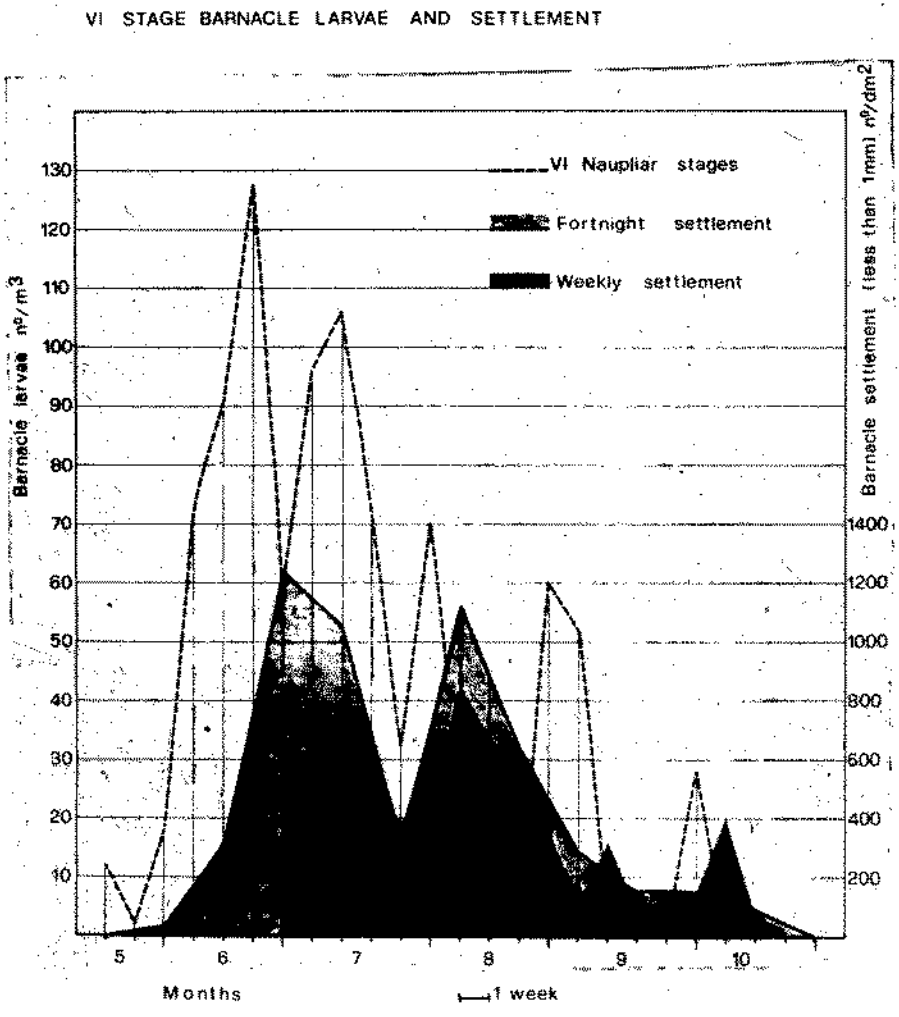

Fig. 5 – VI stage larval density of Balanus and settlement on fortnightly and weekly panels.

Barnacle larvae in Genoa harbour

The study of the relationship between the VI nauplius stage and settled barnacles (fig.5) shows two settlement peaks determined on fortnight panels at the end of june and early august. When considering weekly analysed panels, we find at least four settlement peaks, the same number as of the stage VI larvae.

On this subject it should be noted that a peak of larvae have no numerical comparison value of settlements on the individual panels. The plankton haul is a punctiform datum, whereas the settled panel is representing the integral of everything that happened during the period in question and it has therefore an inertia value vis-a-vis sudden variations in the larval population concentration rate. We can, however, observe that each larvae peak is corresponding to one or two weeks dephased settlement peak. This time stagger is responsible for the lack of correlation between larvae concentration and settlements determined on each sample after the first important initial settlement. As already explained before, larvae generations follow one another but more frequently in spring, then to slow down gradually until autumn. This inertia and competition with other sessile organisms, especially serpulids abounding towards the end of the summer in Genoa harbour (Geraci and Romairone, 1982 a), are probably the main causes for absence of some settlemnt peaks on the panels drown up at fortnight intervals. Larvae density at the initial stage too has an important rôle, especially when the larvae life-span increases as the temperature drops: if no adeguate number of early stage larvae is available, their probability of reaching cyprid stage and settlement are proportionally reduced.

Conclusion

To conclude this analysis of cirripedes larvae in the port of Genoa, we can affirm that most of these larvae found in the outer harbour are belonging to the Balanus genus (averaging 2610 ind/m^3 between may and october 1982) with the following species: Balanus amphitrite, B. perforatus, B. trigonus and B. eburneus.

As to the relationship existing between Balanus larvae and settlement in the outer harbour, later larval stages peaks were observed in 1982 corresponding to as many settlement peaks of which only the first two (june end-early july and august) had a considerable development and were determinant for fouling composition.

As to the existing relationship between nauplius stages, an average reduction of about 98% was observed during the whole period under examination between the first three and the last nauplius stage.

Balanus amphitrite is prevailing by as much as 86% on

all Balanus larvae examined in our study. This species has also the largest numbers of individuals settled on short-term immersed panels during the spring-summer period. The disappearance in autumn of Balanus amphitrite seems to indicate a very fast biological cycle. The rapid succession of several generations between spring and summer of the same year may be confirmed by an examination of the maturity of the ovigerous lamellae and the development of embryo stages during this period.

As to the larvae distribution in the port of Genoa, Balanus (in particular Balanus amphitrite) larvae find their best conditions in the inner harbour waters, characterized by high nutrient values, low oxigen content and little water movement, although serpulid larvae have a greater resistence in these more polluted zones.

A study on Balanus larvae requires, however, further investigations to confirm some hypotesis regarding relationship between larval and sessil life since this complex phenomenon may give rise to numerous biological and other interferences.

Finally, a more detailed examination of the existing relationship between all six larval stages may be interesting, since it would contribute to an evaluation of the adaptation capacity of each species to different environmental conditions in coastal, lagoon and brackish waters where these organisms are most often found in considerable concentrations.

Acknowledgements

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THREE YEARS INVESTIGATION ON MACROFOULING OF A TYRRHENIAN POWER STATION (+)

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ABSTRACT

The macrofouling of the Torvaldaliga power station (80 km north of Rome, Italy) was investigated through the use of asbestos panels and inspection of intake walls from April 1978 to May 1981).
The fouling community, presented high diversity, small biomass, slow development and after one year it showed maximum wet weight up to 50,5 g/dm^2. The Mussel community, which was the main fouling problem, developed 18-20 months after the cleaning of the walls. Antifouling treatment (hypochlorite) was required from March to September.

RESUME'

Pendant trois années (Avril 1978 - Mai 1981), les macrosalissures biologiques de la centrale thermo-électrique de Torvaldaliga (80 km au nord de Rome, Italie) on été étudiés soit au moyen des plaques expérimentales soit avec des observations directs sur les parois des conduites. La communauté fouling installée presentait une diversité specifique assez élevée, une biomasse faible, un développement lente et un maximum de poids humides de 50,5 g/dm^2 après une année d'immersion.
La communauté à Moules, qui dominait et qui presentait le principale problème de fouling dans la centrale, s'installait 18-20 mois après le nettoyage des canaux.
Le traitement anti-fouling (avec hypochlorite) était nécessaire à partir du mois de mars jusqu'à septembre.

(+) Research supported by a contract between University of Genoa and CRTN-ENEL of Milano.

INTRODUCTION.

The use of sea water in power plant cooling systems needs - as it is well known- an efficient prevention of fouling settlement. If biofouling is to be controlled in a practical yet ecologically sound manner, the biological principles governing the development and composition of a fouling community must be understood (Hillman 1977).
The settlement periods, the growth rates, the amount of fouling vary from plant to plant, so that a sound knowledge of these aspects is necessary to select the best schedule for an antifouling system considering both expense and effectiveness. In the Mediterranean region few papers have been published on this topic and most of these are concerned with the Tyrrhenian sea (Relini 1977, 1980). In this paper we have summarized the results obtained during three years investigation on fouling of Torvaldaliga electric power plant with the aim of contributing towards a better understanding of the ecology of fouling organisms and in particular to knowledge of settlement periods in order to determine the best schedule for an antifouling system, based on chlorination. In previous works dealing with Torvaldaliga fouling some systematic groups were described and preliminary data (one year's observation) were reported (Diviacco 1979, Morri 1980, Pisano 1981, Pisano and Canevello 1982, Relini and Bianchi 1979 a, b, Relini et al. 1980).

DESCRIPTION OF SITE.

The electric power station of Torvaldaliga is situated along a rocky shore on the Tyrrhenian coast at 4 km north-west from Civitavecchia and 80 km north to Rome (fig. 1). The sea water for the cooling system of four power groups (one of 200 MW and three of 320 MW) is pumped through two channels (A and B) each partialy divided into two conduits. The channel A, in which the flow rate is about 20 m^3/sec, serves groups 1 and 2 while channel B, which is longer and with a higher flow rate (30 m^3/sec), transports water to groups 3 and 4. In the conduits the water velocity is between 0,4 and 1,2 m/s, lightly higher in channel A; in channel B chlorine was added periodically.
A part of the conduits is situated underwater and protected by two warfs. Before the water enters in the condenser, its velocity is slowed by an enlarged chamber and then flushed through a rotating screen. The water is discharged into the sea later.
During three years research the following water parameters were measured: temperature in basins and outfalls was recorded every day; organic matter as chlorine demand at stations CE and CF was valued every week; salinity and nutrients ($N\text{-}NH_3$, $N\text{-}NO_2$ / NO_3, $P\text{-}PO_4$) mainly at stations CB and CE, dissolved oxygen at all stations were measured every 15 days. The flow rate was determined every day from the num-

ber of operating pumps in each channel. The temperature - range was from 11 to 26,5°C in the basins and always 10° higher at outfalls. The range of salinity was from 36 to 38°/$_{oo}$ and the values of dissolved oxygen were always higher than 6,5 ppm. Sometimes nutrients showed high values probably due to sewage or to chemicals added to sea water circulating in the plant. Seasonal changes of the main parameters are described in the above mentioned previous paper (Relini et al. 1980).

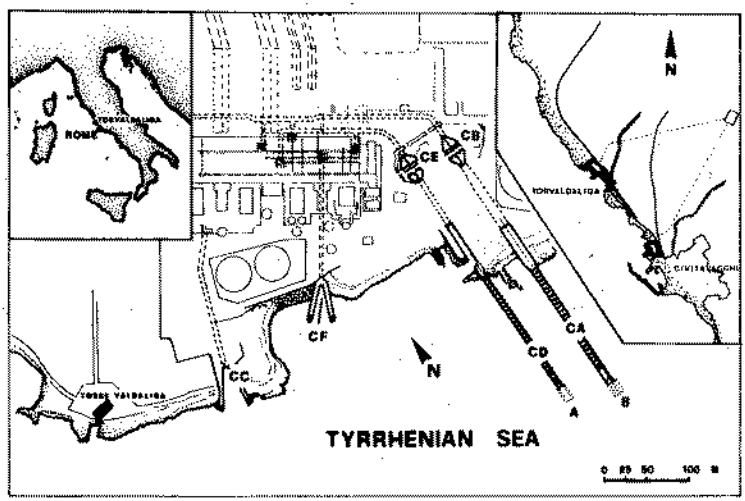


Fig. 1. Location map of Torvaldaliga power station and test sites.

MATERIALS AND METHODS.

Six sites, three in system A and three in B, were chosen for the immersion of panel sets in the following main environmental conditions:

1) Intake channels, characterized by a high water velocity and a general absence of light.
2) Basins at the end of the intakes, characterized by a lower water velocity than station A and the presence of light.
3) Discharge zones (outfalls) characterized by a high water temperature (10°C higher than sea water entering the intakes), the presence of light and high water turbulence.

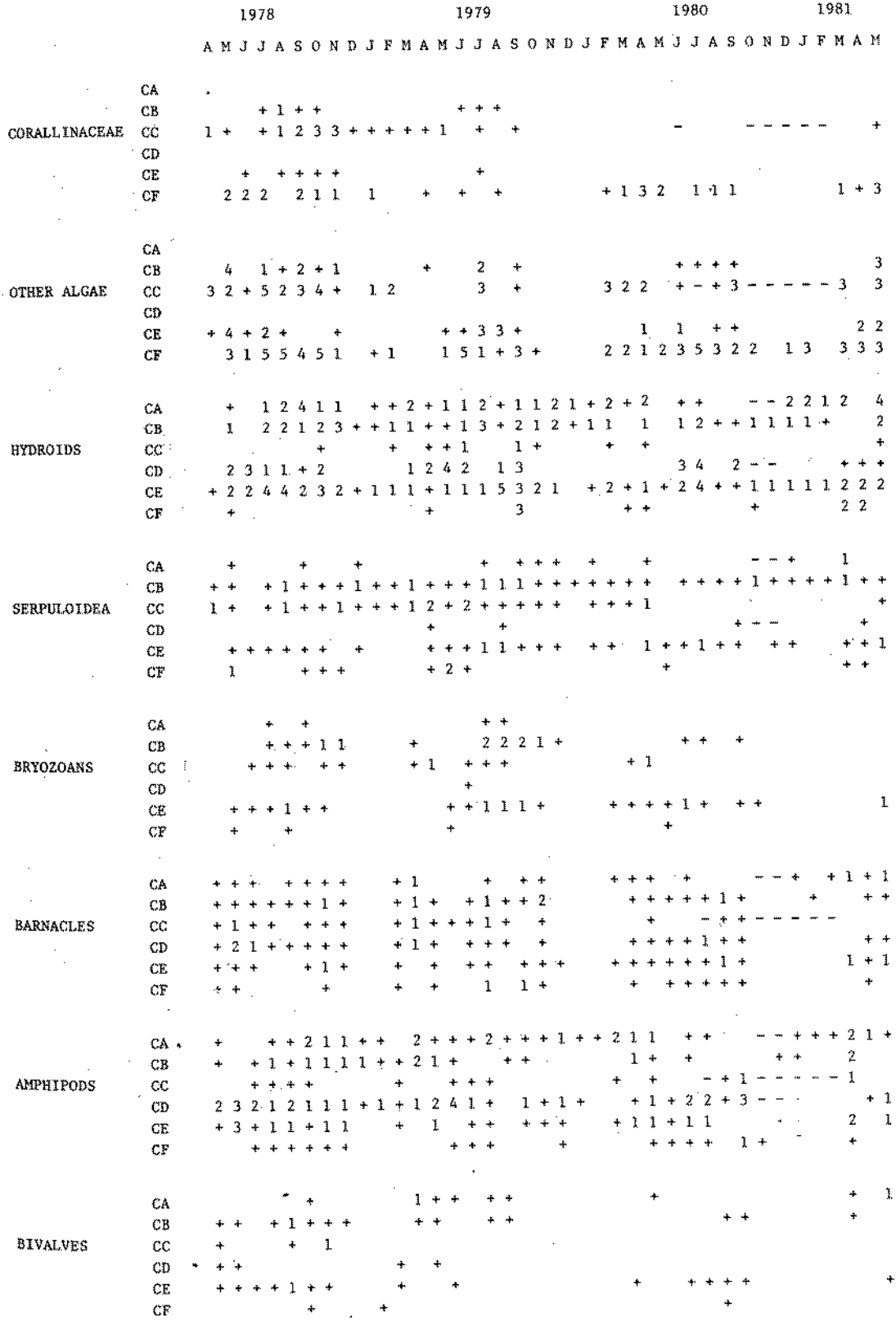
The following six stations were considered:
St. CA: intake conduits of groups 3 and 4, sometimes chlorinated
St. CB: basin of groups 3 and 4, sometimes chlorinated
St. CC: outfall of groups 3 and 4, sometimes chlorinated

St. CD: intake conduits of groups 1 and 2
St. CE: basin of groups 1 and 2
St. CF: outfall of groups 1 and 2

Research carried out over three years between April 1978 to May 1981 through the use of asbestos panels (200X300X3 mm) and the inspection of walls whenever the conduits were opened for servicing. A comparison of the organisms on the fouling plates and the nearby walls of the chamber was made. Fouling samples were photographed before collection and then weighted and examined in the laboratory.
Settlement and recruitment of the fouling was studied on plates immersed for 1,3,6,9,12,29 months. Five panels stood vertically in each horizontal frame at six stations. Asbestos was chosen because it provided a surface similar to the concrete walls of the conduits and chambers and because of the large use of this material in fouling studies in Italian waters (Relini 1980). At the recovery fouled panels were photographed and fixed in buffered formalin 10% in sea water.
Settlement of fouling organisms was estimated by: weight (wet weight of the whole community present on the panels); by counting, when possible, individuals or colonies; and by the use of cover indices (5 = more than 75% of the surface covered by an organism or a group; 4 = from 50 to 75%; 3 = 25 to 50%; 2 = 5 to 25%; 1 = less than 5%; + = negligible settlement).
The water intakes, conduits and basins were examined for fouling settlement when they were emptied for cleaning. Some observations were made on chlorination used at Torvaldaliga to prevent fouling settlement in channel B as recorded in tab. 5.

SPECIES RECORDED.

More than 170 species of animals and 20 of Algae were recorded on panels exposed during three years. The main species are listed in the previous paper (Relini et al. 1980). Only a few species are common fouling organisms, most of the species come from the natural biocoenosis of the surrounding shallow water.
From a quantitative point of view, the main fouling components are: Hydroids Tubularia crocea Agassiz and Sertularella gaudichaudi (Lamouroux); tubicolous Amphipod Jassa falcata (Montagu); Serpulids Pomatoceros lamarkii (Quatrefages) and Pileolaria pseudomilitaris (Thiriot - Quiévreux); Bivalves Mytilus galloprovincialis Lamarck and Anomia ephippium L.; Barnacle Balanus perforatus Darwin; Bryozoans Watersipora subovoidea (D'Orbigny) and Scrupocellaria bertholetti (Audouin); Ascidian Microcosmus polymorphus (Heller) and Algae Amphiroa rigida Lamouroux, Ulva rigida Agardh, Enteromorpha sp. and encrusting red algae (Corallinaceae).


1978
1979
1980
1981
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M
CORALLINACEAE
OTHER ALGAE
HYDROIDS
SERPULOIDEA
BRYOZOANS
BARNACLES
AMPHIPODS
BIVALVES
CA
CB
CC
CD
CE
CF

Tab. 2. Settlement on panels immersed from 3 months to 29 months.

		3M												6M						12M			29M
		1978			1979			1980				1981												
		J	S	D	M	A	N	F	M	A	N	F	M	S	M	N	M	N	M	M	M	M	M
	CA																						
	CB	+	5	+						+				4	1	1	1	+		+	3	3	1
CORALLINACEAE	CC	2	4	4	2		2	1	3	–	–	–		5	3	2	4	–	–	3	+	–	–
	CD																						
	CE	2	5		1	1				1				.	1	1		1	1	1		1	
	CF	4	1	2	2	1		2	2		2	+	4	5	3	1	4	1	2	3	3	2	2
	CA																						
	CB	1	1	+	1			1	+	2		1	2	1	+			+	+				
OTHER ALGAE	CC	5	5	2	3	4	1	4		–	–	–	3	3	2	+		–	–	1		–	–
	CD																						
	CE	4	1			4	+	2	1	+			1		1	2	2		1	1	1	1	
	CF	2	5	4	3	4	3	2	1	5	2	3	4	5	5	3	1	3	4	3		3	3
	CA		+			+								+		+			+	+	+		
	CB		1	1	1	1				+				1	1	1		+		1		+	
SPONGES	CC			+										+	1					1		–	–
	CD													+							+		
	CE	+	1	1		1	+			+	+	+	+	1		4				1	1	+	
	CF																						
	CA		3	1	2	2	3	4	2	1	1	3	5	2	3	4	2	1	4	2	2	4	4
	CB	1	1	2	2	1	3	2	1	2	3	2	1	1	2	1	2	2	1	3	2	1	2
HYDROIDS	CC						+	+		–	–	–			1			–	–			–	–
	CD	1	–		1	1				1	2		2			1	+	1	3	+	+	2	2
	CE	3	2	3	2	3	1	2	2	2	2	1	3	2	1	1	3	2	5	3	1	3	3
	CF	+			+			+							1					1	1	2	2
	CA		+		1	+	+	+	+	+	+	+	+	+	1	+	1	+	1	1	1	2	3
	CB	+	1	1	2	2	1	2	1	1	1	2	+	1	2	1	2	2	3	1	1	5	3
SERPULOIDEA	CC	2	1	1	1	4	+	+	3	–	–	–	+	3	2	2	4	–	–	4	5	–	–
	CD	+			1	+	+			+	+		+		+	+	1	+	+	1	1	1	1
	CE	+	1	1	1	1	1	1	1	+	1	1	1	1	2	1	2	1	2	1	1	2	1
	CF	1	+	+	1	+	+	+	+				+	1	1	+	+	+	+	+		+	
	CA		1			1								1		1				1	+		
	CB	+	2	1		4	2	+	+	+	+		+	3	1	5	1	1	1	3	3	1	2
BRYOZOANS	CC		+	+		1	+		+	–	–	–		+	1	+	+	–	–	1	+	1	–
	CD									+				+				1	+				
	CE	1	2	1		1	1	+	1	2	1		+	2	1	1		1	1	3	2	1	1
	CF	+		+					+								1						
	CA		+		1	1		+		+			2	1	2	2	2	+	4	3	2	5	5
	CB	+	2	1	1	1				+	1	+		2	1	2	2	2		4	2	3	4
BIVALVES	CC	+	1		1	1								1	1	1		–	–	3		–	–
	CD	1	–						1				+	2	1		1	+	1	3	1	2	1
	CE	+	2	+	+	+	+	+	1	1	+		1	2	1	1	4	1	1	5	4	3	5
	CF	+	+			+				+				+	1		1			1	2	1	2
	CA	+	+	1	1	1	1	+	+	+		+	1	1	2	1		+	3	3	2	3	3
	CB	2	1	1	2	+	2	1	+	1				2	1	1		2		3	1	2	
BARNACLES	CC	+		1	1	2	1		+			+		1	2	2	+	–	–	2	2	–	–
	CD	1	–	1	2	+	2	+	1	1		1		2	1	1	1	2	1	3	2	3	3
	CE		+	1	1	1	2	+	1	1	1	+	1	1	4	1	+	1	2	2	1	2	1
	CF	+		+	1	+	1	+	+	2	2	+	1	1	1	1	1	2	1	2	2	3	3
	CA		2	1	2	2	1	4	1		+	3	3	2	4	+	2	+	2	5	1	3	3
	CB	2	2	1	2	1	+	+	1	+	+	+	+	1	3	+	+	+		1		+	+
AMPHIPODS	CC		+	+				+	+					+	+	+	+	–	–			–	–
	CD	5	–	2	2	+	1	1	3	2	2		1	2	2	2	2	2	3	4	3	3	2
	CE	2	+	1	2	1	2	+	4	1	1	1	1	1	1	+	3	+	1	1	+	1	1
	CF	+	+	+	+	+	2		+	+	1			1	+	2	+	+		+			
	CA					+														1	+		
	CB		1	2	1	+							+	1	2	1			+	2	1		4
ASCIDIANS	CC		1	+										1				–	–	+		–	–
	CD													+									
	CE		1	+		+			+					1	1	1		+		1	1	1	2
	CF																			3			

SETTLEMENT PERIOD.

Settlement periods are deduced from monthly panels and sometimes from three monthly panels. In tables 1 and 2 settlement of main organisms during three years is recorded as percentage of the surface covered by each group.
There was no month without any settlement; in the same systematic group different attachments occured from station to station. Sponges, Ascidians and Mussels were more abundant on panels immersed for three or more months.

FOULING COMMUNITIES.

Three main communities occured in three different environments: intakes (staz. CA, CD), basins (st. CB, CE) and outfalls (st. CC, CF), though some differences were found between two stations of the same environment (Tab. 1-2). In the intake stations the community settled after one year was mainly composed of Mussels, tubicolous Amphipods, Hydroids, Barnacles and Serpulids, the same community was described after 29 months. Mussels are dominant on the 29 months panel of station CA, they are not yet dominant on that of station CD, probably in relation to the higher velocity of water in channel A (panels were put in the middle of the stream). In the basin stations on the one year panel the following organisms were dominant: Mussels and other Bivalves (Anomia ephippium) Corallinaceae Algae, Hydroids, Serpulids, Barnacles and sometime Bryozoans. On the 29 months panels Mussels are the major component accompanied by Ascidians (Microcosmus polymorphus), Hydroids, Bryozoans and Serpulids. In the outfalls stations, after one year, the community was composed of Algae (also Corallinaceae) and Barnacles; Hydroids, Serpulids, Bivalves were less important. A community almost identical, according to the qualitative and quantitative point of view, settled on the 29 months panels. The absence of Mussels was interesting. These last stations showed the highest settlement and the most simplified community, probably due to the high temperature and to the water turbulence. The most rich, diversified community, with high biomass, were found in the basins.

WEIGHT OF FOULING.

The amount of fouling was measured as wet weight of the whole community settled on a panel or on the surface of the conduit walls.
During three years of research the wet weight measured on panels immersed up to one year (Tab. 3-4) were lighter than those observed in other Italian power stations in which simplified communities were present in large amounts. In a power station in Liguria up to 160 g/dm^2

Tab. 3. Wet weight (g/dm^2) of fouling settled on monthly panels.

	Intakes		Basins		Outfalls	
	CA	CD	CB	CE	CC	CF
April '78	0.1	0.1	0.1	0.1	0.2	0.1
May	0.1	2.5	0.2	0.4	0.2	1.7
June	0.1	2.5	0.1	0.8	2.5	0.8
July	0.1	0.7	2.3	2.0	2.8	3.5
August	1.8	1.8	2.2	5.4	1.3	3.8
September	5.0	0.4	0.7	1.8	1.0	0.7
October	0.4	0.7	0.8	0.9	1.3	1.3
November	0.3	1.0	0.7	0.3	0.2	0.2
December	0.1	0.2	0.1	0.1	0.3	0.2
January '79	0.1	0.1	0.1	0.1	0.2	0.3
February	0.1	0.1	0.1	0.2	0.2	0.3
March	3.3	1.8	3.5	2.5	1.3	2.3
April	0.3	2.7	3.8	1.3	2.3	0.9
May	0.4	9.8	0.4	1.4	0.8	1.7
June	0.6	1.6	0.6	2.2	1.7	2.5
July	2.3	0.6	2.3	1.5	1.9	2.0
August	0.3	0.4	0.7	2.0	0.3	0.5
September	0.2	1.3	0.5	1.8	0.3	1.2
October	0.8	0.6	0.8	0.8	0.5	0.7
November	0.7	0.7	0.7	0.7	0.9	0.7
December	0.5	0.1	0.3	0.0	0.0	0.0
January '80	0.2	0.0	0.2	0.2	0.1	0.0
February	1.4	0.0	0.6	1.2	0.5	1.9
March	0.9	0.7	0.5	0.8	0.9	1.2
April	1.5	1.3	0.3	1.7	2.1	2.9
May	0.0	0.6	0.2	0.8	0.0	1.4
June	0.5	1.2	0.8	0.8	0.7	1.3
July	0.3	1.3	1.4	1.7	--	1.7
August	0.0	0.6	0.5	1.1	0.5	1.3
September	0.0	0.8	0.2	0.6	1.1	0.7
October	--	--	0.4	0.6	--	0.3
November	--	--	0.1	0.1	--	0.1
December	0.6	0.1	0.3	0.3	--	0.4
January '81	0.4	0.0	0.2	0.2	--	0.5
February	0.1	0.0	0.1	0.1	--	0.0
March	1.9	0.1	1.7	0.8	0.8	1.5
April	0.3	0.4	0.7	0.6	0.0	0.9
May	3.2	1.5	1.3	0.9	2.2	0.2

Tab. 4. Wet weight (g/dm^2) of fouling.

	Intakes		Basins		Outfalls	
	CA	CD	CB	CE	CC	CF
Three months panels.						
June '78	0.3	10.7	1.7	4.8	3.2	1.7
September '78	7.3	---	6.9	5.2	1.3	1.7
December '78	0.8	0.7	3.4	2.1	1.3	0.9
March '79	8.6	4.2	9.4	3.4	2.1	2.3
August '79	3.2	0.4	11.0	3.2	3.3	2.4
November '79	2.6	2.8	3.2	1.9	1.2	2.7
February '80	14.3	0.5	1.3	1.2	1.9	1.7
May '80	0.7	4.4	0.8	3.7	1.7	2.8
August '80	0.3	0.8	1.0	1.3	---	1.7
November '80	0.1	0.7	0.8	0.8	---	0.4
February '81	3.2	0.0	1.3	0.7	---	2.9
May '81	12.2	2.1	1.7	4.9	2.8	3.6
Six months panels.						
September '79	7.0	16.3	14.9	18.9	7.0	2.4
March '79	21.1	2.9	17.8	9.7	5.0	2.4
November '79	2.9	2.9	2.0	3.9	2.9	2.2
May '80	4.2	3.3	5.7	24.6	2.4	1.0
November '80	0.1	1.0	1.7	0.8	---	0.6
May '81	22.6	4.2	6.3	11.1	---	2.2
Nine months panels.						
December '78	3.2	12.3	15.7	12.8	7.3	1.7
Twelve months panels.						
Mach '79	21.3	35.5	25.8	17.8	7.3	4.2
May '80	10.2	13.8	12.3	50.5	3.6	8.7
May '81	34.8	10.2	35.5	43.6	---	12.6
Twenty nine months panels.						
May '81	167.0	13.8	104.2	171.3	---	14.8

was recorded after one year immersion while in Torvaldaliga the highest value was about three time lower (50,5 g/dm^2, st. CE May 1980). High wet weights were observed in Torvaldaliga on panels immersed for 29 months on which 167 g/dm^2 and 171,3 g/dm^2 were reached and a Mussel community settled. Similar values were recorded on the intakes' walls (from a minimum of 55,8 g/dm^2 to a maximum of 358 g/dm^2) and of the basins (up to 156 g/dm^2), after two years. There were some variations in fouling weights in relation to the length of time and seasonal period of exposure. (fig. 2-3).

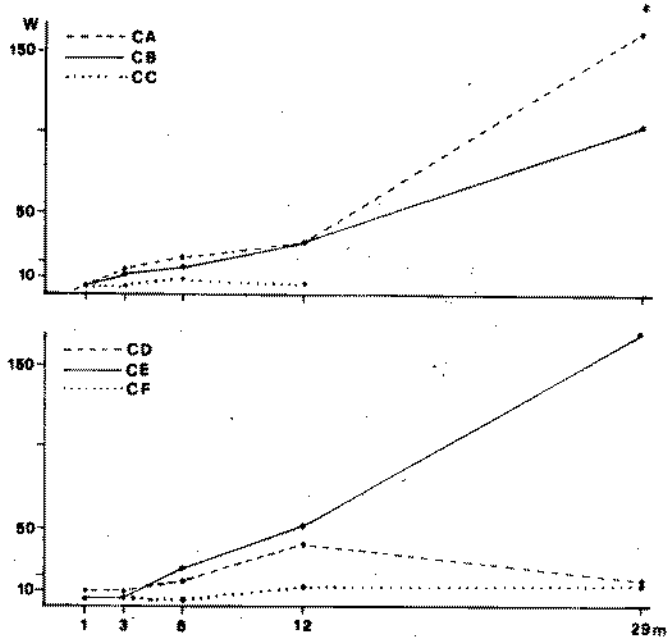


Fig. 2. Distribution of the highest wet weights (W) found with the increasing of exposure time of the panels.

On monthly panels weights were very light (Tab. 3) about 1 g/dm^2 with a maximum of 9 g/dm^2 (St. CD, May 1979) and without differences between stations although different fouling communities occurred.
After three months exposure the highest value was 14 g/dm^2 (St. CA, February 1980) after 6 months 22 g/dm^2 . The amount of fouling was higher during the first six-months period (winter and spring) than during the second period (summer-autumn) and this fact contradicts the pattern of fouling settlement in Italian harbours or in other power station intakes.

After one year, fouling weights of panels immersed in the intakes and in the basins increased respectively up to 30 g/dm^2 and 50 g/dm^2 while at discharge stations they remained about 10 g/dm^2, also after 29 months exposure, because of the stress due to high temperature, turbulence and chemical pollution. With the increase in the time of exposure fouling weights of panels increased at both stations of basins (CE, CB) and only at station CA of intakes in which weights after 29 months were about three times higher than the yearly values.
Differences between stations CA and CD were probably due to the absence of the Mussel community at CD because of the hydrodynamic conditions at this intake.

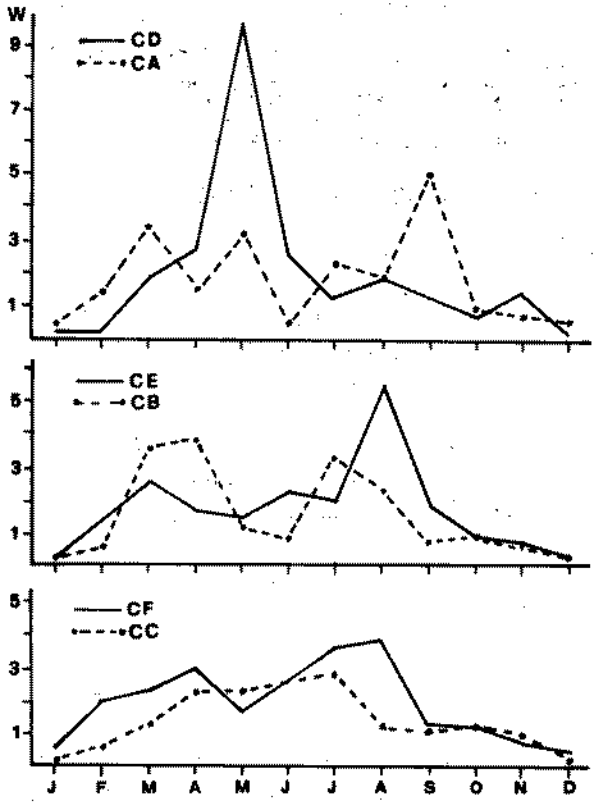


Fig. 3. Distribution of highest wet weights (W) found in each month during three years.

Monthly patterns of fouling settlement as wet weight is represented in fig. 3 in which the highest values found during three years are

recorded. In the intakes and basins there were two main periods of fouling accumulation: one in Spring, due mainly to Amphipods and Barnacles and the second one in the late Summer, due to Hydroids and Bryozoans. In general, these organisms did not represent a dangerous situation although they allow Mussel settlement.

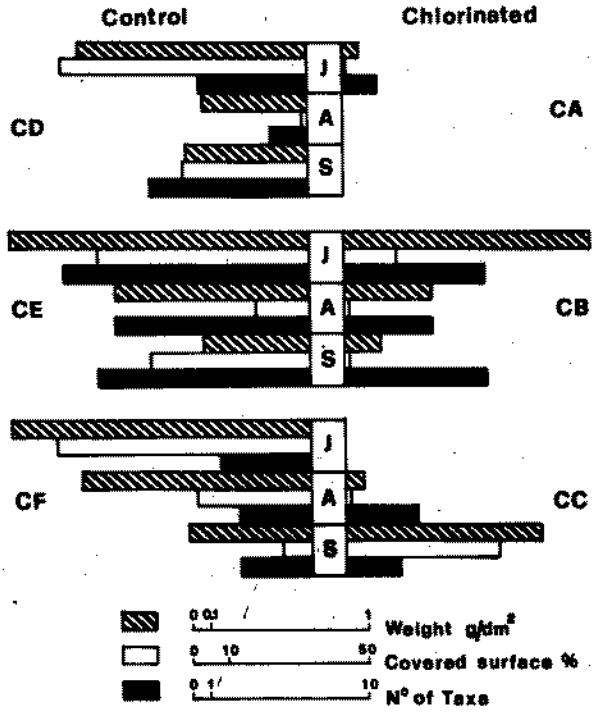


Fig. 4. Comparison between panels exposed to chlorination and not exposed at three environments.

CHLORINATION.

During three years we are able to test chlorine as valid antifouling system only for a short period (July-September) because of an irregular schedule of chlorine supply. For our purpose, in fact, continous addition of chlorine during settlement periods was necessary. Due to this we suggested the use of a chlorination index (Cl.I.): i.e. the ratio between number of days with continous chlorination and days of exposure of panels.
Chlorine was added as hypochlorite at the head of channel B at a rate of 200 l/h measured daily. Residual chlorine (g/l) in the basin and

Tab. 5. Examples of chlorination.

	Monthly average of Chlorine (l/h)	Monthly average of residual Cl (g/l)		Cl.I.
		CB	CC	
April 1978	61,9	59	49	0,43
May	186,6	56	50	0,50
June	102,0	43	60	0,48
July	10,5	4	1	0,03
June 1979	106,8	12,4	10,8	0,32
July	50,8	20,3	16,1	0,19
August	111,4	0,8	0,0	0,28
September	222,9	32,7	29,5	0,28
October	189,1	47,4	17,4	0,73
April 1980	106,1	17,5	29,3	0,30
May	141,5	40,5	6,1	0,54
June	245,6	110,1	32,2	0,70
July	254,3	107,0	27,7	0,77
August	301,0	91,1	38,0	0,77
September	295,2	97,1	44,2	1

outfall was recorded daily during chlorination period.
Chlorine effectiveness in fouling prevention was evaluated from the following items:
1) amount of fouling (wet weight: g/dm^2)
2) cover indices (% of the surface settled by the organisms on 12 dm^2)
3) number of taxa settled.
Stations CA, CB, CC with chlorine were compared (fig. 4) with control stations CB, CE, CF in which no antifouling was added but where only the above mentioned hydrodynamic condition affected fouling settlement. The highest prevention was obtained at the intake station CA. The comparison of the amount of fouling between channel A and channel B in different chlorine content shows that a significative (p 0,005) prevention of fouling was obtained when chlorine was supplied for at least two out of three days or when Cl.I. was 0,67 (see table 5).
On the basis of the results obtained in the Torvaldaliga power station my collegue Dr. C.N. Bianchi has suggested a diagramm (fig. 5) in which he has plotted Cl.I. against the decrease in fouling amount percentage. An index of 0.7 gives rise to a 95% decrease of fouling wet weight on monthly panels.

The pattern of the fouling community continued to be affected by the chlorine for up to six months after the treatment was stopped.

CONCLUSIONS.

The data gathered in the Torvaldaliga power station during the three years (April 1978 - May 1981) on panels immersed from 1 month to 29 months allow some conclusions about the following three main items:
1) fouling composition, its development and seasonal variation;
2) main periods in which fouling prevention is needed;
3) Chlorination.
As already indicated the macrofouling of cooling system in Torvaldaliga is characterized by a large number of species generally represented by a low number of individuals. Such a community with a high diversity has a small biomass and slow development. After one year the maximum wet weight is no more than 50,5 g/dm^2; and the definiti

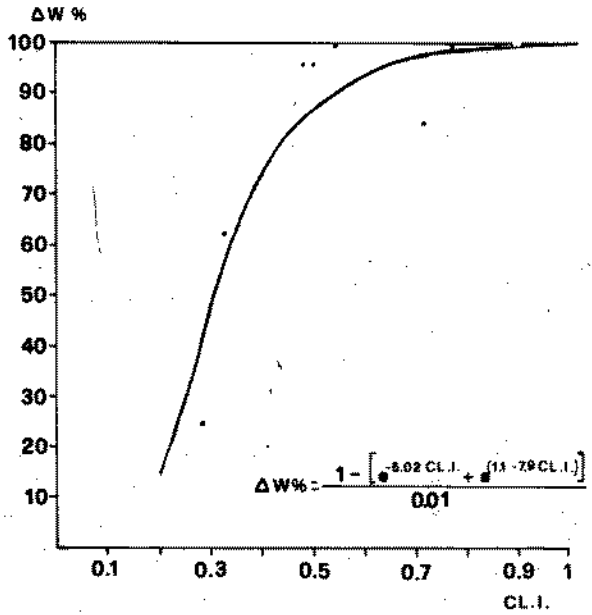


Fig. 5. Per cent decrease of wet weights of fouling (ΔW%) against chlorination index (Cl.I.).

ve community in which Mussel is dominant, has not yet been reached. The Mussel community which is the main fouling problem in Torvaldaliga, develops 18-20 months after the immersion of panels or cleaning of the walls in the intakes.

The explanation for this is that Mussel settlement is not pioneer; Mussels need other organisms on the substrate suitable for attachment.
Three principal communities can be described in three different environments examined: intaked, basins, outfalls, differences between two channels are less important, when chlorine is not added.
The richest community from qualitative and quantitative point of view was found in the basins because of the temperature and turbolence of water the poorest was in the outfalls.
In this last condition Algae were dominant while in basin and intakes the dominant community was charaterized by Mussels.
Though settlement takes place through the year it is possible to recognize a more important periods for prevention.
The main fouling problem is represented by Mussels which settled in Spring reaching a peak in May. In Torvaldaliga a second period of Mussel, settlement in Autumn, as described in other localities, was not found. To prevent settlement of Mussels an antifouling system operating from March to May-June is required. If one wishes to prevent attachment of other main foulers (Serpulids, Barnacles, Hydroids and Amphipods) some of which help Mussel settlement, antifouling treatment may be required also from March to September.
In Torvaldaliga a significative decrease of fouling settlement was obtained when chloride was added at least for 2 days out of three at a concentration 200 l/h of hypochlorite.
A continous chlorination can be decrease wet weight of the fouling which settled on monthly panels of the 77-100%.
In my opinion unsuccessful fouling prevention by the use of chlorination in the most of Italian power plants was due to difficulties in mainteining the established schedule.

ACKNOWLEDGEMENT

The author would like to thank Dr. C.N. Bianchi for valuable help and successfully completing the research summarized in the present paper.

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THE IMPORTANCE OF A PRIMARY FILM OF MICROORGANISMS ON THE SUBSEQUENT ESTABLISHMENT OF A MACROFOULING COMMUNITY

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ABSTRACT

The influence that the primary film of microorganisms which forms on submerged surfaces in the sea has upon the subsequent establishment of a macrofouling community is demonstrated with reference to the barnacle Notomegabalanus algicola. Scanning electron microscopy of recently metamorphosed juvenile barnacles reveals numerous hair-like projections which become entangled with the bacterial and fungal filaments of the primary film. The hair-like projections are composed of mucopolysaccharide and it is proposed that they offer an anchoring mechanism for the newly settled barnacle.

RESUMÉ

L'influence des filmes primaires de microorganismes formés sur des surfaces submergées dans la mer sur l'établissement subséequent de macro-organismes ancrassants, avee référence particuliêre sur la balanne Notomegabalanus algicola est ici déemontrée. L'hexamen an microscope électronique des juveniles de balannes novellement métamorphosées démontre de nombrëuses projectione filamenteuses enchevêtrées avee les filaments des bacteries et des fongies du filme primaire. Ces projections falimenteuses sont constituées de substances mucopolysaccharines. Il est, ici, proposé que ces projections agissent comme ancres pour les balannes nouvellement fixées.

IMPORTANCE OF PRIMARY FILM IN MARINE FOULING

INTRODUCTION

The attachment of the larvae of marine fouling organisms to submerged surfaces is influenced by many different factors. Many workers have stressed the importance that the primary film of microfouling organisms has upon the subsequent development of a macrofouling community (Zobell and Allen, 1935; Zobell, 1939; Miller, Rapean and Whedon, 1948; Liberatore et al., 1972; Dempsey, 1981). Generally most workers agree that the sequence of events following the initial immersion of a surface into the sea begins with the adsorption of organic molecules. This is followed by the settlement of bacteria, diatoms, protozoa, fungus, pioneer macro-organisms and finally secondary macro-organisms.

Zobell (1939) stated that the larvae of any fouling organisms attach to submerged surfaces which are coated with films of bacteria more readily than to bacteria-free surfaces. Subsequent results obtained by many different workers have supported this view (Miller et al., 1948; Wood, 1950; Daniel, 1955; Crisp and Ryland, 1960; Meadows and Williams, 1963; Liberatore et al., 1972; Mitchell et al., 1977) but Crisp (1976) states that although the larvae of many invertebrates settle more readily on filmed rather than clean surfaces, this is a preference rather than a precondition. In some cases, in fact, certain larvae avoid filmed surfaces and favour clean surfaces for settlement (Crisp and Ryland, 1960).

For those larvae which settle preferentially on filmed surfaces, a number of possible roles for the primary film have been suggested. The mucilagenous network could facilitate adhesion by trapping or entangling appendages of the larvae and thereby acting as a holdfast (Zobell, 1939; Knight-Jones, 1951; Dempsey, 1981). Alternatively, some workers have suggested that some components of the primary film could serve as a food source to settling organisms (Zobell, 1939; O'Neill and Wilcox, 1971; Liberatore et al., 1972). Although it is possible that nutrients could be derived from the primary film at a later stage it is unlikely that this could be important at the settlement phase as barnacles and bivalves do not feed at this time (Gabbott, 1976; Lucas, Walker, Holland & Crisp, 1979).

Yongue and Cairns (1971) found that the pH in the primary film micro-habitat can differ by more than three units from that of surrounding water, and that the film could act as a pH buffer. It is noteworthy that this would be important to hard-shelled animals, because deposition of calcium carbonate is pH-dependent. It is also possible that the mucilagenous layer could concentrate certain organic molecules that induce settlement, e.g. proteins originating from conspecific barnacles (Barnes, 1970; Liberatore et al., 1972) or compounds originating from the natural algal hosts of serpulids (Crisp and Williams, 1960). In other organisms bacteria from the primary film could be a prerequisite for metamorphosis. Müller et al. (1976) and Neumann (1979) found

that particular sessile live bacteria triggered metamorphosis in settling scyphozoan planulae.

This paper presents evidence obtained from scanning electron microscopy which demonstrates that in the case of the barnacle, Notomegabalanus algicola at least one of the functions of the primary film is to act as an anchoring mechanism for the metamorphosing juvenile barnacle.

MATERIALS AND METHODS

Polyvinylchloride (PVC) test plates measuring 5 x 5 cm were placed into the sea at a site near to Table Bay Harbour (33°55'S, 18°25'E) and were maintained at a depth of about 2-3 m for periods of up to 7 days. The experiment was repeated several times during a mid-summer period when the seawater was known to be rich in barnacle larvae. After removal from the seawater 5 cm^2 discs were punched out of the test panels and prepared for viewing under a Cambridge S180 Stereoscan electron microscope. Samples were fixed for 3 hrs in 2% gluteraldehyde in seawater, stained for 30 min in 0.5% Osmium tetroxide, dehydrated through 30 to 100% ethanol, dried in a Polaron Critical Point Drier with liquid carbon dioxide for 3 hrs and coated with gold or palladium in a Balzers Vacuum Coater. All S.E.M. work was carried out at an accelerating voltage of 20 kV at magnifications of up to 10,000 times. The test discs were superficially scanned at low power until newly-settled barnacle specimens were found and these were then exmained and photogrpahed at higher magnifications.

RESULTS AND DISCUSSION

Plates 1 and 2 show the composition of the primary film of micro-organisms which developed on a PVC test plate after three days immersion in the sea. The plate had been colonized by numerous coccoid and small rod bacteria and a number of diatoms and choanoflagellates were also present. The entire micro-organism community was entangled into a mass of filaments, possibly of bacterial or fungal origin.

Plates 3 and 4 show recently metamorphosed specimens of N. algicola and a prominent feature of these juvenile barnacles is the numerous hair-like projections found over the entire body surface. From the large number of specimens examined it appeared that these structures were transient in nature and could be found only for a day or so after metamorphosis. Although these hair-like structures have been briefly referred to in previous works on the anatomy of cyprid and juvenile barnacles, no definite function has ever been assigned to them. In her extensive work on the larval structure and metamorphosis of B. balanoides, Walley (1969) made no mention of these structures. They were, however, referred to by Bernard and Lane (1962) who noted that in

B. amphitrite niveus soon after the cyprid exoskeleton was shed 35 to 40 long hair-like projections appeared but persisted only until the shell compartments formed. No function was suggested for the structures. Walker and Lee (1976) describe numerous setae which they found on the surface of the cyprid stage of B. balanoides and to which they assign a sensory function. However, as the setae they describe are only 5-11 um in length they are unlikely to be the same structures as described here.

Examination of Plates 5, 6 and 7 reveals a possible function of these hair-like projections. In the high-power magnification it can be seen that most of the projections point towards the substratum and the tapered ends of many of them become entangled in the filaments of the bacteria and fungi in the primary film. Plate 7 shows that one of the projections almost spirals around a bacterial filament. The plate also shows that at this time the base of the juvenile barnacle is still lifted well clear of the substratum and certainly the process of cementation is not complete at this stage. It does not seem unreasonable, therefore to suggest that the function of the hair-like projections is to form an anchoring mechanism by becoming entangled in the primary film on the substratum. If this is so it would also explain the transitory nature of the hair-like projections. Once the shell plates are fully formed and the cementation process is complete the temporary anchoring mechanism would become unnecessary and hence the hair-like projections disappear at that stage.

Histochemical techniques used to investigate the chemical nature of the projections revealed that they are composed of muco-polysaccharide and that they are not chitin-covered. It is perhaps significant that Baier (1973) discussed unpublished results of Cook, Tosteson, Marshall and Baier in which they found that surfaces immersed in the sea adsorb a layer of glycoprotein which then forms a particularly strong bond with adhesive muco-polysaccharides of certain marine bacteria. It is possible, therefore, that the mucopolysaccharides of the barnacle projections may also form bonds with adsorbed glycoproteins of the substratum.

Thus, in the case of N. algicola it appears that one reason that the barnacles settle preferentially on a surface that has been preconditioned with a layer of primary film is that the entangled bacterial and fungal filaments within the film provide an anchoring point for the hair-like projections produced by the juvenile barnacle at a stage when the barnacle's normal cementing mechanism is not yet operative. It remains to be seen whether a more detailed microscopic examination of the metamor-phosing stages of other barnacle species will also reveal the presence of transient anchoring mechanisms which utilize the primary micro-organism film for this purpose.

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Plate 1 The primary film of micro-organisms on a PVC plate after 3 days immersion. Note numerous bacteria and diatoms.

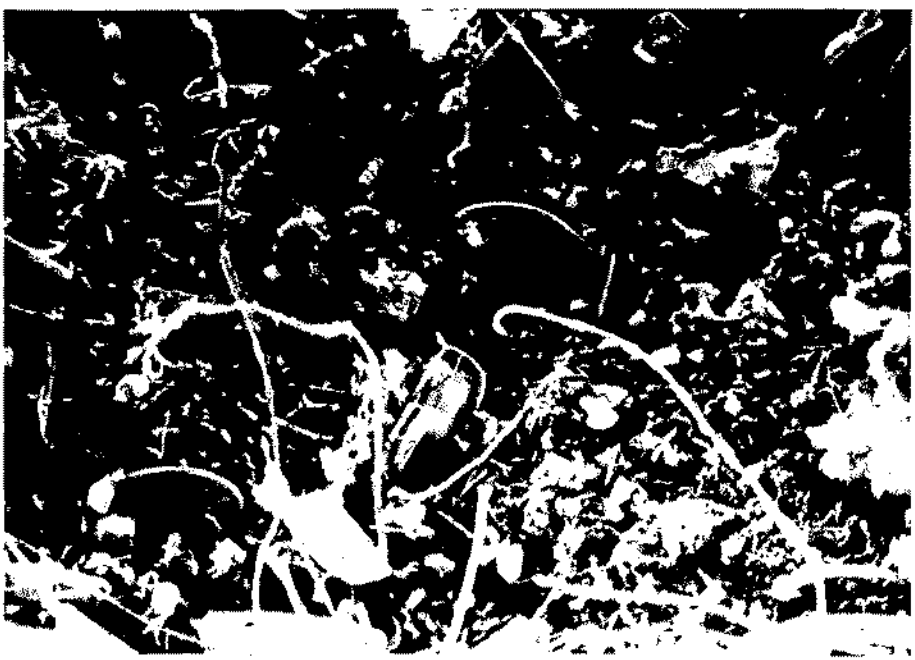

Plate 2 The primary film of micro-organisms on a PVC plate after 3 days immersion. Note numerous entangled filaments - probably of fungal or bacterial origin.

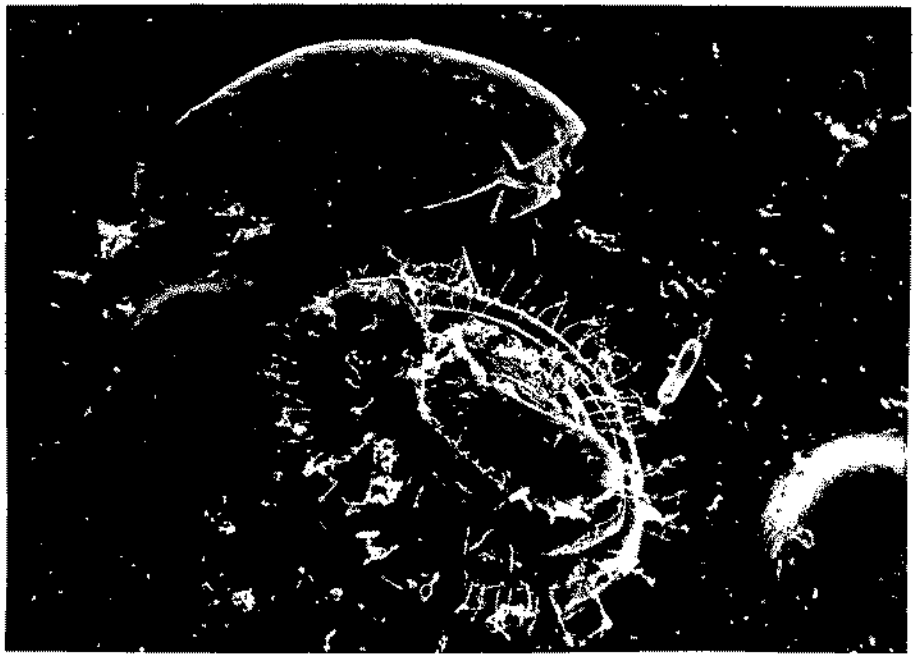

Plate 3 A juvenile N. algicola which has recently emerged from its cyprid shell.

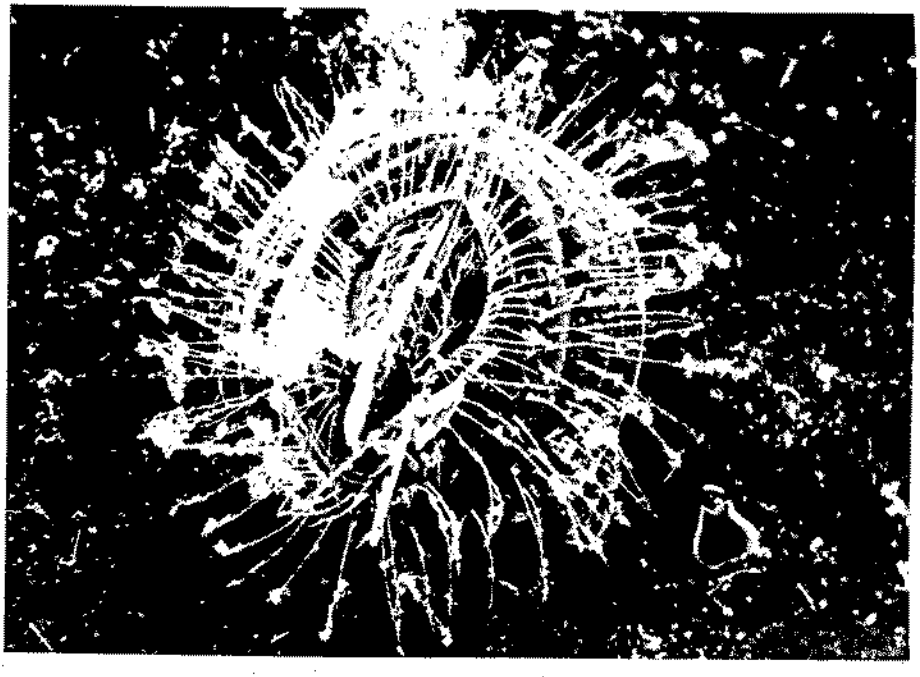

Plate 4 N. algicola juvenile at a slightly later stage than Plate 3. Note numerous hair-like projections on the surface of the barnacle.

Plate 5 A recently metamorphosed N. algicola juvenile. Note how many of the hair-like projections from the barnacle are directed downwards towards the substratum.

Plate 6 A highly magnified view of the base of a juvenile N. algicola to show how many of the hair-like projections from the barnacle become entangled with filaments from the primary film.

Plate 7 A close-up of the base of a juvenile N. algicola to show that the base of the barnacle is not yet fully cemented to the substratum and that some of the hair-like projections are almost spiralled around the filaments from the primary film.

Multivariate Analysis of Three-Dimensional Data in the Study of Succession in Marine Fouling Communities

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ABSTRACT: A multivariate method for analyzing three-dimensional, fouling community data (panels x species x times) is described. The method involves: 1.) hierarchical classification of the typical panels by species data matrix for all samping dates combined, 2.) designation of characteristic species assemblages from the resulting dendrogram, and 3.) analysis of individual panel trends through time. An example of the utility of this method is presented with data from a pollution assessment project carried out in Puget Sound, Washington, U.S.A.

Une méthode multivariate pour l'analization des données communautés en trois dimensiones (paneaux x espèces x temps) s'est décriere ici. La méthode s'involve: 1.) classification hierique des paneaux par d'espèce d'une matrice à données pour toute les dates d'echantillonnage, 2.) la designation des assemblages des espèces characteristique observé du dendrogram resultant, et 3.) l'analyses des tendances des paneaux avec temps. Une example de la utilité de cette methode s'est presenté avec les données d'un projet sur la répartition de la pollution en Puget Sound, Washington, U.S.A.

Contribution no. 1368 from the School of Oceanography, University of Washington.

INTRODUCTION

Multivariate analyses provide community ecologists with an ever-expanding array of powerful techniques for recognizing and summarizing patterns in large, multiple-species data sets. For over two decades ecologists have employed such techniques in elucidating a variety of patterns in the structures of many communities, both terrestrial and aquatic (see review in Gauch, 1982). Community structure, however, is not a static entity; it typically changes through time in response to various biotic and abiotic forces. These changes through time, referred to as community development, are often of more interest to ecologists investigating dynamic systems than community structure at any given instant. In the analysis of community development, the typical site-by-species community matrix is complicated by another dimension, time. Despite early recognition of this complexity, (Williams et al., 1969; Williams and Stephenson, 1973), few simple methods are currently available to the working field ecologist faced with such an analytical problem. It is our intention in this paper to fully describe a method with which we have had considerable success in analyzing such three-dimensional, fouling community data (panels x species x times). In addition, we provide examples with data from five Puget Sound fouling communities to illustrate the utility of the method.

METHODS

Study Sites and Field Methods

Fouling community development was monitored for 100 weeks at five localities in the central and southern portions of Puget Sound near Manchester, Seattle, and Tacoma, Washington, U.S.A. (Table 1, Figure 1). These sites were investigated as part of a National Oceanic and Atmospheric Administration (NOAA)-sponsored pollution assessment project. At each locality, textured, white formica panels (412 cm^2 each) were suspended from stationary docks at 3.0 m below mean tide level. Panels were submerged in July and August, 1980. The percent cover of sessile species growing on the "primary space" (Dayton, 1971) of a given panel's undersurface was estimated by a point sampling technique (Sutherland and Karlson, 1973), using 100 points randomly positioned over the panel's area. This sampling procedure is nondestructive with panels being resubmerged for further development after censusing (Schoener and Greene, 1981). The data set accumulated with these sampling procedures comprises a three-dimensional community development matrix (panels x species x times) with elements of percent cover on each panel, for each species, at each censusing date.

Table 1. General information pertaining to study sites investigated.

Study Site	Location	Initiation Date	Termination Date
Blair	Blair Waterway, Tacoma, WA.	July, 1980	June, 1982
Duwamish	Duwamish River, Seattle, WA.	August, 1980	June, 1982
Hylebos	Hylebos Waterway, Tacoma, WA.	July, 1980	June, 1982
Manchester	Main Basin, Manchester, WA.	July, 1980	June, 1982
Milwaukee	Milwaukee Waterway, Tacoma, WA.	July, 1980	June, 1982

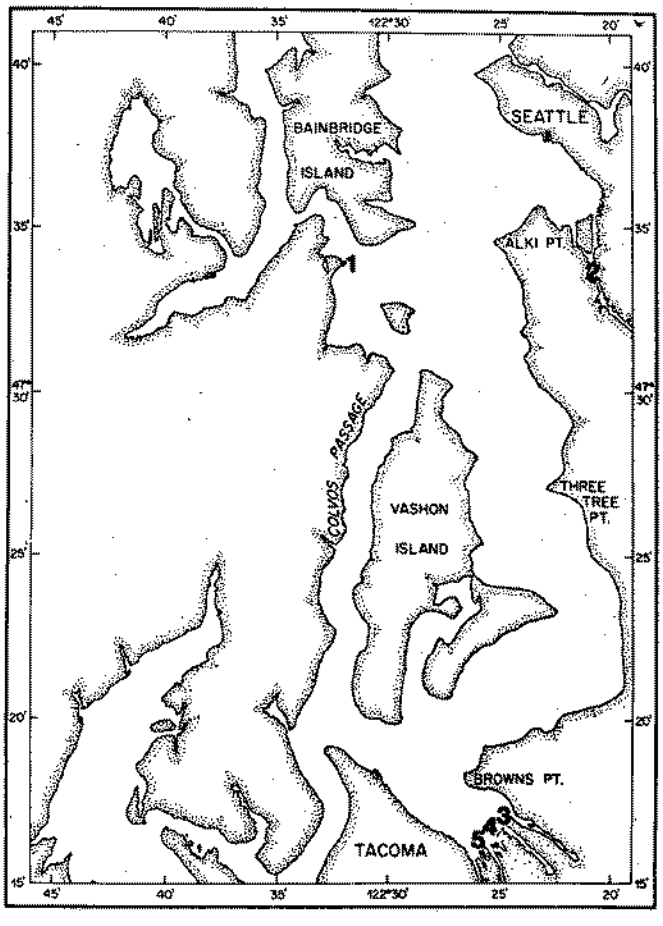


Figure 1. Locations of study sites:
1-Manchester
2-Duwamish
3-Hylebos
4-Blair
5-Milwaukee

Analytical Methods

With 12 panels at a study site, 78 potential sessile species,[1] and eight census dates included, the resultant community development matrix for each locality consists of 7,488 elements. This number can also be viewed as the product of 96 separate individuals (representing the 12 panels for each of eight census dates) and their 78 corresponding attributes (representing the relative abundance of each species). In comparing individuals for similarity in composition, the Bray-Curtis Similarity Index (Clifford and Stephenson, 1975) was used. This index has been found to accurately portray true percent similarity better than most of the other commonly used indices (Bloom, 1981). It is computed as:

$$I_{ps} = \sum_{i=1}^{n} \min (a_{ij}, a_{ik}) \qquad (1)$$

where: a_{ij} is the relative abundance (percent cover) of species i on panel j
a_{ik} is the relative abundance (percent cover) of species i on panel k
n is the total number of sessile species found on panels j and k

The results of this procedure can be portrayed in a 96 individual by 96 individual similarity matrix. Since this matrix is symmetrical above and below the main diagonal, and since the main diagonal elements are each equal to one, the actual elements providing useful, comparative information total

$$4{,}560 = \frac{96 \times (96-1)}{2} .$$

The next step in the analysis is a classification procedure which is both agglomerative and hierarchical (Boesch, 1977). The clustering method we adopted utilizes a flexible combinatorial strategy (Lance and Williams, 1967), with the clustering intensity coefficient set at $\beta = -0.25$ (Clifford and Stephenson, 1975). Computations were carried out with the CLUSTAN© statistical program on the University of Washington's Cyber 170/750 computer. The major output of this clustering program is a dendrogram as portrayed in Figure 2. The 96 individuals are arranged along the abscissa according to their similarity relationships, and the ordinate is a distance scale which relates similarity between clusters at the point of fusion.

[1] Empty space was treated as another species in the analysis since we view it as an essential and interacting element in space-limited systems (Quinn, 1979; Greene and Schoener, 1982).

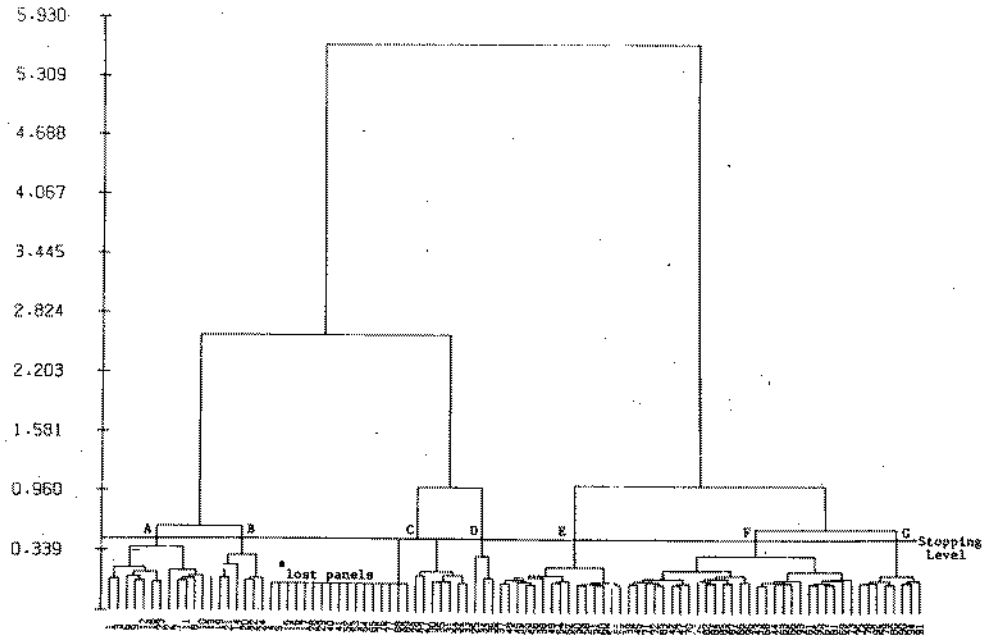


Figure 2. The dendrogram corresponding to the Duwamish River site. The stopping level chosen to designate the "characteristic species assemblages" is indicated. The 96 individuals (12 panels x 8 census dates) are arranged along the abcissa, the ordinate is a distance scale.

At this point in the analysis, a distance level must be chosen to designate characteristic clusters of individuals, or what we refer to as "characteristic species assemblages" (Greene and Schoener, 1982). The choice of an appropriate "stopping rule" to define these species assemblages is typically arrived upon subjectively (Boesch, 1977; Sandland and Young, 1979). The procedure we use is as follows:

1.) to run the clustering program for each census date separately with data from 12 panels

2.) to distinguish between the important clusters of panels on each census date from the resulting 12 panel dendrogram.

3.) to return to the complete, 96 individual dendrogram, and choose a distance level which incorporates as many of these distinctions as possible, while still retaining a convenient number of species assemblages to work with.

Although we recognize the subjectivity inherent in this procedure, we feel it is allowable for the descriptive purposes we intend. Objectivity and repeatability are important considerations, but as Gauch (1982, p. 31) states:

> Objectivity of a study considered as a whole (from observation to final reporting of results), not objectivity of individual steps in the procedure, is the crucial point because subjectivity can be banished from one step only to reappear elsewhere in a worse, more subtle, or complex form.

Thus we justify the use of our procedure until a more consistent, objective procedure is tried and proven.

Once the species assemblages are designated, the remaining procedures are largely bookkeeping in nature. For summarization purposes, the procedures we have been successful with include first, tabulating the average compositions of the species assemblages (Figures 3-7a), and second, tabulating the species assemblages corresponding with each panel over all sampling dates (Figures 3-7b). In the first figures, only species which on the average occupy greater than 10% of the space in a given assemblage are included. Less abundant species have little effect on the results with the clustering method employed (Boesch, 1977), and this relatively arbitrary lower limit is consistent with Sutherland and Karlson's (1977, p. 427) criteria for defining the community's foundation species.

RESULTS

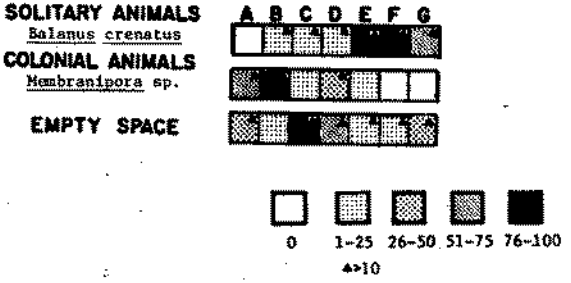
Duwamish River

Community development at the Duwamish River site proved to be the easiest to summarize (Figure 3). By September, only one month after initial immersion, all panels were heavily encrusted by the cheilostome bryozoan, Membranipora sp. Dominance by this bryozoan continued throughout the winter and early spring, although the barnacle, Balanus crenatus, did have a successful, if somewhat light set in the fall. In April, B. crenatus exhibited the heavy settlement typical of this species in mid-spring. By June, the barnacle had surpassed Membranipora sp. in relative abundance on all panels, and this dominance persisted for the remainder of the study.

Manchester

Community development at Manchester was considerably more complex than at the Duwamish River site (Figure 4). Thirteen foundation species had a role in the succession of this community relative to only two in the Duwamish River fouling community. During the fall months, typical early successional species like Membranipora sp. and the hydroids, Obelia sp. and Hydroid sp. 1,

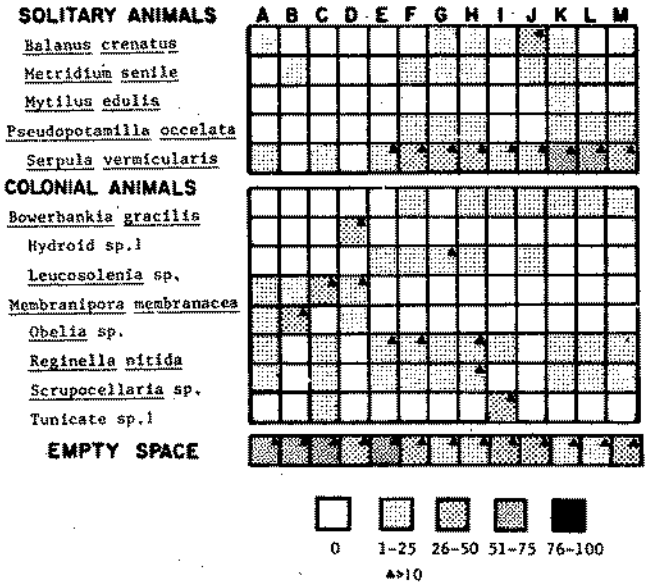
Figure 3. Community development at the Duwamish River site. The upper portion of the figure (a) illustrates the compositions of the species assemblages determined from the cluster analysis dendrogram. Only the foundation species (see Greene and Schoener, 1982) are included in the figure. The coded key corresponds to five relative abundance categories based on mean percent cover; the mean percent cover for a given species being determined from all panels represented in each species assemblage. The lower portion of the figure (b) lists the species assemblage corresponding with each panel on a given sampling date.



Panel	Date 9/80	12/80	3/81	6/81	9/81	1/82	3/82	5/82
1	A	B	D	E	E	F	F	F
2	A	B	C	E	E	E	F	F
3	A	*	C	F	E	F	G	F
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	A	A	C	F	E	F	G	G
7	A	B	C	F	E	F	G	G
8	A	B	C	F	E	F	G	G
9	A	B	C	F	E	F	F	F
10	A	B	D	E	E	F	F	G
11	A	A	C	F	E	F	F	F
12	A	B	D	E	E	F	F	F

\- panel lost
* panel not censused

Figure 4. Community development at the Manchester site (figure format is the same as Figure 3).



Panel	Date 9/80	12/80	3/81	6/81	9/81	1/82	3/82	5/82
1	A	C	A	G	H	M	M	M
2	D	C	E	F	H	F	E	E
3	D	A	E	G	K	L	L	K
4	A	C	A	F	F	M	M	M
5	D	E	F	K	L	L	L	L
6	D	A	E	H	H	K	M	K
7	D	C	E	G	K	M	K	K
8	D	A	B	H	F	M	M	M
9	A	I	I	J	J	J	J	J
10	D	A	E	H	H	M	M	H
11	D	A	E	H	H	M	M	L
12	B	A	E	G	M	L	L	L

slowly colonized the predominantly bare panels. Throughout the winter and spring, the serpulid polychaete, Serpula vermicularis, successfully invaded, grew, and proceeded to dominate nearly all panels. Although a variety of minor species rose and fell in relative abundance at different times and on different panels, S. vermicularis' dominance persisted over the remainder of the study. Only the pattern of development observed for panel nine stands out as distinctly different from the other eleven panels. In late fall and early winter of the first year, this panel was invaded by a compound ascidian. Although this ascidian was absent by late spring, its disappearance opened up space for B. crenatus to recolonize during its heavy spring recruitment. Once established, the barnacle persisted on panel nine for the remainder of the study as a co-dominant with S. vermicularis.

Blair Waterway

Community development at Blair was the most variable of all five sites, especially in the latter portion of the study (Figure 5). The colonization rate was relatively slow at Blair, and no clear dominants emerged on any panels until the summer of 1981. Other than the consistent presence of B. crenatus, panel development progressed along a number of divergent pathways throughout the investigation. By the end of the study, most panels were dominated by either B. crenatus, Metridium senile, S. vermicularis, or various combinations of the three.

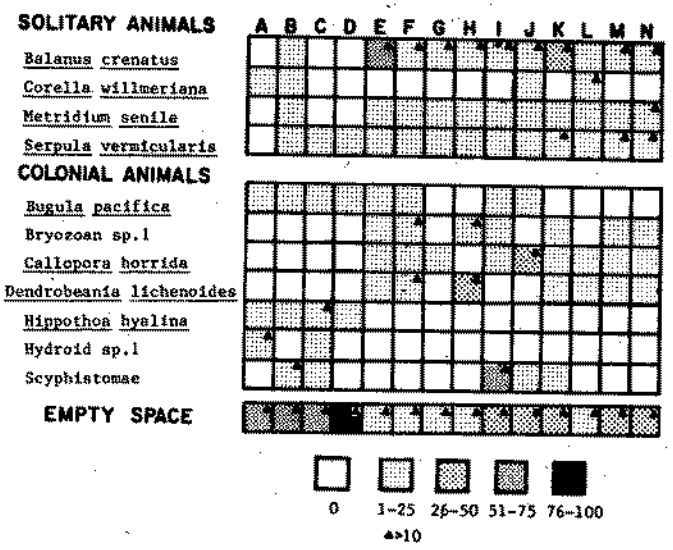
Hylebos Waterway

Community development at Hylebos was similar to that observed at Blair in that it was quite variable, especially in the latter portion of the study (Figure 6). From September, 1980 to March, 1981, most panels underwent similar development, with some panels being temporarily colonized in the fall by rapidly invading, transient species such as Corella willmeriana and Botryllus sp. By March of 1981, all panels were largely barren, and thus characterized by the same species assemblage. During the spring, panels were reinvaded by a variety of species, and by June, space was predominantly occupied by B. crenatus and a number of colonial foundation species: Botryllus sp., Bryozoan sp. 2, Dendrobeania lichenoides, and Reginella nitida. Other than R. nitida on panels 2, 8, and 9, these colonial species declined in relative abundance, until, at the end of study, most panels were dominated by the solitary species B. crenatus, C. willmeriana, or Pseudopotamilla occelata.

Milwaukee Waterway

Community development at Milwaukee progressed more slowly than at any of the other localities (Figure 7). Milwaukee is also

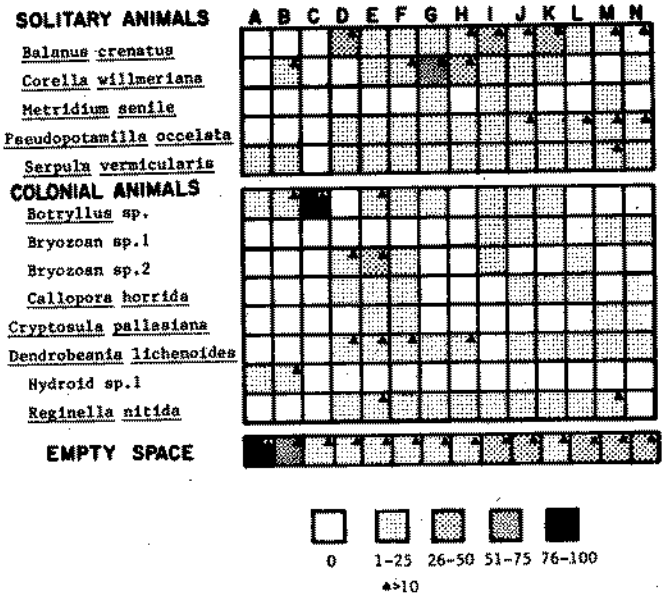
Figure 5. Community development at the Blair Waterway site (figure format is the same as Figure 3).



Panel	Date 9/80	12/80	3/81	6/81	9/81	1/82	3/82	5/82
1	A	C	B	E	G	G	G	G
2	A	D	B	I	J	J	J	I
3	A	C	C	H	L	M	M	N
4	A	D	C	H	F	K	K	N
5	A	D	D	F	F	K	N	N
6	A	C	–	–	–	–	–	–
7	A	D	C	E	F	K	K	K
8	A	D	D	–	–	–	–	–
9	A	A	B	B	L	B	B	B
10	A	D	C	–	–	–	–	–
11	A	–	–	–	–	–	–	–
12	A	C	D	E	G	K	K	H

– panel lost

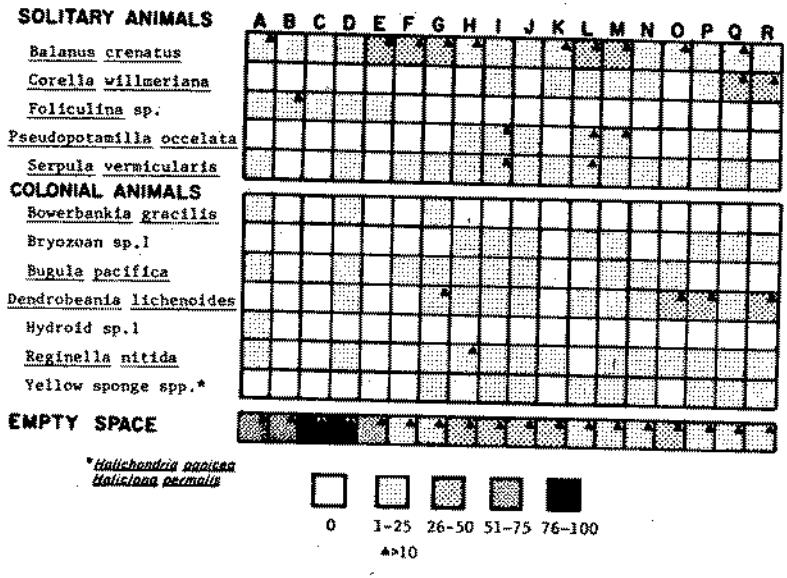
Figure 6. Community development at the Hylebos Waterway site (figure format is the same as Figure 3).



Panel	Date 9/80	12/80	3/81	6/81	9/81	1/82	3/82	5/82
1	A	A	A	A	G	J	J	N
2	A	B	A	D	H	M	M	M
3	A	C	A	D	K	J	J	N
4	B	A	A	I	H	I	I	I
5	A	B	A	D	K	I	K	G
6	B	B	A	D	H	I	K	G
7	A	A	A	D	D	I	I	N
8	A	A	A	E	F	M	M	I
9	A	B	A	E	E	M	M	M
10	B	B	A	D	F	J	J	G
11	A	B	A	L	N	N	L	N
12	B	B	A	D	K	I	K	-

- panel lost

Figure 7. Community development at the Milwaukee Waterway site (figure format is the same as Figure 3).



Panel	Date 9/80	12/80	3/81	6/81	9/81	1/82	3/82	5/82
1	A	F	A	G	Q	K	K	L
2	E	F	F	N	R	I	I	Q
3	A	A	A	O	P	I	I	R
4	A	D	D	N	R	I	I	L
5	C	D	A	O	P	I	I	M
6	B	A	A	H	P	I	I	M
7	B	C	D	G	Q	L	-	-
8	B	D	D	G	Q	L	-	-
9	B	C	D	G	P	H	H	M
10	B	D	D	G	R	I	J	K
11	C	C	D	-	-	-	-	-
12	C	D	A	G	Q	I	-	-

- panel lost

the only fouling community in Puget Sound that we have investigated in which colonial animals failed to dominate the early stages of development (Greene et al., 1983). Throughout the fall and winter of 1980, panels were largely barren with some early colonization by B. crenatus and the spirotrich protozoan, Foliculina sp. In the spring and summer of 1981, there was further recruitment of B. crenatus and addition of the cheilostome bryozoans D. lichenoides and R. nitida as foundation species in the community. Colonies of D. lichenoides were important constituents of the community during the summer, but typically regressed in the fall. The fall of 1981 was also characterized by a decline in B. crenatus abundance and an invasion by C. willmeriana. From this point in the study until the end of our investigation, panels diverged along two major pathways, one dominated by C. willmeriana, the other by B. crenatus. In addition, the solitary, tubiculous polychaetes, P. occelata and S. vermicularis, were also abundant in the latter portion of the study.

DISCUSSION

At the last International Congress on Marine Corrosion and Fouling, we presented our preliminary ideas on variability in fouling community development (Schoener and Greene, 1980). Since that time, we have observed the development of many more panels at a number of localities in Puget Sound (Schoener, 1981; Greene and Schoener, 1982; Greene et al., 1983; this paper). It has become increasingly obvious to us during this period that succession on replicate panels must be treated as a stochastic process (Greene and Schoener, 1982). As such, the major goal of community data analysis becomes distinguishing between the deterministic (i.e., successional) features of the developmental process and the stochastic noise potentially masking these features (Greene and Schoener, 1982). Therefore, as Gauch (1982) emphasizes, it is important that we develop methods to extract patterns from redundancies in the data set while simultaneously reducing stochastic noise. In the final assessment of an analytical method's utility, we ultimately must ask ourselves, "does the method improve our understanding of community processes, or does it only summarize information more compactly?"

As we have shown, the multivariate method just described does effectively summarize information from large, multiple-species data sets. We feel of more importance, however, is its straightforward, conceptual interpretation. Figures 3-7a have equivalent geometrical analogues in multi-dimensional species space. The species assemblages designated in these figures can be interpreted as distinct regions[1] within this n-dimensional hyperspace[2]. Thus, following the transitions through time of each panel, as in Figures 3-7b, can be conceptualized as equivalent to

[1] These regions can be discrete, but typically overlap; the degree of overlap depends on the stopping level chosen for the analysis.

[2] n=community's species richness

observing each panel's trajectory, as a function of time, through n-dimensional species space. This interpretation opens up many potential avenues for exploration. In addition to revealing patterns such as the development of persistent species assemblages (this paper; Greene and Schoener, 1982) and the consistent shift in dominance from colonial to solitary species (see Greene et al., 1983), this method also provides us with an objective approach to address critical problems like community stability and responses to natural and man-made perturbations (Sutherland, 1981; Connell and Sousa, 1983). Specifically, the existence of multiple stable points (Lewontin, 1969; Sutherland, 1974) or global stability (Lewontin, 1969; Holling, 1973) can be objectively defined and analyzed. Likewise, the degree of community resiliency (Holling, 1973) to known perturbations can be objectively measured. The feedback between observation and theory that this method fosters can go a long way toward improving our understanding and predictive capabilities in natural communities.

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FOULING PRODUCTION IN THE WORLD OCEAN

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ABSTRACT

Mytilus mussel communities create some of the most severe fouling problems; their accumulated biomass can reach 15 cm and 40 kg/m^2 in a year. By comparison, warm water coastal fouling communities, often dominated by acorn barnacles and oyster-type bivalves, present a lower profile and weigh only up to about 20 kg/m^2 per year in turbid water, and only about half as much in clear, apparently less fertile water. In the open sea, stalked barnacles sometimes attach and grow to a weight-in-water of about 2 kg/m^2 in just a few months. Below the mixed layer, hydroids and anemones, and occasionally barnacles and tubeworms accumulate slowly.

RÉSUMÉ

Les communautés de Mytilus donnet naissance à des problèmes de salissure considerés comme parmi les plus intenses. Leur accumulation en biomasse peut atteindre 15 cm et 40 kg/m^2 en une seule année. En comparaison, dans les eaux côtières chaudes, les communautés de salissures marines, où dominent souvent les cirripèdes sessiles et les mollusques bivalves, sont moins abondantes; leur poids s'élève à moins de 20 kg/m^2/an dans les eaux troubles et à environ la moitié de ce poids dans les eaux claires et apparemment moins fertiles. En haute mer, les cirripèdes pédoncules s'attachent et peuvent atteindre un poids de 6 kg/m^2 en quelques mois. Par contre, sons la couche melangée, l'accumulation de cirripèdes, bivalves, serpules tubicoles, hydroides et anémones de mer est très lente.

An important penalty of fouling buildup on marine structures is an increase in hydrodynamic drag. Heaf has shown that a 15 cm-thick overgrowth of fouling organisms, if evenly distributed on tubular members of a fixed steel offshore platform, contributes a wave loading of 17.46%, assuming a lifetime wave of 30 m (1). Eikers states that this much wave loading requires an additional 50 mm of steel (2), which for a typical North Sea production platform could mean $1.5 million in additional start-up costs (3). From an engineering point of view then, it is clearly a matter of importance to understand the vagaries of fouling production in the world ocean.

Marine biologists are now able to help. Although quantitative data on fouling production is still in short supply, certain horizontal and vertical trends are becoming more and more apparent.

Using weight and thickness of settlement as production criteria - we know, for instance, that the most productive areas are the shallow, cold water environments of both hemispheres, where Mytilus edulis species-complex mussels are usually the dominants. These mussels are usually superabundant in the plankton in summer, and their special attachment mode (byssal threads) encourages multilayered settlement. Complex physical and biotic factors tend to complicate local settlement patterns but a thickness design allowance of 15 cm is appropriate for cold coastal waters in the upper 25 m of the water column (4-8). In protected embayments, mussel clusters may become somewhat larger (9). A 15 cm thickness of cold water mussels has a wet weight of about 40 kg/m^2 (4-9). The circumpolar ranges of cold water mussels is shown in figure 1.

Somewhat less productive are warm (15° C. mean annual temperature) coastal waters, where barnacles and oyster-type bivalve mollusks are usually the dominants. These dominants attach by cementing themselves directly to the substrate, which results in a lower profile - up to 10 cm for very large species but more often about 5 cm of mostly calcareous biomass, plus an irregular overgrowth of soft-bodied coelenterates, tunicates, and sponges. Multi-layering to greater thicknesses tends to cause suffocation of the early colonizers, and subsequent exfoliation.

In turbid (secchi values of 20 m or less) warm waters, a 5 cm thickness of barnacle/oyster dominated fouling community weighs about 20 kg/m^2 (10-12). However, in clear (secchi values in excess of 20 m), warm coastal waters, a 5 cm thickness of the same dominants produces only about half as much biomass (13-15). Clear waters are apparently less fertile, and therefore less weight productive. The limits of turbid and clear coastal waters as developed by Lepley (16) are shown in figure 1.

Fouling productivity is considerably reduced in the open sea. Stalked barnacles (Lepas and Conchoderma) are usually the dominants in the surface layer. These barnacles are found in all seas (17) and they are occasionally very abundant in tropical and temperate regions. In some parts of the North Atlantic Ocean, for instance, they cover new substrates to a thickness of about 5 cm in just a few months (18). The weight-in-water of stalked barnacles seldom exceeds 2 kg/m^2, however (18).

Below the surface layers, fouling rates diminish progressively with distance from fouling broodsites. Figure 2 shows the quantitative vertical changes in fouling production in coastal regions where currents flow generally parallel with the shoreline. Where current regimes and shorelines are more complicated, this pattern becomes less apparent (19). Figure 3 shows a typical open sea distribution within the mixed layer. This rapid diminution of taxa and biomass with increasing depth has been widely observed (11,18,20).

The least productive regions are below the mixed layers. Plant-like, almost weightless-in-water coelenterates are the dominant - often exclusive - fouling taxa (21-23). They attach and grow very slowly. Substrates exposed directly on the deep sea floor will sometimes also be colonized by small bivalves or barnacles or tubeworms, but even after several years we have observed no more than 0.3 kg/m^2 of biomass accumulation (21,22). This small amount of fouling biomass is easily accommodated by conservative design and careful material selection.

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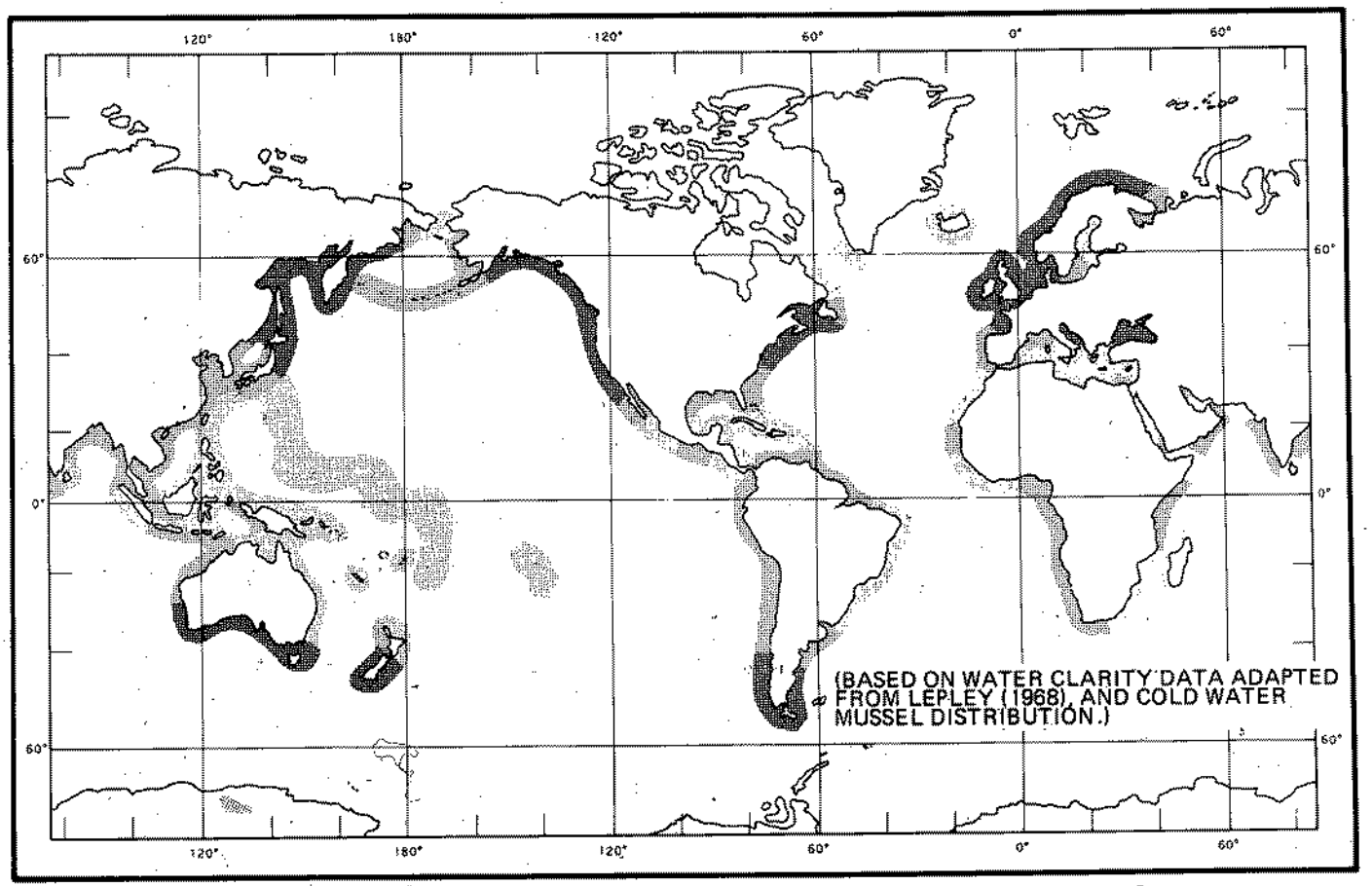
FIGURE 1: BIOFOULING PRODUCTION



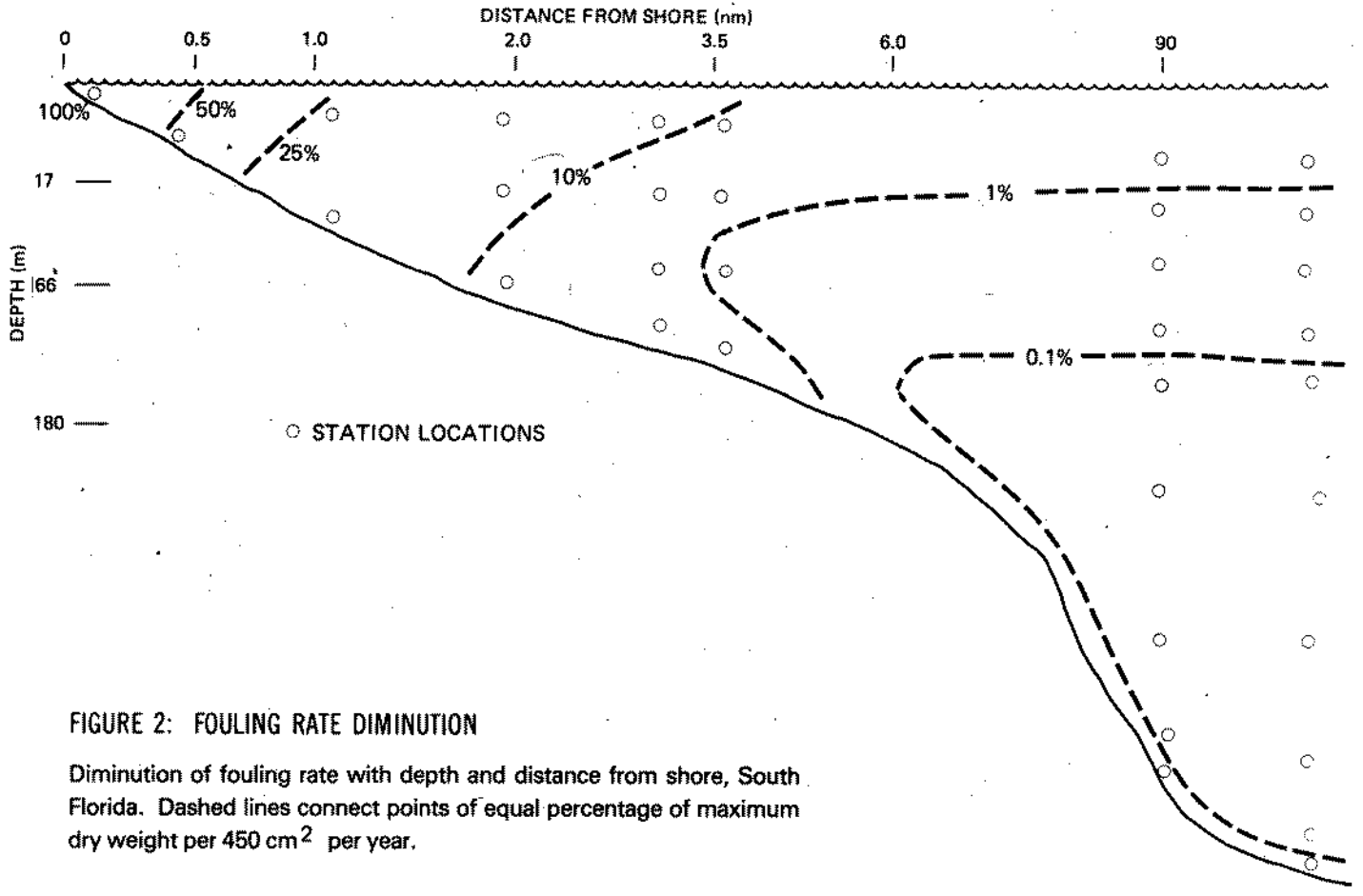


FIGURE 2: FOULING RATE DIMINUTION

Diminution of fouling rate with depth and distance from shore, South Florida. Dashed lines connect points of equal percentage of maximum dry weight per 450 cm^2 per year.

FIGURE 3: OPEN SEA FOULING DISTRIBUTION

Sample depth (m)	Dry weight (gm/m^2)	Principal Taxa: bivalve mollusks	barnacles	marine borers	hydroids
0		X	X	X	X
6	4,065	X	X	X	X
10	630	X	X	X	X
15	594	X	X	X	X
22	401	X	X	X	X
38	330	X	X	X	X
47	127	X	X	X	X
65	226	X	X	X	X
96	424		X	X	X
126	75			X	X
130	----------maximum depth of mixed layer----------				
157	6				X
187	0				
340	0				

Vertical distribution of macrofouling organisms in the upper 340 m. of the water column at a test site 160 nautical miles west of Tampa, Fla., in the Gulf of Mexico

Isolation and Characterization of Adhesive Proteins Secreted by the Sea Mussel, *Mytilus edulis*

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The sea mussel, *Mytilus edulis*, produces a complex attachment device called byssus, which is composed of three primary parts - the stem, byssal thread, and adhesive disc. The adhesive disc provides a tenacious cement, holding the byssal thread to a variety of surface structures. Characterization of the adhesive has been limited due to its extreme resistance to solubilization. Techniques were therefore developed to isolate prepolymerized adhesive proteins from the distal depression of the mussel foot. These adhesive proteins were fractionated by gel permeation and ion exchange chromatography and analyzed by SDS polyacrylamide electrophoresis and electron microscopy and examined by amino acid analysis. Two dominant proteins were observed at molecular weights of 37,000 and 16,500 daltons. The higher molecular weight protein was abundant in histidine, tyrosine and acidic amino acids. The low molecular weight protein was abundant in lysine and acidic amino acids. Electron micrographs showed unique interactions of fibrous and globular units kto form highly organized structures.

In more recent investigations, pepsin digests of adhesive discs have been examined and show one primary protein of approximately 40,000 daltons. This protein exhibits an amino acid profile typical of glycoprotein and also stains positive with periodic acid Schiff reagent. It is suggested that the adhesive disc is primarily a glycoprotein and that adhesive formation occurs via the interaction of a phenolic protein and a lysine-rich protein. The adhesion appears to be associated with the glycoprotein character of the material and the extreme stability related to the extensive crosslinking of the protein components. This work was supported by Grant DE-05800 from the National Institute of Dental Research.

RESUME

Les moules de mer, Mytilus edulis, produisent un organe d'attache complexe appelé "byssus", qui sé compose principalement de trois parties: la tige, le filament de byssus et le disque d' adhésif. Le disque d' adhésif fournit un ciment tenace qui retient le filament de byssus à une grande variété de structure de suface. La détermination des caractéristiques de l' adhésif a été limitée à cause de sa grande insolubilité. Des techniques ont été développéesdepuis pour isoler les protéines d'ashésif prépolymerisées de la dépression distale du pied de la moule. Les protéines d'adhésif étaient fractionnées par pénétration du gel et par la chromatographie par échange d'ions et ensuite analysées par électrophorèse de polyacrylamide de SDS et par microscopie électronique et enfin examinées par l'analyse de l'amino-acide. Deux protéines les plus courantes ont été observées ayant des poids moléculaires de 37.000 et 16.500 daltons. Le poids moléculaire le plus élevé de protéine se trouvait abondamment dans l'histidine, la tyrosine et les amino-acides. Les micrographes électroniques avaient montré des intéractions uniques de fibres et d'unités globulaires pour former des structures très organisées.

Dans les recherches plus récentes, les digestions de pepsine des disques d'adhésif ont été examinées et révèlent une protéine primaire de poids moléculaire environ 55.000 daltons. Cette protéine révèle une caractéristique d'amino-acide typique de glycoprotéine et donne aussi des traces positives avec le réactif d'acide périodique de Schiff. On a suggéré que le disque d' adhésif se compose principalement de glycoprotéine et que la formation d'adhésif s' est faite grâce à l'intéraction d'une protéine phénolique avec une protéine riche en lysine. La force d' adhésion semble être associée avec la caractéristique de protéine du matériel et de l'extrême stabilité relative à l'entrelacement serré des composants protéines.

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Introduction

Marine mussels and other related bivalves attach themselves to littoral substrata by means of a complex structural device termed the "byssus." The process of byssus formation occurs during an intriguing series of events under control of a specialized organ known as the foot. The byssus of Mytilus edulis consists of three primary parts: the stem, the attachment thread, and the adhesive disc. The adhesive disc or plaque provides the cementing interface between the threads and the underlying surface. Studies to elucidate the mechanism of formation and the chemical composition of the bonding substance have included morphological (Tamarin et al., 1976), histochemical (Brown, 1952; Pujol, 1967; Pujol et al., 1970) and biochemical (Pikkarainen et al., 1968; Schwartz et al., 1972; Sherwood et al., 1973; DeVore and Gruebel, 1978; Waite and Tanzer, 1981) investigations.

Adhesive formation appears to result from the interaction of at least three exocrine secretions: a polyphenolic protein, a mucoid substance, and perhaps collagen (Tamarin et al., 1976). In addition, an enzyme component, identified as polyphenol oxidase, has been histochemically detected in the mussel foot (Smyth, 1954). The collagen component does not appear to be a primary constituent of the adhesive disc and probably represents an extension of the collagenous thread into the cementing substance (Tarmarin et al., 1976).

Chemical characterization of the hardened adhesive has been limited due to the extreme resistance of the material to dissolution in all protein solvents (Bowen, 1952). Recently, Waite and Tanzer (1981) isolated acid-soluble dopa-containing proteins from the phenol gland of the foot of Mytilus edulis. These proteins were high molecular weight (135,000 and 125,000 daltons) and rich in lysine, tyrosine, serine, and threonine. In the present investigation, methods are described for extracting prepolymerized adhesive components from the foot of Mytilus edulis. The adhesive components were separated by gel permeation chromatography and analysed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and amino acid analysis. In addition, thread secretions were isolated from the foot and examined by electron microscopy after several incubation time periods.

Materials and Methods

Materials and Supplies

Sea mussels (Mytilus edulis) were provided by Battelle's W.F. Clapp Laboratories, Inc., Duxbury, Massachusetts. After air shipment, the mussels were kept at 12°C in a seaquarium.

Enzymes and chromatography resins were purchased from Sigma Chemical Co., St. Louis, Missouri. Electrophoresis supplies were purchased from Bio-Rad Laboratories, Richmond, California.

Isolation of Adhesive Components

Previous investigations to characterize the sea mussel adhesive disc have been hindered by the resistance of this material to dissolution. Therefore, methods were developed for extracting and maintaining the adhesive secretion in a fluid state. Earlier studies had indicated the participation of polyphenol oxidase in the formation of the adhesive disc (Brown, 1952; Smyth, 1954). Both mushroom and mammalian polyphenol oxidase contain copper as a cofactor for activity. Thus, substances known to inhibit these enzymes via formation of complexes with copper were examined for their ability to inhibit the mussel enzyme and perhaps prevent the setting process. Cysteine at 0.002 M and 0.1 M, glutathione at 0.01 M, and diethyldithiocarbamate at 0.001 M did not affect the activity of the mussel (Engle et al., 1971). Subsequent investigations showed that cyanide (NaCN) at 0.005 M would provide enzyme inhibition (Schwartz et al., 1972). Potassium chloride has been reported to stimulate the secretion of adhesive components by the sea mussel (Tamarin et al., 1976). Based on these findings, adhesive secretions were collected by injecting 100 ul of an equal mixture of 0.005 M NaCN and 0.6 M KCl or 0.6 M KCl alone. Immediately after the injection, adhesive secretions were collected with micropipettes and stored in micro test tubes at -70°C. In other studies, secretions were immediately placed in a proteinase inhibitor cocktail containing 0.05 M Tris-HCl, pH 7.5; 1.0 M NaCl; 0.001 M phenylmethanesulfonylfluoride (PMSF); 0.01 M N-ethylmaleimide; and 0.025 M ethylenediaminetetracetic acid (EDTA) and stored at -70°C.

Digestion of Adhesive Discs

Adhesive discs were collected from the walls of the seaquarium and from mussel shells. The discs were trimmed to remove byssal threads and placed in the proteinase inhibitor mixture desdribed above. Cleaned discs were then washed and placed in 4M guanidine-HCl-Tris (0.1M) at pH 7.2. Discs were extracted in this mixture for 48 hours at 4°C. The disc residue was recovered by centrifugation at 20,000 x g and suspended, after washing, in 0.5 M acetic acid containing pepsin (Sigma) at 1 part per 50 parts wet disc material. The supernatant was saved and stored at -70°C. Pepsin digestion was continued at 4°C for 48 hours at which time additional pepsin (1 part per 100 parts of wet disc material) was added. Digestion continued for an additional 24 hours. The digest was centrifuged at 20,000 x g to separate the pepsin extract from the residue. The residue was washed with distilled water and extracted once again in 4M guanidine HCl. The supernatant was saved and stored at -70°C. Finally, the second 4M guanidine HCl

extract was recovered by centrifugation and stored as above.

Extracts of adhesive discs were examined by SDS polyacrylamide gel electrophoresis and by amino acid analysis as described below.

Fractionation of Adhesive Components

Pooled samples of adhesive secretion were fractionated at 4°C on a Sephadex G-150 column (0.9 x 25 cm), equilibrated and eluted with 0.005 M Tris-HCl, ph 7.2. Fractions were pooled, concentrated, and dialyzed against 0.2 M acetic acid. Acidified samples were placed on a CM Bio-Gel A column (0.8 x 25 cm), equibrated with 0.2 M acetic acid, and eluted at 4°C with a continous gradient of 0.5 M NaCl in 0.2 M acetic acid. Protein fractions were detected and collected as described above, and then pooled and concentrated for further analyses. Samples were stored at -70°C.

SDS Polyacrylamide Gel Electrophoresis

Adhesive secretions, isolated fractions, and digests of adhesive discs were evaluated by SDS polyacrylamide gel electrophoresis using methods described by Anderson and Peterson (1981). Samples were applied to regular and linear gradient 8-20% acrylamide slab gels and electrophoresed at 40 mA for 4-6 hours at room temperature. The gels were then fixed and silver stained using techniques decribed by Morrisey (1981).

Amino Acid Analysis

Samples for amino acid analysis were hydrolyzed in 6 N HCl for 22 hours at 110°C under a nitrogen atmosphere. Analysis was performed using the Dionex D330 amino acid analyzer.

Results

Extraction of Adhesive Secretion

Adhesive secretions were collected after injecting potassium chloride and sodium cyanide into the foot of the sea mussel. Potassium chloride acted to stimulate release of adhesive components from storage vesicles and sodium cyanide prevented polymerization of adhesive components. When sodium cyanide was not included, *in situ* byssus formation occurred. In the presence of sodium cyanide, a viscous and clear fluid was collected.

Fractionation of Adhesive Components

Fractionation of the adhesive secretion on Sephadex G-150 yielded the elution pattern shown in Figure 1. Two major protein fractions

were obtained. Standard molecular weight curves were prepared for each column by chromatographing the following protein markers: blue dextran (2,000,000), bovine serum albumin (65,000) and lysozyme (14,100). Estimated molecular weights of the two primary proteins fractionated from the adhesive secretion were 100,000 and 12,500. The fractions were designated F_I and F_{II}. Subsequent separation of each fraction on CM Bio-Gel A produced elution profiles shown in Figures 2 and 3. As observed, there appeared to be distinct entities in each fraction. Fractionation of F_I on CM Bio-Gel A always produced multiple protein bands. In contrast, F_{II} generally resulted in one protein band (Figure 3).

SDS Polyacrylamide Electrophoresis

Electrophoresis of F_I and F_{II}, unfractionated adhesive, and pepsin digests of adhesive discs producted the profiles shown in Figure 4. Fraction F_I contained a particularly dense protein band at approximately 37,000 daltons and a band at a molecular weight greater than 100,000 daltons. Fraction F_{II} exhibited major bands at approximately 18,500 and 16,000 daltons. The unfractionated adhesive appeared as a composite of F_I and F_{II}. Adhesive secretions collected in the proteinase inhibitor cocktail exhibited banding patterns identical to adhesive secretions collected and stored without proteinase inhibitors.

The adhesive disc digest exhibited a predominant band at approximately 40,000 daltons and numerous, less dense bands at lower molecular weights. The major band stained positive with periodic acid-Schiff reagent (Fairbanks et al., 1971), indicating a glycoprotein composition. In several electrophoretic experiments, this digest appeared to demonstrate retarded movement through the polyacrylamide with the tracking dye being several centimeters behind the tracking dye of other samples. Glycoproteins commonly behave anomalously during SDS electrophoresis, exhibiting decreased mobility (Segrest and Jackson, 1972).

Amino Acid Analysis

Amino acid profiles for the adhesive secretion, F_I, F_{II}, the adhesive disc, and digested disc are shown in Table 1. The adhesive disc was characterized by abundant quantitites of glycine, tyrosine, and basic amino acids. Isolated secretion was abundant in acidic amino acids and rich in histidine and lysine. Thus, fraction F_I resembled the adhesive secretion while fraction F_{II} was rich in lysine and less abundant in histidine and phenylalanine.

Amino acid analysis of the disc digests showed high levels of glycine, other aliphatic amino acids, acidic amino acids and tyrosine. The high aliphatic amino acid content (40.2%) and the

abundant levels of dicarboxylic amino aicds is typical of structural glycoproteins.

The amino acid composition of the disc was similar to that reported by Bdolah and Keller (1976) and by Waite and Tanzer (1981). The profiles presented in Table I showed lower glycine levels and higher levels of phenylalanine and arginine that previous reports.

Discussion

Chemical composition of the adhesive formed by the sea mussel, Mytilus edulis, has been difficult due to the extreme resistance of this substance to dissolution. Previous biochemical investigations have been preimarily limted to amino acid analyses (Engle et al., 1971; Bdolah and Keller, 1976) and determination of hexosamine content (Krivis and Martz, 1972). Morphological studies by Brown (1954) and Tamarin et al. (1976), and histochemical studies by Smyth (1954), Pujol (1967), and Engle et al. (1971) have provided evidence that the adhesive disc is produced by protein secretions of a specialized gland called the "phenol" gland. Recently, Waite and Tanzer (1981) extract "phenol-rich" proteins from excised phenol glands of the Mytilus edulis. These acid-soluble proteins contained high levels of basic amino acids and large quantities of tyrosine, dopa, and hydroxyproline. There appeared to be two high molecular weight polyphenolic proteins with apparent molecular weights of 135,000 and 125,000 daltons. Lower molecular weight protiens were also observed in polyacrylamide gels.

In the present investigation, soluble adhesive proteins were isolated from the distal depression of the mussel foot. Sodium cyanide was used to prevent enzyme catalyzed polymerization. The protein secretion produced two distinct bands when chromatographed on Sephadex G-150. The high molecular weight band was heterogenous as revealed by SDS polyacrylaminde gel electrophoresis. The low molecular weight protein was relatively pure and particularly abundant in lysine with moderate levels of tyrosine (4%). The primary protein of the high molecular weight complex appears to be a 37,000 dalton protein.

It appears that adhesive formation involves reaction of the 37,000 molecular weight protein with the lysine-rich, low molecular weight protein. The low molecular weight protein likely servies as a coupling or tanning agent via an enzyme catalyzed reaction. The high tyrosine content of the adhesive disc suggests that the 37,000 molecular weight protein, the primary component of the high molecular weight complex of the adhesive secretion, also contains a high level of tyrosine. This protein has not yet been isolated in amounts sufficient for chemical characterization. Current evidence indicates that a number of structural proteins are

stabilized by tyrosine and tyrosine derivatives (Andersen, 1974; Waite and Tanzer, 1984; Welinda et al., 1976; Goldberg, 1978 and 1979; DeVore and Gruebel, 1978; and Tidball, 1982). The proposed mechanisms involve formation of tyrosine-derived crosslinks by oxidation of dihydroxy compounds, i.e., tyrosine oxidation to quinones which are the putative crosslinking compounds. Oxidation may be catalyzed by a phenol oxidase. This type of mechanism for forming sea mussel adhesive is suggested by the present investigation wherein the tyrosine-rich proteins react with the lysine-rich coupling protein or the tyrosine-rich proteins undergo intermolecular interaction via dimerization of quinone structures.

Pepsin digests of hardened adhesive discs were primarily composed of a protein constituent with an apparent molecular weight of approximately 40,000 daltons. This protein stained positive with periodic acid-Schiff reagent and contained an amino acid profile similar to other glycoproteins. The amino acid composition was high in aliphatic amino acids (40.2%) and abundant in dicarboxylic amino acids. A glycoprotein composition would tend to support earlier observations showing the presence of significant carbohydrate levels in adhesive secretions (Engle et al., 1971). Tamarin et al. (1976) also suggest that a mucosubstance plays a major role in the adhesion of sea mussel cement. Furthermore, a glycoprotein composition would generally by consistent with the chemical content of other adhesive materials found in nature. For example, barnacle cement has been reported to contain significant amounts of carbohydrate (Walker, 1981) and drosophila glue contains several glycoprotein constituents (Beckendorf and Kafotos, 1976). It is suggested that the glycoprotein component represents a portion of the adhesive complex formed by the interaction of the proteins discussed above. Studies are now in progress to characterize the glycoprotein and to determine its contribution to the adhesive process and to the adhesive structure.

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Table 1. Amino acid composition of adhesive secretion, fractions of adhesive secretion, and adhesive disc (residues/1000 residues)

Amino Acid	Secretion	F_I	F_{II}	Adhesive Disc	Disc Digest
Aspartic Acid	120	123	140	106	142
Threonine	55	52	59	34	63
Serine	55	39	54	60	58
Glutamic Acid	123	131	149	53	100
Proline	39	32	30	34	66
Glycine	44	34	76	115	96
Alanine	55	57	63	50	52
Half-Cysteine	0	0	0	21	
Valine	66	66	47	57	74
Methionine	4	6	4	7	10
Isoleucine	53	57	51	27	81
Leucine	71	72	73	56	99
Tyrosine	39	32	40	96	53
Phenylalanine	68	76	52	58	58
Histidine	104	140	32	71	9
Lysine	72	60	103	67	15
Arginine	33	25	48	110	18

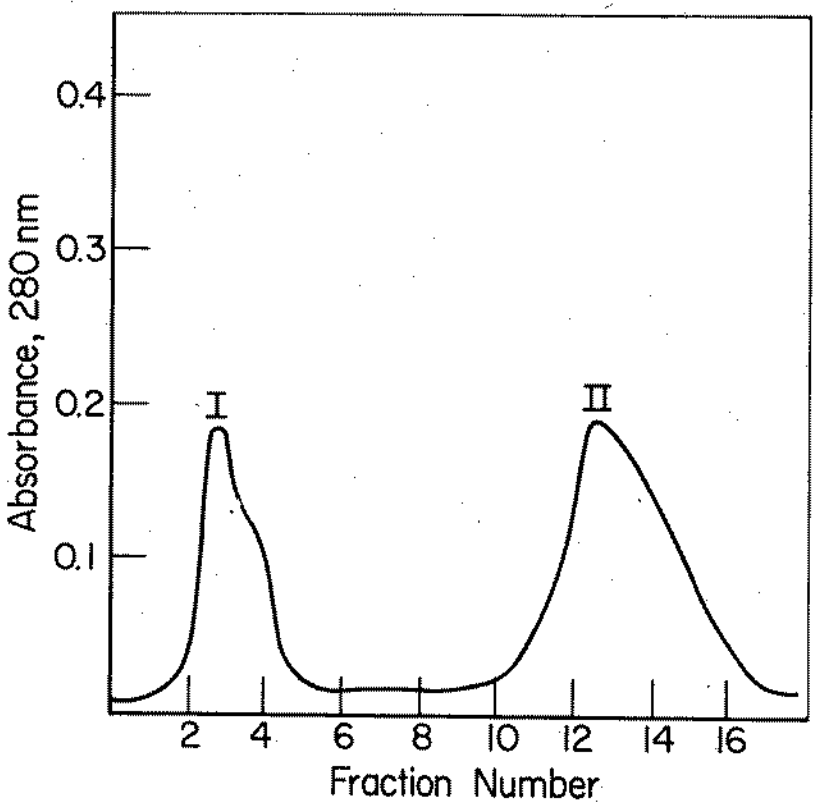

Figure 1:

Elution pattern for the adhesive secretion of Sephadex G-150.

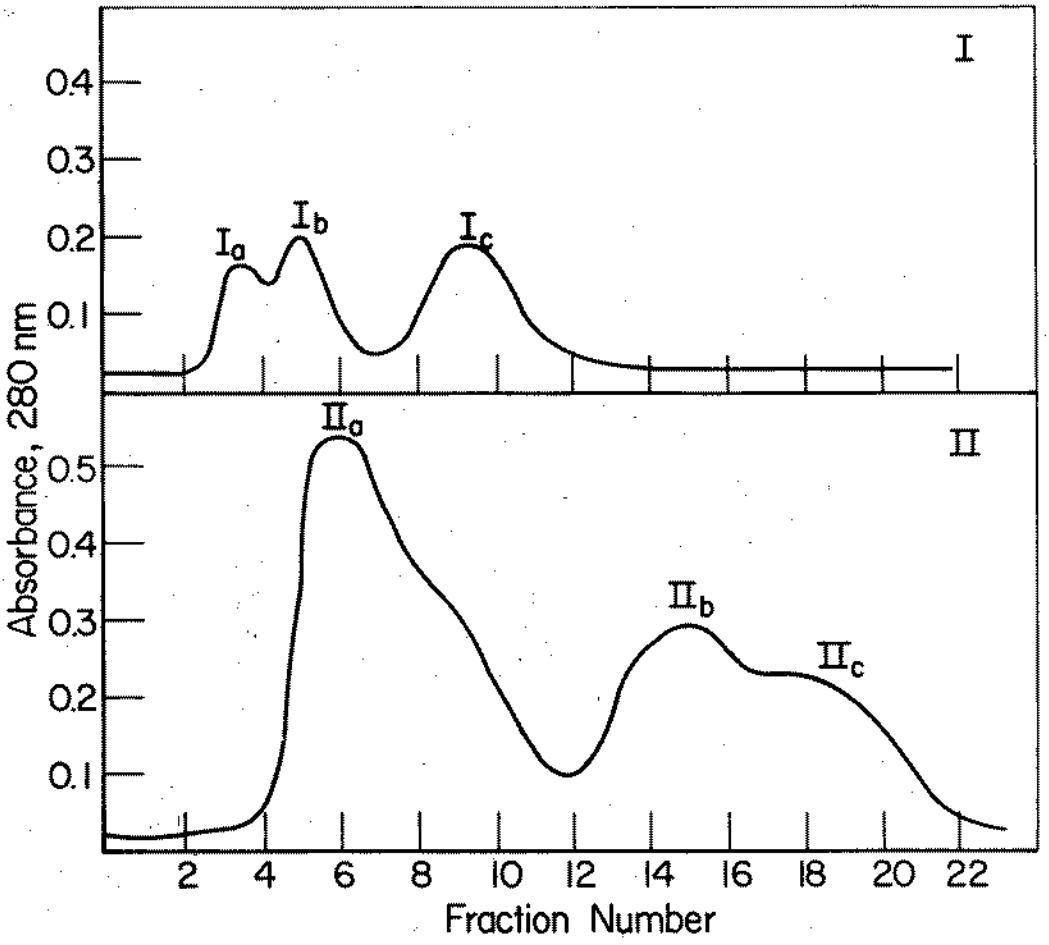

Figure 2: (Top Profile)

Elution pattern of the adhesive protein fraction F_I on Bio-Gel A.

Figure 3: (Bottom Profile)

Elution pattern on the adhesive protein fraction F_{II} on Bio-Gel A.

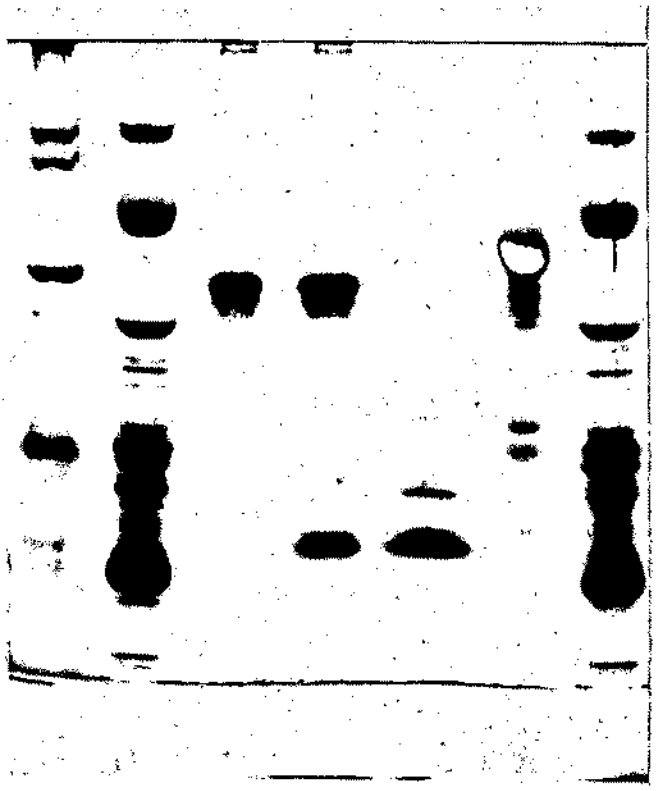

Figure 4:

SDS-polyacrylamide gel of adhesive proteins. (Lane 1) high molecular weight standards; (Lanes 2 and 7) low molecular weight standards; (Lane 3) high molecular weight protein fraction from adhesive secretion; (Lane 4) adhesive secretion; (Lane 5) low molecular weight protein fraction from adhesive secretion; (Lane 6) enzyme digest of adhesive disc.

ANTIFOULING COATINGS

PEINTURES ANTISALISSERES

Foul Play: Some Consequences of Life Histories Among Temperate Bryozoans

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The demographies of colonial animals are often size rather than age dependent. The timing of sexual reproduction in Dendrobeania lichenoides, a marine cheilostome bryozoan, is size dependent. In contrast, the timing of reproduction in Membranipora membranacea, another cheilostome bryozoan, is independent of size and triggered by intraspecific crowding. Size and age independent timing of reproduction may be an adaptation possessed by many colonial invertebrates inhabiting temperate, fouling communities that are characterized by unpredictably available free space. Indeterminacy in the timing of reproduction allows rapid occupation of substrata when available or early onset of reproduction in the absence of available substrata.

Les demographies des invertébrés coloniaux sont souvent dépendant sur la taille plutôt-que l'âge. La reproduction sexuelle en Dendrobeania lichenoides est dépendant sur la taille. Par contre, la reproduction sexuelle en Membranipora membranacea est indépendant de la taille et induit par l'encombrement de la même espèce. La reproduction sexuelle, indépendant de la taille et de l'âge, est peut-être un adaptation des invertébrés coloniaux qui habitent en les communités tempéré sur les bassins le quelles sont characterize par l'espace libre non predictionablement.

INTRODUCTION

Classical demographic theory as developed by Euler (1760) and Sharp and Lotka (1911) is based on the premise that life history characters are age-dependent. In some marine, colonial animals with indeterminate growth, size is often decoupled from age and timing of first reproduction is strictly size-dependant (Winston and Jackson, in press; Grosberg, 1982). This has led to the development of demographic models based on size rather than age (Kirkpatrick, in press; Hughes, in press). Other organisms with size- as well as age- based demographies are fish (Sohn and Crews, 1977) and plants (Wareing and Phillips, 1970; Werner, 1975; Harper, 1977). In other colonial animals, timing of reproduction is size-independent (Yamaguchi, 1975; Grosberg, 1982) and determined by other variables such as the unavailability of empty substrata for further lateral expansion. Size- independent onset of reproduction violates the critical assumption of current, size-based population models for colonial animals.

We will present data on the timing and size of first reproduction in two common bryozoan species from temperate fouling communities, Membranipora membranacea and Dendrobeania lichenoides. These two species display size- independent and size-dependent timing of first reproduction, respectively. The central question addressed here is: What triggers the onset of reproduction in a size-independent species and what are the ecological consequences of size- dependent verses independent timing of reproduction?

RESULTS

Colonies of Dendrobeania lichenoides were collected underneath the floating breakwaters at Friday Harbor Laboratories, Washington, USA in July 1983. Dendrobeania, an anascan cheilostome, forms large (3 cm diameter), foliose fronds firmly attached to the substratum at the base and supported along the blade by rhizoids. Colonies of Membranipora membranacea were collected from kelp blades in the vicinity of the breakwater in July 1983. Membranipora is an encrusting anascan bryozoan that occurs most obviously on algal blades, but is also an important component of the fouling community. Reproductive condition was easily determined in Dendrobeania by noting the presence of brooded embryos on colonies for each of 5 size classes. The size classes in mm were 6-10, 11-15, 16-20, 21-25, 26-30. These correspond to size classes 1-5 in Table 1. Reproductive condition in Membranipora was more difficult to determine because unlike Dendrobeania, eggs and sperm are free-spawned, thus there are no large embryos on the surface of the colony. Instead we determined reproductive condition for each of

5 size classes by noting the presence or absence of eggs within the zooecia using a 10x objective. The size classes in mm were: 1-10, 11-20, 21-30, 31-50, 51-65. Discrete intervals actually sampled within each of these size classes were: 2-6, 15-18, 27-30, 36-47, 55-65. These correspond to size classes 1-5 in Table 1.

Table 1. Comparison of the size at which reproduction begins in Dendrobeania lichenoides and Membranipora membranacea.

Size Class	% Colonies Reproductive			
	Dendrobeania		Membranipora	
	%	n	%	n
1	0	3	43	21
2	43	23	52	25
3	90	10	50	24
4	92	14	0	17
5	100	5	67	3

From Table 1 it is apparent that Dendrobeania has a predictable size of first reproduction: over 90% of colonies exceeding 16 mm are reproductive, no colonies under 10 mm are reproductive. The proportion of reproductive colonies increases consistently with colony size (Spearman Rank Correlation (r_s)=1; $p<.05$). In contrast, it is impossible to predict a size at which colonies of Membranipora will be reproductive: 43% of the smallest colonies censused are reproductive, and only 67% of the largest colonies censused are reproductive. The proportion of reproductive colonies is only poorly correlated with colony size (Spearman Rank Correlation (r_s)= 0.30; $p>.05$). Thus from these data we would conclude that the onset of reproduction in Dendrobeania is strictly size-dependent, and that the onset of reproduction in Membranipora is size-independent and likely governed by other cues.

One factor that drastically affects the relationship between size and reproductive status in Membranipora is intraspecific crowding. Hundreds of colonies often grow on a single algal blade. When colonies contact each other, growth is inhibited at the contact point. As a consequence most colonies are surrounded

on all edges and never reach their full size potential. Thus if we separate Membranipora colonies into two groups, crowded (surrounded on all sides by conspecifics) and non-crowded (not obstructed by conspecifics) and examine the relationship between size and reproductive condition, much of the indeterminacy disappears (Table 2). Comparing reproductive state of crowded and uncrowded colonies in each of the first three size classes we see a significant difference (X^2 Test, $p < 0.01$, $df=2$). Only the first three size classes were compared because larger colonies are not present on crowded kelp blades.

Table 2. Influence of crowding by conspecifics on size at first reproduction in Membranipora membranacea.

Size Class	% Reproductive Colonies of Membranipora			
	Crowded		Uncrowded	
	%	n	%	n
1	80	10	9	11
2	100	13	0	12
3	91	11	15	13
4	-------		0	17
5	-------		67	3

From Table 2 it is clear that crowding by conspecifics triggers reproduction in colonies, irrespective of colony size. In uncrowded colonies, timing of reproduction is probably size-dependent, such that most colonies with a diameter in excess of 55 mm are reproductive. The important fact to note here is that strict size dependence of reproduction is suppressed by intraspecific crowding through obstruction of the colony edge. This phenomenon may well be an evolutionary response to high variance in intraspecific population density and therefore in opportunities for maximal lateral growth. Although Dendrobeania colonies may become crowded, the flexible, foliose morphology permits lateral growth to continue, therefore the consequences of crowding are less severe than for Membranipora.

Because there is often a tradeoff in resources devoted to asexual and sexual reproduction, many colonies stop growing laterally after the onset of sexual reproduction. The ability to adjust the timing of this allocation shift to match the potential for future growth is a tremendous advantage for a

colonizing species. When Membranipora colonizes a clear substratum, all resources are devoted to growth and this allows rapid monopolization of available space. As an example of Membranipora's colonizing abilities, a population of newly recruited colonies were observed to monopolize 79% of the area within 16 days and 95% of the area within 26 days on a series of fouling panels submerged below the breakwater at Friday Harbor Laboratory (Table 3). In this case spatial monopolization was accomplished both by lateral growth of individual colonies and recruitment of new colonies. The rate of colony expansion (Yoshioka, 1982) would potentially allow 5-10 colonies to monopolize this same area in two weeks. In contrast, because of indeterminacy in the timing of reproduction, colonies growing in a crowded habitat can allocate resources to sexual reproduction at a small size and, by initiating reproduction early, produce several generations in a single season.

Table 3. Colonization of conditioned substrata by Membranipora membranacea- percent coverage* of 12 cm diameter circular plates.

DATE	% cover Membranipora per panel						% cover Membranipora $\bar{x} \pm$ s.d.	% empty space $\bar{x} \pm$ s.d.
6/23	0	0	0	0	0	0	0	100
7/8	13	55	79	76	63	66	58.7±24.0	40.0±33.0
7/18	86	87	95	93	90	69	86.7± 9.3	12.0±8.9
7/28	63	98	99	97	96	59	85.3±18.9	13.0±18.2

*Percent cover determined by censusing 100 random points on each panel.

DISCUSSION

Timing of sexual reproduction can be size-dependent or independent in colonies of marine invertebrates other than bryozoans. Yamaguchi (1975) reports size-independent timing of reproduction in Botrylloides violaceaus and Leptoclinum mitsukurii. When colonies are grown on a limited substratum, they begin reproduction, irrespective of size, as soon as free space is exhausted. Grosberg (1983) reports a life history polymorphism in the compound ascidian species Botryllus schlosseri such that both semelparous (one clutch/individual) and iteroparous (many clutches/individual) colonies exist.

The timing of first reproduction is highly size-dependent in semelparous colonies, all colonies with greater than 220 zooids are reproductive and then die. In contrast, iteroparous colonies grow asexually until they cover the substratum and then reproduce independent of size.

Although size-independent reproduction has been observed, crowding-induced reproduction has not been reported previously in colonial invertebrates. It appears that while substratum limitation may generally trigger reproduction in size-independent reproducers, the response to crowding by conspecifics may be more complex and less predictable. For example, it is possible that intraspecific crowding will result in delayed reproduction if energy is allocated to crowding-induced aggression (McFadden, in prep., Francis, 1975; Wellington, 1978; Chornesky, 1983).

Crowding in plants also disrupts normal size-dependent reproductive patterns. The effects of crowding in plants appear to be variable: there are reports of both delays in reproduction by crowding (Solbrig, 1981) and triggering of reproduction by crowding (Harper, 1977).

Life history characteristics shared by many colonial invertebrates in temperate fouling communities are large colony size, indeterminate growth, and perhaps size-independent reproduction. Size- and age- independent timing of reproduction is an advantage for annual species recruiting onto unpredictably crowded or limited substrata. When space is available for growth, these species can grow quickly to occupy large areas and then reproduce; when space is limited, early reproduction can begin, providing an opportunity for multiple generations in a season. We suggest that the ability to quickly occupy large amounts of space will often be associated with indeterminacy in the timing of reproduction. These opportunistic, quickly growing taxa have the greatest potential to be "nuisance" species because of their ability to rapidly dominate space within the fouling community.

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ANTIFOULING PAINTS OF THE SOLUBLE MATRIX TYPE BASED ON WW ROSIN AND CHLORINATED RUBBER

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ABSTRACT

The antifouling formulations studied in this paper are of the soluble matrix type. Dissolution rate of WW rosin was controlled by the incorporation of different quantities of grade 20 chlorinated rubber adequately plasticized.

Tricresyl phosphate, chlorinated paraffin 42 per cent and chlorinated biphenyl 54 per cent were used as plasticizers. In a first series of paints, ten samples were prepared with each plasticizer and were tested on the bottom of three different ships (destroyers).

The second series of samples, including twelve paints, prepared with different plasticizers, was tested on another destroyer.

Results obtained show the possibility to achieve good fouling control during two years.

RESUME

Les peintures antisalissures etudiées dans ce travail correspond au type liant soluble. Le degrée de dissolution de la colophane (WW rosin) est controlé par l'incorporation de différentes quantités de caoutchouc chlorée (degrée 20), plastifié.

Phosphate de tricrésyle, paraffine chlorée (42 %) and diphénile chloré (54 %) sont employés comme plastifiants. Les échantillons préparés (10 peintures dans une première série correspondant à chaque plastifiant) on été essayés dans la carène de trois bâteaux différents (destructeurs).

Une deuxième série de peintures, avec 12 échantillons, est essayée dans un autre destructeur.

Les résultats obtenus indiquent la possibilité d'éviter l'action des salissures marines pendant deux ans.

INTRODUCTION

In previous papers the authors have mentioned the important deterioration of paint systems applied on ships' hulls, produced by fouling settlement. This problem affects also other structures submerged in sea water, as off-shore platforms, pipelines, buoys, etc.

Up to present, antifouling paints are the most satisfactory and economical method for underwater fouling protection. The adequate selection of raw materials is very important in order to obtain efficient formulations.

In the particular case of ships, fouling leads to a reduction of speed and to a greater fuel consumption. This is due to the roughness produced by fouling organisms on the immersed surface, which increases frictional resistance. It is also important to employ paints of good bioactivity to assure long periods between drydockings with no or minimum corrosion on the steel surface.

An efficient paint system for hull protection is formed by an anticorrosive primer, an intermediate paint and an antifouling formulation. Results obtained in service are related to metallic surface preparation, paint application, thickness of the dry film and anticorrosive or antifouling properties of the different products employed.

It is important to obtain anticorrosion protection for a minimum of 5 years and actual researches in the world tend to increase this period. In relation to antifouling paints, it is very difficult to exceed 2-3 years of good bioactivity, since these paints are reactive and must dissolve in sea water for toxicants release.

From this point of view, it is important to remark that previously were developed by the authors antifouling paints of high bioactivity using oleoresinous binders based on WW rosin plasticized with a phenolic varnish. The paints' behaviour was assessed in raft trials and on the hull of different vessels of the Argentine Navy. In both tests, experimental panels exposed in temperate waters remained free from fouling for periods of 24 months or more [1-6].

In the present paper, antifouling compositions based on WW rosin-chlorinated rubber (grade 20) mixtures were studied. Samples obtained dried quickly, however it was possible to apply them with roller or airless spray.

VARIABLES STUDIED

Influence of the plasticizer

Chlorinated rubber films are hard and brittle and show poor adhesion over anticorrosive primers. To avoid that, it is necessary the employ of plasticizers; the type and level of these substances affect some properties of the antifouling films (solubility, hardness, adhesion,

TABLE I

COMPOSITION OF ANTIFOULING PAINTS - SERIES 1 (g/100 g)

Paints.....	1	2	3	4	5
Cuprous oxide.....	70.4	56.3	42.2	28.3	14.1
Zinc oxide.....	7.0	5.6	4.2	2.8	1.4
Calcium carbonate.....	--	15.5	31.0	46.3	61.9
WW rosin.....	8.2	8.2	8.2	8.2	8.2
Chlorinated rubber R-20	8.2	8.2	8.2	8.2	8.2
Plasticizer (*).....	4.4	4.4	4.4	4.4	4.4
Additives.....	1.8	1.8	1.8	1.8	1.8

Paints.....	6	7	8	9	10
Cuprous oxide.....	71.4	57.4	42.8	28.6	14.4
Zinc oxide.....	7.1	5.7	4.3	2.9	1.4
Calcium carbonate.....	--	15.4	31.4	47.0	62.7
WW rosin.....	11.0	11.0	11.0	11.0	11.0
Chlorinated rubber R-20	5.5	5.5	5.5	5.5	5.5
Plasticizer (*).....	3.1	3.1	3.1	3.1	3.1
Additives.....	1.9	1.9	1.9	1.9	1.9

* Paints 1/10 were prepared using tricresyl-phosphate as plasticizer; paints 11/20 and 21/30 had the same general composition but plasticizers were respectively chlorinated paraffin 42 per cent and chlorinated biphenyl 54 per cent.

tensile strenght, etc.

In the present experience, tricresyl phosphate, chlorinated paraffin 42 per cent and chlorinated biphenyl 54 per cent were used as plasticizers. Tricresyl phosphate is of the saponifiable type, while the other two are chemically inert. All of them are compatible with WW rosin and with chlorinated rubber.

In the first series of samples (Table I), 30 paints were prepared. The first group of this series (paints 1/10) correspond to those plasticized with tricresyl phosphate, the second group (11/20) was formulated with chlorinated paraffin and the third (paints 21/30) with chlorinated biphenyl. The remainder components are similar to those indicated in Table I.

Each group of paints was tested on three different ships, identified as destroyers 1, 2 and 3.

In the second series (Table II), another 12 samples were formulated with the above mentioned plasticizers and tested on the destroyer identified with number 4.

The choice of rosin/plasticizer ratio took into account preliminary service trials and results of laboratory tests. These ratios ranged from 70/30 to 60/40, by weight.

It is important to remark that chlorinated biphenyl is considered a toxic substance in some countries. It was used in this research only as having experimental interest and its use is not promoted by the authors.

Influence of matrix solubility

All the antifouling paints studied are of the soluble matrix type. Good sea water solubility is an important property for these paints, which can be achieved with the use of rosin as film forming material. Dissolution rate is regulated by the quantity of plasticized chlorinated rubber incorporated to the formulation. Rosin/chlorinated rubber 1/1 and 2/1 ratios (by weight) were used; the last one corresponds to the matrix of greater solubility. In previous raft trials with the mentioned selected ratios, high toxicant concentration was obtained in the paint film/sea water interphase, forming a lethal layer for fouling organisms.

Influence of the type and content of toxicant

In all the samples, cuprous oxide was used as main toxicant, due to its wide toxic spectrum over marine fouling organisms. Zinc oxide in the first series and a mixture of zinc oxide and cuprous arsenate in the second series were employed as reinforcing toxicants.

Cuprous oxide levels in the first series (Table I) ranged from 70.4 to 14.1 per cent by weight on the dry film (54.4 and 10.9 per cent by weight on the paint). Zinc oxide was incorporated in the proportion of 10 per cent related to the cuprous oxide.

TABLE II

COMPOSITION OF THE ANTIFOULING PAINTS - SERIES II (g/100 g)

Paints.....	31	32	33	34	35	36
Cuprous oxide.....	12.5	25.0	12.8	25.5	13.0	26.0
Zinc oxide.....	1.2	2.5	1.3	2.5	1.3	2.6
Mercurous arsenate.....	1.9	3.8	1.9	3.8	2.0	3.9
Calcium carbonate.....	54.5	38.8	55.5	39.7	56.6	40.4
WW rosin.....	10.9	10.9	14.8	14.8	9.9	9.9
Chlorinated rubber R-20..	10.9	10.9	7.4	7.4	9.9	9.9
Plasticizer (*).....	5.9	5.9	4.1	4.1	5.3	5.3
Additives.....	2.2	2.2	2.2	2.2	2.0	2.0

Paints.....	37	38	39	40	41	42
Cuprous oxide.....	13.0	26.1	13.0	26.0	12.9	25.8
Zinc oxide.....	1.3	2.6	1.3	2.6	1.3	2.6
Mercurous arsenate.....	2.0	3.9	1.9	3.8	1.9	3.8
Calcium carbonate.....	56.8	40.5	56.4	40.2	56.1	40.0
WW rosin.....	14.1	14.1	10.0	10.0	14.6	14.6
Chlorinated rubber R-20..	7.0	7.0	10.0	10.0	7.3	7.3
Plasticizer (*).....	3.6	3.6	5.3	5.3	3.9	3.9
Additives.....	2.2	2.2	2.1	2.1	2.0	2.0

* Paints 31/34 were plasticized with tricresyl phosphate, 35/38 with chlorinated paraffin 42 per cent and 39/42 with chlorinated biphenyl 54 per cent.

In the second series (Table II) cuprous oxide contents varied from 25.0 to 12.5 per cent on the dry film (20.0 and 10.0 per cent on the paint). Zinc oxide and mercurous arsenate were incorporated in the proportions of 10.0 and 15.0 per cent, values referred, respectively, to cuprous oxide content.

The reduction of toxic pigments content in the formulations was compensated by the use of calcium carbonate as extender, just to keep constant pigment volume.

SAMPLES PREPARATION AND APPLICATION

Paints were produced on a pilot plant scale, using ball mills with jars of 28 litres capacity. Binders were previously prepared by dissolution of the resins in a mixture of toluene-xylene[5].

The different components of each formulation were dispersed during 24 hours and cuprous oxide was added 3 hours before the end of the process. The operative conditions of the equipment were adjusted to obtain a product with predetermined characteristics[7]. The final properties were controlled by means of laboratory standarcized methods[8].

For service trials, paints were applied on both sides of each ship (port and starboard side). Painted panels position is shown in Figure 1. Each panel had an area of 12 square meters; after application of two coats of antifouling paint, an average thickness of 80-100 µm was obtained.

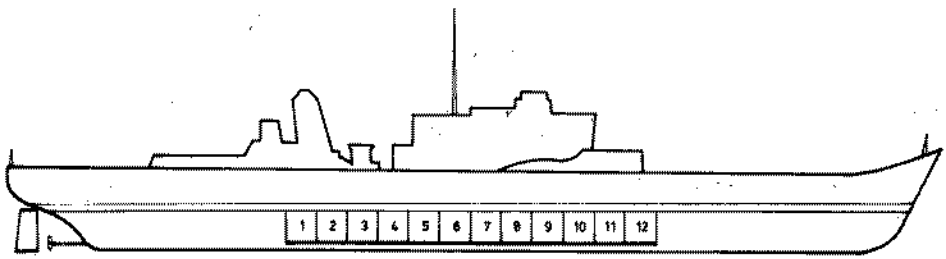

Figure 1.- Position of the panels on the starboard side of a destroyer

RESULTS AND DISCUSSION

Studies of the behaviour of the antifouling paints by means of raft trials or ship trials do not allow conclusions in a shortperiod of time. Furthermore, vessel trials do not offer the possibility to make partial observations on the painted surface during the experiences, except in the case of ships entering drydock due to parti-

cular damages or reparation. Another way of evaluation is with the help of underwater photography or television cameras.

Moreover it is convenient to repeat the experiences in more than one ship, due to the different navigation conditions or itineraries which can modify the results obtained. Important variables to be considered in addition to the paint properties are the speed of the vessel employed, sailing itineraries, duration of the immersion periods, dynamics of the fouling communities in the different harbours, etc.

For these reasons it is also difficult to compare results obtained with the same paints on different ships.

In spite of these problems, continuous research in this area allows, on the basis of the conclusions obtained, to improve the efficiency of the antifouling paints. Laboratory tests do not permit to reproduce the experimental conditions of the marine medium, based on the combined action of sea water and fouling organisms.

Behaviour of paint samples was evaluated by means of the use of a scale of fixation (Table III), which values were: 0 (surface without fouling); 1 (rare), 2 (common), 3 (very common), 4 (abundant) and 5 (completely fouled). Value 1 (80 per cent of efficiency) was fixed as limit of acceptance for an antifouling composition. Paints showing settlement values of 0 or 0-1 (100 and 90 per cent of efficiency) were products of very good bioactivity.

In all cases painted panels were recorded in black and white and colour prints. This methodology permited the comparison of results of different tests and the standardization of the evaluating criteria.

In the first series of tests, the results of which are indicated in Table III, the most demanding trial was that performed on destroyer 1, since it included two periods of intense fouling (Spring-Summer) in a test of 2 years of duration. On destroyers 2 and 3, the experiences were carried out during 9 and 18 months respectively, with only one period of intense fouling.

In this series, value 1 of fouling settlement was exceeded only in paint 20 (destroyer 2, portside). The results indicated that the three plasticizers studied gave paints of similar toxicity.

Related to matrix solubility, represented by the different WW rosin/chlorinated rubber ratios, it can be pointed out that the ratios 2/1 and 1/1 by weight lead to efficient samples, with successful toxicant release.

The different quantities of bioactive material employed (main and reinforcing toxicants) showed that it is possible to prepare efficient antifouling paints in spite of low toxicant content. It was established that paints with 14.1 per cent by weight of toxic material in the dry film are able to control fouling settlement during the experimental period considered.

Since cuprous oxide contents affects significantly the cost of the final product, from an economic point of view it is a remarkable

TABLE III.- FOULING SETTLEMENT - FIRST SERIES OF EXPERIENCES (*)

Paints.....	1	2	3	4	5
Destroyer 1 (Sept. 1977-Aug. 1979), 23 months:					
a) Portside.....	0-1	0-1	1	1	1
b) Starboard side.....	1	0	0-1	0-1	1
Paints.....	**6**	**7**	**8**	**9**	**10**
Destroyer 1 (Sept. 1977-Aug. 1979), 23 months:					
a) Portside.....	0-1	0-1	1	1	0-1
b) Starboard side.....	0	0	0-1	0-1	0-1
Paints.....	**11**	**12**	**13**	**14**	**15**
Destroyer 2 (Dec. 1977-Aug. 1978), 9 months:					
a) Port-side.....	0	0-1	0-1	0-1	0-1
b) Starboard side.....	0	0	0-1	0-1	0
Paints.....	**16**	**17**	**18**	**19**	**20**
Destroyer 2 (Dec. 1977-Aug. 1978), 9 months:					
a) Port-side.....	0	0	0	0	1-2
b) Starboard side.....	0	0	0	0	0-1
Paints.....	**21**	**22**	**23**	**24**	**25**
Destroyer 3 (March 1978-Sept. 1979), 18 months:					
a) Port-side.....	0-1	0	0	0	0
b) Starboard side.....	0	0	0	0	0
Paints.....	**26**	**27**	**28**	**29**	**30**
Destroyer 3 (March 1978-Sept. 1979), 18 months:					
a) Portside.....	0	0	0	0	0
b) Starboard side.....	0	0	0	0-1	0-1

* Key of the Table: 0, without settlement; 0-1, very rare; 1, rare; 2, common; 3, very common; 4, abundant; 5, completely fouled.

fact that even the paints of low toxicant content showed efficient antifouling protection for periods from 18 to 24 months.

In the second experimental series all the samples prepared were painted on the hull of one ship (destroyer 4); this methodology permitted a better comparison of results, since all the antifouling paints were subjected to the same experimental conditions.

Taking into account the main toxicant contents (cuprous oxide), the paints of this series corresponded to the medium and low levels type (25.0 and 12.5 per cent by weight on the dry film). They also included small quantities of zinc oxide and mercurous arsenate in their compositions.

In the formulation of the matrix, WW rosin/chlorinated rubber ratios 2/1 and 1/1 by weight were employed and the same plasticizers of the first series were used.

Fouling settlement values registered on the different panels of destroyer 4 are shown in Table IV. A partial observation was made after 6 months and the final evaluation occurred after a period of 18 months. This test included one season of intense fouling in the case of the first observation and two seasons in the second.

Results corresponding to the first observation indicate that except paint 35 (portside of the destroyer 4, settlement 1-2) in all the other panels the fouling recorded was 0 or 0-1.

In the final observation (18 months) it was noted that the best behaviour is that corresponding to samples plasticized with chlorinated paraffin. Particularly, paints 37 and 38 had settlement 0 (none) on the portside, while the same samples, applied on the starboard side had 0-1 and 1-2, respectively.

It seems that the use of mercurous arsenate as reinforcing toxicant is not necessary for the service conditions of the ships employed. Previously the same conclusions were obtained by the authors for oleoresinous antifouling formulations.

In the observations performed on destroyer 4, clear differences of behaviour of the same paints applied on both sides of the ship were noted. This fact could be attibuted to the following reasons: particular incidence of the sunlight in the harbour, or different drydocking conditions during painting.

In both experimental series, good bioactivity demonstrated by almost all the samples was in close relation with developments previously obtained by the authors. The range of the variables studied did not permit to establish the influence of each of them. Longer periods of immersion seem to be necessary to determine clear differences of behaviour between the studied formulations.

Finally, it is important to point out that only protozoa, diatoms, algae and bryozoa, in little quantities, were observed in some of the patches. Barnacles, an important community in the site of the experien-

TABLE IV

FOULING SETTLEMENT - SECOND SERIES OF EXPERIENCES (*)

Paints.....	31	32	33	34	35	36
Destroyer 4 (Oct. 1978-April 1979), 1st. observation:						
a) Portside.....	0-1	0-1	0-1	0	1-2	0-1
b) Starboard side.....	0-1	0	0	0	0-1	0
Destroyer 4 (Oct. 1978-March 1980), 2nd. observation:						
a) Portside.....	1-2	1-2	2	2	1	0-1
b) Starboard side.....	0-1	1-2	0-1	0-1	0-1	0-1

Paints.....	37	38	39	40	41	42
Destroyer 4 (Oct. 1978-April 1979), 1st. observation:						
a) Portside.....	0	0	0	0	0	0
b) Starboard side.....	0	0	0	0	0	0
Destroyer 4 (Oct. 1978-March 1980), 2nd. observation:						
a) Portside.....	0	0	0-1	0-1	0-1	0-1
b) Starboard side.....	0-1**	1-2**	2-3**	2**	2**	0-1

* Key of the Table: 0, without settlement; 0-1, very rare; 1, rare; 2 common; 3, very common; 4, abundant; 5, completely fouled.

** Loss of adhesion of the antifouling paint due to shock with different objects during navigation.

Figure 1.- Difference between a panel protected with paint 22 (left, 18 months immersion, port-side, destroyer 3, fixation 0) and the reference no toxic paint

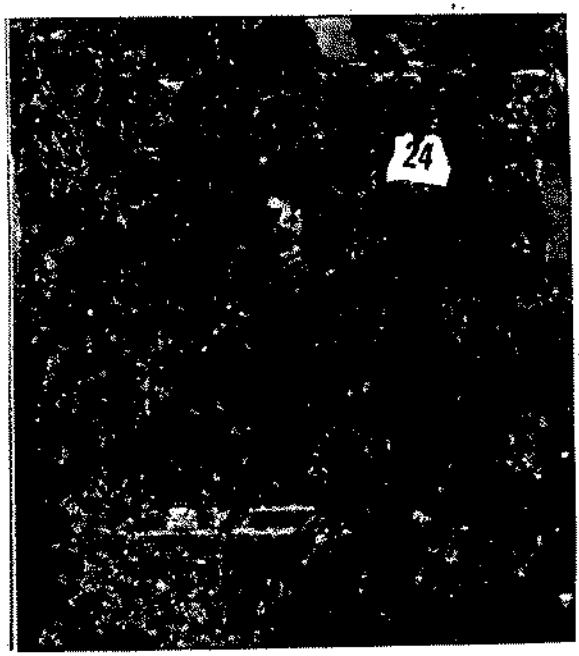

Figure 2.- Inert plate corresponding to fouling fixation after 12 months immersion in the zone of Puerto Belgrano

ces and in South Atlantic ports and generally appearing in raft trials[9-11] were not observed on the antifouling paints tested.

Differences between an experimental sample and the reference no toxic paint is presented in Figure 1.

Figure 2 show the aspect of an inert plate corresponding to fouling fixation in the raft of Puerto Belgrano after 18 months immersion.

CONCLUSIONS

1. Antifouling paints of good matrix solubility in sea water were obtained. The use of different WW rosin/chlorinated rubber ratios (1/1 and 2/1 by weight) makes feasible toxicant release for long periods (18 to 24 months).

2. An adequate fouling control was obtained with paints elaborated with different cuprous oxide contents (between 14 and 70 per cent by weight on the dry film) in the test conditions (type of ship, immersion time, sailing itineraries, etc.).

3. Under the mentioned experimental conditions there were not observed significative differences of behaviour between paints elaborated with and without reinforcing toxicants.

4. Plasticization level employed produces adequate mechanical properties in the antifouling film (good adhesion over the anticorrosive coat and flexibility). Paints formulated with tricresyl phosphate and chlorinated paraffin as plasticizers had the same bioactivity than those including chlorinated biphenyl.

5. Some differences of bioactivity were observed using several vessels for the trials, and also when paints were applied on both sides of the ships.

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BIOACTIVITY OF CHLORINATED RUBBER ANTIFOULING PAINTS TESTED IN SEA WATER

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ABSTRACT

It was studied the influence of different variables of composition, elaboration and testing on the bioactivity of antifouling paints prepared with binders based on WW rosin and chlorinated rubber.

After two years of raft exposure, experimental paints showed differences in their toxic efficiency. The same samples were tested on a destroyer's hull.

Paints formulated with the lesser rosin content had different effectiveness in raft and service trials, while those samples with binders of greater rosin content showed the same bioactivity in both tests.

Relating to the elaboration methods employed in this research, it is possible to remark that paints dispersed in ball mills of different capacity, with a technology previously optimized, had similar and adequate toxic characteristics in raft and service trials.

RESUME

On a étudié l'influence des différentes variables de composition, élaboration et d'essai, sur la bioactivité des peintures antisalissures préparées avec des liants basés en résine colophane (WW rosin) et caoutchouc chloré.

Après deux ans d'immersion des plaques peintes dans le radeau de P. Belgrano, les échantillons préparés présentent différences de toxicité, en rapport avec les matières premières (toxiques et résines) utilisées. Parallèlement les mêmes peintures ont été essayées dans la carène d'un destructeur.

Les formules avec des liants avec une haute quantité de résine colophane ont la même bioactivité dans les deux essais. On trouve des ré-

sultats différents si la quantité de résine dans la formule a été reduite.

INTRODUCTION

In previous papers, the authors have mentioned the important deterioration of paint systems applied on ships'hulls, produced by fouling fixation. Up to present, the use of antifouling paints is the most satisfactory and economical method for underwater protection.

Fouling fixation leads to a reduction of the speed of the ship and increases fuel consumption. This is due to the roughness produced by marine fouling on the immersed surface, which increases frictional resistance. It is necessary to use paints of good bioactivity to assure good periods between drydockings, without deterioration of the painted surface.

An efficient paint system for hulls protection consists of different coats: a wash-primer, an anticorrosive paint, a sealer coat and finally the antifouling. Results obtained are also related to metallic surface preparation, paint application and weathering conditions during painting, dry film thickness obtained and properties of the products used.

The study of antifouling paints in the laboratory is a very complex task, since its principal property, toxic action against fouling organisms, only can be adequately established by immersion in the natural environment. A long time is required to obtain results regarding the effectiveness of the product tested.

Normally, antifouling paints are exposed in raft and in service trials.

In the first case, the protective system is applied on sandblasted or gritblasted steel panels, leaving them to dry under laboratory atmospheric conditions during an adequate period (generally 24 hours for each coat). Then the panels are fixed on frames which are located on the raft anchored at a harbour of well known hydrological and biological characteristics, in order to establish the efficiency of the paints ([1], [2]).

By periodical examination of the panels it is possible to determine changes in the film produced during immersion and especially to establish if antifouling paint avoided the marine fouling attachment.

Experiences on ships' hulls were made after optimizing formulations which accomplished in raft trials ([3]). An important information was obtained when ships of different operative conditions were used, as destroyers, aircraft carriers, tugboats, etc. ([4], [5], [6]).

The purpose of this work is to determine in a raft trial and on a destroyer's hull the behaviour of antifouling paints based on WW rosin and plasticized chlorinated rubber grade 20, and also to establish the correspondence of results obtained in both tests.

Besides, it was compared the effectiveness of paints produced in ball mills of different capacity and operative conditions, starting in each case from the same formulations.

This subject was studied previously by different authors ([7], [8], [9] [10]). Diverse and not always concordant opinions are registered in the mentioned papers.

VARIABLES STUDIED

1. Formulation variables

Binder composition. All the antifouling compositions studied were of the soluble matrix type. An adequate sea water dissolution rate is a very important property of these paints, which can be achieved with the use of WW rosin as film forming material. Dissolution rate was regulated by the quantity of plasticized chlorinated rubber incorporated to the formulation. Rosin/chlorinated rubber ratios 1/1 and 2/1 by weight were used in this work; the last one corresponds to the matrix of greater dissolution rate.

Toxicant type and content. In all samples, cuprous oxide was used as main toxicant, due to its wide lethal spectrum over marine fouling organisms. Cuprous oxide levels mentioned in Table I ranged from 71.4 to 14.1 per cent by weight on the dry film (54.4 and 10.9 per cent by weight on the paint). Zinc oxide was employed as reinforcing toxicant in a proportion of 10 per cent by weight, referred to cuprous oxide content.

2. Testing variables

Raft and ship's hull trials. Aiming the study of antifouling paints behaviour in relation with different hydrodynamical conditions, the samples were applied on raft panels (static test) and on a destroyer's hull (dynamic test). Besides, different zones of the hull of this ship were selected and the paints distributed on port side and on starboard side.

The marine environment where raft was anchored (latitude 38°54'S, longitude 62°06' W) shows tides of 3.6 meters (syzygies) and 2.6 meters (quadratures), with a reduced water turbulence. Surface water temperature varies from 5-10°C in Winter to 20-25°C in Summer. Salinity shows a marked variation (between 26 and 36 g/1000 g); lower values are related with rains in the zone and higher values correspond to saltpetre deposits existing near the harbour ([11], [12]).

Regarding to the vessel employed it is important to mention that it sailed in temperate waters or stayed anchored near the experimental raft.

Testing periods. The destroyer's test, as well as other trials carried out previouly by CIDEPINT researchers, began and finished when the shipowner (in this case the Argentine Navy) decided its entrance to drydock. In this case it was started in September 1977 and ended in August

TABLE I

COMPOSITION OF EXPERIMENTAL ANTIFOULING PAINTS (g/100 g on the dry film)

Paints.....	1	2	3	4	5
Cuprous oxide.....	70.4	56.3	42.2	28.3	14.1
Zinc oxide.....	7.0	5.6	4.2	2.8	1.4
Calcium carbonate.....	-	15.5	31.0	46.3	61.9
WW rosin.....	8.2	8.2	8.2	8.2	8.2
Clorinated rubber R-20	8.2	8.2	8.2	8.2	8.2
Trycresyl-phosphate...	4.4	4.4	4.4	4.4	4.4
Additives.....	1.8	1.8	1.8	1.8	1.8

Paints.....	6	7	8	9	10
Cuprous oxide.....	71.4	57.4	42.8	28.6	14.4
Zinc oxide.....	7.1	5.7	4.3	2.9	1.4
Calcium carbonate.....	-	15.4	31.4	47.0	62.7
WW rosin.....	11.0	11.0	11.0	11.0	11.0
Chlorinated rubber R-20	5.5	5.5	5.5	5.5	5.5
Trycresyl-phosphate...	3.1	3.1	3.1	3.1	3.1
Additives.....	1.9	1.9	1.9	1.9	1.9

1979 (23 months).

Raft trial started simultaneously. The first observation was made after 12 months and final observation, in September 1979, after 24 months. The action of the fouling in the harbour is seasonal (higher fixation in Spring-Summer); two periods of intense action of sessile marine organisms were included in both tests ([11], [12]).

3. Elaboration variables

Paints were prepared using ball mills of two different capacities (3.3 and 28 liters) and also different operative conditions, in order to study parameters related with the scaling up.

Binders were prepared by dissolution of the resins (WW rosin and chlorinated rubber) in a toluene-xylene mixture (1/1 by weight ratio). A high speed mixer was used for this operation and plasticizer was also incorporated to the binders after resins solubilization.

Antifouling paints for raft and service trials were prepared by dis-

persion of the pigments in the different binders, using a porcelain ball mill of 28 liters capacity; duplicates of the same formulations were produced in an equipment with jars of 3.3 liters, only for the case of raft trials.

Differences in the elaboration technologies were the following:

a) A rotational speed of 41 rpm was used in the case of 28 liters ball mills, and 68 rpm for the smaller equipment (3.3 liters) ([13]).

b) With regard to the ball load, mixtures of equal weight corresponding to three different diameters were chosen: in the smaller jars, 14, 19 and 25 mm (1.6 liters) and in the greater jars 19, 25 and 38 mm (14 liters).

c) Paint load was 1.5 kg (1.1 liters) and 11 kg (8 liters) in both mills, respectively.

In the case of the extender, the acidity of the binder and the calcium carbonate particle size distribution achieved in jars of 3.3 liters (before cuprous oxide incorporation) was adopted as reference to define the equivalent dispersion time in the 28 liters mill ([14], [15]).

With regard to toxicant dispersion time in the ball mill of greater capacity, it was selected considering besides the acidity of the binder, particle size distribution of cuprous oxide and the Cu^{2+} content generated during toxicant dispersion, after 3 hours in the ball mill of smaller jars (27 hours of total dispersion time) ([16], [17]).

SAMPLES APLICATION

In order to evaluate the toxicant behaviour of paints by immersion tests in the natural environment, new steel plates (low carbon level) were used in the case of the raft trial. Panels were sandblasted, treated with a vinyl wash-primer and protected with a high anticorrosive coating based on chlorinated rubber-phenolic varnish binder (120-150 µm of dry film). Finally, two coats of the different antifouling samples (80-100 µm of dry film) were applied.

In the case of the destroyer's hull, due to the efficient behaviour of the protective system previously used, the new treatment involved only a pressure water cleaning and the application of an anticorrosive coating of similar characteristics as that used in the raft trial plates. Two coats of the anticorrosive paint were applied, obtaining similar thicknesses to those corresponding to the experimental raft plates.

The painted panels for the service trial had an area of 12 square meters each and they were located on the port side and on the starboard side of the destroyer's hull.

In relation with drying time, 24 hours elapsed between coats, and the launching occurred after 48 hours of the last coat application.

TABLE II

FOULING FIXATION

Paints.....	1	2	3	4	5
Raft test (Sept. 1977-Sept. 1978), 12 months:					
a) Ball mill 3.3 liters..	0-1	0-1	1	0-1	1
b) Ball mill 28 liters...	0	0	0-1	0-1	0-1
Raft test (Sept. 1978-Sept. 1979), 24 months:					
a) Ball mill 3.3 liters..	1	2	2	2-3	3
b) Ball mill 28 liters...	1	1-2	1-2	2	3-4
Service test (Sept. 1977-Aug. 1979), 23 months:					
a) Port side.....	0-1	0-1	1	1	1
b) Starboard side.....	1	0	0-1	0-1	1

Paints.....	6	7	8	9	10
Raft test (Sept. 1977-Sept. 1978), 12 months:					
a) Ball mill 3.3 liters..	0	0	0-1	0	1
b) Ball mill 28 liters...	0	0	0	0	1
Raft test (Sept. 1977-Sept. 1979), 24 months:					
a) Ball mill 3.3 liters..	0	0-1	0-1	0	2
b) Ball mill 28 liters...	0	0	0-1	0-1	1-2
Service test (Sept. 1977-Aug. 1979), 23 months:					
a) Port side.....	0-1	0-1	1	1	0-1
b) Starboard side.....	0	0	0-1	0-1	0-1

RESULTS AND DISCUSSION

The behaviour of paint samples was evaluated by the use of a fixation scale which ranged from 0 (surface without fouling) to 5 (completely fouled). Intermediate values of 0-1 (very rare), 1 (rare), 2 (common), 3 (very common) and 4 (abundant) were also considered. Value 1 was established as the limit of acceptance for an antifouling composition (80 % effectiveness); paints that showed fixation values of 0 or 0-1 (100 and

90 % of effectiveness) were considered as products of very good bioactivity.

In all the cases color prints were taken. This methodology permitted the comparison of results between the different tests and the standardization of the evaluating criteria.

For the raft trial, the immersion period was extended up to 24 months, with a partial observation after the first year immersion. The service trial was performed during 23 months and as it was previously mentioned, started at the same time as the raft trial.

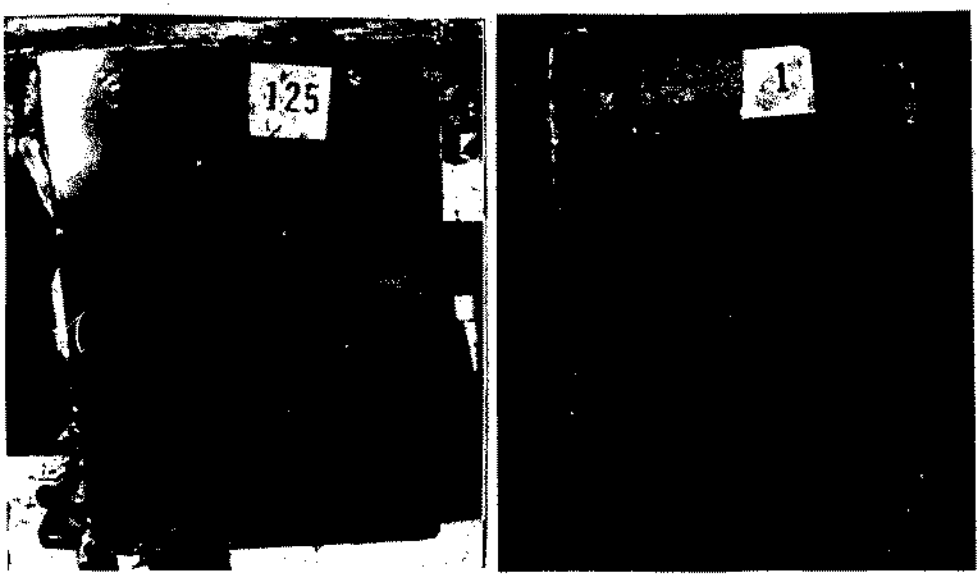

Figure 1.- Panels corresponding to paint 9, elaborated in a ball mill of 3.3 liters (left, fixation 0) and in a ball mill of 28 liters (right, fixation 0-1), after 24 months immersion in sea water (raft test)

After 12 months immersion, results obtained in the raft trial with paints prepared in the ball mill of 28 litres capacity showed that all the samples had an efficient bioactivity, with fouling records of 0, 0-1 and 1 (Table II), thus fulfilling the test requirement.

For the period of two years in the same test, the paints exhibited differences in toxic efficiency, which are related to the binder dissolution rate of the samples. For the binders of lower dissolution rate (1/1 rosin/chlorinated rubber ratio), only the sample 1 had fouling fixation 1; in paints 2 to 5 fouling records varied between 1-2 and 3-4. Paints 6 to 9 corresponding to the binder of higher dissolution rate (2/1 rosin/chlorinated rubber ratio) had fouling settlement 0 or 0-1 and in the case of sample 10 the registered values were 1-2 and 2. The-

re were no differences between paints obtained in a 3.3 or in a 28 liters ball mill.

For a 23 months immersion, all the studied samples showed similar behaviour and good toxicity on the destroyer's test (fixation 0, 0-1 or 1). Results obtained indicate that there were no significant differences of fouling fixation on both sides of the hull. The two binder compositions and the different toxicant content led to paint films with lethal characteristics during the period of test.

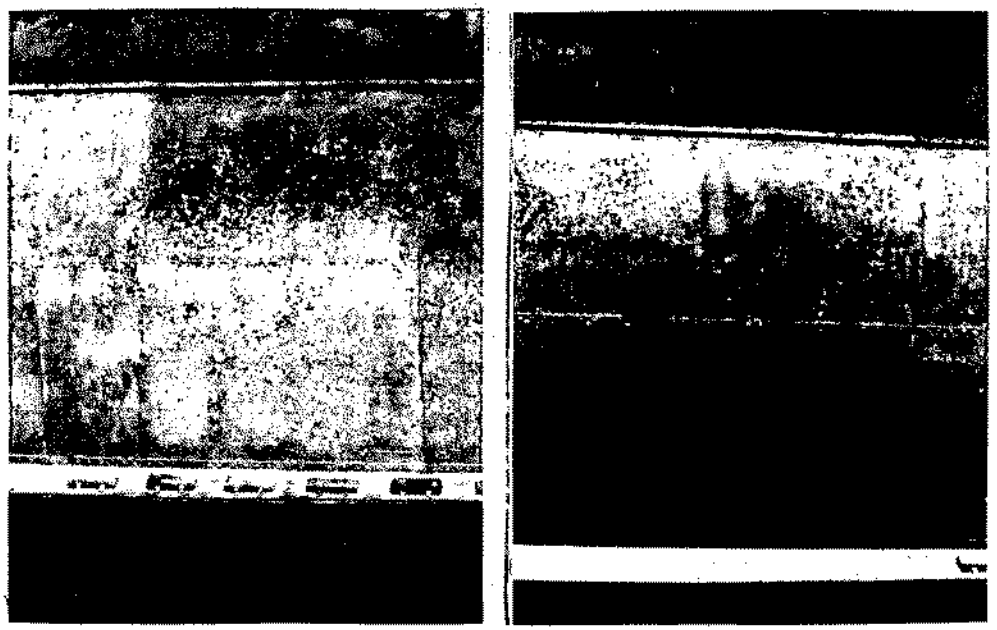

Figure 2.- Panels corresponding to paints 7 (left, high toxicant content, fixation 0) and 9 (right, medium toxicant content, fixation 0-1) tested on the starboard side of a destroyer's hull, after a period of 23 months immersion

The above mentioned considerations permit to establish that paints formulated with less rosin content had different effectiveness in raft and service trials, while those samples with binders of greater rosin content showed the same bioactivity in both tests. These results may be connected with the different dissolution rate of the binders and with the hydrodynamical conditions in raft and service trials.

Painted panels exposed on the experimental raft had, for each sample, a lesser dissolution rate than those on the vessel's hull. From this point of view it is important to remark that the raft trial invol-

ved a greater requirement.

The fact that insoluble rests of paint matrix as well as products of the reaction of some matrix components with sea water remained adhered to film surface, determined a reduction of its dissolution rate.

Toxicant release depended fundamentally on binder dissolution rate. Paints with different cuprous oxide content showed very good bioactivity when this compound was adequately leached. Since cuprous oxide contents affects significantly the final cost of product, from an economic point of view it is important to obtain efficient antifouling paints with the lower toxicant content. For this reason the selection of an adequate binder composition is required.

The use of ball mills of different capacity (3.3 and 28 liters) had no influence on the bioactivity of the paints, since their operative conditions were adequately selected. Thus, it was demonstrated that it is possible to prepare antifouling paints of similar bioactivity, starting from the same formulations, using equipments of different capacity. As previously mentioned, it is necessary to define the operative characteristics of equipments and to control particle size distribution of pigments and acidity of binder, as well as the cupric oxide content generated during dispersion ([15]).

These conclusions are important since they contribute to solve the problems related to the preparation of antifouling paints on the exclusive basis of a formulation or in relation with the change of dispersion equipment in the case of technology transference to the industry.

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DISSOLUTION RATE OF CUPROUS OXIDE AND ITS INFLUENCE ON ANTIFOULING PAINTS EFFECTIVENESS

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ABSTRACT

This paper gives an account of the dissolution rate of cuprous oxide in artificial sea water, evaluating the influence of pH, temperature, chloride ion concentration and dispersion degree of the pigment (different area of cuprous oxide particles by mass unit).

Concerning antifouling formulations, the influence of cuprous oxide content, binder composition and toxicant dispersion time were studied.

Results obtained show that the dissolution rate of cuprous oxide in artificial sea water is directly proportional to temperature and chloride ion concentration and inversely proportional to pH. Behaviour of different samples in raft trials showed that it is possible to define the minimum dispersion degree required to achieve a cuprous oxide dissolution rate leading to saturation concentration of copper complexes in the film. Thus, diffusional process and consequently leaching rate are improved.

RESUME

Dans ce travail on a determiné le degré de lixiviation de l'oxyde cuivreux dans l'eau de mer artificielle. On a evalué l'influence du pH, temperature, concentration de chlorures et degré de dispersion (différente surface des particles d'oxyde cuivreux par unité de masse).

En concernant les formules antissalissures, l'influence du contenu d'oxydé cuivreux, composition du liant et temps de dispersion du toxique a été aussi étudiée.

Les résultats obtenus montrent que le degré de dissolution de l'oxyde cuivreux dans l'eau de mer artificielle est directement proportionnel à la temperature et à la concentration de chlorures et inversement proportionel au pH. Le comportement des échantillons

dans des essais en radeau nous montrent qu'il es possible d'établir le degré de dispersion minimum nécéssaire pour obtenir une dissolution de l'oxyde cuivreux permettant d'arriver à la saturation des complexes de cuivre dans la pellicule de peinture antissalissure. De cette façon le procès diffusionel a été amélioré et aussi le degré de lixiviation du toxique.

INTRODUCTION

Various toxicants have been used for the formulation of antifouling paints ([1]). Metallic copper and some derivated compounds can be mentioned among the most frequently used. Cuprous oxide is the best known of all. It shows an adequate lethal action against fouling organisms and it is not very expensive. Consequently, efficient antifouling paints generally include this compound as fundamental toxicant. Some organometallic substances are also employed.

In order to make these paints effective, from the film is necessary to obtain a constant leaching rate and an adequate degree of toxicant concentration in the paint/sea water interphase ([2]).

In the case of paints of the soluble matrix type, the toxicant lixiviation depends not only on its dissolution rate but also on that of the binder, since film forming substances contribute to obtain an adequate leaching rate ([3]).

As regards the dissolution of the biocide substances inside the film, this stage involves the action of sea water and chloride and hidrogen ions, the subsequent toxicant dissolution and finally its diffusion as copper complexes towards the film surface ([4]). This process implies that the matrix of antifouling paints allows the transference of the dissolved toxicant.

The paints are usually obtained by means of the dispersion of the toxic pigment in a vehicle based on plasticized WW rosin resin, which has acid reaction. The dispersion process is particularly important since an excess or a deffect in grinding diminishes the biocide effectiveness ([5], [6]). On the contrary, an adequate dispersion degree ensures the reduction of the size of pigment particle and its uniform dispersion inside the paint.

In a previous paper ([7]) the influence of the size distribution of the cuprous oxide particles on the toxic effectiveness of antifouling formulations was determined. The toxicant action of paints was verified by immersion trials on an experimental raft.

Taking into account that the behaviour of an antifouling paint is related to the obtention of an adequate steady-state leaching rate, the dissolution rate of cuprous oxide in sea water, considering the medium characteristics and the particle size of the pigment, was determined in this research.

The influence of the toxicant content and binder composition ver-

sus dispersion time on the bioactivity of oleoresinous soluble matrix type antifouling paints was also studied.

VARIABLES CONSIDERED

1. DISSOLUTION RATE OF CUPROUS OXIDE IN ARTIFICIAL SEA WATER

The action of the following variables was studied using a sample of electrolytic cuprous oxide of 99.81 % purity; 0.04 % of cupric oxide and 0.03 % of metallic copper were also observed ([5]).

Influence of pH

The experimental medium (artificial sea water) was adjusted to the following values: 7.61, 7.78, 8.02, 8.20 and 8.41, considering that normal pH values of unpolluted natural sea water varies between 8.1 and 8.2. It is important to remark that pH increases or decreases in harbour due to pollution processes.

The medium was thermostatized at 20±0.5°C and salinity was adjusted to 0.48 M sodium chloride concentration.

Influence of temperature

The dissolution rate was evaluated at 10, 15, 20 and 25°C, keeping pH and sodium chloride concentration at a constant value (8.20 and 0.48 M, respectively).

Influence of chloride ion concentration

The sodium chloride concentration in the experimental medium was 0.48 M, 0.60 M, 0.70 M or 0.80 M; pH was kept at 8.20 and temperature at 20 ± 0.5°C.

Influence of the size and shape of the particles

The mass of a given solid which is dissolved by time unit is a function of the interphase area. This is connected with the size and shape of discrete particles or with their associations in the aqueous medium ([8]). In order to study this variable, cuprous oxide was dispersed at different fineness degree so that particles of different mean size were obtained.

2. TOXIC EFFICIENCY OF ANTIFOULING PAINTS

In order to evaluate the influence of the dissolution rate of cuprous oxide on the bioactivity of the antifouling paints, the following variables were studied.

Influence of binder composition

The dissolution rate of WW rosin was regulated using phenolic varnish (3/1 and 5/1 rosin/plasticizer ratio, by weight) as plasticizer. The binders thus obtained showed satisfactory performances in

previous raft and service trials ([9], [10], [11]), the dissolution rates being of 26.4 and 31.3 $\mu g/cm^2.day$, respectively ([6]).

Influence of the toxicant content

The above mentioned binders were pigmented with cuprous oxide employing 11.4 and 4.0 per cent by volume (45.3 and 18.1 per cent by weight, respectively), calculated on the paint. The products obtained correspond to formulations of high and medium toxicant content type. To keep the total volume of solids of the paints constant, the reduction of the cuprous oxide content was compensated by incorporation of natural calcium carbonate (Table I).

TABLE I

ANTIFOULING PAINTS COMPOSITION (% V/V)

Paints.....	1	2	3	4
Cuprous oxide.....	11.4	4.0	11.3	4.0
Calcium carbonate.....	-	10.6	-	10.6
Additives *	3.0	2.7	3.0	2.7
WW rosin.....	28.0	27.0	31.3	30.2
Phenolic varnish (solids).	10.9	10.5	7.3	7.0
Solvents and thinners.....	46.7	45.2	47.1	45.5
Resin/plasticizer ratio...	3/1	3/1	5/1	5/1

* Surfactants, thickeners and stabilizers.
Resin/plasticizer ratio is expressed by weight.

Influence of dispersion time

A porcelain ball mill with 3.3 liters jars ([12]) was used for the elaboration of samples. In all cased a premixture of various paint compounds was prepared, with the exception of WW rosin and cuprous oxide. Next, toxicant was added and the dispersion was carried out at different times (1, 3, 5 and 10 hours) so as to obtain samples of different particle size distribution. This is related with the operative characteristics of the equipment employed.

Once the dispersion was achieved, the WW rosin solution in a solvent mixture similar to the one used in paint formulation was added. This method allows the reduction of the amount of cupric resinate formed, which diminishes the bioactivity of the paint, since it affects the matrix dissolution rate, according to previous experimental results obtained by the authors ([5]). Viscosity was adjusted at the end of the dispersion process.

The bioactivity of the paints was evaluated by means of test panels, prepared with the different paints, and employing the experimental raft anchored at Puerto Belgrano (38°54' S, 62°04' W). Sandblasted steel panels previously coated with a vinyl wash-primer (SSPC-PT3-64 Specification) and with an anticorrosive paint of high resistance based on chlorinated rubber ([13]) were completed with two coats of the antifouling paints.

METHODOLOGY

In order to evaluate the dissolution rate of cuprous oxide according to the above mentioned variables, the toxicant was previously washed with a toluene-96 % ethylic alcohol mixture to remove the stabilizing material (about 10 per cent by weight).

The sample, dried and weighed was dispersed in neutral distilled water to disaggregate pigment particles. Cuprous oxide particles were kept in suspension by continuous agitation during the test so as to obtain a constant dissolution area.

After having experimented under the already mentioned medium conditions, the dissolution rate of cuprous oxide was calculated taking as starting point the values corresponding to the dispersed toxicant mass and to the mass dissolved in a given period of time.

Dissolved mass of cuprous oxide

The concentration of cuprous oxide attained in artificial sea water was 0.5 ppm at the end of each trial, not to affect the dissolution rate.

The increase of the cuprous oxide concentration during the trial (that is the difference between 0.5 ppm and the value obtained at the beginning of the trial once the dispersion ended) allowed to estimate the dissolved mass of toxicant.

The cuprous oxide concentration in the solution was determined by colorimetry, by means of the sodium diethyl-ditiocarbamate method. As a result of this, it was possible to determine 1 part of copper in a hundred million parts, for concentrations between 0.1 and 1 ppm ([14]).

Dissolution time

To attain the above mentioned 0.5 ppm concentration, the dissolution time of cuprous oxide in each test was graphically determined. Near the final point, the copper concentration was evaluated on aliquots drawn out every 30 seconds.

TABLE II

CUPROUS OXIDE DISSOLUTION RATE IN ARTIFICIAL SEA WATER, mg/s.h

T °C	$[Cl^-]$ mol/l	pH	Dispersion time (hours) 1	2	3	4	5	6	7	8
10	0.48	8.20	30.7	11.8	9.0	7.3	5.5	4.4	3.8	3.4
15	0.48	8.20	40.2	15.8	11.6	9.8	7.0	5.6	4.8	4.4
20	0.48	7.61	159.7	61.6	44.8	36.9	27.3	21.9	19.0	17.0
20	0.48	7.78	116.3	45.2	33.5	27.0	20.3	16.4	14.2	12.7
20	0.48	8.02	78.9	29.7	21.4	17.6	12.9	10.4	8.9	8.0
20	0.48	8.20	52.0	19.9	14.9	12.1	8.9	7.2	6.3	5.6
20	0.48	8.41	35.3	13.7	10.1	8.2	6.1	4.9	4.2	3.8
20	0.60	8.20	72.8	28.2	21.0	17.0	12.7	10.2	8.8	7.8
20	0.70	8.20	101.9	39.2	29.2	23.5	17.7	14.2	12.3	11.1
20	0.80	8.20	133.0	52.0	38.1	30.8	22.9	18.4	16.0	14.3
25	0.48	8.20	65.1	25.1	18.6	15.1	11.3	9.0	7.8	7.0
Particle average diameter, μm...			2.9	7.6	10.6	13.5	18.2	23.1	26.8	30.0

RESULTS

1. DISSOLUTION RATE OF CUPROUS OXIDE

In the determination of the dissolution rate of cuprous oxide in mediums of different characteristics and with samples prepared with different pigment dispersion degree, it was microscopically observed that the particle size distribution was not significantly modified during the trial, for relatively short periods (up to 180 minutes). Therefore in each case the mean initial diameter was evaluated.

Table II and figures 1 to 4 show the influence of the experimental medium characteristics and that of the dispersion time on the dissolution rate of the toxicant. Table III shows the biocide efectiveness of the paint samples on the experimental raft test.

The influence of pH on the dissolution rate of cuprous oxide is considerable (Fig. 1). For example, for a 0.48 M sodium chloride concentration at 20°C and pH 7.61 and 8.02, dissolution rate attained values approximately 3 and 1.5 times, respectively, greater than in the case of pH 8.20, under the same salinity and temperature conditions.

This decrease of dissolution rate when pH increases is due to the formation of basic cupric carbonate on the surface of cuprous oxide particles ([1]).

The increase of sea water temperature in accordance with the atmosphere produces a pH increase, by elimination of carbon dioxide. On account of this fact pH was kept constant in the tests, so as to study only the influence of the temperature. Fig. 2 shows the direct proportionality between temperature and dissolution rate. For example in artificial sea water (pH 8.20 and salinity concentration 0.48 M), at 25°C, the dissolution rate is about 25 per cent greater than at 20°C.

The variation of chloride ion concentration modifies the ionic strength of the medium, being pH and temperature kept constant during the trial (8.20 and 20°C respectively).

Under these conditions dissolution rate of cuprous oxide is directly proportional to chloride ion concentration. For example, a 0.60 M solution (about 0.12 M more concentrated than sea water) led to a dissolution rate 1.4 times greater than the one registered in artificial sea water (0.48 M sodium chloride concentration) (Fig. 3).

The toxicant dispersion times in the ball mill (1, 3, 5 and 10 hours) led to samples of decreasing particle mean diameter and consequently of different specific area (surface per mass unit). Although the dissolution rate of cuprous oxide (expressed in $\mu g/cm^2.day$) is an intrinsec characteristic for a given medium, the dissolution area varies according to the dispersion fineness (different effectiveness).

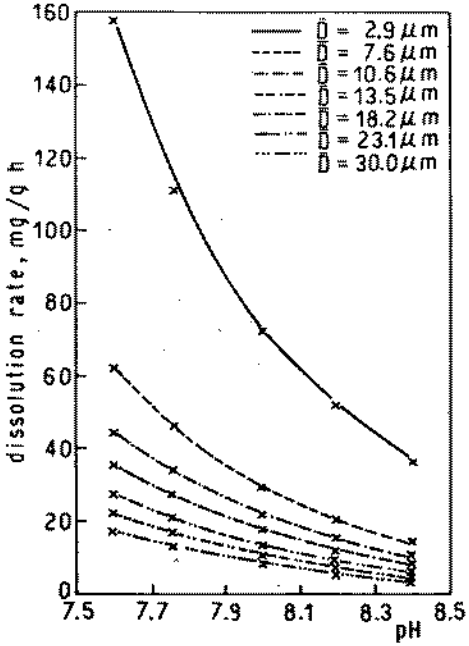

Fig. 1.- Influence of pH on cuprous oxide dissolution rate in artificial sea water (20°C and 0.48 M NaCl)

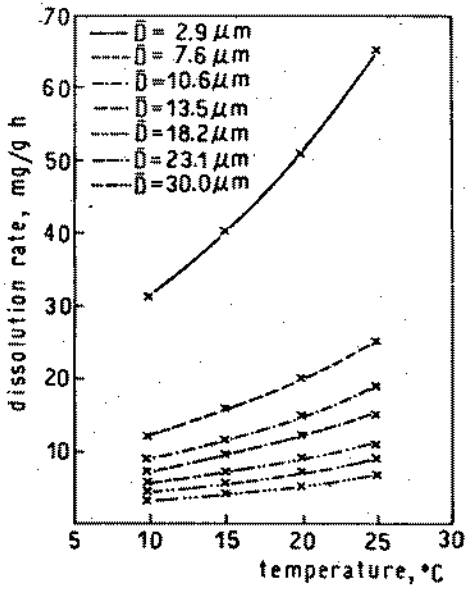

Fig. 2.- Influence of temperature on cuprous oxide dissolution rate in artificial sea water (pH 8.20 and 0.48 M NaCl)

TABLE III.- FOULING FIXATION IN RAFT TRIALS*

Paints.....	Formulation 1				Formulation 2			
Dispersion time..	1 h	3 h	5 h	10 h	1 h	3 h	5 h	10 h
17 months immersion.....	1-2	1	0-1	0	2-3	1-2	0-1	0-1
25 months immersion.....	2-3	1-2	1	0-1	4-5	2-3	1	1
Particle average diameter of Cu_2O, µm.....	28.0	16.5	12.7	8.7	23.8	15.7	11.3	8.2
Cu_2O dissolution rate in artificial sea water, mg/g.h.....	5.6	9.6	12.8	17.6	6.8	10.0	14.1	18.0
Binder dissolution rate in artificial sea water, µg/cm^2.day.....	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4

Paints.....	Formulation 3				Formulation 4			
Dispersion time..	1 h	3 h	5 h	10 h	1 h	3 h	5 h	10 h
17 months immersion.....	0-1	0-1	0	0	1	0-1	0	0
25 months immersion.....	1-2	0-1	0	0	2-3	1	0-1	0-1
Particle average diameter of Cu_2O, µm.....	26.8	15.8	11.9	7.8	24.3	16.1	12.0	8.6
Cu_2O dissolution rate in artificial sea water, mg/g.h.....	6.0	9.9	13.6	19.4	6.4	9.8	13.6	17.5
Binder dissolution rate in artificial sea water, µg/cm^2.day.....	31.3	31.3	31.3	31.3	31.3	31.3	31.3	31.3

* Key of the table: 0, without settlement; 0-1, very rare; 1, rare; 2, common; 3, very common; 4, abundant; 5, panel completely fouled.

Characteristics of the artificial sea water employed in the tests: pH 8.20; temperature, 20°C; concentration, 0.48 M NaCl.

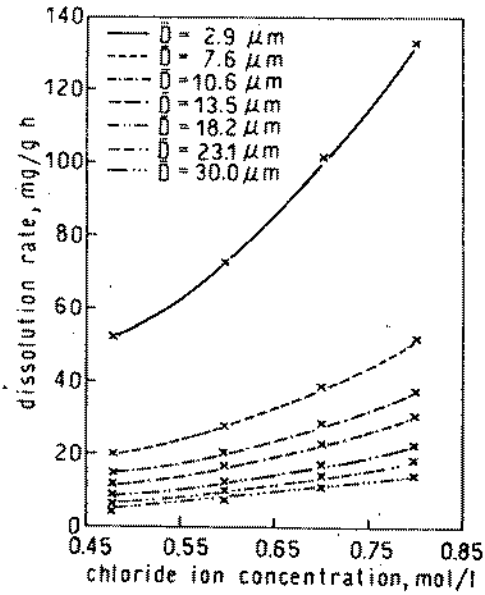

Fig. 3.- Influence of chloride ion concentration on cuprous oxide dissolution rate in artificial sea water (pH 8.20 and 20°C)

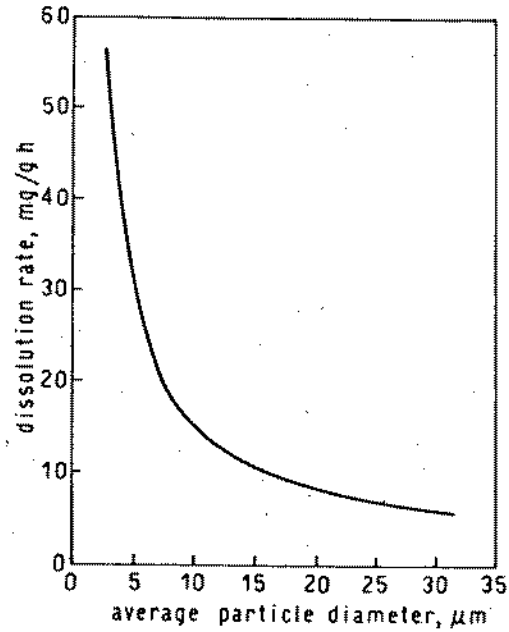

Fig. 4.- Cuprous oxide dissolution rate in artificial sea water (20°C, pH 8.20 and 0.48 M NaCl)

Therefore the rate in mg/g.h is different.

In Figure 4 it is shown that for pigment particles of a determinate average diameter $\overline{D}$ = 2.6 μm, the rate is 52 mg/g.h, while for $\overline{D}$ = 20.0 μm it decreases to 8.0 mg/g.h. This reveals the significant influence of the dispersion effectiveness on the dissolution rate.

2. BIOACTIVITY OF ANTIFOULING PAINTS

The test performed on the experimental raft allowed to evaluate the toxic behaviour of the antifouling paint samples, after 17 and 25 months immersion. Table III lists the values registered for fouling fixation.

After two summer periods of intense biological activity, the first inspection (17 months) showed that the paints corresponding to binders of lower dissolution rate (3/1 ratio and elaborated with high and medium toxicant content, did not fulfil the trial requirements (fouling fixation 1 or less, according to the scale of Table III), when the paints were obtained after very short dispersion times (1 hour for the formulation 1 and 1 and 3 hours for the formulation 2).

However antifouling paints elaborated starting from the same formulation (1 and 2) but with longer dispersion times, showed fixation 0, 0-1 or 1, for the same period.

Samples based on formulations 3 and 4 had fixation values 0, 0-1 or 1 for similar immersion periods. The binder employed in this case (5/1 WW rosin/plasticizer ratio) has greater dissolution rate and consequently films of good bioactive characteristics were obtained.

The second observation, after 25 months, including two summer periods of intense fouling activity, confirms the tendency above mentioned concerning the behaviour of samples elaborated with longer dispersion times (Fig. 5). These results are satisfactory both as regards the two types of binders and the two toxicant contents considered in this research. Samples with binders of lower solubility (3/1 ratio), dispersed during 5-10 hours fulfilled the test requirement (fixation 1 or less); in paints based on a binder/plasticizer ratio 5/1, only samples with lesser dispersion time (1 hour) did not fulfil the mentioned requirement; the other samples showed a very good behaviour in the raft trial.

3. DISSOLUTION RATE OF CUPROUS OXIDE AND PAINT EFFECTIVENESS

The average diameter of the cuprous oxide particles and their dissolution rate become significantly important. They influence on the concentration of chlorinated cuprous complexes formed by solubilization of cuprous oxide inside the film and consequently they modify the transference rate of toxicant toward the paint film/sea water interphase by molecular diffusion. It is important to point out that the diffusion of chloride and hydrogen ions is quicker than that

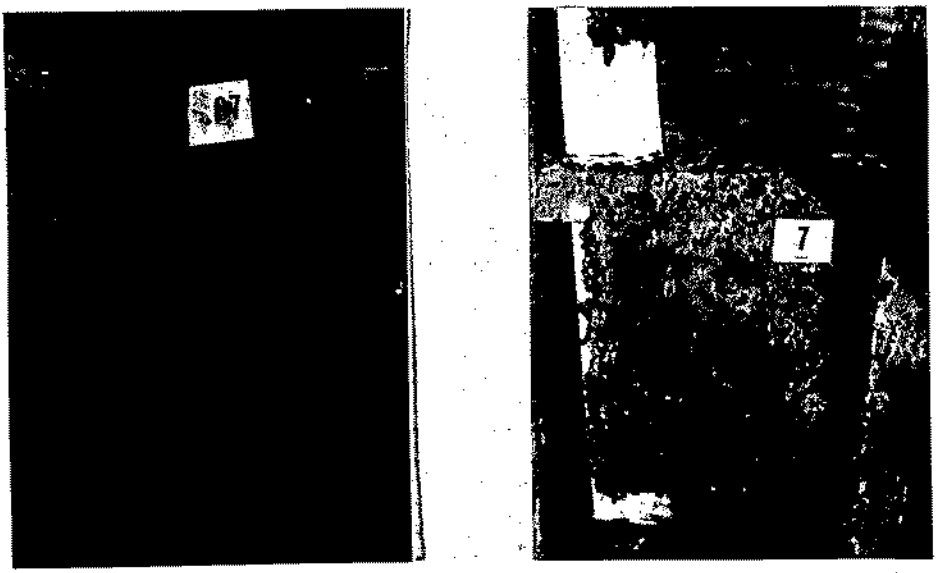

Figure 5.- Panels corresponding to formulation 4 (binder/plasticizer ratio 5/1), medium cuprous oxide content. Left, fixation 0-1, paint elaborated with a dispersion time of 10 hours; right, fixation 2-3, elaboration time 1 hour. Prints correspond to 25 months in the raft at Puerto Belgrano.

corresponding to the chlorinated copper complexes, due to a higher concentration gradient and a smaller molecular size.

Laboratory and raft trials carried out to determine the effectiveness of the different samples of antifouling paints allowed to establish that according to the type of binder formulated (3/1 or 5/1 rosin/plasticizer ratio) it is possible to define the minimum dispersion degree necessary to obtain a dissolution rate of cuprous oxide leading to a concentration of saturation of chlorinated copper complexes and improving the diffusional process, therefore the leaching rate. For example, for a useful life of about 2 years, the binder corresponding to a 3/1 ratio (dissolution rate 26.4 $\mu g/cm^2.day$) required a mean particle diameter no greater than 11 µm (14 mg/g.h for a 0.48 M sodium chloride solution at 20°C and pH 8.20), to obtain a satisfactory bioactivity. Besides, it was observable that the cuprous oxide content had no significant influence under these conditions.

On its turn, 5/1 binder (31.3 $\mu g/cm^2.day$) required a particle diameter no greater than 9 µm (16.0 mg/g.h). The cuprous oxide content proved to be an unimportant variable, as in the previous case.

ACKNOWLEDGEMENTS

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BIO-ACTIVE MATERIALS FOR ANTIFOULING COATINGS

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ABSTRACT

- Today's most economical method to prevent marine fouling on ship's hulls is the use of antifouling coatings which slowly release bio-active materials.
- At present, the great majority of antifouling coatings are based on copper compounds, organotin derivatives, and on their combinations. All existing legislation allows the use of cuprous oxide as bio-active material, and differs marginally for the nature and concentration of organotin derivatives allowed.
- The various steps involved for the introduction of new bio-active materials are described. Such an introduction requires a close cooperation between the bio-active material supplier and the paint manufacturer.
- It is believed that very few new bio-active materials will appear on the market in the future.

- La méthode actuellement la plus économique pour prévenir les salissures marines sur la coque des navires consiste à employer des peintures anti-salissures qui libérent des materiaux bio-actifs.
- La grande majorité des peintures anti-salissures en usage contiennent des composés de cuivre, des derivés d'organo-étain ou des mélanges de ces matériaux. Toutes les législations existantes permettent l'utilisation de l'oxyde cuivreux comme materiau bio-actif, et différent marginale-

ment pour la nature et la concentration des dérivés d'organoétain permis.
- Les différentes étapes nécessaires pour introduire de nouveaux matériaux bio-actifs sont décrites. Une telle introduction necessite que le fournisseur du materiau bio-actif et le fabricant de peintures collaborent étroitement.
- Il est probable que trés peu de matériaux bio-actifs nouveaux apparaitront sur le marché dans le futur.

1. INTRODUCTION

Marine fouling is the result of the settling and subsequent growth of marine organisms on surfaces immersed in sea water. The fouling process and the consequences of marine fouling are well known and will not be described in this paper. Information on these subjects may be obtained from e.g. references (1) to (4).

Many different principles have been suggested which should be able to impede the fouling process: Special surface preparations (5,6,7), electrolytic methods (8), etc. However, the only commercially successful principle in use today for the protection of ship's hulls is the release of bio-active materials from antifouling coatings. The latest development within marine fouling protection - the self-polishing antifouling coatings - is also based on this principle.

The purpose of this paper is to discuss bio-active materials for antifouling coatings. In the first place, the legislation in force in various countries will be reviewed and compared with the bio-active materials actually in use. The long process involved for the introduction of new bio-active materials will then be described. A method for evaluation of their performance in paints will be presented.

2. TODAY'S BIO-ACTIVE MATERIALS FOR ANTIFOULING COATINGS

The antifouling coating industry is dominated by a few large companies and associations. For world-wide supply, the antifouling coatings have to be in accordance with existing legislation of different countries.

2.1 Legislation on Bio-Active Materials - A Survey

2.1.1 *Europe, EEC members*

In 1967, a directive for the classification, packing, and labelling of dangerous substances was issued by the EEC (67/548/EEC). The 6th amendment to this directive (79/831/EEC) requires that substances which have not been marketed within the EEC before September 18th 1981 are to be defined as "new chemicals" and are consequently subject to notification before commercial use. At present a list of the chemical compounds found within the EEC on September 18th 1981 (the socalled European Inventory of Existing Commercial Substances, EINECS) is being prepared by the Commission.

The core of this inventory is ECOIN (European Core Inventory), listing approximately 33,000 chemical substances whose existence within the EEC before September 18th 1981 is documented. A second list called "List of Known Substances" comprises approx. 28,000 chemical substances which have been used commercially before September 18th 1981. Substances from this list, and chemical substances not listed, but known by the individual importer or producer to have been in use before September 18th, have been entered on EINECS at the request of the individual importer or producer. The period within which requests could be made expired on December 31st 1982, and the publication of EINECS is now awaited.

The notification of new chemical substances involves physical and chemical analyses and animal toxicity testings. A survey of the notification requirements is given in Table 1.

Apart from differences in the interpretation of the EEC directive and in implementation dates, each individual member country may have decided on additional restrictions in the use of certain chemical substances.

In *France* the use of organotin compounds in antifouling coatings has been prohibited for boats of less than 25 metres. The prohibition will last for a period of two years, ending on October 1st 1984 (9).

2.1.2 *Europe, non-EEC members*

In *Sweden* a limited number of bio-active materials are allowed for use in antifouling coatings (10). The

compounds are listed in Table 2. As can be seen from the Table, maximum concentrations have been laid down for most of the biocides. In addition, there are certain restrictions in their use when mixed. Furthermore, it can be seen that except for the triphenyltin copolymer all antifouling agents approved in Sweden are covered by ECOIN.

2.1.3 *Japan*

In *Japan* a great number of the existing shipyards belong to the Japanese Shipbuilders' Association. In 1973 the Japanese Shipbuilders' Association and the Japan Paint Industry Association established a list of antifouling agents approved for use in shipyards which belong to the Japanese Shipbuilders' Association. Since 1973 the list has been expanded 4 times (latest in 1979) and today 18 antifouling agents are included. The compounds are listed in Table 3. From the table it can be seen that 10 out of the 18 antifouling agents approved by the Japanese Shipbuilders' Association are covered by ECOIN.

2.1.4 *U.S.A.*

In the *U.S.A.* antifouling coatings and bio-active materials for antifouling coatings are considered as pesticides. The legislative authority for pesticide registration is the Federal Insecticide, Fungicide and Rodenticide Act, FIFRA.

The Environmental Protection Agency, EPA, is responsible for regulating the sale, distribution, and use of pesticides under FIFRA and with certain minor exceptions FIFRA requires that all pesticides must be registered by EPA before they can be sold or distributed commercially.

The procedures for satisfying registration data requirements have not been finally settled yet. However, EPA has issued a PR Notice (PR Notice 83-4 of June 16th 1983 and PR Notice 83-4A (supplement) of June 23rd 1983) announcing an interim procedure called the Owners Submission Method by which applicants for registration under FIFRA may satisfy the statutory requirement to provide data in support of their applications. Each applicant who uses the Owners Submission Method must prepare and submit with his application a list of the data requirements which he believes to be applicable to the product he seeks to register. The list must be based on EPA's proposed regulations in 40 C.F.R. Part 158 "Data Requirements

for Registration", 47 Fed. Reg. 53, 192 (November 24th 1983). The procedures established by this PR Notice took effect on June 30th 1983 and will remain in effect until EPA promulgates final effective rules.

The data required for pesticide registration specified in 40 C.F.R. Part 158 pertain to product chemistry, residue chemistry, environmental fate, toxicology, reentry protection, wildlife and aquatic organisms (plant protection), non-target insects, organism product performance (normally not needed).

With reference to the proposed 40 C.F.R. Part 158, the applicant should select the general use pattern(s) which best cover the use patterns specified in the proposed labelling of the pesticide product. The nine general use patterns on which most data requirements are based appear at the headings in the tables of data requirements. Appendix A in 40 C.F.R. Part 158 contains a list of several hundreds of specific use patterns and the corresponding general use pattern for each. The applicant should next determine which specific types of studies are required for each of the general use patterns of his product by referring to each of the tables of data requirements (e.g. §158.120 Product chemistry data requirements). The tables indicate for each type of study and general use pattern whether data are usually required, conditionally required or not usually required. The footnotes accompanying each table identify the specific circumstances under which each type of study is required.

For the Owners Submission Method the applicant is required to

1) submit a list of data requirements applicable to his product;
2) satisfy each data requirement either
   a) by submitting (or citing) his own valid data;
   b) by citing valid data previously submitted to EPA by another, with the original submitter's permission;
   c) in certain cases by documenting that no data have previously been submitted which would meet the specific data requirements; or
   d) by a combination of these methods.

Before initiating any tests, the applicant should determine whether he is eligible for the "formulator's exemption" of FIFRA: An applicant for registration of an end-use product is excused from the normal requirements of submitting or citing

data on the safety of any ingredient in the applicant's product present solely as a result of incorporation into his product of another product containing that ingredient which is registered under FIFRA and purchased from another producer.

Antifouling coatings containing e.g. copper compounds or organotin derivatives or both are common on the U.S. market.

On the basis of the lists of approved antifouling agents, it may be concluded that antifouling coatings based on copper compounds - primarily cuprous oxide - and selected triorganotin compounds are the most suitable for world-wide supply. In addition to those compounds only a few other biocides may be used.

2.2 Antifouling Coatings in Use

Statistical material from Japan and from other countries confirms that antifouling coatings in use today are based on cuprous oxide, triorganotin compounds and a few other bio-active materials.

A statistical evaluation on 426 paints in use in Japan shows that the major part is based on either a combination of cuprous oxide and triorganotin compounds (40%) or cuprous oxide alone (32%). The figures are listed in Table 4. Significantly fewer paints are based on triorganotin as the only toxicant (13%). Approximately 15% of the paints contain other bio-active materials, the major part in combination with triorganotin (12%). In addition, the figures show that 85% of the paints are of the traditional type (non-polishing), whereas 15% are based on organotin copolymers, i.e. are of the selfpolishing type. The material from Japan is confirmed by the World List of Organotin-based Antifouling Composition (11). Apart from cuprous oxide and triorganotin compounds, a few other compounds are mentioned in the list: copper thiocyanate, copper bronze flakes, isothiazolones.

The world-wide success of cuprous oxide and triorganotin compounds as antifouling agents is explained by their fulfilling the requirements of bio-active materials in antifouling coatings to a very high extent. Incorporated in selfpolishing coatings, these bio-active materials are able to secure a long life protection of ship's hulls. When incorporated in traditional-type coatings, good protection is obtained as well. However, in the case of the

latter type, the paint film is not renewed during service and in certain confined areas early fouling with slime has been recorded. Thus, an effort should be made to find new bio-active materials which are able to prevent fouling with slime.

If the triorganotin compounds and cuprous oxide are not used as directed by the manufacturers, they are potentially toxic to human beings, and the triorganotin compounds are known to be highly irritating to skin and eyes. However, if handled as directed, these compounds are safe in use. The environmental impact of the compounds has been studied intensively. In confined areas with low dilution capacities and a high activity of ships - like in the Suez Canal or in some harbours - accumulation of copper and tin in the bottom sediment has been recorded (12, 13, 14). However, it has also been demonstrated that the concentration of the bio-active materials in the sediment decreases drastically with increased dilution capacity, i.e. by sampling just outside the confined areas.

In spite of the world-wide success of triorganotin and cuprous oxide, as demonstrated in the preceding text, there is still a search going on in order to find new antifouling agents. As it has been the case for many years, part of the research is directed towards bio-active materials providing an even better compromise between efficacy, economy, low mammalian toxicity, and inactivation in the environment. Another direction of research concerns effective slimicides, of special interest in non-polishing (traditional) coatings.

3. NEW BIO-ACTIVE MATERIALS FOR ANTIFOULING COATINGS

In the past, thousands of bio-active materials have been tested for potential use as antifouling agents. The majority of the biocides manufactured have been - and are still being - developed for other end uses, such as pest control in agriculture, wood preservation, preservation in the paper industry, etc.

In the present situation where a world-wide introduction of a new antifouling agent may involve huge investments in order to obtain final approval, it is of the utmost importance that cooperation between the suppliers' development chemists and the antifouling paint experts is initiated at the development stage to ensure that the materials synthesized comply with the requirements for their uses. Thus, any bio-active material under consideration, whether originally designed for use in antifouling coatings or not, must undergo a serious evaluation by the paint manufac-

turers before a decision is made as to its potential use as antifouling agent.

3.1 Evaluation of New Bio-Active Materials

The evaluation of new bio-active materials comprises considerations of technical nature as well as environmental impact. The procedure may involve:

Documentation
Screening by lab. tests and simple raft tests
Further evaluation in paints
Practical testing
Approvals

3.1.1 *Documentation*

On the basis of the information available on the biocides a first evaluation of their potential use as antifouling agents is made. At this stage preliminary investigations as regards possible later approval of the compounds are normally initiated in cooperation with the raw material suppliers.

3.1.2 *First Screening*

As the major part of the bio-active materials under test have been synthesized for other purposes, only limited information is normally available as regards their activity against marine fouling organisms. Consequently, the first screening comprises efficacy testing of the biocides against marine organisms. In the laboratory, LD_{50}-values are estimated with respect to selected marine organisms: Enteromorpha (green alga), Giffordia (brown alga), Achnantes (diatom), nauplii larva and cyprid larva of barnacles, and selected bacteria strains. LD_{50}-values for cuprous oxide and certain triorganotin compounds are estimated for comparison. Secondly, the biocides are tested by the 74% volume toxicity test. The test was presented at the 4th International Congress on Marine Corrosion and Fouling, 1976, by S. Johnsen and V. Rendbæk (15): The bio-active materials are incorporated in simplified test compositions made up with a non-volatile composition of 74% by volume bio-active material and 24% by volume vinyl resin. The compositions are exposed to fouling organisms at different test sites: Denmark, Spain, India, etc.

3.2.3 *Further Evaluation in Paints*

On the basis of the results obtained from the first screening, the most promising biocides are being selected for further evaluation in model paints. Fig. 1 illustrates the matrix on which all model paints are based. The various symbols used are explained in Fig. 2. Basically, 60.5% of the solid volume constitute binder-like ingredients and 39.5% constitute pigment-like ingredients, i.e. the pigment volume concentration (PVC) equals 39.5%. Apart from the additives, the binder-phase is composed of seawater soluble and seawater insoluble binders and/or liquid type biocides, whereas the pigment-phase consists of zinc oxide and/or pigment-like biocides. In Fig. 3 the set of model paints involved in the testing of one pigment-like biocide is shown. The biocide BAM X is tested under the following conditions:

- in different concentrations: 18.5%, 9.25%, 37% solid volume, with a constant total amount of seawater soluble ingredients (TSS) = 74 (paint no. 1, 2, and 3);

- at various TSS levels: 74%, 55.5%, and 37% with a constant concentration of biocide = 18.5% (paint no. 1, 4, 5, 6);

- in combination with a hydrophilic binder (paint no. 7);

- in combination with standard biocides (paint no. 8 to 12).

Seven paints, of which 6 are based on standard biocides, are included as references (paint no. 13 to 19).

The paint properties are evaluated in the laboratory and exposure to fouling organisms take place from raft.

By formulating and testing this set of paints for each potential biocide, the following important properties are clarified:

- compatibility with other paint ingredients, stability in paints (paint no. 1-12)

- activity against fouling organisms (paint no. 1-12 versus 13-19)

- solubility in seawater, leaching rate (paint no. 1-7)

- synergistic effects in combination with standard biocides (paint no. 8-12 versus 13-19)

On the basis of this testing a clear yes or no is given to the potential use of the bio-active material in antifouling coatings. Furthermore, a clear indication of the most favourable formulations of paints based on the experimental biocide is given.

3.1.4 *Practical Testing*

The final stage in the testing of new bio-active materials comprises formulation and testing of final paints. The paints are formulated on the basis of the results obtained from the testing of the model paints. The testing includes exposure from raft, ageing on rotor, and practical testing on ships.

3.1.5 *Approvals*

For promising biocides the entire period of testing will last two years or more. During this period a close contact between the raw material supplier and the paint manufacturer should be maintained. This contact will ensure that the raw material supplier - having to pay all expenses for the initial registrations and approvals - is able to evaluate the market for the biocide at an early stage. In order to cover the expenses for approval, several customers for the new biocide will usually be needed.

4. CONCLUSION

On the basis of the present situation as regards registration of chemical substances in general and legislation on antifouling agents in particular, it is believed that very few new bio-active materials for antifouling coatings will appear on the world market in the future. The development of new antifouling agents must be based on a close cooperation between the suppliers' development chemists and the antifouling paint experts. This will ensure that the materials synthesized comply with the requirements for their end use and thus justify the expenses for approval.

Acknowledgement

I thank my colleagues from Hempel Technology A/S for their help in preparing this manuscript. I also thank the Management of Hempel Technology A/S for permission to present this lecture.

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Identity of substance
Information on the substance in the commercial form
Information relating to the determination of the substance
Information on the manufacturing, use, quantity and distribution of the substance
Physico-chemical properties of the substance
Acute toxicity (peroral, inhalation, percutaneous, skin irritation, eye irritation, sensitization)
Sub-acute toxicity (28 days test)
Mutagenic effects
Eco-toxicity
Degradation
Proposals by notifier as regards classification and labelling
Proposals by notifier as regards special precautions
Proposals by notifier as regards emergency measures
Possible measures after use of the substance
Literature references
Information which the notifier requests to be regarded as confidential

Table 1. Pre-marketing notification requirements of "new" chemical substances as specified in the EEC Council Directive (79/831/EEC).

Table 2. Antifouling agents allowed in Sweden

	Maximum content weight % Sn	listed in ECOIN (EEC)
Organotin compounds:		
A: bis(tributyltin)oxide :	2	+
B: tributyltin fluoride :	4	+
copolymer of tributyltin :	4	+
C: triphenyltin hydroxide :	3	+
triphenyltin chloride :	3	+
triphenyltin fluoride :	3	+
D: copolymer of triphenyltin :	6	-

(Conc. Sn group A + 1/2 x conc. Sn group B + 2/3 x conc. Sn group C + 1/3 x conc. Sn group D ≦ 2%)

	Maximum content weight %	listed in ECOIN (EEC)
Copper compounds:		
copper :	no limitation	+
cuprous oxide :	no limitation	+
copper thiocyanate :	no limitation	+
Sulphur compounds:		
tetramethylthiuram-disulphide (thiram) :	5	+
zinc dimethyldithiocarbamate (ziram) :	5	+

(Conc. thiram + conc. ziram ≦ 5%)

Others:		
zinc oxide :	no limitation	+

Maximum content is calculated for a wet paint based on 50% by weight dry material.

Organotin compounds:	Maximum content Weight %	Listed in ECOIN (EEC)
triphenyltin hydroxide (TPTH)	20 (converted into TPTH)	+
triphenyltin acetate		+
triphenyltin chloride		+
triphenyltin fluoride		+
triphenyltin versatate		
triphenyltin dimethyl dithiocarbamate		
bis(triphenyltin)α,α'dibromo-succinate		
triphenyltin monochlor-acetate		
triphenyltin nicotinate		
triphenyltin alkyd polycondensation		
triphenyltin methacrylate copolymer		
tributyltin fluoride (TBTF)	20 (converted into TBTF)	+
tributyltin methacrylate copolymer		+
bis(tributyltin)α,α'dibromo-succinate		+
tributyltin alkyd polycondensation		
Others:		
cuprous oxide	no limitation	+
tetramethylthiuram disulphide		+
zinc dimethyldithiocarbamate		+
Maximum content is calculated for a wet paint		

Table 3. Antifouling agents accepted by the Shipbuilders' Association and the Japan Paint Industry Association.

BIO-ACTIVE MATERIALS	TYPE OF COATING		TOTAL
	TRADITIONAL (Non-polishing)	SELFPOLISHING (Based on organotin copolymer)	
Cuprous oxide alone (Cu_2O)	135	-	135 (32%)
Triorganotin alone (OT)	41	14	55 (13%)
Cu_2O + OT	126	44	170 (40%)
Cu_2O + others	0	-	0
OT + others	52	2	54 (12%)
Cu_2O + OT + others	7	5	12 (3%)
Total number of coatings	361 (85%)	65 (15%)	426 (100%)

Others: Tetramethylthiuram disulphide and zinc dimethyldithiocarbamate.

Table 4. Bio-active materials in antifouling coatings from Japan.

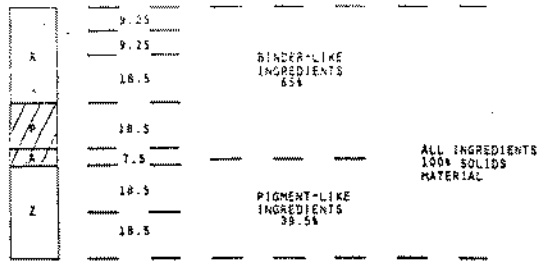

FIG 1. Matrix formula for model paints (volume % solids material).

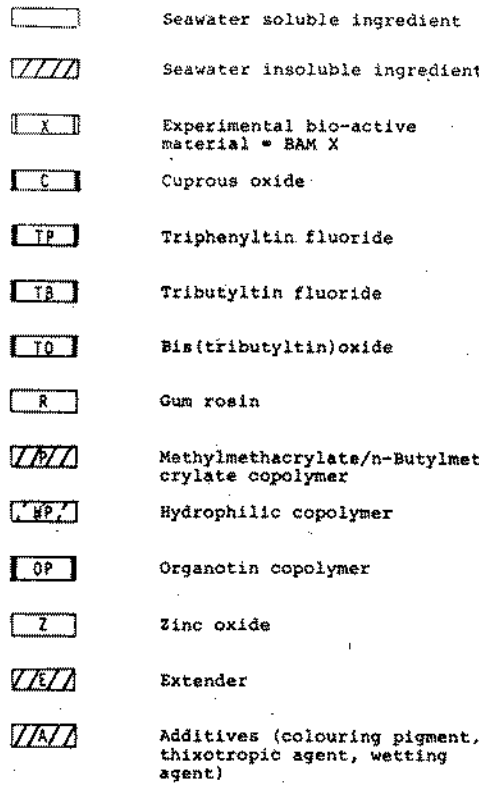

FIG 2. Symbols used for the description of model paints.

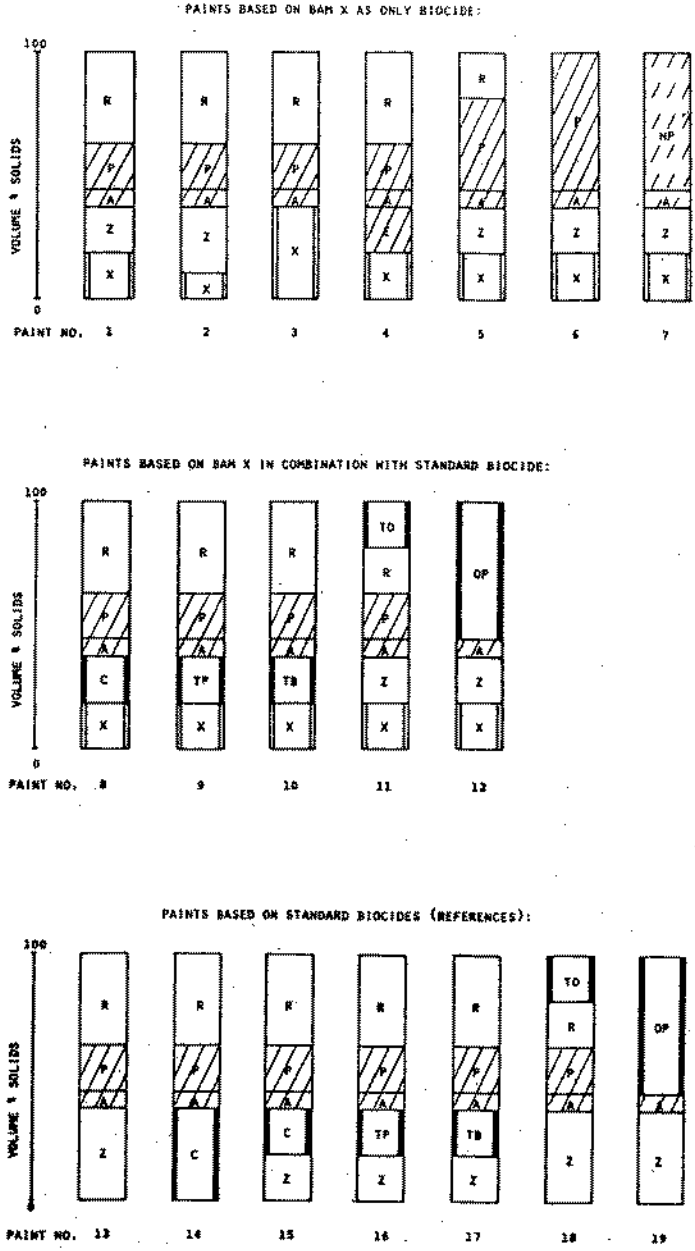

FIG 3. The set of model paints involved for evaluation of one bio-active material BAM X.

A WORLD-WIDE SURVEY OF FOULING ON NON-TOXIC AND THREE ANTI-FOULING PAINT SURFACES

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Abstract:

Four test panels were immersed for two months at each of fifteen sites around the world covering the period January to December 1983. At each site a non-toxic control and three surfaces coated with anti-fouling paint or varnish were assessed for quantitative and qualitative differences in the fouling community which developed. The anti-fouling compositions contained either cuprous oxide or tributyl tin or both cuprous oxide and tributyl tin as biocides.

The results show that in the majority of cases, animal and weed fouling was minimal on the biocide-containing surfaces. However, these surfaces frequently supported a thick diatom slime. Diatoms of the genus *Achnanthes* preferentially colonize the tributyl tin varnish whilst diatoms of the genera *Amphora, Amphiprora* and to a lesser extent *Stauroneis* are commonly found on both the paints containing cuprous oxide. The results are discussed in relation to the relative resistance of fouling organisms to copper and organotin biocides.

Resumé

Quatre panneaux d'analyse furent immergés dans la mer pendant deux mois en quinze localités différentes, à travers le monde, couvrant la période de Janvier à Décembre 1983, A chaque localité un témoin conservé inoffensif et trois faces recouvertes d'un enduit anti-salissures de peinture ou de vernis furent placés à l'intérieur où se développent les salissures biologiques afin d'évaluer des différences qualitative et quantitative. Les mélanges anti-salissures contenaient soit l'oxyde de cuivre, soit d'étain tributyl ou encore les deux à la fois pris comme "biocides". Les résultats montrent que dans la majorité des cas, les salissures des animaux

et des algues était minimale sur les faces contenant du biocide. Cependent ces faces supportaient fréquemment un épais limon de diatomées. Les diatomées due genre *Achnanthes* de preférénce envahissent le vernis d'étain tributyl pendant que ceux des genres *Amphora*, *Amphiprora* et à un degré moindre les *Stauroneis* se trouvaient communément sur les deux faces peintes à base d'oxyde de cuivre. Les résultats sont appréciés par rapport à l'une et l'autre des résistances relatives des organismes sallisures au cuivre et des biocides organotins.

Introduction

Fouling results from settlement and growth of a variety of plant and animal forms. Traditionally, barnacles were the commonest fouling organisms and of greatest economic importance (see Southward and Crisp, 1963). Their larvae settled on ships whilst in port then grew quickly into adults causing increased frictional resistance of ships and disruption of the paint film resulting in corrosion. Modern supertankers with quick turn-round periods and rapid transit between tropical and temperate water favour the settlement and growth of algae rather than barnacles and other animals. The most common fouling algae on ships are species of *Enteromorpha* (green alga, often called "grass") and *Ectocarpus* (brown alga, often called "brown felt"). The dominance of *Enteromorpha* as a cosmopolitan fouling alga resides in its enormous reproductive potential, its highly effective spore attachment mechanism and its ability to withstand widespread fluctuations in environmental conditions (Biebl, 1962; Christie and Shaw, 1968). The development of organotin-containing anti-fouling paints led to *Enteromorpha* becoming less important as a ship-fouling alga than the more resistant *Ectocarpus*. *Ectocarpus* is also cosmopolitan with enormous reproductive potential. Spores germinate and colonize a surface due to growth of horizontal creeping filaments from which erect filaments arise. Following the introduction of copolymer anti-fouling paints (see Evans, 1981) weed fouling became less important. In copolymer anti-foulings a polymer of tributyl tin methacrylate and methyl methacrylate hydrolyses in seawater releasing the biocide tributyl tin in a controlled manner. Other biocides such as cuprous thiocyanate and cuprous oxide are also incorporated into the polymer matrix and are also released as it hydrolyses. Provided the ship is moving, as the polymer hydrolyses the paint surface "polishes" becoming smoother and thereby reducing the frictional resistance of the vessel. Ships coated with copolymer paints can remain free of fouling if operating conditions are optimal. However, this is often not the case and copolymer paints frequently become fouled by diatom slimes (Christie *et al.*, 1976; Daniel *et al.*, 1980) and occasionally by algae and barnacles.

The voyaging patterns of ships are very complex and the geographical location associated with the onset of fouling is rarely known. The present study was initiated to provide data on the fouling communities of the world. The main objectives were:

WORLD FOULING SURVEY

FIG.1. WORLD MAP SHOWING LOCATIONS OF THE TESTING SITES

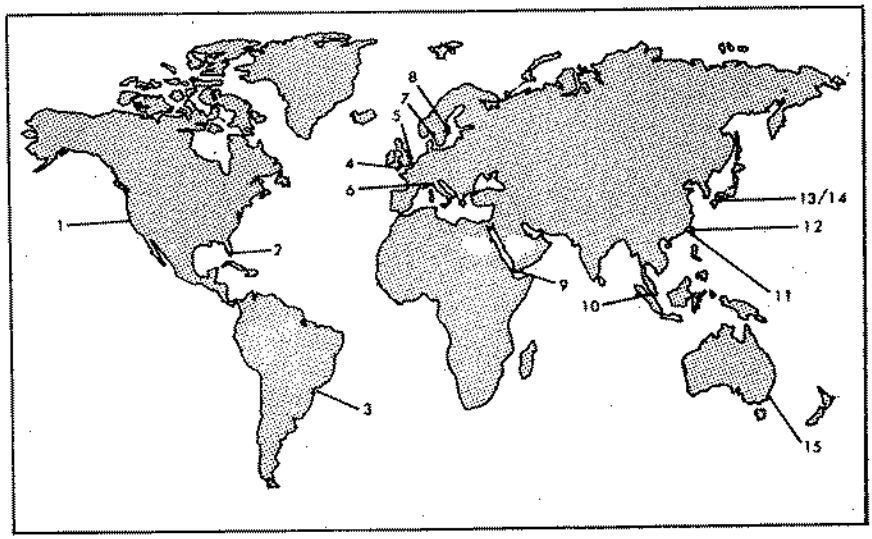


TABLE 1. GEOGRAPHICAL LOCATION OF SITES IN FIG.1.

NUMBER	COUNTRY	SITE
1	United States of America	San Francisco
2	United States of America	Miami
3	South America	Rio-de-Janeiro
4	United Kingdom	Newton Ferrers
5	United Kingdom	Burnham
6	Italy	La Spezia
7	Sweden	Bratton
8	Sweden	Djuro
9	United Arab Emirates	Dubai
10	Singapore	Singapore
11	Hong Kong	Hong Kong
12	Taiwan	Kaohsiung
13	Japan	Tamano (Uno)
14	Japan	Aioi
15	Australia	Sydney

WORLD FOULING SURVEY

TABLE 2: GEOGRAPHICAL CO-ORDINATES AND PHYSICAL CHARACTERISTICS OF TEST SITES

SITE	GEOGRAPHICAL CO-ORDINATES LAT.	LONG.	TEMPERATURE °C* D/J/F	J/J/A
SAN FRANCISCO	37°48'N	122°22'W	12	20
MIAMI	25°45'N	80°15'W	22	27
RIO-DE-JANEIRO	22°53'S	41°17'W	27	—
NEWTON FERRERS	50°18'N	4°02'W	8	16
BURNHAM	51°38'N	0°49'E	6	20
LA SPEZIA	44°07'N	9°48'E	13	22
BRATTON	57°55'N	11°45'E	0	19
DJURO	59°29'N	18°40'E	0	17
DUBAI	24°59'N	55°00'E	23	37
SINGAPORE	1°24'N	103°59'E	29	29
HONG KONG	22°22'N	114°15'E	17	27
TAIWAN	22°36'N	120°17'E	15	33
TAMANO	34°29'N	133°56'E	11	24
AIOI	34°44'N	134°23'E	9	24
SYDNEY	16°40'S	139°30'E	23	14

Footnote:

1) *Approximate seawater temperatures at or near the surface.
Average for December, January and February (D,J,F).
Average for June, July and August (J,J,A).

2) The water was clear at all sites except Burnham and Tamano where it is usually turbid and Singapore where it is turbid during the monsoon (December/January; June/July). At Kaohsiung (Taiwan) there is heavy oil pollution. At Rio-de-Janeiro there is chemical pollution.

1) to obtain an accurate list of marine species which can colonize non-toxic and three standard anti-fouling surfaces at various sites world-wide
2) to determine the seasonality of settlement and growth
3) to investigate the relative anti-fouling performance of copper, organotin and copper plus organotin-containing surfaces
4) to investigate whether geographical location or local differences e.g. clarity of water, salinity, pollution were the most important factors in determining the species composition and quantity of fouling which developed. For this purpose the survey was carried out at two sites each in the U.K., Sweden and Japan.

Materials and Methods

For each test three plastic strips, 8 x 3 cm, were secured to a wooden holder and immersed approximately one metre below the water surface. Half of one plastic strip was untreated and thus represents a non-toxic control whilst the other half was painted with a continuous contact vinyl-rosin anti-fouling paint containing cuprous oxide as the only biocide. A second strip was painted with a clear varnish composed of 60% tributyl tin methacrylate and 40% methyl/methacrylate and thus having tributyl tin as the only biocide. The third strip was painted with a tributyl tin methacrylate/methyl methacrylate copolymer anti-fouling paint containing cuprous oxide and thus having both tributyl tin and cuprous oxide as biocides. This paint is currently in widespread commercial use on ships. The four surfaces are referred to throughout this communication as non-toxic, copper, organotin and organotin/copper respectively. Some of the plastic strips used were black in colour hence some of the non-toxic and varnished panels appear black on the photographs.

Sets of test panels were immersed for two months covering the period January to December, 1983 at 15 sites around the world (see Fig.1 and Table 1). The geographical co-ordinates for each site are given in Table 2. On removal from the water each holder with panels was fixed overnight in 4% (v/v) formaldehyde in seawater. Excess formaldehyde was poured away and the holder sealed in a plastic box and sent to Birmingham for examination. Provided traces of formaldehyde remained in the box, preservation of organisms was good and drying out did not occur. Each surface was assessed for the quantity of fouling on a scale 0-5 where 0 represents the absence of biological settlement and 5 represents a complete cover of organisms which also extend 5mm or more out from the surface. Samples were removed from all the surfaces for microscopic examination. All organisms (macroscopic and microscopic) were identified as far as possible.

Results

Complete sets of test panels (i.e. six) covering 12 months were received from eight sites (San Francisco, Miami, Rio-de-Janeiro, Newton Ferres, Burnham, Tamano, Aioi and Sydney). Five sets of test panels were received from Dubai and Bratton. The quantitative fouling assessments for these ten sites are shown in Fig.2. Data

is not presented for the remaining five sites since the sets of panels received from January to December 1983 number four or less. The major fouling organisms for each surface are listed in Table 3.

At San Francisco there was no seasonal pattern of settlement on the non-toxic surface and colonization was chiefly due to a substantial diatom slime, intermixed with *Ectocarpus* and the filamentous green algae *Ulothrix*. Fifteen genera of diatoms were identified as constituents of the slime. Of these the most abundant were *Melosira sp., Navicula ramosissima, Achnanthes longipes, Navicula spp., Gyrosigma sp.* and *Nitzschia sp. A. longipes* grew throughout the year as an almost pure stand on the organotin varnish. *Amphora coffeaeformis* var. *coffeaeformis* and *Amphora veneta* were most abundant on the organotin/copper paint whilst these species plus *Amphiprora spp.* and small numbers of those species listed for the non-toxic panels occurred on the copper paint.

At Miami, *Balanus amphitrite* grew on the non-toxic surfaces from July to February with maximum settlement and growth in September - October. Algae grew vigorously throughout on the non-toxic panels but were never found on any of the anti-fouling surfaces. The organotin and organotin/copper surfaces were free from fouling except during November and December when a slime of *Achnanthes angustata* developed on the former surface and between September and December when *A. coffeaeformis, A. veneta* and *A. bigibba* were found on the latter surface. In addition to the three *Amphora* species, *Amphiprora spp.* and *Stauroneis sp.* formed slimes on the copper paint.

At Rio-de-Janeiro, heavy fouling of the non-toxic panels was encountered throughout the year with moderate fouling of all the biocide-containing surfaces(Fig.3). Algal growth did not occur except for *Ulothrix* and blue-green algae which were present in trace amounts on all surfaces. All anti-fouling compositions allowed settlement and growth of *Balanus amphitrite* and *Balanus tintinnabulum*, but in reduced numbers compared to the non-toxic control, and all supported substantial growth of the protozoan *Vorticella* and diatom slimes. The slimes from the copper and organotin/copper paints chiefly comprised *A. coffeaeformis* var. *perpusilla* and *A. veneta* with lesser quantities of *Amphiprora sp., Stauroneis sp.* and *Navicula spp., A. longipes* was the only diatom found on the organotin varnish.

At the two U.K. sites fouling differed both quantitatively and qualitatively. At Newton Ferrers settlement was restricted to the summer months (May - October) when ectocarpoid algae were found on the non-toxic controls. No settlement occurred on any antifouling surface except on the organotin varnish which supported the growth of the hydrozoan *Tubularia indivisa* in September/October. At Burnham (Fig.3) there was seasonal settlement of the Australian barnacle *Elminius modestus* (July - August) and *T. indivisa* (September - December). On the copper and organotin/copper surfaces, development of *E. modestus* did not proceed past the juvenile stage in contrast to the organotin varnish where growth was comparable with the non-toxic surface. Small amounts of fouling due to algae and

WORLD FOULING SURVEY

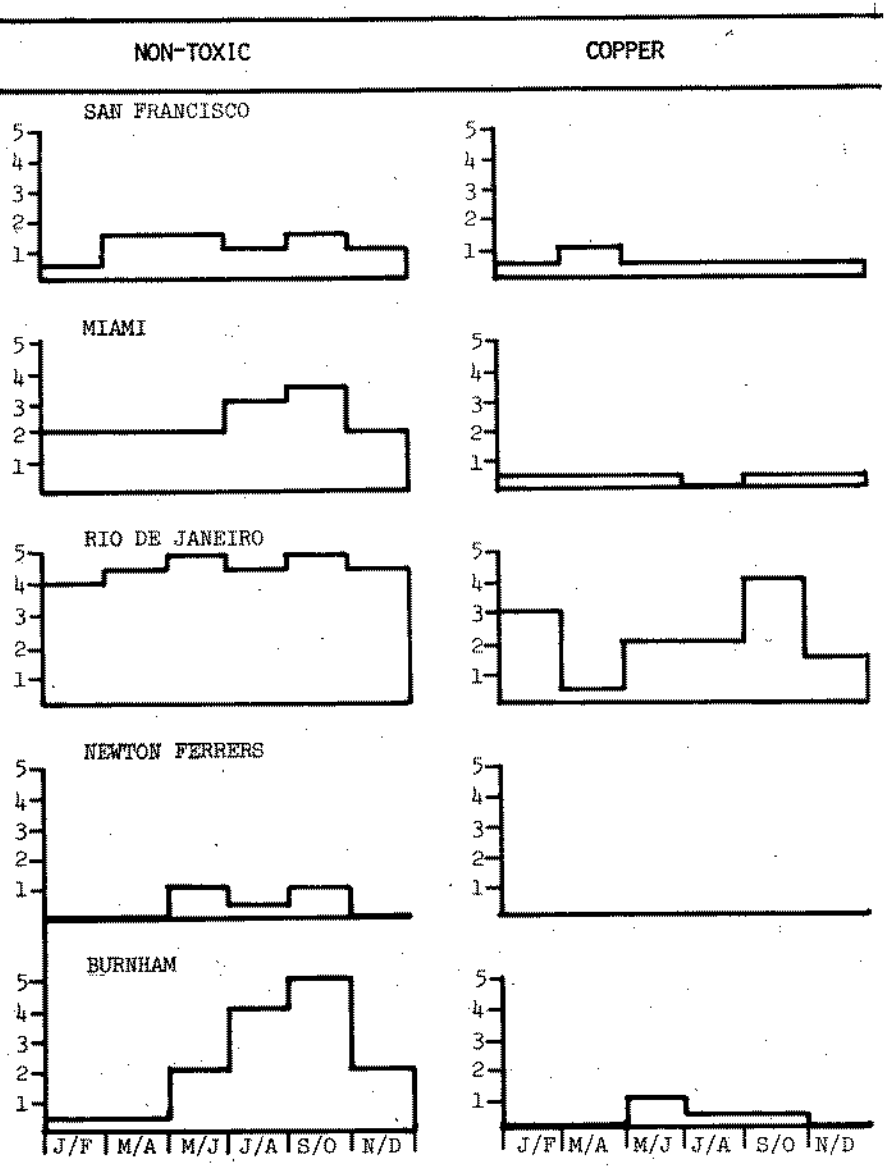
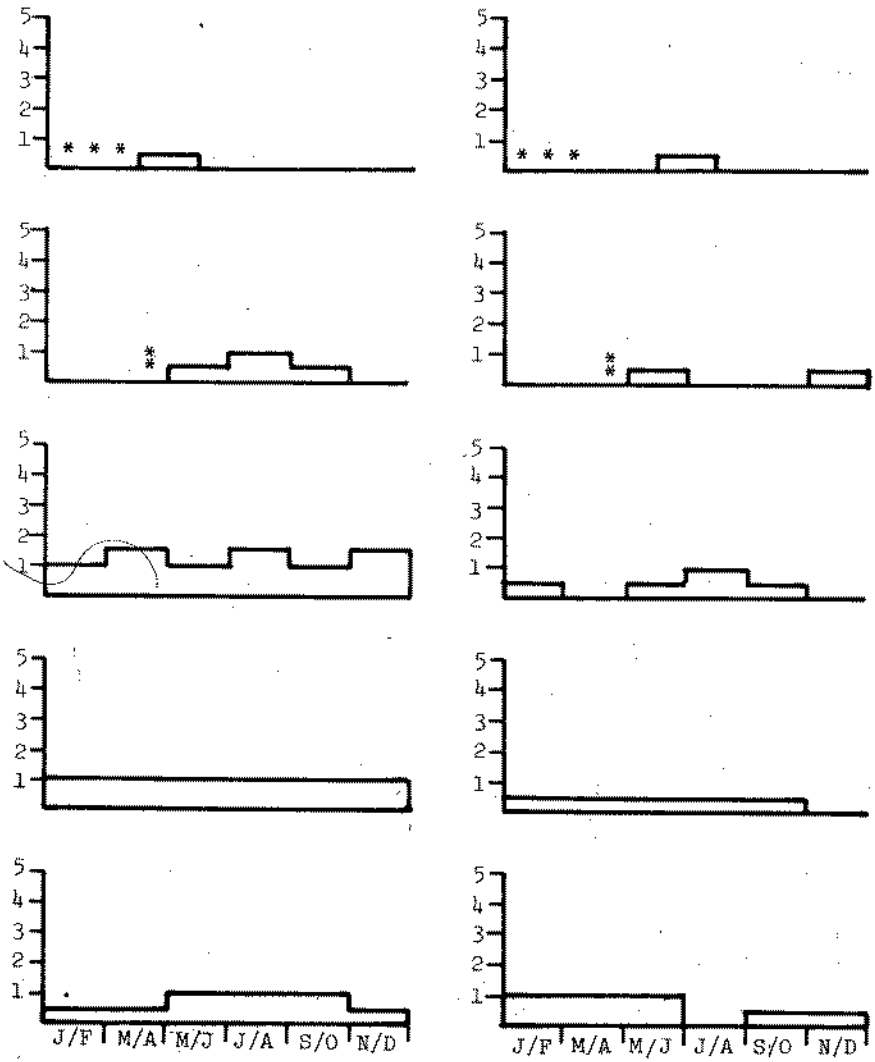


FIG.2. QUANTITATIVE FOULING ASSESSMENT OF FOUR SURFACES THROUGHOUT THE YEAR

WORLD FOULING SURVEY



Footnote:

1. J/F = January/February : M/A = March/April : M/J May/June : J/A = July/August : S/O = September/October : N/D November/December.
2. Total fouling was assessed on a scale 0-5.

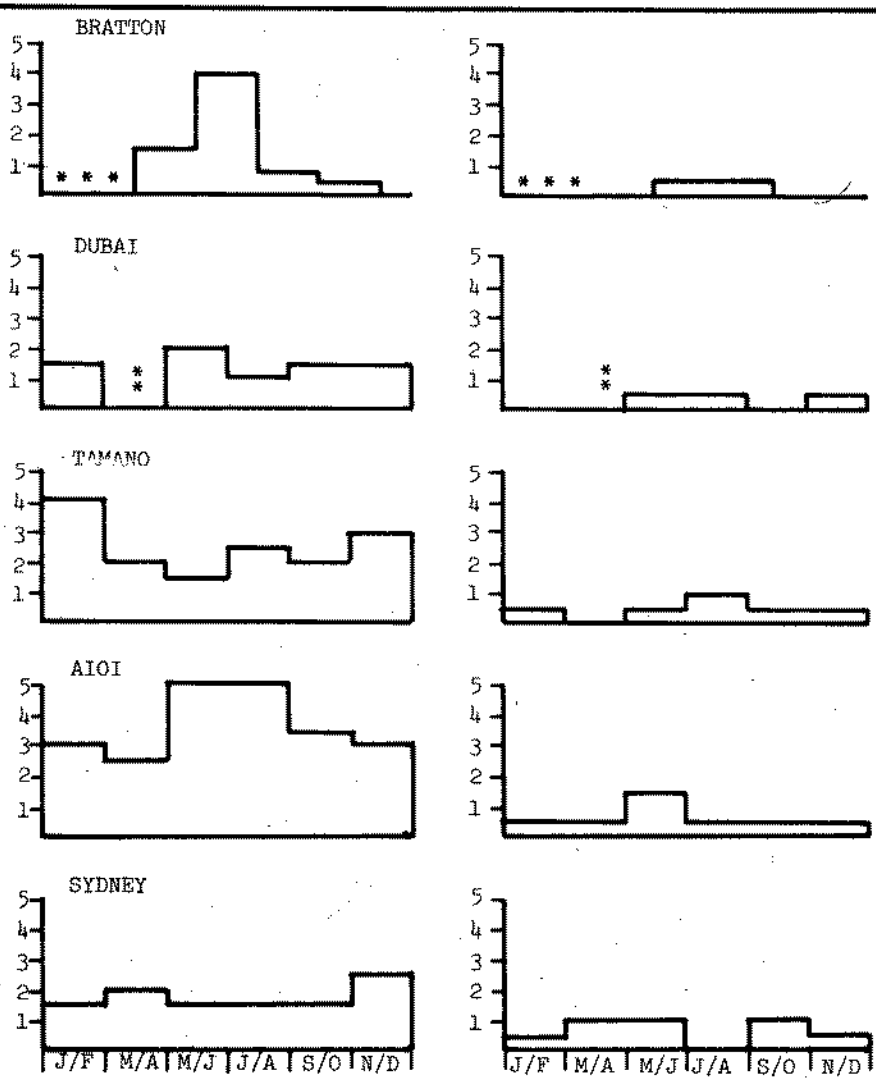
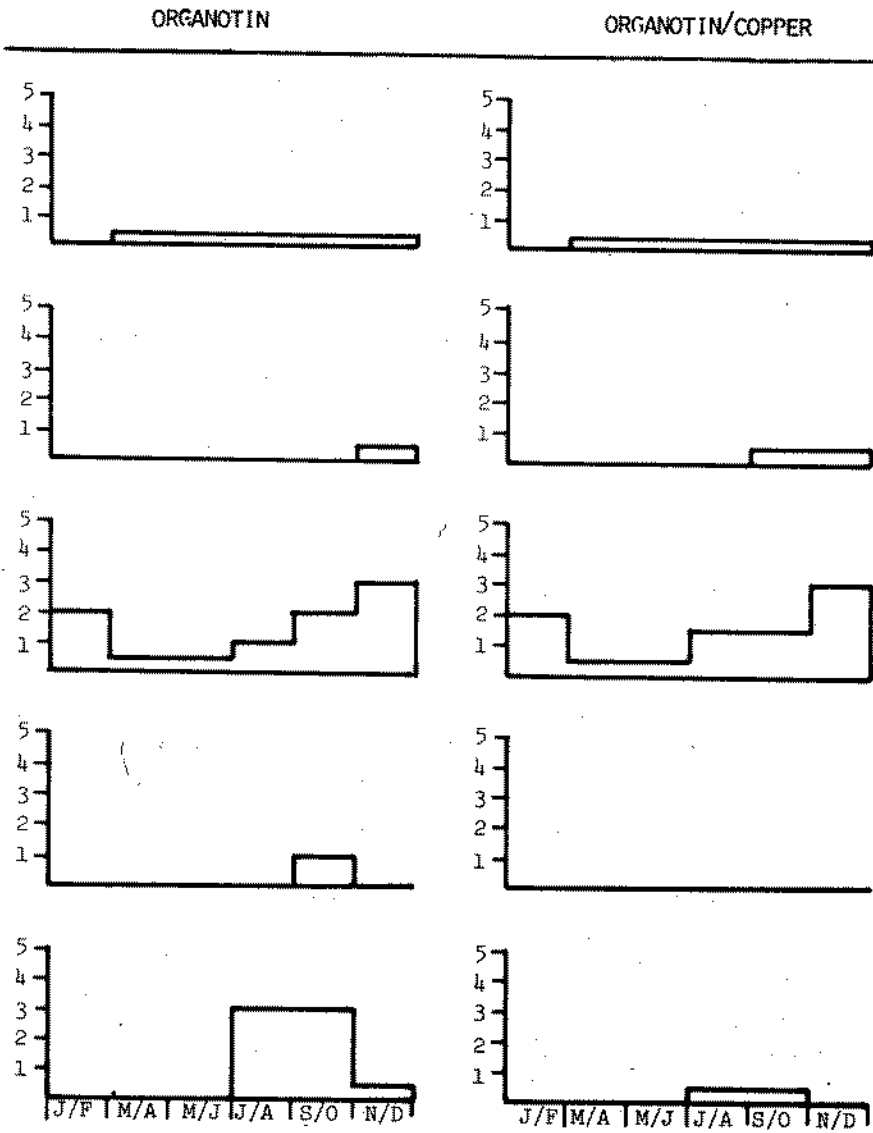


FIG.2. (continued)

Footnote:
*Samples not taken due to the sea freezing at surface.
**Samples lost.

WORLD FOULING SURVEY



Footnote:

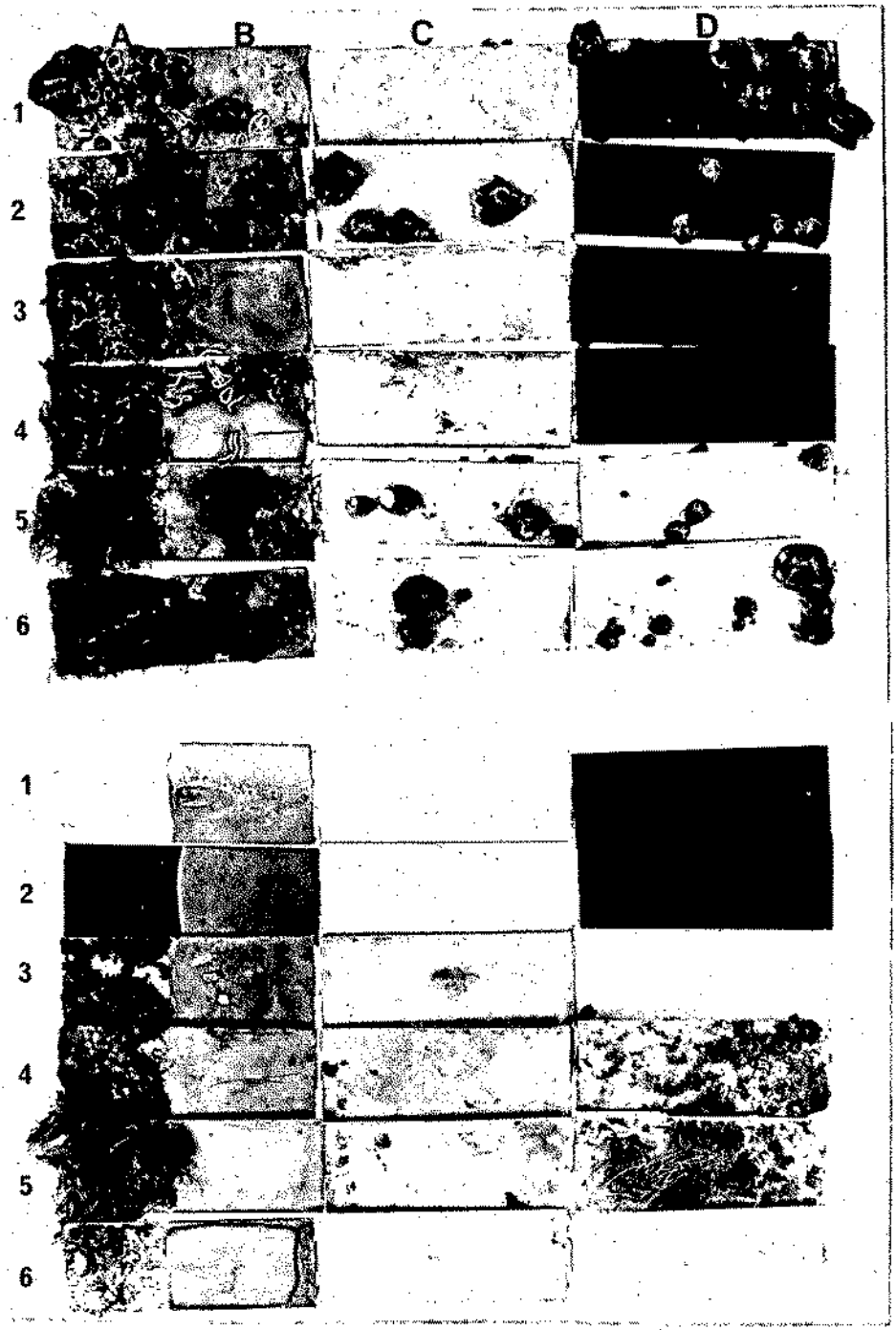
1. J/F = January/February : M/A = March/April : M/J = May/June : J/A = July/August : S/O = September/October : N/D = November/December.
2. Total fouling was assessed on a scale 0-5.

TABLE 3. SEASONAL OCCURENCE OF THE MAJOR FOULING ORGANISMS

SITE	MONTHS	NON-TOXIC	COPPER	ORGANOTIN	ORGANOTIN/COPPER
SAN-FRANCISCO	Jan-Dec	diatom slime, *Ectocarpus*, *Ulothrix*	*Amphora*, *Amphiprora*	*Achnanthes longipes*	*Amphora*
MIAMI	Mar-June	*Cladophora sericea*, *Ectocarpus*, *Callithamnion*, *Enteromorpha intestinalis*	*Amphora*, *Amphiprora*		
	Jan, Feb, July-Dec	*Balanus amphitrite*, weed as above	*Amphora*, *Amphiprora*, *Stauroneis*	*A. angustata*	*Amphora*
RIO-DE-JANEIRO	Jan-Dec	*B. amphitrite*, *Obelia geniculata*, *Bugula neritina*, *Hydroides norvegica*, *Jassa falcata*, *B. tintinnabulum*	*B. amphitrite*, *H. norvegica*, *Vorticella*, *Amphora*	*B. amphitrite*, *Vorticella* *A. longipes*	*B. amphitrite*, *Vorticella* *Amphora*
NEWTON FERRERS	May-Oct	*Ectocarpus*, *Giffordia*		*Tubularia indivisa*	
BURNHAM	May-June	*Ectocarpus*, *Navicula ramosissima*	*N. ramosissima*		
	July-Aug	*Elminius modestus*, *J. falcata*, *E. intestinalis*	*E. modestus* juveniles, *J. falcata*	*E. modestus*, *J. falcata*	*E. modestus* juveniles
	Sept-Oct	*T. indivisa*, *E. modestus*	*Amphora*	*T. indivisa*, *E. modestus*	*Amphora*, *Amphiprora*
	Nov-Dec	*T. indivisa*, *Ectocarpus* *N. pseudocomoides*		*Ectocarpus*, *E. intestinalis* *N. ramosissima*	
BRATTON	April-May	*O. geniculata*, *Ectocarpus*, *Ulothrix*		*Ectocarpus*, *Ulothrix*	
	June-July	*B. improvisus*, *Vorticella*	*Amphora*		*Amphora*
	Aug-Nov	*Ectocarpus*, *Amphora*	*Amphora*		

DUBAI	Jan-Dec	blue-green algae, *B.amphitrite*, ascidian, *Percursaria perousa*, *E.flexuosa*, *E.linza*	*Amphora*, *Amphiprora*	blue-green algae	*Amphora*, *Amphiprora*
TAMANO	Jan-Feb	hydrozoan, diatom slime *Ectocarpus*	*Amphora*	*A.longipes*, *Stauroneis*	*Amphora*
	Mar-Dec	*Ectocarpus*, *E.intestinalis*, *E.linza*, *C.sericea*, diatom slime, *J.falcata*	*Amphora*, *Amphiprora*	*A.longipes*	*Amphora*, *Amphiprora*
AIOI	Jan-April	*Ectocarpus*, *Ulva*, *E.intestinalis*, *Polysiphonia*, *J.falcata*	*Amphiprora*, *Amphora* *Stauroneis*	*A.longipes*, *Stauroneis* *Amphora*	*Amphora*, *Amphiprora* *Stauroneis*
	May-Oct	*B.amphitrite*, *Ectocarpus*, *E.flexuosa*	*Stauroneis*, *Amphiprora* *Amphora*	*A.longipes*	*Stauroneis*, *Amphora* *Amphiprora*
	Nov-Dec	*J.falcata*, *Ectocarpus*	*Amphora*	*A.longipes*	*Amphiprora*, *Amphora*
SYDNEY	Jan-Dec	*Hydroides*, *Spirobis*, *Ectocarpus*, *E.intestinalis*, *Stauroneis*, *Amphora*	*Amphiprora*, *Amphora*, *Stauroneis*, *Navicula*	*A.angustata*, *Stauroneis*, *Amphiprora*	*Amphiprora*, *Stauroneis*, *Amphora*

Footnote:
1) organisms are listed for particular months only when seasonal variations occurred on non-toxic surfaces.
2) within each category the most dominant fouling organisms are named.
3) blank columns reflect absence of fouling organisms (refer to fig.2. for quantitative fouling assessments).
4) diatom slime: slime composed of many genera of diatom.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diatoms occurred throughout the summer on the non-toxic and organotin surfaces. The types most frequently encountered were the algae *E. intestinalis, E. siliculosus, Polysiphonia spp.* and the tube-dwelling diatoms, *N. ramosissima* and *N. pseudocomoides*. A light slime of *A. veneta* and *A. veneta* with *Amphiprora sp.* developed on the copper and organotin/copper paints respectively during the summer months. Observations at Newton Ferrers and Burnham over a number of years have consistently revealed different fouling patterns. At Newton Ferrers, barnacle settlement is never heavy and none were found on the panels in this study. The water is typically clear and algal and diatom growth is usually good during summer but Figs. 3 and 5 show that only light settlement and growth occurred. The results obtained at Burnham follow a pattern observed over a number of years. A heavy settlement and growth of barnacles and hydroids invariably occurs during summer. The water is turbid at Burnham warming up fast in summer as a consequence of large areas of mud and sand being exposed at low tide.

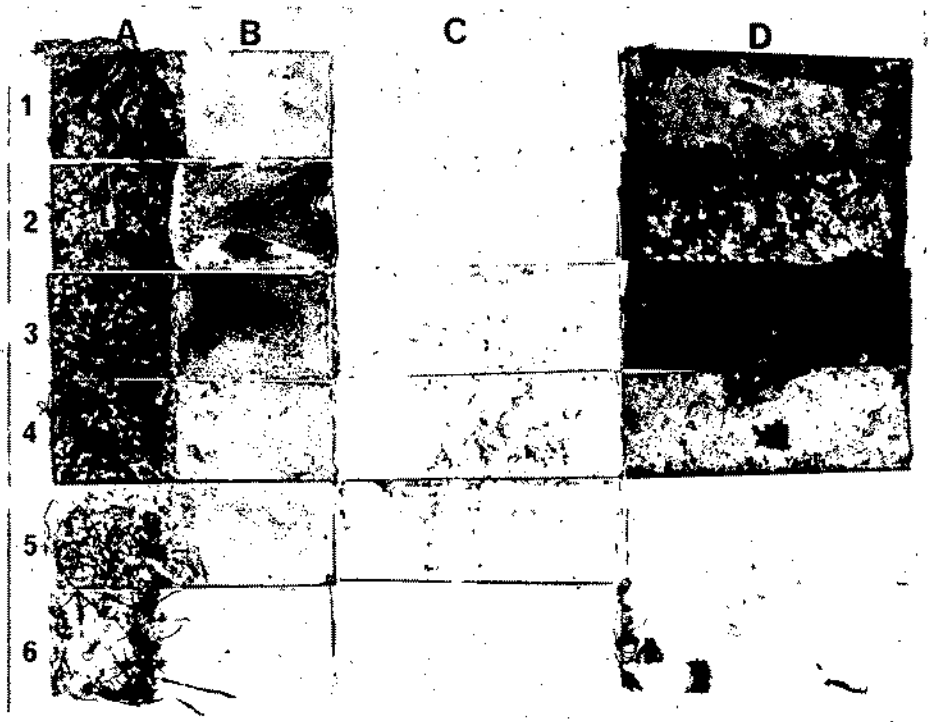
Sites on the East (Djuro) and West (Bratton) of Sweden were included in this survey but during 1983 no biological settlement was found at Djuro. At Bratton, there was a heavy settlement and growth of barnacles *(B. improvisus)* and *Vorticella* on the non-toxic surface during June and July. In the spring and autumn the major type of fouling was algal (*E. siliculosus* and *Ulothrix flacca.*). Little settlement was found on the anti-fouling surfaces. Traces of *E. siliculosus* and *U. flacca* were found on the organotin varnish during April and May and slimes of *A. veneta* and *A. coffeaeformis* var. *perpusilla* were recorded on both the copper and organotin/copper paints.

At Dubai no obvious seasonal settlement occurred. The major fouling organisms throughout the year on the non-toxic surface were blue-green algae from the genera *Oscillatoria, Gleotrichia, Calothrix* and *Spirulina*. These also occurred in reduced quantity on the organotin varnish together with the green alga *Derbesia sp.* Other algae found on the non-toxic panels were *Percursaria percursa, E. flexuosa, E. linza, Derbesia sp. E. fasciculatus* and *Ceramium sp.* Small numbers of *B. amphitrite* settled and grew throughout the year on the non-toxic surface. On the copper and organotin/copper paints light slimes composed of *A. veneta* and *Amphiprora sp.* formed.

FIG.3. PHOTOGRAPHS OF TEST PANELS FROM RIO-DE-JANEIRO (TOP) AND BURNHAM (BOTTOM)

A = Non-toxic; B = Copper; C = Organotin/Copper; D = Organotin.
1 = Jan/Feb; 2 = March/April; 3 = May/June; 4 = July/Aug.
5 = Sept/Oct; 6 = Nov/Dec.

Note: The following surfaces were black (Burnham 2A, 1D, 2D; Rio-de-Janeiro 1D, 2D, 3D, 4D).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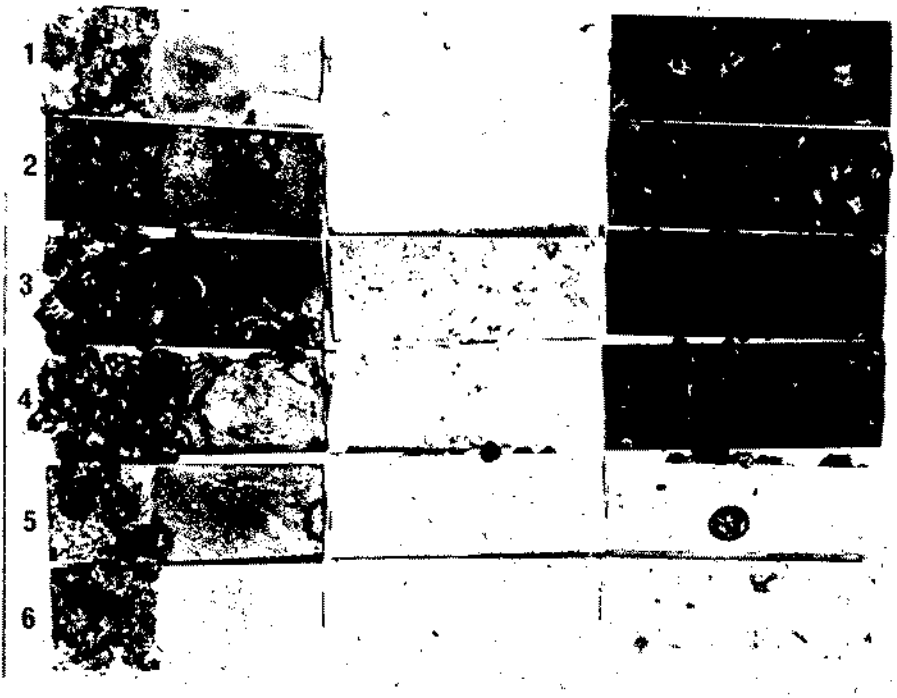
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WORLD FOULING SURVEY

The two sites in Japan (Tamano and Aioi) are both located on the Inland Sea being approximately 80 kilometres apart. At Tamano throughout the year fouling is chiefly due to algae in contrast to Aioi where heavy barnacle (*B. Amphitrite*) settlement occurs between May and October (Fig.4). At Tamano the water is shallow, turbid and flowing whilst at Aioi the water is deep, clear and still. At Tamano, the non-toxic surface supported a growth of hydroids in January/February in addition to a species-rich diatom slime and the algae *E. fasciculatus* and *E. intestinalis*. Predominant members of the diatom slime were species of *Stauroneis, Navicula, Fragillaria* and *Lichmophora*. During the remainder of the year (March - December) a variety of algae in particular *E. fasciculatus* and *Enteromorpha spp.* predominated. The algae were often tightly bound with the mud-binding amphipod *Jassa falcata*. The distributions of diatom species found were similar throughout the year apart from March/April when *A. longipes* was a major component of the slime on the non-toxic surface. *A. veneta* and *Amphiprora sp.* formed slimes on both the copper and organotin/copper paints. Very substantial slimes developed on the organotin varnishes which were predominantly composed of *A. longipes* with lesser amounts of *Stauroneis sp.* In all cases the organotin varnish bore small plants of *E. fasciculatus* and *E. linza*. At Aioi between November and April the major fouling was due to algae and *J. falcata*. The algal flora was richer than that at Tamano and in addition to the most common species listed in Table 3 the following were frequently identified: *Cladophora rupestris, Blidingia minima, C. sericea* and filamentous blue-green algae. During May to October heavy settlements of *B. amphitrite* occurred. Throughout the year a great diversity of both benthic and planktonic diatoms were found on the non-toxic surface. Little seasonal variation in composition of slimes which developed on the anti-fouling surfaces occurred. Members of the genera *Amphora, Amphiprora* and *Stauroneis* were dominant members of slimes on copper or organotin/copper paints. Although *A. longipes* was always present on the organotin varnish other organisms also occurred e.g. a few *B. amphitrite* in May - August, *E. fasciculatus* and the diatoms *A. veneta* and *Stauroneis*.

Moderate fouling occurred throughout the year on non-toxic panels at Sydney. Between November and April there appears to be heavy settlement of serpulid worms of the genera *Hydroides* and *Spirobis*. Settlement of these occurred throughout the remainder of

FIG.4. PHOTOGRAPHS OF TEST PANELS FROM JAPAN: TAMANO (TOP) AND AIOI (BOTTOM).

A = Non-toxic; B = Copper; C = Organotin/Copper; D = Organotin.
1 = Jan/Feb; 2 = March/April; 3 = May/June; 4 = July/Aug.
5 = Sept/Oct; 6 = Nov/Dec.

Note: The following surfaces were black (Tamano 1A-4A, 1D-4D; Aioi 1D-4D.)

the year but in reduced quantity. Algae and diatom slimes were present on all samples particularly *E. siliculosus* and *E. intestinalis*. Of the diatoms, *Stauroneis*, *Amphora coffeaeformis* var. *coffeaeformis*, *Amphiprora* and *Navicula* were most common. There was a small settlement of barnacles of *B. amphitrite* during January and February. Thick slimes developed on the anti-fouling surfaces throughout most of the year. The copper and organotin/copper paints had slimes of similar species composition, the most common diatoms being *Amphiprora*, *Stauroneis*, *A. veneta* and *Navicula*. However, on the organotin varnish *A. angustata* and *A. longipes* were most commonly found, together with *Stauroneis* and *Amphiprora* from May to August.

Four samples were returned from La Spezia covering the period January to June and November to December. The serpulid worm *Hydroides* was the dominant organism on non-toxic surfaces. A few *B. amphitrite* settled during May/June and November/December. The algae *E. fasciculatus*, *Satyosiphon sp.*, *E. intestinalis* and *Chaetomorpha linum* were present on all samples. The copper paints developed slimes of *A. veneta* and *Stauroneis* intermixed with *E. fasciculatus* and *E. intestinalus*. The organotin/copper paints bore only patchy slimes of *Amphiprora* whilst the organotin varnish supported the growth of *Achnanthes subsessilis* and *A. longipes*.

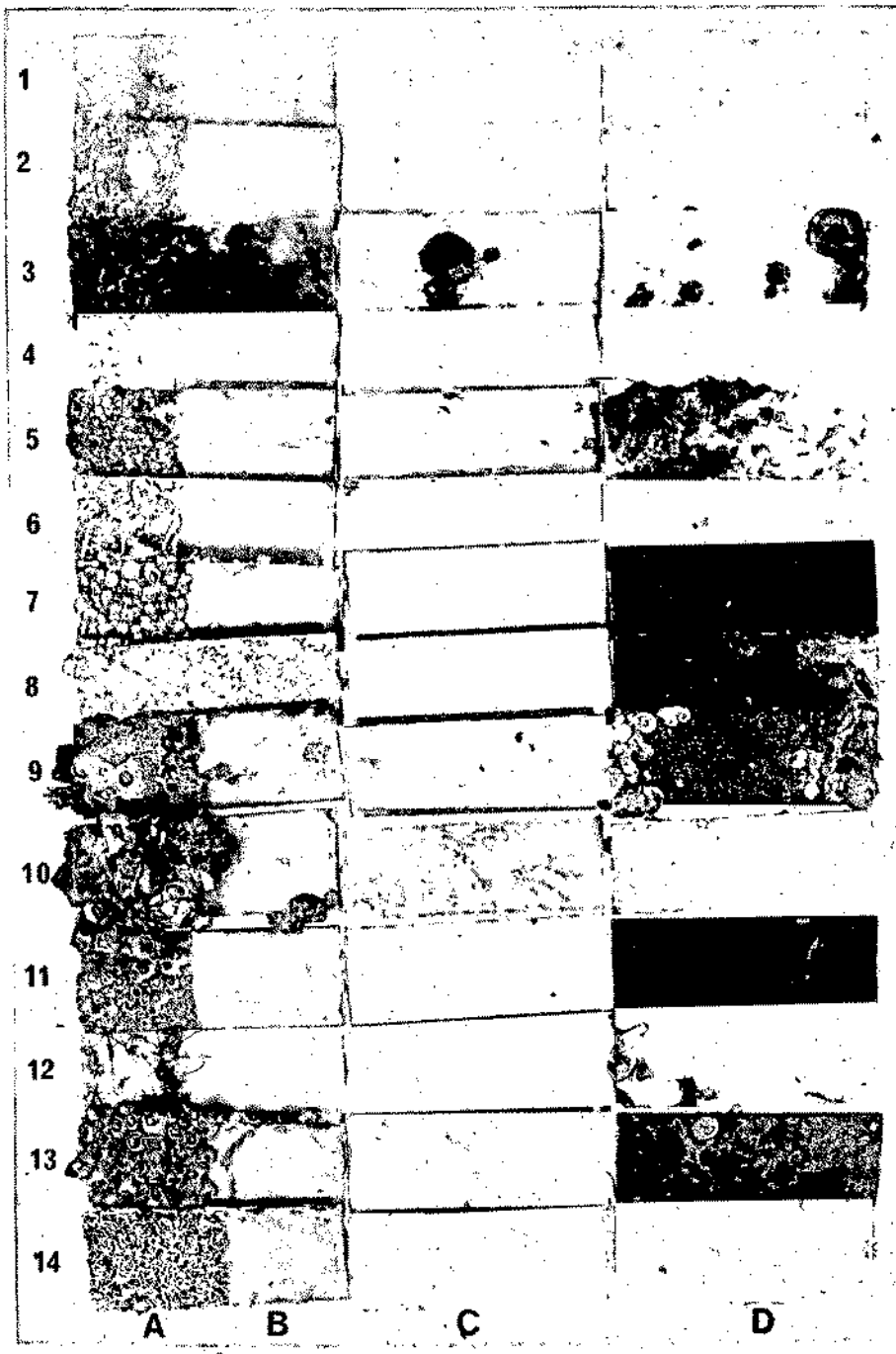
Three samples were received from Singapore covering the period March to August. Heavy animal fouling occurred on the non-toxic surfaces due to *B. amphitrite*, a colonial ascidian and serpulid worms. *Amphora* slimes (*A. veneta*, *A. coffeaeformis* var. *perpusilla*, *A. bigibba* and *A. sp.*) colonised the copper and organotin/copper paints and during March and April the copper paint was found to support many *B. amphitrite* although of smaller size than those on the non-toxic surface (Fig.5). The organotin varnish for the same period (March-April) had a covering of juvenile barnacles in addition to a light slime of *A. subsessilis*.

Only two samples were received from Hong Kong covering the period May - August. The non-toxic samples of both were covered with a massive growth of *B. amphitrite* and *Hydroides* (Fig.5). Thick slimes were present on all the anti-fouling formulations. The organotin varnish supported an almost unialgal growth of *A. angustata*. The slimes on the copper or organotin/copper paints were composed of *Amphora spp.* viz. *A. veneta*, *A. coffeaeformis* var. *coffeaeformis* and *A. bigibba*.

Although four samples were received from Taiwan, three were discarded due to a covering of oil. During January and February the non-toxic surface became covered with a substantial growth of *B. amphitrite* (Fig.5). A slime of *A. veneta* and *Stauroneis* developed on the copper and organotin/copper paints. The organotin varnish supported only a slight slime which contained *Amphiprora*, *Stauroneis*, *Navicula* and *A. longipes*.

Discussion

Growth on surfaces without any anti-fouling protection can be immense, even within a short time as the present study shows(Fig.5).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WORLD FOULING SURVEY

The most commonly encountered fouling types world-wide were *Balanus spp.*, *Enteromorpha spp.* and *Ectocarpus spp.* (also see Fletcher, 1974). The copper paint failed to barnacles at Rio-de-Janeiro (3 samples), Aioi (1 sample) and Singapore (1 sample). The organotin varnish supported barnacle growth at Rio-de-Janeiro (5 samples), Burnham (2 samples), Aioi (3 samples) and Singapore (2 samples). Barnacles were only encountered on the copolymer organotin/copper paint however at Rio-de-Janeiro. Overall, the copolymer paint provided the best anti-fouling surface whilst the organotin varnish was least good.

Marine organisms differ in their tolerance to copper and organotins. Copper leaching rates of 10 $\mu g/cm^2$/day are necessary to prevent settlement of barnacle larvae whilst up to 20 $\mu g/cm^2$/day may be needed to prevent the formation of diatom slimes (Banfield, 1980). There is no published data on organotin leaching rates but it is generally accepted that organotins are approximately twice as effective as copper. In practice, leaching rates of 10 and 4 $\mu g/cm^2$/day for copper and organotin respectively should prevent settlement of all organisms (Milne, personal communication). In experiments where a tributyl tin copolymer with cuprous oxide was dynamically aged at 25°C for two months the copper and tin leaching rates were approximately 10 and 2.5 $\mu g/cm^2$/day respectively. Comparable leaching rates are not available for the copper paint or the organotin varnish but the leaching rate of the former would be in excess of 10 μg copper/cm^2/day whilst that of the latter should be similar to that of the copolymer paint (Milne, personal communication).

In the present study it is not possible to directly compare the performance of a paint world-wide since leaching rates of copper and tributyl tin were not measured. Leaching rate depends on temperature, pH, salinity and speed of water movement. Furthermore, these and other factors such as the level of dissolved organic materials in the water may influence the chemical speciation and hence biological activity of released biocides. The presence of high levels of organic pollution may contribute to the poor

FIG.5. PHOTOGRAPH SHOWING THE PERFORMANCE OF THREE ANTI-FOULING PAINTS RELATIVE TO A NON-TOXIC CONTROL

A = Non-toxic; B = Copper; C = Organotin/Copper; D = Organotin. Each country is represented by one set of test panels chosen for maximum growth on the non-toxic control. Samples marked with an asterisk were chosen from an incomplete set of panels.
Note: Surfaces 7D, 8D, 9D, 11D, 13D were black.

1 = San Francisco, Sept/Oct
2 = Miami, Sept/Oct
3 = Rio-de-Janeiro, Sept/Oct
4 = Newton Ferrers, May/June
5 = Burnham, July/Aug
6 = La Spezia, Nov/Dec*
7 = Bratton, July/Aug
8 = Dubai, May/June
9 = Singapore, March/April*
10 = Hong-Kong, July/Aug*
11 = Taiwan, Jan/Feb*
12 = Tamano, Nov/Dec
13 = Aioi, July/Aug
14 = Sydney, Nov/Dec

anti-fouling performance observed at Rio-de-Janeiro.

Hendey (1951) listed diatoms in order of their resistance to cuprous oxide. *Amphora spp.* and *Amphiprora hyalina* were said to be very strongly resistant whilst *Achnanthes longipes* was said to be strongly resistant. *Achnanthes subsessilis* however, appears to be more resistant to organotin since this was found colonizing organotin-containing varnishes and paints (Callow *et al*, 1978; Callow and Evans, 1981; Blunn, 1982). There is little information available regarding the composition of slimes on ships "in-service". Bishop *et al.* (1974) recorded *Amphora spp.* on Australian naval shipping. Daniel *et al.* (1980) found *Amphora spp.* on supertankers painted with either cuprous oxide or cuprous oxide/triorganotin paints. *Amphora* and *Achnanthes* slimes have both been found on shipping painted with tributyl tin/cuprous thiocyanate and operating in the South China Sea (Callow, unpublished). Although *Amphora* appears to be the most widespread diatom on ships "in-service", slimes have been encountered where other genera are dominant components (Callow, unpublished). In this context the most commonly encountered are *Achnanthes, Stauroneis, Navicula* and *Amphiprora.* These five genera have all been recorded as dominants growing on the copper or organotin/copper paint in the present study. Of these the most common is *Amphora* followed by *Amphiprora.* Both diatoms are very resistant to copper and LD50 values determined in laboratory experiments are similar for *A. coffeaeformis* var. *perpusilla* and *Amphiprora hyalina* (French, personal communication). Daniel and Chamberlain (1981) showed that copper resistance in *A. veneta* is due to immobilization of copper within membrane-bound intracellular bodies, thereby keeping cytoplasmic levels low. *Achnanthes* was the most commonly encountered diatom on the organotin varnish. It is perhaps surprising that *A. longipes* was commonly found on the organotin varnish since Hendey (1951) and Blunn (1982) consider this to be a species strongly resistant to copper. In laboratory experiments where LD50 values were determined, *A. longipes* is only slightly (20%) more resistant to tributyl tin than *A. coffeaeformis* var. *perpusilla.* However, *A. subsessilis* is fifteen times more resistant than *A. longipes* (Wood, personal communication). Thus there appear to be very great difference in resistance to biocides between species within the same genus. The situation is clearly complex and further examination of slimes from ships "in-service" is required as well as data for the relative resistance of organsisms under controlled laboratory conditions. However, in order to correlate laboratory results with field observations and to understand why a particular paint becomes fouled it will be essential to measure the leaching rates of all biocides used in the anti-fouling paint.

Acknowledgements

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WORLD FOULING SURVEY

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POLLUTION OF COASTAL SEA WATER AND SULFATE REDUCING BACTERIA

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Abstract

High corrosivity of sea water is associated with the incidence of organic pollution and the consequent growth of sulphate reducing bacteria that generate hydrogen sulphide. The study was carried out with a view to stipulating the permissible concentration of hydrogen sulphide as well as sulphate reducing bacteria in sea water. Four sites having different pollution status were selected for monitoring various pollution parameters. In polluted waters of proven corrosivity, BOD is very high and hydrogen sulphide value also ranges between 65 to 110 mg/litre. In open unpolluted waters where BOD is within permissible limit of 20 mg/litre, hydrogen sulphide concentration is below 40 mg/litre. A concentration below 40 mg/litre of hydrogen sulphide therefore can be treated as acceptable level.

INTRODUCTION

In recent years there has been an increasing awareness about the corrosion of the waterfront structures induced by sulphate reducing bacteria [Booth, 1964; Nakahara et al., 1977; Postgate, 1979; Salvarezza & Videla, 1980]. An accelerated corrosion of metallic structures as well as unsatisfactory performance of anti-corrosive cathodic protection measures in polluted waters have been earlier reported from Bombay harbour [De et al., 1980]. These authors have attributed this accelerated corrosion to the influx of pollutant species and the consequent anoxic conditions that support the growth of sulphate reducing bacteria like Desulphovibrio desulphuricans and D. vulgaris. These two organisms incidentally are isolated and maintained as pure cultures in the laboratory.

Lately efforts are being made to evolve water quality criteria in respect of coastal waters. In addition to physico-chemical qualities like temperature, pH, dissolved oxygen, the quality of sea water in respect of hydrogen sulphide is also being monitored with a view to stipulating its permissible limit. The aim of the present work was to ascertain the permissible limits for the concentration of hydrogen sulphide and the number of bacteria in polluted sea water.

MATERIAL AND METHODS

For monitoring the sea water quality including the presence of sulphate reducing bacteria, four sites of the following descriptions were selected along the Bombay harbour.

Middle Ground (Site A)	:	Open waters, half a kilometer off the coast.
Harbour Jetty (Site B)	:	Semi-enclosed water along the berth exposed to semi-tidal conditions and reasonably good flushing of water mass.
Wet Basin (Site C)	:	Stagnant sea water subjected to periodical influx of fresh sea water whenever the dock gates are opened as an operational requirement.
Tidal Basin (Site D)	:	A semi-enclosed water close to the shore receiving untreated organic matter through a storm water culvert. A proven corrosive environment.

From each of the above sites weekly collections of sea water samples, one meter below the water surface were made for a period of three years. Basic hydrographical data such as temperature, pH, salinity as well as dissolved oxygen and BOD were collected according to the standard methods [Martin, 1968]. The American Standard Test Method (ASTM, 1977) was followed for monitoring the hydrogen sulphide produced by sulphate reducing bacteria. The values obtained were corrected as suggested by Chaudhari (1967). The detection and enumeration of sulphate reducers were executed by adopting the 'shake tube' technique. A modified iron sulfite agar as recommended by Mara & Williams (1977) was used.

RESULTS

The status of sea water during the monsoon is different than during the non-monsoon period. The data for this period therefore is not commented in the present paper.

Table 1 gives the values in respect of temperature, salinity and pH at 4 sites for a period of one calendar year. It is noted that whereas there are seasonal variations in respect of these water qualities, there are no variations from site to site at a given time of the year.

Fig. 1 (a), (b) and **(c)** depict values of BOD, dissolved oxygen and hydrogen sulphide respectively during the course of one year at 4 different sites. No variations were noted in these values during the course of 3 years when this study was carried out. The values for the year 1980 only are therefore depicted.

Table 1
Physicochemical parameters of Seawater at 4 sites along Bombay harbour

1980	Temperature (°C)				Salinity (parts per thousand)				pH			
Month	Site A	Site B	Site C	Site D	Site A	Site B	Site C	Site D	Site A	Site B	Site C	Site D
Jan	27.0	27.0	27.1	26.7	34.20	33.60	33.30	33.90	7.62	7.49	7.52	7.82
Feb	29.0	29.0	29.2	28.5	34.92	33.65	33.61	33.01	7.82	7.57	7.53	7.36
Mar	28.2	28.1	28.0	27.8	36.54	36.54	36.54	34.29	7.00	7.40	7.40	7.60
Apr	29.8	29.9	30.5	29.3	38.45	38.87	38.45	39.09	7.10	7.00	6.90	7.60
May	31.4	31.2	32.6	31.5	37.49	36.54	36.54	37.18	7.96	7.30	7.83	7.40
Jun	31.2	31.2	31.2	31.2	37.18	33.65	36.54	37.82	7.84	7.96	8.00	7.87
Jul	28.9	28.8	28.9	28.7	21.80	22.12	25.01	21.48	7.63	6.85	6.83	7.51
Aug	28.0	28.0	27.9	27.7	22.77	22.12	22.12	22.45	8.03	7.93	7.90	7.90
Sep	29.4	29.2	28.6	28.0	34.92	35.69	34.42	35.89	7.96	7.95	7.60	7.93
Oct	32.0	31.2	31.0	30.6	33.65	34.29	27.89	34.29	7.68	7.40	7.60	7.40
Nov	28.2	28.6	28.8	28.2	36.54	36.55	34.76	35.20	7.72	7.34	7.26	7.37
Dec	26.4	25.9	26.3	26.0	36.54	37.01	35.57	35.60	7.78	7.80	7.77	7.78

Descriptions of the sites are given in the text.

It is very clearly observed from **Fig. 1(a)** that BOD value is the highest at site D where a culvert carrying organic matter opens into the sea. This value ranges between 35 mg/litre to 90 mg/litre. At site A, away in open waters, BOD values are all along low and are within a stipulated figure of 20 mg/litre (ISI, 1981). In confined waters of the wet basin, site C, BOD values are higher on account of trapped oil and other organic impurities.

Fig. 1(b) depicts values of dissolved oxygen at 4 sites. These values are low at site D where culvert carrying organic waste opens. In open waters at site A, values are higher. In a confined wet basin area which is periodically exposed to open sea, values fluctuate between 1.2 mg/litre to 2 mg/litre.

Fig. 1(c) depicts hydrogen sulphide values at four sites. At site D, close to the culvert, hydrogen sulphide values vary between 65 mg/litre to 110 mg/litre. In open waters at site A, the concentration does not exceed a figure of 51 mg/litre. Intermediate values are noted at two other sites.

The observations made on the basis of the data given above are as follows. In open sea water, close to Bombay coast, BOD values are generally within the range of 10 to 20 mg/litre indicating thereby that the waters are free from organic pollution. In confined water, close to the opening of the culvert, BOD values vary between 39 to 90 mg/litre, almost throughout the year. This area therefore is polluted. In open sea water hydrogen sulphide values are generally below 40 mg/litre. In confined waters where culvert opens, hydrogen sulphide concentration fluctuates between 65 and 110 mg/litre. Open sea water, it is evident has lower BOD value and low hydrogen sulphide concentration. The tidal basin water on the other hand shows higher BOD and higher hydrogen sulphide concentration.

Table 2 gives a relationship between the number of sulphate reducing bacteria present in the sea water and the corresponding value of hydrogen sulphide recorded from that water mass.

Table 2
Showing a relationship between sulphate reducers and hydrogen sulphide concentration

Hydrogen sulphide in mg/litre	No. of sulphate reducing bacteria per ml of sea water	Remarks
25	4×10^2	Likely situation in open waters
40	1.2×10^4	Possible limits
50	3×10^4	Polluted
60	13×10^4	Likely situation near culvert opening

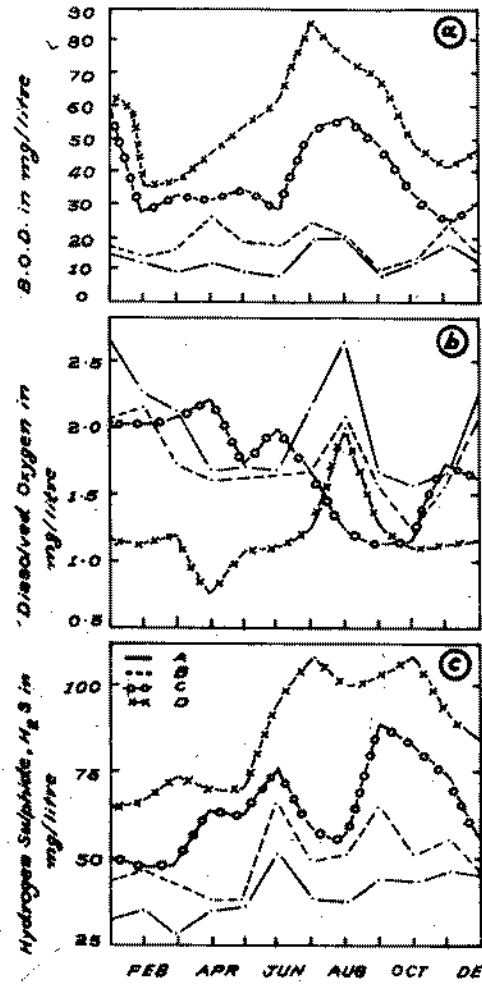

Figure 1(a), (b), (c) :
Showing (a) the levels of Biochemical Oxygen Demand, (b) Dissolved Oxygen Concentration and (c) Hydrogen sulphide at 4 sites in Bombay Harbour

In open waters where hydrogen sulphide concentration is around 30 mg/litre, the bacterial population will be around 400 organisms/ml of sea water. On the other hand at site D, near the culvert where hydrogen sulphide level is always above a figure of 60 mg/litre, bacterial population will be around one hundred and thirty thousand organisms/ml of sea water. The intermediate values are obtained at two other semi-polluted sites.

DISCUSSION

The introduction of pollutant species in the sea water eventually leads to the depletion of dissolved oxygen and the consequent multiplication of sulphate reducing bacteria. As revealed in the present study, there has been a close co-relation between the concentration of BOD, indicative of organic pollution and the concentration of hydrogen sulphide. In the open sea, BOD as well as hydrogen sulphide values are low. On the other hand, in the tidal basin where the culvert opens into the sea, both BOD and hydrogen sulphide values are very high. The published literature dealing with the problem of corrosion behaviour of metals in polluted environments offers comments on the level of dissolved oxygen, biological oxygen demand and the concentration of sulphide. Only a few attempts have been made to correlate the corrosivity with the amount of dissolved hydrogen sulphide and the bacterial population present in the sea water [Nakahara et al., 1977]. Sasaki, et al., (1977 a,b) based on their laboratory experiments have observed a clearcut relationship between the hydrogen sulphide concentration and the corrosion rate of mild steel. They noted that corrosivity was low at 10 mg/litre of hydrogen sulphide, medium at 40mg/litre and the highest at a concentration of 180mg/litre. In tidal basin along Bombay shore, hydrogen sulphide values range between 60 mg/litre to 110 mg/litre during most part of the year. Judging by the above guideline this level of hydrogen sulphide can be considered as conducive to metallic corrosion. That it is indeed so, has been earlier reported by De, et al., (1981) on the basis of the study they carried out on corrosion behaviour of mild steel in the tidal basin. In open, clean waters hydrogen sulphide concentration is around 35 mg/litre during most part of the year. A concentration of hydrogen sulphide below a figure of 40 mg/litre can therefore be considered as a permissible value.

The present work has also shown that the number of sulphate reducing bacteria at concentration of 40 mg/litre of hydrogen sulphide is around 12,000/ml of sea water. This number lies close to the count of 10,000 to 10,00,000/ml associated with serious pollution [Postgate, 1965]. ASTM (1977) guidelines state that sulphate reducing bacteria are absent if the concentration of hydrogen sulphide is less than 50 mg/litre, organisms are considered as present if the value exceeds a figure of 50 mg/litre and further their growth is termed as heavy, if hydrogen sulphide value is as high as 350 mg/litre. In reality at a concentration of 50 mg/litre of hydrogen sulphide the bacteria are not merely 'present' but are in fairly large number to create corrosive environments in the sea water.

The American Standard Test Method (ASTM, 1977) is based upon the iodimetric determination of the hydrogen sulphide produced by sulphate reducing bacteria in a suitable culture medium. Chaudhari (1967) found that the formula recommended by ASTM for calculating hydrogen sulphide value was 'incorrect' and stated that "such values of hydrogen sulphide will be still valid provided they are multiplied ten times".

In our studies related to the monitoring of sulphate reducers in coastal waters, standard ASTM method giving hydrogen sulphide value as well as the extent of anaerobes in the water samples was employed. After repeated samplings monitored for a period of one year, it was realised that the values of hydrogen sulphide obtained were very low even after multiplying the values by a factor of ten as suggested by Chaudhari (1967). After a short incubation period, the culture bottles in reality showed a copious amount of black to grey flocculent precipitate of iron sulphide and heavy cloudiness of the medium accompanied by a very strong smell of hydrogen sulphide. Higher values of hydrogen sulphide were also reckoned by the intense blackening of wet lead acetate paper. The bacterial count executed simultaneously also gave very high values. In this study the estimation of hydrogen sulphide was done according to ASTM method and the actual enumeration of sulphate reducing bacteria was executed by adopting the 'shake tube' technique recommended by Mara & Williams (1977).

Lately a communication received from ASTM conveys that "method D 993-58:31 (1977) will be replaced by a completely new method" and that "the new method will not give user any indication on the amount of hydrogen sulphide that is formed or being formed". It is evident therefore that since the bacterial presence or absence in the sample is related to the amount of hydrogen sulphide generated, the method will also cease to be of any assistance in estimating the sulphate reducing bacteria in water samples [Srivastava and Karande, 1984]. Value of 40 mg/litre of hydrogen sulphide suggested here as a possible safe concentration will have to be suitably corrected when a revised method of calculation of hydrogen sulphide finally emerges.

The authors thank Mr. C.P. De, Director of this establishment for the encouragement he gave during the pursuit of this study.

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RÉSUMÉ

On a fait ces études dans le but de stipuler la concentration permissible de l'acide sulphydrique (Hydrogène sulfuré:H_2S) aussi bien que la bactérie responsable de réduire le sulfate d'hydrogène qui causent la corrosion dans l'eau de la mer. On avait choisi quatre situations de différentes conditions de pollution pour observer sans cesse les divers paramètres de pollution y inclus celui de l'hydrogène sulfuré. Une concentration sous 40 mg/litre de l'hydrogène sulfuré est considérée comme un niveau acceptable.

Studies on Marine Biofouling and its prevention with Special Reference to Fishing Craft

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Results of studies carried out by the authors in Cochin Harbour (Lat 9°58'N, Long 76°16'E) with reference to the biology of fouling, development of antifouling paints with copper acetoarsenite, and Bis (tri-n-butyltin) oxide, effect of electrical polarisation on the initial stages of fouling and the formulation of an antiborer-antifouling elastomeric composition are outlined in this communication. Of all the hydrographical factors, the salinity fluctuations owing to the influence of South-west Monsoon brings out marked changes in the faunistic groups at Cochin Harbour. Several of the major fouling groups are observed to be eliminated during the major part of the monsoon period. Antifouling paint containing copper acetoarsenite (AF1) resisted fouling for over 9 months and the paint containing Bis (tri-n-butyltin) oxide maintained the leaching rate of 1.53 ug tin cm^{-2} day^{-1} even after 12 months. Differences were noticed in the quantum of fouling on polarised and unpolarised panels, the unpolarised panels accumulated more of fouling, however this was not so in the electrically polarised panels owing to the formation of an alkaline environment due to polarisation. Studies have also shown the distinct possibility of combining antifouling and antiborer properties in one matrix by the incorporation of sparingly soluble zinc oxide, special grade silicon carbide and biotoxin in a suitable polymeric matrix.

Biofouling and its prevention in fishing craft

Introduction

Marine biofouling is an economically important problem facing all the maritime nations of the world. Fouling affects propulsion and thereby the economic operation of boats and ships (1). It reduces the efficiency of several underwater marine structures, acoustic devices, pipes and conduit of desalination plants, navigational buoys and OTEC systems. Fouling may accelerate corrosion (2). As estimated by Tighe-Ford (3) the U.S. commercial and military interests alone incur an annual loss of 500 million dollars owing to marine fouling, which does not include the additional costs required by way of increased fuel and docking charges. In India, there are about 16,000 small mechanised fishing craft valued approximately at Rs.809 million (4). The present authors estimated that these craft incur an expenditure of 30 million rupees for fouling prevention per annum. This works out to nearly 4% of the total investment on these craft. The figures will be very high if the bigger trawlers are also taken into consideration. Realising the economic importance of the problem, the authors have investigated several aspects of the fouling problem such as biology of fouling (5), the effects of electrical polarisation on fouling (6) development of antifouling paints (7,8) and an elastomeric antifouling-antiborer composition (9) and our attempts in these lines are reviewed in this communication.

The Cochin Harbour: Cochin Harbour (Lat 9°58'N, Long 76°16'E), a natural harbour situated at the South-west coast of India is an important centre of commercial shipping and fishing activities and forms an ideal location for studies on marine fouling and its control (Fig.1). Located in the Cochin Backwaters, which is a northward extension of Vembanad Lake (a typical tropical estuary) Cochin Harbour receives the full benefit of the South-west Monsoon which brings about pronounced drop in salinity generally during July-October (Table 1). This results in marked changes on the faunistic composition of the backwaters, particularly the fouling organisms. Several of the stenohaline groups which cannot tolerate the low salinity are eliminated during this period leaving only the more resistant forms to survive. For a detailed description of the harbour, attention is drawn to the contribution of Balasubramanyan & Menon (10).

Materials and Methods

The fouling organisms were collected by exposing smooth glass panels 150x100x3 mm fitted on to a grooved wooden rack in two rows of seventeen each. The wooden panels together with the rack were exposed sub-tidally about 30 cm below low water line. Every month one set of panels (34 Nos) was exposed together. One panel each was removed and examined every day. This showed

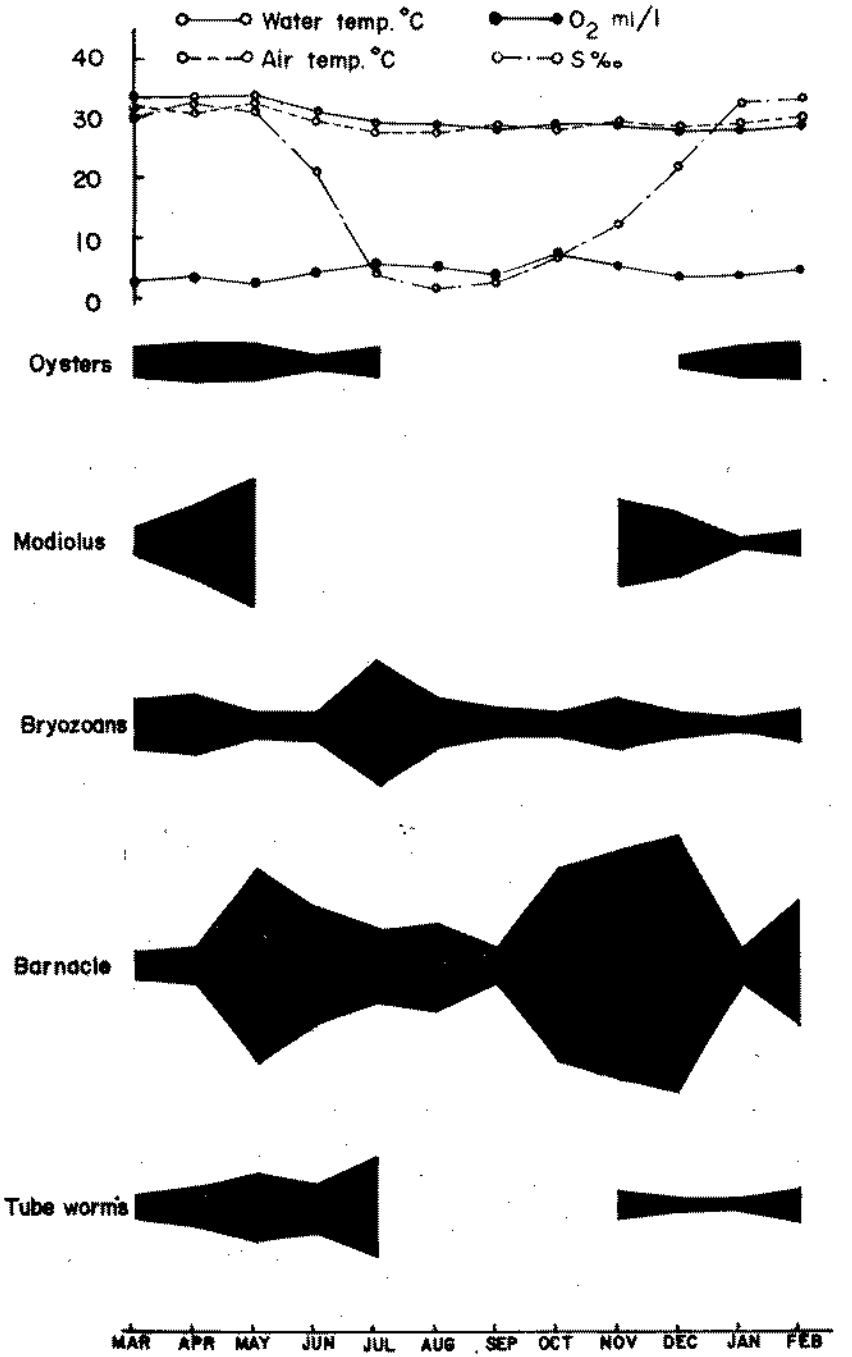

Fig.2. SEASONAL SETTLEMENT OF FIVE MAJOR MARINE FOULING ORGANISMS IN COCHIN HARBOUR

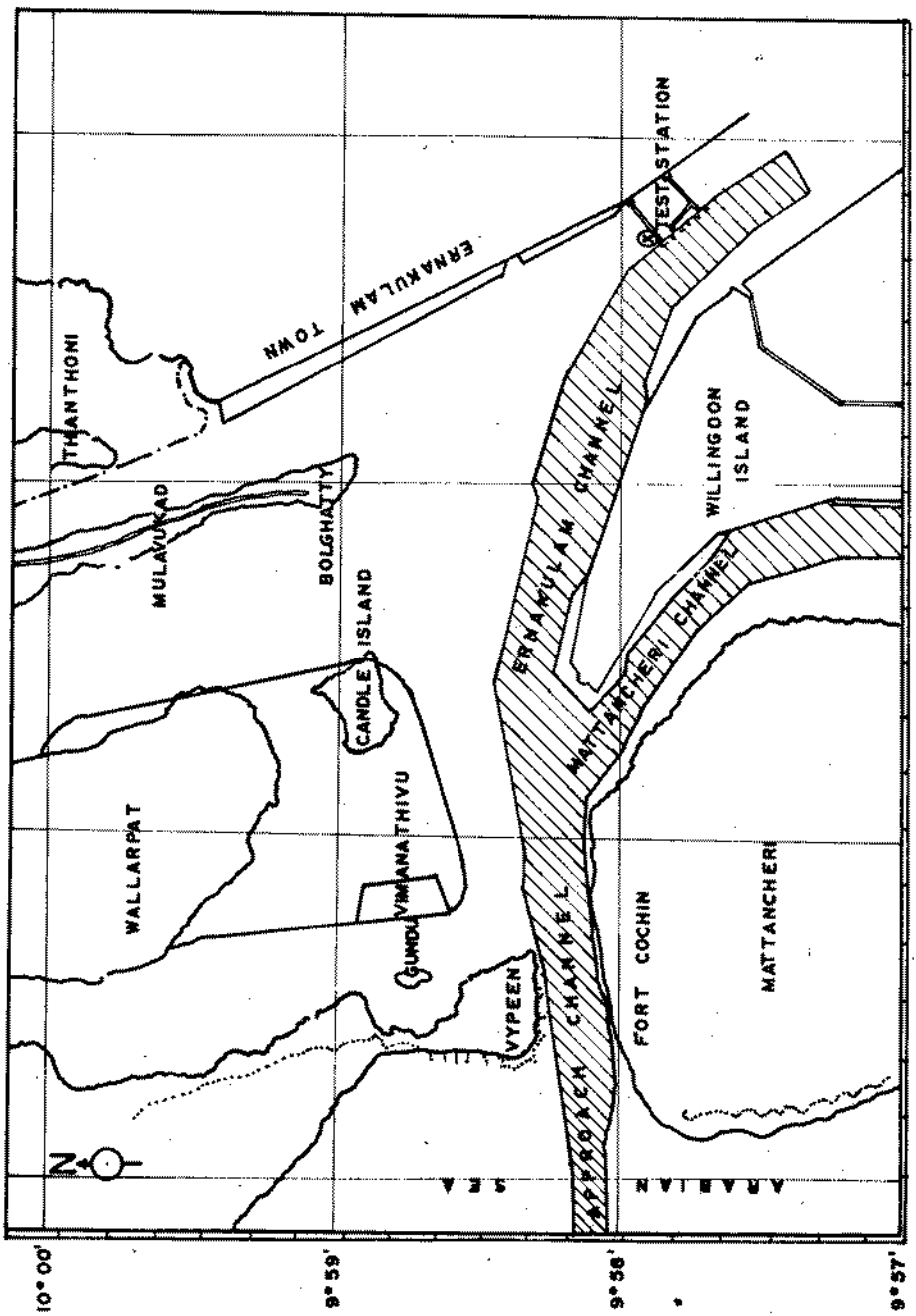

Fig. 1. Cochin Harbour showing the test station

Table 1. Fluctuations in air temperature, surface water temperature, salinity and dissolved oxygen

Month	Air temp. °C	Surface water temp. °C	Salinity ‰	Dissolved oxygen ml/l
March	31.1	31.2	30.3	3.2
April	31.3	31.3	32.2	4.4
May	31.6	31.5	30.7	3.3
June	29.9	29.7	21.2	4.9
July	28.2	28.5	4.5	5.4
August	27.7	28.4	2.1	5.1
September	28.8	29.4	2.8	3.6
October	29.1	28.7	7.2	6.1
November	29.8	29.5	12.5	5.6
December	28.6	28.9	22.4	4.0
January	27.6	27.9	32.5	3.7
February	29.7	29.5	33.4	4.9

the settlement from 1-30 days. Only the monthly averages are presented in Table 2. The seasonal settlement of 5 major

Table 2. Numbers of major fouling organisms settled on test panels at Cochin Harbour

Month	Tube worms	Barnacles	Bryozoans	Modiolus	Oysters
March	15	12	75	12	16
April	30	32	90	118	29
May	117	967	20	352	27
June	66	375	21	-	8
July	257	150	361	-	14
August	-	211	69	-	-
September	-	49	39	-	-
October	-	1110	23	-	-
November	10	1302	62	211	-
December	7	1828	17	113	4
January	4	37	3	3	13
February	22	374	15	8	51

fouling groups, namely, tube-worms, barnacles, bryozoans (encrusting), modiolus and oysters is presented in Fig.2. Fluctuations in air temperature, surface water temperature, salinity and dissolved oxygen are also shown in Fig.2.

Biofouling and its prevention in fishing craft

For studies on prevention of fouling, two paints, one with copper acetoarsenite (AF1) and another with Bis (tri-n-butyltin) oxide (AF2) were formulated. For AF1, several batches of partially soluble matrices consisting of rosin modified with linseed oil and modified cashewnut shell liquid were prepared. The successful formulation consisted of copper acetoarsenite 35.6%, rosin in linseed oil 54.5% and modified cashewnut shell liquid (CNSL) 9.9% by volume. The paint satisfied all physical tests as per ISI 101-1964 and 1419-1959. For AF2, TBTO was used with a resin (limed rosin), plasticisers (dibutylpthalate), fillers (bentonite and calcium carbonate), and driers. The most promising composition in dry state contained limed rosin 36% and TBTO 18% by weight. The satisfactory formulations were subjected to erosion studies following the method outlined in Marine Fouling and its Prevention (2) in the laboratory and for fouling resistance in the sea.

For determination of the effect of electrical polarisation on initial stages of settlement of fouling, test panels of 20x10 cm x 22 SWG aluminium, stainless steel, lead and brass were employed, one set keeping as control and the other set was polarised. Electrical polarisation was effected by galvanic coupling of electrolytic zinc to the test panels. Prior to immersion, the electrode potential of the metal in the natural sea water at 30°C was measured in conjunction with a saturated calomel electrode. Measurements were made with a Philips DC micro volt meter, G.M. 6020. Fouling on cathodically protected panels and unprotected controls was estimated after 1,2,3,6,7 and 14 days.

For the development of an antifouling and antiborer preparation, four toxins were used, namely, bis (tri-n-butyltin) oxide (TBTO), tri-butyltin acetate (TBTA), triphenyltin hydroxide (TPTH) and tri-phenyltin acetate (TPTA) in three different matrices namely, elastomer, FRP and CNSL. The elastomer employed was a synthetic rubber, reinforced with carbon black and marketed under the trade name 'Kotoprene'. FRP composites were prepared by using a layer of surfacing mat and general purpose polyester resin with cobalt naphthenate and methylethyl ketone peroxide. The third matrix consisted of melamine treated cashewnut shell liquid. Total five series of coatings were made, the fourth and fifth being improved designs based on antifouling-antiborer performance of series I to III. In addition to the organometallic compound, this improved design contained a special grade silicon carbide (400 mesh) and reagent grade zinc oxide were also incorporated in the matrix. Coatings were applied to seasoned blocks of Mangifera indica measuring 30x10x3.7 cm and their physical characteristics were determined. The panels were mounted on steel rack and exposed sub-tidally in Cochin Harbour for assessing the antifouling and antiborer properties.

Biofouling and its prevention in fishing craft

Results and Discussions

Fouling organisms:

The major fouling organisms at Cochin Harbour consisted of calcareous tube dwelling polychaetes (Hydroides norvegica) barnacles (Balanus amphitrite communis Darwin) bryozoans such as, Eletra bengalensis stoliczka, Alderina arabianensis Menon & Nair, Electra crustulenta (Pallas) bivalves such as Modiolus carvalhoi, and oyster Crassostrea madrasensis Preston. Apart from these major groups hydroids, mudtube dwelling polychaete, simple and compound ascidians were also encountered. The settlement of the major fouling organisms (Table 2) appears to be very much influenced by the prevailing monsoon over the region. Based on the monsoon the year can be classified conveniently into pre-monsoon (January-May) monsoon (June-October) and post-monsoon (November-December). Table 1 depicts the variation in hydrographic factors during the period of immersion. As is evident from Table 2, that of all the hydrographic factors, the variation of salinity is the most pronounced which brings about marked variations in the settlement of fouling organisms. During the monsoon period tubeworms, modiolus and oysters are eliminated. The influence of salinity in the settlement of several organisms has been demonstrated by several workers (5,10,11,12,13,14,15). The variation in temperature noticed in this study is only 4°C and that of dissolved oxygen 2.9 ml/l while salinity fluctuated between 2.1 to 33.4‰. The quality and quantity of the marine fouling organisms have been the least during the period of low salinity. During monsoon, owing to reduced salinity several organisms die out and the areas left bare by these organisms are found later recolonised by the larvae brought to the site by tides and other movements of the ambient medium during the post-monsoon period when salinity of the estuary becomes identical to the adjoining sea. Thus the alteration of stenohaline and mesohaline conditions existing in this locality exert considerable influence on the fouling organisms.

Soon after immersion the panels were found coated with a slime film chiefly of bacteria. The presence of diatoms in the slime was noticed only after 19 h. Experiments were conducted by us to ascertain the influence of slime film. Slimed and non-slimed control panels were employed. Settlement of barnacles was more on slimed panels when compared to non-slimed controls. This indicates that eventhough primary film favours barnacle settlement, it is not an essential pre-requisite for barnacle settlement. In this respect our studies agree well with those of Weiss (16) Clarke (17), Miller (18), Meadows & Williams (19) Ravindran et al. (9).

Antifouling paints

Leaching rate test results and fouling performance of AF1

and AF2 are presented in Tables 3 and 4. The glycine accelerated leaching rate test (Table 3) for 74 h carried after 1,2,3,4, 5 and 6 months of ageing show that the paint formulation AF1

Table 3. Leaching of AF1 and AF2

Ageing (month)	Interval of sampling (h)	Accelerated leaching rate of AF1 µg Cu cm^{-2} day^{-1}	Leaching rate of AF2 µg Sn cm^{-2} day^{-1}
0	24	1059	1.62
	48	863	
	72	731	
1	24	1213	1.58
	48	868	
	72	461	
2	24	1345	1.60
	48	898	
	72	631	
3	24	1475	1.59
	48	850	
	72	826	
4	24	1031	1.59
	48	671	
	72	490	
5	24	1024	1.56
	48	697	
	72	617	
6	24	915	1.54
	48	623	
	72	638	
7			1.53
8			1.50
9			1.49
10			1.45
12			1.53

maintains its critical leaching rate for 9 to 10 months since 24 h accelerated leaching rate corresponds to 100 days of service in the sea (2). The behaviour of the paint in the erosion test leads to the conclusion that mechanical erosion of the paint film resulting in the exposure of a fresh toxic layer was not operating. The fouling resistance of AF1 in comparison with the best commercially available paint shows (Table 4) the falure of the commercial paint after 3 months, while the AF1 even after 9 months resisted fouling settlement.

Table 4. Evaluation of fouling on AF1 and AF2

Antifouling paint	Months under immersion											
	1	2	3	4	5	6	7	8	9	10	11	12
Commercial	-	-	-	B	B	C	C	D	D			
AF1	-	-	-	-	-	-	-	A	A	B	C	C
AF2	-	-	-	-	-	-	-	-	-			

Key: A = sparse growth; B = 25% surface covered;
C = 50% surface covered; D = 75% surface covered
- = no fouling

In the case of AF2 a leaching rate of 1.53 µg tin cm^{-2} day^{-1} even up to 12 months was discernible (Table 3). This shows that the paint is effective for more than 12 months. Erosion studies carried out as per Woods Hole Oceanographic Institution (2) have shown that the paint film did not erode. Table 4 shows the antifouling performance of AF2. The paint was free from any fouling up to 9 months. Service trials on fishing boats showed a fouling resistance up to 14 months.

Electrical polarisation

Table 5 presents the equilibrium potentials after polarisation and Table 6 the wet weight of fouling complex on different metallic panels. It may be seen from Table 5 that different

Table 5. Potentials with reference to saturated calomel electrode (Electrolyte: Natural sea water, Temperature 30°C)

Metal/Alloys	Equilibrium potential (mV)	After polarisation (mV)
Aluminium	-780	-1000
Stainless steel	-200	- 950
Mild steel	-560	- 750
Lead	-530	- 720
Brass	-220	- 740

metals were polarised to different levels. Therefore comparison of results has been limited to polarised and non-polarised panels of the same metal. No significant differences were noticed in the quality of fouling on polarised and non-polarised panels, but as is clear from Table 6, the quantity of fouling was more on control panels. Electrical polarisation results in the production of an alkaline environment at the metal/seawater interface.

Table 6. Wet weight of fouling (g) settled on metallic panels immersed in Cochin Harbour for 1-40 days

Days of immersion	1	2	3	6	7	40
Aluminium control	0.64	0.37	0.32	0.16	2.60	4.16
Aluminium with zinc	0.54	0.31	0.38	0.13	0.88	1.68
Stainless steel control	0.30	0.49	1.00	0.65	2.63	4.19
Stainless steel with zinc	0.22	0.34	0.32	0.26	1.13	3.00
Mild steel control	0.61	0.47	1.00	0.86	-	9.45
Mild steel with zinc	0.25	0.28	0.50	0.32	-	4.07
Lead control	0.39	0.21	0.30	0.15	1.14	2.25
Lead with zinc	0.20	0.26	0.37	0.31	1.37	1.91
Brass control	0.18	0.11	0.39	0.13	0.58	1.23
Brass with zinc	0.18	0.14	0.39	0.13	0.58	1.14

Depending on the intensity of polarisation extreme alkaline conditions can be created. Mor (20) has reported intense fouling in the pH range 8.2 to 8.5 and gradual declension at pH 9 and above. The decrease in fouling on polarised panels in this study also may be due to the formation of an alkaline environment due to cathodic polarisation. This finding may be of help in the development of second generation antifouling paints.

Elastomeric antifouling-antiborer composition

The physical characteristics of the polymeric coatings with organometallic biocide are presented in Table 7. The physical properties were satisfactory for any marine application. As the coatings were of a high build type applied on wood, the flexibility and adhesion were determined on a third point flexural loading on a span of 30 cm. All coatings had a deflection of 30 mm and the yield point was much higher than this value. A deflection of over tens of millimetre is not usually required and the flexure tests were not carried to ultimate values. The adhesion was satisfactory in all cases. Results of field exposure to evaluate fouling and boring resistance of matrices PM-01 to PM-12 are presented in Table 8. The number of months required for the first settlement of foulers are taken as a good indication of the toxicity at the matrix/seawater interface. On polyester matrices 50-60% and on polymerised CNSL, 20 to 25% coverages of of foulers were noticed. This can be ascribed only to the differences in the migration of the biocide in the two matrices. The biocide got locked up in the matrix of polyester coating while the biocide availability in the CNSL matrix surface was

Table 7. Physical characteristics of polymeric matrices containing organometallic biocide

Matrix no.	Polymeric matrix and biocide	Surface dry (h)	Hard dry (h)	Scratch hardness (kg)	Flexibility and adhesion on 3rd point loading (mm)
PM-01	GP Polyester + TBTO	5	24	3.0	35
PM-02	GP Polyester + TBTA	5	24	3.0	30
PM-03	GP Polyester + TPTA	5	24	3.0	30
PM-04	GP Polyester + TPTH	5	24	3.0	30
PM-05	CNSL + TBTO	12	72	0.9	50
PM-06	CNSL + TBTA	12	72	0.9	45
PM-07	CNSL + TPTA	12	72	0.9	50
PM-08	CNSL + TPTH	12	72	0.9	40
PM-09	Kotoprene + TBTO	1	3	0.9	30
PM-10	Kotoprene + TBTA	1	3	0.9	70
PM-11	Kotoprene + TPTA	1	3	0.9	70
PM-12	Kotoprene + TPTH	1	3	0.9	70

Table 8. Antifouling and antiboring properties of polymeric matrices containing organometallic biocide

Matrix no.	Slime	Algae	Hydroids	Tube worms	Molluscs	Barnacles	Fouling % area	Borers	Adhesion
PM-01	1	-	1	-	1	1	50	3	Binding poor to fair
PM-02	1	-	1	-	1	1	60	3	"
PM-03	1	-	1	-	1	1	60	3	"
PM-04	1	-	1	-	1	1	50	3	"
PM-05	2	-	1	-	-	1	20	3	"
PM-06	2	-	1	-	-	1	25	3	Binding poor blisters formed
PM-07	2	-	1	-	-	1	25	3	"
PM-08	2	-	1	-	-	1	20	2	"
PM-09	3	-	-	-	-	2	25	-	"
PM-10	3	-	-	-	-	2	30	-	Binding satisfactory
PM-11	3	-	-	-	-	2	30	-	"
PM-12	3	-	-	-	-	2	25	-	"
Control	1	1	1	1	1	1	100	1	"

better. Sawada et al. (21) reported that tributyltin and triphenyltin in FRP gel coats prevented fouling until 3 months in Ito Harbour, Japan. However, this was not so in the present studies as is evident from the failure of coatings PM-01 to PM-04 (Table 8). Adhesion was poor in the coatings which resulted in boring of the panels by marine organisms. However, TBTO incorporated in 'Kotoprene' elastomer performed satisfactorily in the field evaluation tests. Barnacles settled on CNSL and polyester matrices within a month and after 2 months on 'Kotoprene'. The slime formation on polyester, CNSL and 'Kotoprene' matrices took place after 1,2 and 3 months respectively. This also shows that the presence of slime is not an essential prerequisite for barnacle settlement. However, it is significant to note that a delayed formation of slime has delayed the settlement showing that slime may have some influence in barnacle settlement. This observation closely agrees with that of Liberatore et al. (22) and Nair & Pillai (23).

The study shows the distinct possibilities of combining antifouling and antiboring properties in one suitable matrix. As is known (2,24,25,26) the action of antifouling paints depends on the toxic availability through diffusion, leaching or contact. Eventhough TBTO has a wide spectrum of activity (27,28) it did not perform satisfactorily in this study owing to the locking up of the TBTO in the matrix and thereby not reaching the seawater/matrix interface. As zinc oxide is moderately soluble in seawater, it was considered on theoretical grounds that incorporation of zinc oside in the matrix would provide pores/paths in the coating system by its slow dissolution in water. Antiborer properties are imparted to the system by incorporating highly abrasive silicon carbide. The modified formulations under series IV and V are prepared and evaluated for physical properties (Table 9), and effective life under seawater (Table 10). In all the

Table 9. Physical characteristics of polymeric matrices containing organometallic biocide, silicon carbide and zinc oxide

Matrix no.	Matrix system	Surface dry (h)	Hard dry (h)	Scratch hardness (kg)	Flexibility adhesion on 3rd point loading (mm)
PM-13	Kotoprene + TBTO+SiC+ZnO	1	3	1.4	45
PM-14	Kotoprene+ TBTA+SiC+ZnO	1	3	1.3	45
PM-15	Kotoprene+ TPTA+SiC+ZnO	1	3	1.5	45
PM-16	Kotoprene+ TPTH+SiC+ZnO	1	3	1.5	45
PM-17	CNSL+TBTO+SiC+ZnO	8	48	1.2	35
PM-18	CNSL+TBTA+SiC+ZnO	8	48	1.3	35
PM-19	CNSL+TPTA+SiC+ZnO	8	48	1.4	35
PM-20	CNSL+TPTH+SiC+ZnO	8	48	1.4	35

Table 10. Biocidal and binding properties of polymeric matrices containing organometallic compound, silicon carbide and zinc oxide

Matrix no.	Months elapsed for settlement of foulers	Area fouled	Marine borers	Nature of adhesion
PM-13	9	Sparse	Nil	Satisfactory
PM-14	7	"	3 at edge	"
PM-15	7	"	5 at edge	Begins to fail
PM-16*	8	"	Nil	Satisfactory
PM-17	5	"	Profuse @	Blistering and flaking
PM-18*	5	"	"	"
PM-19*	5	"	"	"
PM-20*	5	"		
Control	1	100	Completely riddled by borers. The modulus of rupture as residual strength was 6%	

compositions (PM-13 to PM-20) the settlement of foulers was delayed showing better toxicity at the surface of the coating. CNSL based coating (PM-17 to PM-20) could not be continued beyond 5 months as the binding of the coating failed and areas where the fracture appeared developed fouling growth. However the elastomeric coating PM-13 resisted fouling and boring for 9 months. This finding confirmed the view that diffusion of the organometallic compound is considerably enhanced by the vacant, pores/path ways formed as a result of the solubility of zinc oxide.

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*Coating failed in adhesion within 5 months, foulers and borers entered tnrough the area of failure. Observation of foulers beyond 5 months are no longer reliable @The internal damage of timber was over 50%

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Etudes sur les salissures marines et leurs empêchements avec référence spéciale au bateau de pêche

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Les résultats des études suivies par les auteurs au port Cochin (9°58'N, 76°16'E) avec référence avec la biologie des salissures, développement des peintures d'anti-salissure avec acetoarseinité cuivre, et bis (tri-n-butyl étain) oxyde; effet de la polarisation électrique à la phase initiale des salissures et la formulation d'un mélange d'anti-destructeur-anti-salissure elastomérique ont été mentionnés dans ce communiqué. De tous les facteurs hydrographiques, les fluctuations de salinité due à l'influence de sud-ouest mousson apportent des changements nets dans les groupes faunistiques au port Cochin. Plusieurs groupes majeures des salissures ont été observés afin d'être éliminés pendant la grande partie de la période de mousson. La peinture d'anti-salissure contenant le cuivre acetoarsenite (AF1) a resisté la salissure pour plus de neuf mois et la peinture contenant Bis (tri-n-bulylétain) oxyde a maintenu le tav de ladissolution à 1.53 µg etain cm^{-2} $jour^{-1}$ même après 12 mois. Les différences étaient notées dans le quantum de salissure sur les panneaux polarisés et non-polarisés; les panneaux non-polarisés ont accumulés plus de salissure quoique ce n'était pas le même dans les panneaux polarisés électriquement due à la formation d'un milieu alcalin due à la polarisation. Les études ont aussi montré la possibilité distincte de combiner les propriétés d'anti-salissure et d'anti-destructeur en une matrice par l'incorporation parcimonieuse de l'oxyde de zinc soluble, grade spéciale silicium-carbure et biotoxin dans une matrice polymérique qui convient.

Observations of the Inter-relation of Marine Corrosion and Fouling in a Tropical Environment

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Results of one year exposure of carbon steel, stainless steel type 304, aluminium 2S and aluminium M57S to the free attack of marine corrosion and fouling in Cochin Harbour (9°58'N, 76°16'E) are presented. The corrosion rates of metals in the fouled state after a year of exposure were 103, 6.3, 5.6 and 6.4 micron year^{-1} for CS, SS 304, Al 2S and Al M57S respectively. Carbon steel and aluminium alloys were free of any pitting and crevice attack but severe pitting, tunnelling and perforations occurred on SS 304. The fouling load (wet weight per unit area) varied considerably with metals. A maximum load of 21.9 kg m^{-2} was observed on Al M57S in six months but on prolonged exposure the fouling diminished as a result of decrease in salinity of the environment. Based on fouling load on metals investigated, Cochin Port appears to fall under fouled port. Growth curves developed for barnacles, may help in the prediction of fouling in Cochin Harbour. The theoretical growth curves fitted for barnacles on different metals would aid in an understanding of the inter-relation of fouling and corrosion and in the prediction of the quantum of fouling. These results are of considerable importance to the ocean engineering community especially in the tropics.

Introduction

Man's endeavours to exploit the oceans have gained considerable momentum in recent years. Offshore drilling, ocean mining, desalination, offshore metallurgy, alternate food and energy resources are some of the areas of ocean engineering which registered rapid progress. The increased activities coincided

with the demand for a host of materials, equipment and facilities which can withstand the hostile and demanding marine environment. Several factors merit consideration before a material is recommended for ocean service: the structural strength, predicted life expectancy, knowledge of uncertainity and a thorough fundamental understanding of the marine corrosion and marine fouling behaviour. According to Gerchakov & Sallman cited by Schumacher (1) the last mentioned two factors are inter-related which in turn depend upon the interaction of metal, biota and the natural aquatic environment.

Theoretically, microfouling and corrosion are simultaneous processes occurring immediately upon introducing a metallic object in ocean, but in scientific pursuit these aspects have been dealt with by different groups of investigators, generally in isolation.

The net work of interaction and feedback system bringing in biota into metal/seawater interface allows a realistic prediction of performance of materials in seawater. The natural phenomena of biofouling follows a sequence of events like modification of surface by adsorbing biopolymers, the attachment and proliferation of pioneer cells followed by cellular or animal growth and finally mineralisation (2).

The fouling consisting of corrosion product films, precipitated salts, suspended solids deposition and biological growth (3) alters corrosion processes, increases the frictional resistance of ships, destabilises submerged oceanic structures, increases the weight of buoys and navigational equipment, clogs seawater conduits, generates noise which interferes with the sonar operations and increases wave action loadings on structures (4). Tighe-Ford estimated that fouling prevention and antifouling maintenance,cost the maritime industry in USA over 500 million dollars in 1971 (5).

Redfield has reviewed the results of researchers upto 1948 concerning the fouling of the metallic surfaces and the influence of corrosion on the toxicity of metals in a monograph prepared by the Woods Hole Oceanographic Institution (4). An excellent account of macroorganisms in seawater and their effects on corrosion of metals are given by Clapp (6). Extensive investigations carried out at the LaQue Center for Corrosion Technology at North Carolina USA and the works of Efird (7) have brought to focus a unified picture of corrosion and fouling of metals in seawater.

Considerable research efforts to study the simultaneous phenomena of marine corrosion and fouling have been made recently in response to the urgent requirements of high performance materials for Ocean Thermal Energy Conversion Systems and

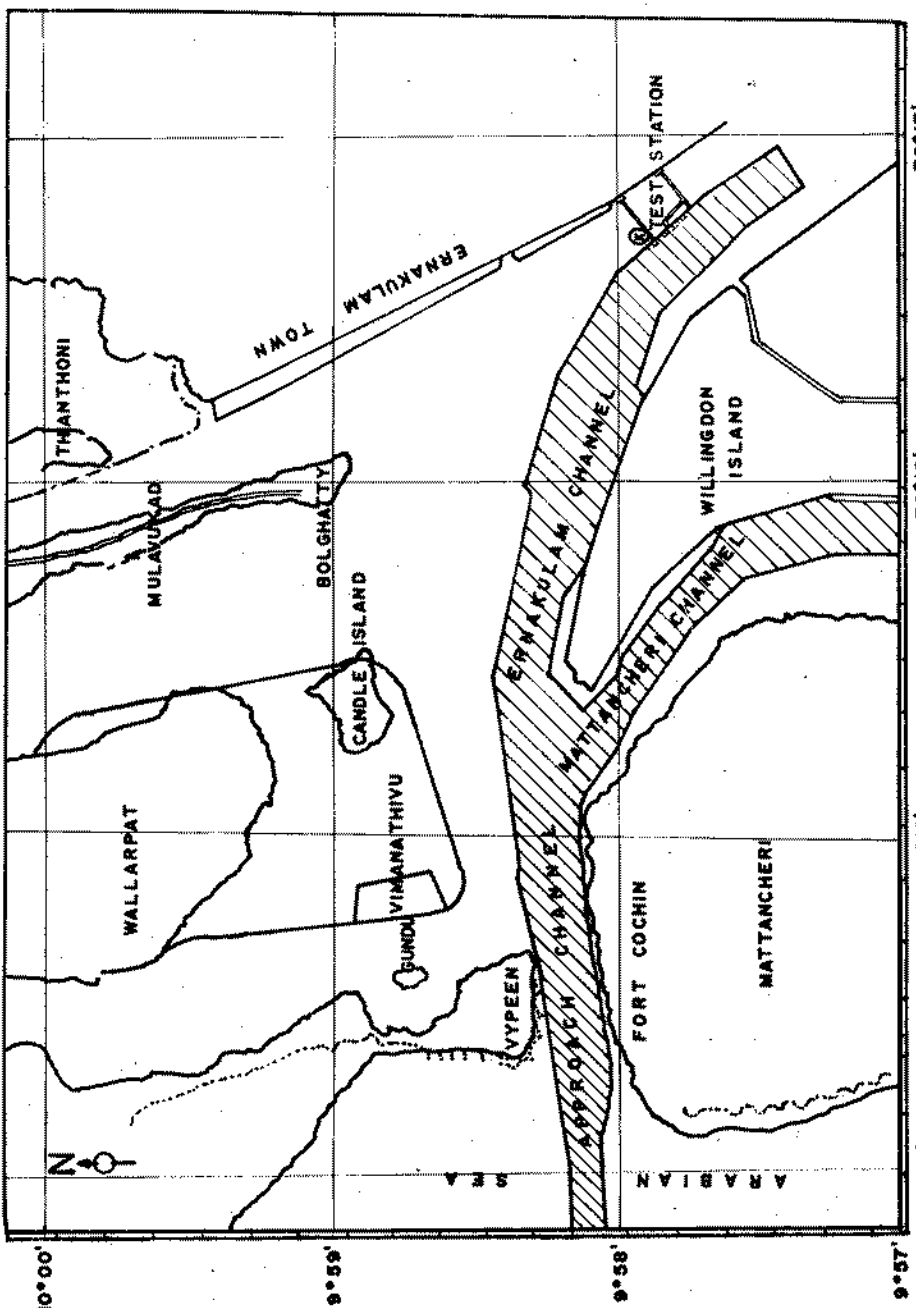

Fig. 1. Cochin Harbour showing the test station

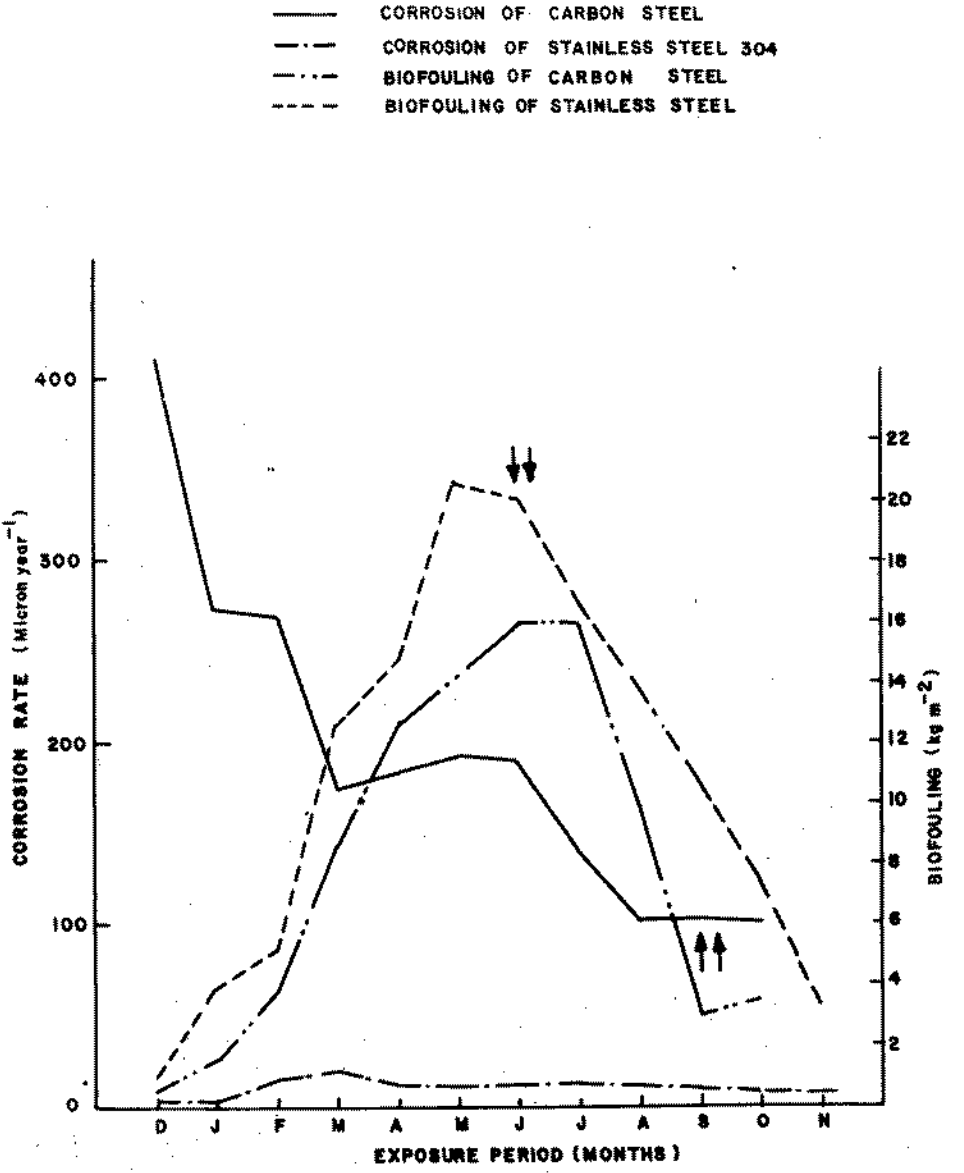

FIG.2. CORROSION RATES OF FOULED FERROUS ALLOYS

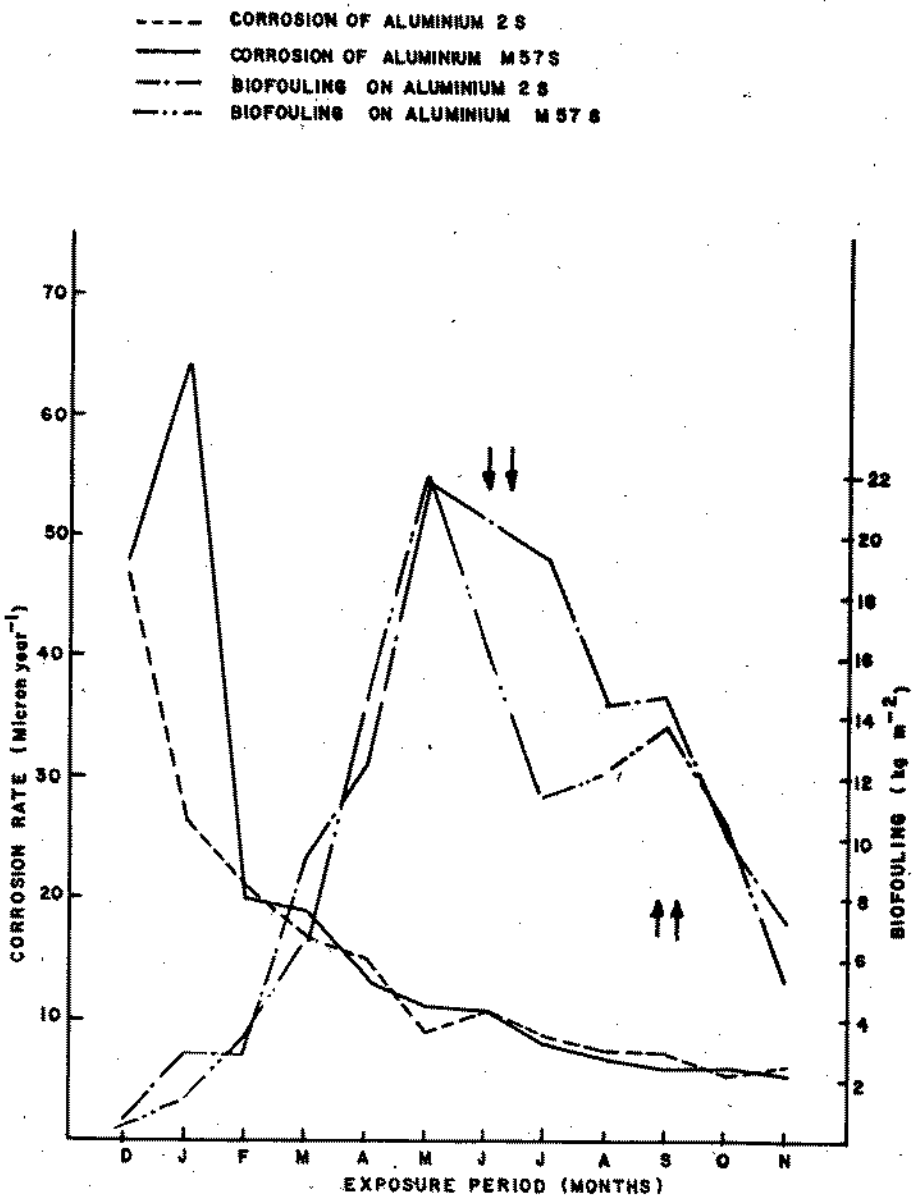

FIG.3. CORROSION RATES OF FOULED ALUMINIUM ALLOYS

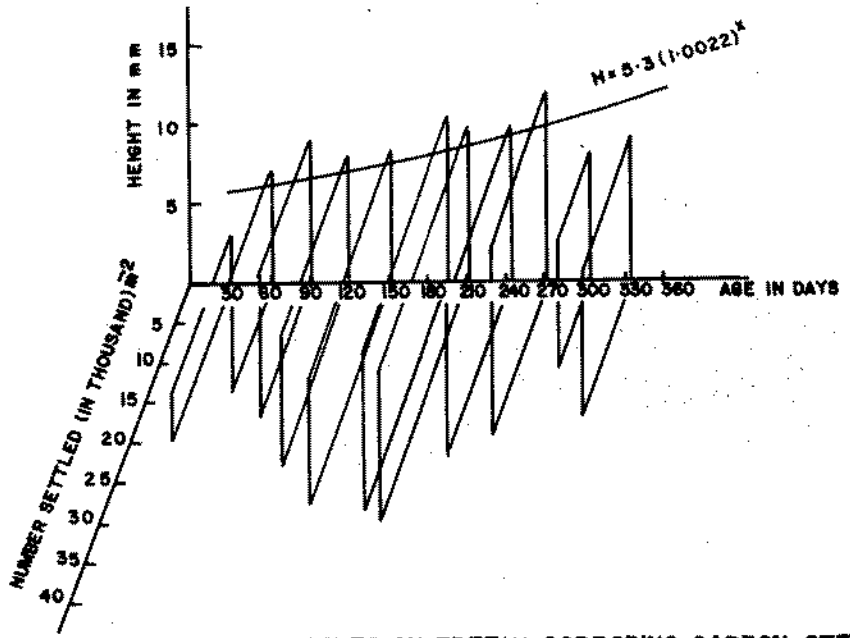

FIG. 4·GROWTH OF BARNACLES ON FREELY CORRODING CARBON STEEL

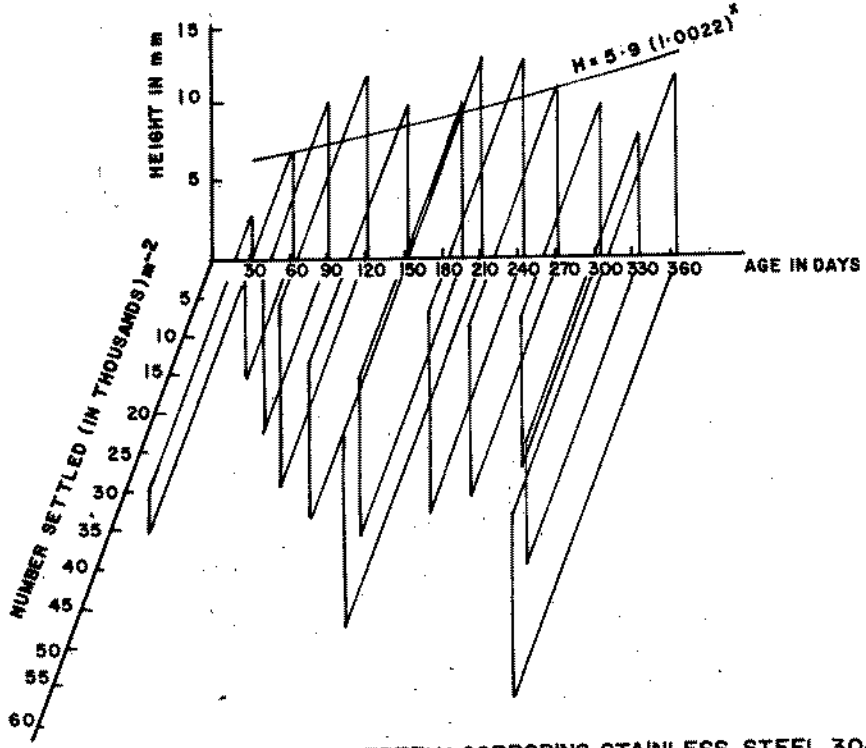

FIG.5. GROWTH OF BARNACLES ON FREELY CORRODING STAINLESS STEEL 304

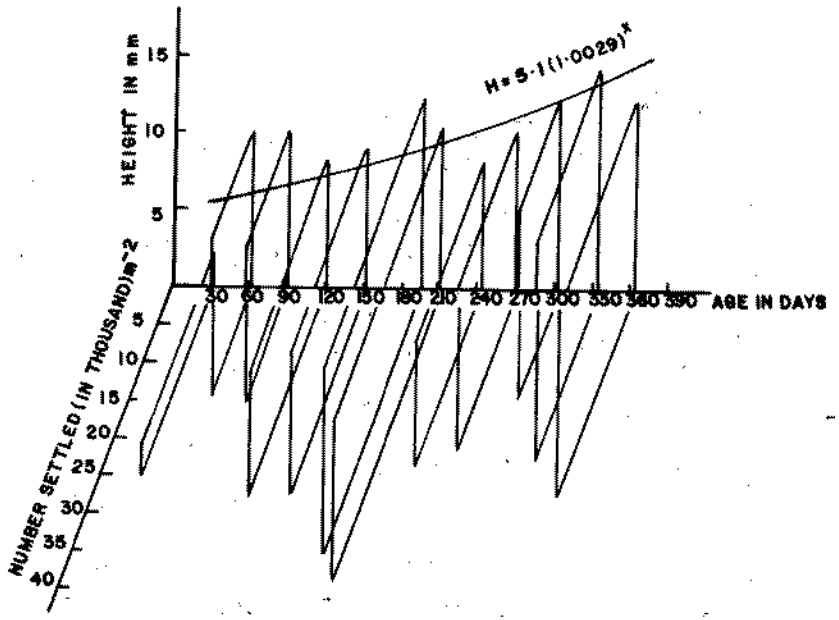

FIG.6. GROWTH OF BARNACLESON FREELY CORRODING ALUMINIUM 2 S

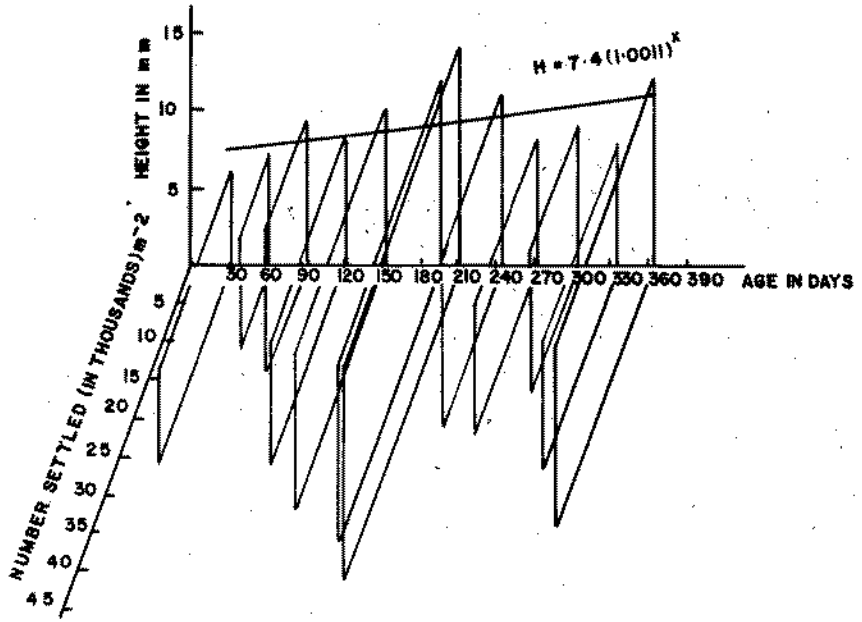

FIG.7. GROWTH OF BARNACLES ON FREELY CORRODING ALUMINIUM M 57 S

subsystems. Fouling in thickness less than 100 micrometre can render an OTEC system inoperative by impairing the heat transfer efficiency (8).

Notable works (9,10) on the distribution of macrofoulers at near shore and offshore site in the Straits of Florida and Bahamas enabled DePalma at the US Navy to develop the growth curve nomogram from which "fearless fouling forecast" could be made within various marine environments. Based on extensive investigations DePalma (11) predicted the effects of macrofouling which may occur on a generalised ocean structure. The problems of macrofouling of hull, heat exchangers and cold water pipes of different designs of OTEC machines and an estimate of maximum organismal growth, weight displacement as a result of fouling were the subject of detailed investigations recently (12).

De et al. (13) who did pioneering works in India, contributed to our understanding of corrosion behaviour of some structural metals at Bombay and Cochin Harbours, but the fouling dynamics in relation to corrosion was not studied by them.

As far as we are aware of no detailed studies on fouling on structural metals, inter-relation of corrosion and fouling and forecast of fouling on submerged metals in seawater in a manner required by the ocean engineers and planners have been carried out in the tropics. This paper presents a part of the results obtained under a comprehensive investigation on the behaviour of structural materials in Cochin Harbour (9°58'N, 76°16'E).

Test programme

The metals and alloys for use in this work were cut to 10x7.5 cm from rolled sheets of carbon steel, stainless steel type 304, aluminium 2S and aluminium M57S. The metal plates were cleaned and weighed as recommended by Ailor (14) and Champion (15) and were mounted on mild steel racks of Carnegie Illinois Steel Corporation design (16) whereby the galvanic action between different specimen or between the specimen and the rack was prevented. The racks containing the metal plates were kept submerged at one metre level from the low water level in the vicinity of Oil Tanker Berth at Cochin Harbour (Fig.1). The plates were thus exposed to the natural assemblage of marine fouling on freely corroding metals. The retrieval of the panels in triplicate were made at monthly intervals and a quantitative assessment of the biogrowth was made. The plates were cleaned off the corrosion products (16), the corrosion rates determined and the relevent data analysed satistically. A detailed account of the test programme is given by Pillai & Ravindran (17).

Results and Discussion

The rates of corrosion and the wet weights of the fouling complex on carbon steel, SS 304, Al 2S and Al M57S as a function

of the exposure period are presented in Fig.2 and 3. Graph representing the age in days on the X-axis, the height in mm on the Y-axis and the number settled per square metre area on the Z-axis of one of the major foulers are given in Fig. 4 to 7.

Corrosion behaviour in relation to fouling

The application of carbon steel as a marine hardware depends much on its corrosion and fouling characteristics which in turn depend upon the environmental factors. At Cochin Harbour, carbon steel corrodes at a rate of 410 micron $year^{-1}$ initially but the rate decreases as the period of exposure increases and attains a value of about 103 micron $year^{-1}$ after 270 days. This near steady state of corrosion is controlled by several factors principally the salinity, dissolved oxygen and water temperature as well as the mass and the nature of the biota on the metallic surface.

Cochin Harbour presents certain unique hydrographic features as there is progressive dilution of seawater owing to South-west monsoon during the period May to July and precipitation from north-east monsoon during August to October. The discharge of fresh water by numerous rivers also lowers the salinity of Cochim backwaters especially during monsoon. An excellent account of the general hydrography of Cochin Harbour is given by Nair (18) and the monthly variations of salinity, dissolved oxygen, surface water temperature and pH pertaining to the period of study by Pillai & Ravindran (17).

The influence of biofouling and calcareous deposits formed on the metallic surface is evident from the slopping trend of the corrosion rate curve as they would restrict the availability of oxygen to the metal surface. Pitting was absent throughout. The biofouling on carbon steel being less adherent, gets sloughed off periodically along with the corrosion film exposing fresh surface of the metal for attack. Based on several tests, Larrabee & Mathay (19) concluded that the corrosion of iron in deaerated unpolluted seawater is 100 to 125 micron $year^{-1}$ for the first year of exposure, Values ranging from 165 to 175 micron $year^{-1}$ were obtained by De et al. for carbon steel at Cochin (13), and Vishakhapatnam (20). Comparable corrosion rate of carbon steel in tropical and temperate waters is in conformity with the reasoning that in tropical waters the fouling is massive and restricts access of oxygen whereas temperate waters contained more of dissolved oxygen, but less of fouling organisms. These factors are self compensating (1).

Type 304 stainless steel showed pitting, tunneling and perforations within a period of 2 months. The crevice attack and perforations occurred beneath barnacle base. The maintenance of passivity which is responsible for the protection of stainless steel is impaired due to the formation of differential

aeration cells (oxygen shields) beneath the settled foulers. The corrosion rate, as determined by the weight loss, was very low: the initial rate is 3.1 micron $year^{-1}$ in one month and attained values of 5.2, 5.7 and 6.3 micron $year^{-1}$ during the 10th, 11th and 12th month of exposure. As the attack was highly localised in nature the average corrosion rate values do not reflect a realistic picture of the deterioration of the metal.

The general corrosion behaviour of aluminium 2S and M57S were comparable as there are very little variations in their corrosion rates after 180 days. Though aluminium alloys are susceptible to crevice and pitting attack in seawater (13,21, 23)aluminium 2S and M57S were free of any localised attack. An adherent heavy mat of fouling on entire surface of the metal in a period less than 2 months and subsequent sumperimposed growth provided considerable shielding to the metal from the environment. In the absence of pitting, corrosion rates based on weight loss determination are meaningful though such computation may give a misleading picture in case corrosion is localised. The observed corrosion rates were extremely low and were in the range of 6.1 to 5.6 micron $year^{-1}$ for Al 2S and 7.6 to 6.4 micron $year^{-1}$ for Al M57S during 300 to 360 days of exposure.

Fouling behaviour of freely corroding metals

The quantum of fouling expressed as wet weight in kg m^{-2} on carbon steel, SS 304, Al 2S and Al M57S as a function of period of exposure is shown in Fig.2 and 3. The upward and the downward arrows in the figures correspond to a period of commencement of fresh settlement of larval forms and the general sloughing off of settled biota with the non-adherent corrosion products respectively. The fouling complex mainly consisted of barnacles, hydroids and modiolus with few (less than 10%) bryozoans, tube worms and oysters but the major share of the weight was due to barnacles.

The graph representing quantum of fouling shows three distinct periods characterised by: (a) a period of increased settlement and progressive luxuriant growth of foulers with the complete coverage of the surface, (b) a period of reduced activity, retarded growth, mortality (70 to 80%) and absence of fresh settlement and (c) a period during which the calcareous structures of the animals remained intact with metals like stainless steel and aluminium alloys, but excessive sloughing off on carbon steel. Fresh settlement of the larval forms also took place during this period.

Maximum fouling loads observed in the present study during the period from December 1980 to May 1981 are 16, 20.7, 21.6 and 21.9 kg m^{-2} on carbon steel, SS 304 ,Al 2S and M57S respectively. The heavy biogrowth on non-toxic corroding metals in terms of mass and unit of exposure period would indicate that Cochin Port

may fall under 'fouled port'. Continued exposure had shown a deline in fouling load per unit area which might be attributed to sloughing off especially from carbon steel, mortality, diminised growth rate etc consequent to environmental changes. Periodical sloughing off from solid corrosion products from carbon steel occurred much before attaining the maximum fouling load.

On non-toxic metals like, Al 2S and Al M57S the fouling load in Cochin Harbour was found to be related to salinity changes and does not appear to depend upon small changes of temperature from 28.8 to 32.0°C at the harbour area. Previous studies (4) have shown that when the water temperature is from 21 to 38°C, biogrowth is prevalent with maximum accumulation from 27 to 32°C. The major role of salinity on settlement of foulers on glass (24) and sand blasted black acrylic sheets (25) was also reported by other workers. While in temperate waters the effects of temperature overshadow that of salinity (4).

The assemblage of foulers, seasonal pattern of species recruitment, larval transport etc are complex functions of several parameters of the environment (26) and the biogrowth appears to depend on physical (4), chemical (4) and electrochemical (27) characteristics of the metal and the nature of the corrosion film (7).

At Cochin Harbour where the salinity influences fouling, the maximum fouling load attained as given in Figs2 and 3 can be taken for engineering computations. This is suggested on the premise of DePalma (11) that once a surface is totally covered, there is a minimal increase of added weight with time and therefore the fouling rates are similar eventhough some are based on 10 to 11 months and others are on a 12 years prediction.

The average density of the biofouling when it was predominently composed of barnacles was 1500 kg m^{-3} while that constituted by oysters showed a density of 1470 kg m^{-3}. Heavy interlace of fouling with superimposed growth on Al M57S caused a maximum fouling load of 21.9 kg m^{-2}. Interesting is a comparison of this value with that of DePalma (11) that a structure totally covered by hard shelled organisms will increase in weight by approximately 17 kg m^{-2}. The painstaking works done by DePalma (9,10,11) were of immense value in predicting the expected mass of fouling organisms and their percentage weight to the hull of the OTEC-1, APL (10-20 MWe) and Spar Configuration (10 MWE and 40 MWe) (12).

While studying the economic implications of fishing fleet management it is reported that Al M57S sheathed fishing boat hull gathered 10 to 15 kg m^{-2} of fouling in a period of 7 to 8 months (28). Observations of heavy biogrowth of varying intensities on fishing trawlers operating in offshore waters and high seas do provide a reflection of the efficacy of antifouling paints available in the country.

Inter-relation of marine corrosion and fouling

Statistical approach to fouling prediction

For a clearer comprehension of complex inter-related and competing phenomena involving unit factors such as time and space, number of occurring individuals and their growth "an analytical graph" was developed by Tatuo Kawahara (29). Following a similar trend, in an attempt to predict the quantum of biogrowth on metals, the three inter-related parameters, namely, the period of exposure, the height and number of barnacles were plotted on a graph. As barnacle fouling is of concern in view of fouling load per unit of exposure time, the regression curves were fitted for the growth in terms of height and basal diameter of barnacles settled on carbon steel, SS 304, Al 2S and Al M57S. These are shown graphically in figures 4 to 7 and the corresponding equations are presented in Table 1. These equations describing two important basic dimensions of barnacle (the shape of

Table 1. Regression equations of barnacle fouling dynamics on freely corroding metals

Metal	Growth rate (mm)	
	Height	Basal diameter
Carbon steel	$5.3\ (1.0022)^x$	$8.4\ (1.0021)^x$
SS 304	$5.9\ (1.0022)^x$	$10.9\ (1.0017)^x$
Al 2S	$5.1\ (1.0029)^x$	$8.8\ (1.0023)^x$
Al M57S	$7.4\ (1.0011)^x$	$10.0\ (1.0013)^x$

which can be approximated to that of a cone) computed with the density of biomass would lead to reliable prediction of the biofouling load that can be expected on submerged metals. This data may also eventually be applicable to evaluate the environmental conditions of near shore ecosystem.

It is emphasised that for the rational design of hardware for ocean engineering projects a fore-knowledge of the materials behaviour in the environment would aid in proper planning and decision making.

Conclusions

Intense pitting, tunneling and perforations of SS 304 under heavy deposits of fouling limit the use of this alloy in ocean engineering. Carbon steel, Al 2S and Al M57S were free from pitting. Fouling in general was very heavy on all metallic surfaces and a maximum fouling load of 21.9 kg m^{-2} was observed on non-toxic metal. Based on the fouling load Cochin Port appears to fall under fouled port. With the help of growth curves meaningful prediction of fouling on different freely corroding metals could be made.

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Les observations sur l'entre-relation de la corrosion marine et des salissures dans un milieu tropical

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Les résultats d'une année d'exposition de l'acier carbone, l'acier inoxydable du type 304, l'aluminium 2S et l'aluminium M57S à l'attaque libre de la corrosion marine et des salissures au port Cochin (9°58'N,76°16'E) ont été présentés. Les taux de la corrosion des métaux dans un état de salissure après un an d'exposition étaient 103, 6.3, 5.6 et 6.4 micron/année pour CS, SS 304, Al 2S et AlM57S respectivement. L'acier carbone et l'aluminium alliage étaient libre de toutes fosses et des attaques crevasses mais des fosses sévères, tunnel et perforations ont eu lieu sur SS 304. Les poids des salissures (poids mouillés par unité aire) étaient variés considérablement avec les métaux. Un poids maximum de 21.9 kg m^{-2} était observé sur AlM57S en six mois mais à l'exposition prolongée, la salissure a été diminuée comme résultat de décroissement dans la salinité de l'environnement. Basé sur les poids des salissures sur les métaux examinés, le port Cochin semble être sous les 'ports des salissures'. La crossance courbe developpée pour les barnacles pourrait aider dans la préatiction des salissures au port Cochin. La croissance courbe théorique convenable pour les barnacles sur les métaux différents pourrait aider dans une compréhension des entre-relations des salissures et de la corrosion et dans la prédiction du quantum des salissures. Ces résultats sont d'une importance considérable pour la communauté des génies océaniques surtout dans les tropiques.

DETERIORATION OF PAINTS AND COATINGS BY MARINE ORGANISM

SAMIR M SAID, N.J. PAUL, DARWISH M GOBAISI,
POWER STATIONS ABU DHABI.

A B S T R A C T

Paints and protective coatings are normally evaluated by the salt spray test or by holiday detectors upon the coated sample. Coatings under polluted sea water conditions have more reasons to fail earlier than can be predicted or evaluated in the laboratory under simulated conditions. There are physical, chemical and electrochemical phenomenon operating as bio deterioration sets in on submerged painted structure. Why and how different coatings are affected at different rates by bio species is a complex relationship based on chemical composition of the paint and the dry film thickness and the porosity of the coating. Different bio species are at action causing failure of the coating. Additional factors such as cathodic protection, chlorination and chemical dosing influence the type and number of organisms causing the deterioration whereby the kinetics of corrosion reaction is altered considerably. Video recordings of some live species and their bio-products throw some light on effective protective action of coatings under submerged conditions.

INTRODUCTION

Paints and coatings are formulated for specific applications and are subjected to approved testing procedures of ASTM in their factories. Whenever failures occur the one common cause attributed is the defect in surface

P.T.O

preparation. Recent extensive work on causes of coating failure have revealed more than one possible cause which could be either physical, or chemical and or electrochemical. Particularly paints meant for under water applications and these intended for polluted sea water conditions have one more cause to fail -- the bio reactions on paints. Although it is commonly assummed that antifouling coatings are effective there are positive evidences to show that even surfaces coated with such compositions have failed under bio deterioration. Thus it is neither the defect of the paint composition nor the defect in coating techniques but that the complexity of the microbe in action that matters.

Again species and their activity vary from zone to zone in various parts of the globe (depending on ecological conditions) and it is rather difficult to drawn one common rule for all cases of failure. The best way is to test the effectiveness of coating under actual field conditions. It may not be surprising that a coating which proved effective in North sea conditions may not work well in Arabian Gulf as we have differences in salinity, water velocity and temperatures, leave apart the type of pollutaries in the sea water. Also there are certain specific applications of paints in contact with polluted sea water and chemicals (as in desalination and power plant near sea side) where antifoulants and such coatings cannot be introduced for reasons of toxicity. The only course left is to depend on certain epoxy formulation which can withstand high temperature, and velocity conditons of operation of this sea water so that the bio species is controlled only by chlorination and chemical dosing.

EXPERIMENTAL DETAILS

For the sea water desalinations units and the power plants millions of gallons of sea water is drawn from the sea and this passes through the coarse screens, sedementation and chlorine treatment tank and band screens before it passes through the pumps and piping systems before reaching the boilers and cooling vessels.

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As in any desalination and power plant one may see wide variety of alloys, metals and materials are used in the fabrication of various components and the clear sea water causes severe corrosion problem particularly at the elevated temperatures and velocities. To add to this, pollutions in coastal waters pose further problems due to the biospecies which flourish under the polluted conditions. This can be seen from the fact that whenever sections of plants and units are opened for annual maintenance debris of biospecies or foul smell of Fes (H_2S) can be noticed in some part or the other. How and when these biospecies managed to escape the barriers and the chlorination treatment no one knows. The astronomical rate of which they multiply even in a short time and their ability to lodge in some crevice or corners to cause severe damage latter is known to all plant operators.

The sole dependance on paints and coatings to prevent this disaster signifies the importance of evaluation of paints and coatings under this polluting or corroding conditions. Either actual objects are coated and the results obtained during next shut down period, or test coupons suspended from a rig in calm sea water condition at sea water entry, and evaluated periodically.

EXPERIMENTAL DETAILS

Large numbers mild steel specimens 12cms x 18cms cut from steel plate of one heat and surface prepared as described by Kenneth Talor etal in their paper on performance of protective coatings.

The surface preparations selected for study was

1. None (mill scale)
2. NACE brush off cleaned
3. NACE 1 white grit blast
4. Hot dip galvanising

The metallurgical analysis of a steel sample as measured by Texas Nuclear alloy analyzer 4266 is given in Table - 1.

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Paints and coatings selected:-

Epoxy
1. amine adduct cured (50% solids)
2. polyamide epoxy with selective pigments (95% solids)
3. epoxy with bleached tar (65% solids)
4. aluminium paste pigmented high build epoxy (80% solids)
5. polyaminoamide

Miscellaneous
6. polyurethane coatings
7. powder glass resin impregnated coatings
8. chlorinated rubber

The first three coatings some of which could be applied both as primer and top coats, were used in different combinations among themselves.

As mentioned in our earlier report[5] only the paint systems that could withstand the severity of temperature and pressure conditions met with in any desalination and power plants were chosen for the study. In about four years in the plant and one and half years of study in sea water various conclusions have been drawn.

EXPERIMENTAL SET UP

Coupons were submerged in calm sea water and drawn out from 15 days to after six months were brought quickly for view under microscope and video recording. Most of the samples were examined under

Zeiss Sterio microscope SR with 5 step magnification

Zeiss universal microscope for reflected light

SONY SMF Trinicon colour video Camera DXC / 1800 PK

SONY Trinitron colour TV CVM 2000 PSE.

Although direct viewing under microscope could not

P.T.O

give magnification beyond 1000X, with the help of video camera and projection on the screen one could get a small area of 3mm x 1mm enlarged to 450mm x 300mm. Thus a magnification upto 10000X in obtained although clarity could be somewhat lost.

Under high illumination of Schott KL 150 (150W) halogen lamp this difficulty was somewhat overcome and the live objects are screened and recorded.

R E S U L T S

From the recording we could come to several conclusions.

1. The type of growth and bio species residue on the various epoxy and other coatings are different possibly influenced by the chemical compositions of the paint.

2. On all epoxies however inpervious they seem to be under other tests (holiday detection, salt spray) Some kind of sessile (attached) growth is observed. The randomness (or the density of growth) and the tenacity of adhesion varying from paint to paint.

3. On the plain mild steel the growth are cocci type (tubular) and on the apparently good coating shell-fish and barnacle (volcano like growth).

4. On the Aluminium paste rich epoxy there have been random growth but of both types which could not be explained. Although earlier reports suggested that certain metals like Zinc, Aluminium, Tin Copper and Mercury could be repellant to bio species. This steel could not be established as even on galvanic anode. Such bio growth was observed by earlier workers as well as by ourselves.

5. The effect of copper salt and copper ion in the vicinity of the coated surface has also been studied.

One of the first pictures in the recording shows a coupon under sea water for over 4 months and the coating (epoxy) was not very satisfactory (both under plant

P.T.O

operation condition as well as in polluted sea water study) even after cathodically protected by galvanic anode was subjected to copper salts deposition onto the surface. The green copper salt could be seen with no bio growth thereafter although under normal condition we have observed bio growth continuing on the old sturucture or the destroyed structure.

Another coupon coated with polyaminoamide was kept close to copper anode (impressed system) so as to receive copper iron environment. There was no growth absolutely of any kind. The same specimen is again kept for prolonged period in calm sea (without impressed current and natural dissolution) to find the effective dose of copper ion requirements. These studies are in agreement with both the reports mentioned in literature although conclusions can be drawn from our continuing samples in sea.

CONCLUSION

Two important conclusions are drawn from several pictures in the video.

1. One can clearly see bright metal exposed (paint removed) at the centre bottom of these micro volcano like structure. If the structure are demolished and the coupon again kept in sea water further growth tend to form on or near the demolished structure. Such preference of location may be due to availability of free corroding metal surface and Iron ions and the lack of porosity in paint elsewhere.

   This explains wherever porosity (micropore) was available on dry film or where the tiny microbe could make its way through the micro capillary and damage the paint until they reach the bare metal surface.

   In a chemical plant steam, or salt could penetrate as long as the pore are direct and is not zig zag or crooked and could be blocked and osmosis is in selective spots. But microbes being extremely small and active and lively could swim through criss cross in the micropore until they make their way to the basis metal.

P.T.O

When live species were photographed under bright illumination they were found to emit some chemical compound (resinous product?). The microbe is always found peeping out in a unidirectional motion while it emits this soap bubble component. This explains why the micro volcanic structure (mainly inorganic and containing calcium carbonate) is soft when it is drawn from the sea and becomes hardened up in air (because of this resinous product) which never becomes soft again even after prolonged immersion in sea water.

This comfirms the view of some of the authors that the bio species when they are sessile emit organic acids which can etch the metal surface (and destroy coatings?) in order the become sessile and the to reinforce their attachment to the surface also produce these resinous product. This is why they could withstand high velocity and pressure conditions as seen in several sea water piping systems.

All these results and recordings are in good agreement with earlier reports. Further study are also underway.

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Analysis by Texas Nuclear alloy Analyzer 4266

Table 1

Analysis of steel sample

Element	--	%
Mo	--	0.00
Ni	--	1.42
Cr	--	0.00
W	--	0.43
Ti	--	0.00
Cu	--	0.88
Fe & C	--	Balance

Table 2

TYPICAL ANALYSIS OF SEA WATER SUPPLY TO ABU DHABI POWER STATIONS

1.ELECTRICAL CONDUCTIVITY AS MICROMHOS AT 20°C	53000 - 55000
2.TOTAL DISSOLVED SOLIDS IN PPM	45357 - 47069
3.pH	8.0 - 8.3
4.TOTAL ALKALINITY AS $CaCO_3$ IN PPM	120 - 125
5.TOTAL CHLORIDES AS Cl' IN PPM	25000 - 26000
6.TOTAL SULPHATES AS SO_4"IN PPM	3400 - 3600
7.TOTAL HARDNESS AS $CaCO_3$ IN PPM	8400 - 8500
8.CALCIUM HARDNESS AS $CaCO_3$ IN PPM	1000 - 1250
9.MAGNESIUM HARDNESS AS $CaCO_3$ IN PPM	7250 - 7400
10.TEMPERATURE	18°C - 39°C
11.IRON CONTENT AS Fe" IN PPM	0.05 - 0.05
12.SILICA DISSOLVED AS SiO_2 IN PPM	0.35 - 0.40
13.ALUMINIUM CONTENT IN PPM Al"'	DETECTED QUALITATIVELY
14.HYDROGEN SULPHIDE CONTENT IN PPM H_2S	0.3 - 1.0
15.APPEARANCE	ALMOST CLEAR OCCASIONALLY TURBID WHEN HIGH WINDS & SAND STORMS ARE PRESENT.

RESUMÉ

La tenue des peintures et protections est normalement vérifiée sur des échantillons soumis à la pulverisation d´eau salée ou par détecteur de porosité. Les revêtements soumis à la pollution d´eau de mer seront détruits plus-tôt que prévu comparativement aux échantillons essayés en laboratoire par simulation, parce que des phénomènes physiques, chimiques et électrochimiques agissent comme ensemble de biodétérioration sur les structures immergées et peintes.

Des relations complexes basées sur la composition chimique de la peinture, ainsi que la porosité et épaisseur de film sec expliquent: Pourquoi et comment des revêtements différents sont endommagés à des niveaux divers par bioespèces. De plus l´action de différentes bio-espèces endommage les revêtements.

Des facteurs additionnels tels que les protections cathodiques, la chloration, les dosages chimiques influencent le type et la quantité des organismes provoquant la détérioration, ainsi que la cinétique des réactions de corrosions.

Des enregistrements video de quelques espèces actives accompagnées de leurs produits biologiques apportent quelques lumières sur l´efficacité des revêtements anticorrosion en conditions immergées.

Fig.1. High build epoxy (Aluminium paste pigmented) 95% solids
D.f.t. 150 m.
45 days in polluted sea water
Magnification: X 0,5

Fig.2. Paint system: Amine adduct cured epoxy (50% solids)
d.f.t.: 150–200 µ.
Magnification: X25
A and B are similar to in Fig. 1 magnified. C- copper ring allowed to leach in sea water.
Figure shows bio grown even in proximity of copper ions (trace).

Fig.3. Paint system: Polyamide epoxy with selective pigments (95% solids)
d.f.t.: 200 µ. Magnification X200
Reproduction: X300
As in Fig. 1 or 2 enlarged. Fan like foundation at the bottom of the volcano.

Fig.4. Paint system: Epoxy amine adduct type (65% solids)
d.f.t.: 200 μ.
Magnification: X300
Description: As in fig. 1 etchings on pain by bio (possible-acid reaction) producing a foundation for superstructure.
Bare Shining metal seen at the centre of the fan, showing bio deterioration of paint.

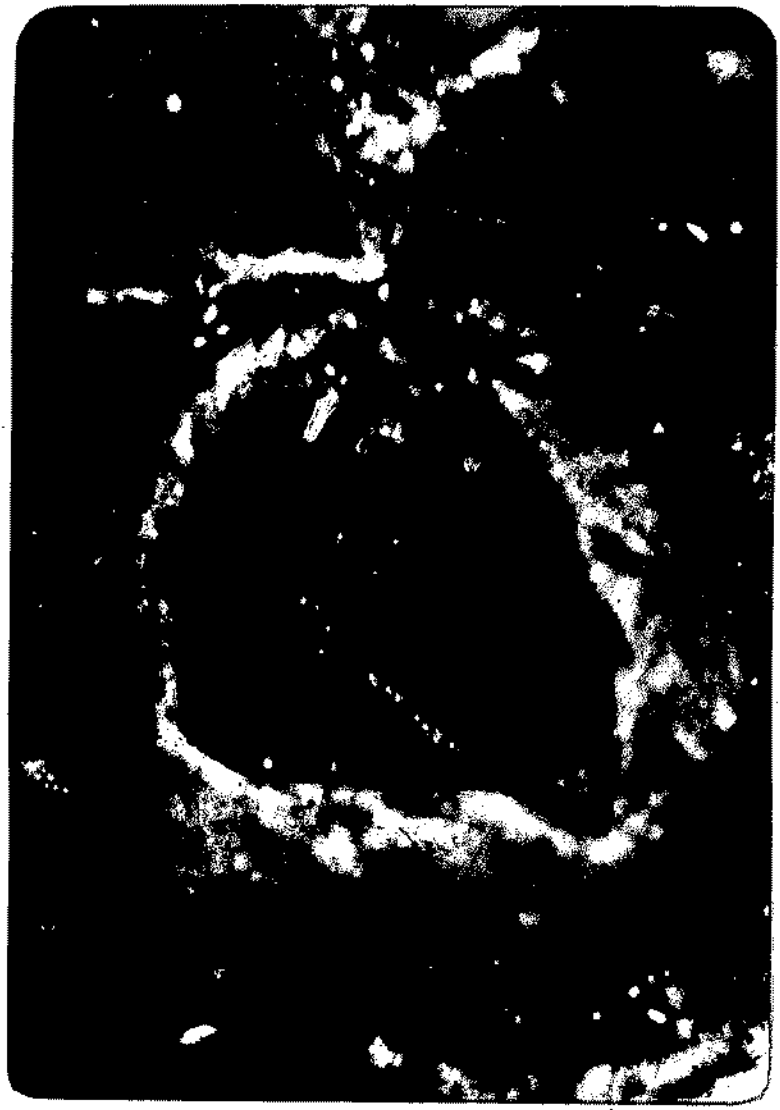

Fig. 5. Paint system: Epoxy with bleached tar (65% solids).
Dry film thickness (d.f.t.: 200 μ.
Magnification: X200
Description: Cross bar protection of entrance of cavity possibly dead bio species can be seen at the bottom of cavity.

ANTIFOULING PAINTS - TODAY AND TOMORROW

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The main function of an antifouling paint is to prevent settling of fouling organisms on the outer hull of ships. Fouling roughens the hull, resulting in increased friction with water, which leads to either an increased use of fuel or to a loss of speed.

The seventies saw the introduction of selfpolishing antifouling paints. These are based on organotin copolymers, which, unlike previous paints, release the biocides in a controlled manner.

One major disadvantage of the selfpolishing paints was their relative higher price compared with conventional and long-life antifoulings. Organotin copolymers with reduced tin content are now under development and their usage should render the selfpolishing paints even more competitive.

The problems of fouling and roughness are examined, alongside with the present choices in antifoulings and their future trends.

PEINTURES ANTI-SALISSURES: AJOURD'HUI ET DEMAIN

La fonction principale d'un antifouling est de protéger les carènes contre les salissures. Les rugosités crées par ces salissures augmentent la résistance à l'avancement ce qui se traduit par une augmentation de la consommation en combustible ou une perte de vitesse.

Les antifoulings autopolissants sont apparus sur le marche dans les années 70. Ils sons à base de résine copolymer et de sels "organoétains", les sels d'étain

s'échappant régulièrement au cours de l'usure de la résine copolymer.

L'inconvénient majeur des peintures autopolissantes était leur prix relativement élevé par rapport aux antifoulings conventionnels et longue durée. Les pourcentages de sels d'étain/résine copolymer nécessaire et suffisant pour obtenir le resultat voulu sont maintenant maitrises et ceci permet aux antifoulings selfpolishing d'être plus que competitifs.

Nous pouvons dire que la gamme actuelle d'antifoulings permet de résoudre les problèmes d'antisalissures et de rugosités de carène.

1. Introduction

The origin of life, neatly explained by Darwin, was a complex development starting from unicellular organisms. The oceans were an intrinsic part of this evolution, and are now bursting with life, from the biggest whales to the smallest bacteria.

Some of the multitude of species populating the oceans are quite happy to swim or to be carried about by currents, but many have to find a hard surface to attach to in order to fulfil their life cycles. Every available space is contested and covered by a variety of barnacles, algae, bacteria etc.. Ships' bottoms are also exposed to this potential settlement of plants and animals, commonly called "fouling".

Antifouling paints have been, for many years, the major way of protecting ships from fouling. As evolution itself, from a fairly simple and humble start, they were developed to the highly sophisticated systems of today.

This paper aims to present highlights of this development and speculate on the future.

2. The Problems

2.1 Fouling

Fouling can be classified into two groups, according to the potential size of the fully grown specimen:

- macrofouling, which includes animals (barnacles, tubeworms, hydroids, molluscs, etc. and plants (green algae, brown algae, etc.)
- microfouling, which includes bacteria and diatoms.

The true extent of fouling capacity was clearly demonstrated by Zobell[1], who immersed a non-toxic 1-inch^2 plate in the sea and counted the number of adhering organisms after 24, 48 and 96 hours. After 24

hours the bacteria population was almost 2 million, rising to about 78 million after 96 hours. The diatom count increased from 940 to over 8000, over the same period. An increase in numbers was also noted for protozoans, larvae of barnacles and other organisms.

The result of such an "assault" can be quite discouraging and figure 1 presents an underwater picture of a fouled hull. On this occasion the antifouling paint was inefficient, numerous barnacles, tube worms and weeds having settled. Details become more apparent in a close-up picture (figure 2).

Figure 1: Underwater picture of a fouled hull.

Figure 2: Close-up picture of fouling

Crisp[2] estimated that the number of known species involved in fouling was in the region of 4,000-5,000. Most other forms of pest control deal with specific animals or pests, but antifouling paints must function against such a variety of species!

All chemicals are potential biocides, if administered in large enough doses to interfere with the metabolism. A biocide is a chemical substance which can inhibit growth or "kill" at very low release rates, of the order of micrograms cm^{-2} day^{-1}.

One of the common antifouling biocides much used in the past and still successfully employed today is cuprous oxide. Most of the classical work has been carried

out on the biocidal activity of copper ions.

Mussels (mollusc family) are relatively easy to control, as little as 1-2 μg $cm^{-2}day^{-1}$ being sufficient. They attach by thin threads called byssus, which are fairly weak and rupture on ships in motion. Their presence on laid up ships is a clear indication of the inefficiency of the antifouling system.

At least 10 μg cm^{-2} day^{-1} copper ions are required for the control of barnacle larvae[3]. The common barnacle, known as the acorn barnacle, is mainly encountered near shores, and like most fouling species, attaches to ships when stationary.

Another species of barnacle, the Gooseneck barnacle, occuring 20-30 miles offshore, is creating problems on slow steaming oil tankers. Once settled, it develops a long neck, lifting away the barnacle to a distance where the released biocide will not harm it. Even if it did, its body will remain attached to the surface by adhesives and physical removal would be the only solution. This is, in fact, true for most fouling species, biocides being needed to "kill" at settlement stage.

Continuing in the brief review of the more important types of fouling organisms, one common algae often found on ships is Enteromorpha, also known as "green grass". Christie[4] showed that settled spores of Enteromorpha are more resistant to toxic action than swimming spores, due to the formation of a thick cell wall around the naked spore, making it difficult for the biocide to enter the cell. Another interesting feature is that re-attachment of fragments of Enteromorpha can occur. This is relevant, especially with respect to scrubbing of existing fouling. Cleaning can create millions of fragments, which can settle yet again, with even greater tenacity.

Slime, a complex mucilageous mixture of bacteria and diatoms, is far more difficult to control by copper alone, needing a release rate of 20 μg cm^{-2} day^{-1} [3]. Particular diatoms like Amphora and Achnantes require even higher rates. Achnantes is commonly found even on highly toxic substrates, containing organotin biocides[5]. Due to its stalked appearance, forming ribbons of cells, it physically rises itself above the attachment surface, rendering the biocides useless.

As primary bacterial films develop within hours of immersion, they were considered to be essential for the settlement of other fouling organisms, but this was disputed by Crisp and Ryland[6]. Data collected by Kirchman and Mitchell[7] indicated that carbohydrates produced by such bacterial films induce larvae of invertebrates to settle, due to the affinity of the carbohydrates to proteins produced by larvae.

2.2 Roughness

Hull design is of paramount importance, as once built, nothing can be done to improve its frictional resistance. However, proper underwater hull management can positively contribute in controlling roughness.

The hull roughness may be divided into two main groups, permanent and temporary. Permanent roughness includes welding seams, valve openings and bulging plates, whilst temporary roughness is the result of corrosion, flaking of old paint, porosity of spent conventional and long-life antifoulings, bad workmanship in the application of the paint, mechanical damage incurred during service and fouling.

A rough hull surface is subject to increased friction, compared with a smooth hull. This leads to an increased use of oil or to a loss of speed.

3. Present Choices

Antifouling paints can be classified into 3 categories: conventional, long-life and selfpolishing.

3.1 Conventional

Conventional paints, also known as 'soluble matrix" paints, have been used for many years. The main binder is rosin, a natural product, which slowly dissolves in sea-water due to its carboxylic acid groups. Rosin is brittle, but despite its plasticisation with various additives, it forms fairly weak films and paints can only be applied in relatively low total dry film thicknesses.

Current biocides used in such paints include cuprous oxide, although in the past, biocides based on lead, mercury and arsenic have been used. Needless to say, such biocides are no longer environmentally acceptable.

The copper release rate is high (figure 3) on immersion in sea-water. As the biocide floods out of the paint film, the rate drops quickly under the 10 μg $cm^{-2}day^{-1}$ (dotted line) required to control macrofouling[3]. Consequently, effective protective life of such paints is short, between 6 and 12 months.

3.2 Long-life

Long-life antifouling paints are also referred to as "insolube matrix". The binder is no longer soluble in seawater as in the case of conventional paints, and as only biocides are released, it is left behind as a porous skeleton.

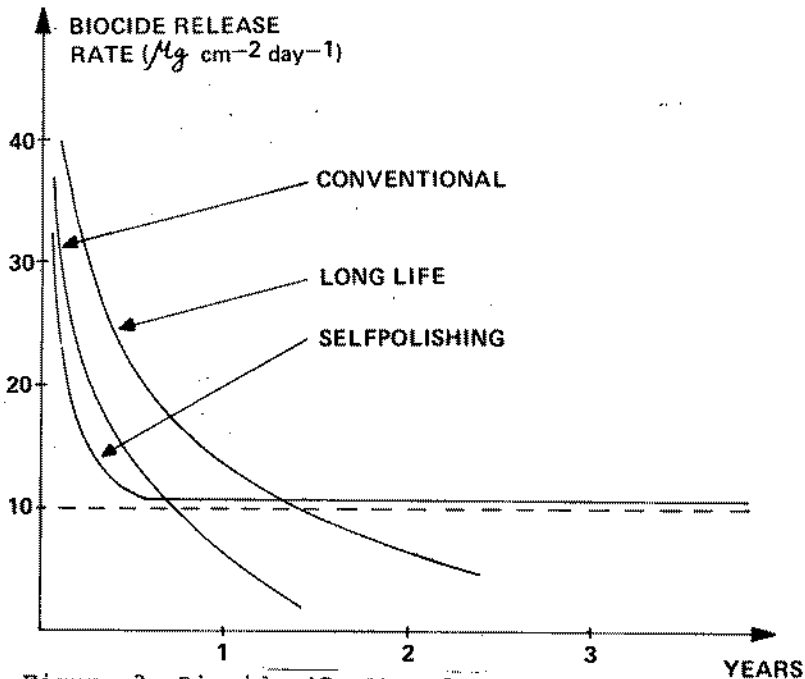

Figure 3: Biocide (Cu_2O) release rate comparison for various types of antifouling paints.

The release rate is exponential and decreases with the increase in the porous layer. Eventually this layer becomes thick and clogged up with fouling and/or insoluble salts. No more biocides can be released and performance drops dramatically (figure 3).

At every drydocking, having removed the fouling infestation, a sealer coat is advisable in order to create a better substrate for the new antifouling. This means that the biocides still present in the film are irrevocably lost, wasting valuable money.

Commonly used biocides include copper oxide and organotin compounds, in different concentrations. Main binders employed are vinyl and acrylic resins and chlorinated rubber.

Effective protective life time varies between 18 and 24 months.

3.2.1 Sandwich Coatings

Conventional and long-life antifoulings have, for a long time, been the only choice for antifouling protection. The porous film left after the biocides leach out presents a weak substrate for the newly applied

coatings. This, together with the accumulation of paint year after year, coat after coat, can give rise to detachment, thus increasing the roughness of the hull. This paint accumulation is also referred to as "sandwich coatings" and presents the most serious cause of increased roughness.

Figure 4 exemplifies a comparison between an ideal situation with perfectly smooth surface and a likely situation, with high roughness. Figure 5 is a typical example of a rough hull due to peeling and flaking.

It is estimated that the average hull roughness increases by about 30 microns per year, although variations from 10 to 40 microns per year have been reported[8].

Although the relationship between hull roughness and oil consumption is complex, it has been estimated that a 10 microns increase in the average hull roughness causes a 1% increase in oil consumption, for ships with an average hull roughness not exceeding 250 microns, and a 0.5% increase for ships exceeding 250 microns.

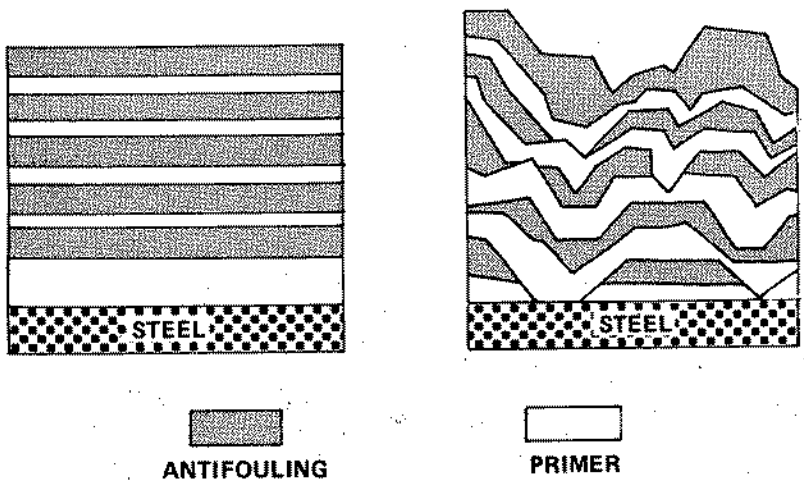

Figure 4: Sandwich coatings after 4 drydockings. Comparison between ideal(left) & real(right) situations.

It is, therefore, important to minimise the increase in hull roughness and selfpolishing paints do that, in addition to providing antifouling protection.

An additional feature of selfpolishing paints is their ability to "smooth". Smoothing is the result of differential polishing rates for peaks and valleys, because the turbulence created by the flow of water is higher on peaks.

Figure 7 illustrates that, after a period in service, the original profile "A" became smoother (profile "B") with the first coat showing in places. In practice alternate selfpolishing coats are of different colours and patterns as those in figure 8 are often observed.

Smoothing is measured by monitoring the profile and the British Ship Research Association (B.S.R.A.) hull roughness gauge is widely used in drydocks.

Briefly, the following advantages are associated with selfpolishing paints:

- predictable performance
- extended drydocking period
- control of roughness and smoothing
- no "sandwich coatings" problems
- fouling control due to linear biocide release rate
- lifetime directly proportional with dry film thickness.

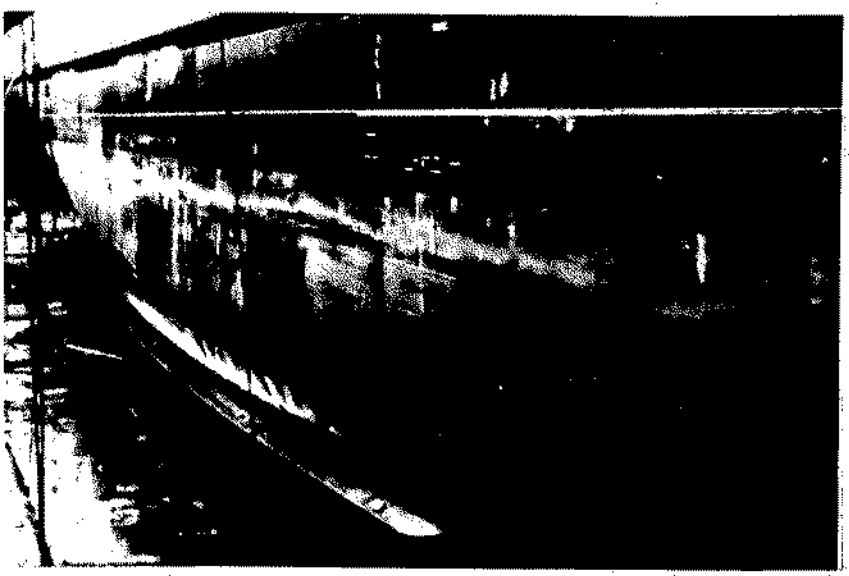

Figure 8: Smoothing observed on ship in drydock.

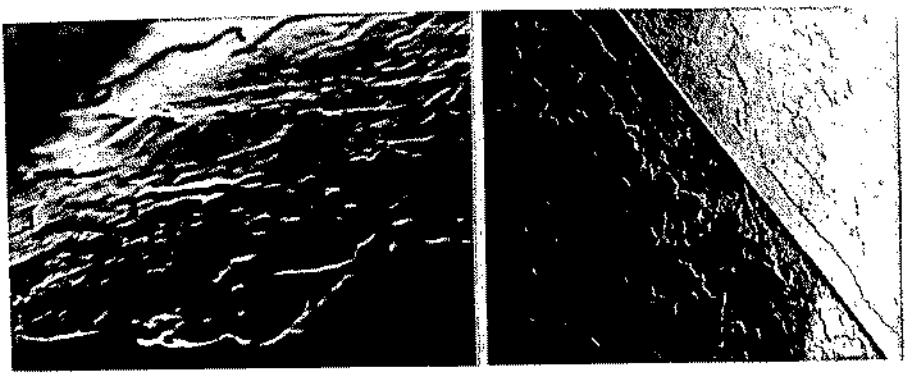

Figure 5: Roughness due to peeling and flaking.

3.3 Selfpolishing

Controversially introduced in the early seventies, selfpolishing paints have been the subject of many articles, patents and reviews.

The major difference with respect to earlier antifoulings is the chemically bound biocide, which is released following hydrolysis in sea-water. Once the biocide is released, as shown in figure 6, the backbone of the polymer becomes water-soluble due to formation of sodium and potassium salts and slowly dissolves and/or is washed away. This surface action is predominant, layer after layer providing the same good and predictable antifouling performance.

Current commercial selfpolishing antifoulings are based on polymers with chemically anchored organotin biocides. As the tributyltin radical is hydrolysed off (figure 6), it reacts with the chloride ions from sea-water to form tributyltin chloride. The linear release of this biocide is responsible for the excellent antifouling performance observed with selfpolishings.

Extra biocides, like cuprous oxide are often used as external boosters. The selfpolishing effect ensures its linear release rate (figure 3), once equilibrium is reached after immersion.

Other common biocide boosters include cuprous thiocyanate, tributyltin fluoride, triphenyltin fluoride and hydroxide, etc..

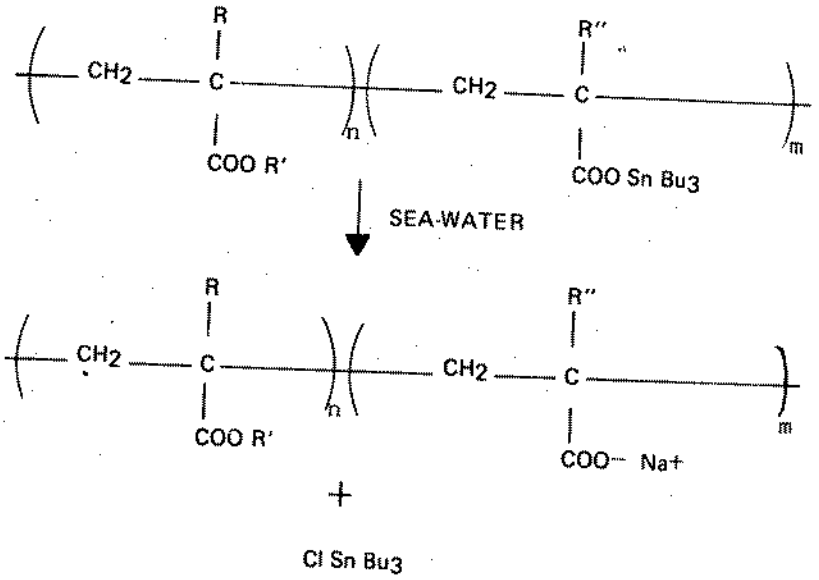

Figure 6: Hydrolysis of organotin polymers.

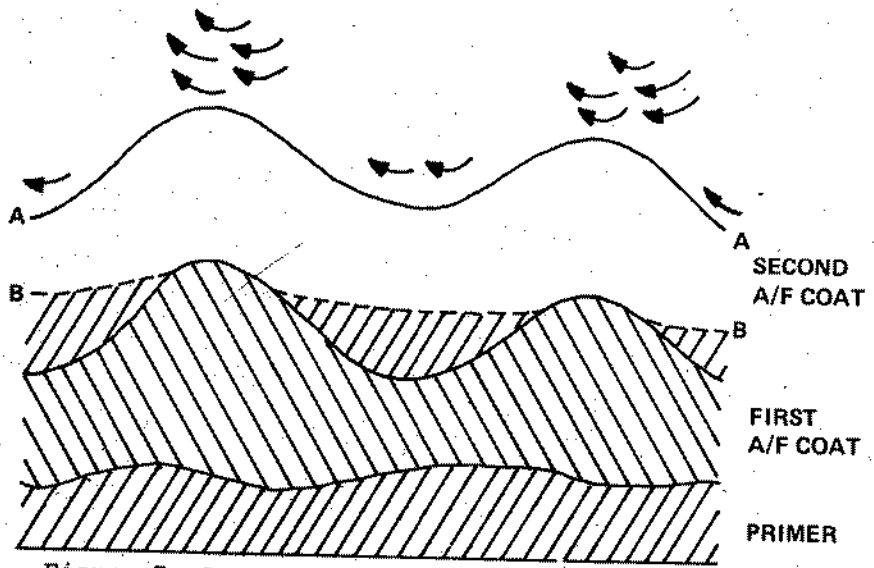

Figure 7: Cross-section of paint system exemplifying smoothing. Profile "B" smoother than "A".

4. Current Research Efforts

The last few years have seen increased research commitments to develop the future generation of antifouling paints. These efforts cover both biocides and polymers.

4.1. Biocides

Some of the most potent biocides, for example organo-lead and organo-mercury, with a wide spectrum of fouling control, had to be abandoned due to health hazards.

As early as 1943 certain organotin compounds had been pinpointed as antifouling agents. The first systematic investigation, carried out by Van der Kerk and Luijten in 1954, highlighted the fungicidal properties of 40 organotin compounds[9].

Intensive work followed and today many organotins are used frequently in antifouling paints, due to their wide spectrum at very low release rates ($1\mu g\ cm^{-2}day^{-1}$)[3]. New organotins are still reported in the race to find a new super-biocide.

It is reasonable to assume that the discovery of such a biocide is unlikely, given various environmental restrictions, not to mention the variety of species such a biocide would have to control.

The future paints would probably contain cocktails of biocides, each giving protection against particular forms of fouling.

Synergism is an important feature to consider, as mixtures of biocides may or may not give an overall improvement over the individual biocides.

Doi et al.[10] reported enhanced antifouling efficiency for selfpolishing paints containing dithiocarbamates. These biocidal compounds, together with the organotin released by the copolymer provide much better protection against slime.

4.2. Polymers

When Montermoso et al.[11] reported, in 1958, the preparation of tributyltin methacrylate and acrylate, as well as their homopolymerisations, they expected to obtain "organometallic elastomers having thermally stable and chemical resistance properties". It is ironic that those monomers have found an extremely useful application due to their chemically labile nature. This is indicative of how new technologies can and often do appear.

The last 25 years have been prolific in research related to organotin antifoulings. Some good reviews have been published [12], [13], [14] but a thorough analysis is beyond the scope of this paper.

Current research interests seem to be directed mainly towards selfpolishing copolymers, although some work is still progressing on non-polishing resins.

Sghibartz [15] reported selfpolishing paints based on copolymers with lower than usual organotin content, including tin-free, based on triorganotin monomers and/or methyl or ethyl acrylate. Alternatively, triorganotin monomers and/or quinolinyl acrylate or methacrylate could be employed[16].

Yamamori et al.[17] described hydrolysable polyester resins obtained by the reaction of a polycarboxylic acid with a hydroxycarboxylic acid salt. Paints based on such polyesters, for example a copper-12-hydroxystearate - 1,6- hexanediol - phthalic anhydride copolymer, are said to be subject to "erosive dissolution". The same authors also suggested the use of 2-hydroxyethyl (meth)acrylate as another means of reducing the tin content in polymers used in selfpolishing paints[18].

5. Future Selfpolishings

The latest developments in organotin copolymers technology made possible the recent introduction of high build or high solids selfpolishing antifoulings.

Such paints can be applied in dry film thicknesses of up to 150 microns per coat. Apart from the usual benefits associated with selfpolishings, high build paints offer the extra benefit of fewer coats for a given total dry film thickness. Consequently application costs are lower, drydocking time is reduced and longer drydocking intervals are possible.

The potential long term savings comparative to the long-life antifoulings are impressive. In an example, starting with a newbuilding and aiming for a 9-10 year protection, the long-life systems would require 5 drydockings (total of 15 coats i.e. 5 sealers and 10 long-life antifoulings) whilst when using a high build paint only 2-3 dry dockings are necessary and a total of 3 coats (figure 9).

In separate developments, some organotin selfpolishing copolymer paints have been designed for application directly on long-life antifouling paints without a sealer coat and without expensive surface preparation.

One coat of 150 microns dry film thickness can provide protection for 24 months.

Selfpolishing paints, having already demonstrated

their worthiness, were the subject of some controversy in some recent articles[19, 20]. The main drawbacks stated included relatively high prices and expensive surface preparation.

However, as indicated above, it appears that self-polishing paints are becoming even more competitive with traditional systems and their share of the market will inevitably increase.

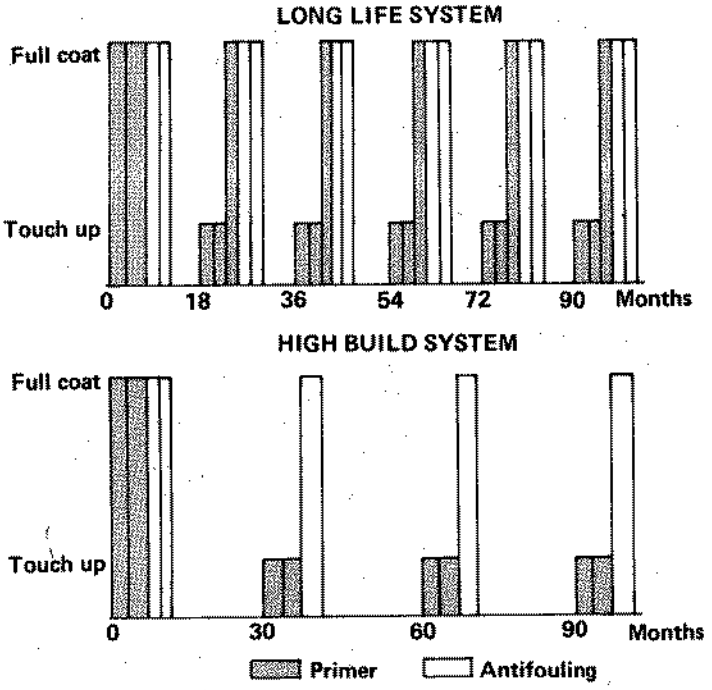

Figure 9: Economical comparison between traditional long-life and high build paints, over 9-10 year period.

6. Fouling Release Coatings

All antifouling paints, despite different mechanisms of action, have one common feature: the use of biocides in order to control fouling.

An elegant and environmentally welcomed development for the future could be the appearance of "fouling release" coatings.

These could be non-toxic surfaces to which fouling organisms could not become attached, for example because of low surface energy.

Krøyer[21] reported coatings based on vulcanised silicone rubber, substantially free from toxic compounds, on which fouling did not attach or become lightly attached and could be easily removed. When the fouled panels were exposed to a rotor test at 30 knots, the fouling washed off. The problem encountered included non-adherence of some of the coatings to the substrate. As mechanical damage is common on submerged objects, especially ships, maintenance may be difficult, as the newly applied coats may not adhere to the existing system.

Griffith and Bultman[22] incorporated powdered Teflon (poly(tetrafluoroethylene)) into fluorinated polyurethane. The resulting coating was used with some success on a tug boat, but more work is necessary to achieve a commercially viable product, long term protection still being out of reach.

Same limitations were reported by Ghanem et al.[23] for coatings made of cellulose acetate and silica-bearing methyl siloxane resins.

An original approach was postulated by Gregor & Gregor[24] and related to membrane technology: as most fouling materials also bear a negative electric charge, a surface negatively charged may discourage microorganisms from attaching. Sulphonate membranes are given as an example: their fixed negative charges generating an electric field.

The "fouling release" concept, still in its infancy, may perhaps be developed to provide the antifouling paints of the future, from new, original approaches or perhaps from the development of the above efforts.

7. Conclusions

The last decade has shown a technological breakthrough in the antifouling field. As research work is intensifying, there is optimism regarding new developments.

The future looks interesting and challenging and will require strong inputs from chemists, paint technologists, biologists, environmentalists etc.

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MICROBIOLOGICAL CORROSION
CORROSION MICROBIOLOGIQUE

MICROBIOLOGICAL CORROSION IN WATER DISPLACED SHIP FUEL TANKS

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ABSTRACT - The corrosion of steel in a water-displaced ship-board fuel tank contaminated with bacteria was analyzed. The effect of microalloying, termal treatment and microbial growth in the sea water in service was compared with laboratory tests. Gravimetric and electrochemical determinations enabled us to evaluate the relative influence of the variables affecting the process. SEM and EDAX were used to analyze their effect on the morphology of the attack.

RESUME - On analyse la corrosion d'aciers dans un recervoir de carburant de bateau déplacé par l'eau de mer, contaminé avec des bacteria. L'effect des éléments d'alliage, du traitement termique et de la taux d'accroissement microbienne dans l'eau de mer en service est comparé avec les résultats des essays de laboratoire. L'influence relative des variables qui contrôlent le processus est determiné à travers des pertes de poids et téchniques électrochimiques. Leur effect sur la morphologie de l'attack est etablie par MEB et EDAX.

INTRODUCTION - The microbial proliferation some times observed in shipboard fuel tanks using sea water for displacement or ballast was reported[1,2] as the cause of metallic corrosion. The sulfur compounds produced by metabolic reduction of sulfate by anaerobic bacteria accelerate corrosion in the storage and fuel circuit. Other inconvenients associated to the biological growth are filter blockage and fuel sour.

The treatment applied when sulfate-reducing bacteria are detected in the tank is the addition of sodium cromate up to a concentration of 0.10 to 0.15% in the sea water[1,2,3]. Even when it stops the microbial proliferation and inhibits steel corrosion its use produces marine pollution[4] during the refueling operations.

One of the greatest inconvenients reported for the study of this subject is the difficulty to maintain viable anaerobic bacteria cultures in laboratory in spite of its prolific activity under field conditions[1,2].

EXPERIMENTAL - The tests were performed on a microalloyed steel Cor Ten B (C 0.14; Mn 1.00; Si 0.24; S 0.011; P 0.020; Cr 0.43; Cu 0.29; V 0.003) and its quenched and tempered form Cor Ten B-QT (C 0.14; Mn 1.13; Si 0.27; S 0.019; P 0.018; Cr 0.47; Cu 0.31; V 0.004) being SAE 1010 used as witness.

Inmersion in the sea water phase of an infected displaced fuel tank in a ship was compared to inmersion and electrochemical laboratory tests. Microbial viability in the tank was determined by means of API reagent in a Gaspak anaerobiosis jar. The activity of sulfate-reducers was evidenced by a black precipitate when hydrogen sulfide is produced in the presence of the ferrous ammonium sulfate which acts as its indicator in the medium. The negative results indicated non-viability.

The laboratory inmersion test in sea water was done in the presence and absence of fuel to establish its effect when the oxygen access is limited under the fuel layer.

Polarizations of the microalloyed steels were performed in uncontaminated and infected natural sea water. This last was extracted from the same ship fuel tank where the test in service conditions was done and metabolites could be present.

A Tacussel PRT 20-2X potentiostat was used applying a potential scan of 2 $mV.min^{-1}$ which produced the same results to the obtained during potentiostatic tests. A conventional Pyrex glass cell with a Pt counter electrode and a saturated calomel electrode, through a Luggin capillary, as reference were used. Deaereation was done by 99.99% N_2 bubbling and oxygen saturation with air. Magnetic stirring at different intensities was applied to evaluate the effect of agitation on the cathodic oxygen reduction reaction. Also static aereated curves were traced.

The corrosion products were eliminated for the loss weight determination and morphologic observations. The picking solution was 50% HCl containing 1% urotropine as iron dissolution

inhibitor.

For the SEM analysis a Philips 505 with EDAX and Jeol JSM-25-S II microscopes were used.

RESULTS

Inmersion tests - The loss in weight did not show evidence of differences in the corrosion rate of the microalloyed steel due to its terminal treatment.

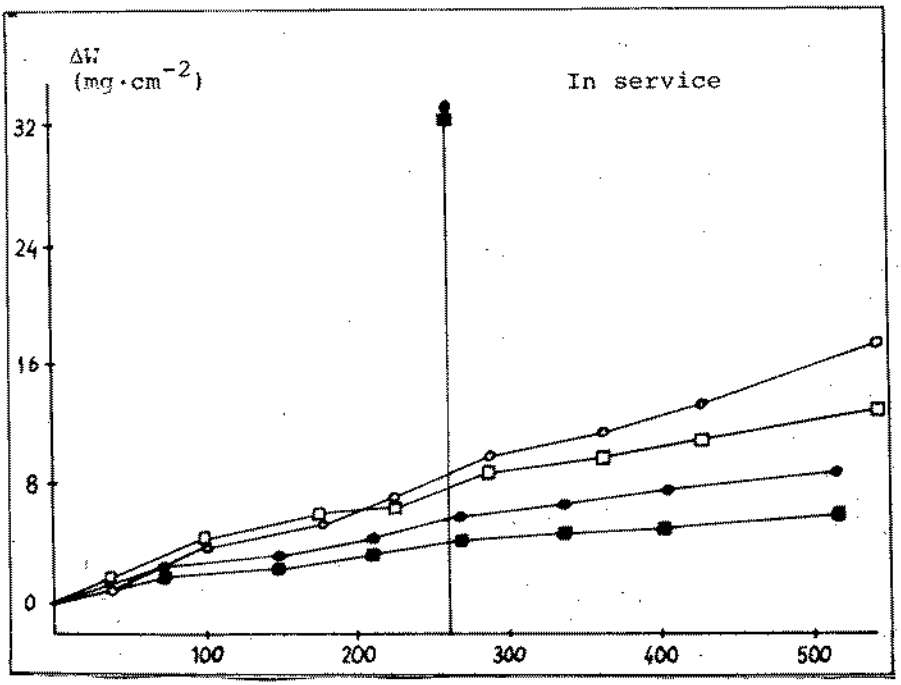


Fig. 1: Weight loss of steels as a function of time of inmersion in sea water ○ Cor Ten B and B-QT, □ SAE 1010; ● ■ Idem, under fuel.

No benefical effect was appreciated of the microalloying elements. The greatest differences were observed in the morphology of attack of the distinct steels having been reproduced in laboratory the type of damage observed in service. In this late the loss in weight is 4 to 6 times the produced in the laboratory inmersions in limited and free access of oxygen conditions, as it is summarized in Table I.

TABLE I : CORROSION RATES OF COR TEN 8-QT AND LOSSES IN WEIGHT FOR 262 DAYS TESTS.

TYPE OF TEST	MEDIUM	SEA WATER	pH	i_c (A. cm^{-2})	ΔW (mg.cm^2)
ELECTROCHEMICAL	STERILE	Deaereated, non-stirred.	7.8	7×10^{-6}	46
		Aereated, non-stirred.	7.8	1.6×10^{-5}	105
		Aereated, stirred	7.8	4×10^{-4}	2,620
	INFECTED	Deaereated, non-stirred.	7.75	9×10^{-6}	59
		Aereated, non-stirred.	7.75	4×10^{-5}	262
		Aereated, stirred	7.75	6×10^{-4}	3,930
Inmersion in laboratory	STERILE	Oxygen limited, non stirred	7.8	----	5
		Free oxygen access, non-stirred.	7.8	----	8
Inmersion in service	INFECTED	Oxygen limited, stirred.	7.75	----	33

Electrochemical tests - Polarization curves of 8 and 8-QT Cor Ten steels are shown in Fig. 2. Very similar behaviour was found in sterile as much as in infected sea water, having greater corrosion rates the termally treated steel than the non treated one. The corrosion currents and ΔW for distinct conditions allowing the evaluation of the variables controlling the process in service are included in Table I, for Cor Ten 8-QT steel.

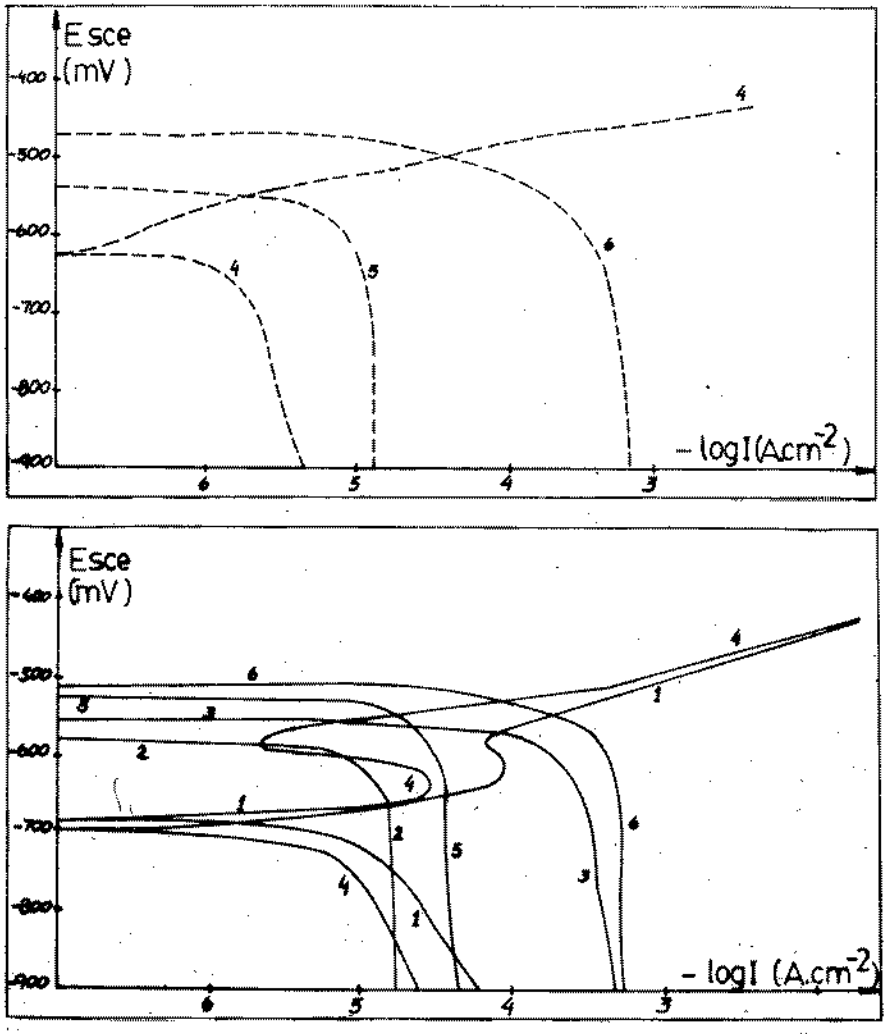


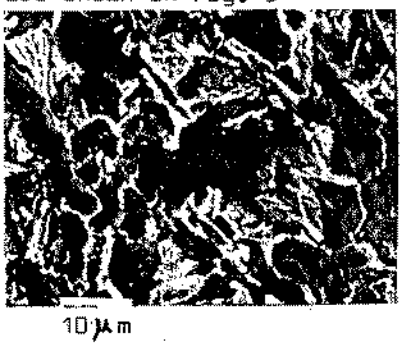
Fig. 2: Polarization of steels in sea water --- Cor Ten B,
—— Cor Ten B-QT.

Sterile	1) Deaereated, non stirred. 2) Aereated, static 3) Aereated, stirred	Infected	4) Deaereated, non-stirred. 5) Aereated, static 6) Aereated, stirred

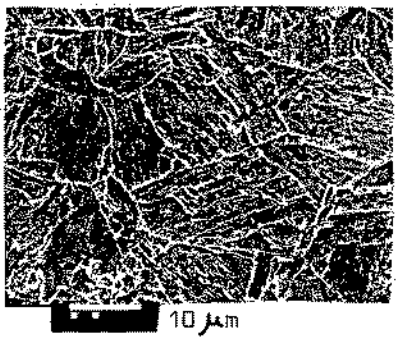
The oxygen reduction produces higher limit currents in stirred sea water than in static one being enhanced the differences due to the termal treatment of the steel in the aereated static tests. On the B-QT steel higher i_L are measured.

In the absence of oxygen the main differences are due to the microbial contamination which reduces the cathodic currents and slightly modifies the anodic curves. No changes were found in the rupture or pitting potential between sterile and contaminated sea water. In order to understand the discrepancy of this result with a previously reported[5] for steels in the same contaminated medium polarizations in 10^{-2} M Na_2S in sea water were done. The values of Ep in fresh solution were in good agreement with those reported, but they changed with time by $S^=$ decomposition, tending in few days to the values obtained in non-contaminated sea water. That would explain the results determined in the water taken from the fuel tank two weeks before to its reception and use in our laboratory. The drop in its $S^=$ content would have cancelled the decrease in the pitting potential of the steels with regard to that obtained in sea water free of $S^=$.

Morphological analysis - Microstructures of both Cor Ten steels are shown in Fig. 3



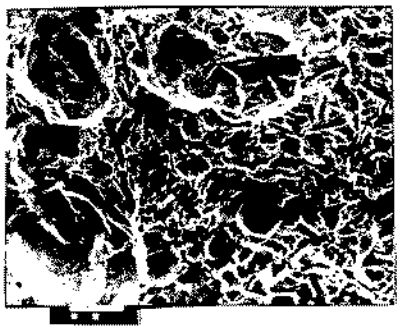
a) Cor Ten B



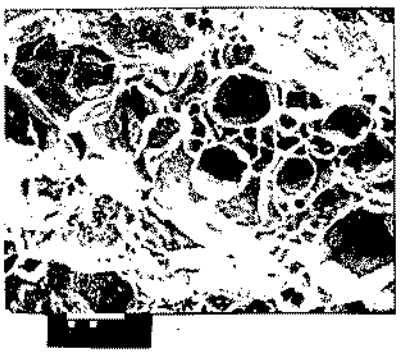
b) Cor Ten B-QT

Fig. 3: Microestructure of the steels.

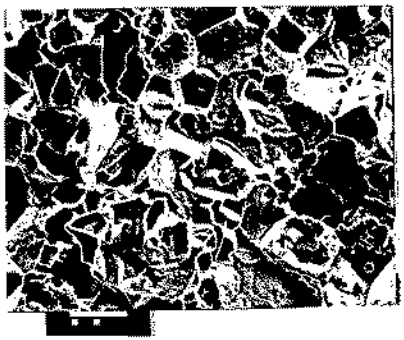
The samples submited to inmersion in service were observed in SEM after pickling. An intense attack is shown in Fig. 4



a) Cor Ten B



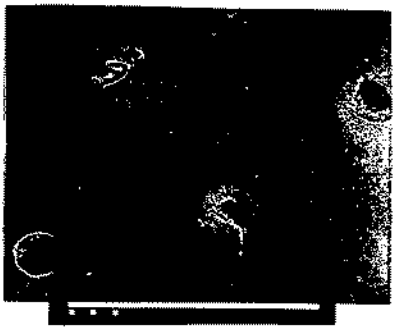
b) Cor Ten B-QT



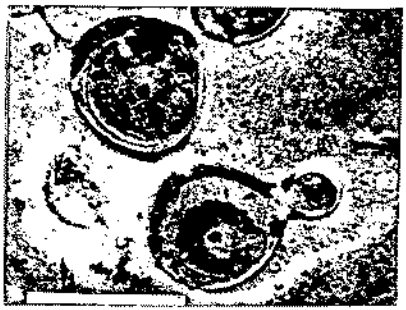
c) SAE 1010

Fig. 4: SEM of steels after inmersion in sea water, in the fuel tank, during 262 days

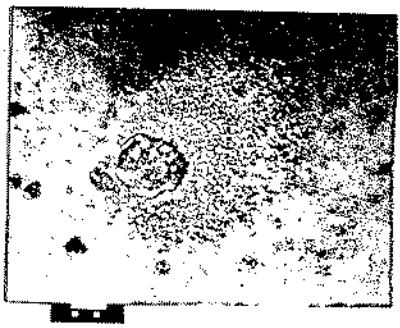
Those polarized were observed with and without the corrosion products in order to analyze the initial stages of the corrosion process. It was electrochemically induced by application of pulses, at -450 mVsce, during different times. It began at non-metallic inclusions and through blistering of the surface, analogously to previous reports for the initiation of the atmospheric corrosion of steels[7,8,6], as can be seen in Fig. 5



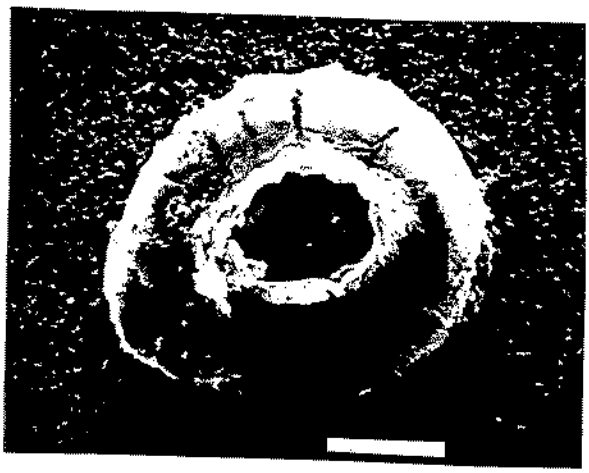
100 µm a)



100 µm b)

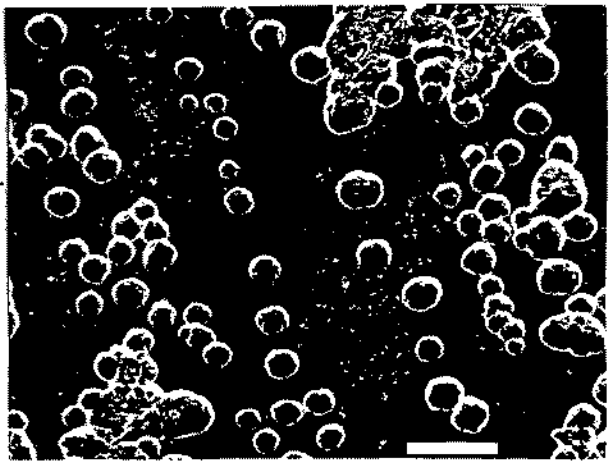


10 µm c)



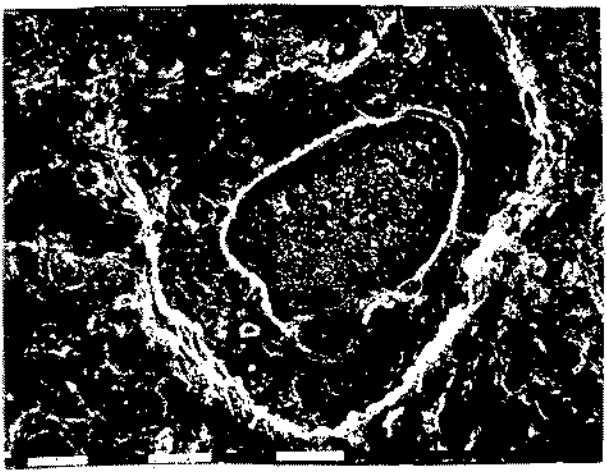
10 µm d)

e) 600 seconds. Pitting distribution and its coalescence



10 μm

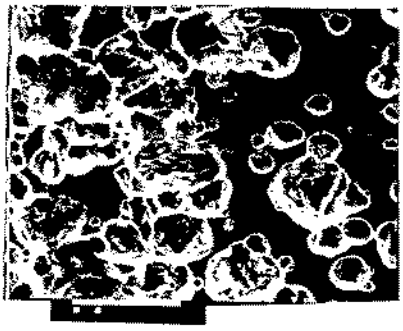
f) Anodic polarization up to -400 mVsce. Blistering under blistering.



20 μm

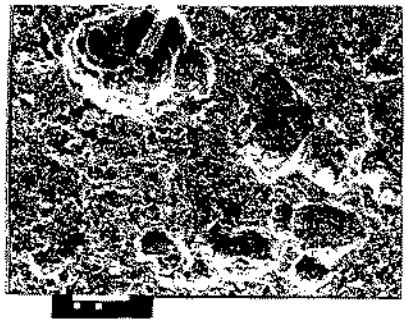
Fig. 5: SEM of the steels surfaces after anodic pulses at - 450 mVsce and polarizations in the contaminated medium. a), b), c) and d) 180 sec. Initiation at non-metallic inclusions and blistering.

The distinct morphologies were found for each steel after pickling as shown in Fig. 6.



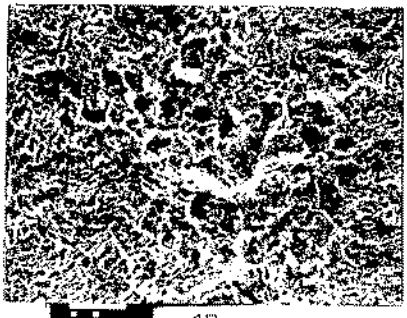
10 μm

a) Infected, Cor Ten B



10 μm

b) Infected Cor Ten B-QT



10 μm

c) Sterile, Cor Ten B-QT

Fig. 6: SEM of steels after polarization in sea water

The magnitude of the attack dependes of the contaminants of the medium including the oxygen and bacteria.

The techniques applied reproduce the damage occurred in service. Deepest pits were observed in the infected medium, as can be seen in Fig. 6.

This results allowed us to extend the field of the previous findings[7,6] because the nucleation seems to be independent of the structure, perlitic-ferritic of the hot rolled Cor Ten B and low C martensitic in the quenched and tempered, after the hot rolling, Cor Ten B-QT.

It was also observed in other metals and alloys during atmospheric corrosion[8]. Other electrochemical attacks initiates through surface blistering of the metal , matrix dissolution around non-metallic inclusions , and selective attack due to the presence of secondary phases[9] .

The influence of the termal treatment is evident form Figs. 4 where the cementite distribution seems to determine the morphology of the attack. The termally treated alloy suffers a more uniform damage than the non-trated one, even when the electrochemical results showed greater corrosion rate for Cor Ten

B-QT than for the other steel.

DISCUSSION - From the results shown in Fig. 1 for the distinct inmersion tests it can be observed that the samples showed losses in weight after 262 days in the water of the fuel tank at least twofold that for 550 days of static inmersion in non-contaminated sea water in laboratory.

The differences in service are:

- the presence of metabolites
- the ship movements
- the lower O_2 concentration under the thick fuel layer.

In Table I the results of the inmersion and electrochemical tests are summarized to evaluate separately each of the factors producing an acceleration in the corrosion of the steel in sea water in the service conditions.

The last column with ΔW was introduced to express the electrochemical results shown in Fig. 2 in the same terms and for the same period as the inmersion ones.

From the electrochemical results, where the different conditions can be independently modified it was concluded that the greatest corrosive effect is due to the agitation, second the oxygen concentration and in last the presence of metabolites. Each of them affected the corrosion rate of the steel in the sea water in distinct magnitudes according to the other parameters. The following mean values of accelerating factors were estimated:

- From sterile to infected media: 1.8
- From free of O_2 to oxygenated media: 3.3
- From static to energically stirred media: 20

The relative magnitud in the losses in weight after the inmersion tests in laboratory are in good agreement with the effect established for the oxygen from the electrochemical results. Bearing in mind the inestability of the sulfur ions produced by the sulfate reducer bacteria a greater influence of microbial activity could be expected in service than the predicted from the polarization results in the infected sea water used. That would increase the discrepancies shown in the last column of Table I among the electrochemical and weight loss results. The discrepancies, previously reported for other systems[10], could be atributed in our tests to the growth of thick layers of corrosion products during the long term inmersions in the almost static media. They would significantly reduce the eficiency of the oxygen reduction on the steels. Being the corrosion rate determining process the O_2 diffussion its decrease would reduce the loss in weight as compared to that measured during polarizations of the bare metal and then estimated

for the inmersion period (262 days).

Considering the above described limitations the electrochemical results provided appropriate means to evaluate the overall influence of the microbial contamination and gentle agitation of the water in the bottom of the ship, in the absence of oxygen, during the inmersion in service. The agreement was also verified through laboratory inmersions.

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CORROSION OF CARBON STEEL INDUCED BY SULFATE REDUCING BACTERIA. EFFECT OF CHLORIDE AND SULFIDE ANIONS.

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ABSTRACT. The corrosion of mild steel in the presence of sulphides either of inorganic or biological origin is studied. The attention is focused on the initiation of the corrosion process which takes place through the replacement of the passive film already present on the metal surface by a poorly protective film of iron sulphide (mackinawite). Localised attack, preferentially pitting, takes place specially in the defective sites of the passive film. Biogenic sulphides derived from the metabolic activity of sulfate reducing bacteria participate in the corrosion process in a similar way than those of inorganic origin. A reaction sequence interpreting the corrosion process is proposed.

RESUME. On etude la corrosion des aciers au carbon en presence des sulfures soit d'origine inorganique, soit d'origine biologique. La recherche est orientée a l'étude de l'initiation du processus de corrosion qui a lieu par la sustitution de la couche passivante d'oxyde sur le metal par une autre couche de sulfur de fer sensiblement moins protectrice. La corrosion localisée, spetiallement par piqûres, peut être originée sur les defauts de la couche passivante. Le comportement des sulfures biologiques derivées de la activité métabolique des bactéries sulfate réductrices dans le processus de corrosion est resembable a celui des sulfures inorganiques. On formule un mechanisme de réaction pour interpreter le processus de corrosion.

INTRODUCTION

The corrosion of iron and steel in the presence of sulphides and sulphur species has been studied intensively due to its importance in relation to different industrial processes such as those of secondary petroleum recovery, pulp and paper industry, heavy water production by the Gidler process, etc. In many cases the presence of sulphides is due to the metabolic activity of sulphate reducing bacteria (SRB) which grow in anaerobic environments at pH values near neutrality such as those generally encountered in waterlogged clay soils. SRB activity is also frequent in sea water specially in polluted environments like those usually found in the vicinity of harbours and fish processing industry.

SRB produce sulphide anions by the desassimilatory reduction of $SO_4^{=}$ anions used by the bacteria as electron terminal acceptors. In neutral media this metabolic activity leads to the production of mixtures of sulphides and bisulphides anions and hydrogen sulphide.

The role of SRB in inducing or accelerating electrochemical corrosion processes has been interpreted on the basis of different mechanisms; considering a) a cathodic effect due to direct removal of atomic hydrogen by enzymatic activity or indirectly by the production of mixtures of sulphides and hydrogen sulphide; b) an anodic effect by the action of sulphides and other sulphur species or c) a combination of both effects.

In spite of the numerous papers related to the participation of SRB in the cathodic reaction postulated for the first time by the cathodic depolarization theory[1], little has been said about the anodic process or about the initial steps of the corrosion reaction.

It is well known in the literature that iron and steel exposed to sulphur species develop firstly a poorly protecting film of mackinawite (an iron rich iron sulphide) that changes later through different chemical and electrochemical paths to more stable iron sulphides. In all cases these iron sulphides are characterized by its marked cathodic effect on the hydrogen reduction reaction leading to an increase of the corrosion rate indirectly.

The present paper is devoted to the study of the electrochemical behaviour of prepassivated SAE 1020 mild steel in the presence of sulphide species either of biological or inorganic origins by means of electrochemical potentiostatic techniques complemented with scanning electron microscopy. It is our intention to give more information on the first stages of the microbial corrosion of mild steel in the presence of SRB in similar conditions as those generally encountered in practical

cases where the metal surface is prepassivated by a mixture of different oxide species.

EXPERIMENTAL

The working electrodes consisted of 1020 steel rods (5 mm diameter) included in an Araldite holder to obtain a circular exposed area of 0.2 cm^2. The metal surface was firstly polished with a fine grained emery paper and successively with diamond paste, alumina paste (1 μm). Each specimen was then rinsed with acetone, twice distilled water and finally dried in air at room temperature. A new working electrode was used in each run. Phosphate-borate buffer (0.1 M KH_2PO_4 + 0.05 M $Na_2B_4O_7$) pH 8.00 with the addition of $Na_2S.9H_2O$ (10^{-3} M < C_{NaS} < 10^{-2} M) was used as electrolyte. The solutions were prepared with twice distilled water and A.R. chemicals. Previous to the electrochemical experiments the electrolyte solutions were purged with purified nitrogen during 3 hours. Measurements were made in a conventional single compartment Pyrex glass cell at 25 ± 0.1°C. The potential of the working electrode was measured against a saturated calomel electrode (Radiometer K-701). The reference electrode contained a KNO_3 salt bridge to avoid the presence of chloride into the electrolyte and was connected through a Luggin-Haber capillary tip. In the text, potentials are referred to the saturated calomel electrode scale. A platinum wire was used as counterelectrode. Potentiodynamic E/I profiles at low sweep rates (0.02 V/min) were applied to the working electrode in the conventional way. The working electrode prior to each run was held at -1.00 V during 1 min 30 sec to achieve a completely electroreduced surface. Current-time transients at constant potential were also recorded. The potential step applied to the electrode was preceded by an electrode pretreatment including the sequence of potential steps depicted in Fig. 3. Scanning electron microscopy (SEM) observations were made using a Philips 500.

Sulphate reducing bacteria isolated from sea water in contact with navy diesel and sludge of ship storage tanks were used. Samples were inoculated in Baars medium of the following composition: KH_2PO_4 0.5 g; NH_4Cl 1g; $CaSO_4$ 1g; $MgSO_4.7H_2O$ 2g; Sodium lactate 3.5 g; distilled water 1 dm^3. The medium was sterilized by autoclaving at 110°C for 15 min and pH adjusted to 7.0 with 0,1 N NaOH.

0.5 g of ferrous ammonium sulphate was added under aseptic conditions after autoclaving .

Incubation was made at 25°C in anaerobic BBL Gas Pack Jars (N° 60465).

Purity of culture was verified from time to time by microscopic observation of the cell morphology (phase contrast microscopy). Diagnostic by ultra violet fluores

cence test of Postgate was also used[3].

Occasionally electrochemical assays were made with artificial sea water[4].

For the electrochemical experiments inocula from cultures in Baars medium were inoculated in erlenmeyers of 500 ml containing 100 ml of solidified Baars medium (1.5% agar) on the bottom and covered with 300 ml of artificial sea water (pH 8.0). The incubation was performed at 25°C in the anaerobic jars. After an adequate growth was obtained (c.a. 48-96 hs) the supernatant liquid was transferred to the electrochemical cell in a nitrogen atmosphere.

RESULTS

The anodic polarization curve of mild steel at 0,02 V/min in phosphate-borate solution shows a first anodic peak at -0,70 V and a second one at -0,30 V (Fig. 1). The

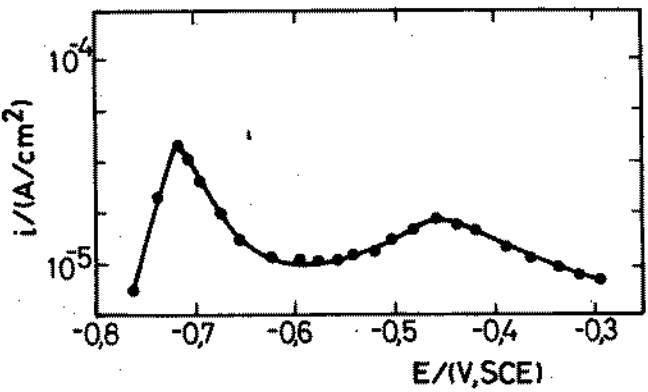

FIGURE 1 - Polarization curve of mild steel in phosphate-borate solution at 0,02 V/min.

main product formed in the potential range of peak I is assigned to the $Fe(OH)_2$ which is electrooxidized to FeOOH in the potential range of the second current peak[5]. The FeOOH layer can be transformed into a complex stable passive film approaching the structure Fe_3O_4/hydrated Fe_2O_3[6].

The metabolic activity of SRB produces mixtures of SH^-/SH_2 as a consequence of sulphate reduction. Table 1 shows the variation of cell number and sulphide levels as a function of the incubation time. It can be noticed that during stationary phase of growth sulphide levels in the order of 10^{-3} M to 10^{-2} M are obtained.

If sodium sulphide is added to carbon steel specimens passivated at -0,64 V ($Fe(OH)_2$ potential range), a marked current rise can be observed increasing its hight as the

TABLE 1

Microorganisms and sulphide concentration vs, time

Time (days)	Microorganism number* (MPN/100 ml)	Sulphide concentration (mol/l)
1	---	0.8×10^{-4}
2	3.0×10^{4}	1.0×10^{-4}
6	1.0×10^{7}	1.8×10^{-4}
8	5.0×10^{8}	5.0×10^{-3}
12	3.0×10^{8}	3.0×10^{-3}

* microorganism growth estimated by the more probable number (MPN) technique[7].

sodium sulphide concentration increases (Fig. 2). General

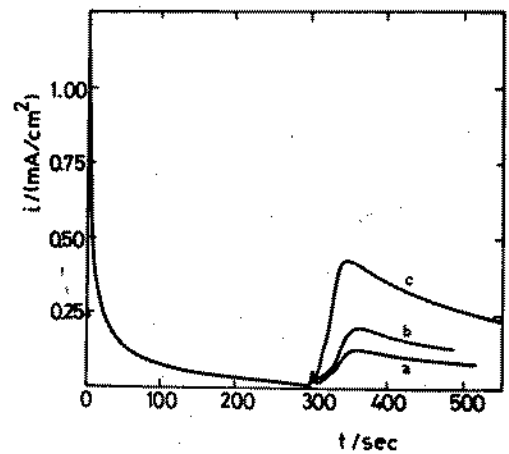

FIGURE 2 - Current transients at constant potential in the phosphate-borate solution. The arrow indicates Na_2S addition. a) 4.5×10^{-3} M; b) 9.5×10^{-3} M; c) 1.4×10^{-2} M. The following electrode pretreatment was used: 90 sec at -1.20 V and then held at the preset potential.

dissolution and pit formation are observed on the metal surface after removing the black film of iron sulphide.

Current values read after 10 min of current transients initiation are plotted as a function of potential in figure 3. In the -0,64 V to -0.60 V range the current increases with the potential. At more anodic potential values than -0,60 V, the current shows a slight continuous decrease as the potential increases.

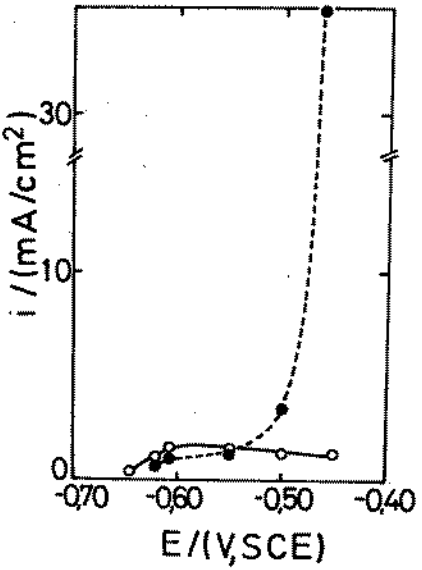

FIGURE 3 - Current density (read from the current-transients at t = 10 min) vs. potential relationship in phosphate-borate solution containing (o) Na_2S $2.5x10^{-2}$ M, (o) Na_2S $2.5x10^{-2}$ M + NaCl 1 M.

To obtain information on the species related to the pitting inhibition, the electrode potential was held at -0.20 V in the neutral buffered solution containing sulphide. After certain time of anodization, potentiodynamic polarization curves in cathodic direction were made. The curves were compared with those corresponding to the buffer solution without sulphide (Fig. 4). In this case, a broad cathodic current peak at -0,50 V corresponding to the electroreduction of the oxide film can be seen[8]. Conversely, in sulphide containing solutions a large anodic peak is observed at -0.50 V. Besides two cathodic peaks appear at -0,68 V and -1,05 V. These results indicate that the pitting inhibition is due to the oxide formation rather than to the sulphide species formed during pitting.

It is known that chloride ions are widely distributed in natural environments (salts, natural waters, etc). Besides culture media usually employed to study the corrosion mechanism of SRB contain different chloride levels. Current transients recorded in the neutral buffered solution containing sodium sulphide at potential values higher than -0.60 V with the addition of NaCl show that chloride ions enhance pitting (Fig. 3) promoting the formation of pits larger and deeper than those observed in the solutions containing only sulphide anions (Fig. 5). At a

constant potential, the anodic current increases with the concentration of sodium chloride (Fig. 6).

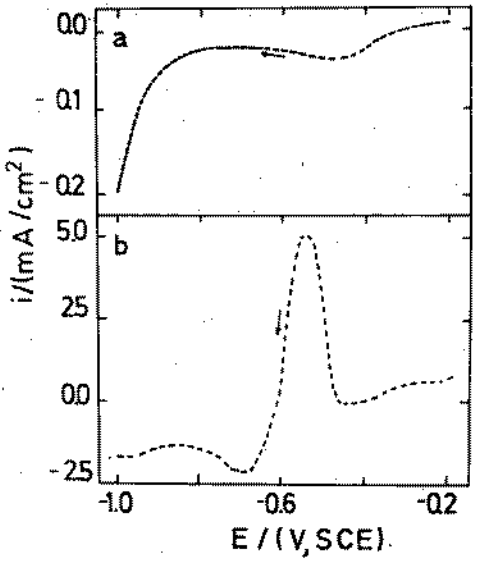

FIGURE 4 - Potentiodynamic polarization curves at 0,02 V/sec in: (a) phosphate-borate solution; (b) phosphate-borate solution containing $2,5x10^{-2}$ M Na_2S. The electrode was held at -0,20 V during 30 sec before the negative scan.

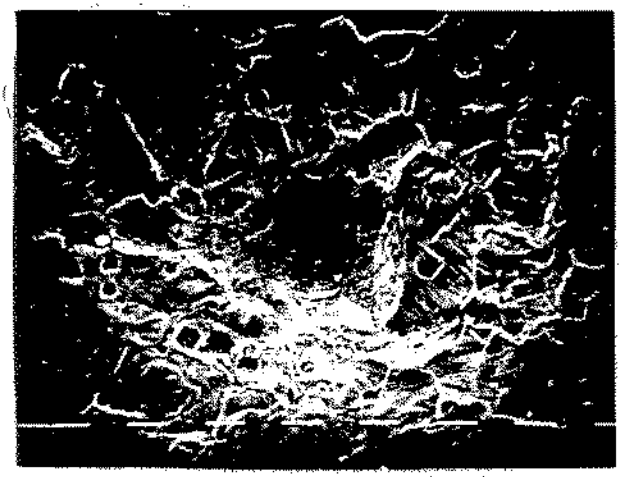

FIGURE 5 - SEM microphotograph of the carbon steel surface after 10 min in $2,5x10^{-2}$ M Na_2S + 0,5 M NaCl at -0,45 V.

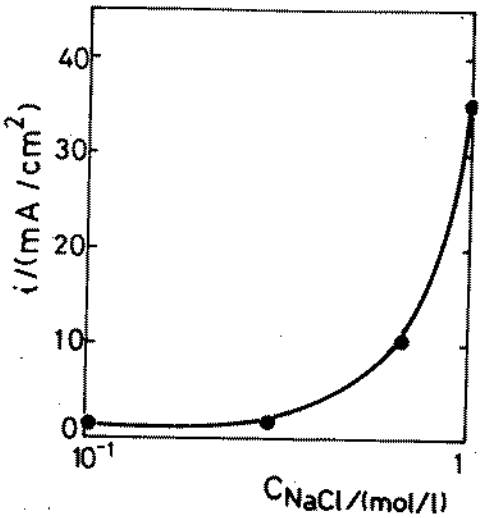

FIGURE 6 - Current density vs. NaCl concentration plot in phosphate-borate solution containing 2.5 M Na_2S. The current density was obtained from current transients at t = 10 min.

To compare the effect of inorganic sulphides with those of biological origin, mild steel electrodes were passivated at -0.62 V in artificial sea water (breakdown potential in this medium was -0.58 V / -0.56 V). The current decreases with time reaching finally a small value (Fig. 7). The addition of 10 ml of a SRB culture to reach a final sulphide concentration of 1.2×10^{-3} M produces a sudden current increase. After the ferrous sulphide has been removed pitting and general corrosion are observed.

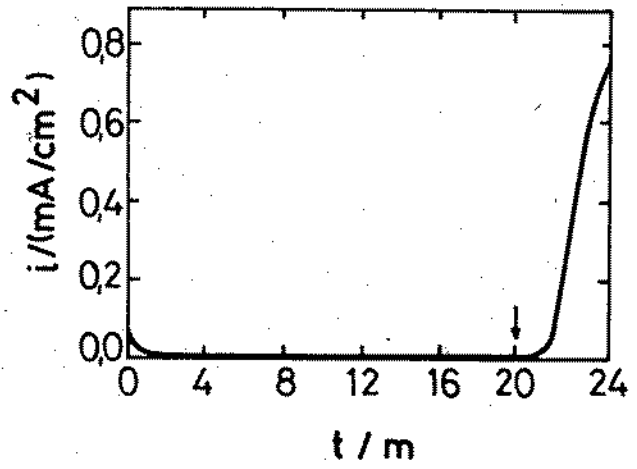

FIGURE 7 - Current transient at constant potential (-0,62 V) in artificial sea water. The arrow indicates the addition of 10 ml of SRB culture. Final sulphide concentration: 1.2×10^{-3} M.

DISCUSSION

According to the Cathodic Depolarization Theory of Von Wolzogen Kuhr and Van der Flugt[1] the corrosion of iron and steel in the presence of SRB is attributed to the capacity of these bacteria to uptake hydrogen through enzymatic activity (hydrogenase). The corrosion process would be accelerated by depolarization of the cathodic reaction: through the remotion of atomic hydrogen from cathodic areas on the iron surface by the bacterial hydrogenase. This reaction will be coupled to the reduction of sulphate to sulphide. The reactions leading to corrosion products will be:

$$S^{2-} + Fe^{2+} = SFe$$

$$2\ OH^{-} + Fe^{2+} = (OH)_2Fe$$

A copious literature has been published since the Depolarization Theory to discuss or convalidate its validity through very different experimental techniques. Detailed discussions on this subject have been made by Davis[9], Miller and Tiller[10] and Iverson[11]. There is no agreement yet on the mechanism although various conflicting theories have been put forward.

Cathodic depolarization was demonstrated experimentally through electrochemical measurements by Horvath and Solti[12] and Booth et al,[13] whereas anodic stimulation by biogenic sulphide rather than cathodic depolarization has been proved by other authors[14,15].

An indirect role of SRB in the corrosion process is proposed by Costello[16] who concluded that cathodic depolarization in cultures of SRB may be attributed to dissolved hydrogen sulphide produced by the microorganisms. The cathodic effect would be expressed by:

$$H_2S + e = SH^{-} + \frac{1}{2} H_2$$

King and Miller attributed to the iron sulphide the main cathodic depolarization and minimized the role of bacteria[17]. According to these authors iron sulphide crystal lattice would act as cathodes for hydrogen reaction. The action of the bacteria would be limited to the remotion of hydrogen atoms linked to ferrous sulphide crystals. In agreement with these results recent observations seem to confirm the depolarizing action of ferrous sulphide on the hydrogen evolution reaction[18].

It is generally agreed that the first iron sulphide formed on iron in the presence of SRB is a film protective to a greater or lesser degree of mackinawite a sulphur deficient sulphide usually represented as $FeS_{(1-x)}$[2]. As a consequence of bacterial growth the film thickens as the sulphur content increases giving to the film poorly

passivating characteristics, King and Miller showed an interrelationship scheme of the different iron sulphides which follow either chemical or bacterial routes in their transformation[19]. The nature and composition of these sulphide films as well as their rates of growth and breakdown are greatly dependent on the pH of the system. Mackinawite is followed later by transformation to greigite although some pyrrothite can be formed. Corrosion rate in the presence of these sulphides follow approximately a first order kinetics. As the corrosion attack in the presence of SRB is mainly pitting attack a little sulphide can cause a disproportionate damage.

The action of SRB on the anodic reaction has been seldom mentioned in the literature if compared with the cathodic depolarizing effect. In the 50's Horvath mentioned an initial anodic depolarization following the initiation of bacterial growth[14]. This effect was observed through electrochemical techniques also employed by Booth et al. who established the formation of a ferrous sulphide film after a certain time of metal exposition to SRB leading finally to an anodic polarization that diminishes the metal dissolution rate[13]. The culture and experimental media used by these authors is a complex one and contains aggressive anions such as chlorides and sulphates that impede the initial passivation of the metal surface. In these experimental conditions it is possible that ferrous sulphide formation as a consequence of the metabolic production of SH^-/SH_2 mixtures derive in an inhibition of the anodic reaction. This composition is nearly a constant characteristic of all media generally employed by different authors in the growth and experimental measures involving SRB.

If we consider that in real situations the iron or steel is generally covered by a passivating film of ferrous hydroxide or ferric oxide the presence of SH^-/SH_2 mixtures result in a marked depolarization of the anodic reaction. Progressive accumulation in the medium of sulphide either inorganic or biogenic lead to a sustitution of the initial oxide or hydroxide passivating film by a less protective film of sulphide which allow dissolution rates appreciably higher than in the original conditions. In its turn, the characteristics of the initial film determines the metal behaviour in the presence of sulphide. We have shown before how for potential values more negative than -0,60 V SCE iron surface is covered by a film of ferrous hydroxide. In these conditions the corrosion process by the action of sulphide can be explained through a competitive process between OH^- and HS^- ions and H_2O and H_2S molecules leading to metal pitting. The following reaction scheme can be used to explain these competitive processes developed in the presence of sulphi

de anions:

$$(H_2O)Fe \rightleftarrows Fe(OH) + H^+ + e \quad (1a)$$

$$(OH)Fe \rightleftarrows Fe(OH)^+ + e \quad (1b)$$

$$H_2O + Fe(OH)^+ \rightleftarrows Fe(OH)_2 + H^+ \quad (1c)$$

$$Fe(OH)^+ \rightleftarrows Fe^{2+} + OH^- \quad (1d)$$

$$(H_2O)Fe + SH_2 \rightleftarrows Fe(SH_2) + H_2O \quad (2a)$$

$$(OH)Fe + SH^- \rightleftarrows Fe(SH) + OH^- \quad (2b)$$

$$(SH_2)Fe \rightleftarrows Fe(SH) + H^+ + e \quad (3a)$$

$$Fe(SH) \rightleftarrows Fe(SH)^+ + e \quad (3b)$$

$$Fe(SH)^+ + SH^- \rightleftarrows SFe + SH_2 \quad (3c)$$

$$Fe(SH)^+ \rightleftarrows Fe^{2+} + SH^- \quad (3d)$$

Species between parenthesis represent adsorbed species. The first sequence of reactions (1a to 1d) corresponds to the ferrous hydroxide film formation and iron dissolution as Fe^{2+}. The second sequence of reactions (2a and 2b) represent species competition. The third sequence (3a to 3d) represent mackinawite film formation and dissolution of iron as Fe^{2+}. This mechanism is similar to that recently published to interpret electrochemical dissolution of SAE 1020 steel in alkaline media containing sulphides[20]. At potential values more anodic than -0,60 V the transformation of ferrous hydroxide to oxide takes place. In these conditions aggressive species (SH^- and SH_2) are only able to attack the metal in the oxide film failures such as those zones of manganesum sulphide inclusions. This indicates the difficulty in migration through the oxide film of sulphur species. Pitting inhibition occurs in the potential region where the oxide film is formed. This suggests the oxide growth underneath ferrous sulphide nuclei as it is explained for the oxide growth on iron covered by salt layers. The presence of chloride into the sulphide containing solutions prevents the oxide growth and, consequently, the electrode repassivation through the OH^- and H_2O displacement from the metal. This explains the enhancement of aggressiveness of chlorides and sulphides mixtures for iron and mild steel.

In many aquatic environments the reduction of sulphate sulphide as a part of the sulphur cycle is accomplish-

ed mainly by the metabolic activities of SRB. This activity is greatly influenced by sulphide concentration already present, temperature, assimilable organic matter, low redox potential values, absence of oxygen and pH values near neutrality. This set of conditions is usually found in soil and water sediments where a high level of sulphate reduction is observed[21]. In this type of solid/liquid interphase a natural enrichment of bacterial nutrients is achieved (ions, organic molecules and colloids) such as that needed to support the growth of an active bacterial population. This bacterial population by means of different physicochemical adsorption effects can colonize the metal surface through glycoprotein production. The metal/medium interphase is in this way substantially modified by the metabolic activity of bacteria leading in some circumstances to the creation of microenvironments optimal for SRB activity. In the solid/liquid interphase the local production of SH^-/SH_2 species can be sufficiently high as to break the original passivating film on the metal surface leading to metal attack.

We can summarize the role of SRB on the corrosion process through the following points:

1. Biogenic sulphide produced by SRB metabolic activity shows a similar behaviour with respect to passivity breakdown of mild steel than those of inorganic origin.
2. The characteristics and the intensity of sulphide action on mild steel is narrowly related with the nature of the passive film already present on the metal.
3. In neutral media sulphide ions lead to the production of a poorly protective film of mackinawite on the metal surface.
4. The mechanism of the corrosion process can be interpreted through competitive reactions between ionic species like OH^- and SH^- and molecules like H_2O or H_2S present in the medium.
5. The anodic breakdown of passivity would be the first stage of the corrosion process. The role of SRB would be indirect through the metabolic production of aggressive species. Cathodic depolarization effect attributed to SRB or to iron sulfides films in the literature would be developed later than passivity breakdown while corrosion process is in progress.

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THE EFFECTS OF MACRO-FOULING ORGANISMS ON STEEL CORROSION AND ITS ELECTROCHEMICAL BEHAVIOR

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ABSTRACT

The different effects of several fouling organisms, barnacles, bryozoan and Corophium, on corrosion of carbon steel, low alloy steels and nickel-base alloy as well as its causes and processes are reported in this paper based on in situ tests of ocean corrosion and electrochemical studies of corrosion.

RESUME

A partir des expériences de corrosion in situ et des tests électrochimiques en laboratoire, nous traitons dans cet article les différents influences exercées par les organisms de la salissure, comme barnacles, bryozoan et Corophium, sur la corrosion de l'acier au carbone, de l'acier au faiblement allié et de l'alliage à base de nickel. Nous étudions également les causes de ces phénomènes et les processus en question.

INTRODUCTION

It is a natural phenomenon that marine fouling organisms adhere to the materials immersed in the ocean, which can affect the working of installations and the corrosion processes of their parts, and lead to a spacial characteristic of corrosion (1, 2). Therefore, it is important to study fouling organism's effects on corrosion of different metal materials and the causes, which will be conducive to the application of their advantages and the prevention of their disadvantages.

The effect degree of barnacles, bryozoan and Corophium, which are some of main macro-fouling organisms, on corrosion of metal materials and the causes are discussed.

MATERIALS AND METHODS

1. Two kinds of specimens, 3C carbon steel and 09CuWSn low alloy steel, were divided into 12 groups and each of them was immersed monthly in a test raft in Xiamen waters, beginning at the end of Jaunary, 1979 for an annual observation. The fouling situations were recorded and temperture, salinity and dissolved oxygen, etc. of seawater were determined on the spot of exposure ance a month during the test.

2. Two other kinds of specimens, A3 carbon steel and 10CrPV low alloy steel which were divided into 8 groups, were conducted to perform an in situ seasonal test, in which, 4 of them in a 121 mesh nelon bag that was changed once each 5 days in order to prevent the mentioned organisms fouling on the panels.

3. Five 1.5cm^2 and five 2.0cm^2 round electrodes were prepared with A3 and 10CrPV steels respectively and sealed with epoxide resin. The electrodes were divided into 2 groups and immersed in the season in which barnacles were breeding, with one inside the nylon bag, and the other outside the nylon bag. The electrodes outside the bag were taken into the nylon bag 2 months later. Cathodic and anodic polarization curves of all the electrodes were determined with potential scanning in natural seawater electrolyte 5 months later.

4. 10 panels of GH30 nickel-base alloy were immersed in breeding season of barnacles. 10 months later, the barnacles on two of the 10 panels were uprooted one by one and the situations of corrosion and barnacle growing were observed. At same time, 10 living barnacles were selected on another panel, in which, 3 bodies were removed and the other were left alive, and exposure was continued for one month. Another panel adhering with some barnacles was put into a beaker with natural seawater and the beaker was covered with 9 layers of gauze, and corrosion situation of the panel was observed after the panel was sterilized in a sterilizing pot in the lab.

RESULTS

1. Reducing of steel corrosion rates by macro-fouling organisms

There is a clear adherence seasonal of organisms, and adherence situations vary with different month groups, espacially on the first several

months of each group though their immersion durations are the same. The fourth group was fouled by barnacles quickly and heavily, and almost entirely covered after it had been immersed for 4 or 5 months because it was immersed in barnacle breeding season (at the end of April)(3), but the panels which were immersed off season (at the end of November) had only about 23% hydroidea and 25% Corophium (Fig. 1). Situations on the other groups were between the above two.

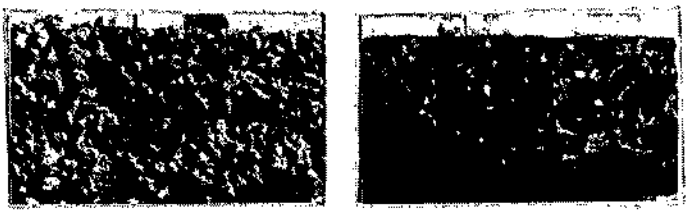

Figure 1. The adherence situations of organisms on 3C panels after 4 month exposure.
Left : Immersed at the end of April.
Right: Immersed at the end of November.

The results showed that 09CuWSn panels immersed at the end of November had the highest corrosion rate, 0.282mm/y; those immersed at the end of April had the lowest corrosion rate, 0.118mm/y (4). Results of 3C panels were similar to those of 09CuWSn. The difference of the corrosion rates was mainly attributed to the different adherence situations of the organisms because all panels had the same exposure duration (one year) in which physical and chemical factors were basically the same for them.

Figure 2 shows that there is a corresponding relation between the annual corrosion rates and the coverage areas of organisms (barnacles and oysters) of each group, the larger the coverage area, the lower the corrosion rate, and vice versa.

It is thus clear that the corrosion rates vary with the different beginning month of exposure and can be reduced appearently due to adherence of those macro-fouling organisms in the immersion tests of carbon steel and low alloy steel with same duration of exposure.

2. The different effects of different fouling organisms on steel corrosion

Panels of A3 steel immersed in 121 mesh nylon bag were not fouled by marine macro-fouling organisms and their corrosion rates rose with tempera-

ture (TABLE 1), as it was pointed out by many researchers that materials, such as steels, had higher corrosion rates in the hot season (5).

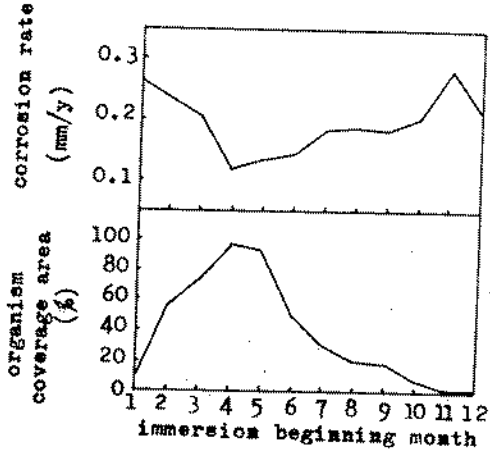

Figure 2. Relationship between corrosion rates and coverage area of organisms on 09CuWSn panels
Above: Corrosion rates of panels immersed in different months.
Below: Coverage areas of barnacles and oysters on the panels immersed in different months 5 months later.

In order to study the effects of several main marine fouling organisms on the steel corrosion, we engaged in a test of immersion according to different breeding seasons of organisms. The results showed that Summer panels had 92% barnacles; Autumn panels, 90% bryozoan and 8% barnacles; Winter panels, 81% Corophium and 15% hydroidea. Summer panels had the lowesr corrosion rates and corrosion rates of the others rose with seawater temperature (TABLE 1). 10CrPV had the same effects.

Normally, Summer panels should have had higher corrosion rates for the higher seawater temperature, but they had the lowest corrosion rates instead owing to barnacle coverage, which showed that integrated coverage of barnacles could effectively reduce corrosion rates of carbon steel and low alloy steel. However, the effects of bryozoan and Corophium were not obvious.

TABLE 1. The mean seawater temperature, dissolved oxygen and corrosion rates (mm/y) of A3 steel in different seasons

season		Summer (June-Aug.)	Autumn (Sep.-Nov.)	Winter (Dec.-Feb.)	Spring (Mar.-May)
seawater temp. (°C)		28.20	23.53	14.01	18.50
dissolved oxygen (ml/l)		4.34	4.85	6.02	5.48
A3 corrosion rate	no macro-organisms	0.263	0.273	0.199	0.227
	with macro-organisms	0.234	0.356	0.248	0.278

3. The causes of reducing corrosion rates of carbon steel and low alloy steels owing to the barnacle coverage.

It is reported by a lot of researchers that barnacle's coverage on steel panels immersed in seawater can reduce corrosion rates, even though the views and explanations are not the same. Some of them thought that adherence of organisms could obstruct diffusion of oxygen to the surface of metal; respiration of organisms could reduce the amount of dissolved oxygen of seawater around organisms, and also the layer of the fouling organisms could weaken the dash of water current against the metal surface (6). Some of them thought that coverage of barnacles could form a close system, it made a anaerobic enviroment on the metal surface, as a result, the anaerobes (mainly the sulfate reducing bacteria) could multiply, which controlled the process of corrosion (5).

In order to investigate how barnacles reduced corrosion rates of carbon steel and low alloy steels, we carefully compared the panels with and without barnacles not long after their exposure, and conducted polarization potential scannings of some electrodes which were made of the same materials as the panels and immersed in the same waters.

When corrosion layer and organisms on the panels, heavilly fouled by fouling organisms not long after their immersion (at the end of April, one year exposure), were removed, we found that there were disk-like areas beneath the bottoms of barnacles, which were basically not corroded and higher than corroded areas (Fig. 3 Left). However, we didn't find similar cases on the panels immersed at the end of November though they had the same exposure duration and also had many barnacles in the next year's breeding season of fouling organisms covered on the scale and other fouls instead of on metal substrata (Fig. 3 Right). It is obvious that the main cause reducing the corrosion rates of those panels immersed in breeding season of organisms were that barnacles adhered directly to the steel's surface and reduced its corrosion area. We ever removed barnacles from the panels immersed in breeding season for 3 months and found they adhered to the surface of those panels very firmly. But adherence can cause uneven corrosion of panels, especially results in deeper etching pits between barnacles that are close to each other.

The barnacles continued growing after those electrodes immersed outside the nylon bag and fully adhering with barnacles (about 2 months) had been removed into the bag, and the electrodes immersed inside the bag maintained no adherence of macro-fouling organisms from the beginning of exposure.

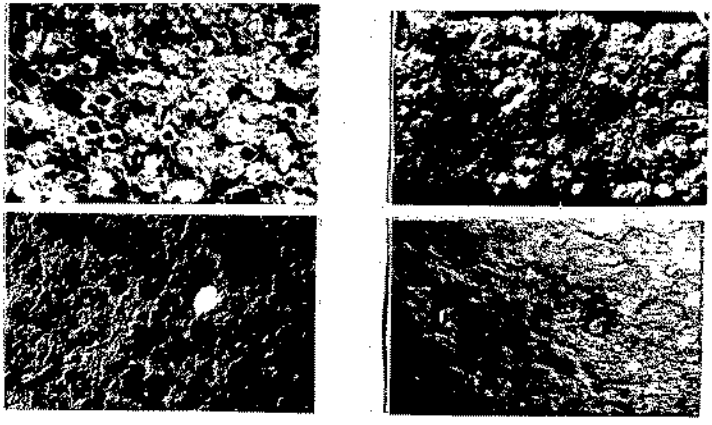

Figure 3. The situations of the adherence and the corrosion on 3C panels immersed in different months for one year exposure duration.
Left : Immersed at the end of April.
Right: Immersed at the end of November.

Slopes of the scanning curves of those electrodes with barnacles were larger than those without barnacles after they had been immersed for 5 months (Fig. 4. Both A3 and 10CrPV electrodes had the same trends). It showed that the slope differences were caused by the barnacles for the test condition were the same except organisms. In other words, the barnacles reduced the corroded area, therefore they reduced the corrosion rates. When compared $1.5cm^2$ electrodes with $2.0cm^2$ ones, we found that the scanning curve's slopes of $1.5cm^2$ electrodes were larger than those of $2.0cm^2$ electrodes wherever with or without barnacles.

When the barnacles, which adhered firmly to the surface of electrodes, were removed, the substratum appeared with metallic luster beneath the barnacle's bottoms, and was not corroded and higher than other areas.

It can be known from the shapes of the polarization curves that anodic process has partly controlled corrosion for anodic products can block further dissolutions of substratum in some degree.

4. Barnacle's effects on corrosion of GH30 nickel-base alloy

As mentioned above, barnacles can reduce corrosion rates of carbon steels and low alloy steels. For stainless steels, barnacles can lead to crevice corrosion, which are universally accepted by corrosion researchers (7). However, there are two explanations among them: 1) corrosion could

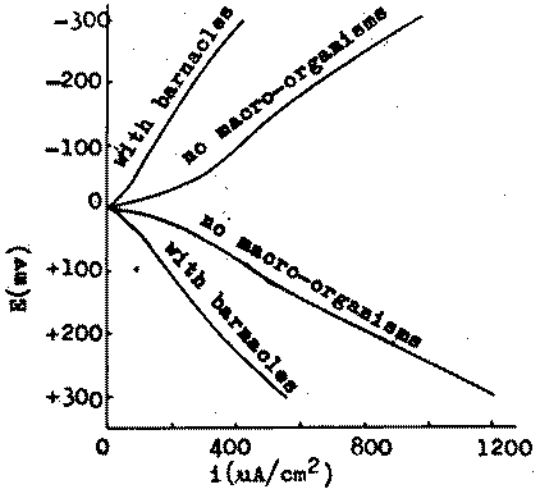

Figure 4. Scanning curves of anodic and cathodic polarization of A3 steel electrodes after five month exposure.
(Rate: 18mv/min)

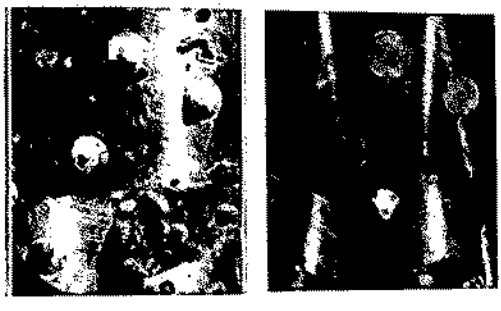

Figure 5. The different effects of dead and living barnacles on corrosion.

Left : 3 shells whose bodies were removed and 7 living barnacles.

Right: The substratum situations beneath 3 barnacle's shells and 7 living barnacles after one month exposure.

reduce the pH of seawater in crevice so that the bottom of barnacle was determined, which was harmful to barnacle's growing and the barnacle died at last; and 2) bacteria that could promote electrochemical corrosion could breed rapidly beneath barnacle, which caused crevice corrosion.

We uprooted the barnacles on two panels that had been immersed for 10 months one by one to investigate crevice corrosion of GH30 nickel-base alloy and found that the substrata beneath 58 firmly adhering living barnacles were not corroded, but most of the substrata beneath the 99 dead barnacles were corroded (only a few newly dead barnacles didn't induce corrosion). Most of the corrosion trails were deeper around the barnacle bottom edges and shallower in center regions, some of them were ring-like trails around the barnacle bottoms whose center regions were not corroded.

There was serious corrosion beneath shells of barnacles whose bodies had been taken away after test panel with shells having one month exposure, but the substratum

beneath those living barnacles were not corroded (Fig. 5).

At the same time, another panel with living barnacles was immersed in a beaker filled with natural seawater and left in lab after they had been sterilized. The corrosion products appeared after half month immersion and no macro-organisms were found in the seawater under microscope, which showed that the corrosion was not caused by micro-organisms.

It is enough, based on the mentioned observation and tests, to prove that nickel-base alloy corrosion induced by barnacles was crevice corrosion, which occured after barnacles had died, and crevice corrosion began at the region beneath the bottom eages of barnacles, then developed to the center regions of the bottoms. Улановский proved that big, living and physically well developed barnacles had unpenetrable bottoms and could firmly adhere to the surface of stainless steel by plating cupper on the panels with barnacles, which also explained that crevice could only insist along the bottom edges of barnacles. Because current movement was blocked in the narrow enough crevice, there were concentration difference of oxygen between inside and outside of the crevice and formed a concentration cell discharged in seawater so that pH of the solution in the crevice reduced and substratum was corroded.

DISCUSSION

1. Substrata of carbon steel and low alloy steel beneath barnacle bottoms were gradually corroded after those regions beside barnacles had undergone corrosion for some time. The adherence trails of barnacles were not found on the A3 panels immersed in breeding season for 3 years when organisms and corrosion layers, which could be removed in large scale, were uprooted.

2. That organisms adhere to steel substrata or corrosion layers are helpful to establish anaerobic envirement, to weaken the dash of seawater current, and to obstruct the diffusion of dissolved oxygen, therefore corrosion rates can be reduced.

CONCLUSION

1. In the corrosion tests of immersion with same duration, corrosion rates of steels vary with different months of immersion.

2. Adherence of barnacles on surfaces of carbon and low alloy steels can reduce corrosion rates, which is mainly caused by the reducing of corrosion area due to the direct adherence of barnacles to the substrata

and causes larger scanning curve slopes of anodic and cathodic polarization. Adherence of barnacles can induce uneven corrosion.

Effects of bryozoa and Corophium on corrosion are not evident.

3. Barnacles can conduct crevice corrosion of GH30 nickel-base alloy, which occur after the barnacles have been dead and begins at bottom edges of barnacles, then develops to center regions of substrata of barnacle bottoms, but living barnacles don't induce crevice corrosion.

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Association Between Early Marine Microfouling and Corrosion of Steel

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ABSTRACT

Polished mild steel test pieces (API 5L grade X65) were exposed to seawater in an established recirculation aquarium for 70-72h. Fouling deposits were examined by both SEM and TEM. The condition of the underlying steel surface was assessed after stripping off this material.

Corrosion proceeded as a localised pitting with the majority of the surface remaining unaffected. Deep pits were confined to a narrow central band on affected specimens. Shallow pitting was more common and more widespread.

The thickness of the macromolecular conditioning film associated with the surfaces varied from 0.16 µm up to 1.6 µm around the corrosion site. Corrosion products were shown to be responsible for this local increase in thickness.

The primary colonising bacteria were found to be mainly cocci, 0.4-1.0 µm in diameter. The distribution of the bacteria across the surface of the steel was not uniform. Densely populated areas were confined to a narrow central band on each of the test pieces; the microbial deposit was continuous and up to 75 µm thick. Most of the remaining surface was very sparsely colonised, often by isolated bacteria. Some areas were colonised to an intermediate extent with large groups of cells. The extent of the bacterial colonisation correlated with the degree of corrosion of the underlying steel. The dense masses of bacteria were always found to be associated with deep pitting corrosion. The steel beneath the sparsely colonised surface was indistinguishable from unexposed steel.

Des pièces test polies en acier doux furent exposées à l'action de l'eau de mer dans un aquarium établi à récirculation pendant une periode de 70 a 72 heures. Des dépôts de salissures biologique furent examinés à l'aide de la microscopie électronique. L'état de la couche d'acier sous-jacente fut evaluée après avoir enlevé ce materiel.

La corrosion se developpe en forme de locales dépressions, la plupart de la surface étant non affectée. Dans la cas des specimens affectés des trous profonds furent confinés à une étroite bande centrale. Plus courantes et generales furent des

dépressions peu profondes.

L'épaisseur du film responsable de nature macromoleculaire associe aux surfaces varient entre 0.16 µm et 1.6 µm autour du point de corrosion. On demontra que les produits de corrosion responsables de l'augmentation de l'épaisseur.

On trouva que les plus importautes bacteries colonisatrices sont des coccus de 0.4 a 1.0 µm de diametre. La distribution des bactéries sur la surface de l'acier n'était pas uniform. Dans tous les cas des regions densement peuplées furent confinées a une étroite bande centrale; le dépôt microbien était continu avec une épasseur maximum de 75 µm. Le reste de la surface était peu colonisée tres souvent par des bactéries isolées. Quelques regions étaient colonisées de forme intermediaire avec des considerables groupes de cellules. L' importance de la colonisation bactérienne était en relation avec le degree de corrosion de l'acier sousjacent. On trouva que les masses denses de bactéries étaient toujours associées avec des profondes dépressions dues a la corrosion. On ne put pas distinguer de différence entre l'acier au-dessous de la surface peu colonisée, et l'acier pas exposé a l'eau de mer.

INTRODUCTION

Both the corrosion and fouling of mild steels exposed to the marine environment occur very much within the same time scale. The corrosion process is initiated immediately on exposure of the material to seawater; fouling, at least by organic macromolecules, commences equally rapidly (Baier, 1980). The subsequent progression of each of these processes occurs over a considerably more protracted time. Though highly dependent upon environmental conditions, uniform corrosion may account for a loss of steel in the region of 0.3 - 2.0µm per day (Chandler, 1974). In natural environments, however, attack tends to be confined to localised sites where it proceeds more rapidly (Carter, 1977). Similarly, observations of marine fouling on a variety of inert surfaces suggest that although the primary colonising bacterial species may become established within a matter of hours (Zobell and Allen, 1935; Skerman, 1956; Dexter, 1976), the succession which occurs within the microfouling community alone is still in a state of flux after 35 days (Gerchakov et al, 1976) or even 100 days of exposure (Edyvean and Terry, 1983). The subsequent colonisation of the surfaces by the macrofouling community proceeds over an even greater period of time. Given these observations, and considering that offshore structures may remain in situ over many years, it is not surprising that exposure times of 100 days have been described as "relatively short" (Terry and Edyvean, 1981).

There is no lack of information regarding either fouling per se or its relationship to corrosion. Much of the avaliable data regarding initial fouling, however, relate to idealised inert surfaces: most frequently glass (Zobell and Allen, 1935; Skerman, 1956; Marshall, Stout and Mitchell, 1971; Corpe, 1973), but also plastics (Dexter, 1976; Dempsey, 1981a). Materials with more specific uses in the marine environment, such as paints (Zachary et al., 1978; Dempsey, 1981b) or alloys of the type used in heat exchanger units (Berk et al., 1981) have also received attention.

Microfouling and Corrosion

The significance of fouling on the corrosion of structural steels has generally only been examined under conditions where a significant biomass has already accumulated. Non-specific effects attributable to alterations in the physical and chemical milieu (pH, oxygen and nutrient gradients) by the metabolism of the fouling bacterial mass have been considered (Miller and King, 1975). Certain organisms have also been ascribed a more direct role in the progress of corrosion. Specifically the activities of the sulphate-reducing bacteria (SRB's) are of considerable concern (see Miller and King, 1975; La Que, 1975; Hamilton, 1983). Similarly, in the macroscopic community, pitting corrosion is apparently enhanced in the presence of hard fouling by barnacles (La Que, 1975). In both of these examples the accumulation of significant numbers of each species is characteristic of late fouling communities.

The information on the relation of fouling to corrosion is by no means complete. A specific area of research where there is a dearth of available information concerns the initial fouling of unprotected structural steels. The importance of a study of this kind is twofold: (1) to elucidate the association, if any, between the initial colonisation by microorganisms and the initiation and progression of corrosion; (2) to determine whether differences exist between this primary colonising microflora and existing data on the microfloras of inert surfaces. The latter point is of significance since it is the metabolic capabilities of the primary colonisers which modify and diversify the environment, and thereby ultimately dictate the form and course of the subsequent ecological sucession. Accordingly, in the preliminary investigation reported in this paper, we have confined our attentions to an examination of the initial fouling of unprotected mild steel test pieces in a laboratory marine system after 70-72 hours of exposure. Particular use has been made of both scanning and transmission electron microscopy.

MATERIALS AND METHODS

(1) Preparation of Steel Test Pieces

Test pieces were prepared from a section of pipe steel (API 5L grade X65). This particular material is not homogeneous, but contains strata of pearlite running parallel to the surface. As a result of the techniques used during their preparation, it is possible that specimens taken from this surface may differ in the extent to which one or other of the phases, ferrite or pearlite, is exposed. Consequently, to ensure reproducibility between specimens, test pieces were prepared from a face cut perpendicular to the original surface. 10mm x 10mm x 50mm bars were cut from the original block of material. Test pieces were removed as 3mm - thick transverse sections, using a low speed cutter (Isomet, Buehler) to prevent work-induced changes in the material. Sections were mounted in acrylic resin and polished through a graded series of abrasive papers. Final polishing was achieved with 6um diamond grit.

Specimens were mounted onto scanning electron microscope stubs

with epoxy resin in such a way as to ensure that only the topmost polished surface was exposed.

Steel surfaces were swabbed with a trifluoroethane degreasant (Arklone, ICI). Examination by scanning electron microscopy revealed the surface to be free of both debris and bacteria after this treatment. In some experiments no further precautions were taken to ensure sterility. Alternatively, after degreasing, specimens were sterilised by immersion in a 2% w/v aqueous solution of formaldehyde for 10 minutes, rinsed in sterile distilled water, rinsed in sterile Analar ethanol and allowed to air dry.

To remove any remaining traces of organic contaminants all specimens were subjected to a mild argon plasma treatment (5cm from electrodes; 20 minutes at 40mA) in a Polaron vacuum-coating unit fitted with glow-discharge rings. This treatment was found to be sufficient to render the specimens freely wettable by water.

Stubs were mounted in a chamber constructed from a modified plastic petri-dish, which had been disinfected previously in 70% v/v ethanol for 24 hours. Two 30mm-long slots cut in the rim of the base of the dish permitted a limited ingress/egress of seawater whilst minimising flow effects.

(2) Exposure to the Environment

Certain organic molecules, and bacteria in general, are known to accumulate in the surface microlayer (neuston) of seawater (see Norkrans, 1980). To avoid contamination of the steel surfaces by these agents the test pieces were introduced into the environment under water rather than via the air/seawater interface. The mounted test pieces were immersed in a beaker of sterile distilled water. The beaker was submerged in the seawater system and the test pieces removed under water.

The test pieces were left in an established and actively aerated recirculating marine aquarium system of 250dm^3 nominal volume (containing a mixed macroscopic fauna) for a period of 70-72 hours. Incubation temperature was c. 10C.

(3) Preparation of Specimens

Biological material: All test pieces were removed into membrane filtered seawater to remove loose debris, fixed for 10 minutes in a 2% w/v solution of glutaraldehyde in sea water, and desalted in distilled water for 10 minutes.

Samples for scanning electron microscopy (SEM) were removed and dried under a stream of oxygen-free nitrogen. Specimens were subsequently sputter-coated with gold (2 minutes, 40mA, argon atmosphere) and examined in a Cambridge S150 scanning electron microscope.

Material for transmission electrom microscopy (TEM) was either frozen in nitrogen slush and lyophilised (freeze dried), or dehydrated through a graded ethanol/water series. The biological film on the steel surfaces was embedded in Spurr resin. This was achieved by straddling the test pieces over each of a series of troughs containing, progressively, 50% v/v resin in ethanol, 75%

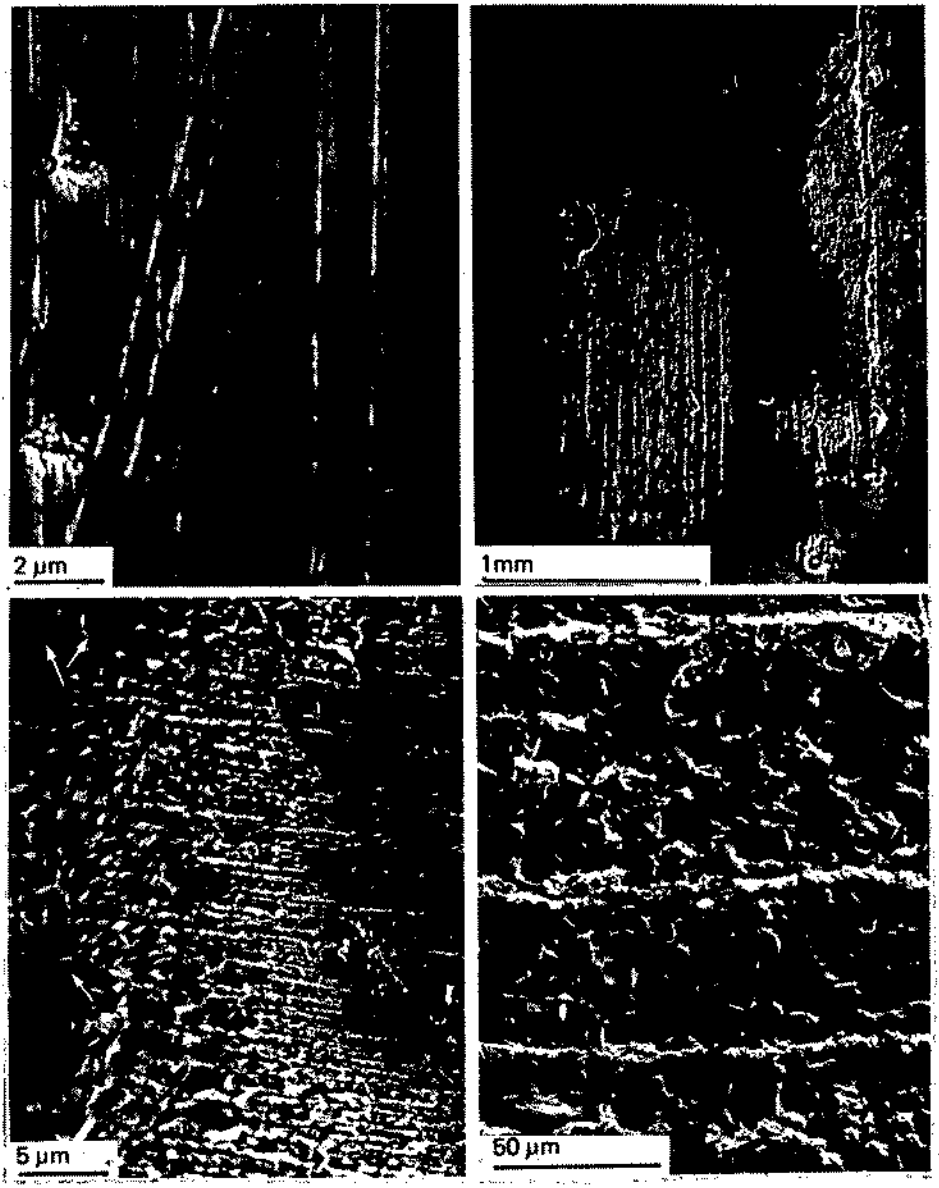

Plate 1: Appearance of Steel. Pre-exposure: fig.1: showing polished lines. Mechanical defects (D) were uncommon. Appearance of Corrosion: fig. 2: shallow (S) and deep (D) pits; fig. 3: advancing edge of corrosion showing etching along polishing lines and grain boundaries (arrowed) as corrosion progresses; fig. 4: corrosion site showing alternating ferritic (F) and pearlitic (P) phases.

resin and 100% resin. The film was finally embedded in 100% resin and polymerised at 60C for 18 hours. Embedded material was detached from the steel surface by cooling the steel in liquid nitrogen, which induced fracturing at the steel/resin interface. Ultrathin sections cut from the embedded material were picked up on formvar-coated grids and stained with 2% w/v uranyl acetate in 70% v/v ethanol followed by Reynold's lead citrate solution. The sections were examined in a Philips 400 transmission electron microscope.

Steel surfaces: The mixed layer of fouling deposits and corrosion products was removed to reveal the condition of exposed steel surfaces by the resin embedding/stripping procedure described above. The stripped surface was sputter-coated with gold prior to examination in the scanning electron microscope.

RESULTS

(1) Appearance of Steel Surfaces

The typical appearance of the steel surface prior to immersion in seawater can be seen at high magnification in figure 1. Surface imperfections were generally restricted to the ubiquitous polishing lines. Minor mechanical defects of the type shown were uncommon.

After exposure, on all of the test pieces, corrosion was found to be confined to a small number of localised pits, the number and extent of which differed even on identically prepared specimens used in the same experiment. The vast majority of the steel surface remained bright and uncorroded over the exposure period. Figure 2, from a resin-stripped surface illustrates the types of pitting which were found. Deep corrosion pits were confined to one particular area common to all the surfaces affected. Shallow pitting was more common and appeared randomly distributed over the surfaces.

In figure 3 the changes in the surface during the transition between the uncorroding and corroding states are evident. Corrosion appears to initiate along the polishing lines. The surface layer of steel is lost and further attack reveals grain boundaries in the steel. The parallel strata of the pearlitic phase becomes visible as the process progresses further (figure 4).

(2) Appearance of Fouling Deposits

Conditioning film: Details of the fouling layer are shown at low magnification in figures 5, 6 and 7. Figures 5 and 6 are light micrographs of the resin-embedded deposit from lyophilised and alcohol-dehydrated specimens respectively. Figure 7 is a scanning electron micrograph of an "air" dried specimen. Corrosion products are visible as dark areas in figures 5 and 6, and appear to form a fragmented layer. The spatial relationship between the corrosion products and the corrosion site can be deduced by comparing figure 5, which shows an embedded film, with figure 2, showing the surface from which it was derived.

Apart from the localised corrosion products, the metal surfaces were found to be entirely covered by a thin "conditioning" film. Peeling and rolling of this film was evident on all of the specimens

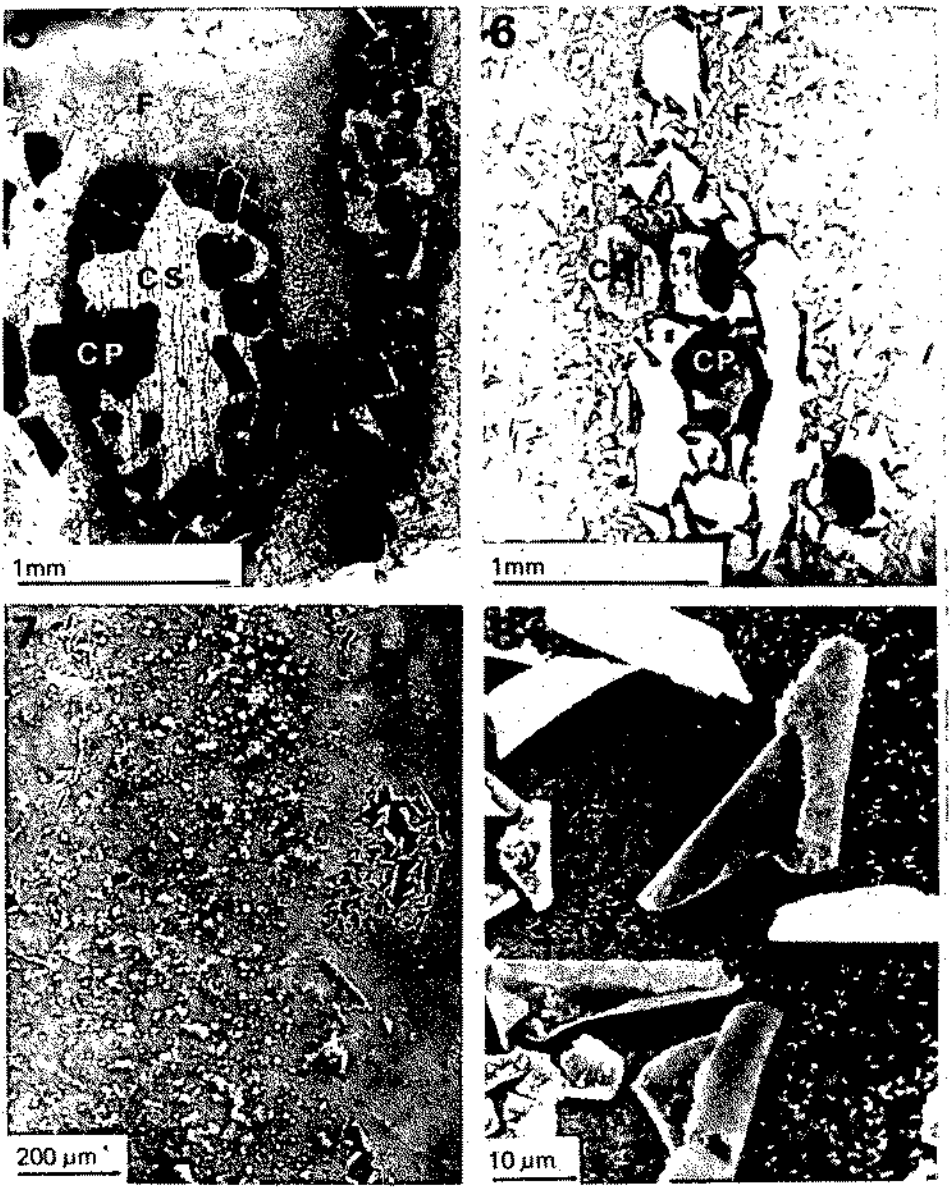

Plate 2: The Fouling Layer. fig. 5: LM of resin-embedded lyophilised film showing peeling film (F), corrosion sites (CS) and corrosion products (CP); fig. 6: LM of resin-embedded alcohol-dehydrated film; fig. 7:(45° tilt) SEM of air-dried film on steel surface; fig. 8: SEM of area from fig. 7 showing film peeling and bacteria colonising exposed steel surface (S).

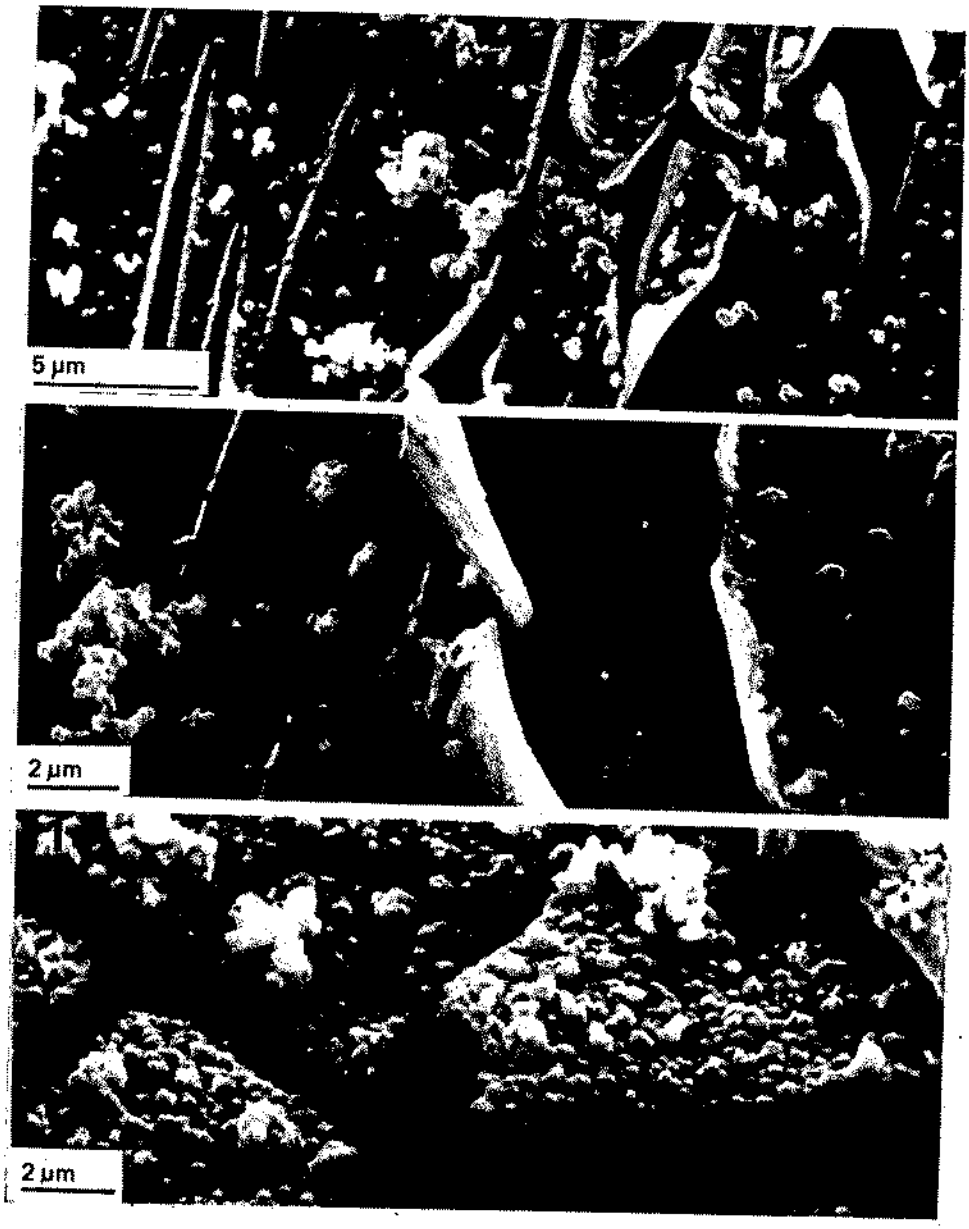

Plate 3: Nature of Conditioning Film. fig. 9:(45° tilt) the film is smooth; cracking follows polishing lines. Colonising bacteria are coccoid and occur singly of in small groups; fig. 10 (45°) shows peeling in more detail; the film is thin and the exposed steel appears etched; fig. 11 (45°) the film from around the corrosion site is thick and textured; few bacteria are attached.

irrespective of the preparation technique used - whether air-dried, lyophilised or alcohol-dehydrated. Figure 8, a high magnification micrograph of an area from figure 7, suggests that bacterial colonisation of the re-exposed steel surface may have occurred. Although this phenomenon was limited to this particular specimen it implies that peeling is not a preparative artefact.

Figures 9, 10 and 11 show the physical nature of the apparently non-corroding area, the film is smooth but exhibits lines running in one specific direction (see also figure 13). Splitting of this film seems to occur along these lines, which correspond to the polishing scratches on the steel. Where the surface of the steel has been exposed it has an "etched" appearance (figure 10) which is considerably different from the pretreated steel (figure 1). Spherical bodies of approximately 0.4µm diameter are closely associated with the film in this area. These bodies were presumed to be colonising bacteria, which was subsequently confirmed by TEM (see later in text). Measurements of the film indicate a thickness in the region of 0.16µm. The film around the corrosion site on the same specimen is shown in figure 11. It differs in character considerably. The surface is globular rather than smooth. Cracking has occurred, but in an apparently random pattern. The most noticeable difference is the thickness of the film, which may approach 1.6µm. The light micrograph (figure 5) indicates that this area is most likely to contain corrosion products and this may account for the observed increase in thickness.

Bacterial fouling: The vast majority of bacteria adherent to the surface were of a coccal morphology, with a considerable variation in cell dimensions, from 0.4 to 1.0µm in diameter (figure 12).

Distribution of bacteria across the steel surfaces was not uniform. Typically, the majority of the surface was only sparsely colonised, to the extent shown in figure 13. However, densely packed masses of bacteria (of the type shown in figure 12) were found but were ubiquitously confined to a narrow band in the centre of the specimens. This region was subsequently found to correspond to active sites of corrosion (see below). Between these two extremes were regions of an intermediate density of cells which were found in close proximity to the band of extensive colonisation. Figure 14 illustrates the delineation between the three types of area.

(3) Association between Microfouling and Corrosion

The surface of the specimen shown above was re-processed by the resin-stripping method in order to reveal the condition of the underlying steel (figure 15). Incomplete permeation of the resin into the film was caused by the gold coating from the specimen's previous examination in the SEM. As a consequence, removal of the gold-coated film was incomplete. Nevertheless, it is clear that the dense accumulations of bacteria visible in figure 14 directly correspond to the underlying areas of deep pitting corrosion, even to the extent that the discontinuities between the pits are

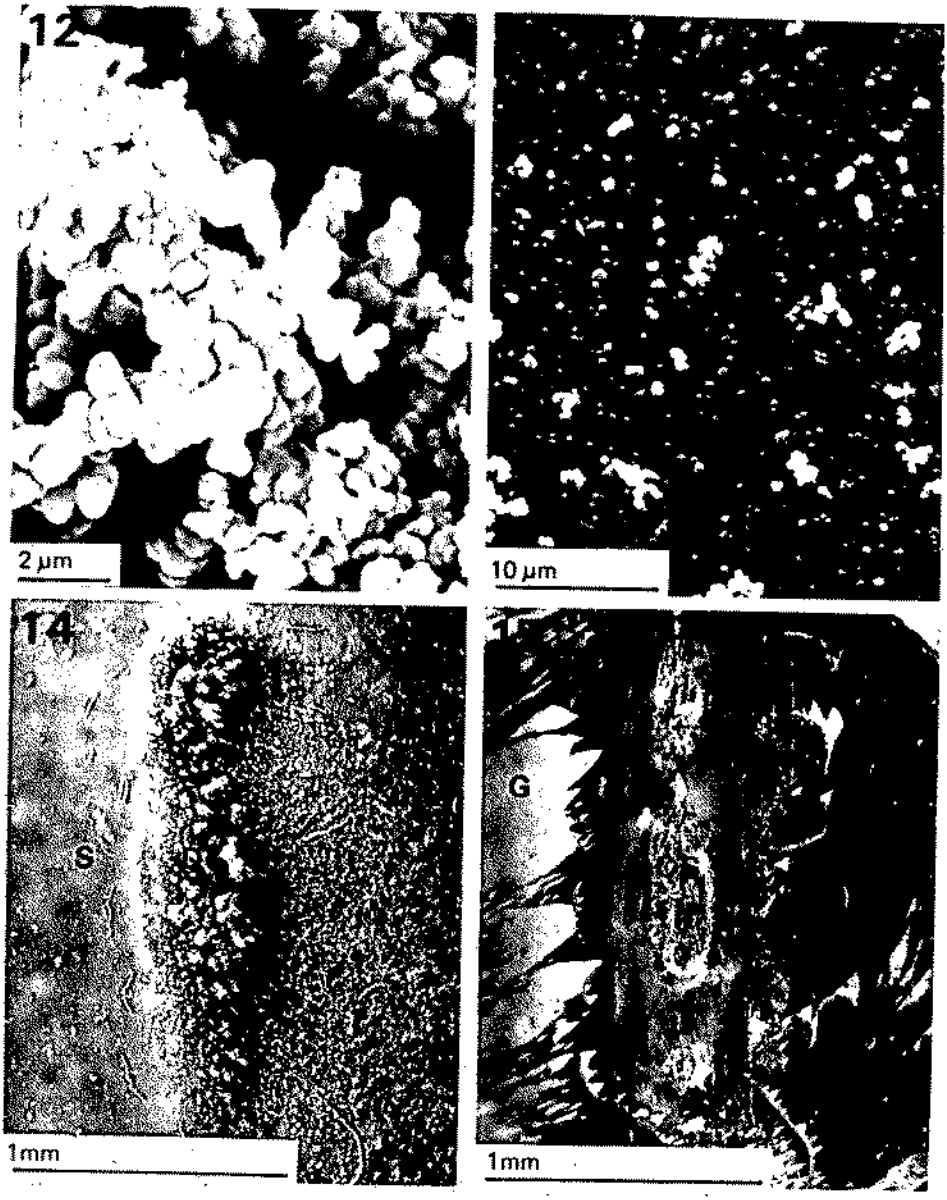

Plate 4: Bacterial Fouling. fig. 12: SEM of bacteria from area of densest colonisation; fig. 13: (45° tilt) SEM of typical area of the surface; bacteria occur singly of in small groups; fig. 14: low power SEM showing uneven colonisation of the surface into sparse (S), dense (D) and intermediate areas (I); fig. 15: the same surface resin-stripped to show location of corrosion sites (CS). Gold remnants (G) are from previous SEM examination.

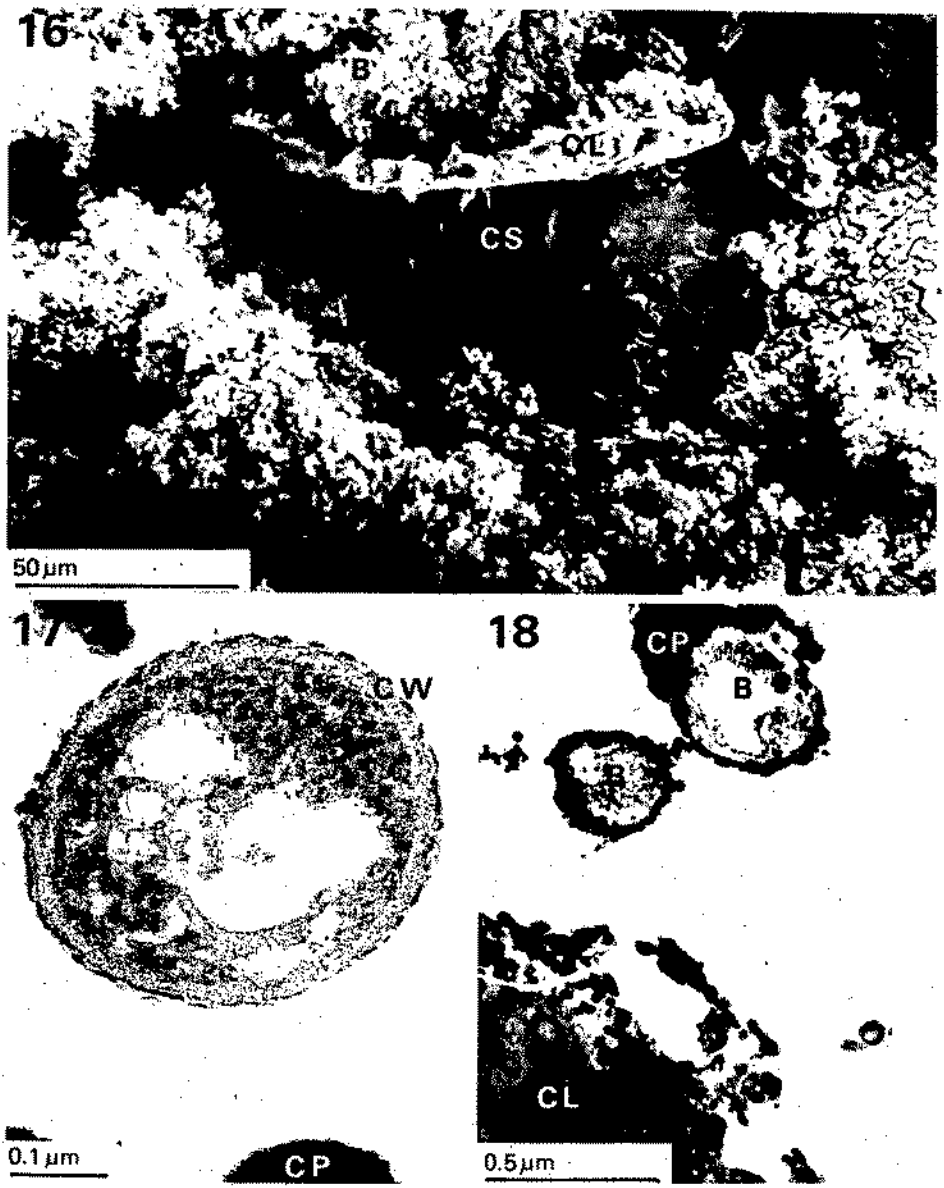

Plate 5: Association Between Microfouling and Corrosion. fig. 16: (45° tilt) SEM of an area overlying a deep corrosion pit (CS), showing thick corrosion layer (CL) and dense masses of associated bacteria (B); fig. 17 TEM of fouling bacterium; the cell wall (CW) is clearly visible as are corrosion products (CP); fig. 18: TEM of fouling bacteria (B) close to the granular corrosion layer (CL); the bacteria are surrounded by similar corrosion products (CP).

colonised to a lesser extent. Figure 16 illustrates the association between the corrosion site and the accumulation of dense layers of bacteria more directly. In this case the deep pits found in the central region of the specimen can be seen overlaid with a dense mass of bacteria which achieved a thickness in excess of 75μm. The layer comprising the conditioning film and corrosion products was found to be in the region of 5-6μm thick.

The intimate association between fouling bacteria and the layer of corrosion products was also demonstrated by transmission electron microscopy on embedded material from the early corrosion sites shown in figure 6. Figure 17 confirms that the coccoid forms seen on the scanning electron micrographs were indeed bacteria. Many of these organisms were found to have electron-dense granules associated with their outer surfaces (figure 18). The corrosion layer itself was found not to be continuous but appears from figure 18 to be formed from irregularly-shaped grains bound together to give the appearance of an intact layer.

An examination of areas beneath sparse and intermediate colonisation on the specimen shown in figure 14 revealed an association between the extent of colonisation and the state of the steel. Below areas of sparse bacterial colonisation the steel was virtually indistinguishable from the steel pre-exposure. In marked contrast, the surface of the steel below the areas colonised to an intermediate extent had an etched appearance similar to figure 10. In some cases the degree of etching was even more extreme, approaching that seen at the advancing edge of corrosion in figure 3.

DISCUSSION

Corrosion of the steel followed a typical pattern. As mechanical polishing produces an amorphous surface layer, the corrosion process could not initiate at preferred sites, such as grain boundaries (Carter,1977), but commenced at mechanical defects (i.e. polishing lines). In common with steel exposed to natural environments in general the subsequent progression of the corrosion process was confined to a localised attack resulting in pit formation. In the absence of major mechanical surface defects, the occurrence of deep corrosion pits at specific sites common to many of the specimens implies the presence of an inclusion within the material. Indeed a metallurgical examination of the steel from which these samples were derived has revealed the presence of manganese sulphide inclusions up to 300μm long running parallel to, or associated with, the pearlite strata (R. Procter, personal communication).

The biological observations from this study are of greater interest. The correlation between both the presence and extent of bacterial colonisation and the degree of corrosion is of particular significance. The heaviest bacterial deposits were always associated with the most corroded sites, the deep pits. The steel beneath areas colonised to an intermediate extent held features in

common with the earliest stages of corrosion, as judged from a comparison with the steel at the advancing edges of shallow corrosion pits. Sparsely colonised areas were characterised by an underlying intact steel surface. It might therefore seem that the colonising bacteria were playing a direct role in both the initiation and the progression of corrosion. Certainly their involvement in the progression of corrosion is most likely, as a result of non-specific processes atributable to the metabolism of the considerable mass of fouling bacteria (Miller and King, 1975). Other observations, however, deny the interpretation of any significant involvement of these bacteria in the actual initiation of the corrosion process. Particularly, it seems unlikely that extensive corrosion sites would form at the same place on different specimens other than as a result of the existence of recurrent flaws in the test material. Furthermore, in certain areas a conditioning film essentially deviod of a significant number of bacteria could be seen peeling away from a steel surface which demonstrated the charateristics of early corrosion. In many such areas propagation of cracks through the conditioning film followed the direction of polishing lines on the steel surface.

It is possible to put forward a more valid hypothesis to explain the observed events. Although the corrosion processes may be initiated on exposure to the marine environment, they progress only relatively slowly. The amorphous surface layer produced during polishing of the steel retards the process further by eliminating the more susceptible corrosion sites. In contrast, the essentially acellular conditioning film also begins to form on exposure of the surface to seawater (Baier, 1980) but forms a continuous layer within only a few hours (Neihof and Loeb, 1973; 1976), before a significant degree of corrosion has occurred. For whatever reasons, the corrosion process appears to localise at distinct sites. Consequently, as the steel surface below the conditioning film erodes, the film progressively loses its mechanical attachment. This may lead to the film peeling or being entirely lost in some areas, particularly where the corrosion process is proceeding most rapidly. To some extent this may be countered by the accumulation of corrosion products in the vicinity of such sites. The transmission electron micrograph of figure 18 illustrates the contribution of corrosion products in film formation. Indeed, although the corrosion film appeared from light microscopy to form an intact layer, this figure shows it to be composed of discrete granules, presumably bound together by the same type of organic material which comprises the macromolecular conditioning film. Transmission electron microscopy also illustrated that the accumulation of the layer of corrosion products occurred independently of significant numbers of bacteria. The scanning electron micrograph of figure 11 is similarly illustrative of this lack of involvement by the initial colonising bacteria.

The evidence found in these experiments suggests that the association of colonising bacteria with corrosion sites is an effect of the corrosion process rather than being its cause. That such

large accumulations of bacteria should be associated with the corrosion sites implies that these areas confer a selective advantage on these organisms. The dense mass of bacteria implies a rapid growth rate and a nutritional advantage might be an appropriate explanation for their accumulation. The possibility has been considered that these organisms were metallo-oxidising bacteria (iron bacteria), based on their occurrence, morphology and the presence of corrosion products on their surfaces (see Cullimore and McCann,1977). Preliminary attempts to isolate the organisms on this premise, using supplemented marine agars containing iron (II) sulphate, have so far proved unsuccessful.

In a more general assessment of the experimental observations, the question arises as to their relevance to the fouling and corrosion of offshore structures. From a materials standpoint, in preparing the steel to give reproducibility between specimens, the surfaces presented for colonisation were unlike any on actual stuctures, in terms of their intrinsic nature (i.e. perpendicular sections) or state (uncorroded, highly polished, unprotected). From a biological standpoint, the use of an aquarium system is open to criticism. Although the resident macroscopic fauna (mussels, barnacles, tunicates, sea-anemones) is a reasonably representative macrofouling community it is more difficult to assess the validity of the resident microflora. However, considering that the aquarium used is deviod of exposed metallic surfaces which could select for organisms with the observed activities, it seems extremely unlikely that the coccal bacteria associated with the corrosion sites are merely charateristic of the laboratory system. Nevertheless, at this stage such an interpretation cannot be discounted, making an examination of evidence from the marine environment a priority. Direct extrapolation from the findings to the field is thus limited. Of potentially greater significance than specific details are the general trends:

(1) the corrosion process appears to actively enhance colonisation and proliferation of bacteria to give dense cell masses up to 75µm thick over a time scale where on inert surfaces only diffuse microcolonies have accumulated (c.f. Dempsey, 1981a);

(2) the greater densities of bacteria over the corrosion site can enhance corrosion for all the indirect reasons previously mentioned (see Miller and King, 1975);

(3) the dense masses of bacteria can bring about a local transition to conditions more usually associated with a later stage of fouling, thereby providing a favourable environment at an early stage for organisms, such as sulphate-reducing bacteria, which have a more aggressive role in corrosion.

In engineering terms, localised corrosion is of greater concern that a more generalised and even attack because it is more likely to lead to structural failure. The ramifications of the points summarised above are that any site prone to localised corrosion, such as inclusions or regions which are subject to stress (see La Que, 1975; Carter, 1977), will be colonised by bacteria several orders of magnitude more rapidly than unaffected material.

Conditions provided by these organisms will ensure that the corrosion process not only remains localised at that site but also that it progresses more rapidly, particularly if secondary colonisers such as SRB's are involved. In this manner a type of positive-feedback mechanism exists whereby the corrosion site, once formed, accelerates its own corrosion making mechanical failure even more likely.

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Interactions between microfouling and the calcareous deposit formed on cathodically protected steel in seawater.

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ABSTRACT- The composition of deposits formed on unprotected and cathodically protected steel in seawater has been studied using scanning electron microscopy and x-ray analysis. Rust products on unprotected steel were progressively depleted of oxygen nearer the steel surface. The scale formed on protected steel had a layered structure, with magnesium hydroxides and carbonates nearest the steel, aragonite above this and calcite furthest from the steel. The formation of these deposits is discussed in the light of studies of the microfouling on the surfaces.

RESUME- On a etudie la composition et la nature de depots se formant, dans l'eau de mer, sur la surface de plaques d'acier non protege et d'acier protege cathodiquement. On s'est servi des techniques de microscopie electronique et d'analyse crystallographique aux rayons X. Les produits de rouille formes sur l'acier non protege ont progressivement reduit la teneur en oxygene a proximite de la surface de l'acier. On a trouve que l'ecaille calcaire formee sur l'acier protege etait stratifiee. Tout contre la surface de l'acier se trouvait de l'hydroxide ou du carbonate de magnesium, par-dessus se trouvait de l'aragonite, et enfin du calcite formait la couche exterieure. On discute la formation de ces depots a la lumiere d' observations faites sur les microorganismes qui encrassent la surface des plaques d'acier.

INTRODUCTION

Marine growth on offshore structures for oil and gas production in the North Sea has been much greater, and the general conditions more severe, than was first expected. This has caused concern over the possible effects of the fouling and a renewed interest

in the relationship between fouling and corrosion (Houghton, 1978 Ralph and Troake, 1930

The corrosion of metal in seawater is a surface microelectrochemical reaction process. This process involves a series of active anodic and cathodic areas on the surface of the metal. The anodic process for a metal M:

$$M \rightarrow M^{n+} + ne$$

involves a dissolution of metal ions from the anode surface. The simultaneous cathodic process on a metal in seawater is:

$$O_2 + 2H_2O + 4e \rightarrow 4(OH)^-$$

In the most simplistic situation the hydroxyl ions generated at cathodic sites, react with the metal ions generated at anodic sites, to produce the characteristic corrosion products.

A range of methods are used to protect offshore structures from corrosion (Bartlett, 1977; Ridler, 1977; Hogkiess, 1978). Above the waterline, in the area subject to atmospheric corrosion, the platforms are coated with paint formulations containing corrosion inhibitive compounds. The submerged zone is protected either by impervious coatings or by cathodic protection, or a combination of both. An additional alloy sheathing is sometimes used to protect the splash zone.

Cathodic protection systems, the main form of protection in the submerged zones, are based on manipulating the electrochemical nature of the corrosion reaction. The anodic reaction is supressed, and the cathodic reaction promoted over the whole metal surface. This is brought about by the introduction of electrical currents from external sources to counteract the normal corrosion reaction. The effect of such an applied current is to reduce the areas that act as anodes; protection being achieved when all the anodes have been extinguished (La Que, 1975). In practice complete cathodic protection is achieved when the potential of the metal has been shifted by about 200 mV more negative than the open circuit (free corrosion) potential.

Two systems can be used as the source of the protective current, a system of sacrificial anodes or the use of an impressed direct current. A sacrificial anode can be any metal which is substantially anodic to the metal to be protected, thus forming a galvanic cell. The anode material normally used is an alloy of zinc, aluminium or magnesium. The anodes have a limited life as they are preferentially corroded. An impressed current system uses a direct current driven through the structure by a D.C. generator. Inert or expendable anodes complete the circuit through the seawater.

MICROFOULING AND CARBONATE SCALE

Both the cathodic protection systems generate a high pH at the cathodic surface, due to the formation of hydroxyl ions by the oxygen reduction reaction, and pH values of up to 11.5 have been recorded adjacent to the protected metal surface (Smith and Mattson 1975). Since the solubility of most inorganic compounds, especially calcium and magnesium carbonates and magnesium hydroxide, decreases with increasing pH, and the carbonic acid equilibrium of seawater will be upset, calcareous deposits will be formed on the protected steel. The thickness of such scales is related, at least initially, to the current density and exposure time (Wolfson and Hartt, 1981) and, once formed, the calcareous scale is practically insoluble at normal seawater pH values. The scale deposits act as barriers to oxygen, and thus help to slow down any corrosion reaction if the protection system becomes ineffective. The high resistance of these scales help to 'throw' the cathodic protection to areas further from the current source (Doremus and Davis, 1967), lengthens the life of sacrificial anodes, and reduces the current density requirements of impressed current systems (Wilkins 1982).

If the cathodic protection systems worked perfectly, then there would be little concern about the effects of fouling on corrosion below the low water mark. However both protection systems have problems with installation and maintenance and have generally shown poor reliability in the hostile environment of the North Sea (Eliassen and Valland, 1979). Thus the calcareous scale becomes very important in protecting the underlying metal when a protection system fails. The scales effectiveness will depend on its composition, porosity, thickness, integrity and other variables. These in turn are affected by the cathodic current density, the flow rate of seawater, temperature and the presence of corrosion product.

The presence of biological fouling may affect the formation and attachment of the scale in many ways. Recent studies have shown that macroalgae, such as Enteromorpha, easily establish on cathodically protected steel and can cause considerable disruption to the calcareous scale when removed (Edyvean and Terry, 1983). Larger algal holdfasts and invertebrate fouling such as barnacles and serpulids appear to grow through any calcareous scale and attach to the steel underneath to the extent of removing bright metal when detached (Terry pers. comm.). Fouling organisms can have a considerable effect on the structure, composition and mineral form of the scale by the large amounts of organic molecules such as polysaccharides and other excretory products they produce (Kitano and Hood, 1965; Kitano et al, 1975; Borowitzka, 1977; Wilkins, 1982). Cathodically protected steel in seawater appears to be more quickly colonised by bacteria and microalgae than unprotected steel (Moss, 1981), and thus there is considerable microfouling activity at the same time as the

calcareous scale is being deposited.

Many studies of cathodic protection systems have been carried out in artificial seawater and there is a need for more research into the effects of biological factors on the formation of calcareous scales (Wilkins, 1982). The deposition of calcareous scales is also important in the corrosion fatigue cracking of steel in seawater (Crisp, 1977, Sterry 1981) and it is only recently that natural seawater, complete with its organic loading, has been used for such tests.

This study examines the effects of the microenvironments created by marine fouling on both unprotected and cathodically protected steel. The physical effects were studied using the scanning electron microscope while EDAX (Elemental detection) and x-ray crystallographic analysis methods were used to determine the physical and chemical composition of both the corrosion products formed on unprotected steel and the calcareous scale formed on protected steel when interacting with the organisms.

MATERIALS AND METHODS

Test plates, 8 x 2.5 x 0.3 cm in size, were cut from BS4360/43A steel. These were polished to a bright metal surface, finished on several grades of emery paper followed by carborundum powder, degreased in Decon 90 surfactant for 30 minutes, rinsed in distilled water, then ethanol, and finally air-dried. The plates were exposed either unprotected or cathodically protected. Cathodic protection was achieved using impressed current to -0.95 v s.c.e. or sacrificial anodes (zinc alloy to U.S. Mil. Spec. or Galvallum 111 aluminium alloy). Test plates were immersed in continuous-flow seawater tanks at the Dove Marine Laboratory, Cullercoats, Tyne and Wear.

SEM observations were made on specimens prepared either from specially prepared SEM stubs cut from steel rod (BS4360/43A), or from samples of the calcareous scale removed from test plates. Fixation was in 4% glutaraldehyde in cacodylate buffer for 24 hours, followed by washing in cacodylate buffer and then through an alcohol or acetone series to 100% and either air or critical point dried (Edyvean, 1982). Specimens were coated in carbon or gold and examined in a JSM T20 SEM. EDAX (energy dispersive x-ray analysis) was carried out using a Cambridge Stereoscan Mk. II SEM with an EDAX 707 attachment. The nature of compounds forming on the steel was determined by x-ray crystallographic analysis carried out by the Department of Crystallography at the University of Newcastle-upon-Tyne or at British Petroleum's research laboratories, Sunbury-on-Thames.

RESULTS

Unprotected steel - Unprotected steel rapidly produced a loose, friable corrosion product which built up with time and gradually became colonised by microalgae. After 100 days immersion the corrosion product was consolidated and considerable algal microfouling was found on the surface (see Edyvean and Terry, 1983). The corrosion product at this stage consisted of three distinct layers. Closest to the steel was a dense, firmly attached, hard black layer, above this was a firm dark brown layer and externally there was a loose red rust layer. These layers could be separated and examined using EDAX and x-ray crystallographic techniques (Figs. 1 and 2). EDAX analysis of the loose 'red' form, furthest from the steel showed iron peaks, with chlorine, silicon and calcium peaks indicating the presence of fouling organisms (Fig. 1A). Analysis of this rust using x-ray diffraction found two types of iron oxides, mainly lepidocrocite (γ $Fe_2O_3.H_2O$) and wustite (FeO), together with sodium chloride (NaCl) (Fig. 2A). The firmer dark brown layer which was highly consolidated, but still easily removed from the steel, showed stronger iron peaks than the outer layer, less chlorine and silicon but more calcium and a strong sulphur peak (Fig. 1B). X-ray diffraction analysis showed the compounds to be goethite (α Fe_2O_3 H_2O) and lepidocrocite (γ Fe_2O_3 H_2O) with lesser amounts of magnetite (Fe_3O_4), iron sulphide (FeS) and sodium chloride (NaCl) (Fig. 2B). The firmly attached black corrosion product closest to the steel surface, showed very pure iron peaks with no other elements standing out from the background, on EDAX analysis (Fig. 1C). This was shown to be almost entirely magnetite (Fe_3O_4) when analysed by x-ray defraction. On the undersurface of this layer some isolated patches of calcium carbonate as aragonite were found, presumably at cathodic sites in the initial corrosion reactions.

Protected steel - Steel specimens in seawater, protected either by sacrificial anodes or by impressed current developed a calcareous scale deposit on the surface. The average thickness of the scale, calculated from weight and surface area data, corrected for the weight of fouling, showed a linear increase reaching around 0.075 mm in 150 days (Fig. 3). The maximum thickness of scale, calculated from micrometer readings of 50 samples was 0.24 mm (SD 0.04) after 150 days. It is unlikely that this linear increase in scale thickness is maintained, although there is no direct comparison, the thickness of scale on sacrificial anode protected steel after 1000 days is 0.231 mm (maximum 0.52 mm including fouling such as small serpulid worms).

X-ray diffraction analysis of samples of scale showed them to be made up of magnesium hydroxides and carbonates and calcium carbonate mostly in the trigonal polymorph, calcite, with some

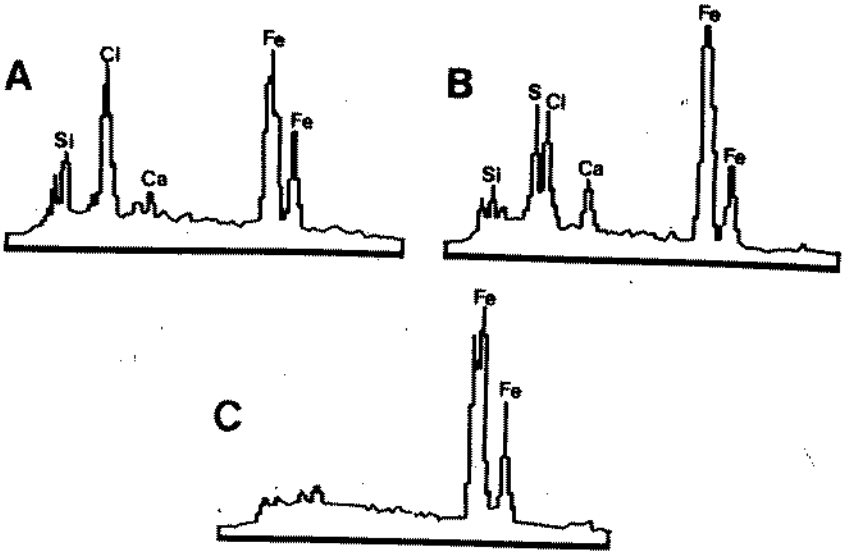

FIGURE 1. EDAX analysis of rust product. A. Outer layer. B. Middle layer. C. Inner layer.

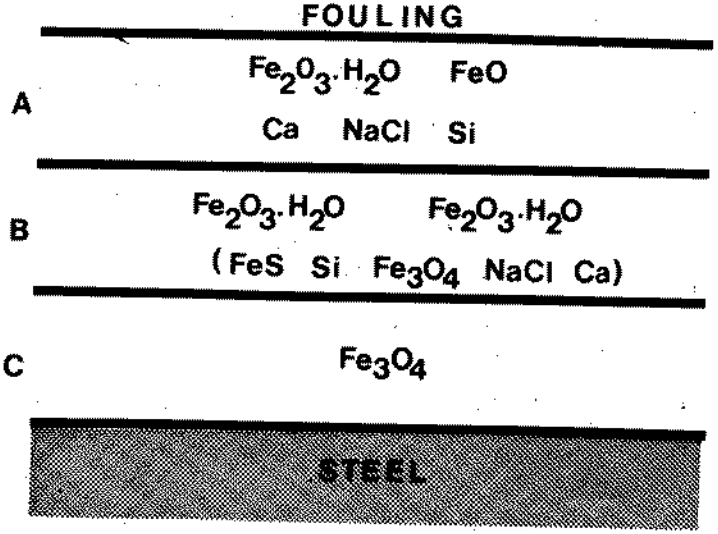

FIGURE 2. Composition of rust product formed on unprotected steel.

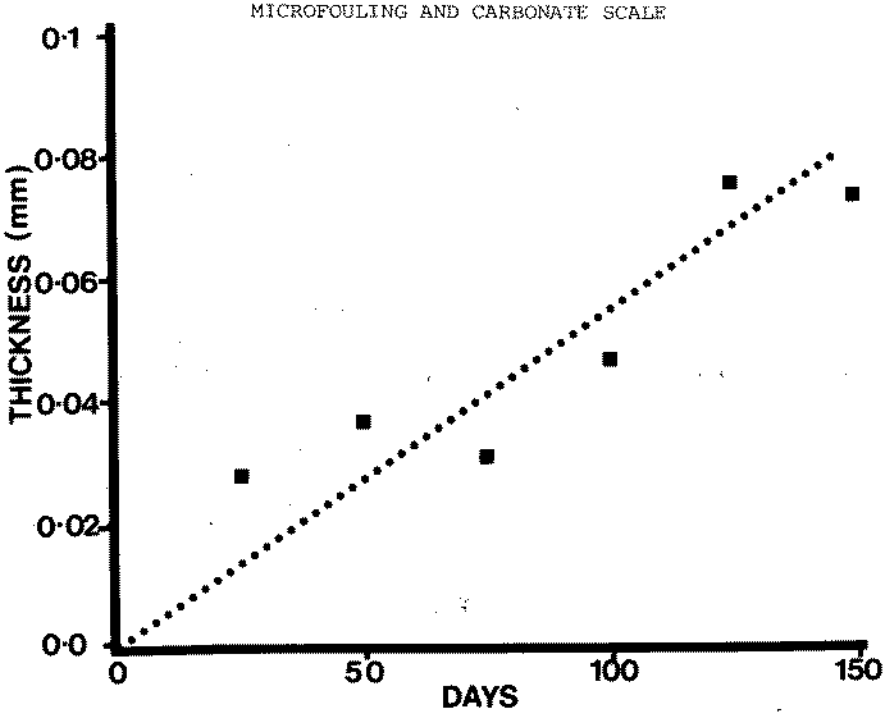

FIGURE 3. Increase in thickness of scale with time (Impressed current protected steel).

in the orthorhombic-pseudohexagonal polymorph, aragonite. Traces of sodium chloride were also found.

EDAX analysis of samples of the scale (Fig.4) shows that magnesium is the dominant element of the scale closest to the metal surface, with the scale furthest from the metal made up entirely of calcium compounds. The distribution of compounds in the scale, determined by x-ray diffraction, is shown in Figure 5. Magnesium hydroxides and magnesium carbonates are deposited first followed by calcium carbonate as aragonite and then calcium carbonate as calcite. An extreeme example of initial magnesium rather than calcium deposition was found in one set of impressed current experiments in which the scale formed in two distinct layers, the lower layer consisting entirely of magnesium hydroxides and carbonates. The upper layer had a lower surface of magnesium carbonate changing to calcium carbonate at the upper surface. The layered structure may be due to a break in the protection current during the experiment. EDAX analysis of cross sections of the scale showed that magnesium and calcium compounds were separated into definite regions rather than a gradation from one to another.

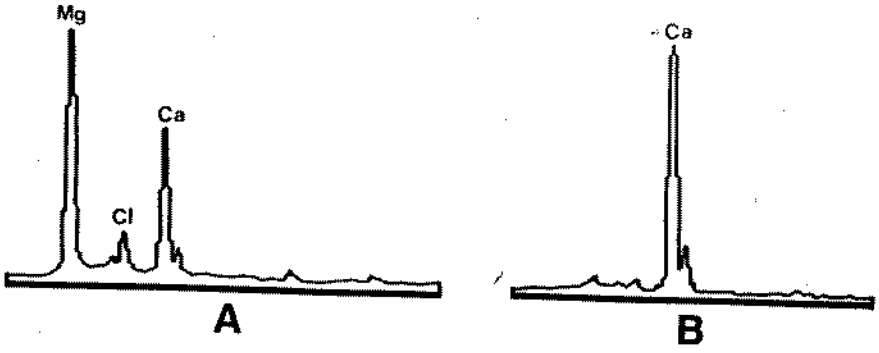

FIGURE 4. EDAX analysis of scale formed on protected steel. A. Nearest steel surface. B. Further from the surface.

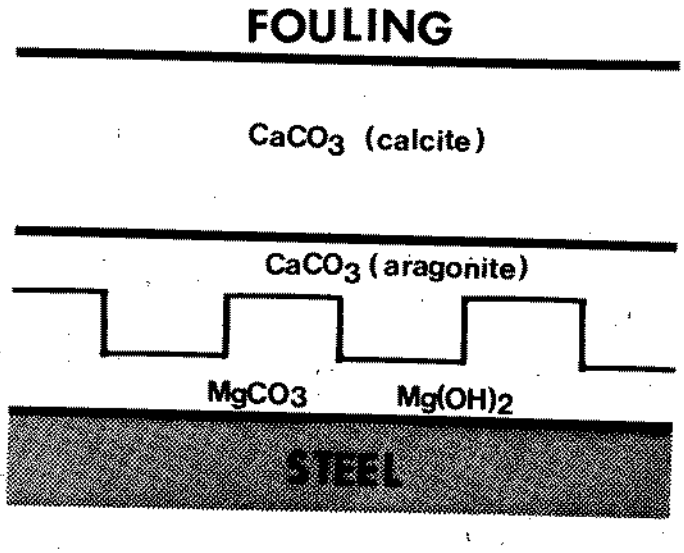

FIGURE 5. Composition of scale formed on a protected steel surface.

MICROFOULING AND CARBONATE SCALE

Fouling - Fouling by microscopic organisms is rapid on both unprotected and protected steel, though species diversity and initial density is greater on protected steel. After 50 days exposure the microfouling on both substrata was very similar and, while there were seasonal differences in the numbers and species present, the microfouling consisted of bacteria, fungi, diatoms and blue-green algae, as well as algal spores and invertebrates such as choanoflagellates and small ascidians. Microfouling organisms produce copious mucilage and often form a dense mat covering the surface. On unprotected steel the fouling organisms, such as diatoms (Plate 1.A) become emeshed in the corrosion product or cover the surface in colonies (see Edyvean and Terry, 1983). This has the effect of binding the corrosion particles in mucilage, trapping silt and organic debris from the seawater and consolidating the corrosion product onto the steel surface.

Fouling organisms readily settle on cathodically protected steel. The calcareous scale forms rapidly and organisms settling on its surface become trapped as more of the scale is precipitated (Plate 1.B). Once entrapped, scale deposition continues round the organisms (Plate 1.C) and may eventually completely enclose them. Large ammounts of mucilage (Plate 1.D) and associated bacteria, will also become incorporated into the scale.

DISCUSSION

Unprotected steel specimins showed high accumulations of corrosion product, consisting mainly of lepidocrocite ($\gamma Fe_2O_3.H_2O$) with some wustite (FeO). After 100 days, the distinctly layered structure observed consisted of an outer layer of lepidocrocite and wustite under which was a firmer layer consisting of gothite ($\alpha Fe_2O_3.H_2O$), lepidocrocite and some magnetite (Fe_3O_4) and iron sulphide. Closest to the metal surface was an adherent layer of magnetite (Fig.2). These compounds are typical of the products formed on rusting steel in both seawater, estuarine water (Booth et al.,1965) and freshwater (Smith and McEnaney,1979). The tendancy for less oxidised products such as magnetite to form close to the metal surface is consistant with findings that these products are formed in low oxygen environments from an initial ferrous hydroxide corrosion product (Smith and McEnaney,1979, Nagayama and Cohen,1962). Smith and McEnaney (1979) also found evidance for a reductive dissolution of lepidocrocite to magnetite. A layer of fouling, even microalgae embedded in copious mucilage could provide the low oxygen conditions for these reactions to take place. Microfouling has a significant role in consolidating corrosion products on steel (Edyvean,1982) and considerable mats of microalgae have been found on the surfacs of corrosion products (Edyvean and Terry, 1983).

Booth et al.(1965) found the presence of iron sulphide and sulphate indicative of microbiological participation in corrosion,

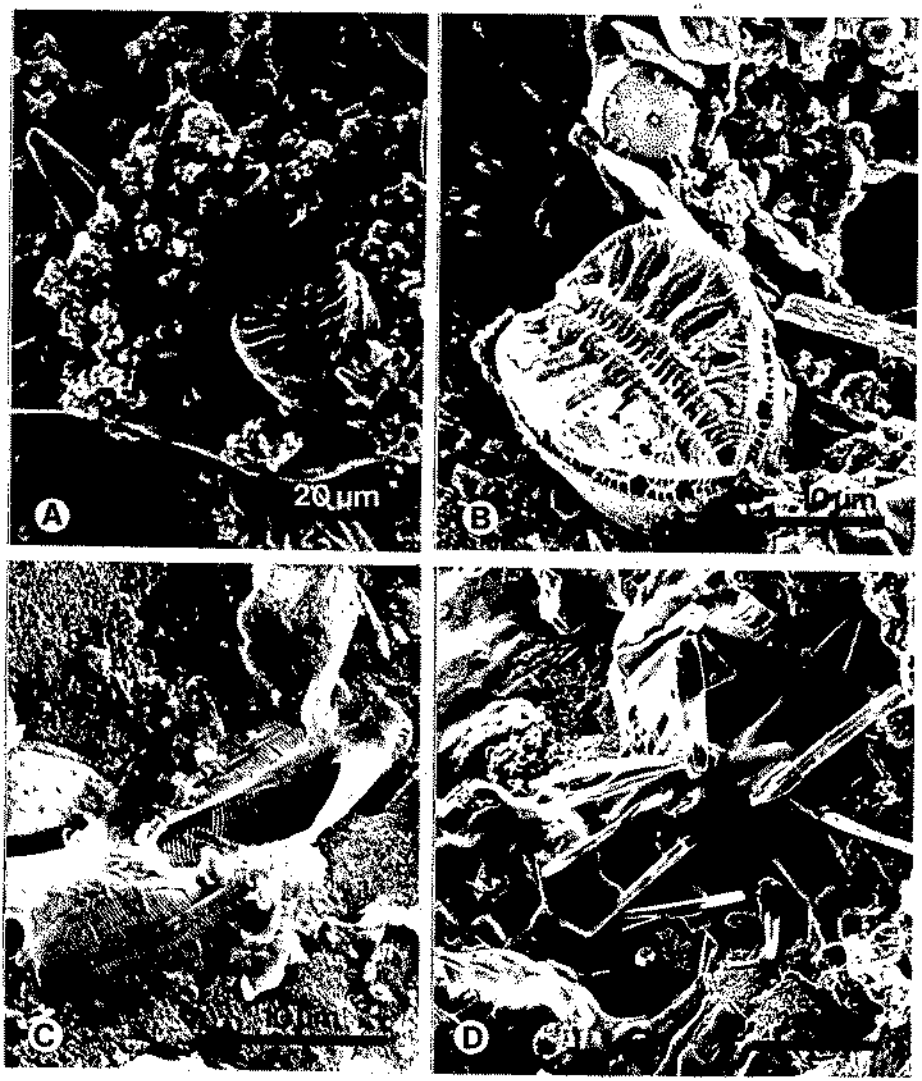

Plate 1 - Microfouling on steel. A. Diatoms (D) trapped in rust product. B. Diatoms (M = Melosira. C = Camplyodiscus sp.) trapped by carbonate scale on protected steel. C. Diatom remains becoming buried in carbonate scale. D. Benthic diatoms producing copious mucilage over carbonate scale. All after 100 days immersion.

usually when heavy biological growth occurred. Both magnetite and iron sulphide can be cathodic to steel, magnetite by 0.5 volts (La Que,1975). Such compounds can cause considerable localised corrosion given suitable conditions. Nagayama and Cohen (1962) found magnetite in active regions of corrosion and an oxide film consisting of an inner layer of magnetite and an outer layer of lepidocrocite in passive regions. A corrosion product consisting of lepidocrocite is therefore likely to be protective, while one high in magnetite and/or iron sulphide is likely to be agressive.

The deposition of calcareous scales is one of the most important factors in the protection of steel in seawater, both naturally, at cathodic sites of galvanic couples and artificially produced by cathodic protection techniques. The scale is not deposited evenly, and its thickness and composition can vary widely with current density and from region to region on the metal surface (Wolfson and Hartt,1981). The maximum thickness of scale found in these results was 0.24mm in 150 days (mean=0.074mm). Assuming a linear growth rate, the data for average thickness (Fig.3) would indicate a thickness of ≈0.2mm after one year at -0.95v(SCE). This agrees well with Wolfson and Hartt (1981) from whose data a rate of 0.44mm per year can be predicted at a potential of -1.03v(SCE) and 0.22mm per year at -0.93v(SCE). These calculations can only act as a guide, as scale deposition is influenced by many factors and is unlikely to remain linear.

The calcareous scale forms as the solubility of its constituent compounds decreases with the increasing pH brought about by the cathodic reaction encouraged by the protection systems. Changes in pH will also influence other aspects of seawater chemistry, chemical forms of metals, their ions and complexes, charge and other characteristics of amphoteric substances. Although, in theory, the higher the current density the better the scale barrier, hydrogen evolution at high current densities will cause the scale to break up (Wolfson and Hartt,1981). High amounts of hydrogen are also detrimental to the steel, entering the metal and causing hydrogen embrittlement and subsequent stress corrosion cracking. Biologically produced anaerobic slimes may also be conducive to hydrogen embrittlement, by containing any hydrogen produced by over protection and thus encouraging corrosion.

Once a scale has formed the magnitude of current density required to maintain a particular potential reflects the protective capacity of the scale (its specific resistivity) and the fractional coverage of the surface (Wolfson and Hartt,1981). A steady state will usually be achieved where the rate of deposition is balanced by the rate of break away or formation of cracks in the deposit. The presence of microalgae and other organic inclusions can alter the specific resistivity and weaken the scale, resulting in a higher current density being required to maintain protection.

Subsequent decay of organic material will provide sites for anaerobic bacteria with possible detrimental effects on the underlying steel. Larger algae and invertebrates can have a considerable effect on the substratum. Algal rhizoids and barnacles may disrupt surfaces and removal of algal hold-fasts has been shown to damage and carry away portions of the calcareous scale (Edyvean and Terry, 1983). Once the protective scale has been disrupted, the conditions under it are likely to provide an environment favourable for bacterial growth and the concentration of aggressive metabolites. Even when not disrupted, the scale cannot be expected to give complete protection and will allow some degree of passage to water, oxygen and ions.

There are many factors which influence the deposition of the scale, such as the ratio of inorganic ions, the rate of crystallisation, pH, fluctuations, temperature and the presence of inorganic solutes (Kitano et al., 1975). The deposition of magnesium rather than calcium nearest the metal surface, can be explained as the result of a higher initial pH at the metal surface which favours magnesium rather than calcium deposition. This magnesium initially deposits as the hydroxide which gradually converts to the carbonate (Digby, 1979). Klass (1958) found that while calcium carbonate was always predominant in the scale, the percentage of magnesium hydroxide increased with increasing applied potential. The deposition of calcium carbonate as aragonite followed by calcite, after the initial magnesium deposition, is more difficult to interpret. The influences determining which polymorph precipitates are complex. It is generally accepted that aragonite is normally deposited in seawater due to the presence of magnesium ions (Kitano et al, 1975; Pentecost, 1980) and this is certainly the case in artificial seawater (Hammonds pers. comm.). Magnesium is thought to retard calcite deposition by entering the calcite crystal lattice causing it to be considerably more soluble than aragonite on which magnesium has no effect. However, seawater is a complex environment and many factors will influence its chemistry. For example, Kitano (1962) found that at low temperature ($\simeq 10^{o}C$, the average temperature in this work), even in the presence of magnesium, 40% of calcium carbonate would precipitate from bicarbonate solution as calcite. At higher temperatures the precipitate was nearly all aragonite. Kitano also reports that if precipitation is rapid, aragonite will form, but if slow then calcite will form (Kitano and Hood, 1965). Kitano gives the following examples of factors influencing the polymorph of calcium carbonate:- the presence of certain inorganic ions in the mother solution (including H^{+}, OH^{-} and CO_2), the influence of organic material, enzymes, bacteria, mechanical conditions and temperature. Unfortunately there are many contradictions in the literature as to which form is favoured by various factors, possible due to slight differences in experimental conditions (Kitano, 1962a).

MICROFOULING AND CARBONATE SCALE

The presence of fouling on the protected steel surface has a considerable influence on the local environment, and this may be the reason that calcite deposits after aragonite. Apart from decomposition processes, by which a variety of enzymes and other compounds are released there is a wide range of extracellular products of living microalgae. These include malic, oxallic, glycollic and lactic acids, amino acids, proteins and polysaccharides (Fogg, 1966). Kitano and Hood (1965) studying calcium carbonate precipitation from solutions containing organic material, found compounds such as citiate, pyrurate, malate, lactate, succinate and glycogen to cause calcite deposition even in the presence of magnesium. Other compounds such as galactose dextrose and acetate did not influence the form precipitated. The mechanism by which some organic compounds act to favour calcite precipitation is thought to be due to a slowing down of the precipitation rate which favours calcite rather than aragonite, though other factors contribute (Kitano and Hood, 1965). Lippmann (1973) considers that the precipitation of calcite in the presence of magnesium and organic molecules may be due either to inactivation of Mg^{2+} ions by complexing with the organic compounds, the organic compounds obstructing nucleation of aragonite, or being selectively adsorbed onto aragonitic surfaces so that growth of aragonite is inhibited.

CONCLUSIONS

Organic material and the activities of marine fouling organisms have a considerable influence on both unprotected and protected steel. The aragonite found initially on protected steel may be due to a combination of an initial high reaction rate, lack of microfouling and the presence of magnesium. Once the microfouling begins to establish the influence of organic compounds increases and this may explain the subsequent calcite deposition. There is a growing realisation that the influence of natural as opposed to artificial test environments must be taken into account when studying all aspects of corrosion and protection. Further study is needed on the formation and nature of scale on protected steel in natural environments.

ACKNOWLEDGEMENTS

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ETUDE ELECTROCHIMIQUE D'UN ACIER DANS DES CULTURES DE BACTERIES SULFATO-REDUCTRICES. I - MECANISME DE CORROSION

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Polarization curves have been obtained for mild steel in sterile culture media with or without sulphides. They have been also plotted in cultures of sulfate-reducing bacteria (Desulfovibrio-desulfuricans). A protective film is formed on steel in the presence of bacteria or sulphides. Initiated or previously existing crevices induce passivity breakdown that promotes a galvanic cell between the bare metal and the protective film.

Nous avons tracé les courbes de polarisation d'un acier dans des milieux de cultures stériles additionnés ou non de sulfures et dans des milieux inoculés par des bactéries sulfato-réductrices (Désulfovibrio-desulfuricans). Il se crée sur l'acier en milieu inoculé ou en présence de sulfures, un film de passivation. La rupture du film par initiation d'une crevasse ou la présence de crevasses déjà existantes, crée un couple entre le métal mis à nu et la couche passive.

I N T R O D U C T I O N

La croissance des bactéries sulfato-réductrices dans un milieu est basée sur une réduction dissimilatrice des sulfates présents, en sulfures. Des composés organiques tels que pyruvate, malate, fumarate, glycérol peuvent constituer les sources énergétiques qui leur sont nécessaires, mais le donneur d'électrons le plus courant est le lactate. La réaction impliquée dans cette "respiration" anaérobie peut être représentée par :

$$2\ CH_3\ CH\ OH\ COO^- + SO_4^{2-} \longrightarrow 2\ CH_3COO^- + 2\ CO_2 + S^{2-} + 2\ H_2O$$

Ces bactéries sont à l'origine de la corrosion métallique en milieu anaérobie. La théorie la plus ancienne et la plus généralement acceptée les mettant en cause est celle de la dépolarisation cathodique proposée par Von WOLZOGEN KUHR et Van der VLUGT ([1]). La réaction générale de corrosion du fer en milieu aqueux désaéré ($Fe + 2\ H^+ \longrightarrow Fe^{2+} + 2\ H$) est rapidement arrêtée après obtention d'un film d'hydrogène sur la surface métallique. Les bactéries stimuleraient donc la corrosion en utilisant l'hydrogène formé sur le fer pour réduire les sulfates ($SO_4^{2-} + 8\ H \longrightarrow S^{2-} + 4\ H_2O$). S^{2-} et éventuellement OH^- réagiraient à leur tour sur Fe^{2+} pour donner FeS et $Fe(OH)_2$. En fait, toutes les espèces de bactéries sulfato-réductrices ne possèdent pas une hydrogénase permettant la réaction de réduction des sulfates par l'hydrogène et il ne semble pas y avoir de relation directe entre l'activité hydrogénasique de la bactérie et la vitesse de corrosion ([2]). COSTELLO attribue donc l'effet de dépolarisation cathodique à l'hydrogène sulfuré formé par les bactéries ([3]).

BOOTH et coll ([4])([5]) proposent un mécanisme de dépolarisation cathodique d'une part par les bactéries, d'autre part par le sulfure de fer formé en solution. Le deuxième facteur étant prépondérant dans le phénomène de corrosion.

Le sulfure de fer est encore incriminé par KING et coll ([6]) qui suggèrent la création d'une pile entre celui-ci et le fer, FeS fonctionnant comme cathode.

Plusieurs auteurs constatent la formation initiale d'un film, partiellement protecteur sur les échantillons ([3]) ([9]). La rupture de ce film, attribuée à la transformation de la couche obtenue ([7])([8]), serait responsable de la corrosion.

Enfin IVERSON ([10]) après filtration stérilisante d'une culture de bactéries sulfato-réductrices et précipitation des sulfures, met en cause un métabolite corrosif contenant du phosphure de fer qui attaquerait les échantillons.

Le travail présenté dans ce mémoire a pour but de rechercher le mécanisme de corrosion de l'acier par ces bactéries en effectuant des études électrochimiques respectivement dans un milieu de culture, stérile, inoculé ou additionné de sulfures.

PARTIE EXPERIMENTALE

Echantillons -

L'acier utilisé est un acier de chaudronnerie A 48. Sa composition centésimale est la suivante : C 0,150, Mn 1,170, Si 0,380, Ni 0,160, Cr 0,150, Mo 0,020, S 0,0014, P 0,008, Cu 0,130, Al 0,028, Fe le restant. Il a un niveau de striction élevé dans le sens travers court (> 65 %) ce qui garantit un faible taux d'inclusions. L'examen métallographique des surfaces ([11]) montre uniquement la présence d'inclusions de type oxydes globulaires. Le nombre moyen d'in-

clusions par champ est de 54 dont 36 d'un diamètre voisin de 2 µ, 15 de 8 à 10 µ et 3 de 20 à 25 µ.

Les échantillons ont été prélevés sur une tôle de 100 mm d'épaisseur, dans une zone comprise entre 3 et 25 mm sous la peau de la tôle, les faces planes des échantillons étant parallèles aux peaux. Les éprouvettes de corrosion sont des cylindres de 7 mm de diamètre et 5 mm de hauteur, la surface en contact avec la solution est de 180 mm^2. Ils sont polis sur papier émeri jusqu'au grain 1200, nettoyés aux ultra-sons, rincés à l'alcool et séchés à l'azote juste avant leur introduction dans la solution. Pour les tracés des courbes E = f(t), il est essentiel d'avoir un très bon état de surface. Un polissage supplémentaire de l'échantillon sur pâte diamant 3µ donne des résultats très reproductibles.

Cellule électrochimique -

La cellule en verre a un volume moyen de 70 ml (fig.1). Entre le couvercle et le corps de cellule, un joint assure l'étanchéité et les rodages comportent des rodets téflon. L'électrode de référence est est une électrode au sulfate mercureux, plongeant dans une allonge de

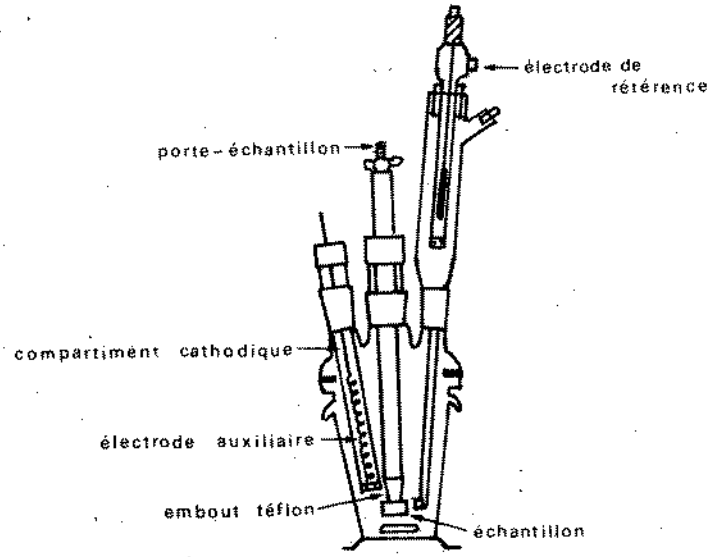

Fig.1 - Schéma de la cellule électrochimique

verre (remplie d'une solution saturée de $SO_4 K_2$) terminée par un capillaire de Luggin. L'électrode auxiliaire en platine est placée dans un compartiment séparé, obturé par un verre fritté. Les échantillons sont montés sur une tige filetée, englobée dans un tube de verre, muni d'un embout téflon.

Stérilisation -

La cellule et le bulleur (utilisé pour le dégazage), l'allonge de la référence, le porte-échantillon et le compartiment de l'électrode auxiliaire sont stérilisés à l'autoclave à 110°C pendant 20 minutes. L'électrode auxiliaire est stérilisée au four à 150°C pendant 1 heure. Tous les éléments sont passés rapidement dans la flamme d'un bec bunsen avant d'être introduits dans la cellule.

Culture de bactéries -

La souche bactérienne utilisée a été isolée d'un circuit incendie et identifiée à l'espèce Desulfovibrio desulfuricans (fig.2). Nous l'avons purifiée par la méthode des dilutions successives dans un milieu gélosé (milieu F de POSTGATE ([12])en tubes profonds ou en boîtes de Pétri incubées à 28°C dans une jarre anaérobie. Nous avons dû ajouter de la kanamycine, à une concentration de 50 ppm dans le milieu gélosé, pour éliminer un contaminant aérobie strict qui était très étroitement associé à la souche sulfato-réductrice et qui résistait en atmosphère d'anaérobiose.

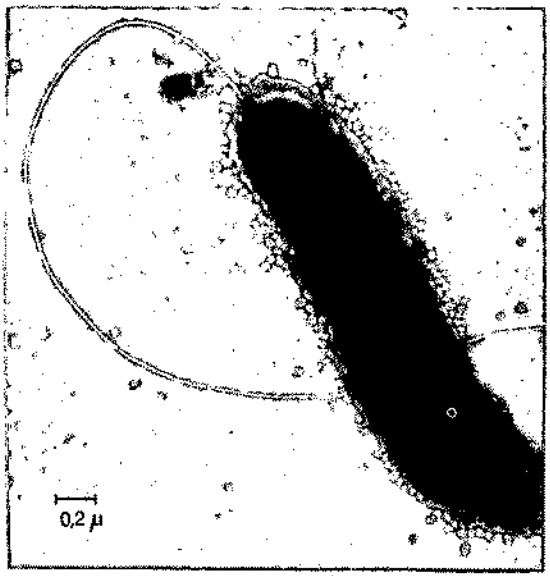

Fig.2 - Photo prise au microscope électronique à transmission (coloration négative)

Les précultures, les cultures, les tracés de courbes de polarisation ont été menés sur le milieu C de POSTGATE ([12]). La composition par litre d'eau permutée est la suivante : KH_2PO_4, 0,5 g ; NH_4Cl, 1,0 g ; Na_2SO_4, 4,5 g ; $CaCl_2 . 6 H_2O$, 0,06 g ; $MgSO_4 . 7 H_2O$, 0,06 g ; lactate de sodium à 50 %, 12 ml ; extrait de levure, 1 g ; $FeSO_4 . 7 H_2O$, 0,004 g ; citrate de sodium. $2 H_2O$, 5 g ; pH final 7,5.

Les précultures sont réalisées en flacon hermétique dans le milieu pendant 3 à 5 jours. Les milieux stériles sont ensuite inoculés dans la cellule d'étude avec 2 ml de préculture.

Au cours des essais, nous avons vérifié la pureté de la souche (absence de contaminants aérobies sur gélose nutritive). Nous avons déterminé la concentration en bactéries sulfato-réductrices par dénombrement en milieu liquide (dilutions décimales à raison de trois fioles par dilution et détermination du nombre le plus probable à l'aide des tables de Mac Grady). Le milieu utilisé pour ces dénombrements est le milieu "Sulfato-réduction NICB", réparti en fioles pénicilline à raison de 4,5 ml par fiole et purgé à l'azote.

La concentration en germes à l'inoculation est de l'ordre de 5.10^6 à 10^7 par millilitre de solution, dans la cellule électrochimique. Au moment de l'inoculation de l'échantillon (après trois jours d'incubation), elle est de 10^8 à 5.10^8. bactéries sulfato-réductrices par millilitre de solution.

Préparation des solutions -

Le milieu de culture est introduit dans les cellules, puis préalablement à toute manipulation, il est dégazé à l'argon stérile pendant 30 minutes. Son potentiel redox est de -0,1 V.

Pour l'étude du milieu stérile, on introduit l'échantillon dans la solution et on désaère à nouveau 5 minutes. La préparation des solutions de sulfures se fait en ajoutant Na_2S au milieu C, le pH étant ajusté avec H_2SO_4 concentré, l'éprouvette est ensuite plongée dans la solution. Pour l'étude du milieu inoculé, on introduit dans la solution C 2 ml de préculture ; la cellule fermée hermétiquement est laissée pendant 3 jours en incubation sans agitation à 28°C. Après ce laps de temps, on introduit rapidement l'échantillon par le rodage central. Le pH de la solution est alors de 7,2, son potentiel redox de -0,5 V.

Tous les essais sont réalisés en étuve thermostatée à 28°C + 0,5°C, les solutions sont agitées (sauf pendant l'incubation) et chaque cellule est maintenue dans une enceinte balayée par un courant d'azote.

Tracé des courbes de polarisation -

Le tracé des courbes de polarisation est effectué avec un ensemble Corroscript SOLEA-TACUSSEL. Les solutions sont fortement agitées pour limiter les phénomènes de diffusion. Les voltampérogrammes sont obtenus par balayage anodique ou cathodique, à partir du potentiel à courant nul de l'échantillon, sur des solutions différentes. La vitesse de polarisation est de 0,45 $V.h^{-1}$. Dans le cas des courbes cycliques, nous avons choisi d'effectuer un balayage retour des potentiels lorsque l'intensité anodique atteinte est de 1 $mA.cm^{-2}$. Un enregistreur EPL_2 muni d'un tiroir potentiel TACUSSEL, type TVED, nous a permis de suivre l'évolution du potentiel de l'acier en fonction du temps.

Dosage des espèces sulfurées -

La somme globale des espèces sulfurées formées, a été obtenue après trois jours d'incubation par dosage avec une solution d'argent en présence de NH_3. La réaction de dosage est suivie potentiométriquement à une électrode à membrane indicatrice d'ions (type PS 3M TACUSSEL).

R E S U L T A T S

I. Allure générale des courbes intensité-potentiel

Les courbes de polarisation de l'acier en milieu stérile, juste après immersion sont données fig.3. La courbe anodique présente schématiquement l'allure des courbes de polarisation obtenues pour les métaux passivables avec cependant une zone passive restreinte. La barrière anodique obtenue à partir d'un potentiel de -0,43 V est associée à une corrosion de l'échantillon qui se manifeste préférentiellement au contact du métal et de l'embout téflon du porte-échantillon.

Si la solution stérile n'est pas désaérée, la courbe $i = f(E)$ est peu différente de la précédente ; par contre, si la solution est saturée en oxygène, la courbe de polarisation se limite à une barrière anodique (fig. 3.a.2).

La courbe cathodique (fig.3.b) tracée en partant du potentiel de corrosion a une pente très élevée. Elle correspond à la réaction de réduction des citrates suivant $H\,Ci^{3-} + e^- \longrightarrow Ci^{4-} + H_2$. En milieu aéré, les courants sont légèrement plus élevés traduisant une intervention de l'oxygène comme capteur d'électrons.

Dans une solution inoculée, après trois jours d'incubation, la concentration en espèces sulfurées est d'environ 10^{-2} M. Les bactéries ont donc réduit environ un tiers du sulfate initial, les sulfures se trouvant essentiellement sous forme de HS^- et H_2S. Si on effectue un dégazage juste avant immersion d'un échantillon, la courbe anodique présente un faible pic d'activité (fig.4.a.2) ; la zone passive est plus marquée et la barrière se rapproche d'autant plus

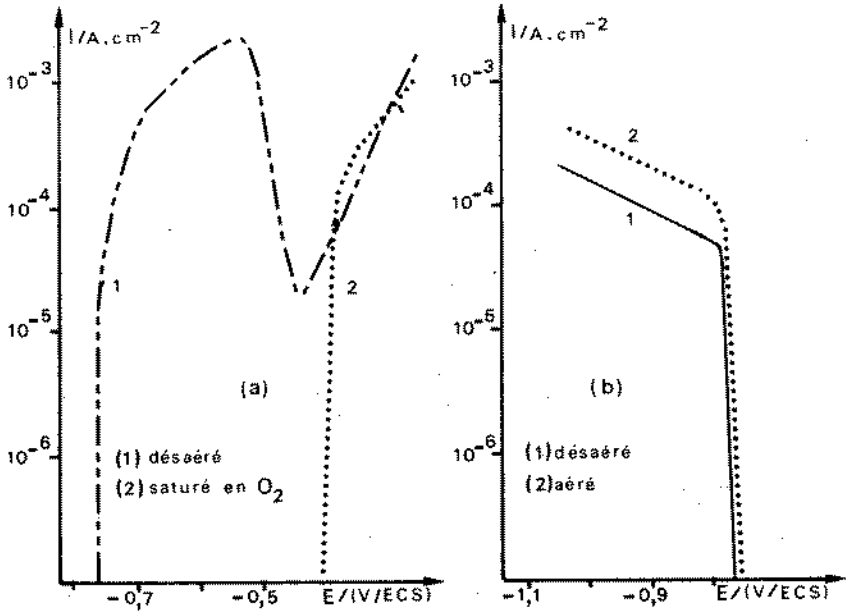

Fig.3 - Voltampérogrammes en milieu stérile, (a) anodiques, (b) cathodiques.

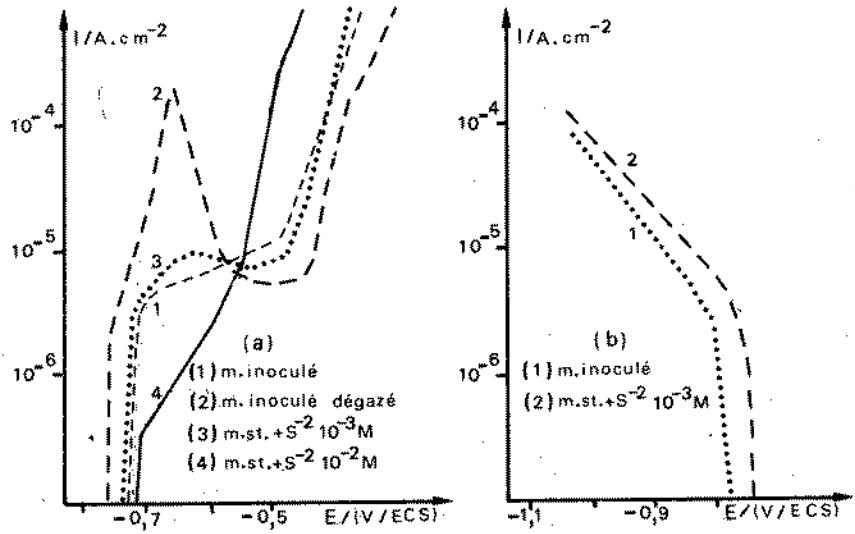

Fig.4 - Voltampérogrammes en milieu inoculé et milieu stérile + S^{2-}, (a) anodiques, (b) cathodiques.

de celle obtenue en milieu stérile, que le dégazage est important.

Si la solution n'est pas dégazée après inoculation, le voltampérogramme anodique se caractérise par la suppression du pic d'activité : la courbe ne comporte qu'un courant limite sensiblement constant caractéristique d'un phénomène de passivation (fig. 4.a.1). Pour un potentiel voisin de E_{ic} (-0,47 V), l'intensité augmente brutalement traduisant une corrosion qui se manifeste sous forme de crevasses visibles à l'oeil nu sur n'importe quelle face de l'échantillon.

Nous avons pensé que le comportement des éprouvettes pouvait être lié à la présence de sulfures solubles, formés en solution par les bactéries. Nous avons donc tracé les courbes de polarisation anodique de l'acier dans le milieu stérile contenant du sulfure de sodium (fig. 4.a et 5.a). La courbe obtenue en milieu Na_2S 10^{-3}M présente une forte analogie avec celle relative au milieu inoculé. Le courant de passivation augmente brusquement avec l'apparition de corrosion localisée à un potentiel E_{ic} (fig.5.b). Dans une solution 10^{-2}M de S^{2-} (fig.4.a.4), le mur anodique se déplace vers des potentiels moins nobles et le changement de pente de la courbe correspondant à E_{ic} est plus difficile à détecter.

Etant donné que la corrosion se manifeste sur les échantillons sous forme de crevasses, nous avons effectué des polarisations cycliques pour étudier ce mode de corrosion ([13] à [15]). Dans le milieu inoculé, on polarise l'acier dans le sens des potentiels croissants, et après avoir atteint sur la barrière une intensité limite (égale ici à 1 mA.cm^{-2}), on change le sens de balayage des potentiels.

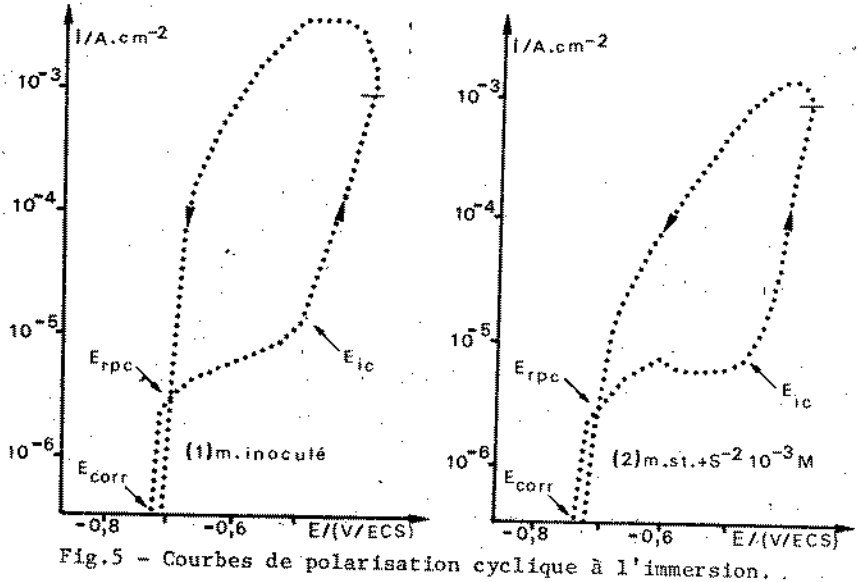

Fig.5 - Courbes de polarisation cyclique à l'immersion.

La courbe retour présente un fort degré d'hystéresis (fig.5.1) et ne rejoint la courbe aller que pour une valeur E_{rpc} (-0,69 V) proche du potentiel de corrosion (E_{corr}). E_{ic} et E_{rpc} sont respectivement les potentiels d'initiation et de repassivation des crevasses. Le fait que E_{rpc} ait une valeur voisine du potentiel de corrosion E_{corr} entraîne une impossibilité de repassivation des crevasses existantes, ou initiées à E_{ic}. Lors du tracé retour, on observe une précipitation importante de sulfure de fer (le métal passe en solution au niveau des zones locales mises à nu et réagit avec les sulfures solubles formés en solution par les bactéries).

De même, dans des solutions de Na_2S (fig.5.2.), la courbe retour ne rejoint le tracé direct que pour une valeur de potentiel E_{rpc} (-0,71 V) proche de E_{corr} quelle que soit la concentration en sulfures. Le tracé retour s'accompagne de la précipitation de sulfure de fer.

Les courbes cathodiques obtenues en milieu inoculé et en présence de sulfures sont peu différentes (fig.4.b). Elles suivent la loi de Tafel (β_c = 135 mV. decade $^{-1}$) et indiquent une polarisation importante par rapport aux courbes obtenues en milieu stérile.

2. Evolution du potentiel de l'acier dans les solutions en fonction du temps

En milieu stérile, l'aspect de l'échantillon et son potentiel évoluent peu, en fonction du temps (fig.6.1.), si les cellules sont maintenues dans une enceinte balayée par N_2. Si cette précaution n'est n'est pas prise, l'oxygène peut diffuser dans la cellule et en présence de citrate et de phosphate, l'acier devient métastable ou passif.

Sur des échantillons immergés pendant quelques jours en milieu inoculé, on observe la formation d'un film de base qui ternit l'acier et d'un faible dépôt noir peu adhérent, précipité localement (le même constat sera fait en milieu stérile contenant des sulfures solubles). Le potentiel de l'acier varie en fonction du temps, en fonction de son état de surface, mettant bien en évidence l'influence de crevasses existantes. Si l'échantillon est rayé volontairement, poli sommairement au papier 1200, on constate une faible évolution du potentiel en fonction du temps ; la valeur finale se stabilise suivant les essais entre -0,62 et -0,71 V (fig.6.3.). Si on polit les éprouvettes avec soin, au papier 1200 ou encore mieux à la pâte diamant 3μ , la variation de E est de 0,3 V (fig.6.2.). Le potentiel E_{max} atteint (-0,46 V) a une valeur proche de E_{ic} obtenu à partir des courbes i = f(E). L'échelle des temps est variable suivant les essais mais une dizaine d'heures est nécessaire pour atteindre E_{max}. WANKLYN et coll ([16]) n'avaient observé qu'une variation de potentiel de 0,1 V en milieu inoculé.

En milieu stérile additionné de sulfures (fig.6.4.), le potentiel E_{max} est plus faible qu'en milieu inoculé. En fait les deux

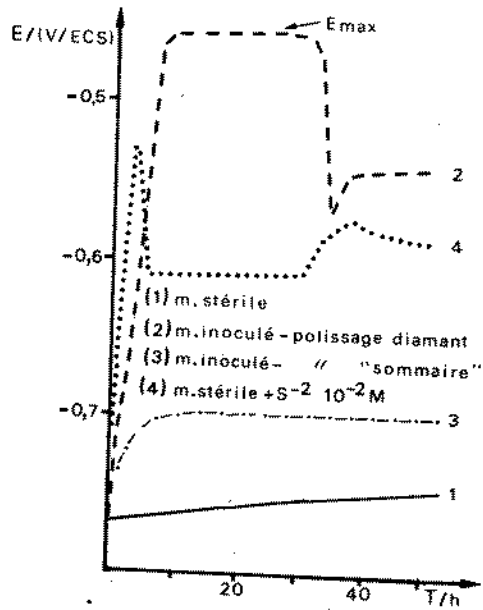

Fig.6 - Evolution du potentiel des échantillons en fonction du temps.

milieux ne sont pas complètement identiques. La solution inoculée contient de l'acétate et du CO_2 produits par les bactéries et son pH augmente de 7,2 à 7,7 après immersion des échantillons.

DISCUSSION

Dans un milieu inoculé avec des bactéries sulfato-réductrices, l'acier est soumis à deux actions contradictoires : d'une part, il peut se passiver, d'autre part cette passivation peut être détruite si des crevasses apparaissent. Des résultats sensiblement similaires ayant été obtenus en milieu inoculé et en milieu stérile additionnés de sulfures, on peut attribuer la passivation à l'action des espèces sulfurés ; celles-ci sont produites dans les milieux de cultures par les bactéries à partir des sulfates présents.

Il est possible d'envisager deux mécanismes de passivation. On peut supposer que les ions HS^- en solution s'adsorbent chimiquement à la surface du métal, gênant ainsi la réaction cathodique de réduction d'H_2S ($H_2S + 2e^- \longrightarrow H_2 + 2\ SH^-$) et stoppant l'attaque de l'acier. D'après Le BOUCHER (17), le dégagement d'hydrogène dans des solutions d'H_2S ne s'effectuerait que par passage préalable des protons de la solution dans le métal, passage catalysé par les ions HS^- adsorbés. D'autre part, comme la répartition énergétique des sites d'adsorption est exponentielle, la courbe cathodique en milieu sulfure ou inoculé suit la loi de Tafel.

On peut supposer aussi, qu'au cours de l'attaque anodique, on dépasse la limite de solubilité du sulfure de fer dans la couche liquide au voisinage de la surface métallique. Ce sulfure précipite alors, assurant une certaine protection. SHOESMITH et coll [18] après étude de la corrosion du fer dans une solution d'H_2S à pH = 7 attribuent la passivation à la formation d'un film de sulfure, la mackinawite. La description des échantillons correspond à ce que nous observons. D'autres auteurs confirment l'obtention de ce sulfure entre pH 6,5 et 8 lors de la corrosion du fer par des solutions d'H_2S ou lors de la réduction bactérienne des sulfates [20] [8].

La disparition de la passivité a fait l'objet de plusieurs hypothèses. D'après MEYER [8], le film de mackinawite perdrait son pouvoir protecteur en se transformant en une écaille de mackinawite de conductivité électronique plus élevée ; simultanément, la diffusion serait plus facile aux joints de grains lors du grossissement des cristallites. Il est possible aussi d'envisager la rupture du film de base comme liée à la transformation de la mackinawite en greigite [19] ou en pyrrhotite et pyrite [8], mais cette éventualité nécessiterait des conditions de pH différentes de celles de notre milieu et des temps plus longs que ceux de nos essais. SHOESMITH [18] pense qu'en milieu H_2S, le film de base se rompt préférentiellement aux défauts macroscopiques de la surface métallique, entraînant alors la corrosion par H_2S des zones mises à nu.

Nous pensons qu'en milieu inoculé, la passivation est due à l'adsorption de HS^- suivie de la formation d'un film de sulfure de fer. La corrosion ultérieure serait liée à la rupture de ce film, quand, après évolution de son potentiel, l'acier atteint une valeur proche du potentiel de crevasse. Le fer mis à nu joue alors le rôle d'anode et il y a couplage avec la zone de métal passivé qui fonctionne comme cathode. Les ions Fe^{2+} engendrés à l'anode réagissent avec les ions S^{2-} en solution pour donner du sulfure de fer précipité. L'attaque étant amorcée par crevasse initiée ou existante, l'échantillon ne peut plus se repassiver et sa valeur de potentiel est celle donnée par le couplage. Ceci explique que dans les courbes E = f(t) données par certains auteurs, les sauts de potentiel soient si faibles [16] [21]. Etant donné le rapport des surfaces défavorables petite anode-grande cathode, les densités de courants anodiques peuvent être très importantes.

C O N C L U S I O N

Nous avons montré que le mécanisme de corrosion de l'acier dans un milieu de culture inoculé avec des bactéries sulfato-réductrices (Desulfovibrio-desulfuricans) est proche de celui obtenu dans des solutions de sulfures solubles au même pH. La corrosion est donc liée à la présence de H_2S et HS^- dans le milieu. Ces espèces provoquent la passivation du métal dont le potentiel évolue jusqu'à une valeur caractéristique où s'initie une crevasse, entraînant la

formation d'une pile : fer (anode) - métal passif (cathode). Dans le cas de crevasses déjà existantes, la passivation s'effectue localement et le métal constitué d'une série d'anodes et de cathodes atteint rapidement le potentiel de couplage.

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PRESENT STATE OF TECHNOLOGY FOR CONTROL OF MARINE CORROSION AND FOULING IN TROPICAL INDIAN WATERS

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Abstract

Sea water corrosion is a complicated phenomenon in the tropical environment owing to complex nature of compositional, physical and electrochemical factors associated with both sea water and metals. Added to these, a factor of marine biofouling and bacterial activity in tropical waters makes the marine deterioration problem more difficult and challenging. The corrosion behaviour of one of the metals, namely mild steel, has been widely studied in tropical waters. The corrosion rates in splash zone and underq uiet sea water have been found to be of higher order than recorded elsewhere under temperate climate. Importance of biofouling and bacterial activity has also been recognised with great concern particularly in tropics. In Indian waters the rate of generation of fouling debris has been found to be of the order of 8 to 9 kg per sq.m. per year. In respect of mild steel, maximum penetrtion due to pitting experienced in various harbours in India is of the order of 1.0 to 1.5 mm/year and is therefore more severe than those reported elsewhere. The discharge of ill-treated orgapic pollutants into the sea has added one more dimension to the problem of sea water corrosion. In this environment, the erratic behaviour of galvanic anodes is of particular concern.

During the past decade, fouling and corrosion preventive technologies involving both marine anticorrosive and antifouling coatings and cathodic protection systems appropriate to Indian conditions have been developed and their performance assessed for a long period. These technologies have shown equivalent performance to those in the developed countries.

Introduction

Sea water is the largest natural reservoir of highly conducting electrolyte and its contact with man-made structural metals results into a most active electro-chemical energy dissipating system. The electrolytic action of sea water permits initiation and propagation of interfacial reactions leading to corrosion processes. These corrosion processes are capable of easy destruction of metals and alloys irrespective of their high strength and toughness.

Much has been said about the sea water corrosion, its mechanism and possible preventive measures. However, despite large amount of literature, many corrosion failures continue to occur under the offshore and onshore field conditions. The mechanism of many of these failures still remains less understood. Sea water corrosion is a single major economical problem having significant impact on the industrial activities, human health and defence efforts and therefore both government, as well as non-governmental agencies are highly concerned about it.

One of the early and extensive studies carried out in major industrial countries like USA, UK, France and Russia relate to the qualitative and quantitative estimation of corrosivity of sea water and its control [1-4]. In India, Naval Chemical and Metallurgical Laboratory [NCML], Bombay with her field stations located at three major ports of naval interest, namely Bombay (18.58°N), Cochin (9.57°N) and Visakhapatnam (17.38°N) has been actively engaged in sea water corrosion studies. The corrosion rates of the major fabrication materials like mild steel for instance, have been found to be varying and the data collected for one water mass is invariably found to be inapplicable to other marine environment. A popular concept of sea water being an invariant fluid characterised by constancy of proportion in its chemical composition, therefore, is only partially valid in context of its corrosive effect on metals. This basic observation is of utmost importance in industrially developing country such as India and must be given due weightage in designing new marine structures. The information generated elsewhere could be adopted only as a guideline and effort must be made to gather appropriate data that is relevant to local conditions. With the advent of offshore oil exploration efforts and fabrication of coastal power generation plants in our country, the assessment of corrosivity of structural steel and structural metals has assumed great importance. The paper elucidates the sea water characteristics relevant to corrosion of structural steel and aims at emphasing the need of creating a pool of information having relevance to our requirements.

Sea Water – A Corrosive Environment

Corrosivity of metals in sea water environment is a relative term since it is influenced by a variety of factors like temperature, salinity, dissolved oxygen, pH, the material itself and application to which it has been put to use. In majority of onshore and offshore marine constructions, structural carbon steel finds maximum application for the reasons that are well known. Plain low carbon steels are reported to corrode in sea water environment at rates ranging from 1 to 30 mils per year (mpy) depending upon the areas, a surf zone to ocean bottom, where these are exposed. Corrosion rate of mild steel is maximum at surf and

splash zones and decreases with increasing water depth. Each of these depths has its characteristic physico-chemical profile. Further, these water qualities also regulate biological activity which in turn influences the metallic corrosion to a great extent. Chemical composition of plain carbon and low alloy steel, has very little influence, if any, on its corrosion rate because the corrosion kinetics is mainly governed by cathodic process comprising reduction of dissolved oxygen in sea water [5]. The limiting rate of oxygen diffusion to substrate at a given temperature, pressure and sea water flow has been universally accepted as a controlling factor of steel corrosion in natural sea water. It is, therefore, appreciated that the consideration such as strength and toughness alone of a given marine design may lead to erroneous predictions with regard to design life.

In calculating the design life, realistic corrosion rate calculated over extended periods are estimated. The data based on prolonged marine exposures are necessary because the corrosion rates averaged for shorter durations have been found to be exceedingly high and therefore unrealistic. In reality on long exposures these values decrease exponentially [1,6] and stabilise to a more or less constant value [Fig. 1]. This constant corrosion rate averaged over extended periods of about 4 to 8 years has been considered realistic for estimating the design life of structures exposed continuously to sea water. Bultman and Southwell [7] have suggested a following empirical relationship which would find use in design life calculations.

$$P_t = P_1 + Rc\ (t - 1)$$

where P_t = average penetration in mils for exposure period t, Rc = stable corrosion rate in mpy and t = time in years.

Bultman and Southwell adopting this formula have predicted average penetration of 32 mils over a period of 10 years in tropical Panama Canal zone. This figure would work out to 71 mils in Indian tropical waters. Corrosivity expressed in terms of average corrosion rate is also misleading because corrosion also manifests itself in the form of ptting that propogates in an unpredictable manner, leading to ultimate perforation. Despite low average corrosion rate higher pitting corrosion is possible [1,6].

Corrosion in Indian Harbours

The exposure study extended over a period of 8 years has revealed that the corrosion behaviour of mild steel at three stations along the western and the eastern coasts of India is similar. Exposed steel panels were covered with heavy deposits of fouling organisms, important among these were barnacles, 'Balanus amphitrite' along the wester coast and mussel 'Mytiloptis sallie' on the eastern coast. The growth of these organisms in Indian tropical waters has been found to be very severe than that observed in temperate water. The average corrosion rate of mild steel was observed to stabilise in 4 years period and was in the range of 6 to 7 mpy. Corrosion manifested mostly in the form of large sized pitting. This pitting characteristics are revealed in Fig.2 and 3. In one year period, maximum penetration due to pitting was found to be of the order of 60 mils. Pitting penetration was about 7 times more than the average penetration based on weight

loss measurements. This higher rate of penetration was attributed to higher temperature (25° to 30°C), higher amount of dissolved oxygen (5.5 ml/litre), tidal movements and heavy settlement of fouling organisms [6].

Table No.I gives comparison of corrosion rates in various parts of the world including India. It is worthwhile to examine the data on average corrosion rates in the context of physico-chemical properties particularly temperature, dissolved oxygen and biofouling growth. It would be observed that corrosion rates of structural steel between the latitudes 8.55°N and 55.8°N are not uniform. Dexter and Couberson [8] state that the global variations in physico-chemical properties influence the overall corrosion behaviour.

These authors have stated that though the seawater possess amazing constancy of proportions in respect of chemical composition, its pH, dissolved oxygen and temperature vary at different geographical locations. The thermodynamic and kinetic parameters related to corrosion reactions therefore are likely to vary causing differences in corrosion rates. The fact that dissolved oxygen is a function of temperature and overshadows the temperature effect on kinetics, it is the oxygen diffusion rate that readily decides the corrosion rate.

Table I
Annual Corrosion rates of mild steel in sea water
at different North Latitudes

Exposure location	North latitude	1 year corrosion rate	8 year corrosion rate
Panama Pacific*	8.55	5.8	2.7
Panama Carribean*	9.21	3.6	0.3
loco solo			
Bombay	18.58	8.0	7.0
Visakhapatnam	17.38	7.02	6.4
Key West,* Florida, USA	24.35	3.7	2.9
Kure Beach *	33.85	5.7	2.2
Emsworth, England *	50.80	4.0	2.1

*Data quoted from reference 7

Bultman and Southwell (7) have attributed the corrosion of mild steel in seawater to a regulating activity of biofouling organisms. This fact has also been noted by De (9). De and co-workers have also pointed out the effect of sulphate reducing bacteria in marine coastal environment as a controlling factor for accelerated corrosion (10, 11). In one of unpublished work of NCML (12) it is noted that simultaneous presence of anaerobic and aerobic bacteria in partially anoxic condi-

tions results into higher corrosion activity. Data on average corrosion rates and pitting distribution of mild steel (Fig. 4 & 5) substantiate this statement.

Biological Growth

Microfouling and corrosion processes commence immediately when a metallic material is exposed in the marine environment. A good information on the quantity and the quality of fouling, including the seasonal rhythm in the settlements of the organisms are of considerable value in evolving and assessing the appropriate antifouling technology. The data is also of immense value in forecasting the nature of impeding fouling at a prospective marine site.

A fairly good amount of information on the etiology of fouling has been collected at the universities and the national laboratories in the country. One of the notable reports on the fouling in Bombay harbour has been published by Iyengar and others [13]. In recent years, the laboratory has collected similar data from the harbours of interest located in either side of the Indian peninsula including the Andaman, Nicobar Islands [14]. The fouling data collected by the immersion of non-toxic test panels in various harbours of India have revealed that most of the Indian harbours are almost '12 month' fouling ports and the density of settlement is comparable with the worst fouling waters in the world.

Table II summarises the values of biomass generated in various ports in India and in the harbours of the world. It can be seen from the table that in tropical Indian waters, the biofouling growth is very heavy. It is obvious, therefore, that some of the marine coatings which offer longer fouling free life in temperate water may not show similar efficiency in tropical waters.

Table II
Biomass Accumulation in Tropical and Temperate Waters

Place	Latitude	Wt/M^2/year (kg)	Reference
Bombay	18.58°N	5 to 6	
(i) Offshore		5 to 6	NCML
(ii) Enclosed water		0.2	Bombay
Madras (Kalpakam)	15°N	8	
Andamans	12°N	7 to 8	
Argentina	38.25°N	0.5	5th International Congress on Marine Corrosion and Fouling
Australia	37°S	0.5	
Florida (USA)	25°N	3.0	
Hongkong	23°N	5.0	

Problems in Offshore Waters

To-date in this part of the world a reasonably good amount of information is generated on the twin problems of marine corrosion and fouling in respect of the moving structures like ship hulls and the stationary water front structures. A similar information in respect of offshore and deep sea waters, however, is seriously lacking, particularly because of our relatively recent exploits in offshore technology. Judging from our experience in near shore waters, it is expected that the severity of marine fouling and corrosion in offshore waters would be equally very high. A severe fouling will not only increase the fluid loading and accelerate corrosion but also seriously impede maintenance and periodical inspection of offshore installations like oil platforms.

The relationship of fouling to the loading of offshore structures is summarised as follows. [15] "Excessive marine growth will increase the sectional area of a member and alter its surface characteristics, but changes tending to increase resistance to waves and currents and to increase the load applied to structures. Where more detailed information is not available, it may be assumed that the growth will increase the diameter of a member by 250 mm." A detailed information on the fouling growth for a given site must, therefore, be gathered so that the growth does not exceed the design allowance and jeopardise the operational efficiency of the structure. The importance of the problem of effect of marine growth on fluid loading has now been very well recognised and investigated. [16,17,18]

A relation between the fouling growth and marine corrosion has been widely reported, particularly on inadequately protected ship hulls and near shore structures. The studies carried out by Bultman and Southwell [7] have shown that the protective advantage of fouling towards reducing the corrosion rate, whenever it exists, is short lived and is diminished considerably when the growth is sufficiently heavy and biologically active to remove oxygen from the immersed surface. For a very long time no attention was paid to the role played by sulphate reducing bacteria in inducing marine corrosion. The studies carried out by Naval Research Laboratory, USA at various offshore sites have revealed that after a sufficient cover of fouling and also corrosion products, anaerobic conditions are established at the metal surface and the corrosion becomes bacterially controlled. In offshore waters the structures are protected by coatings, cladding and cathodic protection system, however, the fouling may contribute to corrosion by damaging the protective coating or by reducing the efficiency of cathodic protection. There is one more aspect of the heavy fouling on offshore structures and this relates to the certification surveys carried out to confirm the integrity of the structures. Heavy fouling on a structure will interfere in the detection of any serious defects such as cracking or corrosion.

One more human activity which relates to the generation of energy, the electrical power, also suffers from the problem of twin evils of marine fouling and corrosion. Power generating plants using nuclear or conventional energy require large quantities of water to remove waste heat. The power plants located along the coast line naturally turn to the sea for their coolant water requirements. The choice of the sea water as a coolant however, is not free from problems, and one of the major constraints is the presence in it of fouling organisms. The fouling

film formed on the condenser tubings not only decreases the transfer of heat but can clog pipes and conduits thereby interfering with the flow of water. It is also reported by Coughlan and Whitehouse [19] that corrosion proceeds faster beneath bacterial plaques than on bare metal.

The problem of marine fouling in intake tunnels has also been lately faced in this country. In the United Kingdom where there are over 300 power generating stations, a good deal of work has been carried out to mitigate this problem. In India only recently this aspect of marine fouling has received the attention. The study carried out at NCML Bombay has revealed that in tropical waters of ours, the fouling in intake tunnels will certainly be one of the major difficulties in smooth running of the power generating plants. It is estimated that in intake tunnels along the Indian coastline, the quantum of growth would be as high as 8 to 10 kgs per square meter every year. For an intake tunnel of an average length of 500 meters, 5 meters in radius, the amount of animal debris that could accumulate every year would be stupendous. For preventing such an eventuality, it is necessary that the settlement of the marine organisms is prevented by suitable remedial measures. One of the most dependable and economical measures has been the use of clorine. The laboratory experiments to assess the chlorine dosage and schedule, keeping in view the major endemic species have been conducted. [20]

Protective Technologies Developed at NCML

Protective technologies against sea water corrosion have been introduced by NCML, Bombay. The anticorrosive systems developed comprise heavy duty anti-corrosive paints, antifouling paints, sacrificial aluminium anodes and Impressed Current Cathodic Protection systems. The performance of these systems has been ascertained in Indian tropical waters. [Table III]

Heavy duty anticorrosive paints :

During the early development efforts, paints based on modified linseed stand oil-phenolic varnish were developed and introduced. Laboratory and service trials indicated that these paints had 12 months corrosion free life. Further efforts led to the development of heavy duty anticorrosive paints based on indigenously manufactured vinyl resins. The polyvinyl chloride and polyvinyl acetate co-polymer films are tough, flexible and highly resistant to many chemicals, acids and alkalies, but suffer from a drawback of poor adhesion to steel substrate. The development efforts at NCML have led to the manufacture of satisfactory resin material through the help of local manufacturers. This resin contains carboxylic end groups in polymer chain that are responsible for satisfactory adhesion. Using this resin, the laboratory has developed vinyl based anticorrosive paints that offer 5 years satisfactory anticorosive life in tropical environments.

Antifouling paints

Indigenous oleoresinous antifouling paints possess limited antifouling life of 9 months in tropical waters. With a view to increasing the ships' docking interval, heavy duty vinyl based antifouling compositions were also developed and subjected to exhaustive laboratory, raft and ship evaluation. The vinyl based underwater

Table III

Performance in Tropical Waters of Underwater Protective Systems Developed at NCML

System	Characteristics	To be used in conjunction with	Performance
Anti-corrosive vinyl coatings	PVC/PVA copolymer, red lead/aluminium pigment. Requires blast cleaned surface	Vinyl antifouling coating, galvanic anodes and ICCP system	5 years
Antifouling vinyl coatings	PVC/PVA copolymer resin, cuprous oxide	Vinyl anticorrosive paints and coal-tar epoxy with a tie coat.	15-18 months
Chlorinated rubber coatings	Anticorrosive (A/c) composition based on chlorinated rubber, non-leafing Aluminium pigment applied over chipped surfaces.	Galvanic anodes and ICCP systems	18 months
Chlorinated rubber A/F paint	Cu_2O used as toxin	Chlorinated rubber A/C	15 months
Self polishing A/F composition	Based on toxic, self-polishing polymer	All A/C systems mentioned above.	More than 18 months
Galvanic Al anodes	Al-Zn-Sn type 70% current efficiency	Any A/C system, used also on bare drilling rigs.	Satisfactory
ICCP system	Based on lead alloy and Zinc reference monitoring.	Vinyl and chlorinated rubber A/c Systems	Satisfactory

paint system including both anticorrosive and antifouling paints has since been introduced into the service by Indian Navy. Self polishing antifouling composition based on polymethyl methacrylate with TBTO has also shown highly satisfactory performance. (21)

Sacrificial aluminium anodes :

The protection offered by anticorrosive composition needs to be augmented by other means. This was achieved in other countries using magnesium or zinc galvanic anodes, none of which could be indigenously produced. NCML ascertained the relative performance of these galvanic materials namely alloys of magnesium, zinc and aluminium, under service conditions. Based on this study, satisfactory aluminium alloy sacrificial anodes were developed.

Impressed Current Cathodic (ICCP) system :

In early seventies, the laboratory developed ICCP system. This involved use of lead alloy anode as current conductor to the seawater environment and an automatic control unit to regulate variable current requirements. The system developed by NCML has been accepted by Indian Navy and is being fitted on all new construction ships.

The technology of cathodic protection and underwater marine paints developed by NCML is progressively being exploited by mercantile marine, port and harbour authorities, coastal power stations using seawater as coolant, desalination plants and offshore oil platforms and drilling rigs.

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RÉSUMÉ

La corrosion dans l'eau de la mer dans les eaux tropicales de l'Inde est plus sévère que celle qu'on a, d'après un rapport, dans les eaux tempérées. On a remarqué que les creux sur l'acier doux qui se trouvait dans les eaux des ports de l'Inde étaient de l'ordre de 1.8 à 1.5 mm/an et le dépôt de l'organisme marin nuisible à la coque était de l'ordre de 8 à 9 kgm/m. carré/an. On a trouvé que les technologies d'empêchement de la corrosion, à savoir, les peintures d'antiorganisme nuisible et anticorrosives aussi bien que les systèmes de protection cathodiques développés pour les conditions tropicales sévères, sont très satisfaisantes.

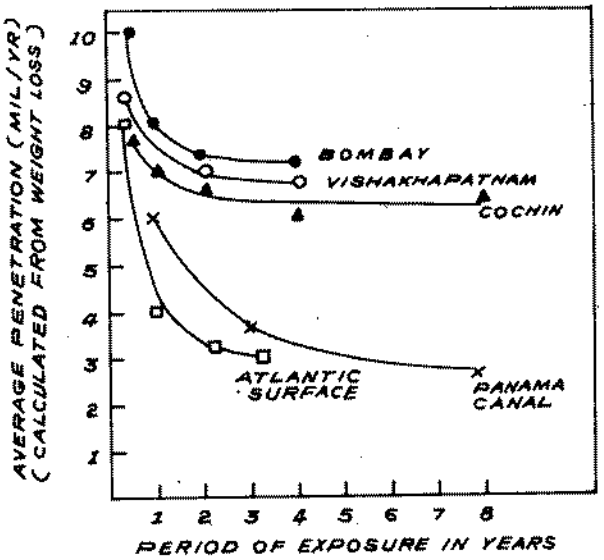

FIG. 1 : MILD STEEL CORROSION RATES (M.P.Y.) AT DIFFERENT LOCATIONS

- BOMBAY
- VISHAKHAPATNAM
- COCHIN

} DATA GENERATED BY NCML BOMBAY (INDIA)

- PANAMA CANAL ZONE
- ATLANTIC SURFACE

} DAT QUOTED FROM SEAWATER CORROSION HAND-BOOK P.17, N.D.C. NEW JERSEY U.S.A.

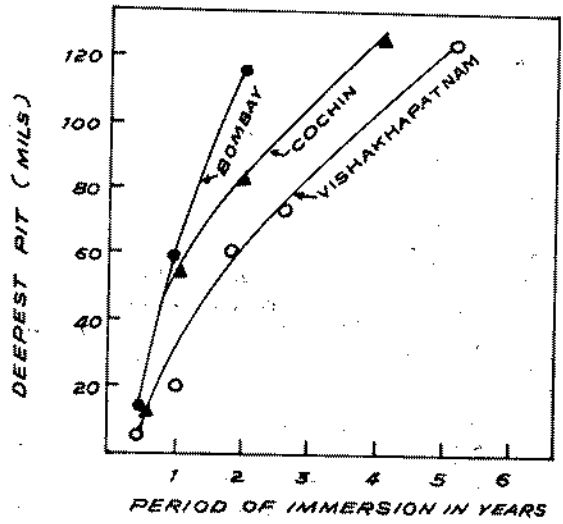

FIG. 2: MAXIMUM PIT DEPTH OF MILD STEEL IN THE INDIAN HARBOUR WATERS Vs EXPOSURE PERIOD

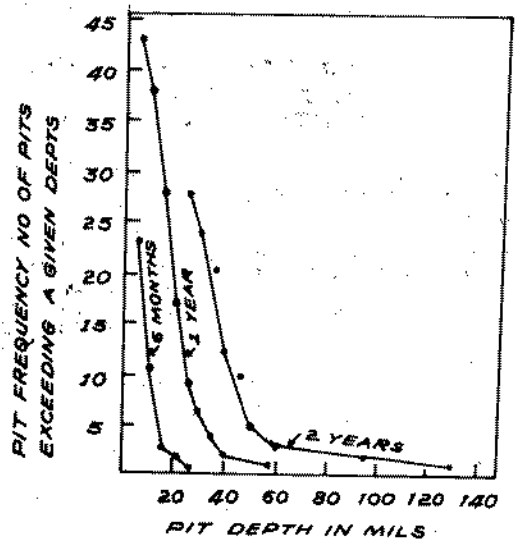

FIG. 3: PIT DEPTH DISTRIBUTION CURVES FOR MILD STEEL IMMERSED IN BOMBAY HARBOUR

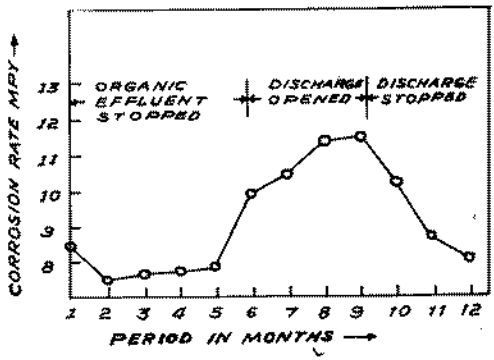

FIG. 4: MILD STEEL CORROSION RATES (MONTHLY AVERAGE) IN HARBOUR WATER MIXED WITH ORGANIC EFFLUENTS

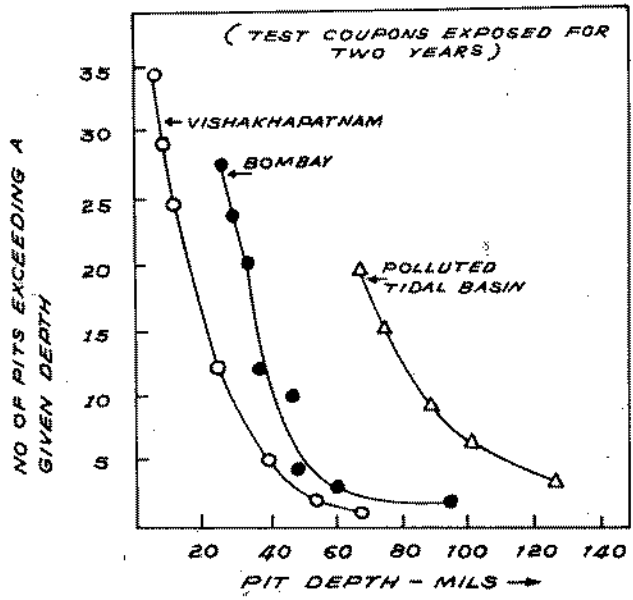

FIG. 5: INFLUENCE OF ORGANIC PIOLUTION ON PIT DEPTH DISTRIBUTION

THE IMPACT OF EXTREME OBLIGATE THERMOPHILIC BACTERIA ON CORROSION PROCESSES

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The contribution of bacterial activity to the corrosion of a high-temperature aqueous system is directly quantified in this paper. An anodic corrosion current of 3.8×10^3 na cm^{-2} and a 1.6 mpy penetration is attributed to the presence of attached obligately thermophilic bacteria. The mechanism for the microbiologically-induced corrosion may be three-fold: in-situ production of acidic metabolites, formation of differential aeration cells and galvanic currents between the substratum and chelated metals.

La contribution de l'activité bacterienne à la corrosion à haute temperature d'un système aqueux est directment determineé dans cette étude. Un courant de corrosion anodique de 3.8×10^3 na cm^{-2} et une pénétration de 1.6 mpy attribuée à la présence de bacteria attachées et obligatoirement thermophilic. Le mécanism pour la corrosion induite microbiologicalement peut être triple: in-situ production de metabolites acidiques, formation de cellules d'aeration différentiel et des courants galvaniques entre le substratum et les metaux déposés.

Introduction

Obligately thermophilic microorganisms which thrive at temperatures from 60 to 90°C have been isolated from a wide variety of natural and manmade thermal habitats, both freshwater and marine. Apart from their ability to reproduce at high temperatures, thermophilic microorganisms resemble their mesophilic counterparts in terms of nutritional requirements, metabolic pathways, and oxygen tolerance (Amelunxen and Murdock, 1978). Large populations may build up on surfaces in hot water systems, even in the presence of extremely low concentrations of organic matter.

The practical significance of bacteria growing in manmade hot water systems has not been adequately explored. In particular, metal surfaces which become covered with fouling films of thermophilic microorganisms may become sites of biologically induced corrosion. The demonstration of a connection between bacterial activity and corrosion of high-temperature aqueous systems, such as heat exchangers, would have enormous practical implications.

In this paper we discuss the isolation of a thermophilic bacterium from a brazed nickel "T" that failed during accelerated corrosion tests conducted at high temperatures. Replicate nickel 201 "T's"brazed with alloy BNi_2 (AMS 4778) were maintained at room temperature, 60°C, and 80°C with distilled water flowing at 0.3 m sec^{-1}. We predicted that at elevated temperatures the rate-determining step in the corrosion process would be accelerated and that failures would result first in systems maintained at 80°C, followed by those maintained at 60°C, and finally those at 20°C. After 21 days of continuous operation, the first leak was observed in the 60°C system. This failure could not be predicted based on rate laws. Examination of the braze joint using scanning electron microscopy revealed that the fillet was blanketed with filamentous bacteria. The presence of the bacteria strongly suggested that they were involved in the corrosion failure (Little et al., 1984). The bacterium has been isolated and maintained in pure culture. This paper discusses experiments designed to quantify the impact of this organism on electrochemical processes.

Methods and Materials

Isolation of bacteria - Bacteria were isolated from the failed nickel "T" by incubating pieces of it at 65°C in a basal salts medium supplemented with 0.1% tryptone and 0.1% yeast extract (both Difco) (Brock and Freeze, 1969). The salts medium contained, per liter: nitrilotriacetic acid, 100ug; $CaSO_4 \cdot 2H_2O$, 60 ug; $MgSO_4 \cdot 7H_2O$, 100ug; NaCl, 8 ug; KNO_3, 103ug; $NaNO_3$, 6890g;

Na_2HPO_4, 111ug; $FeCl_3$, 2.8ug; $MnSO_4 \cdot H_2O$, 22ug; $ZnSO_4 \cdot 7H_2O$, 5ug; H_3BO_3, 5ug; $CuSO_4$, 0.16ug; $Na_2MoO_4 \cdot 2H_2O$, 0.25ug; $CoCl_2 \cdot 6H_2O$, 0.46ug. Final pH of the supplement medium was adjusted to 7.6 with NaOH.

After two days of incubation without shaking, the broth became turbid, and transfers were made to fresh broth. Subsamples of this were streaked onto solid medium of the same composition, plus 3% agar. Isolated colonies were picked and restreaked to obtain a pure culture.

Organisms which attached to metal surfaces were selected by providing a solid substratum, a sterile nickel disc, in the culture tube with the culture medium. The first such tube was inoculated with a pure culture of the isolate. Subsequent inoculations were made by transferring the nickel disc to a fresh tube containing sterile medium and another sterile nickel disc. In this way only organisms attached to the nickel were transferred.

Scanning Electron Microscopy and EDAX - "T's" were removed from the system and the flow-through water was replaced with cacodylate buffered 4% glutaraldehyde which remained in the "T" for four hours, followed by washes of distilled water, acetone, acetone and xylene and finally xylene. The specimens were allowed to air dry, sectioned and sputter-coated for viewing. The alpha-numerics appearing at the bottom of the micrographs indicate the accelerating voltage of the instrument, magnification, measurement indicator, photograph number, Julian date and laboratory acronym.

Measurement of Organic Acids - To test for organic acid production by the bacterium isolated, cells were grown for 24 hours at 65°C in the medium described above, containing 0.1% each tryptone and yeast extract. 0.5 ml samples of this culture were analyzed for volatile and nonvolatile fatty acids by gas chromatography. For volatile acids, samples were acidified and extracted with ether. For nonvolatile acids, methyl derivatives were prepared and extracted in chloroform. A stainless steel column (6 feet by 0.125 inch) containing 10% SP-1000 on 100/120-mesh Chromosorb (Supelco, Inc.) was used in a Hewlett-Packard 5700A gas chromatograph equipped with a flame ionization detector. Acids were identified and quantitated by comparison of retention times and peak heights with those of known standards.

Corrosion Experiments - Ground glass bases from 47 mm Millipore filter supports were joined to the sides of two 1-liter flasks. A 0.1 um pore Millipore filter disc was placed between the two supports and the flasks were clamped together and filled with 800 ml of the basal salts medium supplemented with 0.025% each tryptone and yeast extract. Into each flask was placed an EG&G flat electrode holder containing a 1 cm^2 flat disc of nickel 201. The entire assemblage was autoclaved for 15 min at 121°C and 15 psi. After cooling the flasks were placed into an aquarium maintained at 60°C. Water-saturated air was bubbled into one of the flasks. Previous experiments have shown that oxygen diffused across the membrane and that the water in the two flasks was saturated with oxygen after two hours of aeration. The membrane insured electrolytic conductivity between the discs while maintaining microorganisms on one side of the membrane and an abiotic system on the other side. One flask was inoculated with a nickel disc previously colonized by microorganisms, while a sterile disc was dropped into the flask to be kept in an abiotic condition. The electrodes were then galvanically coupled and the resulting currents measured with an EG&G PARC corrosion measuring device (Model 350). In such a system the measuring device functions as a zero resistance ammeter; i.e., the potential of the galvanic cell is not perturbed by the current measurement. If two identical metal specimens are galvanically coupled and are isolated in identical electrolyte solutions, the current flow between them is zero, even in the presence of active corrosion, because there is no difference in potential. They corrode at the same rate and neither functions as an anode or cathode in reference to the other. If this balance is disturbed by the presence of colonizing microorganisms, a current flows between the two specimens. If the reaction that is taking place at the surface of the working electrode is an oxidation, an anodic current will be recorded; if it is a reduction, a cathodic current results.

Heavy Metal Analysis - Water samples from which the organism was isolated, standards and blanks were acidified to contain 0.15% reagent grade nitric acid. Samples were analyzed with a heated graphite analyzer (graphite furnace) Perkin-Elmer 500 coupled with an atomic absorption spectrophotometer PG 403, using standard methods specified in the Perkin-Elmer manual for drying, charring and atomizing times and temperature.

Results and Discussion

Microfouling and corrosion processes commence immediately upon exposure of a metal to an aquatic environment. These two processes traditionally have been treated separately, and corrosion experiments have been evaluated without consideration of

the impact of attached microorganisms. Gerchakov and Sallman (1979) have reviewed numerous proposed mechanisms for microbiologically induced corrosion. The contribution of a single microorganism to an electrochemical process has never been directly quantified, though.

Figure 1 shows an electron micrograph of the thermophilic bacterium blanketing the failed braze fillet from which it was isolated. The organism forms filaments of variable lengths from 20 to over 200 um. The optimal temperature for growth is 60 - 72°C and the organism is completely inactive below 50°C. It grows very rapidly in the culture medium used in these experiments (doubling time less than 30 minutes at 65°C). Microscopic examination of nickel discs stained for 3 minutes with 0.1% acridine orange, a fluorescent nucleic acid dye (Hobbie et al, 1977), revealed attached bacterial populations of over 2×10^6 cells cm^{-2} after a 6-hour incubation time.

Polarization techniques assume a Wagner-Traud mechanism whereby both anodic and cathodic processes occur with equal probability on all parts of a single metal electrode (Bockris and Reddy, 1970). By galvanically coupling two specimens, the two areas are experimentally separated and the resulting corrosion current can then be directly measured. In the absence of microorganisms, the current flow between the two electrodes was zero (Figure 2). After 20 hours the current at the inoculated electrode began to increase anodically and finally stabilized at 3.8×10^3 na cm^{-2}. For the sake of comparison, a copper electrode galvanically coupled to an aluminum specimen resulted in an anodic current of 8×10^4 na cm^{-2}.

It is convenient and traditional to express corrosion rate as milli-inches per year (mpy) to provide an indication of penetration. Corrosion current can be converted to mpy using the following relationships (EG&G Princeton Applied Research, 1980):

according to Faraday's Law

$$Q = \frac{nFW}{M} \qquad (1)$$

where
Q = coulombs
n = number of electrons involved in the electrochemical reaction
F = the Faraday, 96,487 coulombs
W = weight of electroactive species
M = molecular weight
From equation (1),

$$W = QM/nF \qquad (2)$$

FIGURE 1. Filaments of obligate thermophile in failed braze fillet.

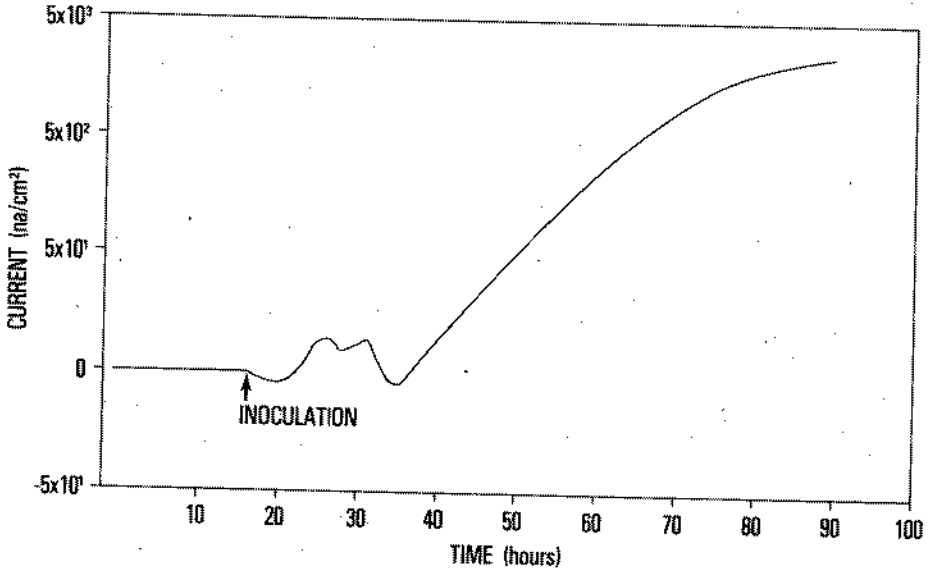

FIGURE 2. Corrosion current vs. time - a quantification of the impact of an obligate thermophile on the corrosion of a nickel electrode.

Since equivalent weight (E.W.) = M/n

$$W = \frac{Q \times E.W.}{F} \qquad (3)$$

and since Q = it from Faraday's Law,

$$W = \frac{it(E.W.)}{F} \qquad (4)$$

Dividing equation (4) by the electrode area and the density gives

$$C.R.\ (cm/sec) = i\ (E.W.)/dFA \qquad (5)$$

Convert seconds to years and centimeters to milli-inches. Convert the Faraday (amp-sec/eq) to microamps.

$$C.R.\ (mpy) = \frac{i(E.W.)(31.6)(10^6)(10^3)}{dFA\ (2.5)(10^6)} \qquad (6)$$

where
E.W. = equivalent weight of the corroding species, g
d = density of the corroding species, g/cm^3

The corrosion rate that can be attributed to the presence of the attached thermophilic bacteria was calculated to be approximately 1.6 mpy. We suggest that the mechanism for the microbiologically induced corrosion may be three-fold: in-situ production of acidic metabolites; formation of differential aeration cells created by respiring colonies; and entrapment and deposition of metals, most notably copper, which may give rise to galvanic currents.

Most heterotrophic bacteria secrete organic acids during the fermentation of organic substrates. The types and amounts of acids produced in nature depends upon the kinds of organisms present and the substrate molecules available. Organic acids from a wide range of bacteria have been shown to enhance corrosion of a number of different types of metal (Staffeldt and Calderon, 1967; Ehlert, 1967; Burnes et al., 1967; Ashton et al, 1973; Webb, 1975). Nickel forms a passivating film in slightly alkaline solutions. Thus acidic metabolites secreted by microorganisms can prevent passivation or destroy an existing passivation film.

Our analyses indicate that two of the major metabolic products of the bacterium discussed here are isobutyric and isovaleric acids. When grown for 24 hours in 0.1% tryptone-yeast extract medium, the bulk concentrations of these t · acids were

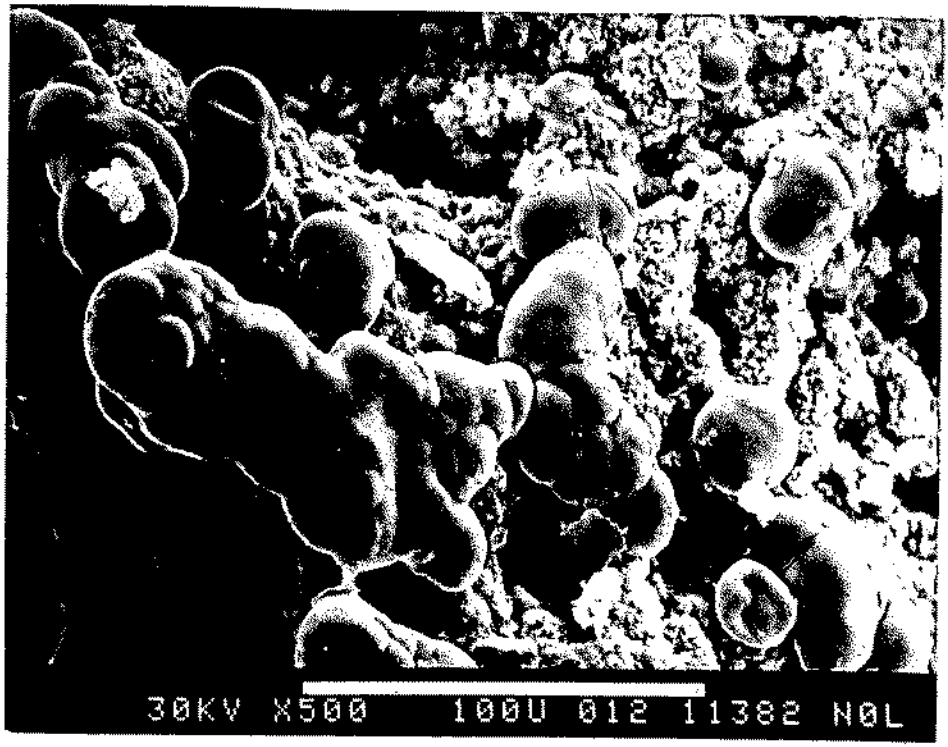

FIGURE 3. A deposit of copper associated with microbial colony.

TABLE 1. WATER CHEMISTRY DATA

	Heavy metal concentration in ppb			
	Cu	Mn	Cd	Fe
Flow-through water before entering system	756.4	151.2	46.0	132.0
Flow-through water after passing through colonized "T"	21.1	77.1	18.6	0

found to be 0.33 mM and 0.35 mM, respectively. However, at the site of production, under the biofilm, the concentrations are undoubtedly much greater, and their impact amplified. The pH of the medium dropped from 7.6 to 7.0.

Oxygen-deficient and even oxygen-free conditions arise beneath biofilms when oxygen is taken up by respiring microorganisms as rapidly as it diffuses to the surface. This situation creates potential differences with subsequent corrosion currents. In addition, microorganisms colonizing surfaces secrete extracellular polymers capable of chelating metals. These exopolymers are capable of entrapping copper, manganese, cadmium, and iron from the flow-through water (Table 1). Figure 3 shows an electron micrograph of a copper deposit associated with the microbial colonies on the failed braze fillet. The absence of other metals in the EDAX spectrum was notable. Reduced copper at such sites will certainly create a copper-nickel galvanic couple.

Involvement of thermophilic bacteria in corrosion failures of high-temperature systems can be expected to occur in marine as well as freshwater environments. This has implications for many marine applications, including heat exchanger systems, oil-drilling equipment, and desalination plants. We are attempting to isolate species of obligately thermophilic bacteria from marine habitats and will test their effect upon corrosion reactions.

Acknowledgments

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ECOLOGICAL ASPECTS OF MARINE FOULING AT THE PORT OF INGENIERO WHITE (ARGENTINA)

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ABSTRACT

The present paper deals with the study of fouling communities at the Ingeniero White harbour, where fouling problems have been detected in cooling systems of a power station and a second, more important one,is planned to be built in the near future.
Data were obtained during two sampling periods (May 1979 - April 1980 and October 1981 - September 1982), using achrylic panels as experimental surfaces.
A short-term series of panels supplied information on settlement cycles of the different fouling species, while the evolution of the fouling community was studied from a long-term series of panels.
Biomass fluctuations of fouling growth along the year were determined, as well as the relationship between these fluctuations and variations in certain environmental conditions.
Results obtained during this research will serve to determine the best schedule of fouling control based on chlorination at the studied power stations, in order to ensure an adequate energy supply to an industrial area of major importance within the country.

INTRODUCTION.

Studies on fouling communities in Argentina were initiated in 1964. Early researches were carried out using experimental rafts and referred mainly to fouling problems on ships' hulls. Some years later, these studies were extended to power stations presenting fouling problems in their cooling systems.

This line of research includes baseline studies in Ingeniero White harbour, where a power station planned to be the main energy-producing station of the Buenos Aires province, of 620 MW, is being built. Given the high industrial development of this area and its importance within the country, an efficient energy supply is of great significance. On the other hand, power stations cooled by sea water which are already working in the province have had serious problems related with fouling growth in their cooling systems. No studies were carried out while these stations were being planned. Now there is a greater awareness of the importance of carrying out environmental and biological studies before the construction is started.

MATERIAL AND METHODS

The results presented in this paper were obtained during two sampling periods, comprising from May 1979 to April 1980 and from October 1981 to September 1982. During the first of these periods, baseline studies were carried out, the results of which were used as reference data for comparison with those compiled during the second.

Sandblasted inert achrylic panels of 300 cm^2 were chosen as experimental surfaces. These panels were placed vertically, in pairs and at different depths (panel A at 4 m, B at 4.80 m and C at 5.60 m), suspended from one of the piers of the harbour. Of the two plates placed at each depth level, one was used to study fouling settlement and the other for biomass determination (dry weight).

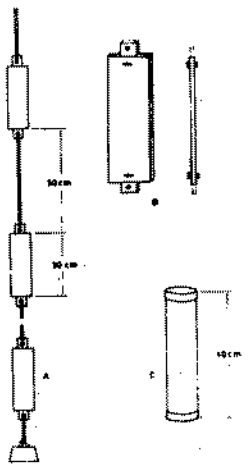


Fig.1.- Experimental panels.

Taking into account time of exposure, panels were classified in two categories:

- Short-term panels, which remained submerged for approximately thirty days and on which recruitment of the main macrofoulers was observed;
- Long-term panels, which were submerged simultaneously and one set was removed every month; these provided information on the evolution of the fouling community.

Retrieved panels were carried to the laboratory, where they were photographed and surveyed under stereoscopic microscope.

Environmental information was supplied by the laboratories of Marine Chemistry and Meteorology (IADO).

Regressions between monthly biomass of fouling growth and mean monthly temperatures were calculated using geometric mean Model II

regression technique, as proposed by Ricker (1973).

Cluster analysis was based on Jaccard's similarity index, using a hierarchic and agglomerative method of complete linkage.

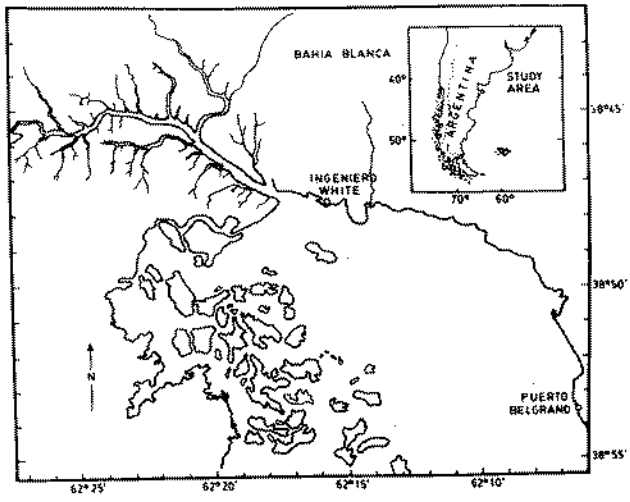


Fig. 2.- Map of area studied

RESULTS.

Environmental aspects.

The area of study is an estuarine environment, typified from a topographical point of view by a high lenght/width ratio and hydrologically by the relatively small inflow of fresh water as compared with the large volume of sea water which inflows during each tidal cycle.

The innermost area where the harbour is actually situated (38°47'S, 62°14'W) can be described as a vertically homogeneous estuary, becoming negative in summers of low rainfall (Freije, 1981). Indeed, salinity values showed a wide range of variation during 1981/82, with a peak record of 38.157°/$_{oo}$ in February and a minimum of 25.804°/$_{oo}$ in late April(fig.3)

Temperatures showed a similar pattern of variation during both periods, with a minimum of about 6°C and peaks above 23°C (fig.4).

The strong tidal currents, the mixture of waters due to turbulence (Freije, 1981) and the continuous dredging of the main channel contribute to the presence of a great amount of suspended matter, with the consequent reduction of water transparency. Measurements of visibility using Secchi disc varied between 0.35 and 0.95 m.

Patterns of settlement on short-term panels

Among the numerous macrofoulers found on short-term panels during the present assay, five groups of organisms were dominant colonizers. These were: coelenterates, polychaete worms, crustaceans, bryozoans

and tunicates. Species belonging to other groups were present only sporadically and always in low numbers.

The analysis of species composition was based almost exclusively on sessile organisms, which are largely responsible for determining the physical structure of the community.

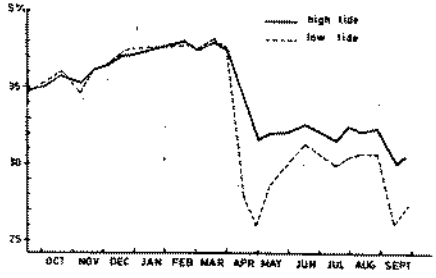


Fig.3. Salinity.Ing. White. Oct.1981-Sept. 1982.

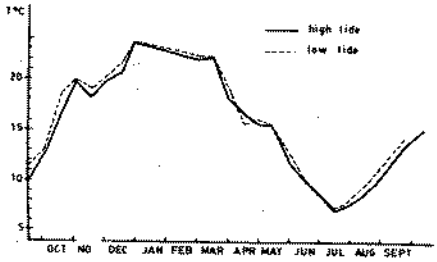


Fig.4.Water temperature. Ing. White.Oct.1981-Sept.1982.

Tubularia crocea

This species, together with some tunicates, typified the community on short-term panels during the warm months. It settles during the whole year and at all depths, with peak abundance in November, January, February and March.

With the exception of November, when colonization was poor, these were also the months of heaviest settlement during 1979/80.

Bastida et al. (1974) compared the pattern of settlement of this species in Puerto Belgrano with the one observed in Mar del Plata, stating that in the latter settlement took place during a longer period of time and specimens reached sexual maturity in a thirty days exposure period. This is also the case of Ingeniero White; probably, the differences registered in Puerto Belgrano are due to the fact that this species presents wide variations in the settlement cycle from one year to another (Bastida, 1977).

According to Bastida & Brankevich (1982), Tubularia crocea shows preference for dimly illuminated levels, so that settlement would be favoured by conditions of low transparency prevailing in Ingeniero White.

Gonothyraea loveni

This hydroid has settled in low densities from October to January and colonization was reinitiated in August, with peak density in September. This settlement pattern coincides with that observed during 1979/80 except for peaks of abundance which occurred in October and November in this last period.

This species has also been recorded for Puerto Quequén (Bastida & Brankevich,1982), where its abundance was grater and the season of settlement more extended, with peak densities during the warm months.

In this harbour, campanulariids show a close association with the nudibranch Tenellia pallida (Bastida & Brankevich, op. cit.).

Spirorbinae

These polychaetes have settled from October to May, with three main peaks in October, January and March and with similar densities at all depths. In spite of their abundance, their presence is not very conspicuous on account of their small size

They have not been recorded in Mar del Plata, while on short-term panels from Puerto Quequén they have been found in increasing numbers over recent years (Brankevich, pers. comm.; Bastida & Brankevich,1982).

Nicolea sp.

It is a chief component of fouling communities in Ingeniero White, colonizing test panels from October to April; no settlement was recorded during March.Peaks of abundance were observed at all depths in November and on the deeper panel, also during January.

No species of this genus have been previously reported in fouling communities of other Argentine harbours.

Polydora ligni

This organism has shown variations in its colonization on short-term panels and has presented no apparent depth preference. Highest densities were observed during the months of October and November. Settlement extended from October to February and was reinitiated in September.

It is one of the most prominent fouling species at the port of Mar del Plata (Bastida et al., 1977), where settlement has varied remarkably along the years. During early researches in the area, this species presented irregular patterns of colonization, while in recent years, heavy growth was registered at all depths and throughout the entire year (Bastida et al., op. cit.).

Polydora ligni has also been recorded for Puerto Quequén (Bastida & Brankevich, 1982), where the cycle has been somewhat more extended; it has not been found in Puerto Belgrano.

Corophium cf. insidiosum

It settled from October to May, always in low densities and at all depths. During the period 1979/80, this organism was present during the whole year except in June. Densities were highest in November and from January to March, though never reaching significant values.

This species has been found along the Argentine coast in all studied harbours (Mar del Plata, Puerto Belgrano and Puerto Quequén). Settlement cycles were found to vary in length, peak densities coinciding with the warm season (Bastida, 1970; Bastida et al., 1974;Bastida & Brankevich, 1982).

Conopeum reticulum

No graph corresponding to this species is presented, since it was registered only sporadically and in low amounts during 1981/82.

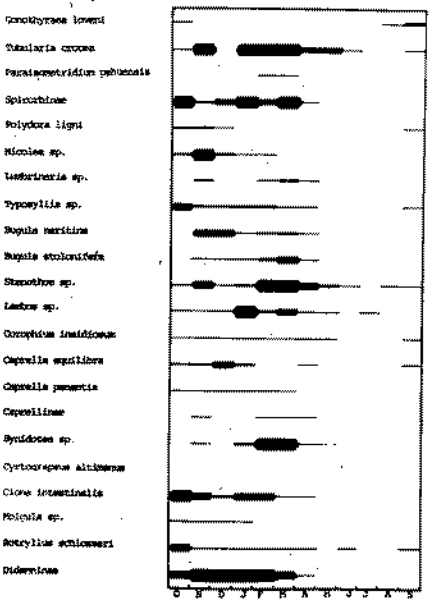


Fig.5.-Settlement cycles on short-term panels, level A. Ingeniero White harbour. October 1981-September 1982

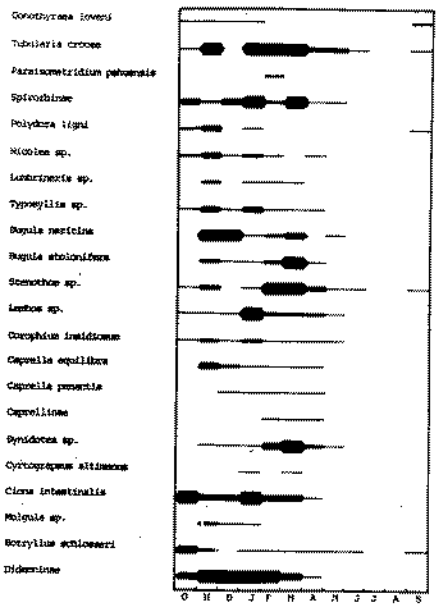


Fig.6.-Settlement cycles on short-term panels, level B. Ingeniero White harbour. October 1981-September 1982

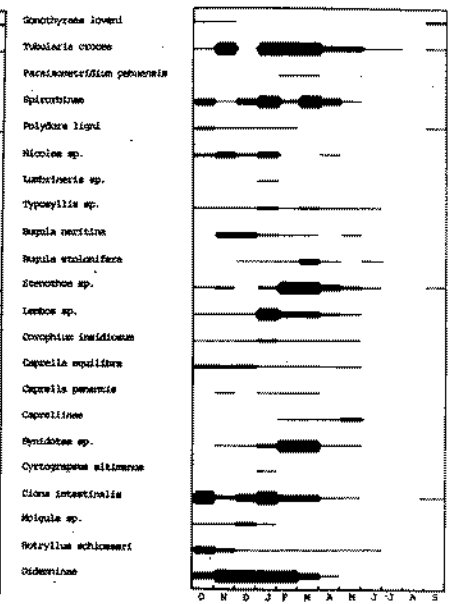


Fig.7.-Settlement cycles on short-term panels, level C. Ingeniero White harbour. October 1981-September 1982

During preliminary studies (1979/80), it settled from August to November, but colonization of test panels was never significant. It seems to show a certain preference for the deeper levels.

Conopeum reticulum has been previously recorded for Puerto Belgrano and mentioned as Conopeum sp. (Bastida et al., 1977) and also for Puerto Quequén (Lichtschein de Bastida & Bastida, 1980).

It has never constituted an important settler on short-term panels, except in Puerto Belgrano, where the colonization period covered the whole year, reaching relatively high densities.

Bugula neritina

This was the most important bryozoan during the present study. Its colonization period extended from November to May, with main settlement during November and December. Preliminary studies in the area indicated a similar attachment pattern, except that peaks occured in January and February.

Although B. neritina has been recorded for the port of Mar del Plata (Bastida, 1970), its presence has been occasional. On the other hand, in Puerto Belgrano colonization has been registered during practically the whole year, with a main settlement from November to May, reaching significant levels of abundance (Bastida et al., 1974).

Bugula stolonifera

It has settled on test panels from November to April, reaching maximum densities during March. During 1979/80, settlement extended over a shorter period of time (November to February), with peak intensity in December and January. This species has shown no preference for a particular depth level.

Coinciding with observations by Bastida et al. (1974) in Puerto Belgrano, B. stolonifera has never reachedimportant concentrations, as those found for B. neritina. In the port of Mar del Plata instead, B. stolonifera is a much more important settler on test panels (Bastida, 1970; Bastida et al., 1977).

Ciona intestinalis

This species, together with the hydrozoan Tubularia crocea and tunicates of the family Didemnidae, has typified fouling communities on short-term panels during certain months. Colonization was similar on the three depth levels. Panel B presented a more regular pattern of settlement, with a main period extending from October to January. Colonization during 1979/80 was less important and extended from August to April.

In the port of Mar del Plata, settlement by Ciona intestinalis has varied remarkably from one year to another (Bastida, 1970; Bastida et al., 1977; Bastida et al., 1980), coinciding with observations on this species for other localities (Keough, 1982; Martínez, pers. obs.).

Didemnidae

These tunicates become remarkably developed on short-term panels and are important components of local fouling communities. They settled at all depths from October to April, with peak intensity in No-

vember, December, January and February.

During 1979/80, these organisms were poorly and sporadically represented, with slightly higher densities in December and January.

These organisms have also been reported for the port of Mar del Plata (Bastida et al., 1977), but never in significant amounts. In Puerto Belgrano they are encountered on test panels from December to September, reaching high densities during February and March.

Botryllus schlosseri

This tunicate was not as abundant as those belonging to the subfamily Didemninae. It was found on test panels from October to June and reappeared in September. Maximum densities were registered in October and November.

The settlement cycle was very similar in the three depth levels.

Botryllus schlosseri was also registered in Puerto Belgrano (Bastida et al., 1974), presenting a similar pattern of settlement as the one observed in Ingeniero White, but a greater abundance.

Bastida & Brankevich (1982) have pointed out that growth of this species is favoured by low salinity values; this fact could account for its importance in Puerto Belgrano and Puerto Quequén and its poor development in the port of Mar del Plata.

Recruitment pattern

Recruitment of sessile forms takes place during a clearly marked period, extending from September to May. During the rest of the year, there is practically no colonization of test surfaces; this situation is evidenced in the dendrogram shown in fig.8.

Within the period of recruitment, two stages can be distinguished, one corresponding to the spring season and early summer (September to January) and another comprising late summer and autumn. This division in two periods is determined by the fact that some species colonize test panels exclusively during the first stage of exposure (e.g. Molgula sp., Polydora ligni, Gonothyraea loveni and Nicolea sp. among others)

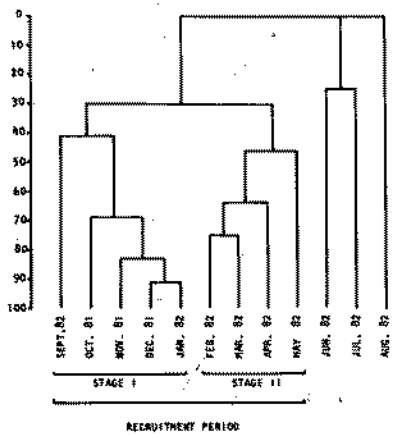


Fig.8.-Cluster analysis dendrogram for Jaccard's similarity index; ordinate is a similarity scale from 0 to 100.

Biomass fluctuations on short-term panels

Biomass values registered along the year showed variations related with a marked seasonality in settlement cycles of the different fouling species.

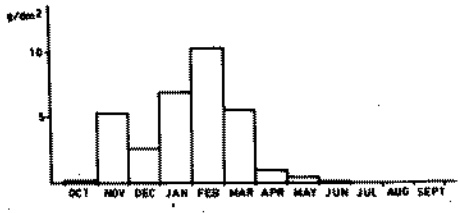


Fig.9.- Fouling biomass (dry weight) on short-term panels (level A).

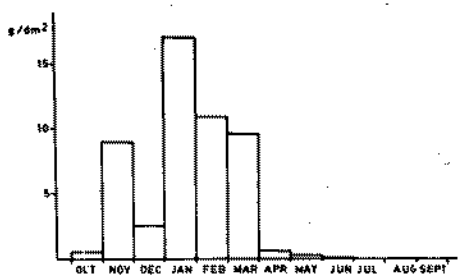


Fig.10.- Fouling biomass (dry weight) on short-term panels (level B).

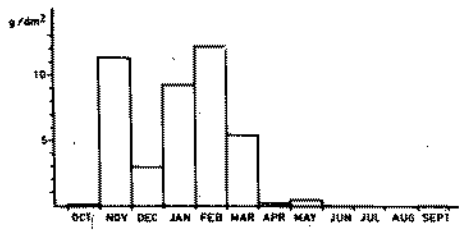
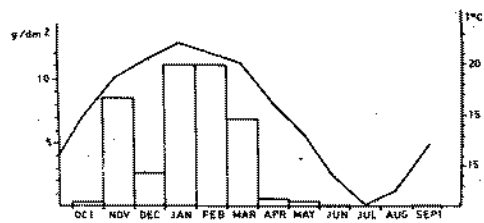


Fig.11.- Fouling biomass (dry weight) on short-term panels (level C).

Fig.12.- Fouling biomass (dry weight) on short-term panels expressed as an average of levels A, B & C and monthly mean water temperatures.



During the warm months, abundant colonization and the large sizes reached by attached organisms determined high values of dry weight, with a maximum monthly mean of 11.17 g/dm^2 during February. In the cold season, on the other hand, there was practically no settlement and consequently, very low biomass values were registered. Colonization was even so poor during July and August that it was impossible to carry out weight determinations in these months; panels appeared covered only by a thin layer of protozoans and detritus.

This marked seasonality which characterizes temperate waters (Crisp, 1965) suggests that temperature is a key factor in the regulation of seasons of settlement and monthly fouling biomass. These processes are affected by critical temperatures acting upon maturation and release of sexual products and upon larval/adult survival; by high temperatures which shorten the duration of the larval stage as well as by temperatures acting upon organisms which form part of the diet of fouling species.

The functional relationship between temperatures (°C) and monthly biomass values (g/dm^2) were examined. This analysis was based on three sets of variates:

- non-transformed field data, X and Y
- ln X and ln Y
- X and ln Y

The product-moment correlation coefficient was used to select the best association between the three pairs of variates. Since the third of these showed the highest correlation coefficient (r= 0.8821**), the appropriate relation was assumed to be of the form:

$$\ln Y = \ln a + b X \qquad (Y' = a' + b X)$$

Since the data are subject both to natural variability and measurement error, we used the geometric mean (GM) Model II method (Ricker, 1973) for estimating the functional relationship between X and Y.

Considering that:

$$v = b / r$$

where v = GM Model II slope
b = Model I slope
and r = absolute value of the correlation coefficient,

then

$$v = 0.4567 / 0.8821 = 0.5177$$

The Y'-axis intercept (a') was estimated from:

$$a' = \bar{Y} - b \bar{X} = -1.5299 - 0.5177 \cdot 14.6682 = -9.1236$$

The calculated regression line would then be of the form:

$$\ln Y = -9.1236 + 0.5177 X \quad \text{(fig.)}$$

The confidence intervals for the Model II slope, as shown by Ricker (1975), were calculated as follows:

$$L1 = v \quad \frac{F\ (1 - r^2)}{N - 2} + 1 \quad + \quad \frac{F\ (1 - r^2)}{N - 2} = 0.6438$$

$$L2 = v \quad \frac{F\ (1 - r^2)}{N - 2} + 1 \quad - \quad \frac{F\ (1 - r^2)}{N - 2} = 0.4163$$

where F = the variance ratio at 95% confidence limit for $n_1 = 1$ and $n_2 = N - 2 = 20$ degrees of freedom

N = number of pairs of variates = 22

The equation relating mean monthly biomass values expressed in weight per surface unit (g/dm^2) with mean monthly temperatures (°C) derived from re-transformation of the original line is:

$$Y = 1.0906 \cdot 10^{-4} \quad e^{0.5177\ X} \quad \text{(fig.14)}$$

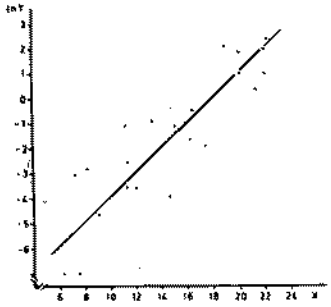


Fig. 13.-Relationship between mean biomass values and mean monthly water temperatures.

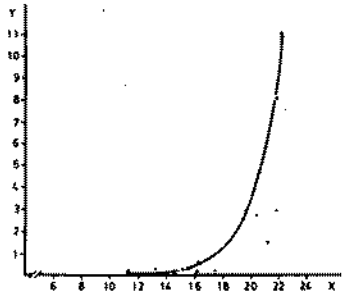


Fig. 14.-Relationship between mean biomass values and mean monthly water temperatures; the curve was obtained from re-transformation of original regression line.

Evolution of the community and biomass fluctuations on long-term panels.

Observations on long-term panels have served a double purpose. For one part, to evaluate biomass fluctuations of fouling growth along the year; on the other hand, to outline the main stages of community development and the influence of these processes on biomass values.

The present assay was initiated in early spring, so that prevailing environmental conditions favoured reproduction, larval development and recruitment of fouling organisms. The slime film became established very shortly after panels were submerged; then followed colonization by dominant pioneer species, such as Ciona intestinalis, Tubularia crocea, Nicolea sp. and tunicates of the subfamily Didemninae.

This first stage is typified by organisms with high rates of growth, coinciding with a proggressive raise of water temperatures. By the fifth month of exposure, these organisms have reached their maximum sizes and peak total biomass values were registered (44.25 g/dm^2 for panel A, 56.17 g/dm^2 for panel B and 48.73 g/dm^2 for panel C). During this period, colonization by other species was conditioned by the ability of larvae to settle on previously occupied substrata and of adult attached forms to avoid the invasion by new settlers (fig. 15).

Bugula neritina, B. stolonifera and Paraisometridium pehuensis, which made their arrival during this stage, were found growing on Ciona intestinalis, as a result of lack of space availability on panel surfaces.

The second important stage in community evolution was heavy mortality of the Ciona intestinalis population and the consequent detachment of species from test panels. This process of detachment of C. intestinalis and other species growing on it led to the appearance of bare areas, which became available for recolonization. It took place during the sixth, seventh and eight months of exposure and was reflected in a marked decline of total biomass, evidenced in fig. 15.

The third stage was characterized by recolonization of available space taking place almost simultaneously with detachment phenomena. During the first period of the third stage, a massive settlement and development of the seaanemone Paraisometridium pehuensis was recorded, but this species was soon replaced by other species of seasonal recruitment. Recolonization coincided with a period of low water temperatures, so that it

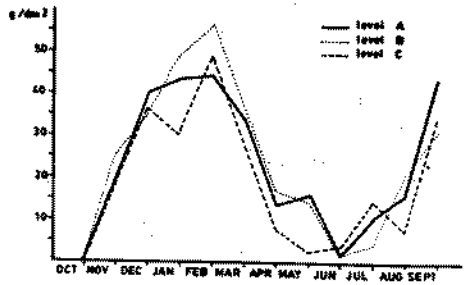


Fig. 15.-Biomass(dry weight) on long-term panels, october 1981-september 1982.

was slow as compared with total colonization rates. Had it taken place during the warm season, the community would have presumably reached its previous condition (at least from a quantitative point of view) in one or two months, as was the case for the port of Mar del Plata (Bastida, 1969) and for Linnhaven Bay,U.S.A. (Otsuka & Dauer, 1982).

The pattern of community evolution observed in Ingeniero White is similar to that described by Sutherland & Karlson (1977) in Beaufort, U.S.A., where a sequence of mortality-recolonization is apparently taking place uninterruptedly. This kind of sequence would typify temperate and subtropical communities, in which recruitment and ability of larvae to invade preoccupied surfaces are variable. There are also variations in the ability of settled adult forms to resist invasion by larvae of potential competitors and on the other hand, organisms are usually short-lived in these environments.

The pattern described above for Ingeniero White is similar in all harbours along the Argentine coast studied upto the present (Bastida, 1971 a & b;Bastida et al., 1977; Bastida & Brankevich, 1980; Bastida et al., 1980).

Finally, it should be pointed out that on long-term panels, the effect of water temperature on weight of fouling growth (total biomass) is detected only during the initial stages of settlement and recolonization, as observed in previous studies(op. cit.)

DISCUSSION

Researches carried out in Ingeniero White during 1981/82 have indicated that among the 37 taxa composing the total number of identified macrofoulers, the dominant groups were coelenterates (hydrozoans and sea-anemones), polychaete worms, bryozoans, certain crustaceans and tunicates.

None of these macrofouling species have presented annual patterns of settlement; in all cases, colonization was seasonal, with variations in the length of the colonizing period.

As expected for an area of temperate climate, peak abundances were recorded during the warm season, while in the cold months settlement was very poor or completely interrupted for most species. This feature of the settlement cycles directly affects annual biomass fluctuations on short-term panels. Similar patterns were observed in previous studies at other Argentine harbours like Mar del Plata, Puerto Belgrano and Puerto Quequén (Bastida, 1971 a & b; Bastida & Torti,1973; Bastida et al.,1977; Bastida & Adabbo, 1977; Bastida & Lichtschein, 1978; Bastida & Brankevich, 1980; Bastida et al. 1980).

No macrofouling species have shown definite depth preferences. During this study, the absence of macroscopic algae was noteworthy and can be attributed to the low water transparency and to prevailing conditions at the depths at which panels were submerged.

It must be pointed out that green algae usually constitute important competing factors on the waterline level or very close to the water surface.

The functional relationship between monthly water temperature and monthly biomass on short-term panels was preliminarily found to fit the

following equation:

$$Y = 1.0906 \cdot 10^{-4} \cdot e^{0.5177\ X}$$

Macrofouling on long-term panels was represented by 56 taxa (belonging to 11 phyla), of which 12 were present during the whole period in which community evolution was studied.

Community evolution on long-term panels can be summarized in three main development stages: a first stage, typified by the rapid establishment of a slime film and the accelerated development of pioneer species, favoured by temperature conditions prevailing during the initiation of the assay, which extends upto the fifth month of exposure; a second important stage was characterized by the death and consequent detachment of the main species forming the community, mainly the dominant form Ciona intestinalis, which took place during the sixth, seventh and eighth month of exposure; then follows the third stage of development, consisting of recolonization of bare areas and which takes place almost simultaneously with the process of detachment; at this stage, the process of recolonization was relatively slow on account of the low water temperatures.

The pattern of community evolution can be defined as a clear sequence of initial colonization-development-death and detachment-recolonization, which takes place uninterruptedly. The length of each developmental stage as well as the intensity of fouling settlement depends on the time of the year, as has been observed in other Argentine harbours and is apparently characteristic of temperate climates.

AKNOWLEDGEMENTS

The authors wish to thank authorities, colleagues and laboratory assistants from the Argentine Institute of Oceanography (IADO), National Institute for Research and Development of Fisheries (INIDEP) and the Energy Agency of the Buenos Aires Province (DEBA) for their help during the course of this research.

We are also grateful to Lic. Victoria Lichtschein for the critical review of this paper and for translating the manuscript.

CHECKLIST OF FOULING ORGANISMS ENCOUNTERED ON TEST PANELS IN INGENIERO WHITE HARBOUR (ARGENTINA)

PROTOZOA
Zoothamnium sp.

PORIFERA (unident.)

COELENTERATA
Tubularia crocea
Bougainvillia ramosa
Gonothyraea loveni
Paraisometridium pehuensis

NEMATODA (unident.)

NEMERTINEA (unident.)

ANNELIDA
Typosyllis sp.
Lumbrinereis sp.
Nicolea sp.
Polydora ligni
Hydroides uncinata
Harmothoe sp.
Halosydnella sp.
Syllidae
Cirratulidae
Spirorbinae
Sabellidae

MOLLUSCA
Lyonsia patagonica
Littoridina australis
Chaetopleura isabellei
Anachis sp.
Ostrea spreta
Crepidula aculeata

BRYOZOA
Bugula neritina
Bugula stolonifera
Crisia sp.
Smittoidea sp.
Alcyonidium polyoum
Bowerbankia sp.

PICNOGONIDA (unident.)

CRUSTACEA
Copepoda Harpacticoidea
Ostracoda
Stenothoe sp.
Lembos sp.
Corophium insidiosum
Caprella equilibra
Caprella penantis
cf. *Noculacia* sp.
Synidotea marplatensis
Sphaeroma sp.
Balanus amphitrite
Neomysis sp.
Cyrtograpsus altimanus
Pilumnus reticulatus
Pachychaeles sp

ECHINODERMATA
Ophiuroidea (unident.)

TUNICATA
Ciona intestinalis
Molgula sp.
Botryllus schlosseri
Didemninae
Ascideacea (unident.)

PISCES
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RESUMÉ

Da communication présente l´étude de"colonies des salissures" au port "Ingeniero White"où les problèmes de salissures ont été détechés dans les systèmes de refroidissement d´une centrale électrique. Les mêmes problèmes seront présentés dans une deuxième station plus importante dont la construction est programmée dans le proche avenir. Les résultats sont obtenus durant deux périodes d´échantillonage en utilisant des éprouvettes acryliques comme surfaces expérimentales. Une série d´éprouvettes à court termesont fournis des informations sur les cycles se sédimentation des différentes espèces de salissures alors que l´évolution de colonies des sallisures a été étudiée par des séries d´éprouvettes à long termes. Les fluctuations de la biomasse relatives à l´évolution de salissure durant l´année sont déterminées,ainsi que la relation entre ces fluctuations et les variations dans certaines conditions d´environement. Les résultats qui sont obtenus durant cette recherche seront utilisés pour déterminer le meilleur control des salissures fondé sur la chlorination aux stations de puissance étudiées dans le but d´assurer les provisions adequates en énergie dans le domaine industriel qui est d´une importance majeure à l´intérieur du pays.

Performance of Copper Base Alloys in Polluted Sea Water

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Abstract :

Pollutants in sea water find their way to sea water cooling system and affect the performance of heat exchangers materials. The present work deals with the effect of sulphide, ammonia, urea, oil. The results show that pollutants contain sulphide species are the most aggressive ones and that the presence of other pollutants with sulphide reduce the harmful effect of the later and the results fit the relation

$$\text{Corr.Rate} = \text{Corr.Rs} - \sum_{i=1}^{i=j} ai \times i + \sum_{i=1}^{i=j} bi \times i \times j$$

The results also show that pollutants react with each other thus reducing the harmful effect of some species. All pollutants studied are harmful to aluminium brass, but in case of 70/30 cupronickel, ammonia, and oil are inhibitors.

Abstract in French:

Les polluants en eau des mers pénètrent dans les systèmes de refroidissement par eau des mers et affectent les performances des matériaux des échangeurs thérmiques. Le présent travail traite les éffets des sulfides, de l'amoniac, de l'urée, et de l'huile.

Les résultats montrent que les polluants qui contiennent les traces des sulfides sont les plus agréssifs, et que la presence d'autre polluants contenant des sulfides réduit les éffets nefastes de ce dernier. Les resultats sont alors exprimés par la relation:

$$\text{Corr. Rate} = \text{Corr. Rs} = - \sum_{i=1}^{i=j} ai \times i + \sum_{i=1}^{i=j} bi \times i \times j$$

Les resultats montrent encore que les polluants réagissent ensemble réduisant les éffets néfastes de certains elements. Touts les polluants étudiés corrodent le bronze d'aluminium, mais, dans le cas de l'alliage Cu - Ni 70/30 l'amoniac et l'huile sont des inhibiteurs de corrosion.

The performance of copper alloys in clean sea water has been examined extensively and documented, and the good performance of some of these alloys will not necessary duplicate when these alloys are exposed to attack by polluted sea water of various degrees of salinity encountered in many harbors. Pollutants vary in their concentration and type from one location to another on the coast, and they also vary in their origin. Effluents discharged from nearby chemical plants, and oil leaking from nearby boats and ships comprise a few of the sources for these pollutants. Other sources of contamination of sea water used in cooling systems is the leakage of the medium to be cooled into the cooling system through pitting, or the decomposition of some of the chemicals added to boiler such as the case of ammonia.

However if polluted water is taken from such sources for cooling purposes in a steam power plant, oil refinery, desalination plant and chemical plants, the corrosion process will be different compared with that in clean sea water. The effect of some of pollutants in sea water has been examined and the adverse effect of dissolved suphide on the corrosion of copper alloys in a queous media is well documented (1-8). Although this deleterious effect of sulphide is known, the mechanism of corrosion by various sulphide species is debatable. In polluted sea water aluminium brass did not perform as well as 70/30 cupronickel. In clean sea water aluminium brass gives service life equals to that given by 90/10 cupronickel alloys (9). 90/10 cupronickel is resistant towards sulphide polluted sea water but the attack rate increases with flow velocity (10). 70/30 cupronickel is the most resistant of the three. Ammonia and ammonium compounds produced as a result of decay of marine organisms usually make copper alloys susceptible to stress corrosion cracking in sea water. Also the presence of ammonia in sea water from sewage contamination has actually been suggested as being responsible for cracking condenser tubes in service (11). 70/30 cupronickel suffers from impingement attack at higher velocities (12). Contamination of sea water with ammonia at levels of 1ppm or more has a pronounced effect on corrosion behaviour of aluminium brass and no observable effect on 70/30 cupronickel (12).

The effect of the above pollutants in combination as well as the effect of other pollutants did not receive the attention of workers as it should. So the present work is planned to study the effect of other pollutants besides sulphide and ammonia and some combinations of these pollutants.

Experimental Procedure

Flow Loop:-

The recirculating loop used in this work is shown schematically in Figure 1. The components of the loop are selected to be corrosion resistant. The pump is stainless steel with a plastic inner coating. The piping is PVC, the valves are made from brass and the testing cell is made from stainless steel of grade 304. It was necessary to incorporate air cooling to remove the unwanted heat. The sea water velocity in the loop was maintained at 1.5m/sec. by adjusting the valve in the velocity control by

pass loop. To reduce the turbulant effect the test cell was tapered (2.5^{o}) as well as an extra length of PVC tube equal to 30 times the cell diameter was connected in the inlet of the testing cell. The specimens were used as pairs of 27x7xlmm, 4mm apart and were connected to the probe of two electrode corrosometer. The specimens themselves were embedded in plastic material with electrical leads. Embedding was done by cold mounting using a mould designed in such a way that the surface at which the embedded specimens had the same curvature as that of the testing cell. The specimens were celibrated with respect to standard corrosometer tips and shape correction factor required was taken into consideration.

Before testing the specimens were descaled in HCl/H_2SO_4 solution (ASTM recommended practice G1-72 for copper alloys) and in some cases the specimens were polished with 320 and 600 emery papers, rinsed in distilled water and dried in warm air. Duration of the test was taken as 100 hrs because the trial runs showed that the corrosion rate reaches the steady state after 50 to 60 hours. Accordingly the corrosion rate was taken after 75 hrs.

Environment:-

Fresh, filtered Arabian Gulf water was obtained for these experiments from Doha desalination plant in Kuwait. This sea water had an initial PH of approximately 8.1 and a salinity of 44 to 60 parts per thousand. The PH was monitored at regular intervals during each experiment and if necessary small additions of NaOH or HCl were made to maintain the PH at 8.2. The temperature of sea water was $50 \pm 2^{o}C$ throughout the experiment which was approximately equal to the outlet temperature of heat exchangers in most of chemical factories in Kuwait in winter season.

The pollutants examined were ammonia (A), urea (U) sulphide (S), oil and combinations of these. Table 1 shows the levels of the various pollutants.

Table (1) pollutants and their levels

Pollutant	Clean	A	U	S	A + U	A + S	U + S	A + U + S	Oil	Oil
level ppm	0	10	10	2	10+10	10+2	10+2	10+10+2	10	20

The sulphide was added as sodium sulphide, and ammonia as commercial ammonia. The pollutants were studied separately and in combination. The materials examined were aluminium brass and 70/30 cupronickel alloy of composition show in Table 2.

Table (2) chemical composition of alloys

material	chemical composition
Al-brass	76Cu, 21Zn, 2Al -
Cupronickel	30Ni, 1Zn, 0.5Fe, 1Mn, RCu.

Experimental Results and Discussion:

Effect of Pollutants on the Corrosion of Al-brass

The effect of different pollutants on the corrosion rate of aluminium brass is shown in some of typical figures 2 to 6. The figures show that all pollutants enhance the corrosion rate, but they vary in their effect. Table 3 shows the corrosion rate after 75 hrs of exposure (steady state condition), together with the relative aggressiveness index for each pollutant with respect to fresh clean sea water.

Table (3). Corrosion rate data of aluminium brass in polluted sea water

Pollutant	Corr.rate MPY	Aggressiveness index
blank	0.06	1
A10	0.22	3.2
U10	0.22	3.5
S2	6.5	110.0
A10+U10	0.085	1.5
A10+S2	1.0	16
U10+S2	0.1	1.6
A10+U10+S2	0.6	10
O10	0.11	2
O20	0.08	1.5

O = oil

The results show that sulphide containing pollutants give the highest corrosion rate, and the presence of 2 ppm sulphide ions in sea water increses the later's aggresiveness by 110 times as indicated by the aggresiveness index. This has been explained in terms of the corrosion product film formed which is loosely adherent (2, 13) and does not provide any protection.

The results also show that both ammonia and urea act as inhibitors when present in combination with sulphide, but urea is a stronger inhibitor compared with ammonia. The present data duggests a relationship between the corrosion rate and the concentration of pollutants as follows:

Corr. rate + Corr. Rs - 9.4A - 1086U + 1.02 A.U .... (1)

Where corr.Rs= corrosion rate when sulphide species alone are present in sea water A, U represent, the concentration of ammonia and urea in terms of ppm. The last term on the right end of the equation represents the interaction coefficient. In its general for equation (1) takes the form

$$Rs - \sum_{=}^{i=i} ai \times i + \sum_{i=1}^{i=j} bi \times i \times j \quad \ldots\ldots\ldots\ldots (2)$$

Where ai represent the interaction parameter between sulphide species and any other pollutant, while bi represents the interaction coefficient between sulphide and two pollutants and so on. Regarding the effect of oil, it is apparent that it is much milder compared with the effect of other pollutants and its aggressiveness index decreases as the oil content increases. This is likely to be because large amounts of oil increase the passive sites on the surfaces. The order of aggressiveness of various pollutants is as follows:

S>A + S>A + U+S>A or U>oil 10 > U+S > A+U or oil 20

The results show that pollutants containing sulphide species are the most corrosive ones and that the greatest corrosive effect of these species when they are present in water alone.

But it appears that the presence of other pollutants with sulphide species has a beneficial effect in reducing the corrosion rate, this is likely to be because some of these pollutants react with sulphide and thus transform it to less corrosive species.

Effect of Pollutants on the Corrosion of (70/30) Cupronickel Alloy in Sea Water

The results are shown in typical figures 7 to 11. From the figures it is obvious that all pollutants affect the corrosion rate, but while ammonia and oil inhibit the corrosion process, the other pollutants increase the corrosion rate. Similarly, the presence of sulphide species in sea water is very deleterious on the corrosion resistance of this alloy.

The most aggresive condition is when sea water contains sulphide species alone and the presence of other pollutants has an inhibitive effect.

Table (4) the data obtaiend.

Table (4). Effect of pollutants in sea water on the corrosion rate of 70/30 cupronickel alloy

Condition	Corrosion Rate MPY	Aggressiveness index
blank	0.05	1
A ammonia	0.01	0.2
U	0.13	2.6
S	15	300
A+S	1.4	28
U+S	08	16
A+U+S	1.7	34
Oil 10	2.06	1.2
Oil 20	.02	0.5

In a similar way the effect of pollutants can be put in the form.

Corr. rate = Corr.Rs - 29.4U - 27.2A + 2.62 AU .....(3)

Equation (3) is similar to equation (1) except for the values of the interaction parameters. The data also shows that the presence of oil in sea water inhibits the corrosion process as it reduces the anodic sites on the surface. The of decreasing the corrosive effect of the different pollutants is as follows:

S>A + S+U > A+S > U+S > U > oil

Generally speaking there is a strong interaction between the pollutants present in sea water and as it is obvious ammonia and urea reduce the activity of sulphide ions by transforming them into less active complexes.

Further assessment of the results about the performance of alunimium brass and 70/30 cupronickel alloy in polluted sea water shows that the former possesses better corrosion resistance in the presence of sulphide species while 70/30 cupronickel is superior in resisting the effect of other pollutants.

But as far as the corrosion process is concerned, the effect of pollutants should be considered with regard to the material involved. For instance, all pollutants are considered corrosive to aluminium brass, but for 70/30 cupronickel some pollutants inhibit the corrosion process such as ammonia and oil while others are corrodants.

Conclusions:

From the above discussion it is eacy to arrive at the following conclusions:

1. Pollutants in sea water affect the performance of copper base alloys.
2. The effect of each pollutant varys from one alloy to another.
3. Sulphide containing pollutants are the most aggressive ones.
4. The pollutants interact with each other and reduce the effect of sulphide species.
5. Al - brass possesses better resistance to sulphide attack while 70/30 cupronickel is superior in sulphide-free sea water.
6. Some pollutants such as ammonia and urea can be used as inhibitors for sulphide attack.

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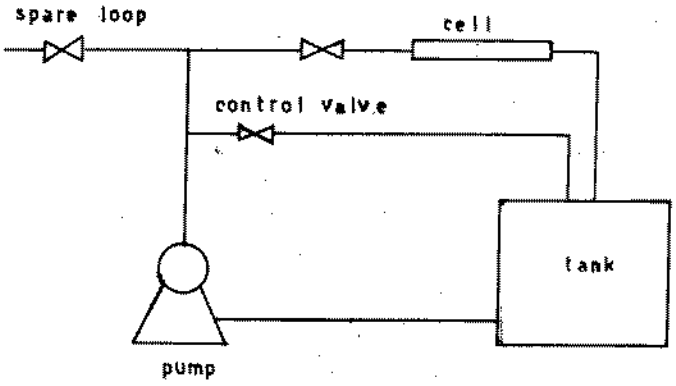


Figure(1)

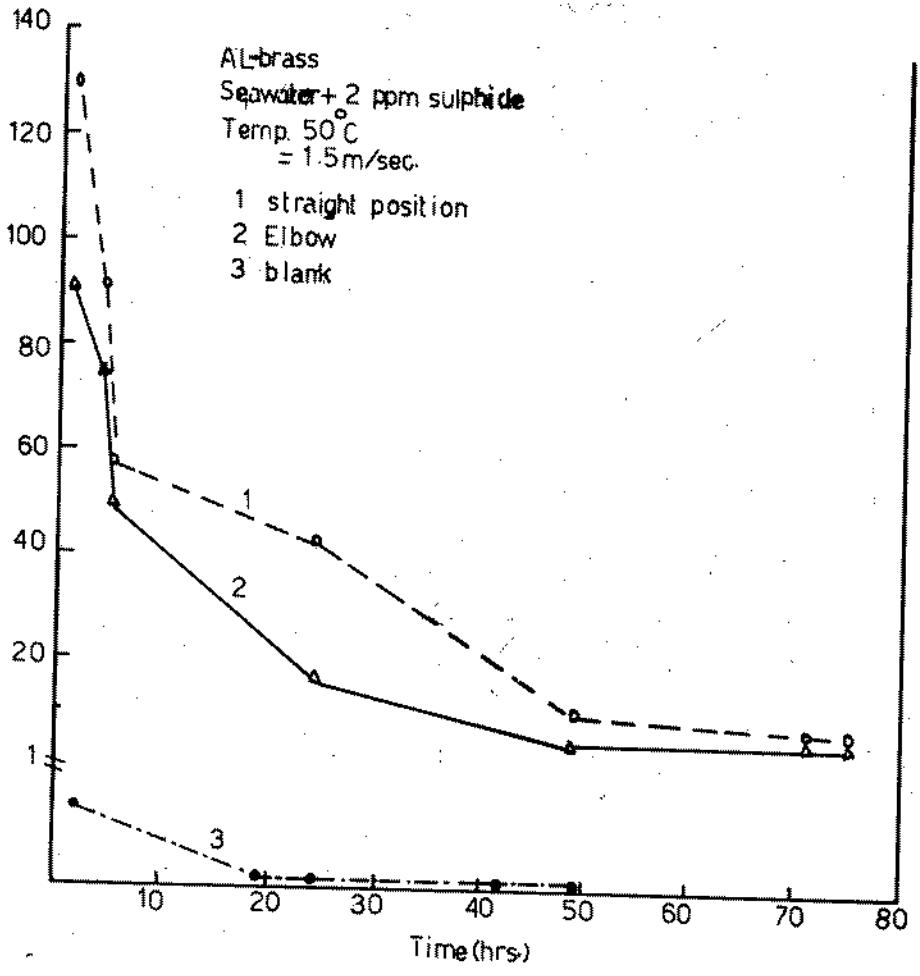
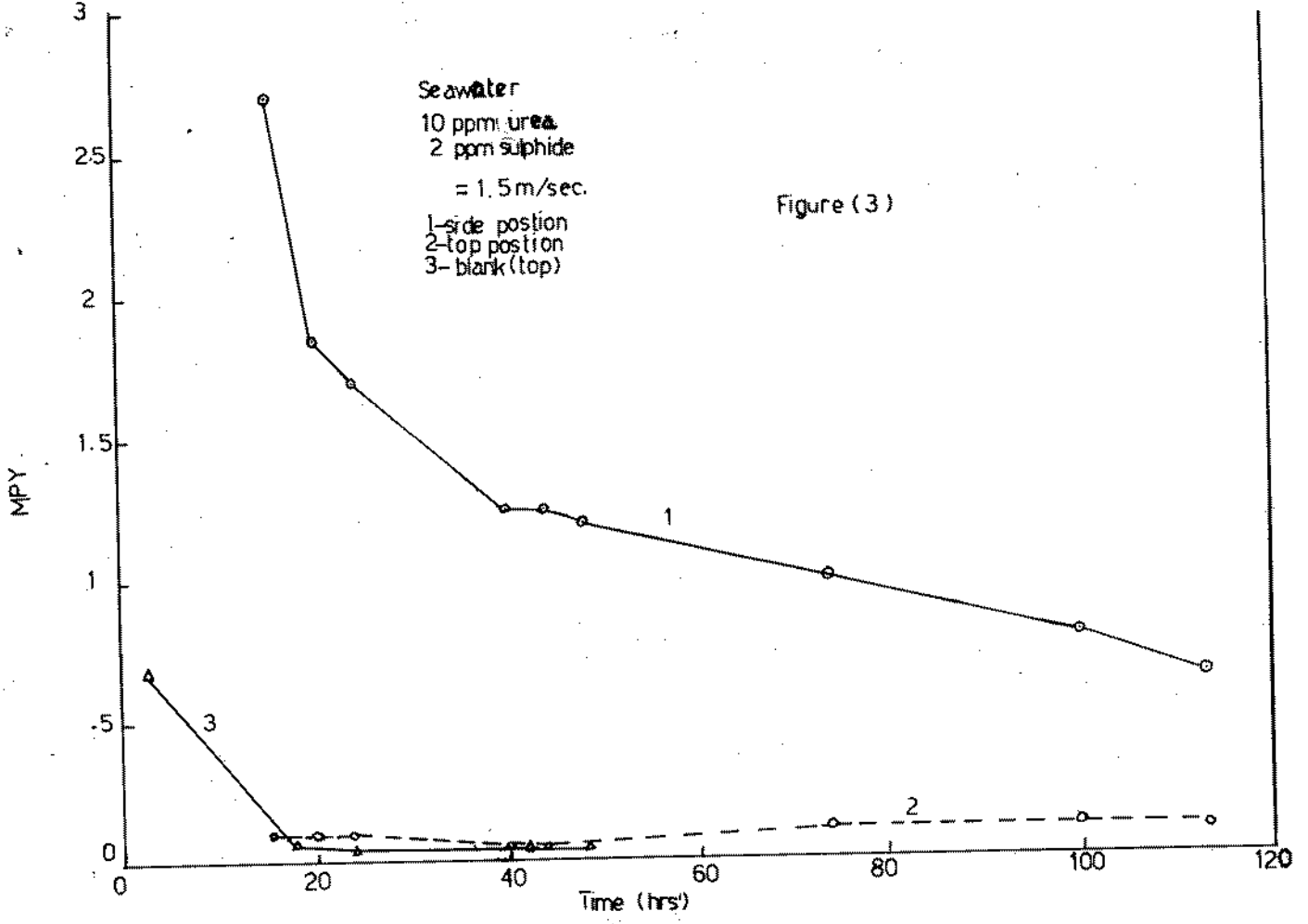
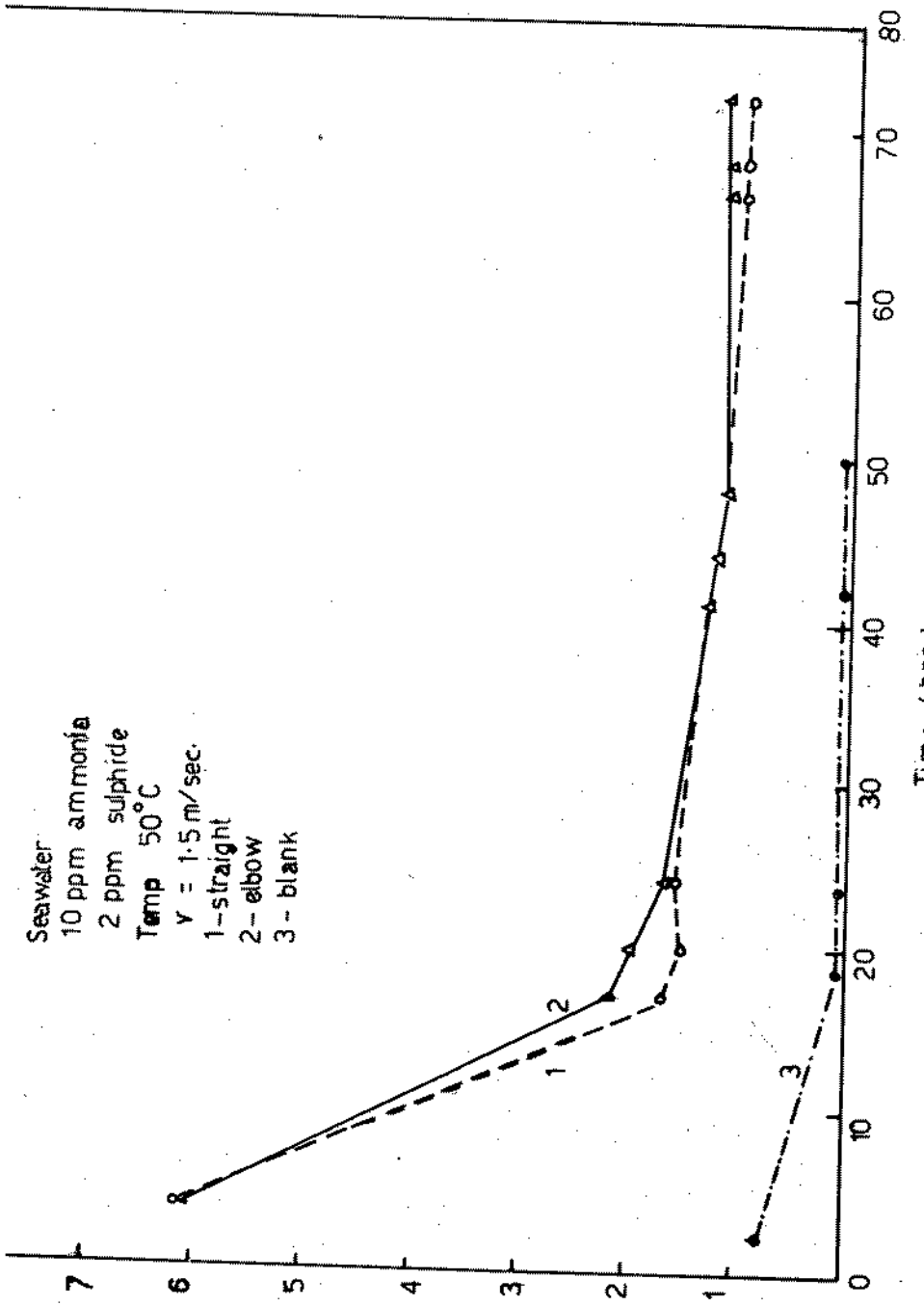


Figure (2)


Seawater
10 ppm urea
2 ppm sulphide
= 1.5 m/sec.
1-side postion
2-top postion
3-blank (top)
Figure (3)
MPY
Time (hrs')
1
2
3
0
.5
1
1.5
2
2.5
3
0
20
40
60
80
100
120


Seawater
10 ppm ammonia
2 ppm sulphide
Temp 50°C
v = 1·5 m/sec.
1-straight
2-elbow
3-blank
MPY
Time (hrs)
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10
20
30
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50
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4
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7

Figure (4)

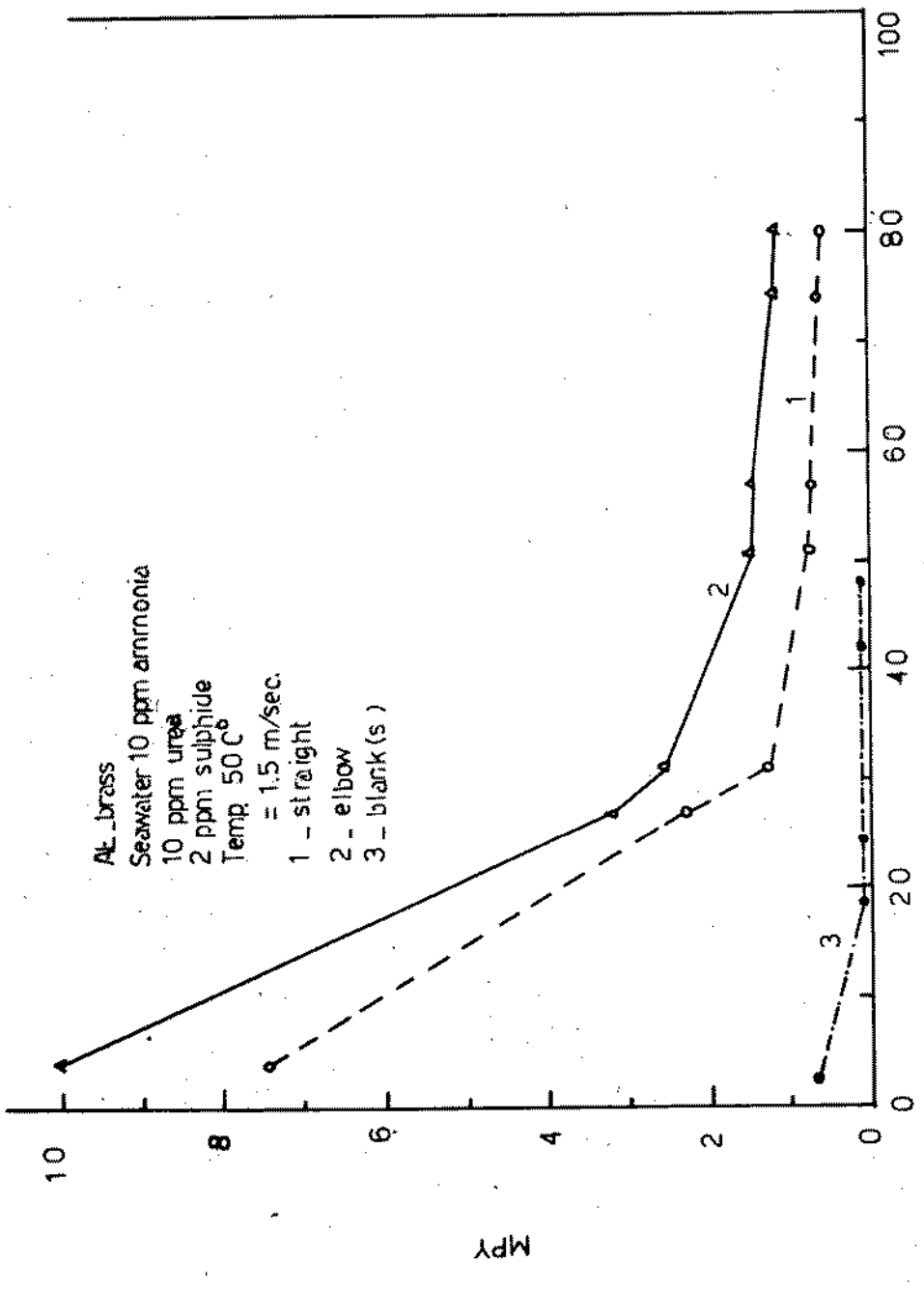

Al. brass
Seawater 10 ppm ammonia
10 ppm urea
2 ppm sulphide
Temp 50 C°
= 1.5 m/sec.
1 – straight
2 – elbow
3 – blank (s)
MPY
Time (hrs)
0
2
4
6
8
10
20
40
60
80
100
1
2
3

figure (5)

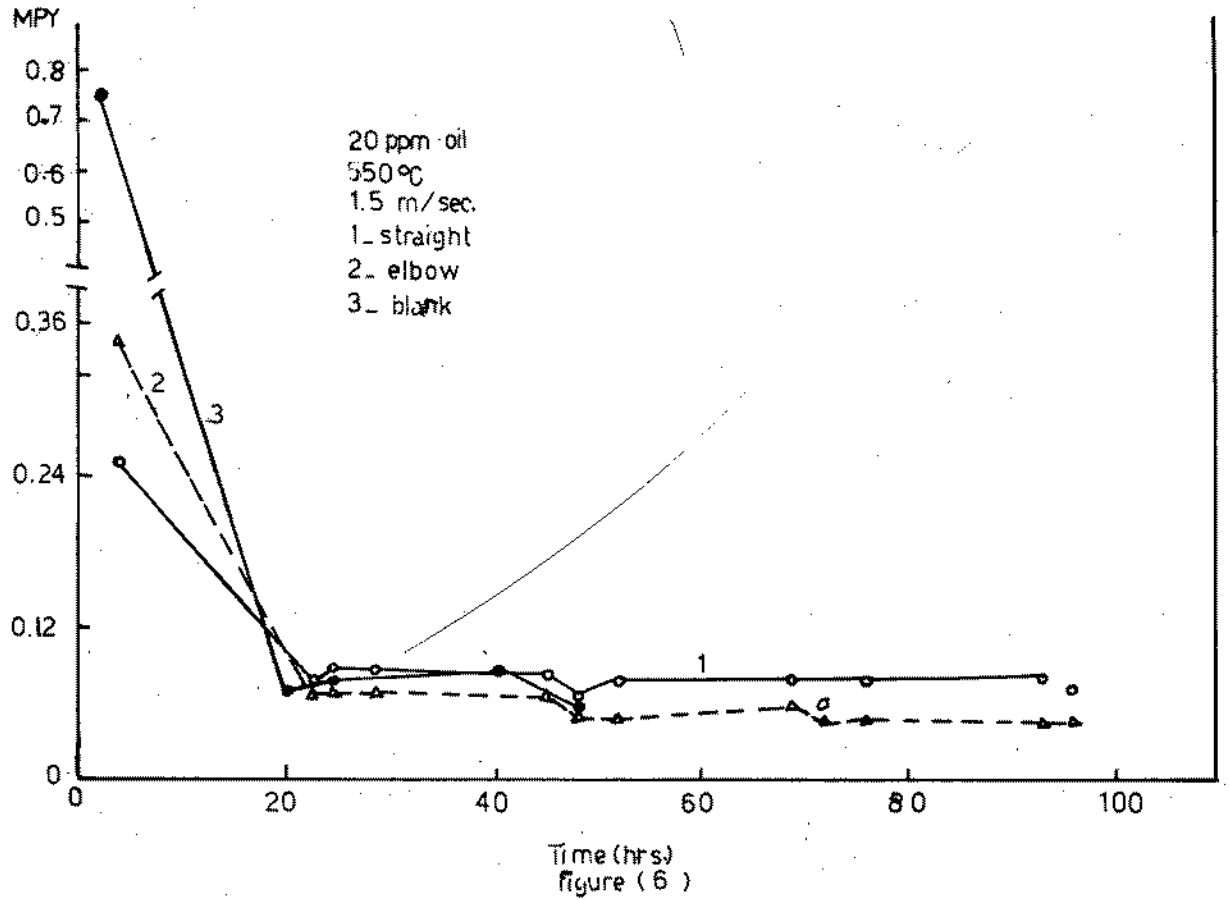
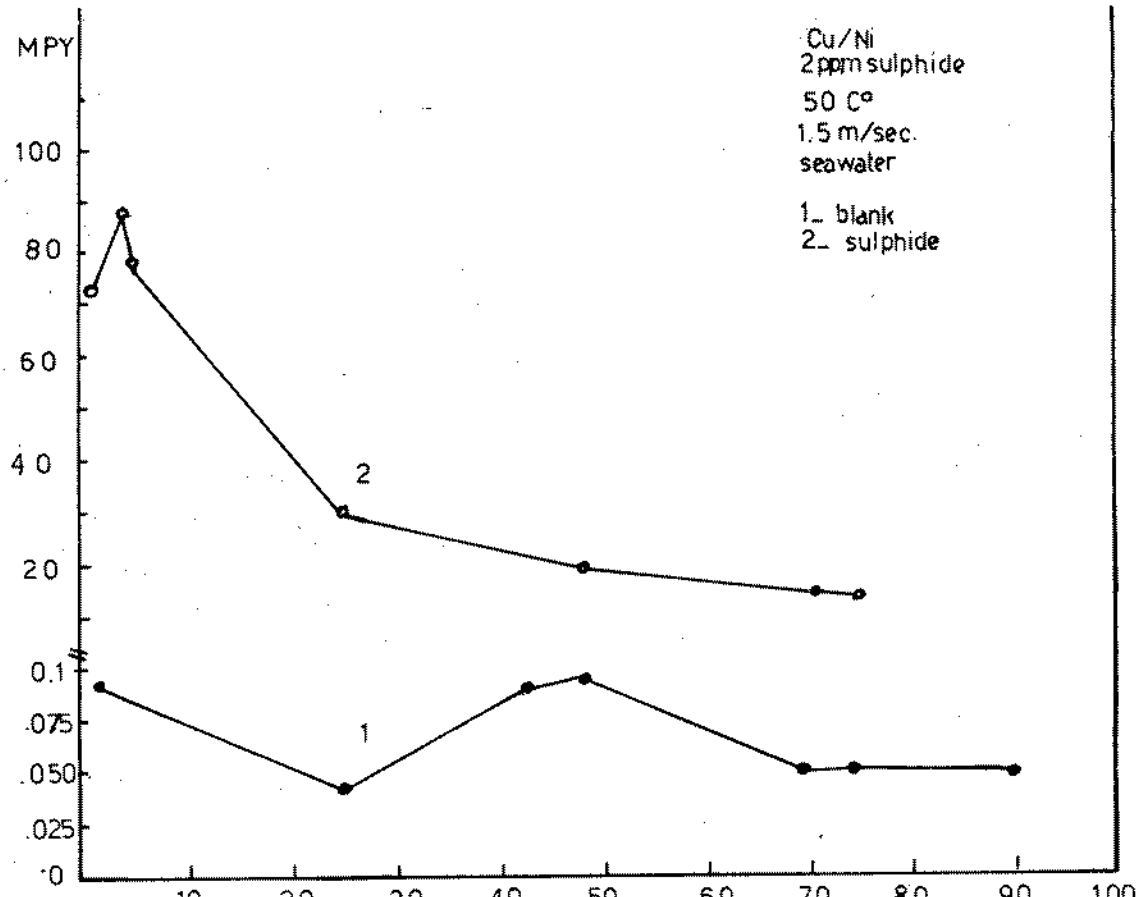

MPY
0.8
0.7
0.6
0.5
0.36
0.24
0.12
0
20 ppm oil
550 °C
1.5 m/sec.
1_ straight
2_ elbow
3_ blank
1
2
3
0
20
40
60
80
100
Time (hrs)

Figure (6)


MPY
100
80
60
40
20
0.1
.075
.050
.025
0
10
20
30
40
50
60
70
80
90
100
Time (hrs.)
Cu/Ni
2ppm sulphide
50 C°
1.5 m/sec.
seawater
1_ blank
2_ sulphide
1
2
Figure (7)

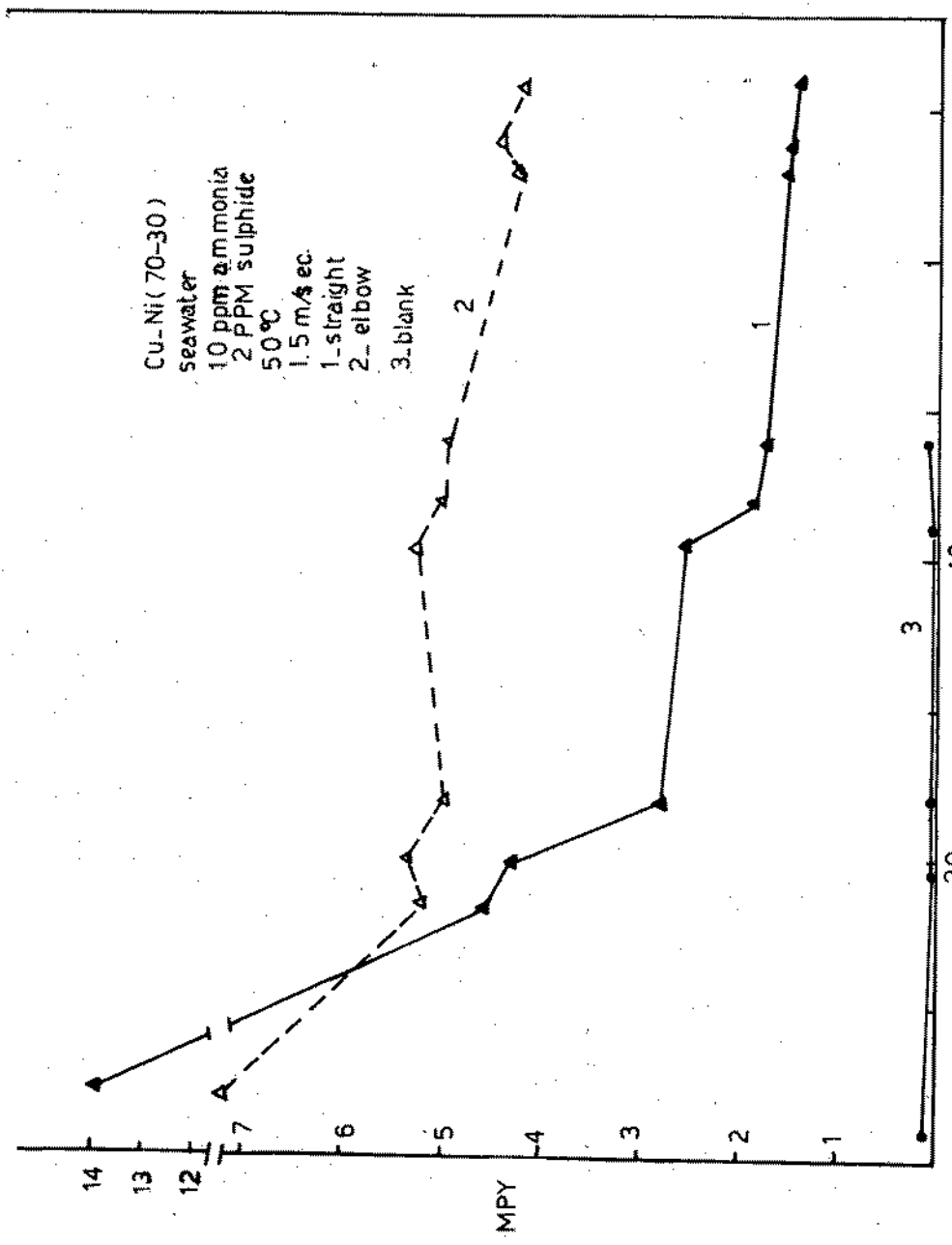

Cu-Ni(70-30)
seawater
10 ppm ammonia
2 PPM sulphide
50°C
1.5 m/sec.
1_straight
2_elbow
3_blank
MPY
time(hrs.)
14
13
12
7
6
5
4
3
2
1
20
40
60
1
2
3

Figure (8)

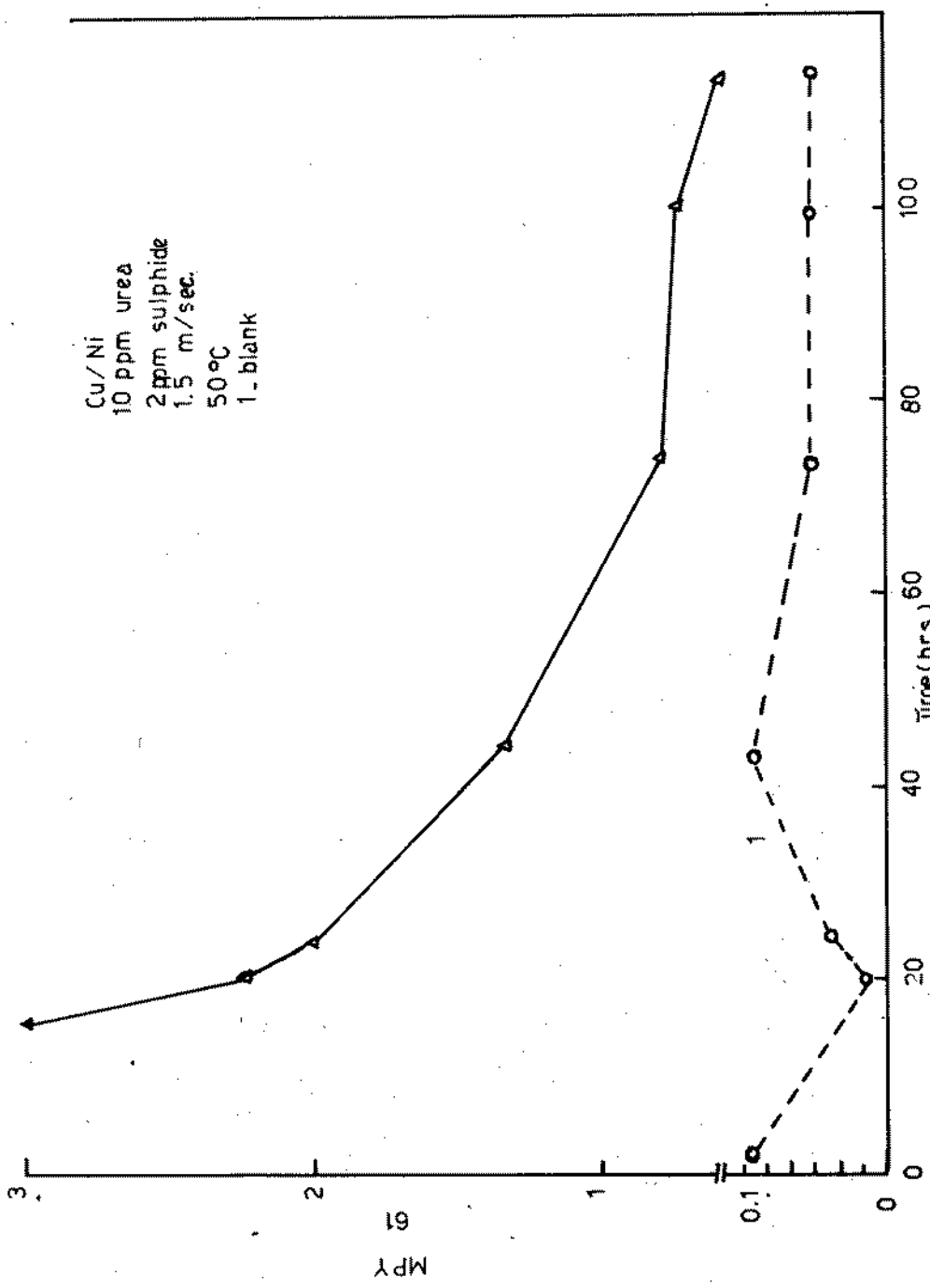

Cu/Ni
10 ppm urea
2 ppm sulphide
1.5 m/sec.
50°C
1_blank
MPY
61
3
2
1
0.1
0
0
20
40
60
80
100
Time (hrs.)
1

Figure (9)

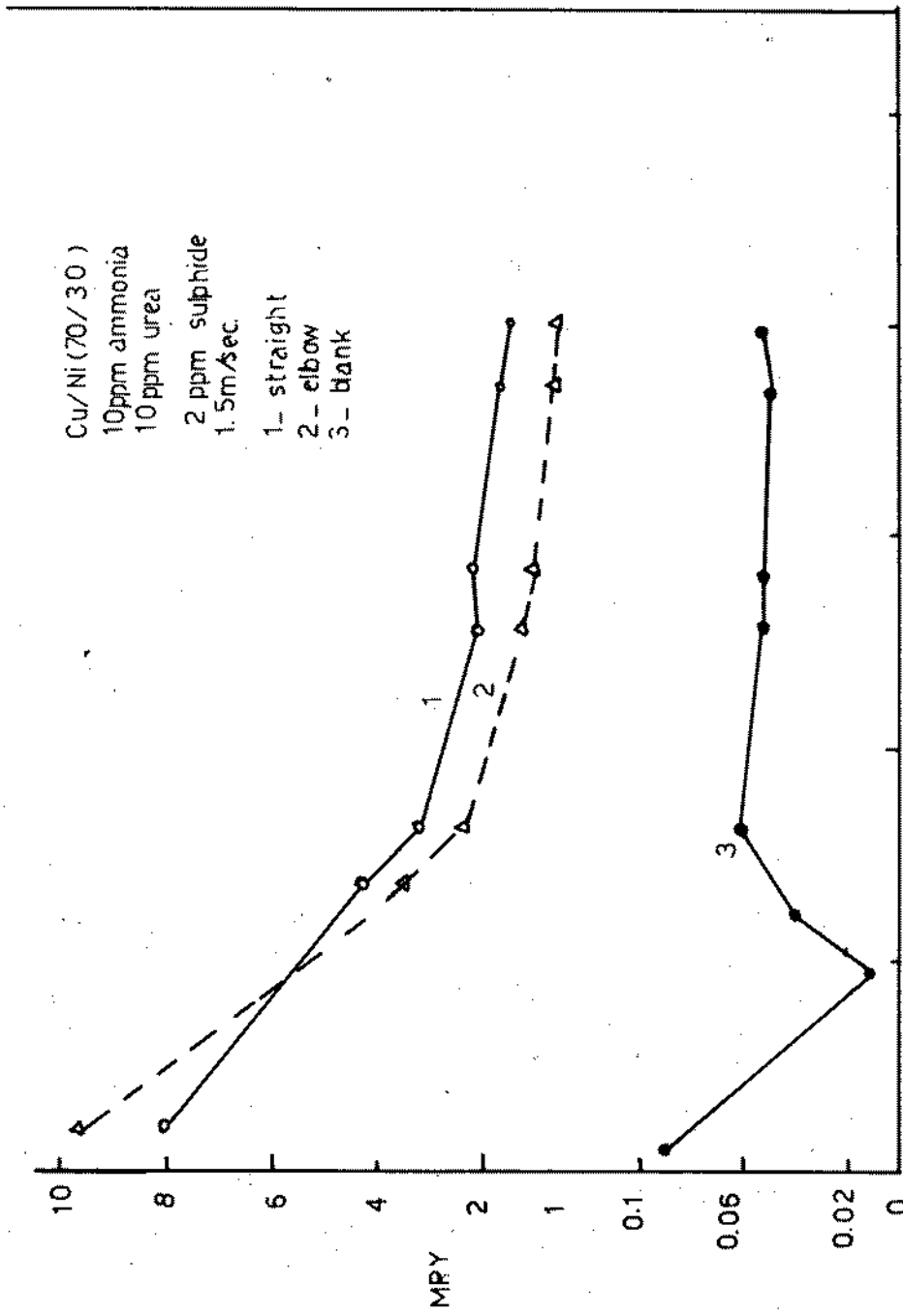

Cu/Ni (70/30)
10ppm ammonia
10 ppm urea
2 ppm sulphide
1.5m/sec.
1- straight
2- elbow
3- blank
MRY
10
8
6
4
2
1
0.1
0.06
0.02
0
20
40
60
80
100
Time (hrs)
1
2
3

figure (10)

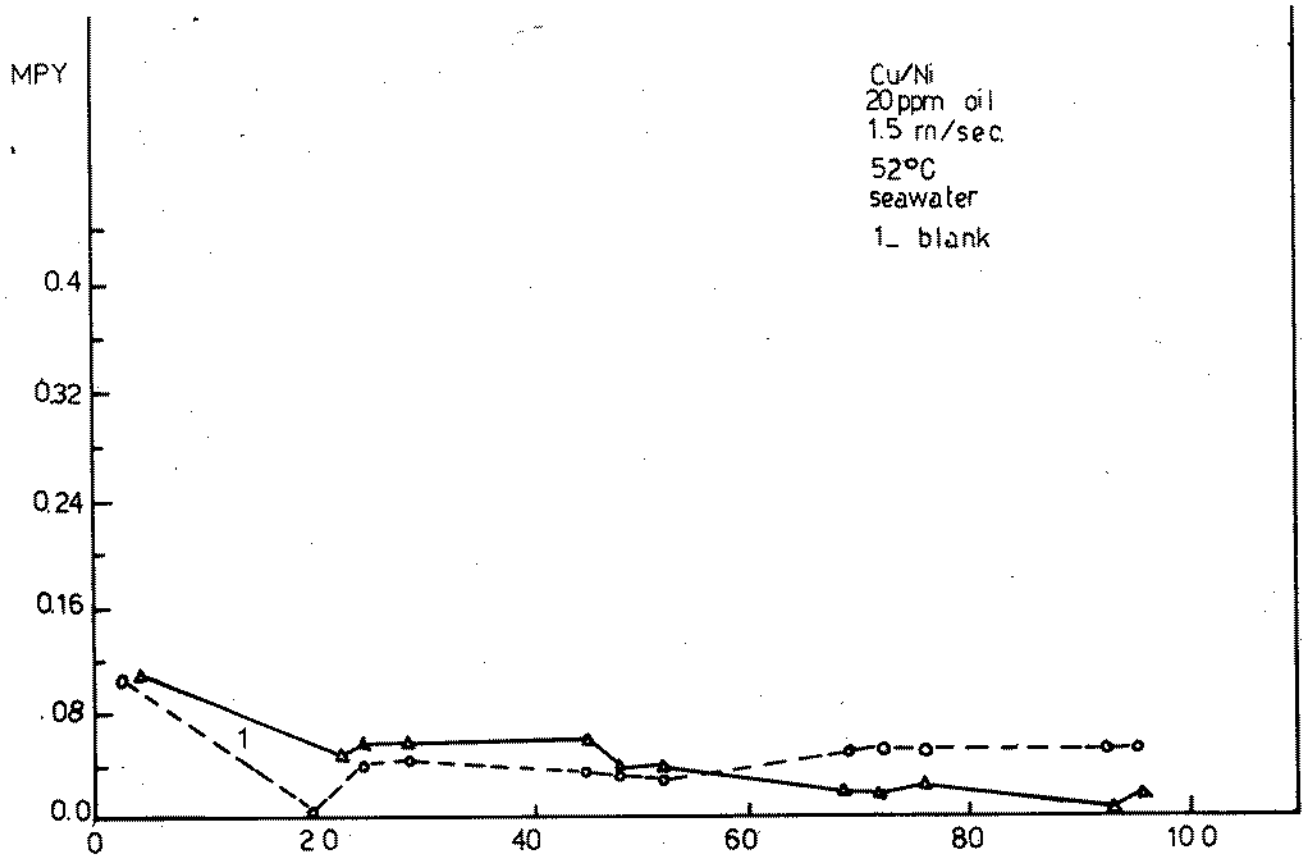

MPY
Cu/Ni
20 ppm oil
1.5 m/sec
52°C
seawater
1_ blank
0.4
0.32
0.24
0.16
.08
0.0
1
0
20
40
60
80
100
time (hrs)

Figure (11)

THE EFFECTS OF TRIBUTYLTIN COMPOUNDS ON SPORE DEVELOPMENT IN THE GREEN ALGA ENTEROMORPHA INTESTINALIS (L) LINK.

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ABSTRACT

The inhibitory effects of a range of tributyltin compounds on spore germination in the marine fouling green alga Enteromorpha intestinalis (L) Link were investigated in laboratory culture. All the compounds had the same basic structure (Bu_3SnX) but differed in the composition of the X moiety. The ED_{50} values for newly settled Enteromorpha spores ranged from 2.7×10^{-5} ppm for tributyltin benzoate to 8.6×10^{-3} ppm for tributyltin acrylate. The toxicity of the compounds appeared to be influenced by the nature of the X group. Using one of these compounds, tributyltin oxide, the progressive increase in ED_{50} values of Enteromorpha spores after increasing post-settlement times was demonstrated.

RESUMÉ

Les effets inhibiteurs d'une gamme de composes du tributyle etain sur la germination en milieu marin, des spores d'algues vertes Enteromorpha intestinalis (L) Link ont été étudies dan des cultures de laboratoire. Tous les composes avaient la même structure de base (Bu_3SnX) mais differaient dans la composition de la partie X. La ED_{50} valeur pour les spores d'Enteromorpha nouvellement fixes s'étageait de 2.7×10^{-5} ppm pour le benzoate de tributyle etain, a 8.6×10^{-3} ppm pour le acrylate de tributyle etain. La toxicité des composes s'averait en relation avec la nature du groupe X. Utilisant l'un de ces composes, l'oxyde de tributyle etain, l'augmentation progressie dans la ED_{50} valeur des spores d'Enteromorpha après augmentation du temps de pré-fixation a été demontrée.

INTRODUCTION

Marine algae are major, world-wide fouling organisms and have been reported on a wide range of structures. They are particularly active colonisers of tankers and container ships, thereby increasing the skin-frictional resistance of the hull through the water and incurring speed or power penalties upon the vessel concerned (Townsin et al, 1976). The number of different algae implicated in large ship fouling is however quite small (Christie, 1973; Benham & Bellinger, 1979) and the bulk of algal fouling can often be attributed to a few species only. The green algal genus Enteromorpha has, in particular, been regularly observed fouling tankers coated with copper-based antifouling compositions (Christie, Evans and Callow, 1975).

In the search for more effective control of Enteromorpha fouling, increasing attention has been given, over recent years, to the development and application of the organotin range of biocides. Both tributyl, and to a lesser extent, triphenyltin compounds are now widely used in the protection of ships hulls (Evans & Smith 1975). Among the tributyltin compounds, the most commonly used in paint formulations are the oxide, fluoride, chloride, sulphide, acetate and methacrylate (usually polymerised with other acrylic monomers)(Evans and Hill, 1983). In the present paper we report on the toxicity of a number of these and other tributyltin compounds toward newly settled spores of Enteromorpha. In addition, using one of these compounds, tributyltin oxide, the increasing resistance of Enteromorpha spores with increasing time after settlement was investigated.

MATERIALS AND METHODS

1). PREPARATION OF ALGAL MATERIAL

Collections of fertile material of Enteromorpha intestinalis (L) Link were obtained from Langstone Harbour, south coast of England, during spring tides in May and June. The material was cleaned with cotton wool and the fertile tips excised and immersed in pasteurised filtered seawater (P.F.S.) to stimulate spore release. Released zoospores were then concentrated using their phototactic response (Fletcher, 1976) and drops of spore suspension pipetted directly onto the surfaces of small 18 mm square glass coverslips. The coverslips were then placed for 2 hours in the dark to allow for settlement and attachment of the spores, after which they were placed individually into the compartments of a repli-dish (10 cm square: 25 compartments) containing serial dilutions of the test compounds.

For the experiment to determine the effect of increasing time of settlement of the spores on their resistance to tributyltin oxide, spore cultures were set up on coverslips similar to the procedure outlined above. After the required time intervals, varying between ½ and 2 hours (see table 2) the coverslips were removed and placed into the repli-dish compartments containing

the dilutions of the test compounds. For the time intervals between 2 and 72 hours the spore cultures were maintained in standard culture conditions (P.F.S.; 15°C temperature; 4000 lux continuous "white" light intensity) prior to their transfer into the test solutions.

2). PREPARATION OF TEST SOLUTIONS AND EXPERIMENTAL PROCEDURE

Table 1 lists the tributyltin compounds used in the present study along with details of their structural formulae. Stock solutions of each of the tributyltin compounds were prepared in acetone from which the serial dilutions were made using P.F.S. Triplicate 3ml samples of each of the dilutions were then placed into the compartments of the repli-dishes. Control solutions using P.F.S., alone and with 0.001% acetone, were also prepared and distributed into the repli-dish compartments.

All spore cultures were then incubated for five days in the above described standard culture conditions after which the percentage number of the spores germinating on each coverslip were determined by random field counting under the microscope. Usually ten fields were counted per coverslip, equivalent to thirty per compound dilution. ED_{50} and ED_{99} values were then obtained by probit analysis.

In addition, the toxicity of the compounds to settling Enteromorpha spores was investigated by pipetting spore aliquots directly into repli-dish compartments containing serial dilutions of the compounds and glass coverslips. After 5 days, microscope counts were made of germlings present on the coverslips relative to those on the controls.

RESULTS

Table 1 presents the results of the toxicity tests. It can be seen that all the tributyltin compounds are highly toxic to the newly settled Enteromorpha spores. Concentrations of 1 ppm of all the compounds were completely toxic to the spores while reduced percentage germination was recorded over the range 10^{-1} to 10^{-6} ppm.
Quite marked variations were recorded in the results of the toxicity tests for the different tributyltin compounds. The order of the ED_{50} values obtained by the probit analyses varied from 10^{-3} to 10^{-5} ppm with the majority either 10^{-3} or 10^{-4} ppm. The most toxic compound recorded was TBT benzoate (ED_{50} 2.7×10^{-5}), followed by TBT dimethyldithiocarbamate (ED_{50} 5.3×10^{-5}) and TBT trichlorophenoxide (ED_{50} 7.4×10^{-5}).
The least toxic compounds tested were TBT acrylate, abietate and dodecanoate (ED_{50}s 8.6×10^{-3}, 2.5×10^{-3} and 2.3×10^{-3} respectively).

Table 2 presents the results of the effect of spore settlement time in Enteromorpha on the resistance of tributyltin oxide. It can be seen that there is a direct relationship between

increasing time after settlement of the spores and the increase in their resistance to the compound. Over the range of settlement times, the critical toxic concentration varied from 2 x 10^{-4} ppm for newly settled spores to 1 x 10^{-2} ppm for three day old germlings.
When the effect on motile spores was gauged however, an ED_{50} of 1 x 10^{-6} ppm TBTO was found to be sufficient to prevent settlement.

DISCUSSION

The present study confirms the high level of toxicity of the tributyltin compounds toward the marine fouling alga Enteromorpha with concentrations in parts per thousand million (and lower for certain of the compounds) inhibiting the germination process of the zoospores. This high level of activity of the triorganotin compounds is consistent with the laboratory tests of Christie (1973) on settled spores of Enteromorpha (LD_{50} 2 x 10^{-3} ppm for triphenyltin chloride) and Rivett (1965) on Chlamydomonas cells (minimum inhibitory concentrations 4.9 - 16.0 x 10^{-3} ppm for a range of tributyl and triphenyltin compounds).

It has been previously reported that, in general, the biological activity of triorganotin compounds, R_3SnX, is dependent primarily on the nature of the R_3Sn group and the X radical has little effect (Sijpesteijn et al 1969; Davies and Smith, 1980). In addition, it is now known that in water, triorganotin compounds form hydrated cations of the type $[R_3Sn(H_2O)_2]^+$, in which the anionic group X is no longer bound to the tin atom (Davies et al 1983) - the biological activity should therefore be independent of X. Blunden et al (1984) have however demonstrated that the use of anionic forms of certain organic chelating ligands when used as the X moiety in TBTX compounds can reduce biological effectiveness of these compounds by chelating intramolecularly to the tin atom.

In this study a range of toxicities were recorded for compounds of tributyltin in which the anion X was varied. The differences noted were very small but nevertheless apparent. The molecules with the highest recorded toxicity were those which possessed an X moiety that is potentially bio-active — benzoate, trichloro- and pentachlorophenoxide, thiocarbamate and tetrachlorophthalate. These compounds had ED_{50} toxicity values varying from 10^{-10} to 10^{-11} M. Most of the other compounds had ED_{50} toxicity values in the 10^{-8} to 10^{-9} M range. It is feasible therefore that these five former anions may be contributing to the toxicity of the Bu_3Sn species by either synergistic means or by promoting the transport of the latter species into the cell.

The data included in Table 1 also indicates the ED_{99} values for the compounds - these values represent a guide to assessing the concentrations necessary for complete control under field conditions (although the relative effectiveness of the compounds is best estimated at the ED_{50} level as this is the most precise parameter which can be calculated). The ED_{99} values would

therefore represent the minimal effective dose and as can be seen in Table 1 this value is several orders higher than the ED_{50} . When expressed in molar terms the ED_{99} values show a less distinct picture with a number of compounds in the 10^{-7} to 10^{-8} range — tributyltin chloride on this basis has the least calculated ED_{99} . (The slope of the probit line, used in deriving the ED_{99} data, gives a reliable estimate of the standard deviation of the tolerance distribution of the population of spores to the compounds).

Increasing the number of Bu_3Sn- species in a molecule did not always lower the toxicity threshhold of the compound. Thus the fumarate, sulphide and oxide had ED_{50} Ms of 2.2×10^{-9} , 1.5×10^{-9} and 0.5×10^{-9} respectively. The tetrachlorophthalate was the exception with an ED_{50} M of 3.7×10^{-10} . The phosphate with three Bu_3Sn- per molecule had an ED_{50} M of 1.0×10^{-9} .

Christie (1973) had documented the increase in resistance of Enteromorpha spores to triphenyltin chloride with increasing time after settlement. This study recorded lower ED_{50} values for tributyltin oxide against motile spores (1×10^{-6} ppm). This value had increased a hundredfold within 30 minutes after settlement and there is then a progressive increase in resistance such that it requires 10^4 times the ED_{50} for motile spores to affect three day old germlings. This progressive increasing resistance of the settled spores to TBTO was undoubtedly due to the gradual production and enlargement of the protective cell wall during the early post-settlement period (Evans and Christie, 1970).

The sensitivity of Enteromorpha spores to the tributyltin compounds tested explains the measure of control that organotin containing antifouling coatings have been able to exert over ship fouling by this alga. However, the search for more effective toxins based upon the active triorganotin centres still goes on with the aim of prolonging antifouling service life and preventing even residual fouling (especially slimes).

ACKNOWLEDGEMENTS

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TABLE 1
Tributyltin compounds examined; their ED_{50} and ED_{99} values against newly settled Enteromorpha spores.

TBT Compounds	Formula	Molecular Weight	ED_{50} ppm	ED_{50} Molarity	ED_{99} ppm	ED_{99} Molarity
Abietate	$C_{19}H_{29}COO.SnBu_3$	591.5	2.5×10^{-3}	4.2×10^{-9}	4.8×10^{-1}	8.1×10^{-7}
Acrylate	$CH_2:CHCOO.SnBu_3$	361.1	8.6×10^{-3}	2.4×10^{-8}	7.3×10^{-1}	2.0×10^{-6}
Benzoate	$C_6H_5COO.SnBu_3$	411.1	2.7×10^{-5}	6.6×10^{-11}	5.3×10^{-3}	1.3×10^{-8}
Chloride	Bu_3SnCl	325.5	2.5×10^{-4}	7.7×10^{-10}	2.2×10^{-3}	6.8×10^{-9}
Dimethyl dithiocarbamate	$(CH_3)_2NCSS.SnBu_3$	410.2	5.3×10^{-5}	1.3×10^{-10}	7.4×10^{-3}	1.8×10^{-8}
Dodecanoate	$CH_3(CH_2)_{10}COO.SnBu_3$	489.3	2.3×10^{-3}	4.7×10^{-9}	6.6×10^{-1}	1.3×10^{-6}
Ethane sulfonate	$C_2H_5SO_3.SnBu_3$	399.1	1.4×10^{-3}	3.5×10^{-9}	4.2×10^{-2}	1.0×10^{-7}
Fumarate	$Bu_3SnOOCCH:CHCOO.SnBu_3$	694.1	1.5×10^{-3}	2.2×10^{-9}	5.9×10^{-1}	8.5×10^{-7}
Hendecanoate	$CH_3(CH_2)_9COO.SnBu_3$	475.3	4.9×10^{-4}	1.0×10^{-9}	1.7×10^{-2}	3.6×10^{-8}
Linoleate	$CH:CH(CH_2),COO.SnBu_3$ $CH_2CH:CH(CH_2)_4CH_3$	569.5	3.6×10^{-3}	6.3×10^{-9}	3.6×10^{-1}	6.3×10^{-7}
Methacrylate	$CH_2:C(CH_3)COO.SnBu_3$	376.1	3.2×10^{-4}	8.5×10^{-10}	1.2×10^{-2}	3.2×10^{-8}

TABLE 1 (contd.)

TBT Compounds	Formula	Molecular Weight	ED_{50} ppm	ED_{50} Molarity	ED_{99} ppm	ED_{99} Molarity
Naphthenate	Cycloparaffins-gen.form. C_nH_{2n-1} COO.$SnBu_3$	490-590	3.7×10^{-4}	6.9×10^{-10}	1.9×10^{-2}	3.6×10^{-8}
Oxide	$Bu_3Sn.O.SnBu_3$	596.1	3.2×10^{-4}	5.4×10^{-10}	4.2×10^{-3}	7.0×10^{-9}
Pentachloro-phenoxide	$C_6Cl_5O.SnBu_3$	555.3	2.6×10^{-4}	4.7×10^{-10}	1.3×10^{-2}	2.3×10^{-8}
Phosphate	$(Bu_3Sn)_3.PO_4$	965.1	9.8×10^{-4}	1.0×10^{-9}	4.8×10^{-1}	5.0×10^{-7}
Salicylate	$C_6H_4(OH)COO.SnBu_3$	427.1	5.3×10^{-4}	1.2×10^{-9}	4.2×10^{-2}	9.8×10^{-8}
Sulphide	$Bu_3Sn.S.SnBu_3$	612.1	9.1×10^{-4}	1.5×10^{-9}	6.5×10^{-2}	1.1×10^{-7}
Tetrachloro-phthalate	$Bu_3Sn.OOC.C_6Cl_4COO.SnBu_3$	882.0	3.3×10^{-4}	3.7×10^{-10}	3.9×10^{-2}	4.4×10^{-8}
Trichloro-phenoxide	$C_6H_2Cl_3O.SnBu_3$	486.5	7.4×10^{-5}	1.5×10^{-10}	1.9×10^{-2}	3.9×10^{-8}
Xylenoxide	$C_6H_3(CH_3)_2O.SnBu_3$	411.2	1.0×10^{-3}	2.4×10^{-9}	4.6×10^{-2}	1.1×10^{-7}

TABLE 2
Effect of increasing settlement time on resistance of Enteromorpha spores to tributyltin oxide.

Settlement time/hours	ED_{50} ppm	ED_{99} ppm
Motile spores	1×10^{-6}	7.1×10^{-4}
1/2	2.2×10^{-4}	5.2×10^{-2}
1	2.4×10^{-4}	6.5×10^{-2}
2	3.2×10^{-4}	3.0×10^{-2}
10	2.4×10^{-3}	1.7×10^{-1}
24	5.3×10^{-3}	2.2×10^{-1}
48	8.7×10^{-3}	2.3×10^{-1}
72	1.0×10^{-2}	2.0×10^{-1}

ECOLOGICAL ASPECTS OF MARINE FOULING AT THE NECOCHEA POWER STATION (PUERTO QUEUQEN,ARGENTINA)

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ABSTRACT

The present paper deals with the study of fouling communities at the Necochea Power Station(Puerto Quequén,Argentina).

Data were obtained during one sampling period(October 1978-September 1979)carried out in two different areas(Water Intake and Pump Room)using achrylic sandblasted panels as test surfaces.

A short-term series of panels supplied information on settlement cycles of the different fouling species,while the evolution of the fouling community was studied on the basis of a long-term series of panels.

Biomass fluctuations of fouling growth along the year were determined,as well as the relationship between these fluctuations and variations in water temperature.

The results of this research constitute the reference for the efficient development of control systems being implemented at present.

INTRODUCTION

Proceeding with research relating to fouling communities at Puerto Quequén(Quequén Harbour,Argentina)and their influence on the working of the power station located there,a new study on test panels has been carried out during another annual period.Information already compiled last year(Bastida & Brankevich,1980,1981,1982)has been compared and enlarged by means of this survey,allowing a deeper study of attachmant cycles of species not considered up to the present and widening the general knowledge of these communities.The results of these researches constitute the reference for the efficient development of control systems being implemented at present.

MATERIALS AND METHOD

Results presented in this paper were obtained in the course of a period extending from October 1978 to September 1979.The same methodology as that employed over the last years(Bastida & Brankevich,1980, 1981,1982)has been applied during this assay.Biological samplings were obtained by means of sandblasted achrylic panels of 300 cm^2,placed vertically at three depth levels labelled B,C and D.Level B is the shallowest and was influenced by daily tidal fluctuations,whereas level D is the deepest(Bastida & Brankevich,1980,1981).Each panel consisted of two plates attached back to back;one was used to analyze attachment cycles,while the other was destined to determine biomass (dry weight).Test panels were placed in two areas with different environmental characteristics:

- External area(Water Intake,constant light);
- Internal area(Pump Room,no light).

Two types of panels were used in each of these areas and were classified taking into account their exposure time:

- Short-term panels:submerged for approximately twenty days,they allowed observation of recruitment periods of the different fouling species;
- Long-term panels:submerged simultaneously at the beginning of the assay-one set being removed monthly-they provided information on community evolution.

Environmental data(sality values,water temperature and pH)were obtained using classical methods.Correlations between these variables and fouling growth were established on the basis of biomass samplings and values of water temperature obtained throughout the assay,by means of the "r" coefficient or "Pearson's product moment"(Sokal & Rohlf,1979). The degree of affinity between faunal components on different sets of panels was analyzed on the basis of qualitative studies,applying Jaccard's similarity index(C.C.)(Stirn,1981).Cluster analysis dendograms for each tested group were made with the values thus obtained.

RESULTS

Environmental aspects

The surveyed area(38°35'S Lat.,58°42'W Long.),whose general characteristics were defined in previous papers(Bastida & Brankevich,1980, 1981)is an estuarine environment with a strong marine influence.

The water temperature curve shows annual variations similar to those registered in the previous years(Bastida & Brankevich,1980,1981)(fig.1). The highest mean temperature was 21°C in February and the lowest mean temperature was 10°C in July.Highest and lowest absolute values ranged from 22°C to 8.9°C.Average sality values varied between 6°/₀₀ and 27°/₀₀, and absolute ones between 4°/₀₀and 32°/₀₀(fig.2),while pH remained stable (8 to 8.3)and no important pollution phenomena were recorded.

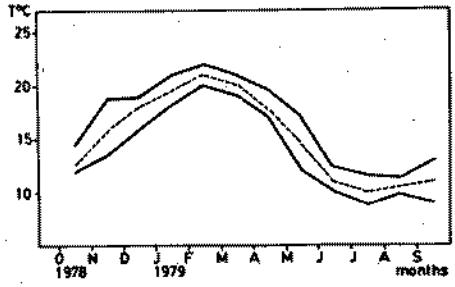

Fig.1.Water temperature.Puerto Quequén.Oct.1978- Sept. 1979.

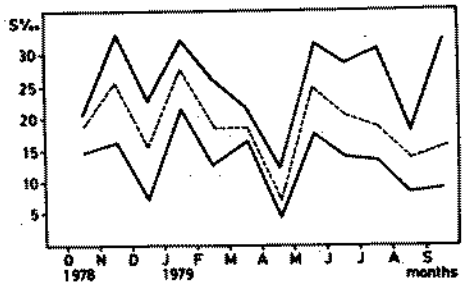

Fig.2. Salinity. Puerto Quequén. Oct.1978-Sept.1979.

Settlement patterns on short-term panels

Settlement cycles of different microfouling and macrofouling species in the surveyed area,with special emphasis on the best represented ones, were obtained in the course of this new study.The microfouling category is made up of organisms ranging from bacteria (not dealt with in this paper)to invertebrate larval stages,young forms of macroscopic algae and small invertebrates such as copepods.All other organisms of larger size are classed in the second category(macrofouling species).Just as in previous years(Bastida & Brankevich,1980,1981,1982)community development of both the external and internal areas(labelled E and I,respectively)has been analyzed separately.

Microfouling

Diatoms

This group has displayed the greatest species diversity and the highest numerical abundance.A total of 27 genera and more than 36 species were identified(Table I);the settlement cycles of the six main fouling diatoms are showed in fig.3.The genus *Navicula* proved to be the most abundant during the assay and was present on test panels throughout the year.*Achmantes longipes*,*Melosira moniliformis* and *Synedra* spp. can be cited next in order of importance.The first two species presented an annual cycle,while *Synedra* spp.has been recorded seasonally.A greater tendency to colonize both the external and the upper levels has been observed in this group,this characteristic being directly related to light requirements.Comparisons made with respect to previous studies(Bastida & Brankevich,1981)have indicated a high degree of coincidence between settlement cycles.

Green algae

Three genera belonging to this group have been identified in the course of the present research(Table I),and graphs corresponding to two of them have been presented.The genus *Enteromorpha*(fig.3)has proved to be the most important one.It was observed throughout the yearand showed a marked preference for the upper outer levels.It was never recorded in the internal area.This genus has been included within the microfouling category due to the fact that during this assay specimens never reached significant sizes on short-term panels.Two species of the genus *Scenedesmus* were recorded(Table I),*Scenedesmus quadricauda* (fig.3)being the most frequent one.This species,of fresh water origin,has settled at all depth levels,both in the inner and outer areas.

Protozoans

Protozoans comprise a total of eight genera(Table I),of which *Zoothamnium* turned out to be the most important one all along the experimental period(fig.3).Like in previous studies(Bastida & Brankevich,1981),this colonial ciliate has shown an attachment cycle extending throughout the assay with sporadic interruptions and no preference for any of the surveyed areas.The genus *Coturnia*(fig.3)presented an irregular cycle,being more frequent at upper levels.

Crustaceans

Harpacticoid copepods were the most abundant microcrustaceans recorded throughout the present study.This class has been represented by eight genera(Table I),shown jointly on the graph(fig.3)for practical purposes. Settlement took place during the whole experimental period,proving more abundant in late spring and summer.These organisms have shown a slight preference for outer panels.

Macrofouling

Hydroids

The two hydroid species recorded at Puerto Quequén(Table I)showed a marked difference both in preference for areas and in the length of colonization cycles.*Gonothyraea loveni*(fig.4)has been present almost throughout the year,dispaying a tendency to settle on the outer panels. *Tubularia crocea*(fig.4)presented a typically seasonal attachment cycle and a preference for the non-illuminated outer area,with peak settlement during March and April.Both species show an increase in density in direct proportion to rise in depth.

Polychaetes

The two species which displayed greatest abundance within this group (Table I)were *Mercierella enigmatica* and *Polydora ligni*.The former(fig.4) presented a seasonal settlement cycle(November/May)showing a slight preference for the inner area,while *Polydora ligni*(fig.4)tended to colonize the outer panels during the whole year,with a peak settlement period in summer.

Bryozoans

This group is typified by two species(Table I),*Cryptosula pallasiana* being the most important one.This species has settled on test panels from December to June(fig.4)and has shown a tendency to attach to the lower panels of both sampled areas.

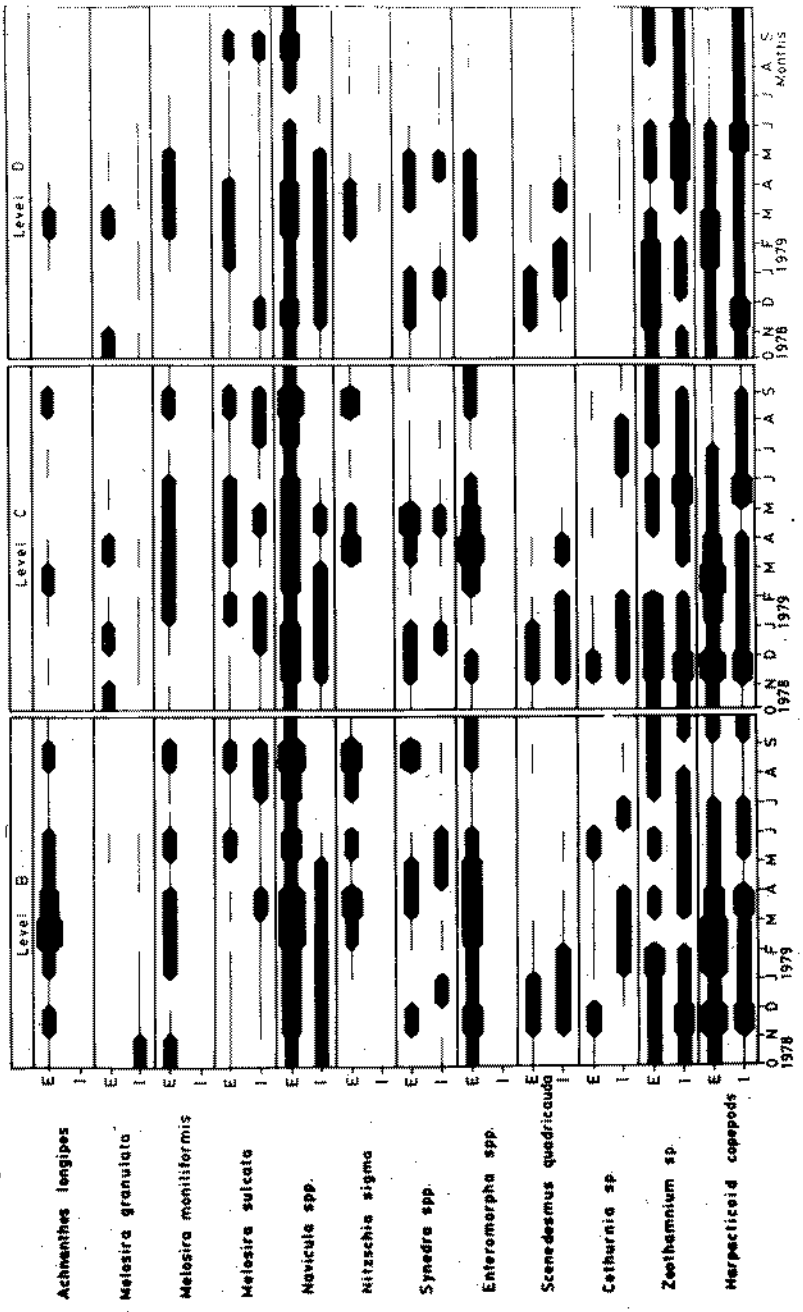

Fig.3.Settlement cycles on short-term panels.Puerto Quequén.

Molluscs

Three species pertaining to this group(Table I)were recorded during the present research.*Mytilus platensis* and *Brachydontes rodriguezi* (fig.4)were the most harmful to the normal working of cooling systems at the power station.Their attachment cycle was irregular,unlike that recorded in previous studies(Bastida & Brankevich,1980,1982),although October was found to be one of the most important months in the course of the clonization cycles.As stated in previous papers(Bastida & Brankevich,1980,1982),given the fact that *Tenellia pallida*(fig.4)feeds on *Gonothyraea loveni*,its presence has depended on colonization by the latter.

Crustaceans

Macrofouling crustaceans have been mainly typified by five main species:*Sphaeroma* cf.*serratum*,*Corophium insidiosum*,*Balanus amphitrite*, *Cyrtograpsus altimanus* and *Cyrtograpsus angulatus*(fig.4).As recorded in other ports(Bastida,1971 a & b;Bastida,1977;Bastida,1980),*Shaeroma* cf. *serratum*(fig.4)has significantly colonized the upper level.The amphipod *Corophium insidiosum*(fig.4)was the species presenting peak density on the test panels observed.Its settlement cycle takes place almost throughout the year and presents a peak attachment period from October to March,with preference for outer panels.No doubt,the crustacean species deserving more consideration due to its harmful effects is the barnacle *Balanus amphitrite*.This organism presents a characteristic seasonal settlement cycle(fig.4)extending from January to April, with peak density in February.It displays a preference for the upper external level.As verified in previous studies(Bastida & Brankevich, 1980,1982)this organism shows a tendency to settle on well-illuminated levels.It is rarely found in the internal area.Decapod crabs of the genus *Cyrtograpsus*(fig.4)are characterized by a preference for colonizing lower levels and by their presence in both sampled areas.

Tunicates

Two species of tunicates have been encountered(Table I),*Botryllus schosseri* was the most important one(fig.4).Its preference was mainly limited to the lower panels in the external area,with a seasonal cycle extending from February to March.

Biomass fluctuation on short-term panels

External area

The general biomass(dry weight)curve corresponding to external short-term panels(fig.5)showed marked fluctuations during the year.Maximum values occurred in March(0.51 g/dm^2)coinciding with periods of highest attachment of fouling organisms.Minimal values were recorded in cold months-June and July-scarcely reaching values of 0.03 g/dm^2 and 0.04 g/dm^2.Analyzing the biomass curves for each outer level,it can be observed that highest values correspond to levels C and D(fig.7);these values also coincide with peak values on the mean biomass curve(fig.6).

Internal area

Generally speaking,biomass(dry weight)values on short-term internal panels were similar to those observed in the external area.The highest value was recorded in March(0.70 g/dm^2).An abrupt fall in biomass values

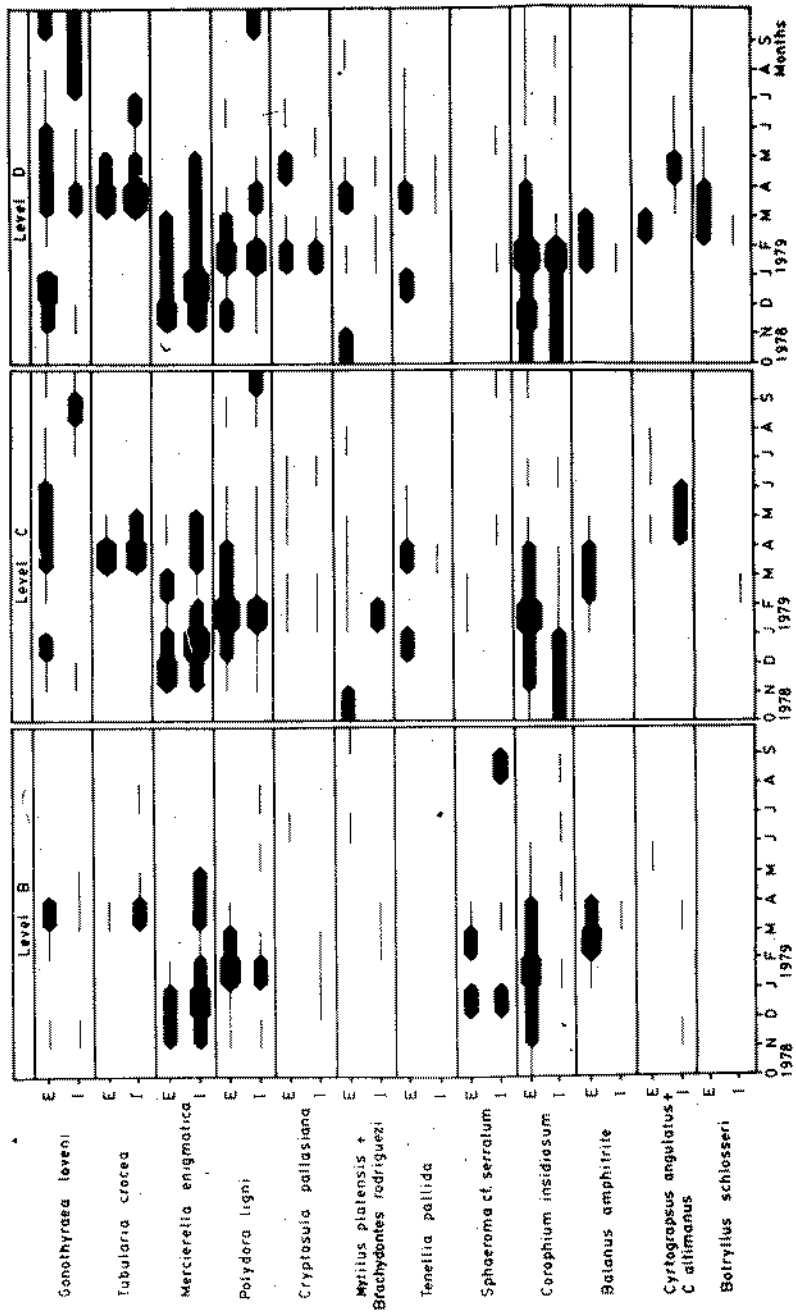

Fig.4. Settlement cycles on short-term panels. Puerto Quequén.

was verified in February.This fall is related to absence of settlement on panels due to interruption of water flow in the canal where test panels had been placed.The actual minimal value was registered in June (0.05 g/dm^2)Observing the graph corresponding to biomass at each level (fig.8),it is evident that level D is the one which brought greatest influence to bear on the high values recorded during March.This is directly related to the remarkable development achieved by *Tubularia crocea* on the above mentioned panel.

Comparison between both areas

Comparing biomass(dry weight)curves obtained in both sampled areas (figs.5 and 6),it can be seen that fluctuations taking place throughout the assay agree for the most part,except in February,due to already expounded causes.Although months of maximum and minimum attachment coincided in both areas,some differences in dry weight recorded for each of them should be pointed out.In December,there was greater colonization of the inner area by *Mercierella enigmatica*,which resulted in an increase in biomass,and a similar situation was observed in January.During March,the inner panels presented superior biomass values of 0.19 g/dm^2,but in this case due to the remarkable development of *Tubularia crocea*,especially at level D.During the rest of the assay no differences were detected,except for August when there was a larger gathering of detritus on the inner surfaces.

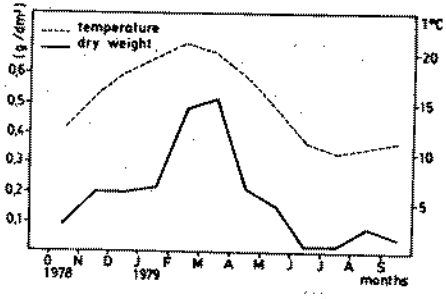

Fig.5. Fouling biomass(dry weight) on short-term panels expressed as an average of levels B,C & D and monthly mean water temperatures. External area.

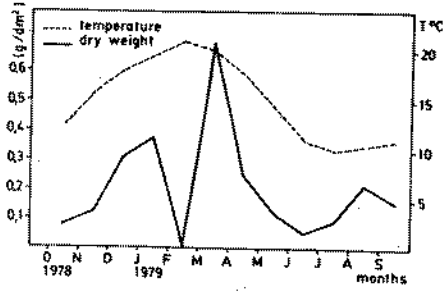

Fig.6. Fouling biomass(dry weight) on short-term panels expressed as an average of levels B,C & D and monthly mean water temperatures. Internal area.

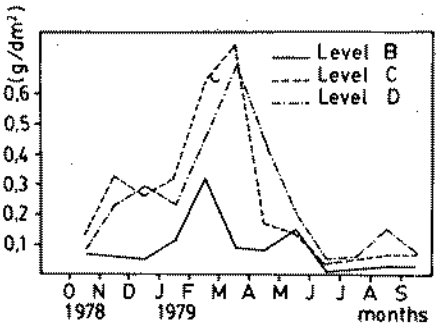

Fig.7. Fouling biomass (dry weight) on short-term panels. External area.

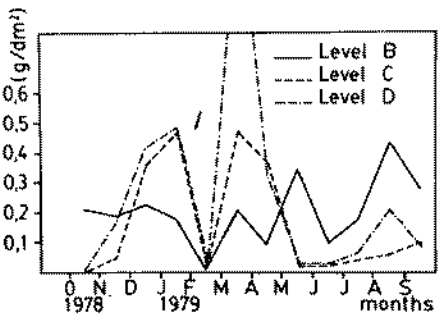

Fig.8. Fouling biomass(dry weight) on short-term panels. Internal area.

Correlation between biomass on short-term panels and water temperature

In order to verify the apparent relationship between biomass(dry weight) and water temperature shown in figs.5 and 6,statistical tests were carried out.The analysis was accomplished using dry weight values in both areas and mean monthly water temperatures recorded simultaneously.Correlation coefficient "r" or "Pearson's product-moment"(Sokal & Rohlf,1979)was selected to verify the relationship between the above mentioned variables.Taking into account in the first place monthly biomass values in the external area,the calculated "r" value was 0.9062, indicating a clear correlation between temperature and biomass.Even if the biomass value corresponding to February was considered abnormal and consequently discarded,the "r"value for the internal area was 0.7935,indicating a positive correlation for this area.

Biomass fluctuation on long-term panels

External area

Analyzing the biomass(dry weight)curve corresponding to long-term outer panels(fig.9),the lack of correlation between water temperature and community evolution can be clearly seen.Water temperature only exerts its influence during the initial stages of community development.Biomass values in this area diplayed a gradual increase during the first nine months of exposure,while a significant rise occurred in the tenth month,with values of 59.70 g/dm^2.It is worth mentioning that such a high dry weight value had not been recorded previously in the surveyed area or in other studied ports,like Mar del Plata and Ingeniero White.This can be attributed to the unusual development of *Mercierella enigmatica* which formed a layer of more than 6 cm.at lower levels.Moreover,due to the fact that this species form calcareous tubes,its weight is not significantly modified by the procedures used in dry weight determinations.Analyzing biomass values at each level (fig.10)it is possible to observe variations along the year,as shown

in the general graph.Comparisons of the three depth levels show that heavy settlement on panel D was largely responsible for high values recorded in July.This significant development of *Mercierella enigmatica* was also noted on the shallower levels,but in no case attaining such values as those recorded on level D.

Internal area

Biomass(dry weight)analysis in the internal area has been more complex than in the external one. The first four months presented a gradual biomass increase(fig.9), as observed on the external panels.From February onwards,inner panels have presented minimal settlement,thus producing much lower dry weight values than those expected for that season.This alteration in the sequence of evolution can be attributed to the interruption of water curculation in the canal where panels were located during the fifth,sixth,seventh and eighth months of exposure. Furthermore,the last four months of the assay did not display a similar pattern as that recorded for the external area.Lower biomass values were recorded for almost every month and especially in the course of the tenth month,when there was a significant difference due to an unequal development of *Mercierella enigmatica* in both areas.Biomass curves at each level(fig.11)have been similar to that shown in the general graph.Highest dry weight values were recorded at level D.

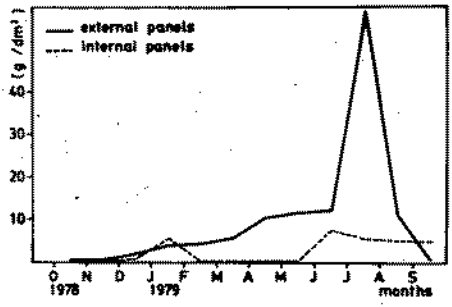

Fig.9. Fouling biomass(dry weight) on long-term panels expressed as an average of levels B,C & D.

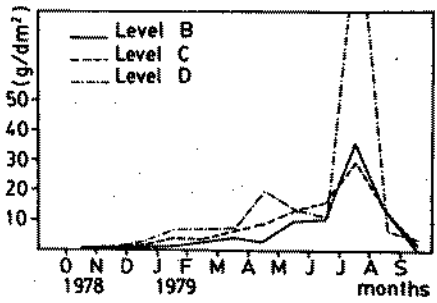

Fig.10. Fouling biomass(dry weight) on long-term panels. External area.

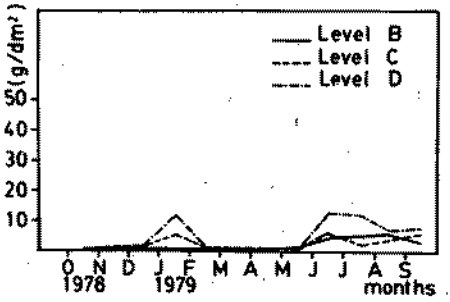

Fig.11. Fouling biomass(dry weight) on long-term panels. Internal area.

Comparison between both areas

Previous researches(Bastida & Brankevich,1980,1982)have indicated a close relationship between biomass(dry weight)fluctuations on short-term panels in both sampled areas.This similarity in values could not be recorded during the present study,though it should be considered that a large part of the panels were subject to abnormal conditions. This fact renders comparisons between fouling development in both the internal and external areas meaningless.

Community evolution

Analysis of community evolution was based exclusively on the long-term outer series.The inner area was not taken into account due to difficulties recorded in water inflow.Three main evolutional stages have been recorded:

- Settlement,gradual biomass(dry weight)increase correlated with water temperature;
- Biomass increase due to development of dominant species;
- Community decay from the tenth month onwards,detachment of main species and recolonization.

In some cases,different species typified each of these stages, depending on the depth levels(B,C or D)and on the species light requirements.For example,the algae *Enteromorpha*constituted one of the first settlers at levels B and C,whereas the hydrozoan *Gonothyraea loveni* was the first colonizer at level D.From November onwards(second month of exposure)new settlers joined the community,thus increasing specific diversity and coinciding with a progressive rise in water temperature.The highest specific diversity was attained in March(sixth month of exposure),suggesting a certain degree of community stability.This stability,however,only refers to diversity of component species,since quantitative development,especially that of *Mercierella enigmatica* settlers,increased uninterruptedly until July (tenth month of exposure)when a peak biomass value(dry weight)of 59.70 g/dm^2 was recorded.Detachment processes were initiated in July,and continued throughout August and September.Recolonization by species surveyed during the first phases of this assay(*Enteromorpha, Polydora ligni* and *Gonothyraea loveni*)was recorded parallel to detachment of others.This marked the beginning of a new stage in community evolution.Considering the time of year when the assay was carried out (October/78-September/79)and the remarkable growth of *Mercierella enigmatica* on long term panels,it can be stated that the community took ten months to reach its full development.From the tenth month on,detachment and resettlement of test surfaces took place.

Affinity between panels on the short-term series

In order to determine the degree of affinity between data obtained on short-term panels,Jaccard's similarity index(Stirn,1981)was applied. Microfouling and macrofouling were analyzed together at the first stage (fig.12)and separately at the second,identifying different cluster patterns which are not evident in the general graph.The first tendency noticed in clustering of samples related to their origin(external

and internal areas).This arrangement is verified in those dendrograms corresponding to the whole community(fig.12)and to microfouling(fig. 13).The second clustering pattern observed was based on similarity between sampling dates.It was recorded in the general cluster analysis dendrogram(fig.12)and in that corresponding to macrofouling(fig.14).

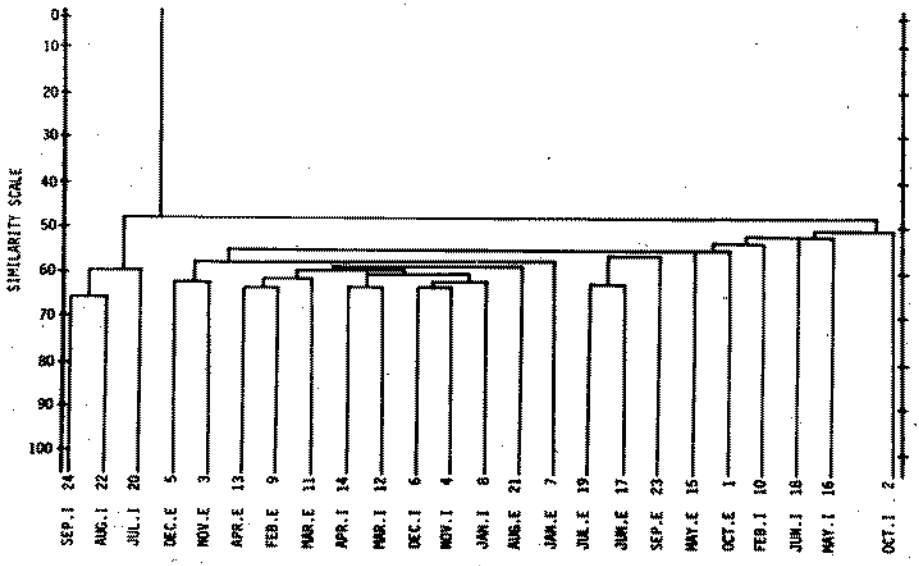

Fig.12. Cluster analysis dendrogram for Jaccard's similarity index; ordinate is a similarity scale from 0 to 100. Microfouling & Macrofouling.

Considering that trends observed in the general dendrogram(fig.12) are a result of interaction between microfouling and macrofouling,both have been considered in the present analysis.By analyzing organisms identified during the sampling period in both areas,we were able to determine that,as regards microfouling,36% of species were recorded only in the external area,19% in the internal one,and 45% in both.With respect to macrofouling,percentages were as follows:27% in the outer area,8% in the inner one,and 65% common to both.Recorded percentages point to a greater differentiation between both areas when considering microfouling(45% of organisms common to both areas)as opposed to a greater homogeneity in distribution of macrofouling components(65% of organisms common to both areas).The corresponding dendrograms(figs. 13 and 14)show this difference in settlement by microfouling and macrofouling organisms on inner and outer panels.The light requirements of a large part of microfouling organisms(mainly diatoms)and the total

absence of light in the inner area constitute the main causes of this arrangement.This requirement is unimportant among macrofouling organisms since there is no significant diversity in their plant components, and therefore no marked preference for any given area.

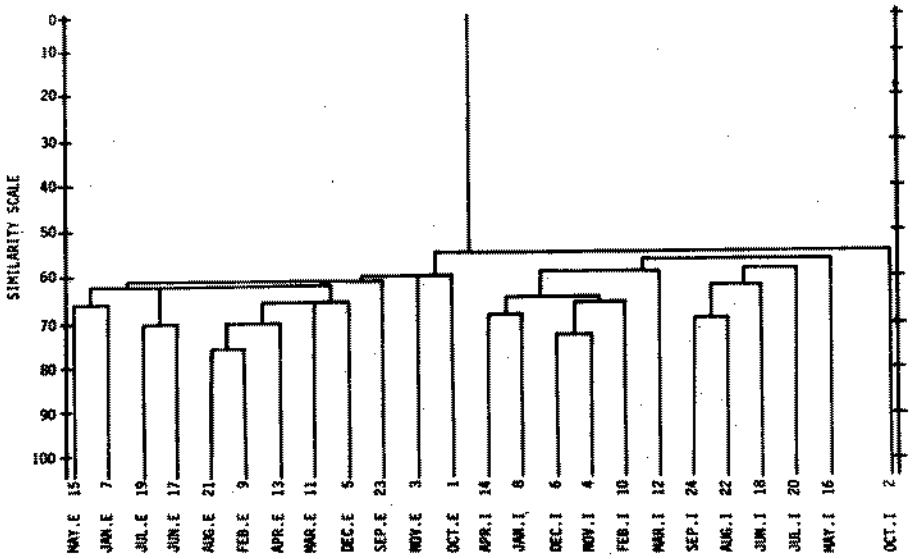

Fig.13. Cluster analysis dendrogram for Jaccard's similatity index; ordinate is a similarity scale from 0 to 100.Microfouling.

Analyzing the frequency of appearance of community components along the year,it can be seen that most organisms pertaining to the category of macrofouling(fig.4)have presented a seasonal settlement cycle. This situation has noticeably influenced clustering of samples.This characteristic becomes less evident for microfouling,which has presented fluctuations in its degree of abundance along the assay,but no interruptions in colonization(fig.3)

DISCUSSION

A total of 77 taxa belonging to 9 phyla were identified in the course of the present study.

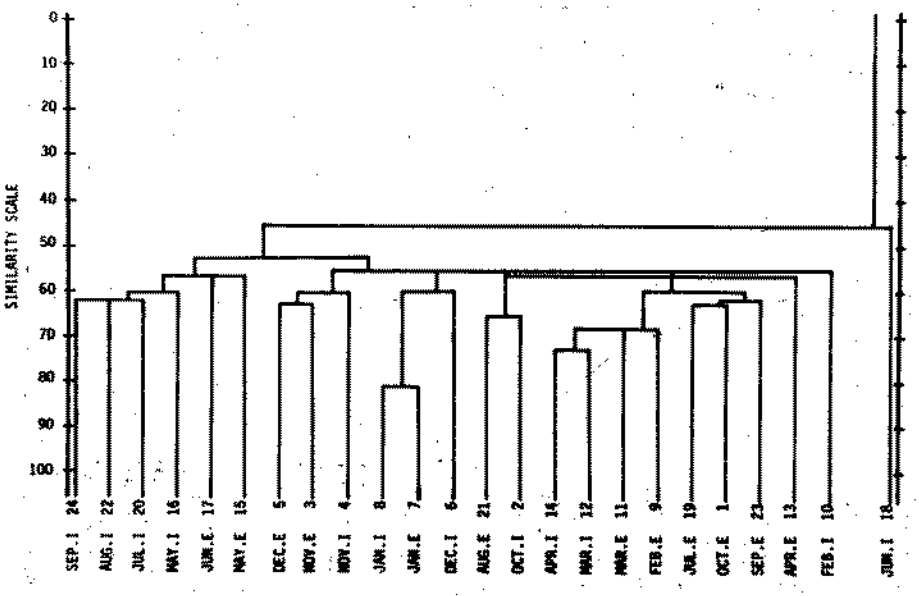

Fig.14. Cluster analysis dendrogram for Jaccard's similatity index; ordinate is a similarity scale from 0 to 100.Macrofouling.

When considering the two fouling categories-microfouling and macrofouling-it was found that the first group presented the greatest specific diversity.Attachment cycles of most microfouling organisms were annual, whereas macrofouling displayed seasonal colonization patterns,as recorded in other harbours such as Mar del Plata and Puerto Belgrano (Bastida,1971 a & b;Bastida,1977,1980).This characteristic of macrofouling is mainly based on variations in water temperature along the year,which exercise a direct influence on settlement periods.This constitutes a typical feature of temperate areas,where peak abundances are recorded during the warm months.On the other hand,most macrofouling species are rare in the course of the cold season.Differences in community composition recorded for both areas-internal and external-referred to microfouling plant components,which were more abundant in the outer,better illuminated,area.Macrofouling species did not display reamarkable differences in either area,given the fact that their plant components present a low specific diversity.These characteristics determined clustering of samples,microfouling and macrofouling being analyzed separately.Analysis of monthly biomass(dry weight)values and water temperature in both areas showed a direct correlation between both variables,as visualized in the corresponding graphs and statistically tested applying correlation-coefficient "r" or "Pearson's product-moment".This relationship was not recorded for biomass on long-term panels,where water temperature influenced the

first stages of community evolution,but not subsequent development.

Community evolution has displayed characteristic similar to those observed at other ports(Bastida 1971 a & b;Bastida,1977,1980).Three main stages were identified:

- Settlement,establishment of slime film with attachment of pioneer organisms,especially *Enteromorpha* at levels B and C and *Gonothyraea loveni* at level D.
-Community development with marked increase in biomass,which reached its highest value in the tenth month.
- Community decay from the tenth month onwards,detachment of main species and beginning of a new settlement period.

This outline of community evolution at Puerto Quequén is similar to that recorded by Sutherland and Karlson(1977)in the area of Beaufort,where these authors have verified a clear uninterrupted mortality-settlement sequence which is apparently characteristic of subtropical and temperate fouling communities.

ARNOWLEDGEMENTS

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We are also grateful to Prof.María Cristina Sarasa for translating the manuscript.

TABLE I
CHECKLIST OF FOULING ORGANISMS
ENCOUNTERED ON TEST PANELS IN QUEQUEN HARBOUR
(ARGENTINA)

ALGAE
Chrysophyta
Achnantes longipes
Amphora sp.
Asterionella japonica
Chaetoceros spp.
Cocconeis sp.
Coscinodiscus spp.
Cyclotella sp.
Cymbella ventricosa
Epithemia cf.*argus*
Gomphonema sp.
Grammatophora marina
Gyrosigma sp.
Licmophora abbreviata
Melosira granulata
Melosira moniliformis
Melosira sulcata
Melosira varians
Navicula grevillei
Navicula spp.
Nitzschia closterium
Nitzschia obtusa
Nitzschia sigma
Nitzschia sp.
Odontella aurita
Odontella mobiliensis
Odontella sinensis
Pinnularia sp.
Pleurosigma spp.
Rhabdonema cf. *arcuatum*
Rhoicosphenia curvata
Stephanopyxis turris
Surirella spp.
Synedra spp.
Thalassiosira ovalis
Thalassiothrix sp.
Triceratium favus
Cyanophyta (unident.)
Chlorophyta
Enteromorpha spp.
Pediastrum sp.
Scenedesmus acuminatus
Scenedesmus quadricauda
Rhodophyta
Callythamnium sp.

PROTOZOA
Dinoflagellata
Prorocentrum sp.
Silicoflagellata
Dictyocha sp.
Ciliata
Cothurnia sp.
Favella sp.
Lacrymaria sp.
Zoothamnium sp.
Folliculinidae (unident.)
Suctoria
Acineta sp.
Rhizopoda
Bolivina sp.

COELENTERATA
Gonothyraea loveni
Sagartentus bandae
Tubularia crocea

NEMATODA (unident.)

ROTIFERA
Colurella sp.

ANNELIDA
Halosydna
Mercierella enigmatica
Polydora ligni
Phyllodocidae (unident.)
Syllidae (unident.)
Errantia (unident.)

BRYOZOA
Bugula flavellata
Cryptosula pallasiana

MOLLUSCA
Brachydontes rodriguezi
Mytilus platensis

Tenellia pallida

CRUSTACEA
Copepoda
Ameira sp.
Amphiascus spp.
Dactilopodia sp.
Harpacticus sp.
Laophonte sp.
Nitocra spp.
Paralaophonte sp.
Tisbe sp.
Isopoda
Idotea sp.
Sphaeroma cf.*serratum*
Amphipoda
Corophium insidiosum
Gammaridae (unident.)
Cirripedia
Balanus amphitrite
Decapoda
Cyrtograpsus altimanus
Cyrtograpsus angulatus

TUNICATA
Botryllus schlosseri
Ciona intestinalis

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SURFACE MICROLAYER PROPERTIES AFFECTING DRAG PHENOMENA IN SEAWATER

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ABSTRACT

On a souvent remarqué que les pellicules microbiennes vaseuses et des autres formes de micro-encrassement superficiel augmentent d'une manière significative la résistance à l'avancement des objets qui sont en contact avec de l'eau de mer qui coule. À l'occasion, les pellicules de micro-encrassement fournissent des propriétés de reduction de cette résistance à l'avancement aussi bien que l'amoindrissement simultancé du rendement calorifique à cause de la stablisation des strates de limite laminaires plus épaisses. L'investigation actuelle identifie les caractéristiques chimiques et physiques des surfaces matérielles spécifiques associées aux phénomènes de mer. L'investigation met ces caractéristiques en comparaison aver les micro-strates superficielles des pellicules microbiennes vaseuses aussi bien que des peaux naturelles des mammifères marins (marsouins, orques épaulards). L'investigation évalue les perspectives pour la réduction significative de résistance à l'avancement par des macromolécules amarrées à la surface des matériaux techniques déjà largement utilisés pour la construction maritime. On a construit une nouvelle cellule d'écoulement de point mort qui permet la testage de laboratoire des concepts différents de réduction de résistance à l'avancement aux numéros Reynolds de quelques ordres de magnitude plus bas qu'il faut pour écouler dans une conduite. Pour fournir des éléments d'information de comparaison on a accompli les mesurages de la texture superficielle, la chimie superficielle, et l'énergie superficielle de la peau des marsouins et des orques épaulards vivants. Les résultats courants suggèrent que, plutôt que d'avoir une surface de réduction de résistance à l'avancement soit active ou passive, les mammifères marins sont libres des conséquences qui intensifie cette résistance associées aux microstrates de materiaux d'encrassement fortement liées. En contrepartie, la modification intentionnelle de surface des matériaux fabriqués par l'homme qui ont des revêtements polymères orientables semblent capables de donner des avantages de réduction vraie de résistance à l'avancement.

INTRODUCTION

It has often been noted that microbial slime films and other forms of surface microfouling significantly increase the drag of objects in contact with moving seawater (1,2,3,4).There is a reported instance where a drag penalty was observed from the presence of microfilm even in the presence of a substantial amount of macrofouling and its associated gross increase in surface roughness (5). Moreover, it is generally observed that almost all commonly used toxic anti-fouling coatings do not prevent the formation of bacterial slime films, although one Japanese firm is presently marketing an AF (Anti-Fouling) coating system that claims prevention or reduction of bacterial slime films using a combination of TBTO (Tri-butyl tin oxide) and a thio-carbamate, producing a powerful biocide, and achieving significant reduction in drag penalties (6). Where macrofouling is reduced or controlled by chlorination, or by the use of inherently bio-incompatible metal alloys as in heat exchangers, not only do bacterial slime films remain a significant problem in terms of drag effects, but concurrent loss of heat transfer efficiency has been noted due to the stabilization of thicker boundary layers (7).

The present investigation is identifying the surface chemical and biophysical features of microbial slime films as well as the natural "skins" of marine mammals such as porpoises and killer whales that are associated with drag phenomena in seawater. The long range objective is to evaluate the prospect of non-toxic control of bacterial slime films, and derive sufficient understanding of the processes to determine if drag reduction can be achieved using surface effects, including the "tethering" of molecules known to produce a drag reduction when present in bulk sea or freshwater.

It has been our thesis that the sequence of initial events in the exposure of an artificial surface to a biological fluid follows a universal order, and is fundamentally affected by the short range forces that dominate surface interaction and determine the strength of adhesive bonds that may be formed (7). Thus, the first event upon exposure, be the biological fluid a coastal or deep-sea environment, a freshwater lake, the fluids of the oral cavity, or even flowing blood, is the spontaneous adsorption of an organic layer, dominated by glycoproteins and insoluble polysaccharides. The initial surface is then modified and forms a new substrate that is then colonized by the arriving pioneer bacteria whose exudates, in turn, further change the surface (8,9). Supported by the work of other investigators, we have established that the strength of adhesion biological attachment is a function of the surface energy of the substrate, with surfaces dominated by closely packed methyl groups offering the weakest bonds, followed closely by surfaces rich in hydroxyl groups (10,11). In this work we argue that this principle may allow exploitation by non-toxic means, and point to the apparent success of fast swimming marine mammals in achieving both micro- and macrofouling control. On the other hand, we also attempt to show that the low-drag hypothesis that has

been advanced under the name of Gray's Paradox (12) for porpoises is not tenable on the basis of skin surface properties (13). This finding has not dissuaded us from continuing efforts to seek a relationship of drag reducing phenomena and surface properties. The Thom's effect, and the physical models that have been advanced to explain it, including the work of Zimm, et al (14,15) suggest that energy transfer in the bulk fluid near solid walls is related to changes in configuration of highly coiled molecules, and of significant effect, in steep streamline bending, such as may be seen in stagnation flow (16). We have developed a novel stagnation flow chamber to test our hypothesis on a laboratory bench scale and allow us to study drag reduction events at lower Reynold's numbers that are required to observe similar effects in straight line pipe flow.

METHODS AND MATERIALS

Methods for the determination of surface energy and surface chemistry are the same whether bacterial films deposited on artificial substrates or the compliant surface of the marine mammals are to be studied. We follow here the techniques that we have successfully employed in other applications, including studies of surfaces of teeth (17), and of materials in contact with blood (18). Measuring the contact angles of a series of ultra-pure liquids of known surface tension, one may plot the cosine of these angles against the liquid's surface tension. The intercept at the $\cos \theta = 1$ axis of the least square fit of the data gives a numeric value that has been termed the critical tension or critical surface energy by Zisman, who--with coworkers--developed the technique (19). The concept provides an empirical description that closely relates to the substrate's surface energy, and has proven to be an excellent predictor of wettability and adhesion. Such a Zisman plot from contact angles made by liquids applied to a bacterial film allowed to form on germanium exposed to flowing seawater is shown in Figure 1.

The identification of the dominant chemical bonds of the molecules that make up the outermost layer of the surface to be studied, the thickness of the film, the orientation of molecular dipoles as reflected by measurements of contact potential, and the surface energy of adsorbed organics and bacterial films can readily be ascertained using germanium prisms. Small, optically flat trapezoids of Ge are transparent to infrared energy, and allow attenuated total reflection (ATR) spectroscopy. The spectrum represents an "inside-out" view of an adsorbed film; i.e., the information is from the substrate, the germanium, looking outward to see the medium of exposure. Analysis is non-destructive and permits subsequent tests, also non-destructive, such as ellipsometric determination of film thickness, and measurement of contact potential. Only the application of the test liquids for determination of critical surface tension must modify the affected areas. These methods are summarized schematically in Figure 2.

The marine mammals examined were available through the courtesy of the Niagara Falls Aquarium, Niagara Falls, New York, the Marineland Seaquarium, Niagara Falls, Ontario, Canada, and a few tests were performed at the US Naval Ocean Systems Center, Hawaii. The porpoises, located at all three locations, were of the species Tursiops truncatus, while the killer whales (Orcinus orca) were examined only at the Marineland facility. The mammals were housed in seawater tanks that required the addition of chlorine and considerable water filtering and recycling. The oxidative effect of the relatively high chlorine levels--estimated to vary between 0.5 and 2ppm residual--may have affected the rate of desquamation of epithelial cells over that in the natural environment. The killer whales, in particular, not only appeared to lose skin material readily when rubbed by hand, but skin cell aggregates were found also floating in the tank. Porpoises, at any of the facilities visited, appeared to have a far lower rate of desquamation. As discussed in the results section below, neither visual nor microscopic examination revealed obvious differences in skin appearances or properties at any of the facilities, leading us to infer that our observations may reasonably reflect findings for animals in their natural, open ocean environment.

At all three locations the analyses were performed when the mammals were either lifted from their tanks during scheduled semi-annual water changes, or when the animals were left in their tanks as draining took place (see Figure 3). Every attempt was made to minimize the duration of exposure and the measurements were typically accomplished in 12 to 19 minutes per animal. A total of 7 porpoises were examined for all three variables measured, with additional tests performed on porpoises at the Hawaii facility.

Skin roughness samples were taken by preparing replicates using Rubberjell (trademark), a polysulfide preparation used in dental practice for the replication of intra-oral surfaces. Sufficient material was mixed to allow application of a patch about 2 by 2 cm. Curing time was based on prior testing on human skin and curing appeared nearly as rapid in seawater as in air. Patches were applied predominantly on dorsal and lateral surfaces, the mammal's ventral side being generally less accessible. Patches were peeled off after 3 to 5 minutes curing time, marked to identify the fore and aft direction, and subsequently examined by SEM (Scanning Electron Microscopy). Selected samples were cast in dental stone for subsequent analysis by a surface roughness analyzer (positive replicas courtesy of Dr. H. Flynn, Department of Restorative Dentistry, State University of New York at Buffalo; roughness measurements and microtopograms courtesy of the US Naval Ship Research and Development Center, Annapolis, Maryland).

The molecular composition of the skin was determined using ATR (Attenuated Internal Reflection) IR-Spectroscopy described above. Polished germanium plates or prisms measuring 50x20x1mm were gently touched and lightly rubbed to the animal's skin, only slight contact being necessary to

transfer the materials. The plate's high surface energy ensures the attachment of any exudates, desquamating cells or materials adventitiously present. Prisms were air-dried when samples were taken from still-wetted skin, wrapped in Kimwipe tissues, and taken to the lab for detailed analysis. ATR-IR spectra were run on a Perkin-Elmer Model 710B spectrophotometer using a Wilkes ATR adapter; contact potential was measured using an Am-141 electrode and a Keithley electrometer, while film thickness measurements were performed on Rudolph & Sons thin film ellipsometer 43603-200E, with data reduced using a program developed at the National Bureau of Standards.

Contact angle measurements were made using the technique mentioned above, viewed with a Rame-Hart Contact Angle Gonimeter. For the in vivo tests, contact angle was estimated, and photographed using a macro lens with ring flash adapter. Not all photos taken gave acceptable results; however, estimates were felt to be reliable because of their self-consistent nature. For any given substrate, the contact angles typically decrease with the decreasing surface tension of the liquid--the substrate becomes more and more wettable to fluids of lower surface tension. Because 11 liquids were available and drops were applied in decreasing order until spontaneous spreading (i.e., spontaneous wetting) was observed, a range of angles was thus defined.

The stagnation flow chamber is a modification of the DePalma flow cell (Calspan patent # 4,175,233) which was developed to allow the observation and study of the formation of adsorbed films, including bacterial slime films, under predetermined flow conditions that can be adjusted to be laminar, transitional, or turbulent in nature. The stagnation chamber is shown in Figure 4, and the measurements were made using a Marriotte flask to provide a constant head for the flow, using electronic switches to measure the time required to allow a fixed volume of water--about 750 ml--to pass the cell. The stagnation chamber was tested for its capacity to reflect drag reduction effects from bulk additions of polyox (poly-oxyethylene) to tap water. Concentrations ranging from 31ppm to 500ppm of polyox showed increasing drag reduction that followed the classically observed patterns; i.e., greatest effects at the highest concentration, with the 500ppm concentration approaching a maximum effect asymptotically (20). Results are shown in Figure 5, where the 0ppm value reflects baseline flow, and the cell dimension of an equivalent straight pipe flow where Reynold's numbers range from 3700 to 4300, which is less than typically demonstrated in drag reduction using bulk additives in standard pipe flow.

RESULTS AND DISCUSSION

An artificial substrate exposed to marine, estuarine, or fresh water rapidly and spontaneously acquires an adsorbed organic film that typically is glycoproteinaceous in nature, renders the surface hydrophylic if not

originally in that state, and leaves the surface negatively charged. Bacterial attachment follows this induction period (20) with more or less typical pioneer organisms shown in Figure 6. These tend to be rod-shaped bacteria which quickly leave an ovoid ring of exudates. These in turn are followed by a prosthecate, oligotrophic bacterium. Film thicknesses now tend to approach 20 to 200nm, and this attachment phase then overlaps the subsequent arrival of the macrofouling larvae and algal species. The macrofouling stage has not only been extensively studied and described, but has been effectively suppressed by various anti-fouling coating systems, which almost exclusively rely on toxic mechanisms (22).

It is the lack of success of toxic coatings to inhibit or prevent the living process of biofilm formation that stimulated studies of this type, and further elicited the question of whether extremely fast-swimming porpoises and killer whales have biofouling resistance or even drag-reducing skin characteristics. Visual inspection of the bottlenose dolphin Tursiops truncatus and the killer whale Orcinus orca reveals a skin that is remarkably smooth, free of hair or appendages. A finely formed system of ridges and grooves, oriented transversely to the flow, become apparent, particularly on the forward dorsal surface during the brief contraction when the animal exhales. Skin replicates examined under the SEM further verify the visual impressions, as can be seen in Figures 7a and 7b. The quadrilateral shapes are desquamated cells transferred to the replicating polymer. While loose cells and cellular debris may contribute to micro-roughness, we believe that the small fraction of the total area thus covered suggests that ablative processes are unlikely to account for the skin's fouling resistance. A microtopogram was prepared from a stone-cast positive of the polymer replicates to magnify the observed grooved features and compare them to the more or less random roughness of the underlying structural detail. As Figure 8 shows, the wavelength is about 0.3mm to 0.4mm, and the trough to crest wave height averages about 10μm. For a porpoise swimming at 8.2ms^{-1} (16 knots), the roughness height of these features should remain well within the boundary layer thickness; hence a hydrodynamic role of these features must remain speculative. The groove orientation at right angles to the flow further suggests that a functional significance must be sought along other paths.

The outermost molecular composition i.e., the nature of the covalently bound groups of the molecules in direct contact with the marine environment, was determined by ATR infrared spectroscopy. A typical spectrum obtained from analysis of a germanium prism touched and rubbed lightly over the porpoise's skin is shown in Figure 9. Not only do these samples show remarkable consistency in their spectral features from differing places on the animal, but inter-animal variation is very low, and the similarity to samples from killer whales is also striking (Figure 10). The outermost skin layer is essentially glycoproteinaceous with little or no evidence of lipid or saccharide components. While fatty tissues exist in ample quantity below epidermal layers, examination of skin segments from both a

deceased porpoise and our in vivo studies showed neither fats nor mucus secretions to be detectable surface characteristics of these species.

Contact angle measurements made both on the animals in vivo and on material transferred to the germanium prism again show a remarkable intra- and interspecies consistency. A Zisman plot, shown in Figure 11 for Tursiops truncatus, gives a critical surface tension between 22 and 30 mNm^{-1}, the range consistent with minimum bioadhesion. Histologic analysis of skin sections from biopsies of living animals and deceased porpoises (13) show the uniform outermost epidermal layer to be parakerototic and fully nucleated, including the most superficial squamous layer that appears to be actively producing the pure proteinaceous extracellular coating that is visible spectroscopically. This structure, (Figure 12) and the interdigitating rete pegs that extend into the collagenous, fibrous tissue of the dermis, have the properties of mucosal skin, such as the interior surface of human cheeks, and inside portion of human lips Mucosal surface and the endothelial linings of blood vessels exhibit a similar range of critical surface tensions. and these surfaces have a similar requirement to remain free of fouling debris as well as drag enhancing accumulations of bacteria and their exudates (21).

Based on three surface-specific analyses, namely surface roughness and texture, molecular composition of the outermost layer, and determination of critical surface tension, we infer that the essentially mucosal surface of the porpoise and killer whale confers to the animal non-toxic, highly fouling resistant properties.

To investigate the relationship of surface-specific characteristics to hydrodynamic properties, and to study the range of drag increasing to drag reducing phenomena, we employed a novel stagnation flow chamber that permits the observation of a range of effects on a laboratory bench scale.

Measurements were made on substrate ranging from substrates with well-developed bacterial films, via typical fish mucous secretions, cetacean surface material transferred onto germanium plates, and finally to known and experimental drag reducing agents deposited onto test substrata. Table 1 summarizes a series of measurements that began with an attempt to determine if a known drag reducing agent, in this case simply deposited onto the stagnation flow cell substratum, had measurable drag-reducing effects. It has been our working hypothesis that the intrinsic molecular property that microlayer drag in seawater confers drag reducing benefits when added as a bulk constituent can be preserved when one end of the molecule is tethered to the surface. As the positive numbers in the right-hand column of the table show, such effect is observed, although we hasten to add that in this case it may simply derive from ablation and resuspension of the polyox (poly oxyethylene). This appears to be confirmed by the diminished spectral intensity of the accompanying ATR infrared spectra.

It is of interest to note that a test of Emulsan, a commercially

advertised drag-reducing agent of quite different composition, produces a significant drag increase when deposited as a film, although it may be significant to note that in this case spectral evidence indicates far less material loss from the substratum during the tests.

The last two measurements summarized on Table 1 show drag increases observed due to biofilms collected on flow cell substrata exposed to laboratory seawater aquaria. In this case the surfaces of the substrata were modified to give low critical surface energy regimes. Although the data vary, drag increases are noted in both cases.

Present work is evaluating the drag effects of marine and estuarine biofilms, from barely formed bacterial colonies to thick films rich in entrapped diatoms and filamentous algae. Examples of the latter, allowed to form in nutrient rich, warm, shallow estuarine waters taken from 1 m below the surface show significant drag increases, as is expected, and has been seen in other studies (21). Films formed in cold, generally oligotrophic coastal waters show greater variability and at times exhibit actual drag reduction when tested in stagnation flow, as summarized in Table 2. While there have been literature references to observation of drag reduction from such biofilms (21), including that of fish mucous secretions (22), it remains to be established that the effects are indeed surface-specific.

In this work, we varied the critical surface tension of the substrata used in the flow cells used to collect the biofilm under uniform shear rates. Detergent-washed glass has a critical surface tension of about 40 to 50 mNm^{-1}, while the other half of the samples used glass slides to which poly dimethylsiloxane was covalently bound, giving a critical surface tension in the minimally bioadhesive range of 20 to 30 mNm^{-1}. While these data represent work in progress, initial results appear to follow our previous findings, where film detachment under high shear rates was commonly observed for substrates having critical surface tensions of 20 to 30 mNm^{-1}. The drag reduction observed for these substrata subjected to stagnation flow under high shear may be related to the partial ablation and resuspension of bacteria and other film components.

It is the aim of our continuing effort to determine if the working hypothesis advanced earlier, namely that the drag-reducing property of some molecules can be preserved when one end, or one series of branches, is "tethered" to a surface. We have been evaluating an experimental coating material in the stagnation flow regime. When the candidate drag-reducing polymer was surface deposited, though still capable of some ablation or resuspension, drag-reducing effects were variable, and at times absent entirely. A version where the material was irreversibly bound to a supporting mesh but left one surface having many "dangling" polymer strands exhibited good drag reduction when compared to its "passive" side, as summarized in Table 3.

CONCLUSIONS

Typical engineering materials placed in contact with seawater will acquire not only a film of organic matter that spontaneously adsorbs from dissolved or colloidal matter in the sea, but will subsequently acquire a bacterial film even when precautions have been taken to create a surface environment hostile to macrofouling organisms. Once well developed, such biofilms will induce a drag penalty on ships, as has been shown in field trials as well as laboratory experiments using rotating discs. Using the principles and methods of surface chemistry and physics, we have shown that fast swimming mammals, the porpoise and the killer whale, can escape or minimize the penalty by virtue of having a smooth, mucosal surface that is predominantly glycoproteinaceous, and has a critical surface tension that is consistent with offering the least propensity for microbial as well as macrofouling. On the other hand, we could not detect, and are inclined to reject the hypothesis, that these mammals possess a surface-specific drag-reduction mechanism. Testing biofilms grown under predetermined shear rates on substrata of different critical surface tensions, we find in early results under stagnation flow that biofilms give apparent drag reduction when formed on low bioadhesion substrates, and extract a drag penalty when tightly anchored to high energy substrates. While we are only at the beginning of more extensive exploration of this phenomenon, it does suggest that control of drag-producing slime films may be achieved on the basis of surface energy modification. Achieving drag reduction using a surface-specific mechanism appears at present a more difficult objective, but present theory does not totally contradict early and suggestive experimental findings.

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TABLE 1

DRAG REDUCTION EXPRESSED AS FLOW INCREASE
IN STAGNATION FLOW CELL

DRAG AFFECTING CONDITION	FLOW $CM^3\ SEC^{-1}$	Re	% CHANGE (-) 0 DRAG REDUCT. (+) 0 DRAG INCR.)
POLYOX low flow	41.32 40.78	3600	+1.32
POLYOX med. flow	50.18 48.91	4300	+2.59
POLYOX high flow	73.51 72.29	6300	+1.69
POLYOX high flow	75.71	6500	+2.55
POLYOX modified cell	65.29 64.81	5700	+0.73
EMULSAN	71.54 73.48	6300	-2.73
BIOFILM ON poly di-methyl siloxane	71.36 71.05	6230	-0.44
BIOFILM on 3 Hept	64.81 64.77	5670	-0.036

TABLE 2

DRAG EFFECTS OF MARINE AND ESTUARINE BIOFILMS ON SEVERAL SUBSTRATA IN STAGNATION FLOW

SUBSTRATE	FLOW $3-1$ cm s	SOURCE AREA WATER TYPE	PER CENT CHANGE > 0 drag reduct. < 0 Drag incr.
Glass	70.23	coastal ocean	-1.3
Glass	70.19	coastal ocean	+0.5
Glass	71.05	coastal ocean	-1.2
Glass	62.85	estuarine	-9.4
PDMS*	69.04	coastal ocean	+1.5
PDMS	66.92	coastal ocean	+5.7
PDMS	66.03	coastal ocean	+2.3

*PDMS - poly dimethylsiloxane

TABLE 3

EXPERIMENTAL DRAG REDUCING COATING IN STAGNATION FLOW

CONDITION	FLOW $3-1$ cm s	% CHANGE * 0 drag reduct.	NO. OF REPS.
no coating	70.61	0	6
streaked rough coating	70.13	+0.68	6
buffed	71.21	-0.85	6
no coating	65.17	0	6
streaked, rough	67.29	+3.26	6
light buffing	71.07	+9.05	6
vigorous buffing	69.98	+7.38	6
no coating	71.48	0	6
streaked, rough	67.02	-6.24	6
light buffing	69.22	-3.16	6
vigorous buffing	68.85	-2.63	6
"active" side of mesh	67.59	+3.88	6
"passive" side	65.07	0	6

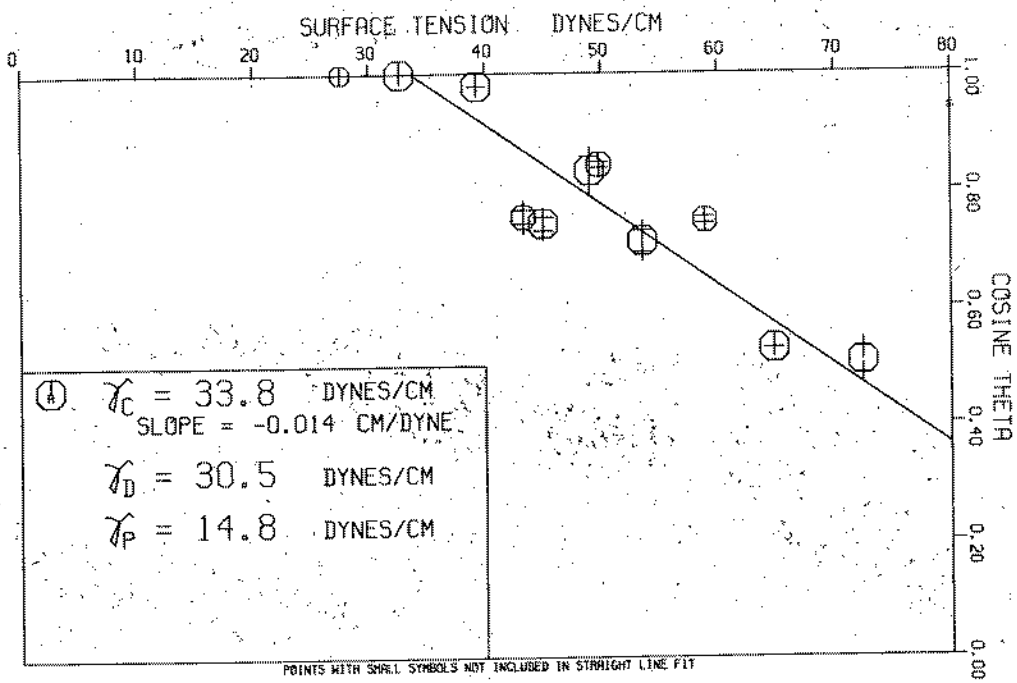


Figure 1 ZISMAN PLOT FOR GERMANIUM INTERNAL REFLECTION PLATES EXPOSED TO OCEAN WATER

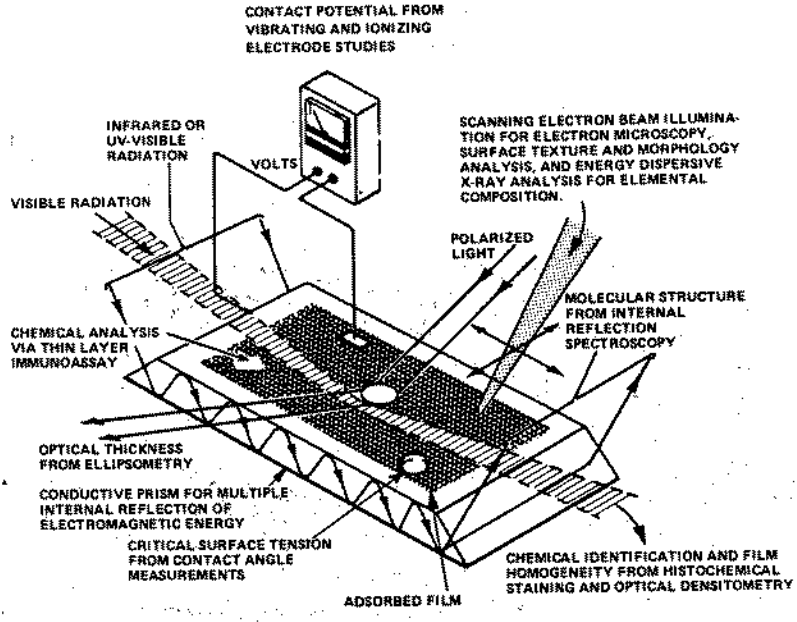


Figure 2 SCHEMATIC OF NONDESTRUCTIVE ANALYTICAL TECHNIQUES USED IN CHARACTERIZING PRIMARY ADSORBED FILMS AND DEPOSITS OF BIOLOGICAL OR MINERAL MATERIALS

Figure 3 PORPOISES DURING IN VIVO SKIN SURFACE ANALYSIS

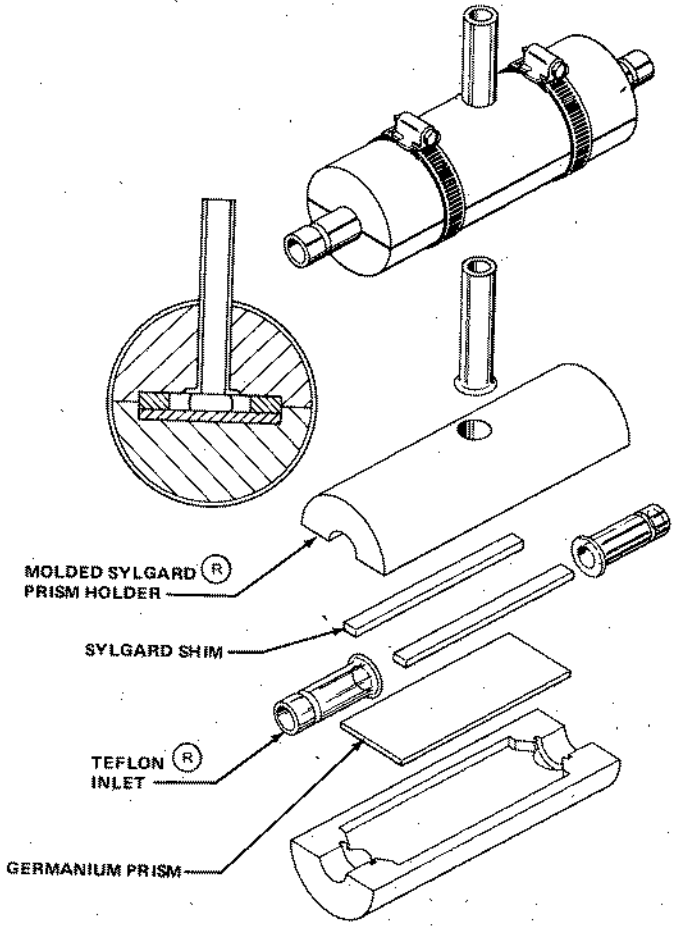


Figure 4 SCHEMATIC OF STAGNATION FLOW CHAMBER

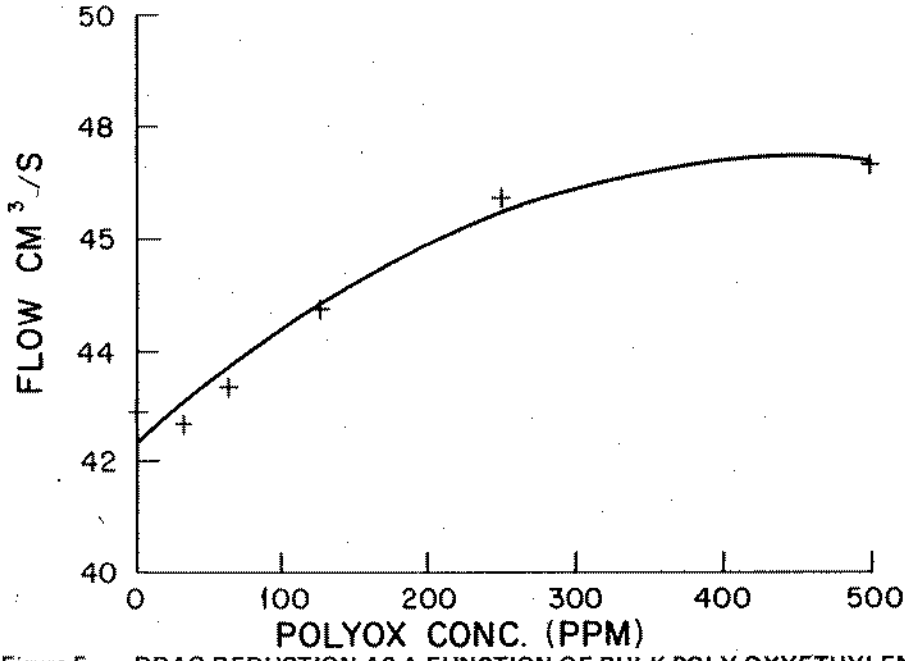
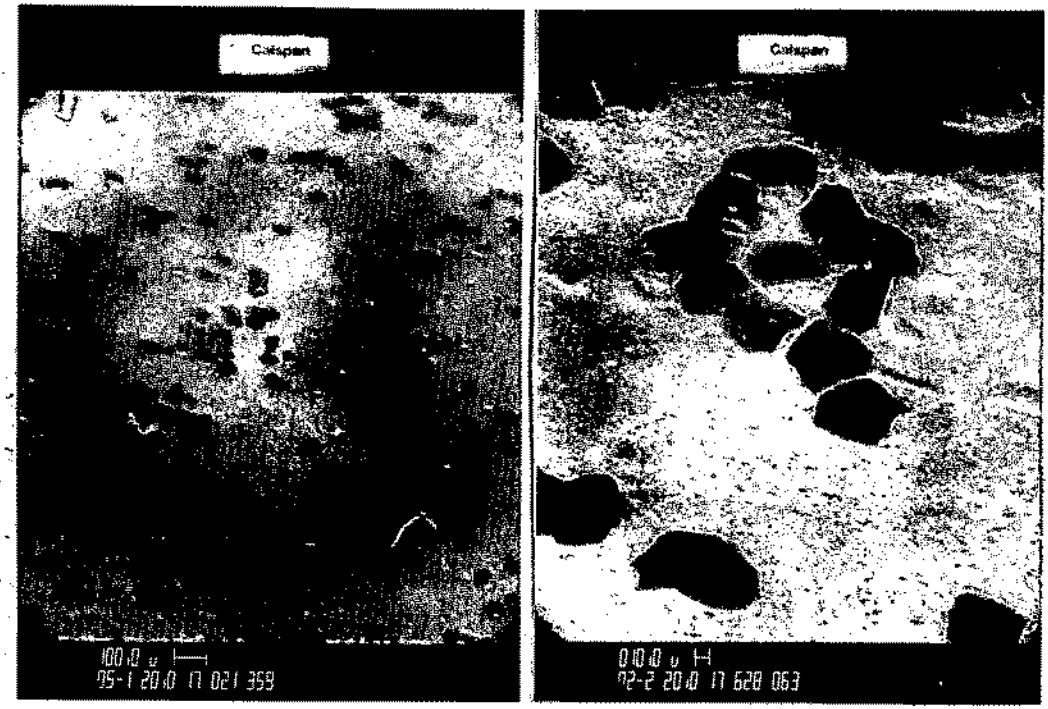


Figure 5 DRAG REDUCTION AS A FUNCTION OF BULK POLY-OXYETHYLENE CONCENTRATION IN STAGNATION FLOW

Figure 6 "PIONEER" MARINE BACTERIA ATTACHED TO TEST SUBSTRATE AFTER THE INDUCTION PERIOD



(a) (b)

Figure 7 VIEW OF NEGATIVE POLYMER REPLICA OF PORPOISE SKIN SHOWING SMOOTH TEXTURE AND TRANSFERRED DESQUAMATED CELLS

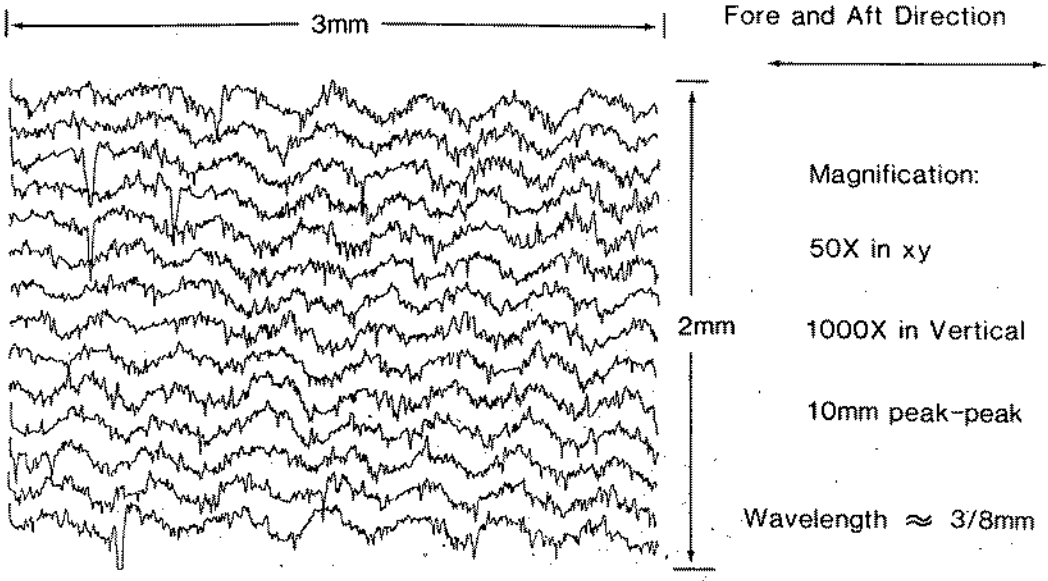


Figure 8 MICROTOPOGRAM OF POSITIVE REPLICA OF PORPOISE SKIN AFT OF DORSAL FIN

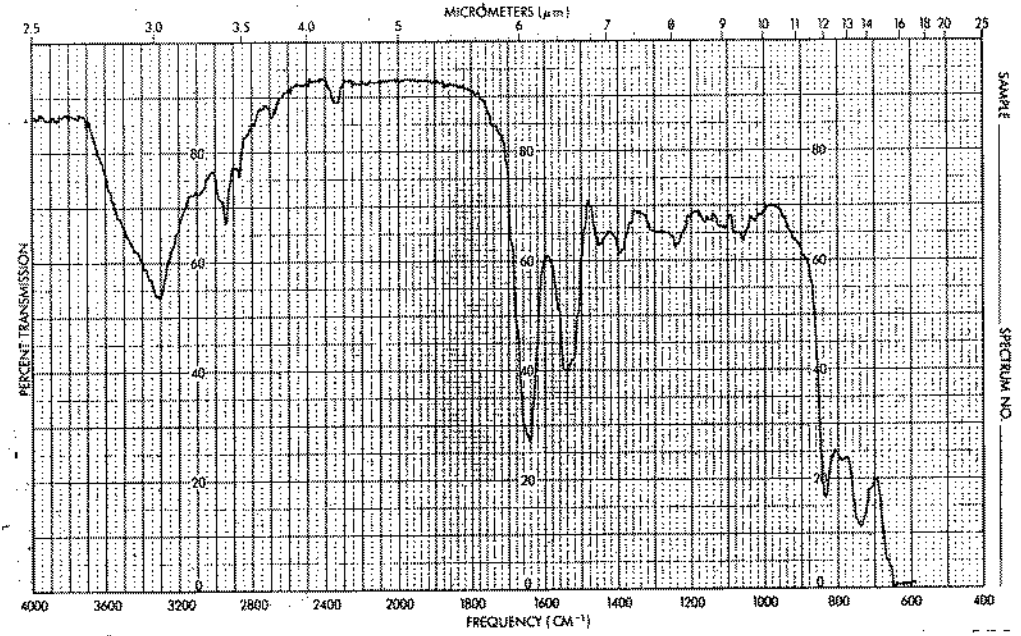


Figure 9 GERMANIUM PRISM TRANSFER OF SKIN "EXUDATE" FORWARD OF DORSAL FIN OF PORPOISE (TURSIOPS TRUNCATUS) "MISTY"

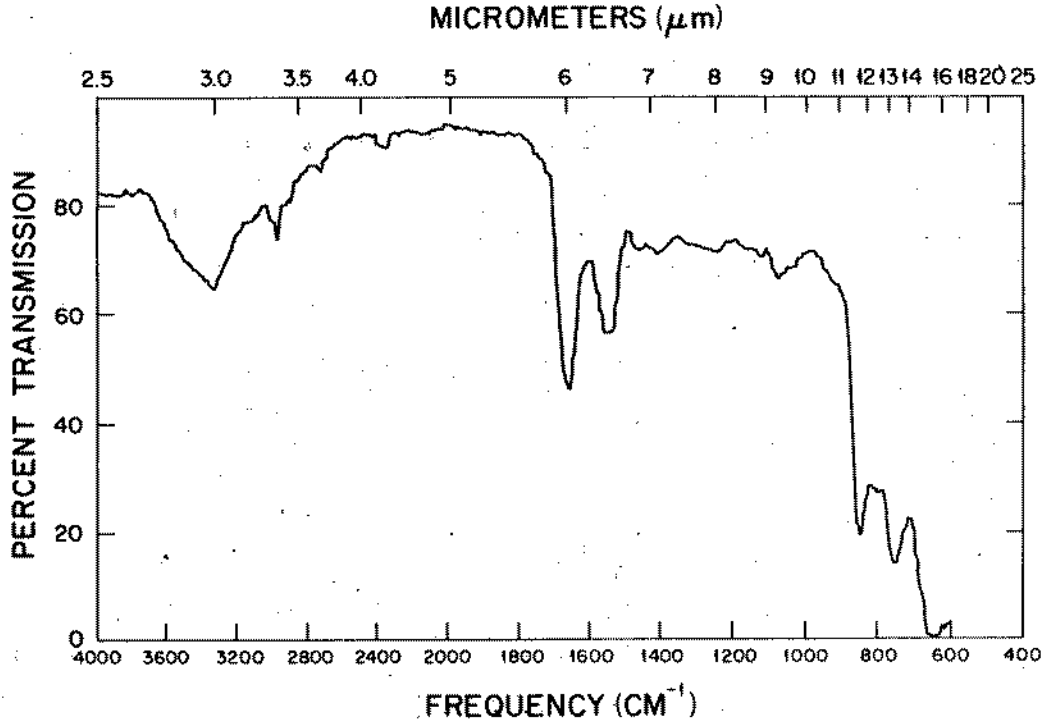


Figure 10 SKIN EXUDATE OF KILLER WHALE, ORCINUS ORCA, LATERAL SIDE NEAR FLIPPER

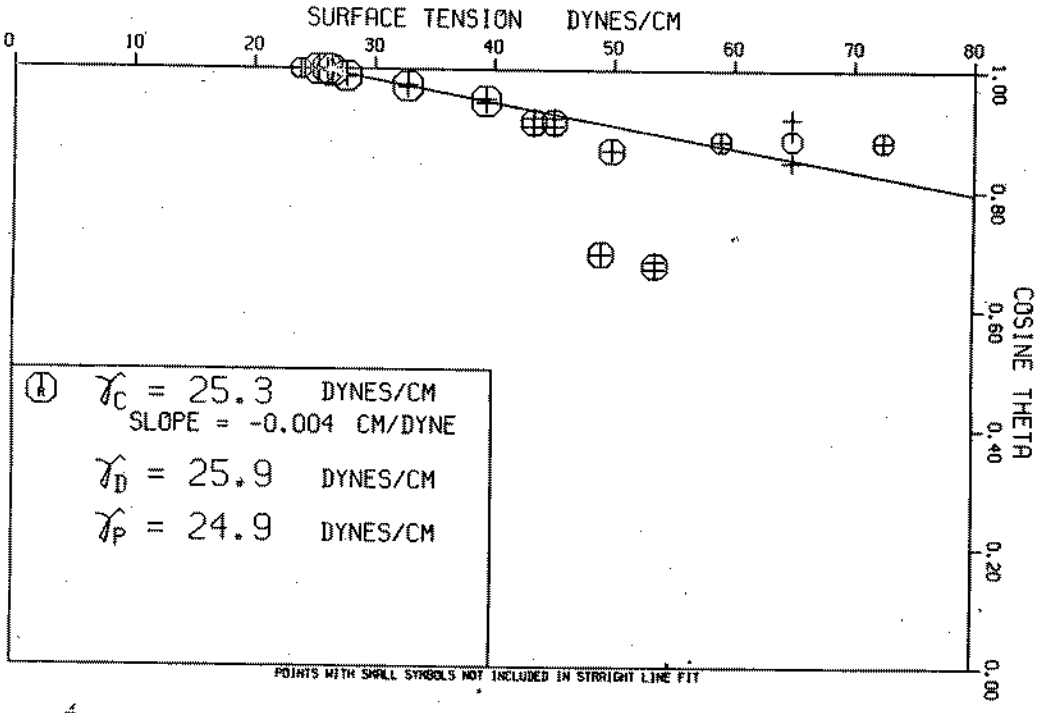


Figure 11 ZISMAN CONTACT ANGLE PLOT OF FILM TRANSFERRED FROM SKIN OF TURSIOPS TRUNCATUS

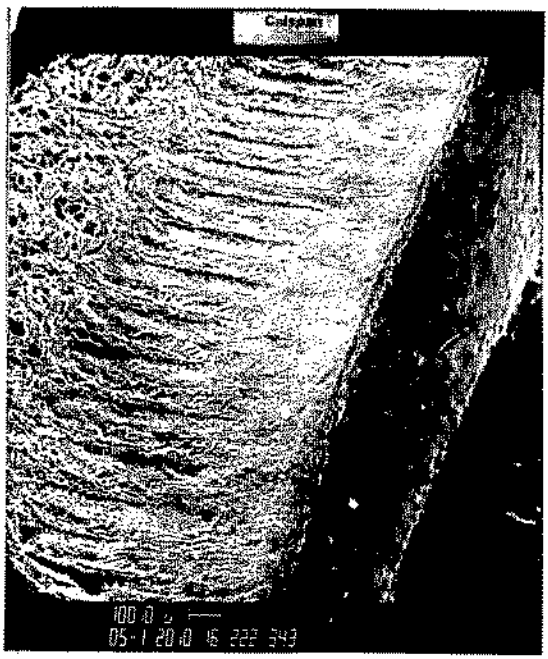


Figure 12 CROSS-SECTIONAL VIEW OF SKIN SEGMENT OF DECEASED PORPOISE SHOWING NUCLEATED CELLS AND STRUCTURE OF RETE PEGS

MICROFOULING SURVEY OF ATLANTIC OCEAN WATERS

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Abstract

Une unité testeuse pour micro-encrassement autonome et portative a été installée à bord du USNS Lynch pour une croisière trans-atlantique, mars-avril, 1983, de Charleston, Caroline du Sud, USA, à Glasgow, Ecosse, Royaume-Uni, les stations intermédiaires dans la mer Sargasso et les îles Canaries. Le débit moyen contrôlé des écoulements d'eau de mer était maintenu par des cellules spéciales qui contient soit de l'acier inoxydable dépourvu d'inclusions de qualité d'échangeur de chaleur ou du même acier modifié par un revêtement superficiel mince de dimethylsilane (tension superficielle critique = 22 dynes/cm). Des spécimens encrassés pendant une semaine de chacun des cinq partages de la croisière étaient mis en comparaison l'un à l'autre et avec les plaques testeuses exposées à l'encrassement microbiens pendant la durée du voyage de 38 jours. Pour chaque partage de al croisière, on a documenté des dispositions d'encrassement distinctement différentes; on a aussi constaté les apports à l'encrassement total relatifs du voyage entier. Dans l'ensembel, le micro-encrassement des plaques en acier originairement sans revêtement était plus prononcé d'une manière significative que le micro-encrassement des plaques enduites de silane de basse énergie superficielle. Ceci confirme le potentiel minimal d'encrassement observé auparavant des matériaux nontoxiques de tension superficielle critique de la gamme de 20-30 dynes/cm. La plus grande densité d'encrassement, principalement par des petites diatomées de l'ordre Pennales (l.) et de la matière particulaire riche en calcium, était observé au port de Las Palmas, îles Canaries. La méthode testeuse s'est montrée comme simple, vigoreuse, peu coûteuse, et capable de donner des éléments d'information excellents pendant des durées très courtes, ce qui recommande son extension aux enquêtes mondiales sur le mincro-encrassement.

Microfouling of ocean waters

A self-contained, portable microfouling test unit was installed aboard the USNS Lynch for a trans-Atlantic cruise, March-April, 1983, from Charleston, South Carolina, USA to Glasgow, Scotland, UK with intermediate stations in the Sargasso Sea and Canary Islands. Controlled-shear-rate flows of seawater were maintained through special cells containing either clean heat-exchanger grade stainless steel or that same steel modified with a thin surface coating of dimethylsilane (critical surface tension = 22 dynes/cm). One-week-fouled specimens from each of five cruise legs were compared with each other and with test plates exposed to microbial fouling for the entire 38-day duration of the voyage. Distinctly different fouling patterns were documented for each segment of the cruise, and the relative contributions to the total fouling for the entire voyage were ascertained. Overall, microfouling of the originally uncoated steel plates was significantly more severe than for the plates coated with the low-surface-energy silane, confirming the previously observed minimal fouling potential of non-toxic materials with critical surface tensions in the 20-30 dyne/cm range. The greatest fouling density, mainly by small pennate diatoms and calcium-rich particulate matter, was observed in the port of Las Palmas, Canary Islands. The test system was shown to be simple, hardy, inexpensive and capable of yielding excellent data over very short periods, recommending its extension to worldwide microfouling surveys.

Introduction

The macrofouling potential of ocean and coastal waters has been the subject of ongoing study for over 30 years (1,2). Practical applications of this science include forecasting the performance of anti-fouling coating systems, predicting the degree of impairment of devices such as hydrophones or environmental sensors, and gathering intelligence about the likely area a drifting object may have traversed, and the time it has been in the water. Currently available anti-fouling coatings are successful in reducing macrofouling for periods up to 24 months, but are not able to significantly limit the formation of biofilms, which form on an exposed material after an induction period (3). These films begin as pioneer bacteria attach to and enrich the pre-existing adsorbed organic layer with their own exudates and in turn, serve as substrata for subsequent arrivals. The primary layers of the biofilm ultimately enmesh filamentous algae, capture diatoms, and allow larval settlement where the surface environment is free of hostile toxic materials (4).

To determine if environmental or geographic differences exist for bacterial film formation as they do for macrofouling communities, we took advantage of the transatlantic survey cruise of the USNS Lynch (T-AGOR-7) in March and April 1983. Biofilms were allowed to form on two types of test substrata installed in simple, rugged flow cells that

were fed from a small reservoir replenished daily with ocean surface water collected from the bow of the vessel. Films from each leg of the five leg survey were collected, as well as a cumulative biofilm formed during the entire cruise. Subsequent analyses were performed in the laboratory; these include scanning electron microscopy coupled with energy dispersive X-ray analysis, determination of film critical surface tension, examination of the effects of substrata surface energy, and identification of classes of bacteria using newly developed in-situ immunofluorescent techniques. Our results suggest that flow cell technology readily lends itself to the rugged shipboard environment and that the available analysis methodology, especially the immunofluorescent technique, is of sufficient sensitivity to meet the study objectives.

Methods and Materials

A Portable Biofouling Unit (PBU) was designed and assembled for the cruise. The PBU consisted of a standard size (approximately 5 gallons) Coleman cooler containing a submersible pump; the pump fed a flow manifold constructed from PVC tubing. Four flow cells could be exposed simultaneously with this system. Figure 1 shows the unit on board ship.

Twelve Calspan-designed flow cells (U.S. Patent #4,175,233) were utilized in this survey. Six cells contained medium-energy AL-6X stainless steel test plates; the other six cells contained AL-6X stainless steel plates pre-coated with a low-surface-energy silane (polydimethylsiloxane).

At all times, four flow cells were up and running: one "full cruise" cell containing medium-surface-energy plates (AL-6X stainless steel), one "full cruise" cell containing low-surface-energy plates, one short-term medium energy cell, and one short-term low energy cell. The PBU was filled from daily bucket dips over the bow of the moving ship. Each day, 25-50% of the volume was drained and the unit topped with fresh seawater. Flows through the test cells were maintained at approximately 350 milliliters/minute, which gives a shear rate of $1000s^{-1}$ and approximates the rates in source heat exchangers.

The test plates were prepared and the flow cells were constructed in Calspan's Advanced Technology Center laboratory in Buffalo, New York prior to the cruise. "Medium energy" plates were prepared by treating detergent-washed stainless steel coupons (5 cm x 2 cm x 0.1 cm) with a radio-frequency glow discharge (RFGD) technique. The plates were then exposed to ambient clean room conditions (67°F, 55% relative humidity) for one day, at which point they had achieved a surface energetic "steady state." Low energy test surfaces were obtained by coating RFGD-treated stainless steel coupons with dimethyldichlorosilane, which covalently couples with the RFGD-activated metal surface to give a coating that is methyl-rich and that has a critical surface tension of

approximately 22 dynes/cm. The medium energy steel plates have critical surface tensions averaging 30 dynes/cm.

After the desired exposure terms at sea, the cells were immediately and gently flushed with a 2% glutaraldehyde solution in seawater in order to biologically fix the acquired fouling film. After fixation, the cells were gently flushed with triple-distilled water in order to remove any excess glutaraldehyde and loosely adherent matter. The cells were allowed to air dry in a vertical position, while still assembled, and then were stored in ziplock polyethylene bags until delivery to the Calspan surface science laboratory.

Analyses included contact angle measurements (to determine the critical surface tensions and surface energy components of the acquired films), scanning electron microscopy (to record film morphology), and energy-dispersive x-ray analyses (to determine the elemental compositions of the films). One plate from each cell was used for contact angle analysis; the other plate from the cell was used for SEM/EDX-ray analysis. The latter plate then became the subject of the *in situ* immunofluorescence assay.

The immunofluorescence technique used for this study comprised the following steps:

1. Culturing of marine bacteria in aquaria to establish strains for which antibodies are to be prepared.

2. Selection and growth in pure culture of five marine bacterial species.

3. Antisera production for the following isolates: *Achromobacter sp* *Comamonas terrigena*, *Pseudomonas putrefaciens*, *Pseudomonas sp* and and *Vibrio alginolyticus*. Antibodies were produced by injection of bacterial suspension in incomplete Freund's adjuvant into rabbits, and serum antibody titer was determined by tube agglutination assays (5).

4. Immunofluorescence assays followed the methods of Mouton et al. (6). Appropriately treated bacterial smears were treated with serial dilutions of the rabbit antisera, and incubated with goat anti-rabbit ImmunoGlobulin (IgG) conjugated with fluoroscein isothiocyanate.

5. A phase contrast microscope equipped for incident-light fluorescence was used to examine stained bacterial smears, and a two-fold serial dilution of each antisera exhibiting a 4+ fluorescence was selected as the working titer for subsequent assays.

6. Exposed test plates previously fixed, rinsed, and dried were sequentially stained with each of the five antisera in indirect immunofluorescent assays. Total numbers of microorganisms in the

microfouling films were determined by acridine orange staining and epi-illumination microscopy (7).

Results and Discussion

The ship's track for the five leg survey is given in Figure 2. Leg 1 of the 38 day voyage, which began March 10, 1983 in Charleston, South Carolina, included a transit of coastal waters before traversing the Gulf Stream and entering the waters of the Sargasso Sea. These waters are of high salinity and temperature with little seasonal variation, and are nutrient starved, oligotrophic environments. During Leg 2, the vessel essentially remained in Sargasso Sea water. Seas were calm during Legs 1 and 2, as may be expected during a transit of the "horse latitudes." Rougher seas were recorded for Leg 3, which ran from approximately 20°N latitude to the Canary Islands, about 30°N.

Leg 4 was spent in port (Las Palmas), where the water was visibly polluted. The final segment of the cruise, Leg 5, stretched north from the Canary Islands to Scotland; seas were intermittently rough during this period.

Composite water samples were taken for each leg of the cruise and were analyzed for selected parameters upon return to Buffalo, NY, USA (Table 1).

Results of the surface analyses of the exposed flow cell plates are given in Table 2. Each leg of the track can be differentiated by the morphology of the fouling film on the medium-surface-energy steel plates: Leg 1 - coccoidal bacteria are dominant, exudate patches observed; Leg 2 - rod-shaped bacteria dominate, some inorganic deposits occur; Leg 3 - almost no attached bacteria; Leg 4 - surface littered with calcium-rich structures; Leg 5 - dense organic deposits, many rod-shaped bacteria. The plates that were exposed throughout the entire cruise were covered with a film that was dominated by the calcium-rich particles observed for Leg 4. Figures 3a-3f are representative photomicrographs that corroborate these observations.

The films adhering to the low-surface-energy plates were not identical in appearance to those adhering to the medium energy plates because, as we have observed in many prior fouling studies (8), biofilms do not form as strong an attachment to low energy surfaces as they do to other surfaces. Minimum biological adhesion has generally been observed on materials having initial critical surface tensions between 20 and 30 dynes/cm (9). This is further verified by number density differences of adherent bacteria observed as well as by the film roughnesses and particle distributions for the films from the cruise. Films on the low energy plates tended to be more loosely organized. In addition the calcium-rich structures acquired during Leg 4 were clumped and less evenly distributed over the low energy test plates.

The critical surface tension (γc's) of the fouling films reflected the nature of the films: organic-dominated films had γc's in the 20's or low 30's dynes/cm; inorganic-dominated films had γc's of 40 dynes/cm or above. The γc's of the low energy plates from Leg 4 maintained a value in the 20's, because the original fouling resistant coating allowed only marginal and uneven distribution of the inorganic particles. There was, thus, significant "show through" of the coating film beneath (Figure 4). Additional seawater exposure of the adherent inorganic particle clumps during Leg 5 (in the full-cruise test cells) apparently caused de-aggregation and a more complete surface coverage (Figure 5). The γc for these plates was 49.0 dynes/cm, reflecting the presence of the more complete inorganic "blanket."

The utility of immunofluorescent reagents in identifying the bacteria present in naturally produced microfouling films was also examined (Table 3). Bacterial species in these films could be identified with the antisera originally developed from species in a laboratory seawater aquarium system. Achromobacter was detected on plates from each leg of the cruise in proportions up to 28% of the total number of cells, as was Vibrio alginolyticus, which was found in proportions up to 20%. However, antisera to the other seawater microorganisms detected these species far less often. Pseudomonas putrefaciens was found in three of the cruise legs in proportions up to 24% but was not detectable in plates exposed for the full cruise. Pseudomonas sp.I was found in only two of the cruise legs, including the second cruise leg, where it formed 39% of the total adherent microorganisms. Comamonas terrigena was found in only small numbers in the second leg and on plates exposed for the entire voyage. Consistent with the analyses by electron microscopy, high numbers of adherent bacterial cells were found on the medium-surface-energy plate exposed for the entire length of the cruise while significantly fewer (approximately one-half) cells were found on the corresponding low-surface-energy plates. The smaller number of adherent microorganisms seen on the low-surface-energy plates is in good agreement with previous findings indicating that biofouling occurs to lesser degree on these surfaces (8,10,11).

While the antisera employed in these studies were, with the exception of Pseudomonas, species specific, the possibility exists that there may be bacteria in microfouling films possessing cross reacting antigens which may give false positive reactions. In natural microfouling films, the potential complexity of the seawater microflora together with the high proportion of fluorescing cells point to the greater likelihood of finding cross-reacting species. In the case of Pseudomonas, the cross reactions seen between Ps. putrefaciens and Ps. sp.I makes it impossible to distinguish between these two species in immunofluorescence assays. On the other hand, the cross reacting antigenic determinants may play a role in bacterial adhesion mechanisms. Polyclonal antisera directed toward these components may therefore be useful in studying adhesion processes.

Conclusions

Both population density and diversity of marine microbes from ocean surface waters that form micro-biofouling films appear to vary as a function of geographic origin. This undoubtedly reflects the micro-ecology of the near-surface environments, is likely to be coupled closely to the water masses that are sampled, and suggests a number of interesting applications. Possible applications include ecological investigation, water mass tracer studies, forecasting the micro-fouling potential, and intelligence gathering. We believe we have established that sufficient sensitivity is available to study these phenomena in greater detail using fairly simple and quite rugged shipboard sampling devices, which principally rely on simple inexpensive flow cells that can hold a wide variety of test substrata under controlled shear rates, and shoreside analytical procedures that may be novel but that can readily be replicated. Our findings further corroborate prior work that the microbial attachment process, strength, and resistance to fluid shear forces is strongly influenced by the surface energy of the available substrata; this may point the way toward possible nontoxic micro-biofouling control.

Acknowledgments

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Table 1

CHEMICAL ANALYSIS OF COMPOSITE SEAWATER SAMPLES
FROM USNS LYNCH CRUISE, SPRING 1983

Parameter*	Leg 1	Leg 2	Leg 3	Leg 4	Leg 5
pH	7.85	8.05	8.15	8.10	8.10
Conductivity	49000	>50000	50000	50000	50000
Salinity	35	39	37	36	36
Total Organic Carbon	<2	<2	<2	<2	<2
Total Inorganic Carbon	26	25	22	21	23
Phosphate-P	0.96	0.74	0.62	1.05	3.00
Sulfate	5200	6000	5400	5800	5200
Calcium	154	193	180	175	168
Magnesium	1020	1150	940	920	930
Copper	0.07	0.06	0.06	0.07	0.08
Iron	0.06	0.07	0.09	0.08	0.06
Manganese	0.03	0.04	0.02	0.03	0.04
Nickel	<0.04	<0.04	<0.04	<0.04	<0.04
Zinc	0.13	0.21	0.03	0.32	0.07

*Conductivity is presented in μmhos; salinity values are in parts per thousand; all other values are in parts per million (mg/l).

Table 2

SURFACE ENERGY AND MORPHOLOGY OF FOULING FILMS ACQUIRED ON TRANSATLANTIC CRUISE

Pre-Exposure Surface	Cruise Segment	γc^*	γd^*	γp^*	SEM/Edax Observations
Medium Energy Stainless Steel	Leg 1	27.6	27.5	10.1	Regularly spaced dark deposits (exudates, slimes); some rod-shaped bacteria, but most are round (coccoidal); exudate patches not always encircling denser bacterial groups.
	Leg 2	26.5	29.2	10.4	Mixed mode of deposits: some grainy organic-dominated patches; white plate crystals; more rod-shaped bacteria than coccoidal; no exudate patches observed.
	Leg 3	34.5	21.8	17.8	Very few bacteria observed; no mineral deposits observed.
	Leg 4	40.7	25.2	19.1	Littered with calcium-rich inorganic "fibers" (~2μ x 0.5μ), very evenly distributed; very few bacteria observed.
	Leg 5	34.9	26.3	11.2	Some dense organic deposits; many rod-shaped bacteria, some coccoidal; scattered smooth spheroidal inorganic particles (3-4μ diameter).
	Full Cruise	45.3	32.9	15.2	Littered with calcium-rich fibers (see Leg 4), piled up parallel to flow (in ridges).
Low Energy Coating on Stainless Steel	Leg 1	32.6	23.8	12.7	(Cell was plugged during exposure)
	Leg 2	31.3	31.0	5.0	No bacteria or mineral deposits observed; one clump of particles observed, pollen-like.
	Leg 3	27.6	28.7	4.6	Very few bacteria observed; biofilm is more ruffled than on medium energy plates; scattered smooth inorganic spheres (4-5μ diameter).
	Leg 4	27.9	29.6	3.8	Clumps of inorganic fibers, calcium-rich, uneven distribution; very few bacteria; some bacterial colonization in deep scratches on plate.
	Leg 5	26.6	24.3	4.0	A few dense organic deposits; very few distinguishable bacteria; few spheroidal inorganic particles.
	Full Cruise	49.0	5.8	73.8	Calcium-rich particles everywhere, but not piled up in ridges; clumped.

* γc - critical surface tension, obtained by a Zisman plot of contact angle data;
γd - dispersive component of the surface energy ($\gamma d + \gamma p$), calculated from contact angles and dispersive nature of diagnostic liquids.
γp - polar component of the surface energy, calculated in the same manner as γd.
All values are in dynes/cm.

Table 3

Immunofluorescent Determination of Microfouling Organisms in the Atlantic Ocean

Cruise leg[a]	Surface energy of test plate	Achromobacter sp.	Comamonas Terrigena	Pseudomonas putrefaciens	Pseudomonas sp.I	Vibrio alginolyticus	Total number of adherent microorganisms /1000 mm^2
		percent bacterial cells					
1	low[b]	ND	ND	ND	ND	ND	ND
	medium[c]	6	0	0	0	13	274
2	low	1	7	1	0	11	423
	medium	0	0	15	39	6	538
3	low	4	0	0	0	4	177
	medium	15	0	24	0	20	283
4	low	13	0	0	0	0	239
	medium	16	0	0	15	4	311
5	low	28	0	0	0	0	407
	medium	0	0	3	0	1	493
Full cruise	low	16	2	0	0	0	433
	medium	5	2	0	1	1	836

[a] From the March-April, 1983 voyage of the USNS Lynch
[b] dimethyldchlorosilane coated stainless steel
[c] radio frequency glow-discharge treated stainless steel

ND - not detected

Figure 1 PHOTO OF THE PORTABLE BIOFOULING UNIT ON BOARD THE USNS LYNCH. THERE ARE FOUR FLOW CELLS IN OPERATION AT THE LEFT END OF THE UNIT.

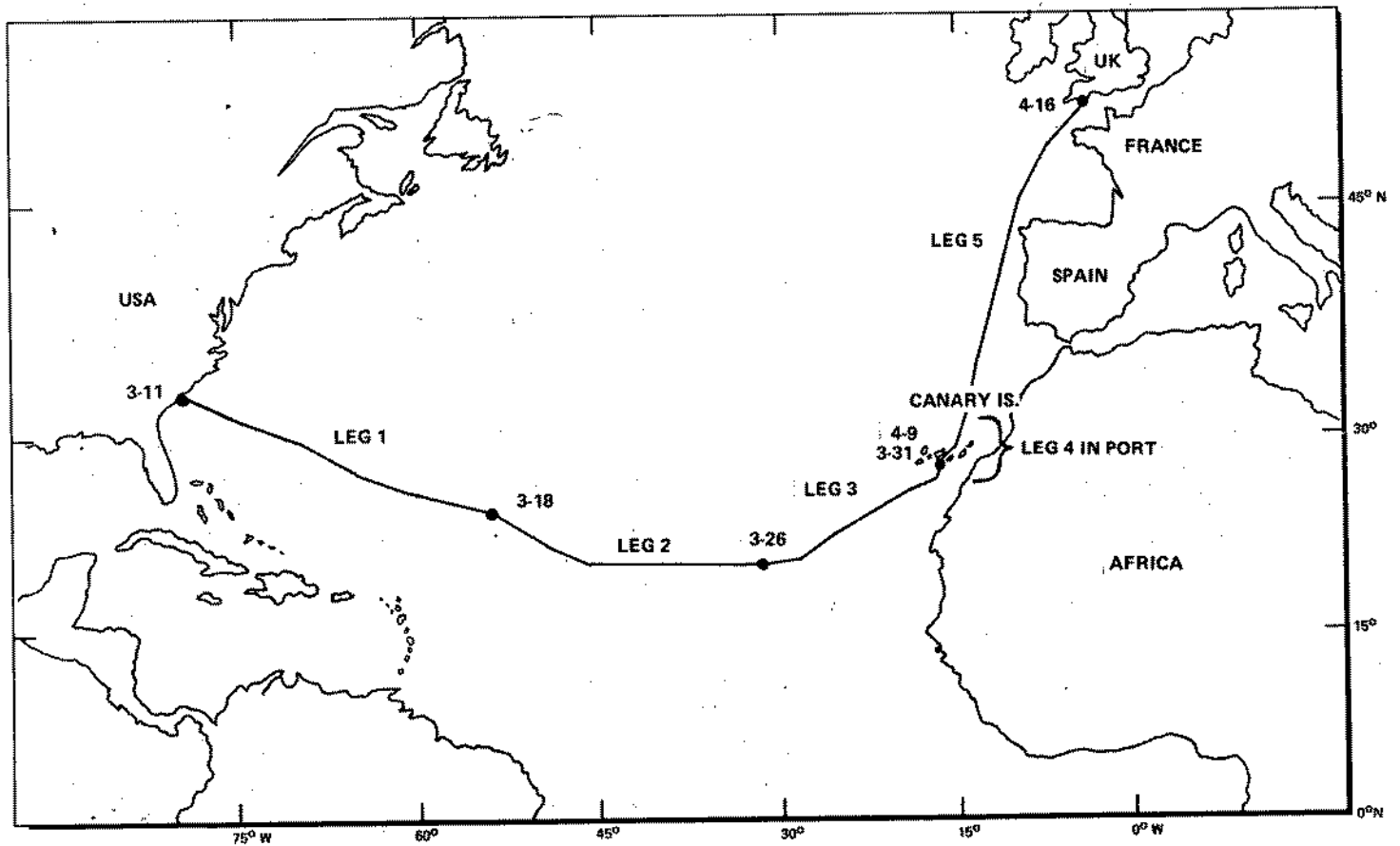

Figure 2 CHART OF USNS LYNCH CRUISE TRACK, SPRING 1983

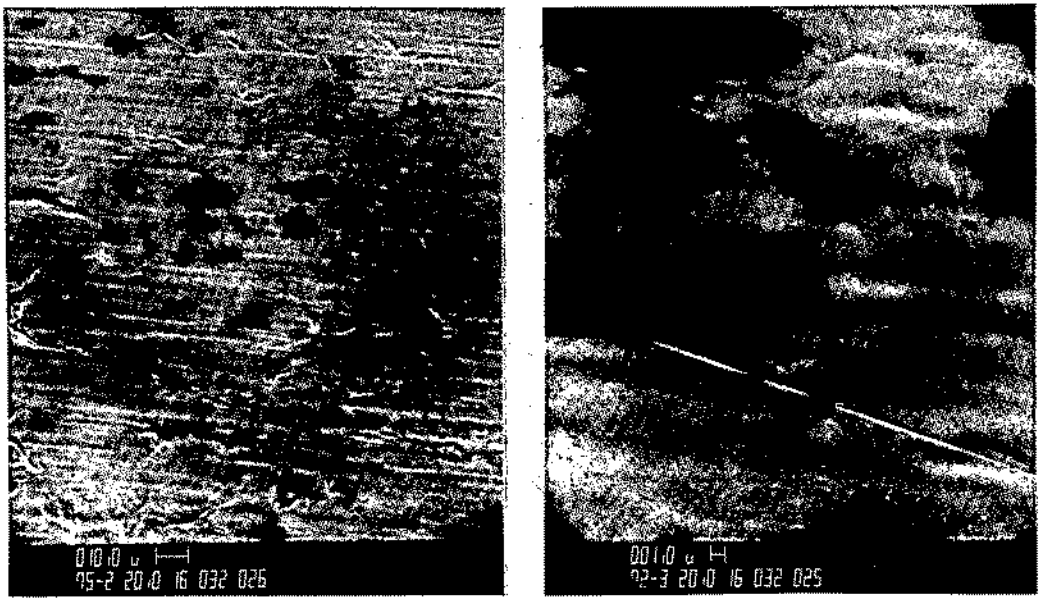

Figure 3a FOULING FILM ON MEDIUM-SURFACE-ENERGY STAINLESS STEEL PLATE AFTER LEG 1 OF THE USNS LYNCH CRUISE. NOTE THE PREDOMINANCE OF COCCOIDAL BACTERIA (ROUND SHAPES, APPROX. 1m IN DIAMETER). LEFT PHOTO - 500X; RIGHT PHOTO - 2000X MAGNIFICATION.

Figure 3b FOULING FILM ACQUIRED ON A MEDIUM ENERGY STEEL PLATE DURING LEG 2 OF THE CRUISE. - LEFT - 500X; RIGHT - 2000X.

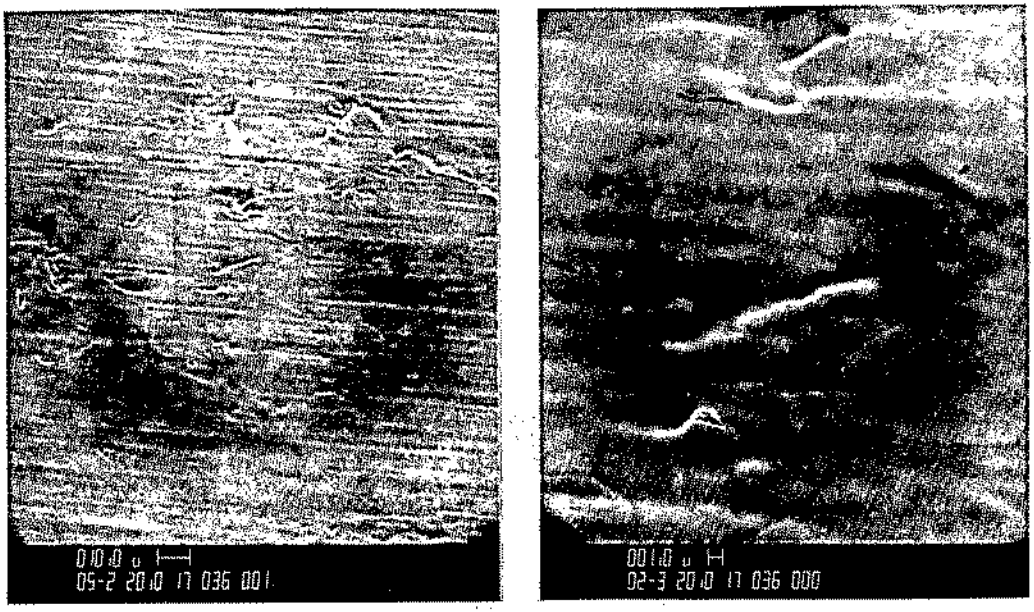

Figure 3c THE STAINLESS STEEL (MEDIUM ENERGY) PLATES WERE RELATIVELY CLEAN AFTER EXPOSURE DURING LEG 3. LEFT - 500X; RIGHT - 2000X.

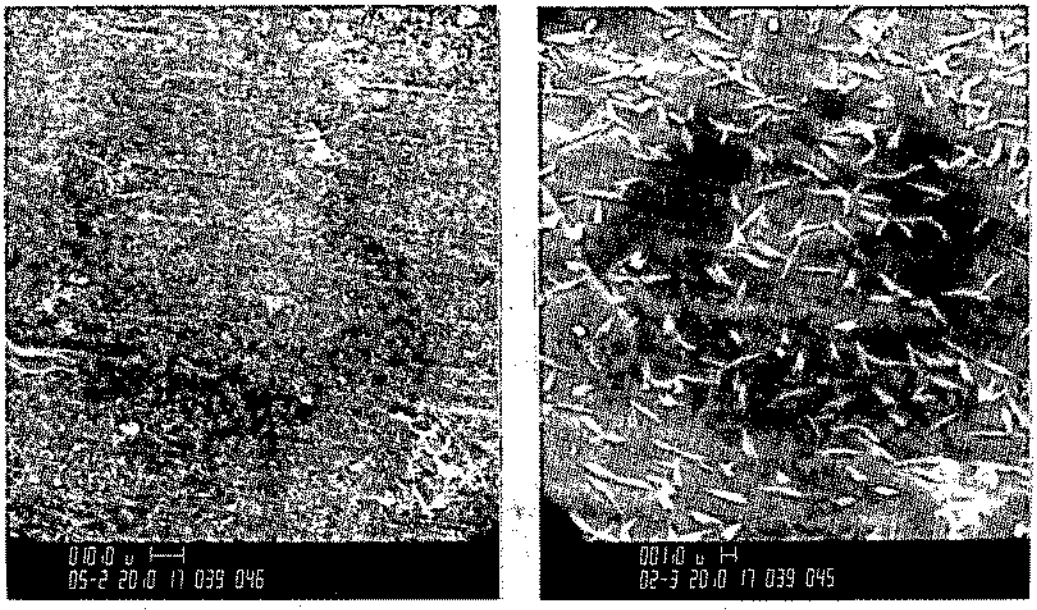

Figure 3d THESE PARTICULES, ADSORBED ON THE STEEL PLATES DURING THE STAY IŃ PORT AT LAS PALMAS (LEG 4), ARE CALCIUM-RICH. LEFT - 500X; RIGHT - 2000X.

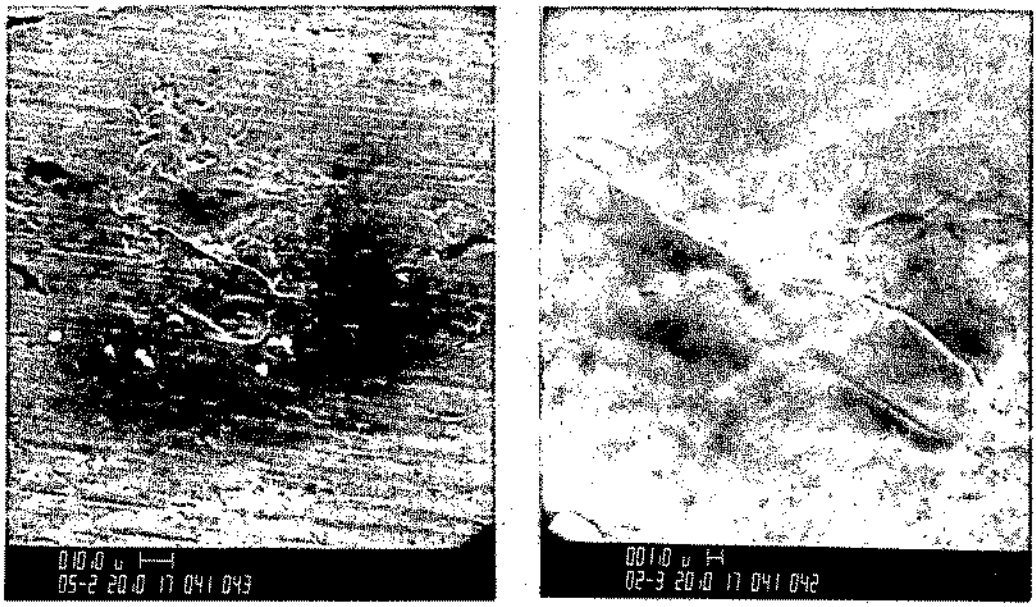

Figure 3e FOULING FILM ACQUIRED ON A MEDIUM ENERGY STEEL PLATE DURING LEG 5 OF THE CRUISE. LEFT-500X; RIGHT - 2000X.

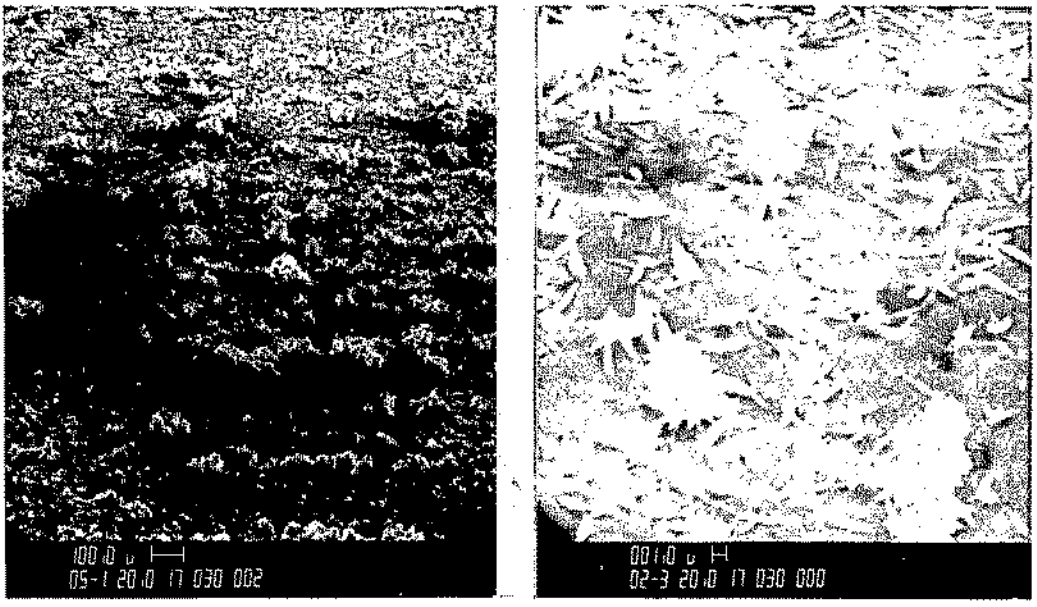

Figure 3f THIS IS THE FILM THAT WAS BUILT UP OVER THE ENTIRE CRUISE (LEGS 1-5). IT IS DOMINATED BY THE CALCIUM-RICH PARTICLES FROM THE LAS PALMAS PORT. LEFT - 50X; RIGHT - 2000X.

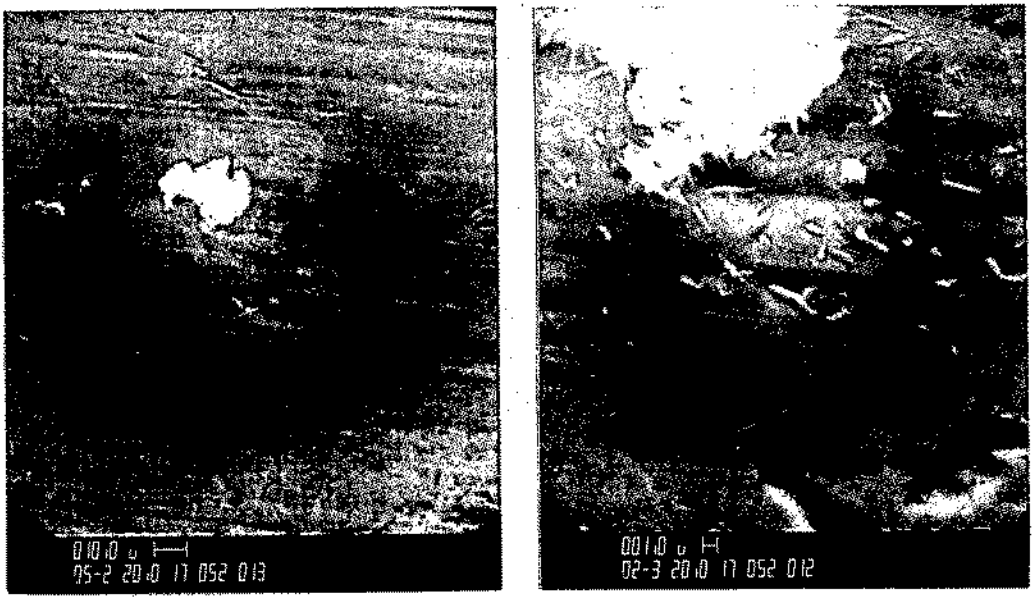

Figure 4 THE CALCIUM-RICH PARTICLES THAT WERE OBSERVED ON THE MEDIUM-SURFACE-ENERGY STEEL PLATES FROM LEG 4 (SEE FIGURE 3d) WERE ALSO PRESENT ON THE LOW-SURFACE-ENERGY PLATES (PICTURED HERE). BUT, NOTE THE RELATIVE ABSENCE OF PARTICLES OVER MOST OF THE TEST SURFACE. LEFT - 500X; RIGHT - 2000X.

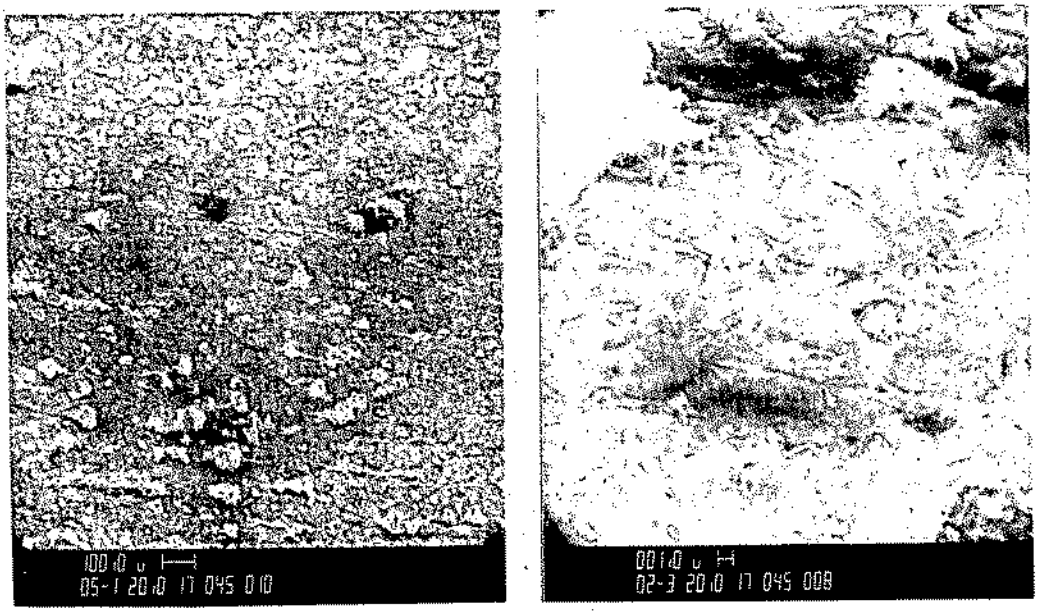

Figure 5 AFTER ADDITIONAL EXPOSURE IN THE FLOW CELL, THE CLUMPS OF PARTICLES ACQUIRED DURING LEG 4 WERE BROKEN APART AND PARTICLE DISTRIBUTION OVER THE LOW ENERGY SURFACE BECAME MORE EVEN. THIS TEST PLATE WAS IN THE "FULL CRUISE" FLOW CELL. LEFT - 50X; RIGHT - 2000X.

Compositions anti-salissures pour des structures immergées fixes

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Abstract

Epoxy based mastics with polyamide hardener are applied on large scale for corrosion protection of submersed marine structures, due to their "water displacement" property.

These mastics, acting on the impermeability of the cross-linked epoxy structure and with the addition of a biocide, are highly efficient as an anti-fouling agent.

Leaching rate of the toxic component is ensured by a diffusion mechanism in the insoluble polymeric matrix through the introduction of slowly reacting substances. As biocides are used organostannic compounds at relatively small amounts clearly inferiors to the average of 25% used in conventional antifouling compounds.

Résumé

Les mastics époxyde à durcissement polyamidique, grâce à leurs propriétés de "water displacement", trouvent une large application dans la protection anti-corrosive de structures marines immergées.
En intervenant sur "imperméabilité" de la structure epoxyde réticulée et en y introduisant un biocide, on a trouvé que ces mastics peuvent exercer même une action anti-salissure remarquable et prolongée.
La lixiviation du composant toxique se fait à travers un mécanisme de diffusion, dans la matrice polymère insoluble, ce mécanisme est rendu possible par l'introduction de substances ayant un caractère réactif lent. Comme biocides, on emploie des composés organostanniques dans des quantités relativement basses, de toute façon nettement inférieurs aux moyennes de 25% en poids employé dans les compositions anti-salissures traditionnelles.

INTRODUCTION

La fixation du fouling sur des structures immergées dans la mer a toujours été, depuis les débuts de l'histoire navale, un problème sérieux et important.
En effet, sur la carène des navires, le fouling provoque une augmentation de la résistance au mouvement avec, comme conséquence, une plus grande dépense en combustible et en abri dans les cales, mais, sur les structures métalliques, en général, il crée également des problèmes concernant une corrosion plus forte.
Malgré certaines interprétations bien connues au sujet de cette dernière assertion, toujours basées sur des expériences faites sur des surfaces limitées et relatives donc à un fouling compact, il suffit de rappeler les essais classiques faits après de notre Comité C.O.I.P.M. (1) et, de façon particulière, ceux qui ont été faits à La Rochelle sur un même acier et une même eau de mer avec et sans fouling, pendant un an où, à part la différence macroscopique de la tôle, on a mis en évidence des pertes de poids de 3,1 g/dm^2 sans fouling contre 12,0 g/dm^2 en présence de fouling (2).
Tout cela a stimulé, au cours de ces dernières années, les recherches sur les peintures et les systèmes antifouling en général pour attendre des protections efficaces pendant des temps longs. De plus, le problème a impliqué même le premier voile biologique (slime) aussi bien pour sa propre résistance au mouvement de la carène que pour le conditionnement qu'il crée sur la surface immergée à l'égard de la fixation successive du macrofouling et à l'égard de ces mêmes caractéristiques électrochimiques pour des supports métalliques déterminés (aciers inoxydables par ex.) (3).
Enfin, il y a un dernier aspect du problème relatif à la pollution de l'écosystème qui imposerait l'exclusion des substances toxiques dans les compositions antifouling, ou du moins la réduction au minimum de leur diffusion dans le milieu acqueux comme telles. Selon cette ligne de recherche, la protection tend à devenir, en même temps anti-corrosive et antisalissure.
Les études faites, au course de cette dernière dix ans, ont porté, en effet, à la réalisation de compositions qui, tout en étant exemptes de toute espèce de substance biocide (y compris les composés de l'étain et du cuivre) sont également capables de maintenir la surface immergée libre de fouling et de slime grâce à un état intrinsèque particulier ayant une énergie libre superficielle minimum (4).
On a atteint ces résultats en observant des états naturels tels que ceux des parois intérieures des artères et de la muqueuse buccale, de la peau de certains mammifères marins (orque marine et marsouin) et des plumes des oiseaux aquatiques (5). Même dans ces cas, il y a une manifestation d'états d'énergie libre superficielle minimum, où cesse toute adhérence et, de façon typique, le ciment de la base des

balanes ne fait pas prise (4).
Il existe, cependant, un secteur particulier, tel que celui des structures immergées fixes (plate-formes off-shore, sea lines, poteaux etc.) où le problème du fouling n'est, ni ne peut être résolu par ce qui vient d'être dit, puisqu'une fois immergées, la réfection anti-salissure n'est plus prévue.
Ces structures, en effet, pour lesquelles on peut retenir comme résolu, du moins virtuellement, le problème de la protection anti-corrosive, sont encore soumises, au moins deux fois par an, à des raclages mécaniques faits par des plongeurs pour éviter que l'impact de la houle puisse provoquer des dégâts irréparables à cause de la grande augmentation crée par le fouling même. La résolution du problème anti-corrosion a été obtenue en employant des méthodes de protection active (anodes sacrificielles ou systèmes à courant imprimé) intégrées avec des enduits anti-corrosion du type "barrière" ayant une imperméabilité élevée ou avec des enduits ayant une très grande épaisseur (6) surtout pour les parties proches de la ligne d'eau sujettes à l'alternance de la houle et des marées (splash zone).
Parmi ces derniers enduits, il faut regarder avec une attention particulière les mastics époxydes expressément étudiés et formulés pour des applications sous-marines (7).
Cette recherche a voulu prendre ces mastics comme point de départ pour en obtenir, grâce à d'opportunes modifications, un système anti-fouling efficace dans le temps, en maintenant les propriétés d'application en immersion.

Recherches expérimentales et résultats

Les mastics époxydes, dont les premiers résultats en milieu marin en tant que protecteurs anti-corrosion ont été obtenus par Jorda en 1963 (8), présentent deux prérogatives particulières et précisément les propriétés polaires de grande adhérence au support avec déplacement complet de la phase aqueuse (Water displacement) à l'interface avec le support (9) et les propriétés rhéologiques qui les rendent applicables même dans de grandes épaisseurs sans risques de crevasses et retraits sur la couche finie.
Leur application, prévue sur des supports sablés, est faite à la main après le mélange de la peinture avec le produit d'addition.
La facilité d'application et de retouche les rend, quand la réticulation est terminée, une base possible d'accrochage pour d'éventuels cycles anti-fouling dans les cas où la structure peut être maintenue pendant assez longtemps hors de l'eau. Mais, là où cela n'est pas possible, le problème de la protection anti-fouling des structures fixes immergées, présente, comme nous l'avons dit, des remarquables difficultés n'ayant pas une solution pratique facile même si la fixation d'organismes incrustants devient moins critique que dans le cas

des carènes des bateaux où il est nécessaire de maintenir minimes les coefficients de frottements.
Actuellement, on se limite, pour ces structures, on le répète, à des opérations de nettoyage mécanique malheureusement coûteuses pour conjurer les dommages que la houle peut provoquer aux installations.
A la lumière de cette situation, on a retenu remarquablement intéressant du point de vue pratique examiner la possibilité de rendre antisalissure les mastics époxydes sous-marins avec une adjonction de biocides sans en altérer les susdites caractéristiques physiques et rhéologiques.
Les caractéristiques d'imperméabilité et d'insolubilité de la matrice époxyde réticulée déconseillaient déjà, au départ, de recourir à la simple dispersion d'un biocide dans le mastic époxyde sous-marin, même si à des concentrations tellement élevées qu'elles constituent un contact continu entre les particules (10); tout en tenant compte du fait qu'une résine époxyde est en mesure d'occlure des pourcentages élevés de substances étrangères (11). Des essais préliminaires dans ce sens ont, en effet, donné des résultats négatifs après peu de mois d'immersion.
Au contraire, on a pu constater comment, en intervenant sur l'imperméabilité de la structure réticulée grâce a l'introduction d'un biocide dispersé dans une phase réactive à l'égard de l'eau de mer et comme telle, capable de devenir, lentement, hydroperméable, il était possible d'arriver à une composition antisalissure efficace. On pense que le mécanisme d'action peut être supposé ainsi: le mélange d'un mastic époxyde sous-marin avec une substance hydroperméable sous la forme de minuscules petits canaux qui permettraient une pénétration lente de l'eau vers l'intérieur, favoriserait même la lixiviation du biocide qui, par diffusion, attendrait l'extérieur à travers un véritable taux de lixiviation. Ce taux de lixiviation serait donc lié au mécanisme de lente lixiviation du toxique qui, contrairement à ce qui arrive dans les peintures anti-salissure n'intéresserait qu'une phase réactive hydroperméable dispersée dans toute l'épaisseur du mastic. Un modèle de lixiviation du toxique est schématisé à la figure 1.
Le biocide introduit dans le véhicule réactif hydroperméable est constitué par des composés organostanniques du type R_3SnX (*) qui, au contact de l'eau de mer, subissent une dégradation (12) en formes ioniques hydratées actives $(R_3Sn(H_2O)_2)^+$.
Le choix de ces composés a été dicté par leur efficacité biocide sur la quasi-totalité des organismes du fouling (14,15,16) et par

(*) R= phényle ou butyle; X = Cl,F,OH,OAc. - La toxicité de ces composés ne dépend que de la nature du radical organique, tandis que le groupe X aurait en effet modeste (13).

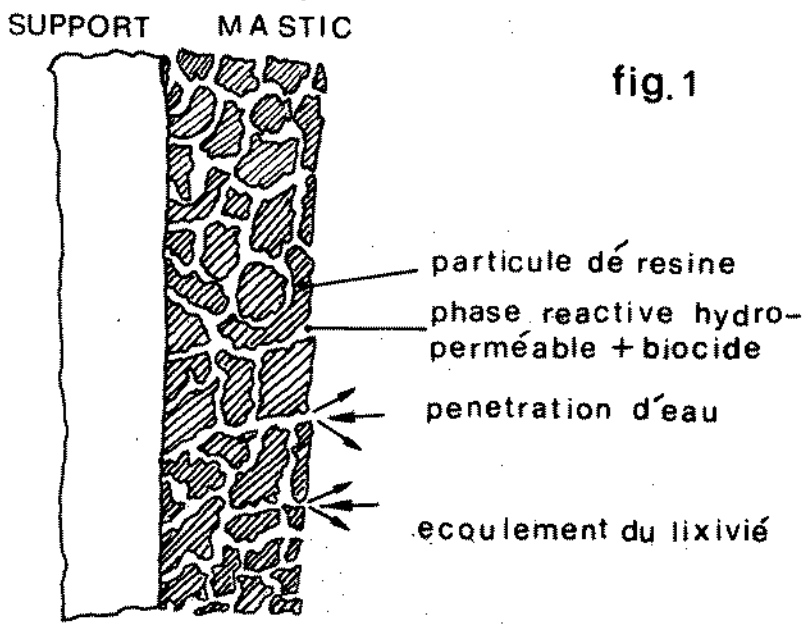


fig. 1

leur inaltérabilité en la présence d'éventuels systèmes de protection cathodique comme cela est confirmé par de récents essais de champ à cet égard.

Dans cette recherche, on a préféré les composés triphénilyques car ils manifestent des valeurs de solubilité dans l'eau de mer inférieures à celles des composés tributilyque. On passe, en effet, d'une valeur de la solubilité de 6 et de 8-10 ppm respectivement pour l'oxyde et pour le fluorure de trybutil-étain (17) à une valeur de 1 ppm pour le fluorure de triphenyl-étain (18).

Le choix des composés organostanniques entre la gamme possible de composés biocides employés dans les compositions anti-fouling nous a été conseillé non seulement par leur ample spectre d'éfficacité à des concentrations relativement basses sur les organismes du fouling mais aussi du fait que, dans des laps de temps plus ou moins longs sous l'action de l'eau de mer vivante, des rayons ultra-violets et des micro-organismes, ils subissent une dégradation jusqu'à des dérivés inorganiques de l'étain (atoxyques) selon le schéma proposé par Smith (13) fig. 2.

Il faut encore observer que la toxicité de ces produits dans le pourcentages que l'on retrouve dans le compositions anti-salissures, est extrêmement bas au niveau humain et limité au seul risque de contact avec les yeux ou avec la peau (19).

Nos essais de champ ont été faits auprès de la Station Expérimentale

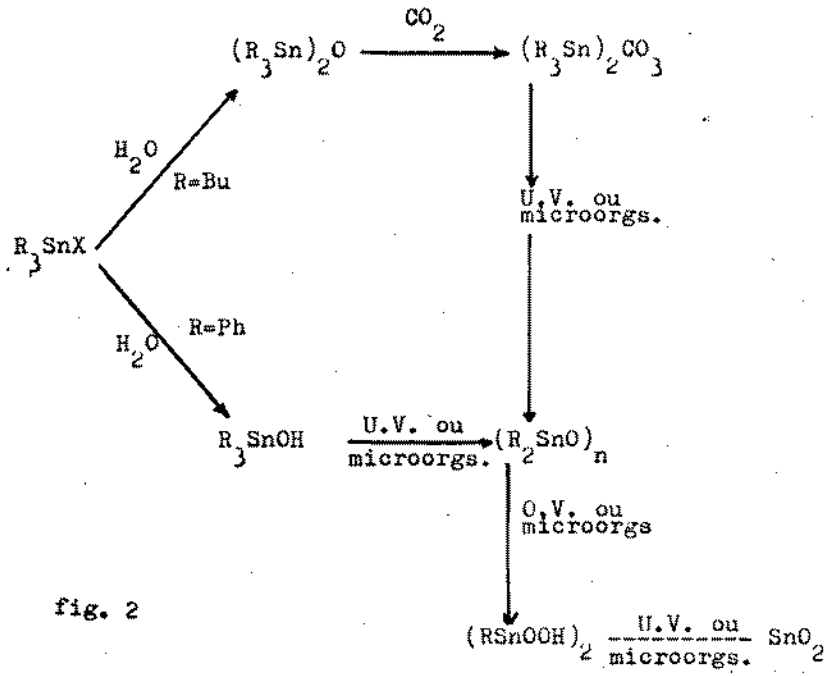


fig. 2

Schema de dégradation des composés organostanniques selon Smith.

de notre Institut située actuellement à l'embouchure de levant de 14 Avamport de Gênes où, dans le nappe d'eau donnant sur la Station même, sont amarrés les radeaux expérimentaux.
Les conditions de la mer comme mouvement et comme propriété des eaux sont beaucoup plus aptes à ces genres d'expériences que les eaux intérieures.
Les principales caractéristiques hydrologiques et biologiques sont reportées dans le Tableau I - II.

TABLEAU I

CARACTÉRISTIQUES PHYSICO-CHIMIQUES DE L'EAU DE MER

Salinité (°/₀₀)	= 37,76
Oxygène dissous (q)	= 6,64 ml/litre
Poids spécifique (σ_{20})	= 28,95
Conductibilité électrique	= $5{,}33 \cdot 10^{-4}$ µS/cm
Concentration ions hydrogène (pH)	= 8,2
Alcalinité	= 2,604 milliéquivalent g/litre
Anhydride carbonique totale	= 2,297 m Mol/litre

TABLEAU II

ESPÈCES SESSILES DOMINANTES
DANS LE «FOULING» DU PORT DE GÊNES

Spongiaires :	Sycon raphanus (O. Sch. m.) Lewconia crambessa (Heck.)
Hydraires :	Tubularia mesembrianthemum (Allman) Eudendrium racemosum (L.) Ventromа halecioides (Ald.)
Plathelminthes :	Thysanozoon brocchii (Grube)
Bryozoaires :	Bugula avicularia (L.) Zoobothryon verticillatum (D. Ch.) Pedicellina cernua (Pall.) Membranipora sp.
Polichètes :	Hydroides norvegica (Gunn.) Spirorbis laevis (Quatr.) Sabella pavonina (Savigny) Polydora ciliata (Johnst.) Spirographis spallanzanii (Viviani)
Mollusques :	Mytilus galloprovincialis (Lam.) Ostrea edulis (L.)
Cirripedes :	Balanus trigonus (Darw.) Balanus amphitrite (Darw.) Balanus eburneus (Gould.) Balanus perforatus (Brug.)
Tuniciers :	Ciona intestinalis (L.) Styela plicata (Les.) Didemnum maculosum (M. Edw.) Botryllus schlosseri (Pall.)

Le mastics anti-salissures, après avoir passé l'étude et les contrôles aussi bien physiques que biologiques faits au laboratoire, étaient étalés, en immersion, en couches de 300 à 500 microns sur des supports sablés ou, déjà préliminairement, traités avec des primers zinguants époxydes ou époxy poliuréthanes avec des épaisseurs maximum de 40-50 microns.

Toutefois, pour les expériences à long terme, on a préféré employer comme support les panneaux en amiante ciment précomprimé du type autoclavé qui se démontra tout à fait compatible avec les mastics mêmes, permettant ainsi de dépasser, dans ces essais à long terme, le problème anti-corrosion, résolu de façon différente dans les cas pratiques d'installations off-shore, sea lines, etc.

Les panneaux étaient, de toute façon, installés sur opportunes structures rigides fixées à 150 cm de profondeur. Ces mêmes panneaux d'amiante-ciment servaient de panneaux atoxiques de contrôle pour la fixation des organismes du fouling au cours des temps.

Les compositions ont été préparées en prenant comme base una résine époxy du type "Epikote 1001" opportunément épaissie avec une charge inorganique et en employant comme durcissant une résine polyamidique. La phase hydroperméable et à lente réactivité avec l'eau marine était

additionnée avec le biocide (tryphényle substitué) et tout était incorporé dans la résine époxy pendant le mélange des deux composants de base (résine + durcissant) en des proportions comprises entre 40% et 65%.
Le biocide résulte, par rapport à la composition totale, dans des proportions variables entre 12% et 16% en poids.
Au tableau III, on reporte 5 des compositions qui ont fourni les résultats les plus significatifs. Le biocide est exprimé comme étain.

Pourcentage en poids de biocide (exprimé comme étain) et de phase hydroperméable présents dans les 5 compositions qui ont fourni les résultats les plus significatifs.

Tableau III	% Sn	% phase hydroperméable	% mastic
Composition 1	3,86	50	46,14
" 2	4,50	55	40,50
" 3	5,14	50	44,86
" 4	5,14	60	34,86
" 5	3,86	60	36,14

Les essais physiques préliminaires de contrôle pour chaque type de composition étaient référées, tout d'abord, à la capacité de réticulation et d'adhérence au support mouillé par l'eau de mer, en exécutant sur les échantillons les essais prévus par les normes ASTM (20). Ce n'est que lorsque ces essais résultaient veritables que l'on contrôlait l'absence de crevasses et de retraits sur des épaisseurs plus grandes.
Il a été, ainsi, possible d'arriver à délimiter les pourcentages les plus corrects de phase hydroperméable et de biocide par rapport au mastic, en écartant aussi bien les valeurs trop basses (inférieures à 30%-35%) que les trop élevées (supérieures à 60%-65%).
En effet, dans le deux cas, on rencontrait defauts ou excès sur le taux de lixiviation contrôlé avec des méthodes réalisées au loboratoire (21-22) ainsi que des anomalies sur le modèle physique supposé, ce qui, dans le deux cas, donnait lieu à des compositions avec un effet anti-salissure limité dans le temps.
Certains essais biologiques préliminaires ont été, également, étudiés et mis au point dans notre laboratoire, ce qui a permis d'arriver à des conclusions intéressantes sur le mécanisme anti-fouling possible de ces compositions.
Ces essais étaient réalisés dans des conteneurs particuliers où le fond et les parois étaient partiellement enduits avec les compositions d'experimentation et où, dans l'eau de mer coulante, on pla-

çait soit des pediveligers de Mitilus gallo-provincialis ou des trocophoras de Hidroides elegans ou même de petites moules ayant une longueur de 2-5 mm qui, comme on le sait, peuvent bouger sur un support.
Ainsi, a-t-il été possible de mettre en évidence une action répulsive typique soit sur les larves que sur les petits individus adultes de la part des mélanges biocide-phase hydroperméable.
Sur les panneaux en essai de champ depuis désormais 36 mois, on exécutait, périodiquement, en plus des contrôles visuels et photographiques dont aux figures 3, 4, 5, 6, quelques contrôles analytiques sur la quantitè d'étain résidu présent sur de petits fragments de mastic prélevés de façon opportune sur les panneaux servant pour l'experience.
La quantité de biocide encore présente, aux diverses echéances, dans les 5 compositions type du tableau III, déterminée et exprimées comme étain, sont reportées dans le graphique de la fig. 7.

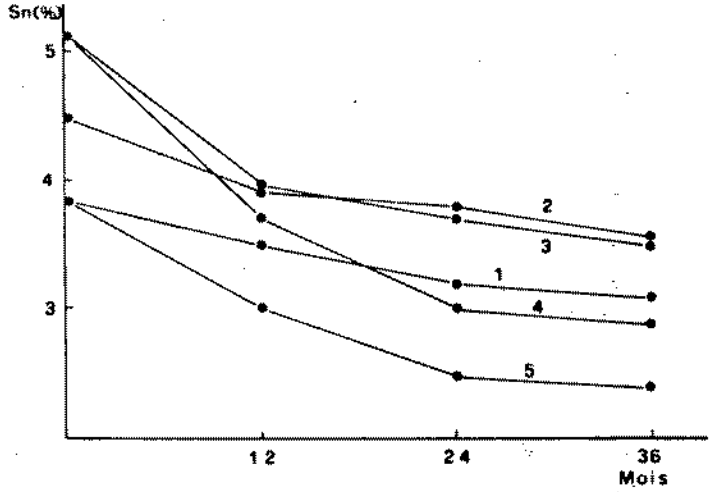


Fig. 7 - Pourcentage de biocide résidu, exprimé comme étain, dans 5 compositions antisalissures.

D'après une analyse de ce graphique, on peut mettre en évidence ce qui suit:
1) La vitesse de lixiviation du biocide est liée au pourcentage de phase hydroperméable présente dans la composition: lente et régulière pour le compositions où cette phase est comprise entre 50% et 55% (voir tableau III,compositions 1, 2, 3); plus rapide et marquée pour les compositions à 60% (Tableau III, formulations 4 et 5).
2) Quand le pourcentage de biocide devient inférieur à 3%, correspondant à 8% - 9% de composé triphényle substitué, la composition commen

ce à perdre son efficacité antisalissure.
Parmi les techniques que l'on peut employer en plus des recherches analytiques normales, on a jugé opportun de s'en tenir à la spectroscopie par fluorescenze de rayons X (EDS).
Cette enquête avait lieu sur d'opportunes sections transversales d'échantillons en obtenant l'image à electrons secondaires émis par l'échantillon lui-même frappé par un faisceau d'électrons primaires accélérés à 25 KV. En sélectionnant dans le multi-canaux la ligne $L\alpha$, caractéristique de l'étain, on a pu visualiser dans la figure 8, la distribution de celui-ci dans un petit section représentée par la figure 9.
Il est evident (relativement une composition (F2)) une certaine raréfaction de l'élément dans les zones proches de la surface (à droite dans la photographie). Une confirmation est également, mise in évidence par les données analytiques reportées au Tableau IV.

TABLEAU IV: % d'étain reporté à % de silicium, terme de comparation au moyen de EDS sur la zone de la fig. 8.

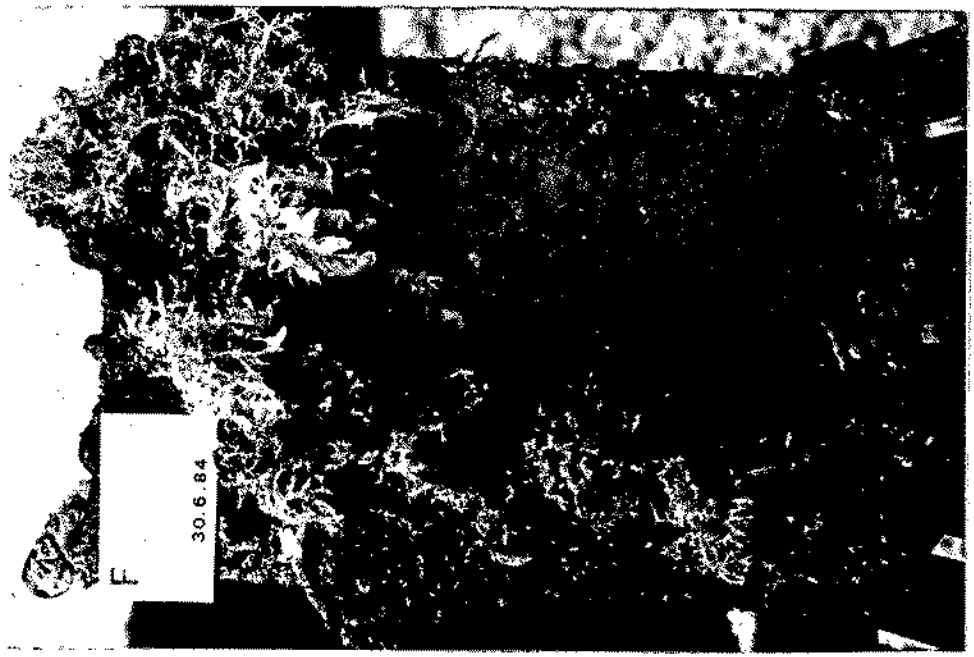
Zone intérieure	Zone Intermédiaire	Zone Extérieure
Si % 8.388	Si % 57.744	Si % 63.087
Sn % 91.611	Sn % 42.255	Sn % 36.917

Conclusions.

Comme cela est mis en évidence par le graphique de la figure n. 7 et par la documentation photographique, les résultats des essais, après 36 mois d'immersion dans l'eau de mer, apparaissent plus que satisfaisants. Les limites normales des durée des compositions antisalissures formulées par les techniques traditionelles ont désormais été dépassées avec l'avantage d'avoir réduit à un maximum de 16% la quantitè de biocide - dans les normales compositions antisalissures , le pourcentage d'organostannique à une valeur d'environ 25% (23) - et d'avoir à diposition un produit qui peut être appliqué sur des supports qui sont déjà en immersion; ici, quànd la composition aura épuisé sa toxicité, il est facile de superposer de nouvelles couches.

Nous tenons a rémercier ici M.me Maria Laura SCOTTI et M.me Silvana FRANCO pour la collaboration donnée à la realisation de ce travail.

Fig. 3 - 4: Compositions F1, F2 à 36 mois d'immersion.



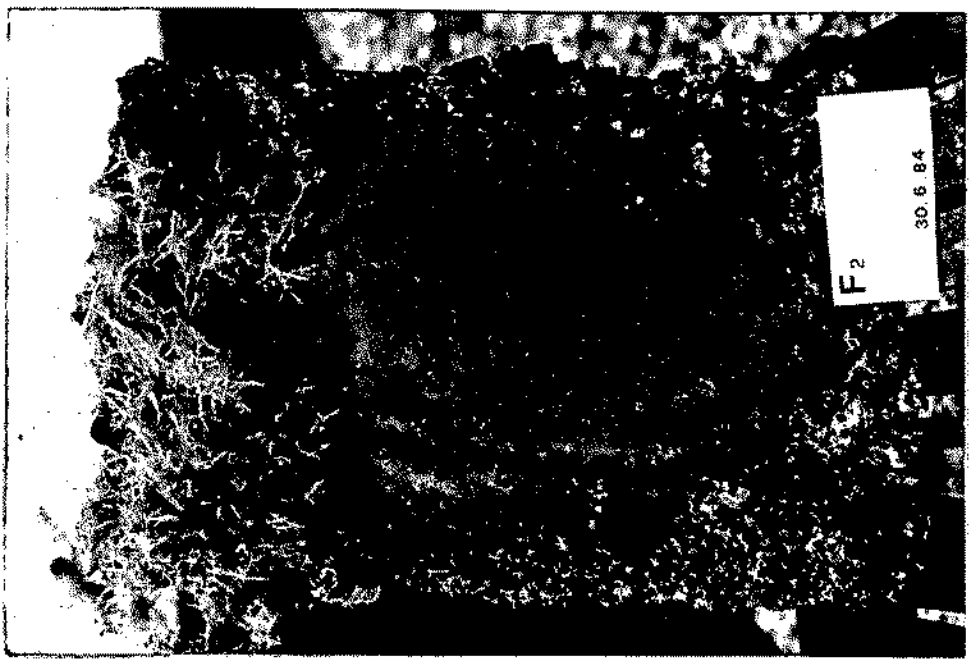


Fig. 5-6 : Compositions F3 à 36 mois d'immersion et relatif contrôle atoxique.

Fig. 8: Images à électrons secondaires d'une section de mastic anti salissure.

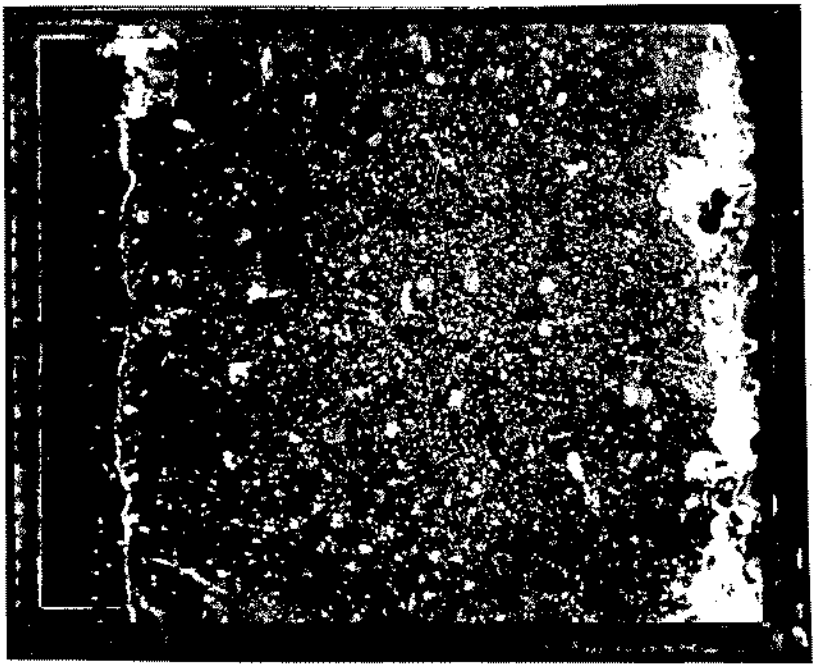
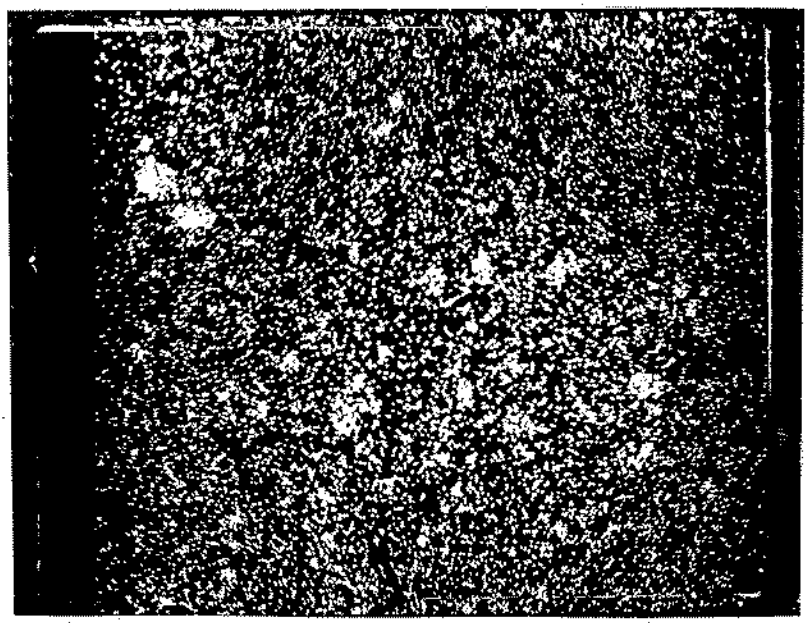


Fig. 9: Distribution de l'étain dans la zone représentée par la figure n. 8.



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