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SESSION II Marine biology
Tuesday 8th November

MUSSELS IN THE OFFSHORE FOULING OF ITALIAN SEAS
Giulio Belini and Nanuela Montanari

INTERACTIONS WITHIN FOULING BIOFILMS: STUDIES USING A MODEL SYSTEM
K E Cooksey and R E Murray

LAKE TIMSAH AS A BARRIER FOR THE SERPULOID (TUBE WORMS) MIGRATION ALONG THE SUEZ CANAL
A F A Ghobashy, I M Shalaby and S H Shalla

THE BIOFOULING COMMUNITIES NO THE PIER PILLING AT XIAMEN, CHINA
Z G Huang and R X Cai

TOWARDS THE STANDARDISATION OF THE TEST EXPOSURE PROCEDURE IN THE TROPICAL SEA
A A Karande

FOULING OF SEAWATER FILTRATION SYSTEMS OF OFFSHORE OIL PRODUCTION PLATFORMS
R G J Edyvean and J L Lynch

ECOLOGY OF FOULING COMMUNITIES AND THEIR IMPACT ON MARINE WOOD BORING ORGANISMS IN PORTONOV COASTAL AREA - SOUTH EAST COAST OF INDIA
R Radhakrishanan and R Natarajan

MICRO-ORGANISMS CANNOT SETTLE SUCCESSFULLY ON THE SURFACE OF THE SPONGE *HALICHONDRIA PANICEA*
B Wolfrath and D Barthel

SELECTION OF ANTI-FOULING MATERIALS AND ITS ANTI-FOULING MECHANISM
Haruo Shimada

DIVERSITY AND SEASONALITY OF MICROBIAL AGGREGATION-ADHESION ENHANCING (AAE) BIOFOULING MACROMOLECULES IN COASTAL SEAWATER

T R Tosteson

AN ACCOUNT OF FRICTIONAL RESISTANCE INCURRED ON OCEAN GOING RESEARCH VESSELL ORV SAGAR KANYA DUE TO FOULING

A B Wagh and K Nandakumar

MUSSELS IN THE OFFSHORE FOULING OF ITALIAN SEAS

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ABSTRACT

Fouling of some offshore platforms situated in the North Adriatic, Ionian and North Tyrrhenian Seas was investigated on the basis of direct observations, sampling and panels immersed for periods of 1 to 12 months. UP to 10 m depth, Mussels (*Mytilus galloprovincialis* Lam.) are the main fouling organism on all offshore structures examined and they represent 80 to 95% of the wet weight of total fouling.

In the Adriatic Sea Mussels after one year can reach a shell length of 6-7 cm and a wet weight of 90 kg/m^2; in the Ionian Sea the values are 3.5 cm and 6 kg/m^2, in the Tyrrhenian Sea 6.2 cm and 26 kg/m^2.

INTRODUCTION

The increasingly pressing need for information on the fouling of offshore structures in the Mediterranean Sea led us to publish a series of data gathered about ten years ago, but which are still relevant to the present environmental situation.

We focused our attention on Mussels because these Bivalves represent the principal and dominant component within the first ten meters of depth of the fouling on the various structures examined.

This paper also completes a series of publications concerning a vast area of research carried out by the Istituto per la Corrosione Marina dei Metalli for AGIP, and it relates to a series of observations of fouling made at several platforms producing natural gas. In fact, after a first general study (Relini et al. 1976) a series of papers was produced on the most important systematic groups i.e.: Barnacles (Relini & Matricardi 1977), Hydroids (Montanari & Morri 1977), Amphipods and Polychaetes (Relini et al. 1977) but not Molluscs.

On the whole, the detailed literature available on the fouling of offshore structures for the seas surrounding Italy is rather limited and is made up of works by Relini (1975, 1976) based on his observations carried out at 200 m depth in the Ligurian Sea, that of Ardizzone et al. (1980) off the coast at Fiumicino (the Tyrrhenian Sea), De Palma (1963) (Southern Sardinia), Dalla Venezia (Venice lagoon, 1976, off the coast of Ancona 1981).

With regard to the general problems of the effects of fouling on offshore structures, we make reference to De Palma (1967) and Edyvean et al. (1987), Marine fouling of offshore structures (1981).

MATERIALS AND METHODS

The observations were carried out on offshore platforms situated in the North Adriatic Sea (Ravenna), the Ionian Sea (Crotone) and the upper part of the Tyrrhenian Sea (the Ligurian Sea, at Genoa) by means of samples and survey photography.

The environmental characteristics and the experimental conditions are shown on Table 1, while Table 2 shows the hydrological parameters. A more complete study was made at Ravenna and Crotone where asbestos cement panels (200x300x4mm) were used and were immersed for graduated periods of time (1,3,6,9,12 months) in special structures placed at different depths. Each structure was fastened to a ballast cable which brought together seven panels positioned radially with the longest side in a vertical position, making a total of 120 panels at Ravenna and 80 at Crotone. For further information about the immersion techniques and the characteristics of fouling at Ravenna and Crotone see Relini et al. (1976).

The fouling and the Mussels were quantified in terms of density values (numbers of individuals/dm^2) and wet weight. The Mussels were also subdivided into size classes (model 1 cm) based on shell length.

RESULTS

1) The Adriatic Sea

The data that is available for the Adriatic Sea was collected off the coast at Ravenna on two platforms named AGO-A and PCW-A.

a) The settlement of Mussels

The descriptions supplied of the settlement relate to panels situated at various levels of depth for periods of one month, three months, six months and one year.

Platform AGO-A (Table 3)

- One-month panels - The panels that remained immersed for the month of June at three different levels show the maximum number of monthly presences (522 individuals/dm^2), corresponding to 97% of the monthly settlement ascertained during the year. The maximum settlement of Mussels (93%) was located at the first level (surface zone), while the remaining percentage was recorded at the second level (-9m). At the third level (-20m) only sporadic presence was noted. Regarding the dimensions of the individuals, it was noted that the length of the shell never exceeded 5 mm.

- Three-month panels - The total number of Mussels settled on the three-month panels was about 4 times higher than that found on the one-month panels. The panels with the highest settlement were those immersed from May to July: 1869 individuals/dm^2 at the first level and 246/dm^2 at the second level. A moderate number of individuals (59/dm^2) was also ascertained on a panel immersed at the first level in the months from November to January. On the remaining panels the values were extremely moderate and in particular the third level settlement was always very low. The valves of the individuals found reached maximum lengths of 30 mm. Nevertheless, the most representative class was the first (10 mm), which was also the only one present at 20 m depth.

- Six-month panels - The first-level panel which remained immersed from May to October showed a higher settlement compared to the one which remained immersed from October to April (2462 individuals/dm^2 vs. 115) and contained Mussels which reached the fourth class. It is to be noted that the total settlement on the two six-month panels is about six times higher for the first level than for the second level (2577 individuals/dm^2 vs. 430).

- One-year panels - Among the panels that remained immersed for the entire annual cycle, the one with the most settlement was that which was situated near the surface (first level) with 9724 individuals/dm^2. Among these were found individuals up to the sixth class, even if 95% of the population belonged to the first class. The maximum size (70 mm) was reached only at the second level (-9m) where there were also the most individuals of the sixth class.

Platform PCW-A (Table 4)

- One-month panels - The settlement of Mussels occured in two periods during the year: from May to September with a peak in June (375 individuals/dm^2), which corresponds to about 86% of the monthly settlement ascertained during the year; and from October to January with a settlement density which was much lower. Also at this station the panels immersed near the surface (first level) had the highest settlement (79.5%), those situated at -5m (second level) still showed a certain degree of settlement (20%) while those at -11m (third level) were very thinly populated.

- Three-month panels - Also at this platform the panels with the most settlement were those immersed in the period from May to July: (1500 individuals/dm^2 at the first level, 763 at the second level). With regard to the panels exposed from November to January, the number of individuals observed was considerably higher compared to the AGO-A platform: 747 individuals/dm^2 at the first level and 291/dm^2 at the second level. With regard to the sizes of the individuals, only on the panels immer-

sed from May to July were Mussels found belonging to the third and fourth class of length.

- Six-month panels - In contrast to the difference noted for AGO-A, at this platform the panel immersed in the second period of six months had a higher population (3688 individuals/dm^2) than that immersed in the first semester (2344/dm^2). The same phenomenon was seen even more markedly on the six-month panels immersed at the second level. These observations confirm the existence of two distinct periods of Mussels settlement. It must be emphasized, however, that the maximum sizes are reached only by those Mussels which settled on the panel immersed in the first period of six months.

- One-year panels - First of all, it is to be noted that the number of individuals found on the panel which remained immersed for one year at the first level of PCW-A (4999 individuals/dm^2) does not correspond to reality since a part of the organisms became detached from the panel during the with-drawal of the samples. On the panel immersed at the second level (-5m), 3069 individuals/dm^2 were found, which was greater than for the panel immersed at the second level (-9m) of AGO-A. The individuals reached a maximum size (seventh class) only at the second level, at which a higher number of individuals of the sixth class was also observed.

b) The role of Mussels in the determination of the Biomass in Fouling

Table 5, which reportes the wet weight in relation to the entire surface of the panel (12 dm^2), shows that the values of the biomass reflect an increase in the accumulation of fouling with time. In fact, the highest weights (i.e. above 10 kg) were recorded on panels positioned at the first level of the platform AGO-A and on the first and second levels of the platform PCW-A after 12 months of exposure.

The diminution of fouling with depth is connected to the fact that the Mussels are present at the first two levels and predominate especially on the multi-month panels, where they form compact layers of considerable thickness, while on the substratum placed near the sea bottom the majority of organisms found were: Barnacles, Serpulids and Hydroids. These, however, because of their quantity and structure, had little influence on weight (Fig. 1).

With the increase in the period of immersion there was also an increase in the accumulation of fouling and thus of weight; this can be demonstrated by comparing the values for the fouling weight settled on the panels immersed for a longer period with those found on panels immersed for only one month.

Taking as an example the three-month panel immersed at the surface of AGO-A from May to July, we note that it had a fouling weight equal to 795 g, while the corresponding one-month panels immersed in May, June and July respectively yielded a total weight of 315 g (5+170+140). As can be seen, this value is lower by half than that provided by the corresponding three-month panel, even though the surface available for settlement was renewed three times.

This observation can be made each time we compare the weight provided by a six-month or one-year panel with that provided by the sum of substrata that remained immersed at the same period but for a shorter time. In conclusion, there does not seem to be a substantial difference between the two platforms in the accumulation of fouling on the panels partially immersed at the first level, while at the other levels the differences are considerably more because the depths of the two platforms are different in relation to the position of the sea bottom.

This is particularly evident for the one-year panels for which, transposing the values of Table 5 (which are expressed in grams per panel = 12 dm^2) into kg/m^2, the following values were obtained:

AGO-A surface	PCW-A surface	PCW-A - 5 m	AGO-A - 9 m	PCW-A - 11 m	AGO -A - 20 m
99.3 kg/m^2	90.9 kg/m^2	88.3 kg/m^2	49.5 kg/m^2	20.2 kg/m^2	5.3 kg/m^2

Examining the importance of Mussels in the formation of the biomass, it can be observed that it increases with the duration of the immersion, and this is especially true for the panels that remained immersed during the summer months.

Table 6 shows (for the multi-month panels only) both the total fouling weight and the weight of the Mussels found; in this way it was possible to calculate the share of the organisms in the formation of the biomass which, in the case of the 9 and 12 month panels partially immersed or placed at the intermediate depth, could reach over 90%.

As already reported for the wet weight, the Mussel biomass was almost equal on the partially immersed substratum of the two platforms, while it was substantially different at the other depths.

If we express the weight of the Mussels in relation to the annual panels in kg/m^2, we obtain the following values:

AGO-A surface	PCW-A surface	PCW-A - 5 m	AGO-A - 9 m	PCW-A - 11 m	AGO-A - 20 m
96.6 kg/m^2	86.6 kg/m^2	84.2 kg/m^2	40 kg/m^2	11.6 kg/m^2	4.9 kg/m^2

The above values show how the weight of the Mussels is similar up to -5m while it is reduced twofold times at -9 m, eightfold times at -11 m, eighteenfold times at -20 m.

2) The Ionian Sea

The data available for the Ionian Sea was collected off the coast at Crotone on a platform named "LUNA-A". The descriptions of the settlement refer to one-month, three-month, six-month, nine-month and one-year panels immersed at various depths (Table 7).

a) The settlement of Mussels

Platform LUNA-A

- One-month panels - The presence of Molluscs was somewhat low, in particular the Mussels appeared only at the first and second levels during the Spring months.

- Three-month panels - Settlement was still scarce and only on the panel immersed from April to June were appreciable numbers of Mussels counted at the first level (37 individuals/dm^2); at the second and third level a lower number of Mussels was found accompanied by other Molluscs.

- Six-month panels - Only the panals of the second six-month period showed a considerable amount of settlament. It was especially Mussels which enriched the first level with 105 individuals/dm^2. At the second level there were still some Mussels (61/dm^2), while at the third level the settlement of Molluscs and Mussels in particular was noticeably more scarce.

- Nine-month panels - At the first level the settlement of Mussels comprised 11 individuals/dm^2. At the second (-14 m) and third (-20 m) levels the presence of Mussels could still be noted even if in smaller amounts that at the first level (0 m). At the fourth level (- 65 m) *Mytilus* were absent.

- One-year panels - An appreciable settlement of Mussels was recorded only at the first level with 379 individuals/dm^2, the length of whose valves reached 35 mm. At the second level the Mussels were 93/dm^2 and at the level three 25/dm^2. At the fourth level the panel was completely covered with *Pionodonta* in which the individuals reached a size of up to 40 mm in diameter. On the whole, the largest development of Molluscs was found at the first level with seven species amounting to a total of 596 individuals/dm^2 of which about 90% were *Mytilus galloprovincialis*. At the second level there were nine species of Molluscs, with 298/dm^2, of which 90% consisted of *Mytilus* and *Saxicava* in equal numbers. At the third level the number of Molluscs was still fewer (105 individuals/dm^2) and consisted of 55% of *Saxicava* and 26% of *Mytilus*. The fourth level showed the dominance of *Pycnodonta*, with no Mussels.

b) The role of Mussels in the determination of the Biomass in Fouling

Table 8 reports the wet weights (in grams) with reference to the biomass of the entire surface of the panel. The values of the weights are also reported in the diagrams illustrating the dominant communities on the multi-month panels (Fig. 1). These allow us to make an immediate comparison between the surfsces of the panel occupied by the fouling and the wet weight of the same.

The examination of the monthly weights demonstrates that the greatest weights were reached at the different experimental depths in the months from March to September, even if the monthly settlement was always very scarce. In fact, adding together all the monthly weights obtained at Crotone at different depths, the total of 215 g is reached; this value was lower than that reported in only one month at Ravenna (August, PCH-A, first level = 300 g/panel). On the three-month panels of the platform at Crotone the weight values of the fouling reached a maximum of 106 g per panel while at Ravenna the maximum was 2140 g/panel. For the six-month panels, it was noted at the two intermediate levels the maximum accumulation was reached in the first period, while at the first level the highest weights were in the second period. The values at the fourth level of fouling weight were similar for both periods. The panels immersed for 9 months showed the greatest weights at the two intermediate levels, while the one-year panels showed equally high weights at the first three levels.

Regarding the increase of the fouling weight, there was an increase in weight as the period of immersion continued at all levels. For example, if we consider the panels at the first level immersed in June and which remained for increasingly long periods of time, the following weight increase was observed (Fig. 1): the one-month panel = 1 g; the three-month panel = 6 g; the six-month panel = 18 g; the nine-month panel = 166 g; the one-year panel = 816 g.

As already reported for the platform at Ravenna, also at Crotone the fouling weight on a substratum immersed for a long period of time was always higher than the sum of weights reached in the corresponding single months or groups of months. Bearing in mind the reduced total number of Mussels present at Crotone, the wet fouling weight was hardly influenced by these bivalves.

The influence of the Mussels on weight was only at the first level for panels immersed for nine months or more; in particular, for the one-year panel out of 816 grams of fouling, 492 grams were made up of Mussels (60.2%). Calculating these values in kg/m^2 the following results are obtained: 6.8 kg/m^2 (fouling) 4.1 kg/m^2 (mussels).

3) The Tyrrhenian Sea

Fouling was examined at the landing platform (oil pipe terminal) for super oiltankers situated offshore at the port of Genova-Multedo. After obtaining appropriate photographic documentation, samples were taken. Specifically they were gathered from the girders on a surface of 30x40 cm at various depths, up to 34 m, a level which, according to the divers, was the lowest limit for the presence of Mussels.

The fouling communities at various levels are described, while all the data concerning the Mussels is collated in Table 9.

- Level 0 m - Near the surface (that is the area which is partly affected by the time), the fouling was characterized by the dominance of Mussels (1059 individuals) of sizes between 17 and 62 mm in shell length and *Balanus perforatus*. Other organisms were rather scarce.
- Level -5 m - Mussels still represented the biggest fraction with 434 individuals of sizes from 11 to 78 cm. Other different organisms also appeared; in particular, they were Bernacles (*B. perforatus* and *B. trigonus*), Bryozoans (*Schizoporella, Celleporina, Aetea*), Hydroids (*Sertularidae*), Serpulids (in particular *Salmacina*), Ascidians (*Styela plicata*), red Algae (also encrusted), various Decapods Crustaceans (*Porcellana, Pilumnus, Pachygrapsus*), Sponges and Bivalves (*Anomia* and *Ostrea*).

- Level -11 m - The Mussels were of larger sizes (23-101 mm in length) even though in reduced numbers (34 individuals). The epibiont organisms were well developed and were represented above all by the two species of Barnacles, some Serpulids (*Serpula*), colonial Ascidians (*Botryllus* and *Didemnidae*), Sponges, Bivalves (*Ostrea*). At this level Sea-Anemone (*Aiptasia diaphana*) began to appear whose numbers increased with depth.

- Level -34 m - The community was much rarer: 21 Mussels were present in sizes from 17 to 101 mm. In all probability they had migrated down after having settled at upper levels. Barnacles, Actinians, Sponges, Serpulids (*Salmacina, Serpula, Pomatoceros*) and Hydroids were still well-represented. Bivalves (*Ostrea, Anomia* and *Arca*) and sea-urchins (*Arbacia lixula*) were also sampled.

The samples taken from the platform showed that the Mussels at tide level in one year reached a valve length of 62 mm even if 37.5% of the population belonged to the third class (from 20 to 30 mm in length). The Mussel settlement, which reached 26 kg/m^2 at the surface, diminished with depth. The discovery of large individuals below 5 meters was connected to the fact that there had been no periodical cleaning operations of the platform.

An opportunity to have access to additional data about fouling in the Ligurian Sea presented itself with a meteo-oceanographic buoy of the series ODAS (Oceanographic Data Acquisition System). This enabled us to register an intense accumulation of Mussels even at a considerable distance from the coast.

The ODAS "ITALIA 1" buoy was immersed by the Istituto per l'Automazione Navale del CNR of Genoa in September 1980 and it remained in the position 43°38'08" latitude N 09°43'04" longitude E at 104 km from the coast where the water had a depth of 500 meters until July 25, 1983. On that date it was transferred to the position 43°51'07" latitude N and 09°06'06" longitude E at 63 km from the coast where the water had a depth of 1000 meters. The hull of the buoy was not cleaned in any way at this occasion.

In October, 1986 the buoy was taken away for repairs, towed into the Port of Genoa and put in dry-dock to be cleaned. We make reference here to a series of observations reported in Grassia and Piattelli (1987). When the buoy was put into dry-dock it was possible to observe the presence of Mussels weighing about 600 kgms. This represents a value lower than the real one because during the whole period that the buoy was in position, some Mussels were occasionally taken away from the surface. The Mussels were concentrated on the two opposing faces of the hull of the buoy in a more or less uniform way and decreased with depth up to 40 meters.

On the stainless steel mooring cable which had diameter of 15 mm the Mussels had formed a compact sleeve around the cable of more than 30 cm in diameter. The maximum size reached by the Mussels was 10 cm in valve length.

CONCLUSION

The data collected showed the undisputed role of Mussels in the formation of fouling on the offshore structures examined in the three Italian Seas, at least in the first 10 m of depth. Nevertheless, the importance of Mussels assumed a different character in relation to the eutrophic state of the waters. In fact, in the Adriatic Mussels form the largest biomass (up to 96.6 kg/m^2) and show a more rapid growth (see Table 10) since the waters of the middle Adriatic are more similar to those of the ports or the brackish.

At Ravenna the Mussels show two periods of settlement over the period of a year; the first and by far the most important is in the Spring-Summer period, reaching a maximum in June and the second is in Autumn with a peak in November-December. The Mussels prove to be dominant after three

or four months on the panels immersed in May and after six months on those immersed in October. As the length of time that the substratum is exposed increases,there is also an increase in the accumulation of fouling and in particular of the Mussels and thus of the weight. In general, one can say that the share of the weight of the Mussels expressed as a percentage of the total weight of fouling relates directly to the immersion time and inversely to the depth (the maximum of about 1 meter). It is safe to say that in one year the accumulation of Mussels on a single platform (limited to the calculation of 20 girders with a diameter of 80 cm and 10 meters of depth, and not considering the beams) is of about 50 tons.

Finally, a maximum estimate for all the structures used for the extraction of natural gas off the coast at Ravenna (probably about 360 girders with an average diameter of 80 cm) leads one to suppose that in one year there is an accumulation of about 900 tons (a figure which can only be an approximation). At the time of our research, this enormous mass of Mussels was removed annualy by divers who scraped the surfaces and then threw the Mussels back into the sea with serious environmental consequences. They are now collected and put on to the market with considerable (also economic) advantage.

At Crotone the period of settlement and of greatest growth of Mussels is Spring and dominance is reached after 6-8 months, depending on the moment of immersion of the substrata. It is, however, limited to the first 8-10 meters of depth.

Both in the Ionian Sea and the upper part of the Tyrrhenian Sea (Gulf of Genoa) it is possible to reach similar accumulations such as those found at Ravenna but the period of time necessary is much longer.

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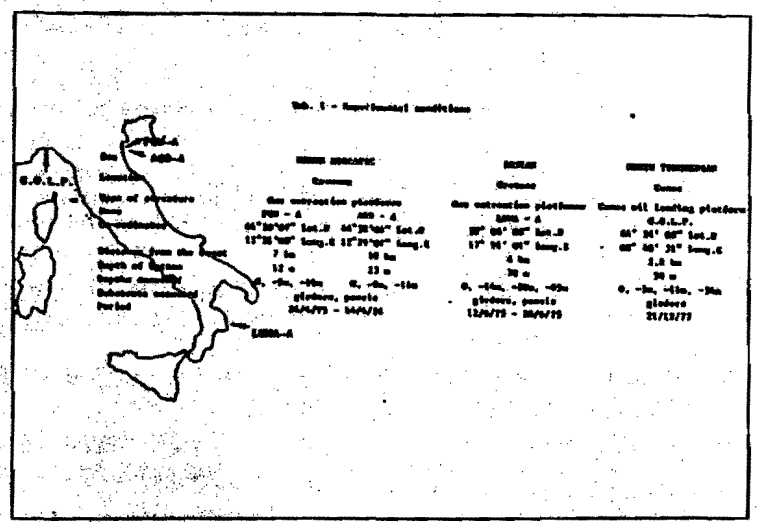


Tab. 2 - Hydrological data of the environments

	RAVENNA				CROTONE				GENOVA
	PCM - A		ACO - A		LUNA - A				Open Sea Dabini et al.1972
	- 2m	- 8 m	- 2 m	-12 m	- 2 m	-15 m	-30 m	-60 m	- 2 m - 8 m
T °c	13.32 ± 7.02	13.17 ± 5.00	13.88 ± 6.67	12.83 ± 3.95	17.12 ± 3.66	16.97 ± 3.54	16.01 ± 2.98	15.67 ± 2.47	17.19 ± 16.53 ± 0.38 0.29
S ‰	32.84 ± 2.39	35.53 ± 0.95	33.73 ± 2.28	36.98 ± 0.34	38.18 ± 0.24	38.22 ± 0.19	38.25 ± 0.19	38.33 ± 0.13	36.92 ± 37.15 ± 0.15 0.25
O_2 mg/l	9.47 ± 1.63	7.06 ± 1.87	9.06 ± 1.79	7.36 ± 1.97	7.41 ± 0.32	7.35 ± 0.23	7.43 ± 0.10	7.36 ± 0.23	8.10 ± 0.06
pH	8.32 ± 0.14	8.20 ± 0.11	8.28 ± 0.14	8.16 ± 0.14	8.14 ± 0.09	8.18 ± 0.08	8.18 ± 0.06	8.19 ± 0.06	8.22 ± 0.04
N-NO_2 µg/l	6.52 ± 5.22	6.12 ± 3.96	4.72 ± 3.70	2.79 ± 1.67	3.66 ± 2.89	3.92 ± 2.23	3.43 ± 1.23	4.16 ± 1.84	0.8 ± 1.30
N-NO_3 µg/l	103.40 ± 98.07	61.08 ± 41.25	76.26 ± 68.76	25.26 ± 14.08	22.26 ± 13.83	23.68 ± 12.73	22.82 ± 14.90	27.23 ± 20.36	16.2 ± 11.97
P-PO_4 µg/l	4.39 ± 2.02	6.45 ± 4.27	4.44 ± 3.38	6.23 ± 6.47	4.29 ± 2.20	2.42 ± 1.74	2.60 ± 1.55	4.52 ± 2.67	3.47 ± 4.83

Tab. 3 - Settlement of Mussels (no/dm²) of different size-classes at AGIP-A platform (Ravenna)

Period	Month	I LEVEL 0 m							II LEVEL -9 m								III LEVEL -20 m							TOTALE
		1°	2	3	4	5	6	TOT.	1	2	3	4	5	6	7	TOT.	1	2	3	4	5	TOT.	
1M	MAY	1						1	1							1	1					1		3
1M	JUN	489						489	33							33	+					+		322
1M	JUL	1						1	1							1								2
1M	AUG	+						+	+							+								+
1M	SEP	+						+	+							+								+
1M	OCT	+						+	+							+								+
1M	NOV	11						11	+							+	+					+		+
1M	DEC																							11
1M	JAN																							
1M	FEB																							
1M	MAR																							
1M	APR																							
Total		501						501	35							35	1					1		538
3M	JUL	1822	45	2				1869	239	7						246	+					+		2115
3M	OCT	+						+	+							+								+
3M	JAN	59						59	6							6	+					+		65
3M	APR	+						+	3							3	2					2		5
Total		1881	45	2				1928	248	7						255	2					2		2185
6M	OCT	2082	276	94	10			2462	111	73	62	21	1			268	3	3	+	+		6		7638
6M	APR	113						115	159	3	+					162	2				8	10		287
Total		2197	276	94	10			2577	270	76	62	21	1			430	5	3	+	+	8	16		2925
12M	APR	9269	254	120	51	29	1	9724	359	4	5	10	18	20	1	410	27	+	+	3	6	36		10170

° 1, 2, 3... represent length classes: 1 = 0-10 mm, 2 = 10-20 mm, ...
+ = no/dm² < 1

Tab. 4 - Settlement of Mussels (no/dm²) of different size-classes at PCW-A platform (Ravenna)

Period	Month	I LEVEL 0 m							II LEVEL -5 m								III LEVEL -11 m							TOTALE
		1°	2	3	4	5	6	TOT.	1	2	3	4	5	6	7	TOT.	1	2	3	4	5	6	TOT.	
1M	MAY	3						3	13							13	+						+	16
1M	JUN	320						320	52							52	3						3	375
1M	JUL	+						+	+							+								+
1M	AUG	2						2	+							+	+						+	2
1M	SEP	+						+																+
1M	OCT								+							+								+
1M	NOV	1						1	10							10								11
1M	DEC	19						19	11							11								30
1M	JAN								+							+	+						+	+
1M	FEB																							
1M	MAR																							
1M	APR																							
Total		345						346	86							86	3						3	434
3M	JUL	1429	65	6				1500	629	116	18	+				763	16	+	+				16	2270
3M	OCT	+						+	+							+	+						+	+
3M	JAN	747						747	291							291								1038
3M	APR	6						6	10							10	1						1	17
Total		2182	65	6				2253	930	116	18	+				1064	17	+	+				17	3334
6M	OCT	1809	421	105	9			7344	53	12	22	22	3			112	3	4	11	1			19	2475
6M	APR	3604	64	+				3668	800	95	4					899	2						2	4569
Total		5413	485	105	9			6012	853	107	26	22	3			1011	5	4	11	1			21	7044
12M	APR	4701	140	90	34	29	3	4999	2943	58	13	15	11	17	15	3069	1	2	+	5	14	4	61	8129

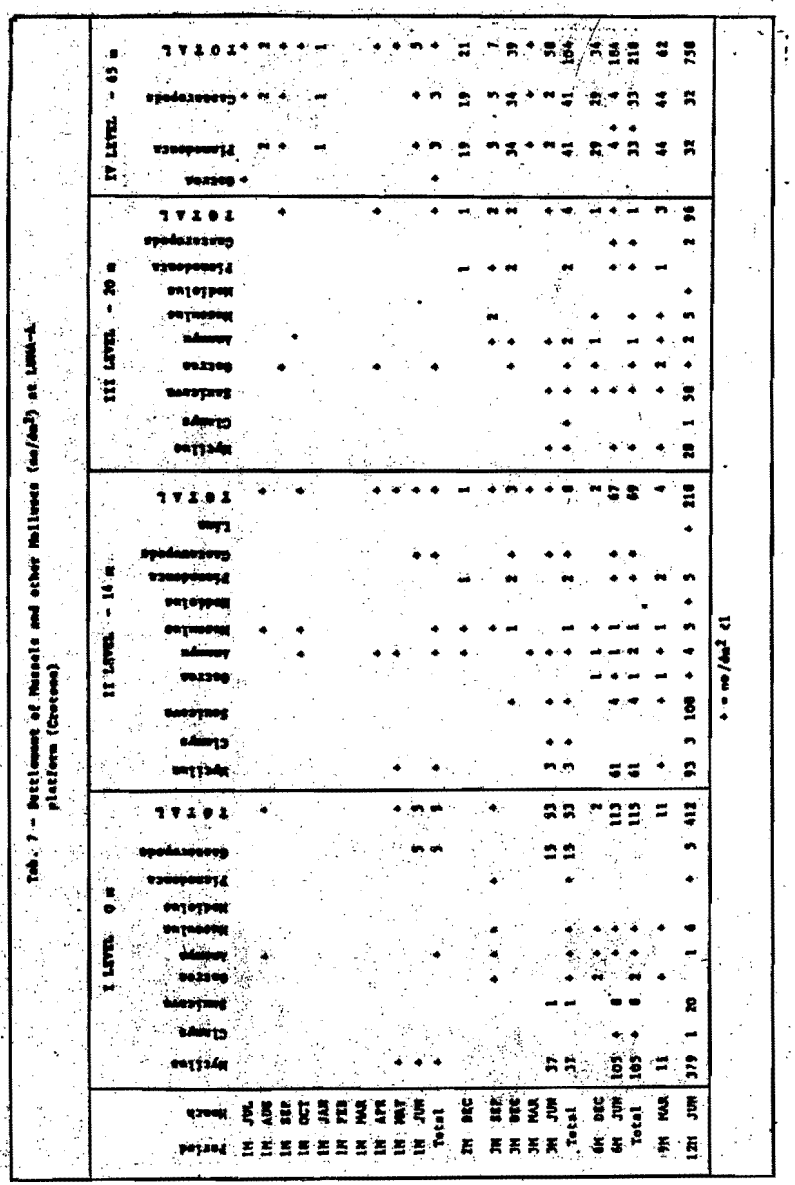
° 1, 2, 3... represent length classes: 1 = 0-10 mm, 2 = 10-20 mm, ...
+ = no/dm² < 1

Tab. 5 - Wet weight of fouling (g/12 dm^2) settled on panels immersed at Ravenna

Period	Month	Platform AGO-A			Platform PCW-A		
		LEVEL			LEVEL		
		I 0 m	II -9 m	III -20 m	I 0 m	II -5 m	III -11 m
1M	MAY	5	10	20	30	15	10
1M	JUN	170	230	7	80	180	20
1M	JUL	140	70	5	20	65	30
1M	AUG	190	25	5	300	230	50
1M	SEP	5	80	10	50	60	60
1M	OCT	160	60	8	80	80	25
1M	NOV	20	10	2	5	55	30
1M	DEC	<1	8	<1	10	10	5
1M	JAN	<1	<1	<1	<1	7	<1
1M	FEB	<1	<1	<1	<1	<1	<1
1M	MAR	<1	1	1	<1	2	<1
1M	APR	<1	1	20	5	10	2
3M	JUL	795	386	37	1395	2140	174
3M	OCT	243	144	11	379	330	68
3M	JAN	23	120	10	179	180	95
3M	APR	<1	11	16	25	18	16
6M	OCT	5010	2389	320	7041	3074	434
6M	APR	60	200	61	1197	1534	280
9M	JAN	6338	3491	397	6802	5621	1306
12M	APR	11916	5949	820	10909	10601	2426

Tab. 6 - The role of Mussels on the wet weight of fouling (g/12 dm^2)

Period	Month	Platform AGO-A						Platform PCW-A					
		I LEVEL 0 m		II LEVEL - 9 m		III LEVEL - 20 m		I LEVEL 0 m		II LEVEL - 5 m		III LEVEL - 11 m	
		Total fouling weight	Weight of Mussels / % of total	Total fouling weight	Weight of Mussels / % of total	Total fouling weight	Weight of Mussels / % of total	Total fouling weight	Weight of Mussels / % of total	Total fouling weight	Weight of Mussels / % of total	Total fouling weight	Weight of Mussels / % of total
3M	JUL	795	500 63%	386	85 22%	37	+ +	1395	1066 76%	2140	1450 67%	174	3 1.7%
3M	OCT	243	+ +	144	+ +	11	+ +	379	1 +	330	+ +	68	+ +
3M	JAN	23	+ +	120	+ +	10	+ +	179	+ +	180	+ +	95	
3M	APR	41	+ +	11	+ +	16	+ +	25	+ +	18	+ +	16	+ +
6M	OCT	5010	3713 74%	2389	1765 74%	320	45 14%	7041	6710 95%	3074	2600 84%	434	313 72%
6M	APR	60	15 25%	200	40 20%	61	1 1%	1197	907 76%	1534	1168 76%	280	1 +
9M	JAN	6338	6212 98%	3491	3256 93%	397	257 65%	6802	6359 93%	5621	5147 91%	1306	1154 88%
12M	APR	11916	11600 97%	5949	4797 81%	820	584 71%	10909	10391 95%	10601	10100 95%	2426	1398 57%



Tab. 7 – Settlement of Mussels and other Molluscs (no/dm²) at LUNA-A platform (Crotone)

Period	Month	I LEVEL sup.	II LEVEL - 14 m	III LEVEL - 20 m	IV LEVEL - 65 m	TOTAL
1	JUL	1	5	5	1	12
1	AUG	2	10	15	1	25
1	SEP	1	5	12	1	19
1	OCT	2	4	4	1	11
1	JAN	2	1	1	1	5
1	FEB	1	1	1	1	4
1	MAR	15	1	1	1	18
1	APR	35	1	1	1	38
1	MAY	13	5	8	1	27
1	JUN	15	6	4	1	26
Total		87	39	52	10	188
2	DEC	16	4	6	1	27
3	SEP	6	35	30	2	73
3	DEC	5	30	25	5	65
3	MAR	106	1	1	1	109
3	JUN	80	7	15	19	121
Total		197	73	71	27	368
6	DEC	18	193	183	25	419
6	JUN	180	22	16	30	248
Total		198	215	199	55	667
9	MAR	166	279	330	129	904
12	JUN	816	853	840	586	3095

Tab. 8 – Wet weight of fouling (g/12 dm²) settled on panels immersed at Crotone.

platform LUNA-A

Tab. 9 - Mussels sampled on 12 dm^2 surfaces at different depths at Genoa Platform.

GENOA OIL LANDING PLATFORM (G.O.L.P.)															
	no/12 dm^2 of Mussels	length classes											% of most common length class	Weight of Mussels g/12 dm^2	Weight of Mussels kg/m^2
		1*	2	3	4	5	6	7	8	9	10	11			
0 m	1,059	1	230	398	278	112	33	1	/	/	/	/	37.5% (20-30 mm)	3,129	26.1
- 5 m	434	/	89	216	96	11	20	13	5				48.2% (20-30 mm)	2,123	17.7
-11 m	34	/	/	2	/	/	/	8	12	10	1	1	35.3% (70-80 mm)	1,200	10.0
-34 m	21	/	1	1	/	/	1	3	/	2	10	2	52.4% (90-100 mm)	1,240	10.3

* 1, 2, 3 represent Length classes: 1 = 0-10 mm, 2 = 10-20 mm ...

Tab. 10 - Summary of data on fouling of offshore structure

SEA	ADRIATIC	ADRIATIC	IONIAN	TYRRHENIAN
Locality	Ravenna	Ravenna	Crotone	Genova
Offshore structure	PCH - A	AGO - A	LIRA - A	G.O.L.P.
Distance from coast	7 km	18 km	6 km	2.8 km
Depth of bottom	12 m	23 m	70 m	30 m
Level of sample	surface	surface	surface	surface
Substrate	panels	panels	panels	girders
Maximum age of Mussels	12 months	12 months	12 months	12 months
no of Mussels/12 dm^2	59,988	116,686	4,549	1,059
Maximum Length	60 mm	60 mm	35 mm	62 mm
% of most common length class	94.0% (0-10 mm)	93.3% (0-10 mm)	78.0% (0-10 mm)	37.5% (20-30 mm)
Weight of Mussels g/12 dm^2	10,391 g	11,600 g	654 g	3,129 g
Weight of Mussels kg/m^2	86.6 kg	96.6 kg	5.4 kg	26.1 kg
Mussels as % of total fouling weight	93%	97%	80%	90%

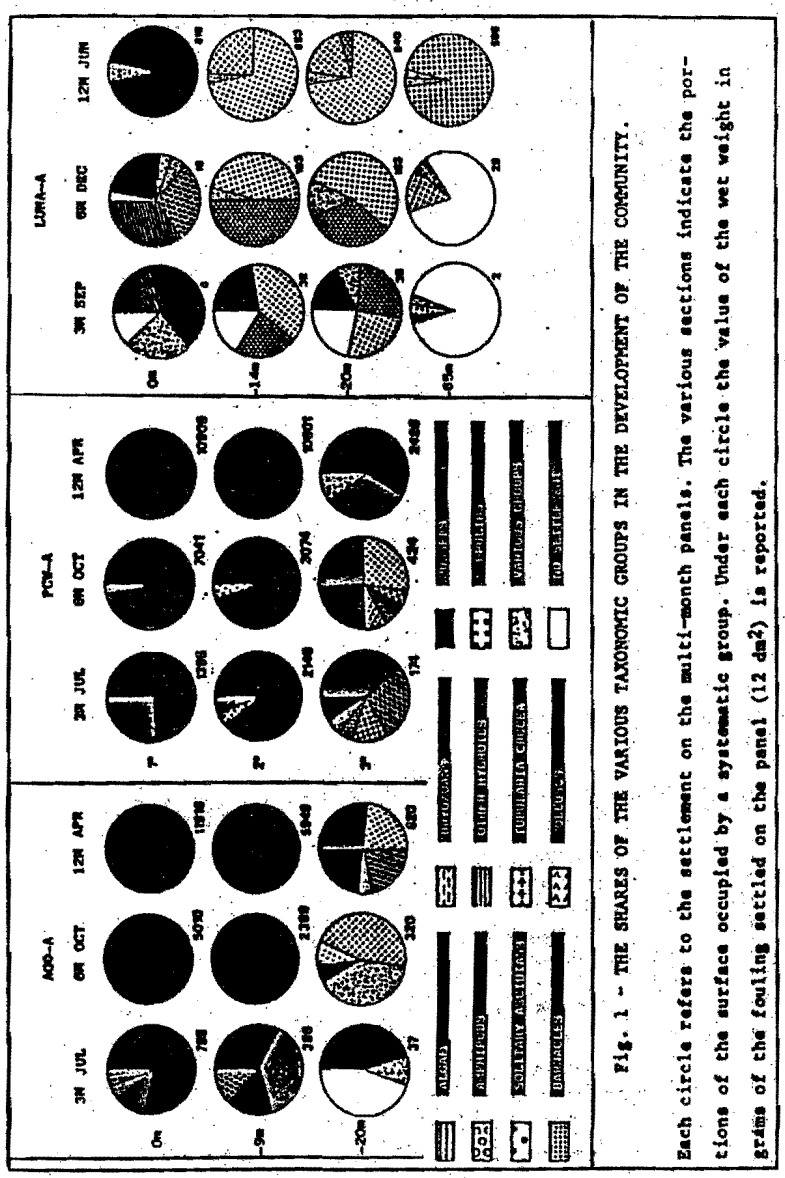


Fig. 1 - THE SHARES OF THE VARIOUS TAXONOMIC GROUPS IN THE DEVELOPMENT OF THE COMMUNITY.

Each circle refers to the settlement on the multi-month panels. The various sections indicate the portions of the surface occupied by a systematic group. Under each circle the value of the wet weight in grams of the fouling settled on the panel (12 dm^2) is reported.

INTERACTIONS WITHIN FOULING BIOFILMS: STUDIES USING A MODEL SYSTEM

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ABSTRACT

A procedure is described whereby mixed algal/bacterial biofilms can be constructed in the laboratory. They are used to show how cell-cell interaction can be measured in terms of *in situ* bacterial film growth at the expense of algal photosynthesis. Artifacts are documented arising as a result of harvesting biofilms from surfaces before metabolic measurements are made. Suggestions how mixed films can be used to enhance biofouling research are included.

INTRODUCTION

Biofilms are collections of organisms and their products at liquid-solid interfaces. It is the formation of this coherent film on the surface of man-made equipment that heralds the equipment's subsequent decay in performance. This can be attributed largely to the fact that surface-associated individual cells do not change the environment at the surface whereas the presence of a coherent film does. Since there is a diffusional resistance within the biofilm and between the biofilm and the bulk aqueous milieu, biofilms are properly termed microenvironments, and as such, their study presents us with specific problems.

Biofilms are ubiquitous on wetted surfaces, and where those surfaces are illuminated, phototrophs as well as heterotrophs are found. In these circumstances, often the major component of the biomass is diatoms (1,2). Biofilms appear to be particularly successful in inhospitable, and especially oligotrophic, environments. It is probably their apparent success in such circumstances, together with the problems they cause, that has lead to a considerable number of comparative studies concerning the relative metabolic activities of cells on surfaces and similar populations in suspension (8).

Most of the early studies used natural and uncontrolled mixtures of organisms (3,4). Later, when problems were realized concerning interpretation of results with unreproducible populations in mixed films, researchers moved to the use of pure cultures. In model systems, there is no doubt that axenic cultures were extremely useful, but Nature rarely exists as a pure culture! Studies with such cultures miss an extremely important facet of the metabolic operation of the biofilm i.e. the potential for interaction between like and unlike organisms. Although it is well-known that planktonic

organisms are capable of interaction (5,6), little has been published until recently on the potential for organism-organism interactions within films. This is largely because of the difficulty in sampling such films. Not to be discouraged by this, several workers scraped cells from surfaces, homogenized the resultant suspension and used this to perform replicated experiments (7,8). Whether the results contained artifacts when obtained in these types of experiments could not be assessed.

To investigate the potential for interaction between organisms on surfaces, we have attached axenic heterotrophic bacterial and photoautotrophic diatom cells to surfaces separately and in known ratios and measured the ability of the bacterial cells to incorporate ^{3}H-thymidine into DNA as a measure of their potential for cell division. Also, compared here are the *in situ* ^{3}H-thymidine incorporation rates for cells in biofilms and in suspensions of cells made directly from those films.

METHODS

Microbial Cells

The diatom, *Amphora coffeaeformis* was grown at 100 Einsteins m^{-2} sec^{-1} in ASP-2 medium modified to contain 0.25mM Ca (9). The marine bacterium, *Vibrio proteolyticus*, (10) was grown in the same ASP-2 medium supplemented with 0.033% (w/v) yeast extract and 0.17% (w/v) peptone (11).

Attachment of Cells to Substrata

Amphora were added to sterile polystyrene petri dishes in a suspension containing 1-2 x 10^{6} cells. The calcium concentration of the medium was increased to 2.5 mM (12) and the cells allowed to attach for 4h. The diatom film was rinsed twice with 10 ml medium to remove unattached cells. The *Vibrio* was washed by centrifugation and resuspended in ASP-2 medium before being attached to petri dishes in a similar manner to the diatoms. The procedure is summarized in Fig. 1.

Thymidine Incorporation

The incorporation of ^{3}H-thymidine into bacterial DNA (13) was used as an indicator of bacterial growth. Details of the procedure for attached cells have been published (11). In some cases, the radioactivity in attached algal/bacterial mixtures was not counted, and instead the films were radioautographed using the procedure described by Brock and Brock (14), after

staining with the DNA-specific stain Hoechst 33258 (15). The stain allowed bacteria to be counted under epifluorescent illumination and an assessment of the degree to which silver grains in the radioautography experiments were associated with diatom nuclei.

Harvesting the Attached Films and Subsequent Experiments Using ^{3}H-thymidine Incorporation

Biofilms were harvested by scraping the surface of the petri dishes gently with a sterile rubber policeman. The detached cells were shaken in ASP-2 medium containing 0.25mM Ca and transferred to glass vials where they were incubated for 30 minutes with ^{3}H-thymidine (final concentration was 20nM) as described previously (11).

RESULTS

Figure 2 shows that the algae or bacteria alone took up little ^{3}H-thymidine into the cold trichloracetic acid insoluble fraction of the cell. This fraction is considered to be largely DNA (17). When an attached bacterial-algal mixture was incubated with ^{3}H-thymidine, considerable labelling of the film took place, in fact, the total incorporation was several fold greater for the mixture than for the sum of that taken up by the individual films. Radioautography of the radiolabelled films showed that the radioactivity has associated only with bacteria. No labelling of the diatom nuclei was seen. This observation was facilitated by he bright blue fluorescence of the nuclei when viewed microscopically with combined phase contrast and epifluorescent illumination. Note that as the algal film proliferated (16 to 70 hours), there was an increased rate of incorporation of ^{3}H-thymidine into the bacterial cells. Table 1 further documents the dependence of bacterial thymidine incorporation on the algal metabolism and abundance. Where the films were incubated in the dark, the algae did not grow and neither did the bacteria (cf lines 2 and 3, Table 1).

Figure 2 represents an experiment similar to that presented in Figure 1, but here the incorporation of ^{3}H-thymidine by intact and disrupted films was compared. Except in the case of the algae, cells from the disrupted film incorporated 4 to 5 times more thymidine than cells in a film. When these figures are converted to potential bacterial cell growth (Table 2), using the conversion factors favored by Fuhrman and Azam (13), the artifact caused by the disruption of the biofilm is highlighted. In each case, disruption of the film caused an increase in

the predicted bacterial growth rate. By microscopical examination, disrupted films contained a barely detectable level of damaged cells.

DISCUSSION

Biofilms in nature are visibly "patchy" when examined microscopically and are thus difficult to sample in a replicate manner. We have shown that to remove a large area of film and use this as a source of experimental material for replicate experiments causes considerable artifacts. In our experiments, sufficient organic carbon was released from bacterial or algal cells when they were scraped gently form a surface to support a five-fold increase in bacterial growth in the absence of other added nutrients. These conclusions are based on the finding that the ^{3}H-thymidine labels only DNA significantly in these bacteria (16). The uptake of radioactivity by the diatoms was shown not to be associated with the nucleus of the cell and was in fact not statistically different from formalin-killed control cells. The increase in bacterial growth rates in the absence of added nutrients but in the presence of diatoms was considerable. After 32 hours, bacterial cells were growing 16-fold faster in the presence of diatoms than in their absence. This emphasizes the degree of interaction between the heterotrophic bacterium and the autotrophic diatom. In these films, the ratio of bacteria: diatoms was 40:1, so the influence of diatoms on the overall energy budget of the film was considerable. It appears therefore, that in an illuminated and oligotrophic situation, diatom photosynthesis and subsequent release of photosynthetic products to the extracellular space may be one of the most important factors in the ecological success of the film.

The two component biofilms we have described can be used as models for research in the area of cell-cell interactions in fixed films. Produced as described here, the biofilms represent a cellular monolayer, so that they mimic only "young" biofilms i.e. films up to approximately 10 µm. In thicker films, where light penetration is reduced, diatoms in the upper layers may provide the electron donors for sulfate-reducing bacteria lower in the film.

There appears to be no obvious restriction, other than physiological, on the type of organisms that can be accommodated into a film of this type. For instance, we see no reason why a facultative fermenting organism should not be incorporated into a film together with a sulfate-reducing bacterium. Such a consortium could be used for

studies in microbially-assisted corrosion. Other possibilities for these films include biocide testing and their inclusion in continuous flow reactors.

ACKNOWLEDGEMENT

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TABLE 1 Influence of Algal Abundance and Illumination on Growth Rate of Attached Bacteria in Mixed (Algae and Bacteria) Biofilm Communities

Time Elapsed Since Formation of Biofilm (Hrs)	Incubation Conditions	Total Algal Cells/Dish	Moles of Thymidine Incorporated/ Bacterial Cell
16	light	1.0×10^{6}	0.8×10^{-21}
32	light	2.4×10^{6}	4.2×10^{-21}
32	dark	0.7×10^{6}	0.3×10^{-21}
70	light	7.4×10^{6}	17.0×10^{-21}

Light was 97 μ Einsteins m^{-2} sec^{-1} from cool white fluorescent lamps

TABLE 2 Bacterial Growth in Intact and Disrupted Biofilms

Community	Growth (g. carbon $n^{-1} \times 10^{-8}$)
Intact: bacteria	2.8 ± 1.2
Intact: bacteria and diatoms	5.6 ± 0.4
Disrupted: bacteria	12.2 ± 2.8
Disrupted: bacteria and diatoms	27.5 ± 11.3

[1] Converted from ^{3}H-thymidine incorporation rates (Fuhrman & Azam (13), J.H. Paul, pers comm.); n=8, ±1 standard deviation. Films were incubated under constant illumination for 48h. before addition of 20nM ^{3}H-thymidine. All figures are corrected for the uptake measured in Formalin-killed controls. Incubations with radiolabelled compound were terminated by the addition of a final concentration of 5% Formalin.

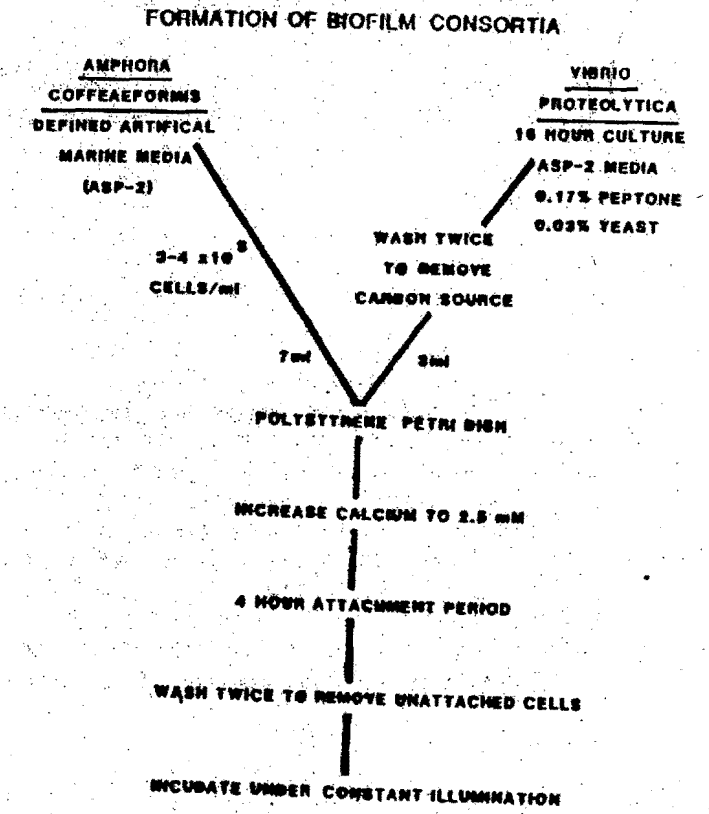

Figure 1. Protocol for the formation of mixed algal-bacterial films.

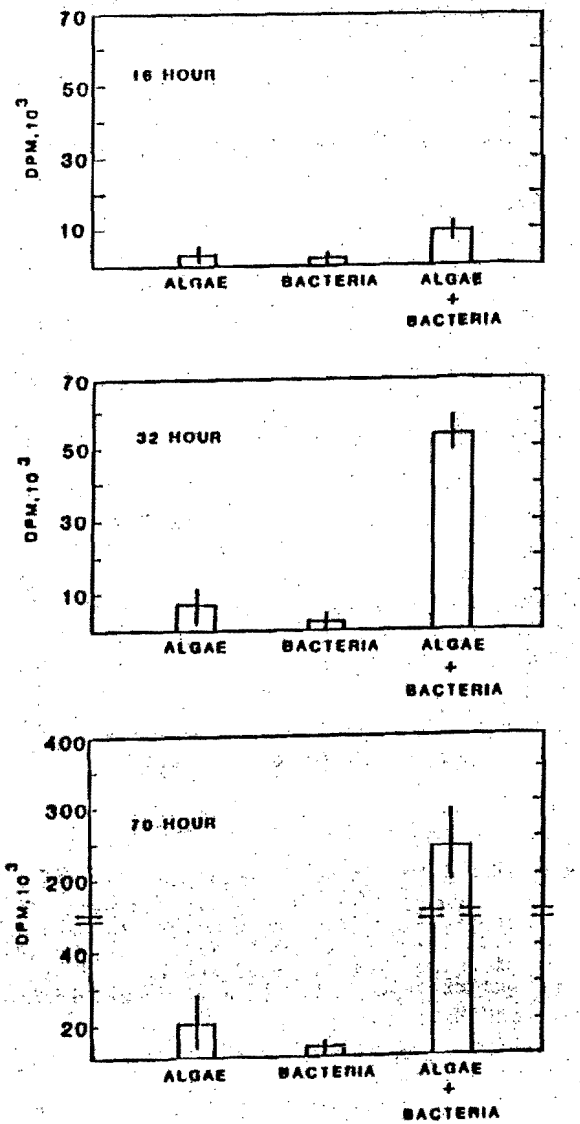

Figure 2. Incorporation of ^{3}H-thymidine into the cold trichloracetic acid insoluble fraction (DNA) of biofilms made from diatoms alone, bacteria alone and a 40:1 mixture of bacteria and diatoms. Bars represent ± one standard deviation.

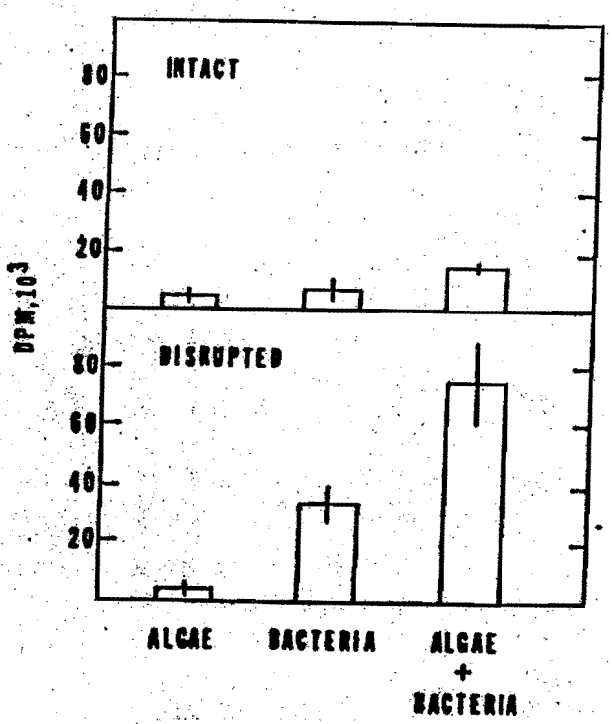

Figure 3. Incorporation of ^{3}H-thymidine by intact and disrupted algal, bacterial and mixed algal and bacterial films. Each petri dish contained 5.25×10^{6} algal cells and/or 1.95×10^{6} bacterial cells. Bars represent ± one standard deviation (n=8). Other details as in Table 2.

Lake Timsah as a barrier for the serpuloid (tube worms) migration along the Suez Canal.

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ABSTRACT

Six serpuloids live at Lake Timsah. Their origin and water quality influence their distribution in the Lake. *Hydroides dirampha* and *H. elegans* are abundant in the Lake but the latter as well as *Spirobranchus tetraceros* predominate in the oil polluted area. *Pomatoleios kraussii*, the Red Sea form, fails to cross the Lake to the North. *Pileolaria (Simplicaria) pseudomilitaris* and *Janua (Neodexiospira) pseudocorrugata*, on the other hand, do not live at the Red Sea and their southern appearance ceased at the borders of the Lake Timsah. Preference of the worms to certain hydrographic ranges is statistically discussed.

INTRODUCTION

Lake Timsah, although comparatively small in surface area (almost 50 Km2), it contains different water qualities. This would have an effect on the migration of organisms between the two big seas (Mediterranean and Red Sea) as it lies nearly at the middle of the Suez Canal. It is characterized by a marked saline stratification (Por, 1978), receiving salty bottom water from the south (Bitter Lakes) and diluted water from the Nile through Ismilia Canal which supply the whole Suez Canal region by drinking and irrigation waters. Ghobashy, Shalaby and Shalla (1986) observed that the lake contains different water qualities and the hydrographic discrepencies appear to be causing those differences.

RESULTS

THE HYDROGRAPHY OF THE LAKE

Salinty, surface temperture, pH and other parameters including chemical-oxygen demand (Cod) and turbidity at five location of the Lake (Figure 1) were determined during 1983-1984. Table I gives the ranges of salinity and of other parameters at the five locations during the period of investigation.

Table 1: The hydrographic conditions of the lake during 1983-84.

Location	I	II	III*	IV	V
Ranges of Salinity:	27.9-40.1	27.3-39.8	28.85-41.0	27.3-39.8	28.7-38.4
Temperture	17.0-30.0	16.0-30.0	17.0-29.0	17.0-30.0	16.5-30.0
pH	8.05-8.40	8.05-8.30	8.10-8.30	8.10-8.30	8.10-8.45
Turbidity	clear	often turbid	turbid	clear	clear

* This location is highly polluted with oil.

Serpuloids of the lake

The superfamily Serpuloidea is represented in the Lake Timsah by two families Serpulidae and Spirorbidae and six species; *Hydroides dirampha*, *H. elegans*, *Spirobranchus tetraceros*, *Pomatoleios kraussii*, *Pileolaria (Simplicaria) pseudomilitaris* and *Janua (Neodexiospir) pseudocorrugata*.

Table 1 illustrates the distribution of these serpuloids in the world as well as in Egyptian marine localities.

Species recorded	World distribution				Egyptian distribution		
	Atlantic	Pacific	Indian	Med.	Alx.	S.Canal	Red Sea
Hydroides dirampha	+	+	+	+	+	+	-
H. elegans	+	+	+	+	+	+	-
Spirobranchus tetr.	+	+	+	+	-	+	+
Pomatoleios krau.	-	+	+	-	-	-	+
Janua pseudo.	+	+	+	+	-	-	-
Pileolaria pseudo.	+	+	+	+	-	-	-

\+ present , - not recorded.

Occurrence of the worms in the lake and in other areas

A- *Hydroides dirampha* (Morch): Figure 2 shows that this species is abundant at the southern locations (I and II) and less common at the other locations. its highest record was found in May 1984 at location II (1080 worms/2 liters of fouling). Its greatest settlement took place from March to June and the tube length reached its maximum value (7-10 cm) in the same period.

B- *Hydroides elegans* (Haswell) : It is exclusively the most abundant tube worm in the lake (Ghobashy and El Komy, 1981a) as well as in other Egyptian waters (Ghobashy, 1984). Figure 3 shows that its occurrence was particularly great at locations I, II& III. About 10000 worms/2 liters of fouling were collected at the location II in April 1984. Although very common in Mediterranean, it has not been recorded before in the Red Sea. Ghobashy and El Komy (1981 b) found the species decreasing southernly along the Suez Canal.

C- *Spirobranchus tetraceros* (Shmarda) : Although present in all locations but the catch was always small. Like *H. elegans*, it showed a marked resistence to oil pollution and its appearance in location (III) was remarkable(Figure 4). It is common in Red Sea (Pixel, 1913; Fauvel, 1933; Fishelson, 1969, Hove, 1970 and Mohomed, 1971) and at Suez Gulf (Potts, 1928) , but apperently failed to invade Mediterranean and only six specimens were found attached to ships comming from the Indopacific through Suez Canal (Zibrowius, 1970).

D- *Pomatoleios kraussii* (Baird) : Rare in all locations. While 86 worms were found in location I, 33 specimens were collected from the other

parts of the lake during the whole period of the study. Not recorded in Mediterranean.

E- *Janua (Neodexiospira) pseudocorrugata* (Bush) : New record in the Egyptian waters. All the worms (30 specimens) appeared among the fouling of location I. Clearly not yet established in the lake.

F- *Pileolaria (Simplicaria) pseudomilitaris* (Thiriot-Quievreux) : Over 14000 worms were collected from all locations(Figure 5) . Wide spread in the world.

Influence of hydrographic values on worms distribution

To compare between the five locations investigated in respect to the quantity of each species, considering the different hydrographic affinities of the lake; salinity temperature and pH, the statistic method adopted was the two way analysis of variance (ANOVA). The significant range value was obtained from the Dancan"s table according to the degree of freedom of the error and the significant level taking in consideration the number of means including in the comparison of the range test. The Dancan"s value was compared with absolute difference and if the difference was equal or higher than the value , the difference becomes significant.

The analysis of the data obtained for the relation between values and *H. diramphe* counts showed that there is a significant difference between the number of the worms in the locations: I & III, II & III and II & IV (Table 2).

Table 2: Distribution of *H. diramphe* with respect to salinity value.

	Number of the worms in the locations						
Salinity ranges	I	II	III	IV	V	Total	Mean
25-	600	920	320	28	880	2748	549.6
30-	805	1480	272	120	800	2477	695.4
35-	1922	2144	160	812	618	5656	1131.2
40-45	0	0	0	30	0	30	6
Total	3327	4544	752	990	2246	11911	2382.2
Mean	831.7	1136	188	247.5	594.5	2977.7	

The analysis showed that the salinity range 35-40% is the most favoured range for the settlement of this species.

Considering the influence of temperature on the distribution of the same species, similar statistical analysis proved that 25-30 C is the leading range for this worm to flerish. Nevertheless, pH has no significant influence on the distribution of the worm in the lake.

Regarding *H. elegans* which is thriving in the lake and in particular in oil polluted location (III), table 3 the distribution of this species with respect to salinity

Table 3: Distribution of *H. elegans* with respect to salinity values.

	Number of the worms in the locations						
Salinity ranges	I	II	III	IV	V	Total	Mean
25-	1240	2960	14080	160	6360	28800	5760
30-	12345	17740	19541	3360	8080	61066	12213.2
35-	8340	25320	11760	11242	13495	70157	14031.4
40-45	0	0	0	1745	0	1745	349
Total	21925	50020	45381	16507	27935	161768	32353
Mean	5481.25	12505	11345.25	4126.75	6983	40442	

The analysis showed the significant preference of the oil polluted location (III) and clearly the species preferred the salinity range 35-40 ‰. It was also found that the temperature range 25-30 C was significantly favoured to the worms to flourish. However, no influence for pH on the distribution of the worm in the lake was indicated.

Table 4: Distribution of *Spirobranchus* with respect to salinity values.

	Number of the worms in the locations.						
Salinity ranges	I	II	III	IV	V	Total	Mean
25-	0	5	75	6	4	90	18
30-	13	10	24	4	10	61	12.2
35-	6	16	40	44	25	131	26.2
40-45	0	0	0	0	0	0	0
Total	19	31	139	54	39	282	56.4
Mean	4.75	7.75	34.75	13.5	9.75	70.5	

The data emphsized the preference of this form to settle at location (III) but no salinity range was significantly preferred. Similarly neither the temperature no pH has an effect on the distribution of the species in Lake Timsah.

The same treatment was applied for *Pomatoleios* which settled scarcely but nearly solely in location (I) and no hydrographic parameters showed any influence on the distribution of the species in the lake.

The remaning two species were either too scarce (*Janua*) or quite fairly distributed in the whole lake.

DISCUSSION

Despite its small area Lake Timsah embraces different water bodies; the water at the South is more or less higher in salinity and lower in pH than of the North. This in effect has controlled the distribution of serpuloid

populations in the lake. Two species; *Hydroides elegans* and *Spirobranchus tetraceros* proved to be indicators for the oil polluted water. *H. dianthe* preferred locations I & II (at the South) because of their higher salinity over the others.Pomatoleios, the Red Sea form practically restricted its settlement to the southern part of the lake. Generally speaking the Mediterranean and Red Sea species succeeded to reach the lake but some failed to cross it to the opposite side of the canal. *H. elegans*, the most abundant fouling organisms in Egyptian Mediterranean harbours(Ghobashy, 1984) and in Lake Timsah, is rare in the southern part of the Suez Canal (Ghobashy and El Komy, 1981). For unknown reasons, *H. dianthe* did not appear in the Red Sea despite its success to reach the southern part of the lake. Similarly *Spirobranchus* and *Pomatoleios* failed to be established in Mediterranean and in the northern part of the canal.

These findings illustrate that Lake Timsah works as a barrier for migration the Serpuloids along the Suez Canal.

Por (1978) claimed that fouling organisms moved to the western seas just on ships and did not consider them among the Lessepsian migrants. One can ask, what about these serpuloids which can not cross the Lake Timsah to the other side of the Suez Canal despite of tremenous number of ships sailing every day along the canal and from one world hemisphere to the other. It is thus difficult to accept that fouling organisms migrate only in such a passive way, i.e. on ships and ignoring the role of their free moving larvae for their dissemination.

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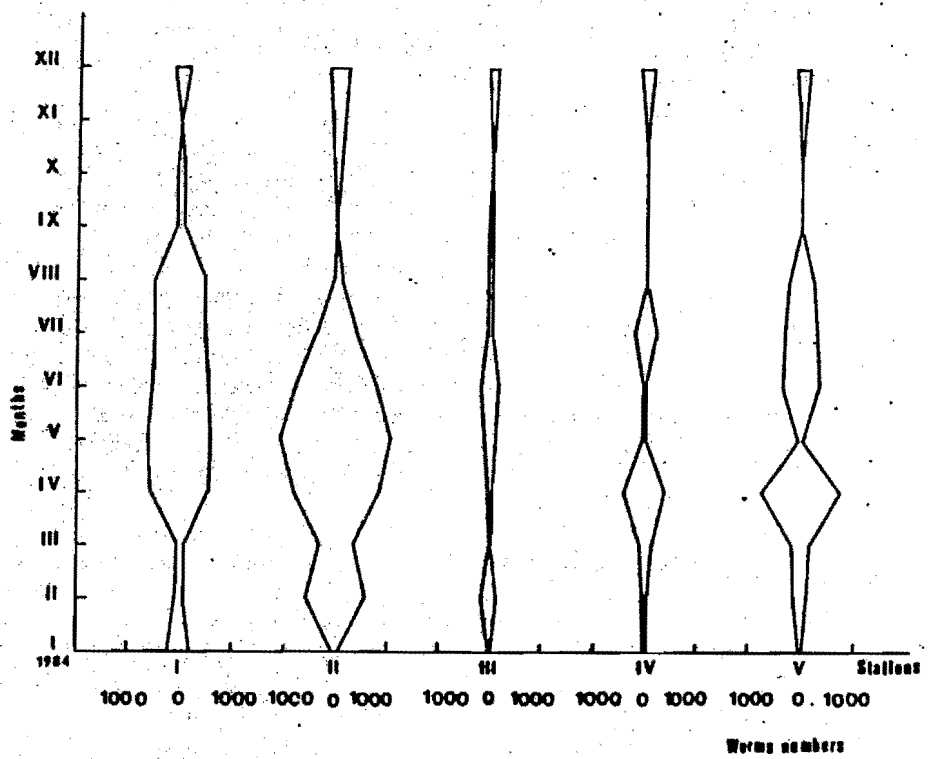

Figure 2: Monthly settlement of *Hydroides dirampha*

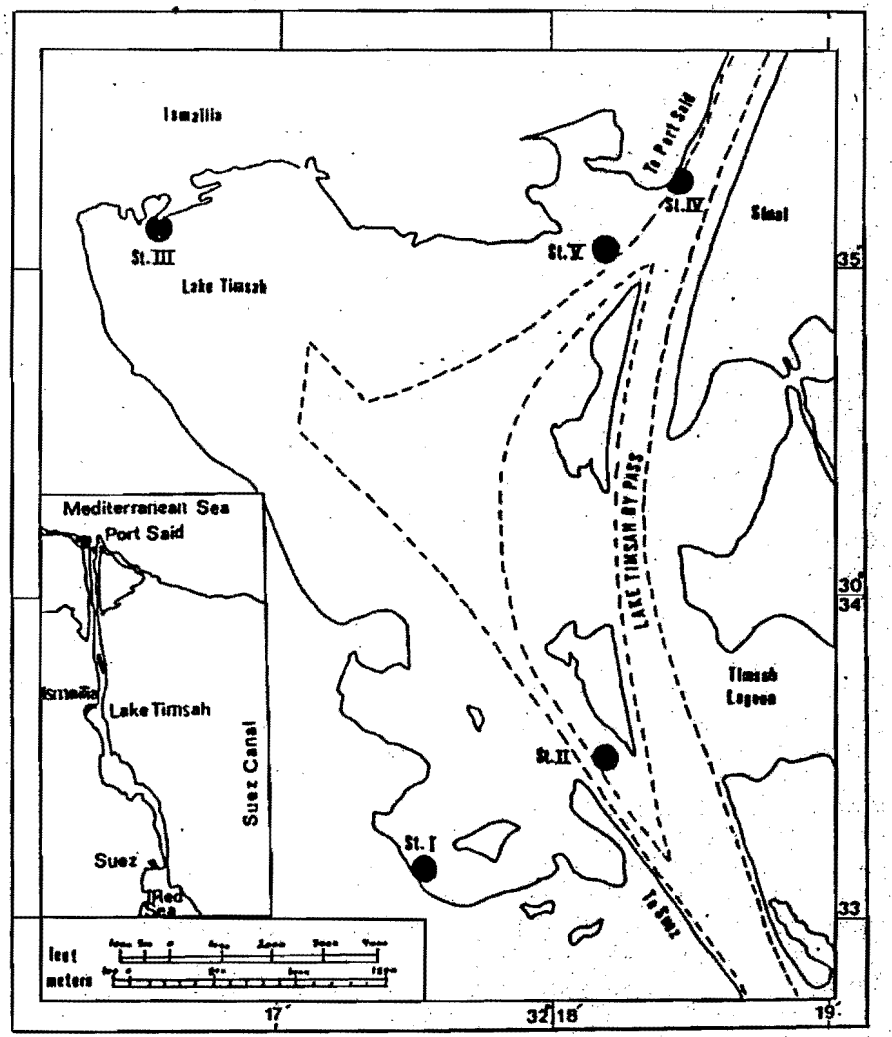

Figure 1: A map to show the Lake Timsah and the position of the five stations.

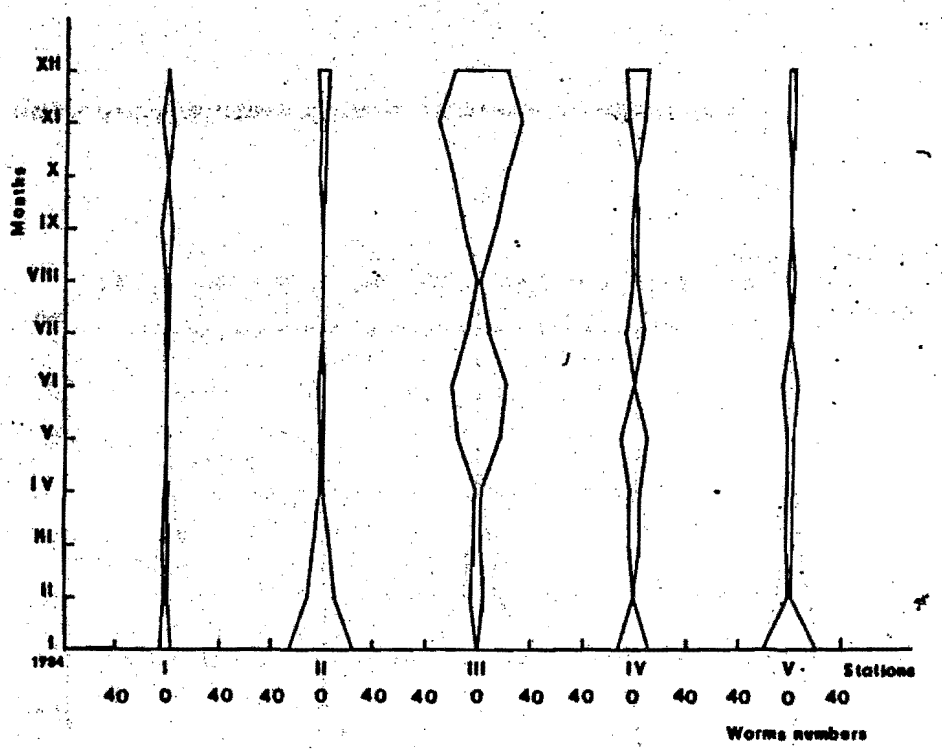

Figure 4: Monthly settlement of *Spirobranchus tetraceros*.

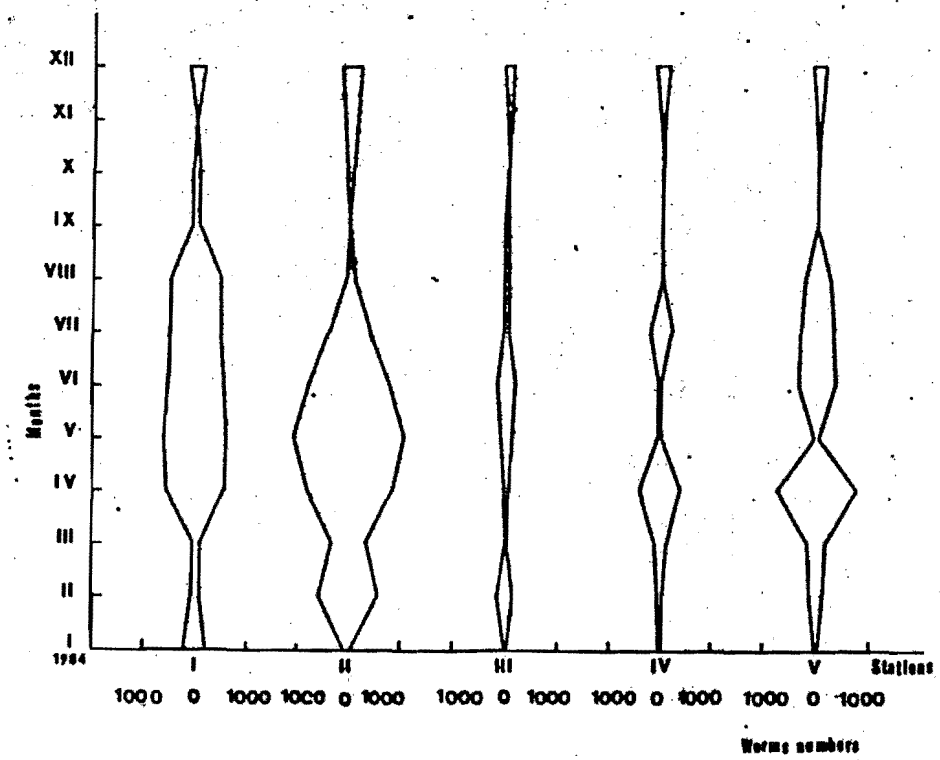

Figure 3: Monthly settlement of *Hydroides elegans*.

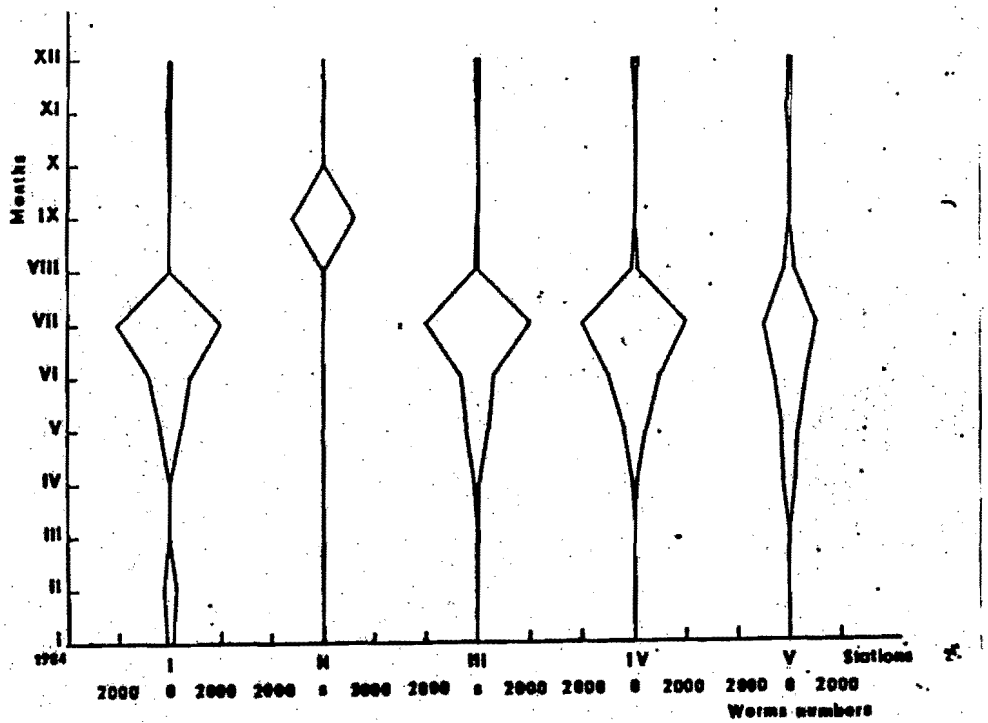

Figure 5: Monthly settlement of *Pilaelaria (Simplicaria) pseudomilitaris*

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THE BIOFOULING COMMUNITIES ON THE PIER PILLING AT XIAMEN, CHINA

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ABSTRACT

The biofouling communities on the tidal zone of concrete pilling of pier at Xiamen were studied. 120 species were found. The species increase from upper tidal zone to lower tidal zone. The dominant species in upper tidal zone is Euraphia withersi and in middle tidal zone is Ostrea echinata. There are various species in lower tidal zone. The highest record of wet weight is 10.3 kg/m^2. The wet weight in middle tidal zone is the heaviest and that in upper tidal zone is the lightest. The biofouling communities on the pilling can be generally divided into five vertical distribution zones. The climax communities on the pilling is relatively stable, but species successions still exist during interyears and interseasons. Such successions grow more apparent from upper tidal zone to lower tidal zone.

INTRODUCTION

Compared with the communities in natural rock tidal zone, the biofouling communities on the tidal zone of pier pilling have clearer vertical zonation. Their interrelation to tide level is closer meanwhile. They are one side of coastal ecology study (Morton, 1983). And the fouling organisms on the pier pilling are one of the parameters of coastal engineering design (The Bureau of Ship Inspection, China, 1984).

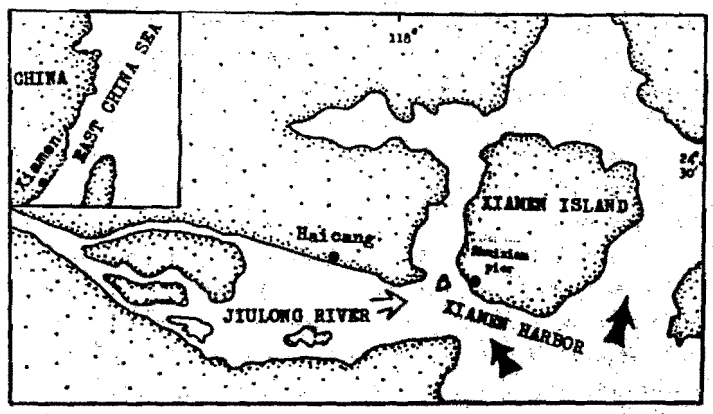


Figure 1. Map of Xiamen harbor showing the sampling site locations The influence of the Jiulong Rive, with fesh water run-off (white arrows) and the oceanic water of full salinity (blask arrows).

Since 1960, the authors have, from the side of time and space, taken a series of stydy on the species, quantities, attaching season and distribution of fouling organisms in Xiamen Harbor (24° 26′ N, 118° 4′ E), China, and their relation to environment (Huang et al. 1984) This paper is the part of distribution topic, about tidal zone . The part about sublittoral zone will be reported in other paper. It took five years (1960-1964) to finish the field investigating tests and an in site check was done in 1984, after finishing the analysis.

MATERIAL AND METHOD

Xiamen Harbor is a semi-esturial harbor in subtropical zone, tide of which is semi-diurnal tide. Its possible largest tide range reaches 723 cm and average tide range is 390 cm (Sun et al.,1981). In order to describe conveniently and according to the division by the author in the past (Huang et al., 1960), the tidal zone is divided into three zones in line with tidal levels: lower zone (tide level below 2 m), middle zone (2.0-5.5 m) and upper zone (5.5-7.2 m),

The concrete pilling of Shuixian Pier, Xiamen (Built in the 30s) was taken as the main site of the investigation and test. In the four seasons:spring, summer, autumn and winter, vertical distribution, thickness and cover area of the climax community on the pilling were measured on the spot and seasonal succession of species was recorded. Then quantitative samples (30 x 30cm) were scraped in each representative tide zones so as to do species identification and measurement of densities and wet weights of all species.

In order to find out the vertical distribution and succession of communities in the initial stage, during the whole test, a 10 cm wide 8 m long China fir lath was vertically fixed on the concrete pilling surface, from upper tidal zone to sublittoral zone. It is recovered and replaced by a new one seasonally or monthly.

RESULTS

1. The climax communities on concrete pier pilling

Species: 120 species are recorded totally. The distributions of these species in each tidal zone are apparently different. There are more and more species from upper tidal zone to lower tidal zone. Only 1-6 species are found in upper zone but as many as 22-45 species in lower zone. The highest records of quantities of 30 major species in 33 quantitative samples are enumerated. Each tide zone has its outstanding representative species. Among sessile or attaching species, Euraphia withersi is the only representative and dominant species in upper tidal zone. Its largest density reaches 23, 648 ind./m^2. The typical species of middle tidal zone is Ostrea echinata. There are quite many species with large quantity in lower tidal zone. Among them, the appearences of Spongia (Pachychalina variabilis, etc.), Hydroids (Obelia and Tubularia), Anthopleura pacifica, Dakaria subovoidea, Pomatoleios kraussii, Polydora ciliata and Corophium acherusicum in large quantity are all very typical signs of lower tidal zone.

Quantities: The highest wet weight record of fouling organisms on the pilling is 10.3 kg/m^2. Quantities differ greatly in different tidal zones. The wet weight is heaviest in middle zone, less

TABLE 1. THE HIGHEST AMOUNT RECORDED OF COMMON FOULING ORGANISMS ON SHUIXIAN PIER PILLING

Science names	Amount ind./m^2	Amount g/m^2	Sampling levels(m)	Sampling months
CHLOROPHYCEAE				
Ulva linza		115	1.5	FEB
PORIFERA				
Pachychalina variabilis		1038	0.5	FEB
Suberites carnosa		513	0.5	FEB
Rhizaxinella gracilis		744	0.7	FEB
COELENTERATA				
Tubularia mesembryanthemum		24	1.2	DEC
Obelia spp.		24	0.7	JUL
Lytocarpus sp.		30	0.5	FEB
Telesto sp.		2280	1.2	DEC
Anthopleura pacifica	1815	124	1.2	DEC
ECTOPROCTA				
Dakaria subovoidae		1743	2.0	APR
Electra devinensis		107	0.7	DEC
Cabarea boryi		41	0.5	FEB
POLYCHAETA				
Salmacina dysteri		88	1.4	OCT
Serpula vermicularis		247	1.4	OCT
Pomatoleios kranssii	1250	38	1.6	APR
Polydora ciliata	2625	550	0.5	FEB
SIPUNCULA				
Phascolosoma arcuatum	450	113	2.3	OCT
MOLLUSCA				
Ostrea echinata	2550	6107	4.3	OCT
Ostrea cucullata	1375	3441	1.4	OCT
Xenostrobus atrata	425	58	4.3	OCT
Perna viridis	132	281	1.2	DEC
Barbatia virescens	75	28	2.0	APR
Tectarius granularis	96	2	6.5	Nov
Tectarius vilis	32	1	6.5	NOV
CRUSTACEA				
Euraphia withersi	23648	1867	5.6	NOV
Balanus amphitrite amphitrite	9600	1278	4.3	DEC
Balanus reticulatus	1125	215	1.4	OCT
Corophium acherusicum	33000	66	1.2	DEC
Nanosesarma minutun	900	23	2.8	APR
OPHIUROIDEA				
Ophiactis savignyi	1800	123	0.5	FEB
ASCIDIACEA				
Didemnum sp.		125	0.7	DEC

heavy in lower zone and lightest in wooer zone (table 2). Large quantitied oysters in middle tidal zone are of long life cycle and thick calcareous shell and can attach layer by layer. So there is heavy wet weight. In upper tidal zone, Euraphia withersi is of small size and does not attach by layers, so the wet weight is light. In lower tidal zone, though there are many species, the wet weight is not so heavy as that in middle tidal zone because species with heavy calcareous shell are not dominant.

TABLE 2. THE CLIMAX FOULING COMMUNITIES ON THE SHUIXIAN PIER FILLING, XIAMEN

Tidal zones and levels (m)		No. of sampling	No. of species	Wet wt. (kg/m^2)	Dominant species
Upper (5.0–6.0)		4	1–6	0.60	Euraphia withersi
Middle	(4.0–4.5)	3	3–11	4.53	Ostrea echinata Balanus albicostatus
	(2.0–3.0)	4	3–21	5.61	O. cucullata B. amph. amphitrite Enteromorpha spp.
Lower	(1.4–2.0)	6	8–27	3.93	Spongia Dakaria suboviodae O. cucullata B. reticulatus
	(0.5–1.0)	3	22–45	3.05	Hydroids C. cucullata Perna viridis B. reticulatus

Vertical distribution: From upper tidal zone to lower tidal zone it can be generally divided into 5 zones.

1) Littorinid-Ligia zone: In the main, their is no sessile-living species from above 6 m level in the upper tidal zone. But littorinids (Littorina scabra, Tectarius vilis and T. granularis) and Ligia exotica (Isopoda) can be distributed to higher than 7 m.

2) Euraphia withersi zone: Tide level from below 6 m to 4.4 m. The species are simple and in large density.

3) Ostrea echinata zone: Oysters are distributed from below 5.1

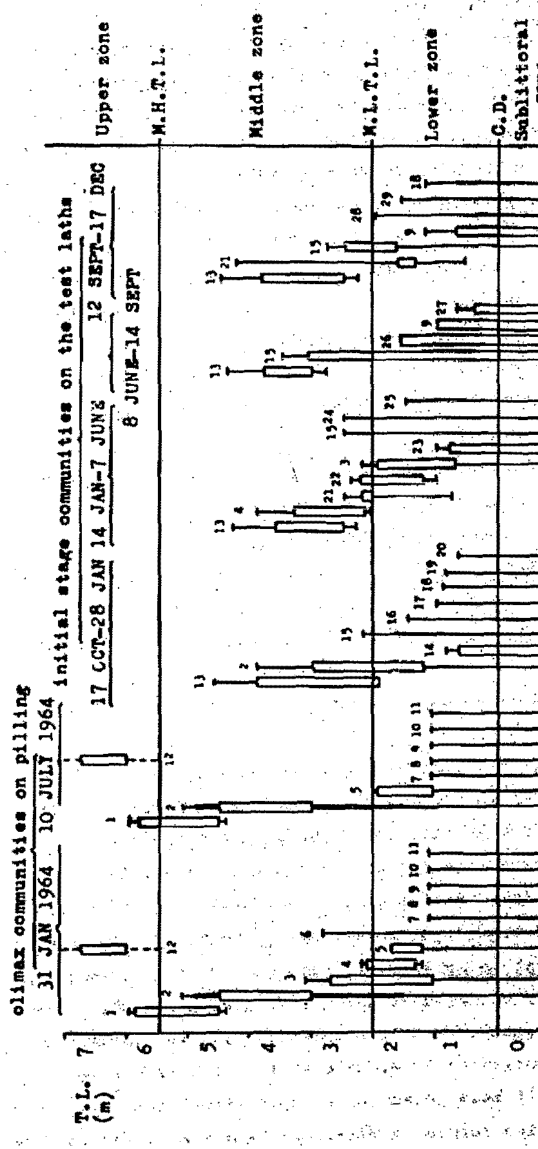


Figure 2. The vertical distribution of major species on pier pilling of Shuixian, Xiamen.

1. Euraphia withersi; 2. Ostrea echinata, O. cucullata; 3. Dakaria subovoidae; 4. Ulva and Enteromorpha; 5. Halichondria sp. and Rhizaxinella gracilis; 6. Chirona amaryllis; 7. Telesto sp.; 8. Hicksonella guishanensis; 9. Obelia and Campanularia; 10. Nellia oculata and Caberea boryi; 11. Salmacina dysteri and Sabellastrate sp.; 12. Littorinopsis scabra, Tectarius granularis and T. vilis; 13. Balanus amphitrite amphitrite; 14. Lytocarpus and Obelia; 15. Balanus reticulatus; 16. Membranipora amoyensis; 17. Megabalanus rosa; 18. Halocordyle disticha; 19. Pomatoleios kranseii; 20. Corophium acherusicum; 21. Ostrea echinata; 22. Spirorbis foraminosus; 23. Tubularia mesembryanthemum; 24. Ostrea cucullata; 25. Anthopleura pacifica; 26. Membranipora tuberculata; 27. Perna viridis; 28. Electra devinensis; 29. Sabellastrate sp.

m through to sublittoral zone.

4) Dakaria subovoidea-Spongia composite zone: The zone from below 3.2m to 1m is a composite zone of Dakaria subovoidea (Bryozoa), Spongia and other species. The vertical distrbution ranges of various species are different and have remarkable seasonal variation and mutual succession phenomena (Table 2). For example, Dakaria subovoidea begins to occur in the second and last ten days of October and grows flourishingly in February and March. It is of a blood red colour, almost completely covering the pilling surface from 2.7 to 1.0m tide level. It grows black and dettaches successively in the second ten days of May and completely disappears in June and July. For another example, Spongia exist all the year round, but grow particularly flourishingly in summer and can develop to higher tidal level.

5) Telesto-Bryozoa zone: From below 1.2 m, there occur various species of Coelenterata (Telesto sp., Hicksenella guishanensis, Anthopleura pacifica, Lytocarpus, Halocordyle distisha, etc.), Ectoprocta (Nellia oculata, Cabellastrate boryi, etc.), sedentary polychaetes (Salmacina dysteri, Sabellastrate sp., etc.) and Spongia. These species usually assume inlaid distribution and can be distributed to the deepest 15 m bottom of Xiamen Harbour. It means that dominant species in this zone are also shallow water species of sublittoral zone (Table 2).

2. Communities in the initial stage on the test lath

The five groups of vertical laths placed in different seasons lasted for a month and a half, three months (representing summer, autumn and winter respectively) and half a year respectively. The result shows that, like pilling climax communities, lath communities in the initial stage also have very apparent vertical zonation. But differences exist as follows: (1) In initial stage communities, species which grow fast and have short life cycles are the major. (2) There are less species and less quantity. (3) The upper limit of vertical distribution is lower than that of climax community and organisms haven't been found in the upper tidal zone. (4) Initial stage communities formed in different time are different from

TABLE 3. THE BIOFOULING AMOUNTS ON FIVE GROUP TEST LATHS IN EACH TIDAL LEVELS AT SHUIXIAN PIER

Months	Groups	Periods	Tidal leves (m) / Amount	0	1	2	3	4 - 5
One	I	1 SEP / 15 OCT	No. of species	4	3	2	1	
			Cover (%)	94	51	81	3	
			Wet wt. (g/m^2)	804	308	905	2	
Three	II	8 JUN / 4 SEP	No. of species	14	14	2	2	1
			Cover (%)	84	84	95	62	15
			Wet wt. (g/m^2)	1309	1309	1950	220	30
	III	12 SEP / 7 DEC	No. of species	3	7	2	2	1
			Cover (%)	100	98	98	90	15
			Wet wt. (g/m^2)	3100	1169	1910	1603	4
	IV	17 OCT / 29 JAN	No. of species	6	6	3	1	1
			Cover (%)	100	13	80	69	2
			Wet wt. (g/m^2)	279	152	1475	794	8
Six	V	14 JAN / 7 JUN	No. of species	10	6	7	2	
			Cover (%)	100	82	55	50	
			Wet wt. (g/m^2)	460	210	156	183	

each other. It is described concretely in the following (Table 3).

Group I (1 Sep.——15 Oct.): 7 species are found and the highest level of distribution is only 2.92 m. There is not bio-attachment at all in upper tidal zone. The major species in middle tidal zone are B. amphitrite amphitrite and O. echinata and in lower tidal zone are B. reticulatus, Membranipora tuberclata and Lytocarpus sp..

Group II (8 Jun.——4 Sep.): Just during the three midsummer months, 19 species are found and the highest tide level of distribution is 4.4 m. There is no bio-attachment in upper tidal zone either. B. amphitrite amphitrite predominates in the upper levels of middle tidal zone and the density of B. reticulatus in the lower tidal zone. Except B. reticulatus (5,000ind./m^2), Lytocarpus sp. is of large quantity and there are Halocordyle and Bryozoa too. Different tidal zone vary in growing speed as well as in species.

For example, the diameter of B. reticulatus in middle tidal zone is 12.5 mm, but reaches 15.0 mm in lower tidal zone.

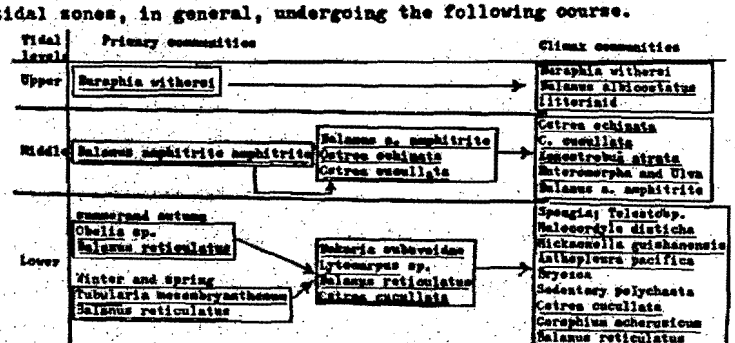
Group III (12 Sep.——17 Dec.): Just during the three autumn months, the result is very much the same as the community on summer lath.

Group IV (17 Oct.——29 Jan.): During three months in autumn and winter. The upper limit of bio-distribution is 4.6 m. From below this level to sublittoral zone, it can be divided into three quite clear biological zones: 3.9-1.9 tide level is B. amphitrits amphitrite zone (its density is 19,800ind./m^2 and cover area is 75%), 1.9-1.2 m is O. cucullata zone and 0.65 m below is Obelia sp. zone (cover area 85%).

Group V (14 Jan.——7 Jun.): This group lasted for 6 months of the first half of the year. The community is apparently different from those of the above group. There are many species in the community and the zonation is clearer. From top to bottom, the biological zones are B. a. amphitrite zone (3.6-2.5m), Ulva-Enteromorpha zone (2.05-1.20 m), Dakaria subovoidea zone (1.95-0.15m) and Tubularia mesembryanthmum zone (0.8m to sublittoral zone).

3. Community succession

The study on initial stage and climax biofouling communities shows: The climax community on pilling is in a relatively stable state, But has some change with time. Its formation, development and succession proceedings are absolutely different in various tidal zones, in general, undergoing the following course.



DISCUSSION

The biofouling community on the pier pilling in Xiamen Harbour is a transitional community between esturine and open sea: Xiamen Shuixian Pier is influenced by the open sea high-salinity water as well as by the dilution of Jiulong River. The salinity fluctuates greatly(3.4-31.8‰). The pilling biofouling community characterizes between open sea community and typical esturial community. For example, Euraphia withersi shows a band-like distribution in upper tidal zone at piers of Xiamen. It is also a dominant species in upper tidal zones southeast coastal inner bays and low-salinity waters in China (e.g., Aotou Pier at Da Ya Bay, Zhanjiang Pier, Guangdong, and Beihai Pier, Guangxi). This species is rarely found at offshore islands or open and high-salinity waters. For another example, Balanus uliginosus is a dominant species in lower tidal zones of all coastal esturial low-salinity waters of China. It almost completely covers the middle and lower tidal zones of Haicang Pier at Jiulong River Mouth, but is scarce at Shuixian Pier. Bugula neritina, Styela plicata and Hydroides elegans are widely distributed in southeast coastal inner bay high-salinity waters of China, but almost doesn't occur at Shuixian Pier.

Vertical distribution issue: The vertical distribution of organisms in tidal zone is closely related to tide level (Bastida 1980, Cai 1983, Haderlie 1976, Huang et al. 1960, 1980 and Morton 1983). The vertical distriouution ranges of various major fouling organisms on Xiamen pier pilling are greatly different (Table 2). They are not completely limited by different zones or levels of tidal zone. Therefore, we think that, in ecological studies on tidal zone, especially on that of shore with wide tidal range, quantitative sampling sites must depend on the distribution range of dominant species and, on the vertical distribution of major dominant species, it's better to measure on the spot.

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TOWARDS THE STANDARDISATION OF THE TEST EXPOSURE PROCEDURE IN THE TROPICAL SEA

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The present paper is an attempt to give an overall picture of the biofouling growth in Indian tropical harbour and discusses the recent field and laboratory results, as a first step towards the standardisation of the marine exposure procedure.

It is observed that larval recruitment of one or more members of each endemic animal taxa occurs almost simultaneously on the three varying areas within first 15 days of exposure. No biological succession, as evidenced by the simultaneous recruitment of the species of so diverse taxa as hydroids, bryozoans, cirripedes and ascidians, is noted.

In the present study 8 different kinds of substrates were used as test coupons and 4 different criteria were adopted to qualify fouling growth. If mere weight of the biofouling generated on these surfaces is considered then teflon coupons show a least amount of biofouling. The maximum weight is generated on asbestos but it is only marginally more than that noted on glass or bakelite. On teflon it is not the recruitment but a sustained adhesion of balanids that is affected. On the otherhand, if one considers the area covered by the fouling organisms as a criterion, then teflon appears to be no way better than most of the other surfaces, particularly if this fouling happens to be due to soft bodied species of bryozoa or ascidia.

The field experiments were carried out to ascertain the extent of variability of biofouling in relation to coupon size and number. The study show that sample size of 12 coupons, each of 180 sq.cm, is adequate for the near shore waters of Bombay.

Introduction

There is no unique solution to the problems of marine biofouling. Measures adopted with advantage in one situation or in one biotic environment may not be equally effective in other situations. Use of widely different materials and configurations, coupled with differences in quality and quantity of the biomass generated on immersed structures, separate one problem from the other. In this context therefore, biological studies become the most essential component of research affort.

In studies on monitoring of biofouling, it has been now an accepted practice to immerse non-toxic coupons from an exposure raft or a rack suspended from jetties or other water front installations. Several marine laboratories engaged in this task have developed raft exposure procedures suited to their individual requirements. Despite of extensive work reported from India on biofouling, one aspect of the exposure studies that has hitherto remained neglected is the standardisation of the exposure procedures. Unless there is a uniformly accepted method which stipulates the number, size, substrate and the disposition of the test coupons, it is not possible to compare biofouling results from any two laboratories located even in a small geographical region. The present paper is an attempt to give an overall picture of the biofouling growth in Indian tropical water, and discuss the recent field experiments carried out as a first step towards the standerdisation of the exposure procedure.

The importance of the surface quality and dimensions of the

test coupons is emphasised by several workers engaged in the studies on biofouling or the development of community structure of attached epibenthic marine organisms[1-5]. Jackson[6] has observed that "environmental or regional comparisons of fouling studies based upon data from different size substrate are subjected to serious misinterpretations". In Indian tropical waters where biofouling growth is almost a twelve monthly phenomenon[7,8] and where per unit weight generated due to this assemblage is two to ten times more than in temperate waters[9], an assessment of the importance of both the process of biological succession and the influence of varying substrate size is a worthwhile exercise.

Material and Methods

The study was carried out in the near shore waters along the Bombay coast (lat. 18° 55′ N; Long. 72° 50′ E) for a period of over one and half years during 1985-86. Three different sizes of perspex (polymethyl methacrylate) coupons were immersed for periods 15, 30 and 60 days, 1 meter below the low tide level. Dimensions and areas of coupons were 45, 90 and 336 cm^2. Twenty coupons in duplicates were immersed at the beginning of every month. To obtain the estimate of species abundance and area occupied on each panel, the grid method adopted earlier by Winston and Jakson[10] was used. The papers published earlier[11,12] give the details of the methods adopted here.

Observations

Fouling biomass

At this study station, fouling settlement is very severe all through the year and on no occasion it was less than 4 kg/M^2/month. The amount of debris generated within one month's period was far more than what is recorded in one year's period at most of the places in the world. At the end of 10 to12 months, the maximum growth built was around 10 kg/M^2 and after this period, the organisms either due to overcrowding or as a result of death got dislodged. The coupons immersed for a period of about 20-24 months might generate as much as 16 kg/M^2 of fouling in a situation like cooling seawater inlets of the power stations in tropical waters of India. The amount of debris that is likely to get dislodged and enter the flow of water can be placed around 3 to 4 kg/M^2/month.

The thickness of the fouling growth formed as a result of biological activity is of considerable interest to the maintenance engineers. A heavy accumulation as thick as 10 inches on structural elements of the offshore installations has been earlier reported in the north sea. Figure 1 illustrates the possible increase in the biofouling growth at various time intervals. It is estimated that at most of the sites along the Indian coasts, a fouling growth in about two years period will be around 4 inches thick.

Enumeration of the Recruitment

Table 1 summarises the data analysed for ascertaining the extent of recruitment of each animal taxa in the development of biofouling community on different substrates during 15,30 and 60 days. Encrusting bryozoans: The recruitment of six bryozoan species was monitored during this investigation. The average numbers of bryozoan colonies appearing on small, medium and large substrates were 19.62%, 17.4% and 14.28% respectively during the first 15 days. After an extended exposure of 60 days, however, the number of colonies developed on the largest substratum was significantly less than that on two other substrates. During the first 15 days, when the species composition was almost the same, no significant differences in the

: 1 - The recruitment rate of four animal taxa in the development of biofouling community on different areas during 15 to 60 days in Bombay harbour

	Average number of colonies											
	Bryozoans			Hydroids			Balanids			Calcareous polychaetes		
	15 days	30 days	60 days	15 days	30 days	60 days	15 days	30 days	60 days	15 days	30 days	60 days
	19.62±3.15	19.99±2.36	27.77±3.56	79.94±2.27	61.3±3.91	18.77±1.02	* -	9.62±2.26	2.59±1.33	1.11±0.69	9.33±1.58	Inadequate
	17.4±3.35	21.28±3.42	26.11±2.3	68.13±4.37	85.53±3.91	7.21±1.02	8.22±0.63	16.84±6.28	2.03±0.44	* -	7.21±2.0	- * -
	14.28±1.26	18.4±2.72	11.65±0.31	52.77±1.58	15.55±4.6	7.22±3.37	3.46±1.17	4.66±0.92	1.73±0.39	1.11±0.69	3.33±1.71	- * -
	t=0.48 NS	t=0.31 NS	t=0.39 NS	t=2.40 (P < 0.05) S	t=6.46 (P < 0.0001) HS	t=1.45 NS	t=2.53 (P < 0.05) S	t=1.08 NS	t=0.46 NS	t=0.62 NS	t=0.83 NS	
	t=1.60 NS	t=0.44 NS	t=4.41 (P < 0.002) S	t=9.40 (P < 0.00005) HS	t=7.57 (P < 0.00005) HS	t=1.10 NS	t=2.53 (P < 0.05) S	t=1.96 NS	t=0.62 NS	NS	t=2.60 (P < 0.05) S	
	t=0.87 NS	t=0.65 NS	t=5.95 (P < 0.0002) S	t=3.50 (P < 0.02) S	t=1.69 NS	t=0.002 NS	t=0.93 NS	t=1.94 NS	t=0.54 NS	t=0.65 NS	t=1.40 NS	

Key - S = Significant
NS = Not significant
HS = Highly significant
* = Inadequate growth

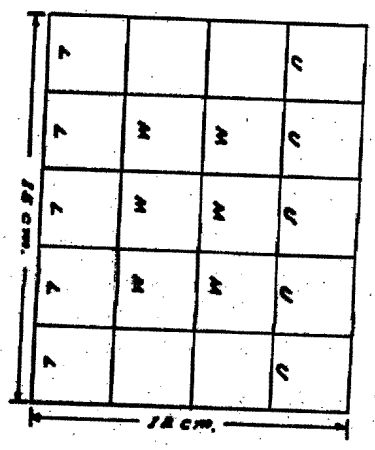

Fig.1 - Illustrates three arbitrary upper (U), middle (M) and lower (L) portions of test coupons immersed at the field station to study the choice of organisms for the settlement.

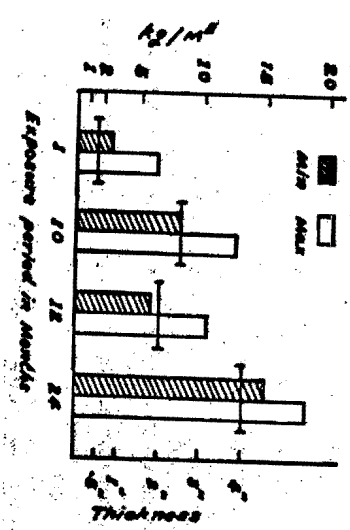

Fig. 2 - Biofouling growth (weight and thickness) on coupons exposed for various durations.

colony growths were noted from substrate to substrate. The limitation of space became apparent only after 30 days exposure. The growth on the smallest substratum was significantly slow than on the medium and the larger substrates. This happened despite the fact that there were no significant differences in the total number of colonies on these substrates. However, though there were no differences in the number of colonies, the differences in the species composition depending on sizes of coupons were evident. The reduced bryozoan growth on smaller coupons might as well be due to higher number of hydroids present on these coupons as earlier noted by Nishihira[15] and Rao & Ganapati[16]. By the end of 60 days immersion, however, the colonies fully covered all the surfaces, there being no significant differences in the areas covered from coupon to coupon.

Hydroids: The hydroids appeared in large numbers during the early 15 days exposure when the substrates were fresh. However, with passage of time, hydroids were found to be progressively reduced on all the three substrates. It is further evident from Table 1 that the recruitment of hydroids was significantly higher on the smaller surfaces than on the larger ones. And this was particularly true when the surfaces were clean during the first 15 days.

Cirripedes: Generally, larger the substrate more is the recruitment of balanids during the early 15 days immersion. With extended exposures no significant differences in the recruitments from substratum to substratum were observed.

Polychaetes : Hydroides norvegica - does not seem to prefer any particular substrate in terms of area and if the coupons exposed for a period of 15 days are any indication, there are no significant variations in the larvae recruited on the three substrates.

Site Selection Amongst Fouling Larvae

The larvae of several sedentary organisms, it is reported, choose specific sites for the settlement on the under water substrates[15-17]. Most of the sedentary organisms being gregarious, explore the surface before attaching to the sites of choice. Many species of hydroids select rather smaller areas than the larger ones for the attachment. It is, therefore, necessary that for a given environment this aspect is given due consideration in the studies related to monitoring of biofouling with the help of test coupons. It is noted by us for instance, that brown mussels do not settle on small size coupons.

With a view to examining the possible site preferences amongst the sedentary organisms, test coupons of 180 cm^2 were immersed and the settlement choices of hydroids, balanids and both erect and encrusting bryozoans were recorded. 10 to 15 coupons were immersed at this site for a period of 30 days during monsoon, premonsoon and post-monsoon months.

Each test coupons as illustrated in Figure 2 was arbitrarily divided into three zones, namely upper, lower and middle portions. Each of these zones covered 45, 45 and 54 cm^2 respectively of the total area of the coupon. Each coupon was divided into 180 cm^2 quadrates and the number of individual organisms occupying each of these squares were examined under microscope for the census. The statistical significance of their preferences to these areas was determined by adopting Student 't' test[18]. It was observed (Table 2) that none of these organisms showed any significant choice for any of the three positions of the test coupons.

Table 2 - Showing statistically ascertained variations in choice of positions on coupons by four animal taxa.

Coupon portion	Barnacles		Hydroids		Erect bryozoans		Encrusting bryozoans	
	Mean number	't' test	Mean colonies	't' test	Mean colonies	't' test	Mean colonies	't' test
Upper	31.5±1.44		36.1±1.47		9.3±1.20		11.9±1.19	
Significance level, U x L		t = 1.23 NS		t = 0.15 NS		t = 0.46 NS		t = 0.58 NS
Lower	25.1±4.19		36.6±2.58		10.6±1.17		10.7±1.68	
Significance level, L x M		t = 1.05 NS		t = 0.14 NS		t = 1.41 NS		t = 1.20 NS
Middle	31.85±3.09		37.2±2.78		7.6±1.77		13.2±1.21	
Significance level, M x U		t = 0.10 NS		t = 0.34 NS		t = 0.79 NS		t = 0.76 NS

Key - NS = Not significant
U = Upper portion
L = Lower portion
M = Middle portion

Surface Quality and Fouling Stability

A variety of materials are being used as test coupons in biofouling monitoring and assessment. Keeping in view that the organisms show varied responses towards these substrates, 8 different materials were used for preparing the test coupons and these were immersed in sea water for periods of 30 and 60 days under identical conditions in Bombay harbour. The coupons were examined under stereomicroscope and the counts of balanids and encrusting bryozoan colonies were recorded. Two other observations made were the percent area of each of the test coupons covered and the dry weight of the biofouling after 60 days immersion.

Table 3 summarises the results of the census made on 8 different kinds of surfaces. Dolgopal Ukaya [3] had earlier observed that "resting of steel panels reduced the value and reliability of the data" on biofouling he collected off the Crimean Coast of Black Sea. In the present study also, the data on mild steel coupons was not considered for the biogrowth comparison.

Dry biofouling weight : Table 3 will reveal that if mere weight of the biofouling generated on these coupons is considered, then the teflon coupons show the least amount of biofouling. While maximum weight is generated on asbestos coupons, it is only marginally more than that noted on glass and bakelite.

Calcareous growth : The number of balanids settled on teflon coupons is several times less than that settled on asbestos or bakelite. It is observed that on teflon, it is not the recruitment of balanids that is affected but it is the lack of adequate adhesion that dislodges the young, growing individuals. There is also a notable difference in the number of balanids settling on perspex, glass and asbestos coupons.

Table 3 - Ranking of some commonly used coupon materials as anti-adhesion surfaces.

	Criteria for the ranking			
	Dry wt. kg/m^2 (60 days)	Area covered (%) (30 days)	Barnacles No/m^2 (30 days)	Bryozoan colonies/m^2 (30 days)
Teflon	0.33 (A)*	90 (E)	607 (B)	1063 (E)
Rubber	1.55 (B)	15 (A)	646 (D)	600 (A)
Perspex	2.3 (C)	85 (B)	258 (A)	850 (C)
Slate	2.27 (D)	90 (F)	580 (C)	1111 (F)
Bakelite	4.48 (E)	65 (D)	14080 (F)	720 (B)
Glass	4.61 (F)	80 (C)	6746 (E)	1013 (D)
Asbestos	4.7 (G)	95 (G)	15586 (G)	1152 (G)
Mild-Steel	- -	- -	304 (-)	1514 (-)

* Anti-adhesion ranking A > B > C > D etc.

Encrusting growth: Differences are generally not noted in the numbers of bryozoan colonies developing on various kinds of substrates.

Area covered: Because of heavy settlement of encrusting bryozoans in this environment and also because of their ability to spread in sheet like colonial forms, all surfaces including teflon get readily covered.

From Table 3 it is very clearly evident that the ranking of the material as a good or a bad antifouling surface will vary with the criterion used in fouling assessment. For instance, if one considers mere weight of the biomass generated, then teflon emerges as the best fouling-free surface. On the other hand, if one considers the area covered by fouling, then teflon is not found to be any better than the rest of the surfaces. This situation in the present case, however, is due to cheilostomes which are light in weight but have good capacity to spread. Asbestos surface, however, has been found to be highly fouled, no matter which criterion is used for the assessment.

Table 4 gives force (Nm^{-2}) required for dislodging a bivalve Mytilopsis sallei from various surfaces. From these values reported earlier[20], it is evident that the forces required for the two polar surfaces, namely slate and glass are greater than that required for teflon.

Table 4 - Force (Nm^{-2}) required for dislodging M. sallei from various surfaces

Experiment	Force required (Nm^{-2}) ($\times 10^8$)			
	Slate	Glass	Perspex	Teflon
1	0.09	0.08	0.03	0.02
2	0.09	0.06	0.02	0.03
3	0.09	0.07	0.03	0.02

Coupon Area and Sample Size

With a view to determining the coupon size, field experiments were carried out in Bombay harbour. The coupons of three different dimensions in various numbers were periodically exposed to analyse the variations in the biofouling assemblage. In this study parameters like variability in species count, seasonality, as well as the size of the coupons and the exposure periods were examined in a cheilostome dominated environment. The data were statistically analysed and "trumpet" diagrams as suggested by Fellier[21] and English[22] were drawn to determine the sample size of the coupons.

Variability in Species Count

Figure 3 illustrates trumpet diagrams showing the upper and lower 95% confidence limits (expressed as percentage of the mean) plotted against sample size (number of coupons). The data represent mean number of sessile species on panels and is based on variance observed for 20 coupons held submerged for 30 and 90 days. The trumpets which indicate the width of the confidence intervals around the mean as a percentage mean, the confidence intervals generally span ten percent on either side of the mean and are further reduced by increasing the sample size beyond 10 to 12 coupons. The benefit of using more than 12 coupons will decrease with the increased sample size.

Figure 4 shows trumpet diagrams for the data collected during monsoon periods. In this case also, the sample size of 12 coupons appears to be adequate. The seasonality, therefore, does not seem to influence the similarity of biofouling growth.

Figure 5 illustrates trumpet diagrams representing mean number of sessile species on two different sizes of the test coupons (180 cm^2

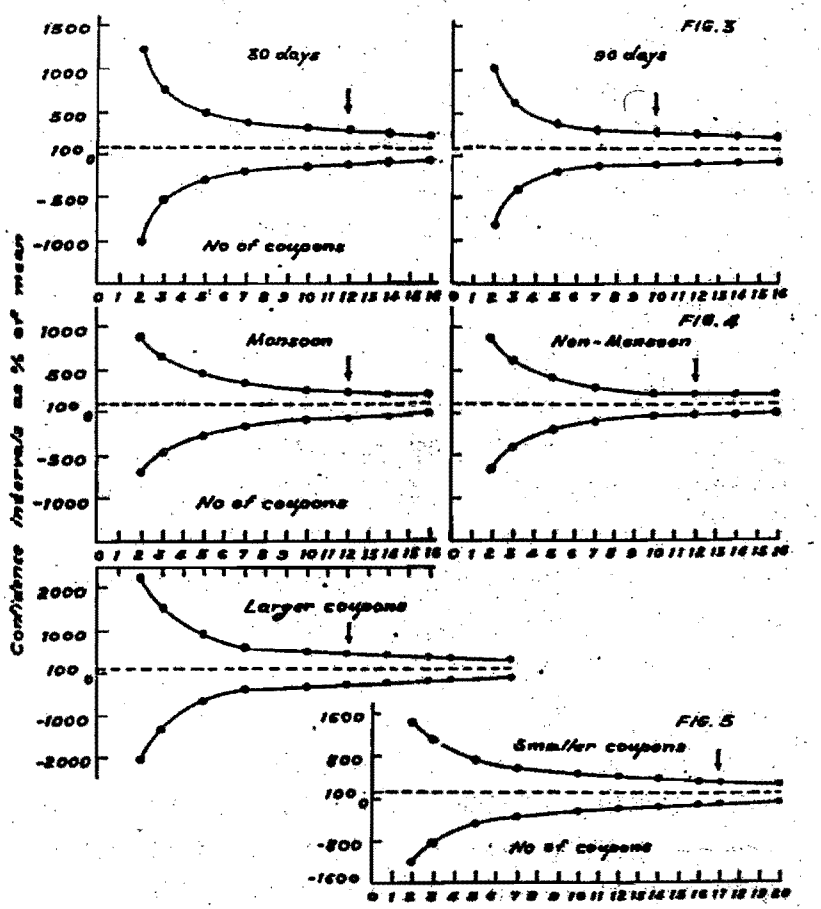

Figure 3 : The upper and lower confidence limits (expressed as a percentage of the mean) plotted against sample size (number of coupons), variance observed after 30 days and 90 days.

Figure 4 : Variance observed during monsoon and non-monsoon months.

Figure 5 : Variance observed on large and small coupons.

and 90 cm^2). It is observed that in respect of larger coupons, the confidence intervals are generally reduced to 10 percent at the sample size of 12 coupons. In respect of smaller coupons, the confidence intervals of 10 percent is reached beyond sample size of 17 coupons. It is evident, therefore, that with the decreasing size an increasing number of test coupons are required for obtaining statistically valid results.

Discussion

The present study was carried out with a view to ascertaining the extent of availability of substratum area and any specific portion of it mattered in the development of epibenthic community. The influence of surface quality on bioaccumulation was also studied.

It was observed that, the larval recruitment of one or more members of the four animal taxa occurred almost simultaneously on all the three substrates within first 15 days of exposure. As a matter of fact, the species composition, if not number, barring four erect bryozoans and a colonial ascidian, all through 60 days immersion, on all the three substrates, remained generally the same.

The common encrusting bryozoans appeared uninterruptedly throughout the study. Members of cirripedes, hydroide and serpulids appeared regularly on all the three substrates immersed. No biological succession, as evidenced by the simultaneous recruitment of the species of so diverse taxa as hydroids, bryozoans, cirripedes and others, was observed in the present study. Almost all the species continued to appear on the substrates but ultimately disappeared as a result of cheilostome species that were dominantly present at the site. In this case bryozoans did emerge as a climax community but it did not develop through a process of succession as the term implies. A

situation like this is typical of Indian waters. The emergence of a climax or rather dominant community at a given place is not through a process of succession but is entirely dependent upon the preponderance of one or two species endemic to that water. Climax communities of balanids, mussels or serpulids are identified in the Indian waters. It is not the discrete selection of the raw surface or the selective preference for the covered surface or the biological succession that decides the emergence of climax community. It is a rich larval density, a superior spreading ability and the overgrowth capacity that decide the emergence of climax community in Indian tropical waters.

It was observed that serpulid Hydroides norvegica showed no preference to any particular area. Balanus amphitrite preferred a larger area to smaller one and hydroids very conclusively preferred smaller area to larger one.These variations in preferences,as a matter of fact are necessary for ensuring maintenance of epibenthic diversity patterns in the nature.

In the field exposure studies related to the assessment or the forecasting of the biofouling growth, the test coupons of widely varying sizes are being employed. This has created problems in comparison of data collected from different localities. It is believed that the differences cited in fouling biomass may not be entirely due to the variations in larval densities or the hydrographical conditions but may be because of the size of the coupons.While this might be true, the present study has also brought out that the sizes of the coupons may not be that important particularly in an environment such as this, where one animal taxa, cheilostome for instance, prevails dominantly. The dominant species irrespective of area, emerges as a sole space occupier. It is, therefore, necessary that while ascertaining the

significance of size and the number of test coupons, the characteristics of the environment in respect of interrelations between the dominantly present species and the others are adequately understood.

While reporting on the density of fouling in various world harbours, it has been a practice to mention the weight of the biomass that is generated on the test coupons. The test coupons used by these workers, however, may not be of the same size and material. This creates difficulties in comparing the data from different localities. The present work has very clearly brought out that the nature of the substratum does influence the development and the sustenance of the fouling growth. Besides, it is not only the nature of the substrate but also the kind of criterion one chooses to qualify fouling that would introduce variations. For instance, it is observed that if one considers the weight as a criterion, then teflon emerges as a least fouled surface. On the other hand, if one considers the area covered by the fouling colonies as a criterion, then teflon appears to be no way better than most of the other substrates. Particularly if such a fouling happens to be mostly due to bryozoans, then identifying teflon as a fouling prone surface could be misleading and erroneous. Bryozoans are excellent surface occupiers but their contribution in terms of weight is very small.

In reality, teflon does not permit sustained adhesion of calcareous organisms. For instance, balanids which contribute to weight, get easily dislodged from teflon surface. In our earlier study it was observed that a very small tensile force was required to pull or dislodge balanids from teflon surface than say, from slate or a glass substraum . It is observed that teflon is no effective antifoulant or repellant but it certainly is a very poor substrate for the adhesion.

The present study has very clearly suggested that if not the recruitment of the larvae, the sustained adhesion and growth of the fouling organisms depend on the nature of the substratum. This aspect therefore, must be taken into consideration in choosing the material for test coupons. A uniform choice of coupon material as well as uniform criteria of assessment will permit the comparison of fouling data generated even at distantly located geographical sites.

The field experiments were also carried out in Bombay harbour and the variability of fouling in relation to coupon size and number was examined. The similarity values calculated by Bray-Curtis equation for 15 coupons experienced at this site revealed a close similarity between the coupons. Schoener & Greene[23] who experimented in temperate waters have reported similarity amongst coupons numbering between 5 to 10. In the present case the similarity noted has been due to the dominent presence of cheilostomes which by their sheer competitive ability and reproductive capabilities constitute a major component of the bio-growth. In other tropical situations where balanids, mussels and others compete for space, the similarity amongst the coupons will be difficult to obtain. Both Boyd[24] and Sutherland[25] found in their studies the fouling community being extremely variable. The importance of the biotic environment and particularly of the dominant organisms endemic to the environment cannot be therefore ignored.

In the present study use of trumpet diagrams was made and a species count was used as a parameter for deciding the sample size of the coupons. It was observed that an advantage of sample size beyond 12 test coupons will decrease with the increasing number of coupons.An

immersion of 12 coupons each of 180 sq.cm. is considered as quite adequate, since variability in species counts between panels is negligible.

During this study it was also felt that the areas/dimensions of the coupons as well, would influence the sample size. This turned out to be true. Whereas for larger area (180 sq.cm), the number of coupons required was 12, for a small area (90 sq.cm), this number came to around 17 coupons.

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FOULING OF SEAWATER FILTRATION SYSTEMS OF OFFSHORE OIL PRODUCTION PLATFORMS

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ABSTRACT

Large volumes of seawater are filtered on offshore oil production platforms for use in reservoir pressure maintenance. The unique characteristics of seawater, in terms of chemistry and biology, can cause severe problems to the filtration systems and associated pipework. Fouling blockage is most severe during planktonic blooms, while in the longer term, biological corrosion of the system can be a considerable problem. Direct observations of filtration systems are combined with reports from the literature to provide an overview of seawater filtration problems offshore.

INTRODUCTION

Filtered seawater is used extensively in waterflood operations in the offshore oil production industry. Waterflooding pumps seawater into oil bearing strata in order to maintain pressure and force oil to the surface. Water injection is now used much earlier in the oil winning operation to maintain a constant pressure in the reservoir. Systems are working up to 500,000 barrels (80,000 m^3) of water per day (using several injection wells (Cubine and Ramdolf, 1973) and nearly all North Sea fields are now water injected.

Seawater carries contaminants such as clays, sand, bacteria and plankton, which can cause blockage of the well and reservoir, and increase the corrosion and fouling of well casings, other pipework and equipment. Filtration is used to remove these contaminants and it is the maintenance of the filters and associated system that can cause problems.

The typical specification for water quality is removal of 98% of all particles above 2um (Mitchell and Finch, 1981). This is usually accomplished in two stages, coarse filtration to 80-150um, and fine filtration to 2-10um. There are several types of filters used offshore, the main ones being; downflow sand, dual or multi-media, precoat, cartridge (backwashable or disposable) and tubular backwashing filters (Cubine and Randolf, 1973; Kaiser,1983) but the most important factors are efficiency, ease of operation, size and weight.

Considerable problems have been found with the filtration process, especially during planktonic "bloom" periods (Edyvean and Pearson, 1982). A better understanding of the characteristics of seawater and the waterflood systems in total would do much to improve both the quality of the water and the efficiency of filtration. Factors such as corrosion, blockage, bacterial growth, precipitation of both organics and scale and the effects of hydrogen sulphide should be taken into account when designing for filtration. These factors are discussed below using data from several seawater filtration systems.

MATERIALS AND METHODS

Regular raw seawater particle count data was analysed from two oil production platforms in the North Sea; Beatrice, 15 miles offshore in the Moray Firth in shallow water, and Thistle, 100 miles offshore, north-east of the Shetland Islands in deep water. The effects of suspended solids on 200um. and 80um. wire weave mesh and polypropylene cartridge backwashable coarse filters, and

2-5um. polypropylene cartridge fine filters removed from various North Sea sites, were studied using light and electron microscopy and chemical analysis.

RESULTS AND DISCUSSION

SEAWATER CHARACTERISTICS

To consider seawater as a 3.5% solution of sodium chloride is a considerable oversimplification. Almost every known element can be detected in seawater and there are considerable variations in pH, salinity, dissolved oxygen concentration and solids loading from site to site (Dexter and Culberson, 1980). Open seawater is considered very clean, containing 0.2 to 0.8 mg/l suspended solids in the North Sea (Mitchell, 1978, Carlberg, 1979). However, the presence of organics; either as living organisms (especially plankton) or the dissolved and particulate material derived from them, results in considerable filtration difficulties. The chief planktonic organisms which affect filtration systems in the North Sea are copepods, dinoflagellates and diatoms (Edyvean and Pearson, 1982). These all have durable exoskeletons, considerable amounts of external mucillage and internally stored lipids. They range in size from a few microns to a few millimetres (Edyvean and Sneddon, 1985). The amount of organic material in seawater can change considerably and rapidly during seasonal (spring and autumn in temperate waters) "blooms" of plankton, and it has been reported that more than 90% of suspended matter in seawater can be organic, (Matthews et al.1984). Not only is there a general increase during these periods but there can also be large very localised differences (Edyvean and Sneddon, 1985).

Particle count data in raw seawater at two sites in the North Sea

are shown in Figure 1. There is considerable variation, both between sites and with season at either site. The solids loading in seawater from Thistle, a considerable distance offshore, is low during the winter, but shows peaks during spring, summer and autumn, corresponding to various plankton "blooms". The solids loading in seawater from Beatrice, an inshore field, is considerably higher, reaching 14.32×10^6. against 2.1×10^6. for Thistle with peak loadings during the winter. These winter peaks, due to land run off and storm action stirring up shallow bottom deposits, masks any peaks which could be attributable to planktonic blooms.

EFFECTS OF SEAWATER ON THE FILTRATION AND ASSOCIATED SYSTEM

A diagram of a typical system is given in Figure 2. Seawater is lifted from the desired depth by pumps or gas lifts, chlorinated and may have scale inhibitors added. Coarse filtration is followed by deaeration, fine filtration and the addition of biocides and corrosion inhibitors. The injection pumps may be preceeded by guard or polishing filters. One important fact that the figure does not emphasise is the considerable amount of pipework in the system which has to be kept free from corrosion and fouling.

SEAWATER UPTAKE

The first problem is in the water uptake pipework. Growth of larger fouling organisms, can restrict or even plug uptakes and will increase the solids loading of the water. Chlorine is usually used to prevent this fouling and should be introduced at effective levels of around 0.5mg/l as soon as possible (McCune,1982; Mitchell, 1978). Other antifouling measures in the seawater intakes include biocidal paints and linings, and copper/aluminium biocide anode systems (Anon,1978).

FILTRATION

As filtration is being used to prevent the water blocking the reservoir rock, the problems that are being avoided in the reservoir are transferred to the filters.

The life span of fine (5um) disposable cartridge filters from one North Sea platform is shown plotted against season in Figure 3. The short life time, shown by the short lines and smaller intervals between lines is evident in April and May and late August and September, corresponding to increases in plankton.

Blockage of both coarse and fine filters is due to the large amounts of lipid and mucilage produced by planktonic organisms which "cements" inorganic and organic particles onto the filter (Edyvean and Sneddon, 1985). Scanning electron microscope examination shows the pores of coarse filters to be initially bridged by copepod and dinoflagellate exoskeletons held in place by this cement (Figure 4). This then gathers other particulate material and lipids and the blockage is compounded (Edyvean and Pearson, 1982). Any movement inherent in the filter will enhance entrapment and crushing of plankton, and very rigid (eg. wedgewire) filters, which do least damage to the plankton, will release the least lipid material into the system (Edyvean and Pearson, 1982). Blockage of fine filters is by smaller particles, again cemented by lipid material which has passed the coarse filters. Typical organisms and material which cause problems in the fine filters are shown in Figure 5.

FILTER FOULING

Inadequate biocide treatment of the filters will cause considerable impairment of their function due to insitu growth of bacteria, algae or other organisms on, or within, the filter media.

PROBLEMS AFTER FILTRATION

CORROSION

Corrosion, bacterial growth, and scale deposition will reintroduce particulate loading which can be worse than the original seawater. The most frequent causes of corrosion failures in water systems are due to Hydrogen sulphide (H_2S), oxygen and bacteria (Case,1961). Oxygen is removed using gas stripping or vacuum deaeration and chemical scavengers to a level of 0.1ppm or less. Prior to deoxygenation, corrosion resistant or nonmetallic pipework is required. After deoxygenation, steel can be used with care. However, deoxygenation of s system which is still rich in organic material can provide ideal conditions for anaerobic bacteria (Carlberg, 1979). These bacteria produce H_2S and iron sulphides in the system and considerably enhance corrosion. Unless controlled, they can reach the reservoir and turn sweet produced water and oil sour on breakthrough and it has been noted that virtually all seawater floods eventually become sour (Carlberg, 1979).

SYSTEM AND RESEVIOR PLUGGING

There are several mechanisms by which the injection well and surrounding reservior rock can become blocked (Barkman and Davidson, 1972). Seawater systems are prone to blockage by the formation of colloidal iron (from water or corrosion) in the presence of organics, and by the organics themselves, especially waxes derived from copepods (Mitchell and Finch, 1981).

Blocking of filters by organic material and particulates from seawater is almost inevitable. However, design and operational factors can considerably lengthen their lifetime.

To achieve maximum efficiency filtration systems should be designed for a particular site (Cappi and Blagden, 1981). Information of the type shown in Figures 1 & 3, together with detailed analysis of the variation in particulate material at a given site can be of considerable use in the design of an efficient system.

Whatever the system, the first aim should be to take the water from an area of least solids loading. There is considerable advantage to be gained by selecting the depth from which the water is taken and by being aware of other operations (such as the discharge of drilling muds) on the platform. Small, though often expensive, modifications of the seawater intake can result in ten fold increases in filter working life (Anon, 1978). Water from about midway between the surface and seafloor is thought to give the lowest solids loading (Carlberg, 1979) and 200 ft. below sea level has become standard in the North Sea (Mitchell, 1978). Automated sampling and analysis of seawater from various depths, together with some knowledge of the effects of various plankton and other particulates on the filter system, could be used to take advantage of diurnal depth variations in plankton loading. However, no filter system is likely to be able to cope with a dense cloud of plankton drifting past the water uptake and emergency shutdown procedures should be available.

Control of corrosion and bacterial activity after filtration is essential, as is a full knowledge of any added chemicals (some corrosion inhibitors will provide nutrients for bacterial growth). Precipitation of insoluble compounds (scales) can also be a problem where a change in the chemistry of the water due to, for example, chemical additions or mixing with formation water, causes an incompatibility (Case, 1960; Mitchell, 1978). H_2S

corrosion is very variable and not necessarily proportional to measurable H_2S concentrations. It has been observed that corrosion is usually mild in clean systems regardless of the amount of H_2S present while in fouled systems, corrosion can be severe despite very little detectible H_2S in the water (Case,1961). The corrosion product in clean systems tends to be a thin uniform and protective film, while in a fouled system bacterial activity produces deep pits filled with sulphide corrosion product (Case, 1961). Apart from direct action, iron sulphide produced in these conditions forms a galvanic cell with the steel and this accelerates corrosion. Chlorine is effective in controlling sulphate reducing and other bacteria providing no growths are allowed to develop on surfaces. Such "biofilms", imbedded in protective mucillage, are highly resistant to biocides (up to 2000 ppm of chlorine is needed to be effective (Bessems, 1983)) and "slugging" with other biocides then becomes is necessary for their control (Mitchell, 1978; Bessems, 1983).

CONCLUSIONS

Seawater filtration should not be considered in isolation from the rest of the waterflood system. As much information as possible should be gathered about the seawater characteristics at the site prior to a filtration operation and systems should be designed to enable water to be taken from varying depths when required. Filters can be designed to do minimal damage to planktonic organisms thus reducing the "cement" released into the system. Adequate biocide, corrosion and scale inhibition should be carried out and recorded. Efficient monitoring, designed into the system at an early stage and constantly used will help to maintain the system in optimum condition.

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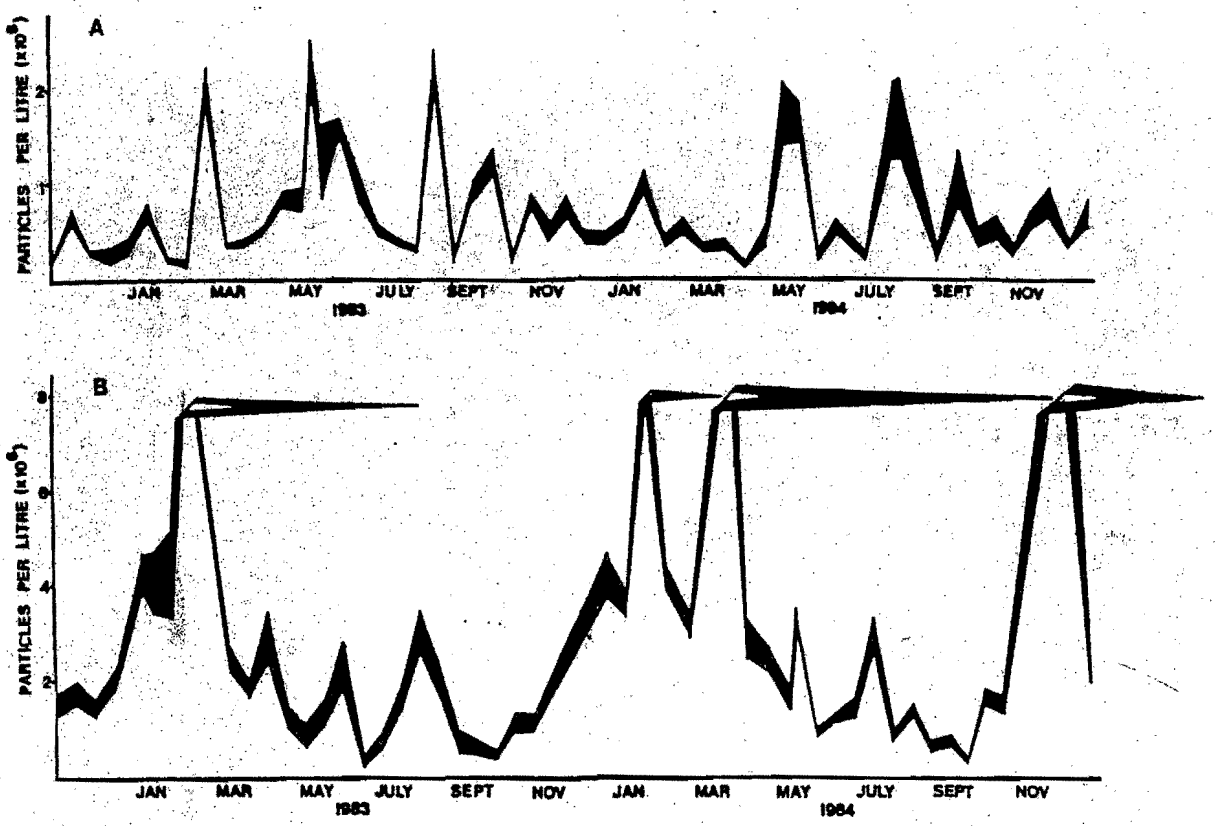
FIGURE 1. Particulate material >2um in raw seawater from (A) the Thistle field (100km. offshore), and (B) the Beatrice (15km offshore) North Sea fields. (shaded areas represent particles > 5um).

FIGURE 2. Schematic of a typical Seawater Filtration and waterflood system.

FIGURE 3. Fine filter life (5um disposable cartridge), in terms of throughput against season. From a Northern North Sea location.

FIGURE 4. Scanning electron micrographs of coarse filter blockage (80um fibre filters). A = Low magnification showing dinoflagellates, copepods and other blockage material. B = High power magnification showing part of a copepod, interlocked dinoflagellates, diatoms and other blocking material.

FIGURE 5. Scanning electron micrographs of fine filter blockage material (removed from the filters). A = general low power view, B and C = Foraminiferans, D = a coccosphere, E = a diatom , F = a small dinoflagellate and associated mucilage.



Schematic of a
Typical Seawater Injection
System

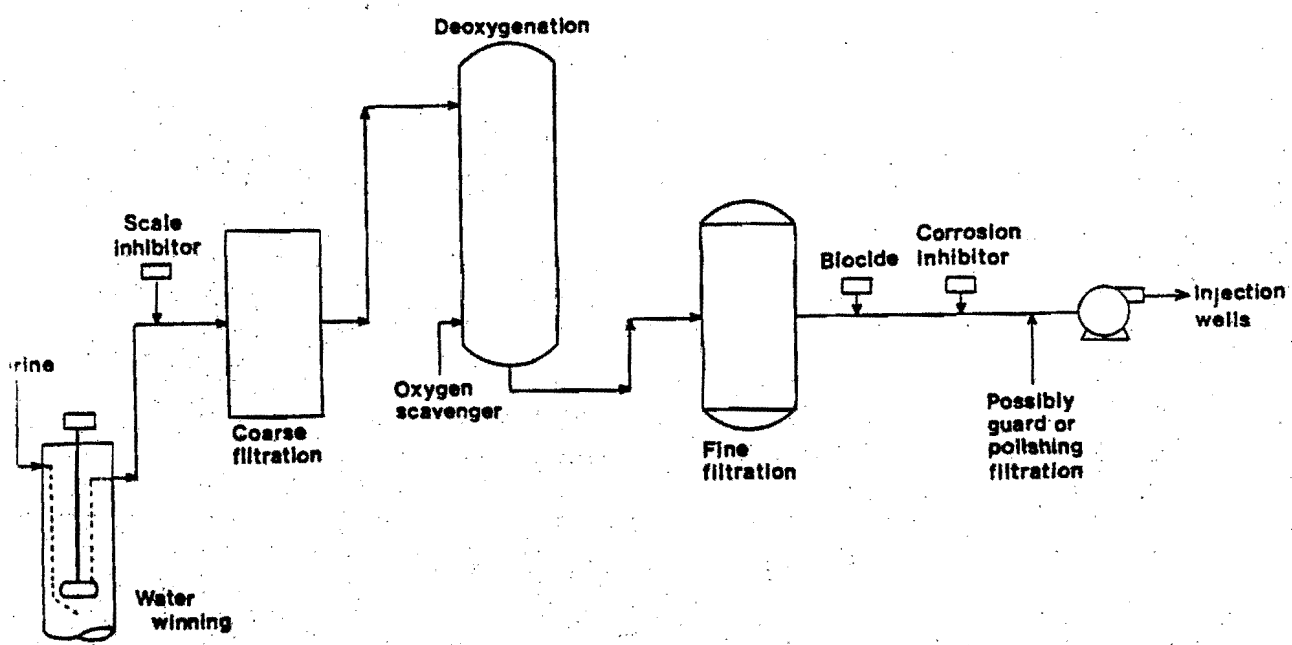


FIGURE 3

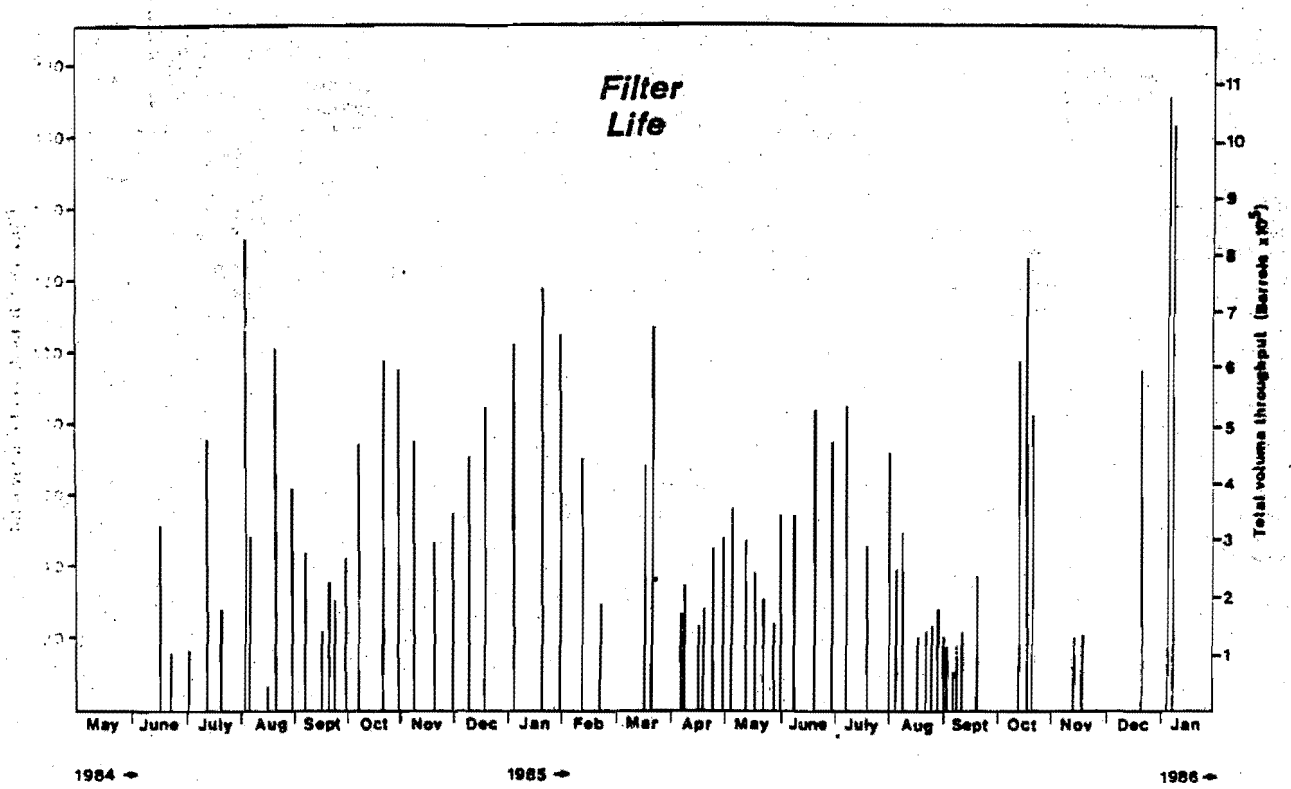


FIGURE 4

FIGURE 5

ECOLOGY OF FOULING COMMUNITIES AND THEIR IMPACT ON MARINE WOOD BORING ORGANISMS IN PORTONOVO COASTAL AREA-SOUTH EAST COAST OF INDIA

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ABSTRACT

Studies on the abundance of fouling communities indicate that the intensive settlement occurred during summer and premonsoon periods. The intensity of the attack of the marine wood borers are relatively higher in various mangroves such as Rhizophora mucronata, Rhizophora apetala, Avicennia marina and Avicennia officinalis where the rate of fouling is more. In contrast in Exoecaria agallocha, the wood boring activity is less where the fouling rate is more.

INTRODUCTION

The damages caused by fouling and wood boring organisms to underwater timber construction in marine environment is well known and is of great economic significance to all maritime countries having an expanding shiping and fishing industry. It has also been reported that these communities are capable of extending their destructive activity to living mangrove vegetation as well (Bowmann, 1917; Sivickis, 1928; Rehm and Humm, 1973; Estevez and Simon, 1975 and Day, 1975).

Mangrove ecosystems are also the shelter houses of many endangered species and also the varied faunatic and floretic features are suitable to act as reserves for rare species (for eg. Crocodiles; Whitaker and Whitaker, 1979 and Rollet, 1981). This significant ecosystem is the only immediate available substrata for foulers and marine wood borers. Studies on these organisms are fewer and different. Most are references to their presence in larger faunas (Van Name, 1920; Tabb and Manning, 1961 and Courtney, 1975), new distributional records (Ganapati and Lakshmana Rao,

1959; George, 1963; Nair and Dharmaraj, 1980 and Santhakumaran, 1983).

In India mangroves cover about 7,000 Sq.Kms. Sixty percent of the area lies in Bengal and 17 percent in Andaman-Nicobar islands. The world's largest mangrove, the Sundarbans in the Gangetic - Brahmabutra delta covers 10,000 Sq.Kms. of which about 4,000 Sq.Kms. lie in India and the rest in Bangaladesh. In Tamil Nadu, (South India) Vellar-Coleroon estuarine complex (Pitchavaram) is the biggest one which covers 2,640 hectares. Previously only preliminary surveys have been made (Nair and Dharmaraj, 1980; Meher-Homji, 1985) on the geographical-geomorphological set up of the area. The present study was carried out for one annual cycle to determine the nature of the fouling organisms associated with wood borers and certain aspects on the intensity of wood borers in various vegetations of mangrove swamps. In addition the selectivity of the species of vegetation by different borers, were also extensively studied.

STUDY AREA

The Portonovo is located on the coromandal coast, 250 Km. south of Madras. The mangrove of Pitchavaram (at 11°25'N; 79°47'E) is well preserved at the northern extremity of the Cauvery delta in South Arcot District (Fig.1). There are numerous channels in the mangrove linked with the sea on one hand and the rivers coleroon, Vellar and Uppanar on the other. This area offers great potentialities for mariculture and extensive fishing is carried out mainly by indigenous crafts like catamarans and wooden canoes. Considering the wide use of this estuarine complex and also the increasing the likely hood of activity of borers this Portonovo coastal area was selected for this study.

MATERIALS AND METHODS

Settlement of foulers and wood borers was determined by using wooden blocks of Mangifera indica (mango) (15 X 10 X 10 Cm in size). These blocks were conditioned for a period of fifteen days by soaking in filtered estuarine water (Nair, 1962). Subsequently two series of experiments were conducted. In one series, one string with 2 blocks of M.indica was tied vertically to a rectangular frame and anchored with a stone at the bottom of the water. In another series three strings each with two numbers of blocks were immersed at the same time and each string was taken one by one at an interval of one month during the post monsoon period (January-March). In the similar way, as many as 9 strings were employed during summer (April-June), pre monsoon (July-September) and monsoon (October-December). This would give an idea of the settlement for the respective period of immersion from one month to three months during each season. The use of this system of blocks furnished a fairly accurate record of the settlement of these organisms month by month and also during the different seasons of the year (Nair, 1965).

The collected wooden blocks were immediately sprayed with 5 percent formaline to collect foulers and wood boring crustaceans. The remaining organisms were isolated by scrapping and cutting wooden blocks into smaller pieces. The weight of the fouling materials were asscessed with the aid of a sensitive balance to study the occurrence of wood boring sphaeromids in relation to fouling materials. Salinity was determined twice per week by Strickland and Parson (1968) method. Temperature was recorded twice per week with the aid of a precalibrated thermometer. Surface water samples

were collected by a clean plastic buckets and were immediately filtered by using Whatman No.42 filter paper and a vacuum pump. The filtered water samples were deep frozen (in high density polythene bottles) in the laboratory for further analysis. Inorganic phosphate-phosphorous and total nitrite-nitrogen were estimated following the standard methods prescribed in APHA (1975).

The activity of the wood borers in various mangrove vegetations were studied by tagging method. Fifty numbers of living prop roots in each type of vegetation on which attack of borers was not observed previously were selected and tagged at the beginning of the severe attack season (Summer - see results) and were cut at the end of that season. The samples were wrapped with moist cotton and brought to the laboratory, and animals survived even for 48 hours in the same burrow (Pillai, 1961; John, 1968; Estevez, 1978).

RESULTS AND DISCUSSION

The present study indicates that forty six species of fouling organisms (Table 1) and six species of molluscan wood borers belonging to the family Teredinidae, one species of Pholadidae and 4 species of Sphaeromidae are causing much damage to the timber structures in different seasons (Table 2).

It is interesting to note that the incidence and intensity of different fouling and wood boring organisms show variations and fluctuations in different seasons and also in various mangrove vegetations. Among foulers, Rhizosolenia sp. and Biddulphia sp. are abundantly seen during

the month of April. Among macroalgae, Chaetomorpha Enteromorpha and Cladophora were seen abundantly during June to August. Barnacles were the dominant foulers in all seasons. Amphipods, isopods, tanaids and crabs were abundantly seen in mangrove vegetation and associated with other wood borers. Crossostrea sp. also seen throughout the season. The above observation revealed that the Pitchavaram mangrove is an annual fouling area unlike in sub-oceanic islands of Andamans (Karande, 1978). During the months of December through February the abundance of wood borers are comparatively less. This may be due to the paucity of the planktonic diet. The hydrographic relationship (Table 3) revealed that the increased temperature in conjunction with the increased salinity enhances the secondary productivity of zooplanktons (Edmonson, 1971). Correspondingly inorganic phosphate content values also increase upto 0.5 ± 0.06 µg at/l (Table 3). Total nitrate-nitrogen contents increased during the months of November through April, which effects an increase in the faunal production. This faunal increase is a prime factor for the intensive attack of the wood borers. More over different wood borers depend upon these microfoulers as their prey.

The 3-monthly cumulative attack of wood borers (Table 2) reveals that the intensity of attack increases during summer and pre monsoon seasons. This may be due to the accumulation of microfoulers like marine fungi and bacteria which facilitates intensive attack by sphaeromids by the conversion of wood cellulose into cellubiose since the digestive enzymes of sphaeromids have no effect on cellulose of wood as reported by George (1963). Three species of sphaeromids were found to occur abundantly during the summer

season when the fouling rate was high (Fig.2). During the monsoon season, the fall in fouling rate which results in a scarcity of food for wood borers (Estevez, 1978) may be one of the factors responsible for the low settlement of sphaeromids.

The intensity of attack of wood borers varies in different type of mangrove vegetations. Sphaeroma sp. bore into the living prop roots from the outside, producing a multitude of minute holes which give the stem a lace like appearance. The results of the present study revealed that S. terebrans attacks intensively on R. mucronata, where as S. anandalei on A. marina and E. agallocha.

Observations on the pattern of vertical distribution in the roots of live mangrove trees show that the wood boring sphaeromids always attack intensively in the intertidal zone and consequently the burrows encountered in the intertidal area of prop roots have been more in numbers. The ability of the sphaeromids to tolerate the prolonged exposure to air (John, 1968), their wide salinity tolerance (Cheriyan and Cheriyan, 1968), competition for space with the molluscan wood borers which are very active in deeper levels (Nair, 1966), might be some of the factors which increase their intensity in the intertidal zone.

During the present investigation, different vegetations were subjected to fouling diatoms, macroalgae, annelids, oysters, modiolus and barnacles. This showed a decrease intensity of fouling on the selected vegetations in the order of R. mucronata, A. marina, A. officinalis, E. agallocha, S. apetala and S. maritima. The ship worms exhibit high attack in R. mucronata, R. apetala, A. marina and A. officinalis (Table 4) where

the rate of fouling is more. Nair (1962) also reported in Western Norway that the stations which registered heavy fouling showed serious ship worm infestation. In contrast in the case of E. agallocha, the ship worm attack is less even though the fouling rate is comparatively higher. This may be due to the chemical nature of the latex present in it as observed by Turner (1976) in Dalbergia. From the above observations, it can be concluded that the incidence and intensity of the wood borers and the other associated foulers vary according to the nature of the timbers or vegetations. A proper understanding of these organisms and the different aspects of biology is basic formulate measures for the conservation of mangrove forests and other water front timber structures against their attack. The action and growth of these pests could be controlled only by a unified efforts of specialists belonging to fields such as ecology, biology, taxonomy and biochemical toxicology.

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No.	Species	Jan	Feb	Mar	Apl	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
20.	Lepas anatifera var indica anandalei	C	C	VC	A	A	C	C	R	R	R	R	R
21.	Lepas anserifera Linnaeus	C	C	C	VC	VC	A	C	R	R	R	-	-
22.	Cirolana fluviatilis Stebbing	C	A	A	C	VC	A	C	C	C	C	C	VC
23.	Tanais philetaerus Stebbing	R	C	A	A	VC	C	C	VC	C	R	R	R
24.	Parorchestia morini Peethambaram	-	A	A	A	VC	C	R	R	-	-	C	VC
25.	Ptychognathus altrimanus	-	A	A	A	VC	C	-	-	-	-	C	VC
26.	Melita zeylanica Stebbing	C	A	A	A	VC	C	R	R	R	R	C	VC
27.	Maera othonides Walker	C	A	A	A	VC	C	C	R	R	R	C	VC
28.	Parhyale hawaiensis (Dana)	C	A	A	A	VC	C	C	R	R	R	C	C
29.	Grandidierella megnae Chilton	C	A	A	C	VC	C	R	C	C	R	C	C
30.	Corophium triaenonyx Stebbing	C	A	A	C	VC	A	C	A	R	C	C	C
31.	Thalamita crenata	C	VC	VC	VC	VC	A	C	C	VC	R	C	C
32.	Sesarma quadrata	C	C	VC	VC	VC	A	C	C	C	R	R	R
33.	Sesarma minuta	C	C	VC	A	A	C	C	C	R	R	R	R
34.	Sylla serrata	C	C	VC	A	A	A	C	C	C	R	R	R
35.	Portunus pelagicus	C	C	VC	A	VC	C	C	R	R	R	R	R
36.	Musculus cumingianus (Dunker)	C	C	VC	C	C	R	R	-	-	-	-	-
37.	Mytilus viridus Linne	C	C	VC	A	C	C	C	C	R	R	R	R
38.	Crossostrea madrasensis Preston	C	C	VC	A	C	VC	C	C	C	C	C	C
39.	Cerithidea fluviatilis (Potiez and Michand)	C	C	C	VC	A	C	C	R	R	-	-	-
40.	Cerithidium rubra Bruguiere.	R	C	C	C	VC	A	C	R	R	R	R	R

Contd....

Table 1.

Seasonal abundance of fouling organisms in portonova coastal area during January 1980 - December 1980

No.	Species	Jan	Feb	Mar	Apl	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1.	Coscinodiscus ecentricus Ehrenberg	VC	A	A	VC	C	VC	C	R	R	R	R	C
2.	Coscinodiscus gigas Ehrenberg	A	R	C	R	R	VC	R	R	R	R	A	VC
3.	Rhizosolenia alata Bright well	R	R	C	R	C	-	C	VC	A	R	R	VC
4.	Rhizosolenia robusta Norman	C	A	R	VC	R	-	C	VC	A	A	C	C
5.	Rhizosolenia setigera Bright well	-	-	R	C	A	A	VC	C	R	-	-	C
6.	Biddulphia sinensis Gray	-	-	C	VC	A	VC	R	-	-	-	-	-
7.	Biddulphia mobidiencis Gray	-	-	C	A	VC	VC	R	-	-	-	-	-
8.	Nitzschia closterium (Ehrenberg)	-	-	-	-	A	C	C	VC	VC	R	-	-
9.	Oscillatoria princeps Drouet	-	-	C	VC	C	C	-	-	-	-	-	-
10.	Lyngbya sp.	-	R	C	C	C	C	VC	VC	-	-	-	-
11.	Trichodesmium thiebautii Gomont	-	R	C	R	R	-	-	-	-	-	-	-
12.	Enteromorpha intestinalis (Linnaeus)	-	-	C	VC	A	A	VC	VC	C	C	-	-
13.	Chaetomorpha linum (Miller)	R	R	C	R	VC	A	A	A	VC	R	R	-
14.	Cladophora expansa var glomerata	-	-	R	R	VC	A	A	A	VC	R	-	-
15.	Sea anemones	-	-	R	R	C	VC	A	C	-	-	-	-
16.	Nereis sp.	C	C	C	VC	VC	A	R	R	R	R	R	R
17.	Hydroides norvegica Gunneres	R	C	C	VC	A	A	A	A	A	VC	-	-
18.	Balanus amphitrite amphitrite Darwin	C	C	C	VC	VC	A	C	C	C	C	C	C
19.	Balanus variegatus Darwin	C	C	VC	A	A	A	C	C	C	C	C	C

TABLE - 2 The 3-monthly cumulative abundance (number of specimens) of marine wood borers in long term wooden blocks in Pitchavaram mangrove area during 1980.

Season	Series	Period	St	Sa	Sat	Sw	Ms	Tf	Lp	B.c	B.car	Nh	Bt
Post Monsoon	B1	Jan	22	..	..	..	..	10	2	..	..	4	8
	B2	Jan - Feb	27	..	..	..	17	42	14	12	17	12	4
	B3	Jan - Mar	38	..	..	6	87	104	31	17	61	16	..
Summer	B4	April	29	32	29	22	61	130	12	21	12	3	3
	B5	Apr - May	42	100	33	37	131	357	32	32	36	8	14
	B6	Apr - June	84	116	44	48	272	401	57	80	47	7	22
Pre monsoon	B7	July	13	12	2	7	12	72	27	127	56	..	..
	B8	July - Aug	29	17	8	..	27	112	62	164	87	..	..
	B9	July - Sep	41	68	17	..	34	164	88	187	112	..	..
Monsoon	B10	October	8	1	..	..	..	5	12	13	4	7	13
	B11	Oct - Nov	19	..	..	..	..	13	27	37	21	19	28
	B12	Oct - Dec	21	..	..	..	..	2	32	67	51	32	32

St - S. terebrans
Sa - S. anandalei
Sat - S. anandalei var travencorensis
Sw - S. walkeri
Ms - M. striate
Tf - T. furcifera
LP - L. pedicellatus
B.c - B. campanellata
B.car - B. carinata
Nh - N. hedleyi
Bt - B. thoracites
.. - Absent

No.	Species	Jan	Feb	Mar	Apl	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
41.	Telescopium telescopium (Linnaeus)	C	VC	VC	C	VC	A	A	A	A	R	C	C
42.	Nerita plicata Linne	C	VC	A	C	C	C	C	C	C	C	R	R
43.	Littorina Scabra Linne	C	VC	A	C	C	C	C	C	R	R	R	R
44.	Littorina undulata Gray	C	C	VC	A	C	C	C	C	R	R	R	R
45.	Perna viridis	R	C	VC	A	C	C	C	C	R	R	R	-
46.	Modiolus (Modiolus) striatulus Henley	R	C	A	VC	C	C	C	C	R	R	R	R

A = Abundant (75 - 90%)
VC = Very common (50 - 75%)
C = Common (25 - 50%)
R = Rare (Less than 25%)
- = Absent.

Table 3 - Mean hydrographic nutrient factors of Pitchavaram mangroove during January 1980 - December 1980.

Month	FACTORS			
	Temperature (°C)	Salinity (%)	NO_2-N (µg at/l)	Inorganic Phosphate (ug at/l)
January	26.5 ± 1.2	14.7 ± 3.6	1.7 ± 0.4	0.50 ± 0.06
February	25.5 ± 1.2	18.1 ± 2.6	0.73 ± 0.26	0.39 ± 0.01
March	28.2 ± 4.6	21.5 ± 2.6	0.5 ± 0.27	0.16 ± 0.03
April	33.4 ± 0.8	23.6 ± 5.6	1.0 ± 0.01	0.18 ± 0.12
May	33.2 ± 0.9	27.8 ± 4.7	0.14 ± 0.08	0.27 ± 0.08
June	31.0 ± 2.6	28.1 ± 4.5	0.44 ± 0.26	0.107 ± 0.04
July	30.0 ± 1.6	14.6 ± 2.7	0.73 ± 0.7	0.3 ± 0.047
August	29.9 ± 1.9	14.6 ± 3.3	0.32 ± 0.15	0.15 ± 0.03
Septemper	30.3 ± 1.7	13.5 ± 4.3	0.41 ± 0.11	0.34 ± 0.05
October	30.2 ± 1.0	5 ± 0.73	0.54 ± 0.16	0.34 ± 0.05
November	28.7 ± 1.3	3.7 ± 1.1	1.29 ± 0.6	0.48 ± 0.11
December	24.8 ± 0.9	5 ± 1.3	0.59 ± 0.11	0.22 ± 0.03

Table 4. Intensity of Wood borers in various mangrove vegetations

Sl. No.	Wood borers	VEGETATION						
		Rhizophora mucronata	Rhizophora apetala	Avicennia marina	Avicennia officinalis	Suaeda apetala	Suaeda maritima	Exoecaria agallocha
1.	Sphaeroma terebrans	A	A	R	A	VC	C	R
2.	Sphaeroma anandalei	R	R	A	R	C	C	A
3.	Sphaeroma anandalei var. travencorensis	R	R	..	..	C	C	..
4.	Martesia striata	A	A	R	..	..	R	..
5.	Teredo furcifera	A	A	R	..	..	..	..
6.	Lyrodus pedicellatus	R	R	A	A	..	..	..
7.	Nausitora hedleyi	R	R	..	..	..	..	..
8.	Bankia campanellata	..	..	A	C	..	..	..
9.	Bankia carinata	A	R	C	C	..	..	..
10.	Bactronophorus thoracites	A	A	C	C	..	C	..

A = Abundant (75 -90%)

VC = Very Common (50 - 75%)

C = Common (25 - 50 %)

R = Rare (Less than 25%)

.. = Absent

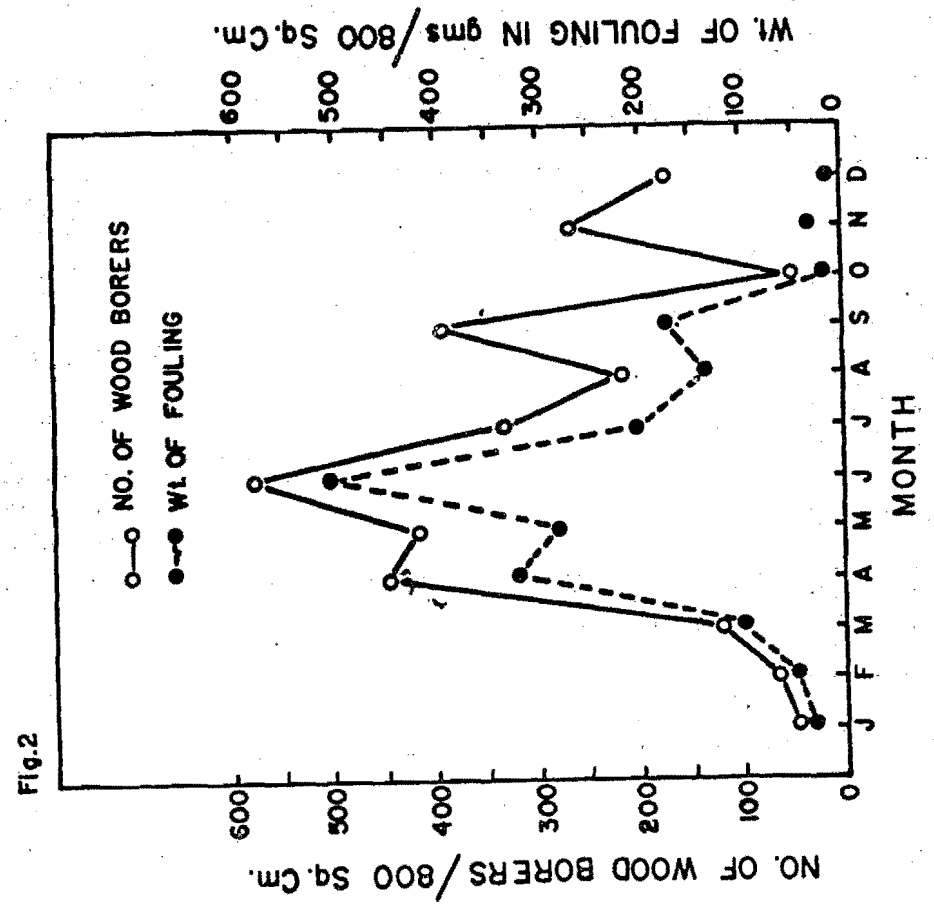
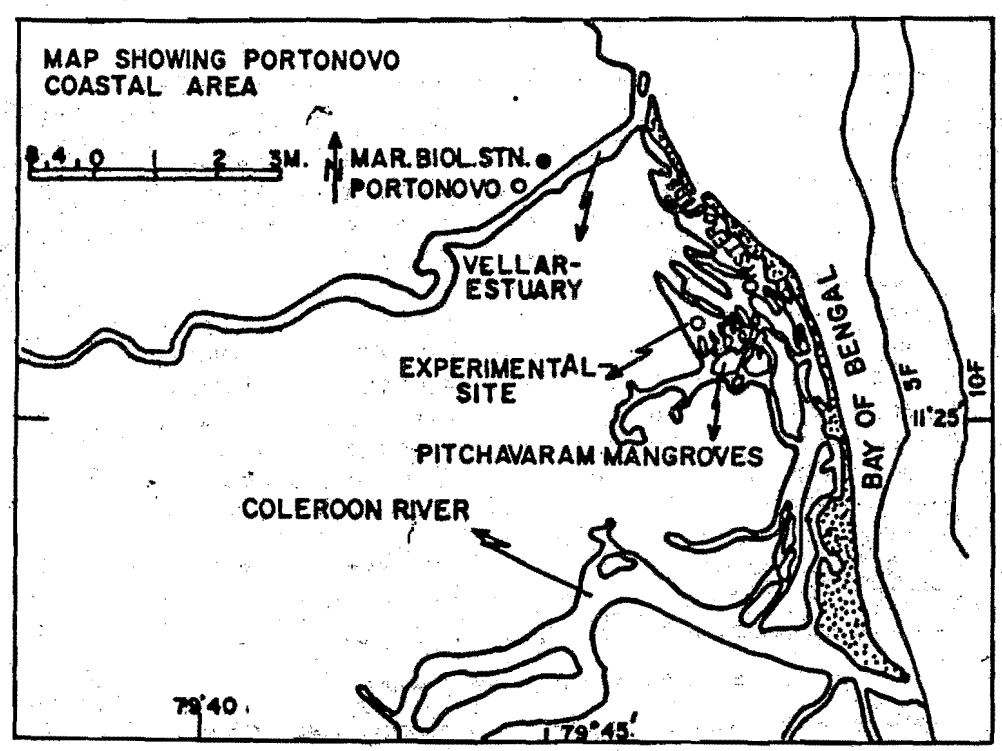

Wt. OF FOULING IN gms/800 Sq.Cm.
600
500
400
300
200
100
0
NO. OF WOOD BORERS
Wt. OF FOULING
J F M A M J J A S O N D
MONTH
NO. OF WOOD BORERS/800 Sq.Cm.

Fig.2

Fig. I


MAP SHOWING PORTONOVO
COASTAL AREA
0 1 2 3M.
MAR. BIOL. STN.
PORTONOVO
VELLAR-
ESTUARY
EXPERIMENTAL
SITE
PITCHAVARAM MANGROVES
COLEROON RIVER
BAY OF BENGAL
3F
10F
11°25'
79°40'
79°45'

Contribution pertaining to section II, biology and ecology of fouling organisms

Title: Micro-organisms cannot settle successfully on the surface of the sponge *Halichondria panicea*

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Abstract

The sponge *Halichondria panicea* Pallas usually harbours a large variety of infauna and inflora organisms in its tissue, especially in its water conducting channels. A number of organisms attempt to settle on the surface of this sponge as well, but are prevented from succeeding, as the sponge regularly peels off its outer tissue layer, thus keeping its surface clean. The micro fouling community found on the sponge peels comprises faunal, floral and microbial elements and shows a clear seasonal variation. Among the faunal elements are motile and sessile ciliates, nematodes and copepod larvae. The phytal compound is constituted by pennate and centric diatoms of both planktonic and benthic origin, but includes fine filamentous algae as well. By shedding its surface tissue, the sponge prevents the firm, long term establishment of these organisms and subsequent settling of larvae of other sessile macrobenthos, thus inhibiting further fouling processes.

Introduction

Halichondria panicea Pallas is a cosmopolitan sponge species occurring in coastal habitats with very varied conditions of light, currents and particle load. Notably, it forms extensive populations in waters with high amounts of suspended particles

like the North Sea and the Baltic Sea. Furthermore, *Halichondria panicea* has an extensive associated fauna wherever it occurs (Anger 1972, Bongers 1983, Costello and Myers 1987, Frith 1977, Peattie and Hoare 1981), which is partly inquilinistic like the hydrozoan *Dipurena spongicola* (Anger 1972) or the nematode *Leptosomatum bacillatum* (Bongers 1983), partly motile epifauna like *Caprella linearis* (Peattie and Hoare 1981).

In view of these manifold plant and animal associations it is noteworthy that the surface of *Halichondria panicea* is hardly ever fouled by other organisms. This is even more surprising, as the species has only weak antimicrobial activity, and does not produce bioactive substances inhibiting growth of algal propagation stages or preventing settlement and development of larvae of fouling organisms (Thompson et al. 1985). The production of mucus ,preventing settlement of fouling organisms on the surface of other sponge species, does not take place either (Thompson et al. 1985, own observations).
This study describes the seasonal variation in the organisms trying to settle on *H. panicea* and the sloughing off of surface tissue as the prime strategy of the sponge to prevent permanent detrimental surface fouling.

Methods

Between August 1987 and January 1988, specimens of *Halichondria panicea* were collected from various stations in Kiel Bight by either diving or dredging. Clean pieces of about 1-2 cm^3 volume with at least one osculum were cut off and tied to a glass slide following the method by Barthel and Theede (1986). The slides were inserted into small racks and kept in a 90 dm^3 aquarium at T/°C 13-15 and S·10^3 15-18. The running sea water was roughly freed of large particles by means of a sand filter, but contained small plankten and also detritus, part of which sedimented within the aquarium. The sponges were fed twice a week with a few drops of Liquizell, a suspension of finely powdered dry algae (Dohse Aquaristik Bonn) distributed in the aquarium.

Whenever tissue sloughing occurred, the tissue flakes were carefully collected from the aquarium and inspected for the adhering organisms.

Results

The sponges in the running sea-water aquarium attached to the glass slides within a few days after preparation and started to grow. Within two weeks the first tissue sloughing took place. The surface tissue changed its colour from yellow to brown and started to separate from the body at the rim of the osculum. In the course of 7 days, this process extended over the whole sponge surface, the shedded tissue falling off in large fragile flakes.

Microscopic inspection showed the flakes to consist of a dense layer of sponge tissue and spicules together with fragments of copepod carapaces and antennae, diatom frustules and sand grains. The shed tissue was inhabited by a variety of motile and sessile organisms: Either pennate benthic diatoms or chain fragments of planktonic centric diatoms were usually dominant (Fig. 1), but filamentous red and green algae were observed as well. The faunal community consisted of nematodes, motile and sessile ciliates (Fig. 2 and 3), turbellaria and larvae of copepods and polychaetes. Very conspicuous were large, red or green *Spirulina*-like bacteria which slowly moved through the tissue flakes.

The composition of the surface fouling community clearly changed with season (Table 1). While some groups like nematodes, motile ciliates and the green and red filamentous algae where present during the whole investigation time, other components, like larval stages of copepods and polychaetes occurred only at times, reflecting the seasonality of these species or stages respectively. There was no time during the investigation, when the sponge tissue would be free of potential inhabitants.

After the first tissue sloughing the process was repeated in experimental sponges regularly every three weeks for as much as 7 times (end of the experiment). During this period, sponges were

growing, some reached about three times their original size within 5 months. In order to check whether the sloughing would occur in detritus-free sea water, four *Halichondria panicea* were transferred after the first sloughing to an aquarium filled with filtered sea water. No further sloughing took place in these specimens during 3 months.

Discussion

In the presence of sedimenting material the sponge *Halichondria panicea* can clean its surface by sloughing off the outermost layer of tissue, thus getting rid of adhering debris and microorganisms. When no or little sedimentation takes place, the sponge stops sloughing.

This simple but effective strategy counteracts the obstruction of the ostia by sedimentation as well as by overgrowth by epizoic organisms. As potential settlers are present during the whole year, even in the winter months, this protective mechanism would have to be functional even at times, when *Halichondria panicea*'s overall metabolism is low. Metabolically, this is rather costly, as especially small sponges may loose a considerable part of their body substance, but the great advantage is that sloughing is apparently optional and only takes place when necessary. Most other adaptations in this direction are more permanent, like the production of antimicrobial and bioactive substances found to prevent fouling in some sponges (Thompson 1985, Thompson et al. 1985, Walker et al. 1985, Bakus et al. 1986). These mechanisms may, in the long run, entail as much or more energy expenditure than tissue sloughing.

Even in the absence of any other pronounced defence mechanism, sloughing off affected tissue seems to function sufficiently well to enable *Halichondria panicea* to survive even in coastal waters with an extremely high particle load like the Western Baltic Sea, where it occurs in extensive populations with high biomasses even in microhabitats with low current velocities and high sedimentation rates (own observations). The capacity to slough off tissue quickly enough to prevent being fouled is apparently

limited by low current velocities and high temperatures, as the bacterial infections in *Halichondria panicea* observed by Hummel et al. (1988) under these conditions obviously could not be fought off by the sponges: The combined effects of insufficient ventilation and establishment of a bacterial population in a time much shorter than that necessary for sloughing were lethal in this case. However, bacterial infections of sponges in the field seem to be quite rare (reviewed by Lauckner 1980) which indicates that bacteria can only develop when the sponges are weakened by unfavourable physico-chemical conditions.

The process of tissue sloughing has been found in other organisms before: Filion-Myklebust and Norton (1981) observed epidermis shedding in the brown seaweed *Ascophyllum nodosum* and Wahl (1987) found it to occur in the didemnid tunicate *Polysyncraton lacazei*: In both cases surface cleaning seems to be the main effect of the shedding process. In sponges, tissue sloughing has not been observed in the field at all, but Connes (1967) observed a similar process in the sponge *Thethya lyncurium* Lamarck. In the laboratory, this sponge reacted with tissue sloughing when microorganisms started to invade its surface. The process included mass formation of new spicules and was usually restricted to certain areas. After the loss of the original surface tissue, a layer of chiaster spicules lay bare and formed a protective layer. Such a change of surface structure could not be observed in *H. panicea*, probably because this species lacks special spicule layers below the surface that could be exposed.

It may be that tissue sloughing is widespread among sponges and that we only lack the respective observations due to difficulties with sponge cultivation in the laboratory. The chances to observe tissue sloughing in the field are slim, as it seems to occur only when sediment and micro-organisms become too disturbing, and currents and detritus feeders will very quickly destroy the tissue flakes.

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Table 1: Appearence of organisms in the sloughed off tissue flakes of *Halichondria panicea* during different times of the year. Amounts or numbers found: +++: very high, ++: high, +: small, -: not present.

Season / Content	September/ October	November/ December	January	February/ March	April
Copepods/ Exuvia	++	++	+	+	++
Nauplius	++	+	-	+	++
Tintinnids	+	-	-	-	-
Ciliates					
sessile	-	+	++	+++	+
motile	+++	+++	+++	+++	+++
Nematodes	+++	+++	+++	+++	+++
Turbellaria	-	-	+	++	+
Polychaete-larvae	-	-	+	++	-
"*Spirulina*-like bact.	+++	+++	+++	++	+
Ostracodes	-	-	+	++	++
sponge-spicules	++	++	++	++	+++
Diatoms					
pennate	+++	++	-	+	++
centric	++	++	+++	+++	+++
filamentous fine algae					
green	++	++	++	++	+++
red	++	+++	++	++	++
diatom frustules	+++	++	++	++	+++

Figure legends:

Figure 1: *Halichondria panicea*, tissue flake, September 1987. The tissue flakes contain many sponge spicules and pennate benthic diatoms are present in high numbers. Magnification 288x

Figure 2: *Halichondria panicea*, tissue flakes containing motile ciliates, November 1987. Magnification 461x.

Figure 3: *Halichondria panicea*, tissue flake inhabited by a sessile ciliate. A large, *Spirulina*-like bacterium is visible at the left hand side of the photo. Magnification 461x.

WOLFRATH & BARTHEL

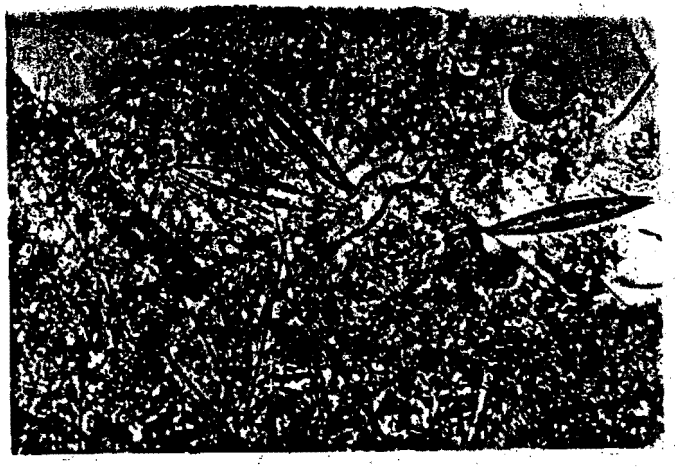
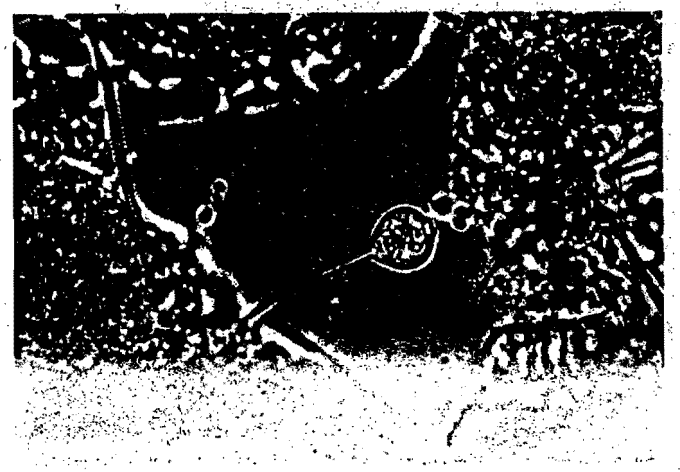


Fig. 1

Fig. 2



SELECTION OF ANTI-FOULING MATERIALS AND ITS ANTI-FOULING MECHANISM

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Abstract
In order to select the most suitable metal and economical material which can prevent the attachments of such marine animals as barnacles, mussels and etc, we immersed various kinds of non ferrous metals in sea water for 1.8 years and made clear the attachment behaviour of marine animals on these materials. From the obtained results, it was found that Cu-Ni-Mn alloy including appropriate amounts of Mn is effective for this purpose. In this paper, its anti-fouling mechanism is discussed by theoretical approach.

1. Purpose of this study

Prevention of various kinds of marine construction and heat exchanger exposed to sea water from the attachments of such marine animals as barnacles,mussels and etc is most significant for ocean application. Accordingly, with the aim of selecting the most sutitable metal materials for this purpose, we immersed various kinds of non ferrousn metal materials in sea water for long term and made clear the attachment behaviour of various marine animals on these materials. In addition, we tried to clarify the mechanism of preventing or promoting such marine animals attachments.

2. Experimental procedures.

Metal specimens are all non ferrous metal materials which are commercial pure Ti sheet, pure Cu,various kinds of 90Cu-Ni alloys whose Ni element is replaced partly by Mn-Fe, Ti, Al, Mn-Al, Mn, Mn-Nb, Mn-Si. Pure Cu and the other Cu alloy metals are molten in our laboratory and hot rolled to the sheets. The purpose of bearing various metal elements to Cu-Ni alloy is to change their corrosion resistance in sea water and to change the dissolution rate of Cu into sea water. Their surface was polished with emeri paper after mechanical shaping, and they were degreased with acetone and held in decicater. Their dimention is 5-10mm x100mm x 3mm. these metal specimens prepared in this way were set in the exposure equipment with float and held steadily at the depth of 1m from sea water level for 1 year and 10 months from February, 1984 to December, 1985.(Fig.1) The sea water immersion test was carried out in the sea area with the depth of 11m at the distance of 30m from the pier of Kimitsu Work of Nippon Steel in Tokyo Bay. The sea water showed the lowest temperature of 9℃ in winter season and the highest temperature of 23℃ in summer season. The pH value of sea water was 8.2 and the amounts ofdissolved oxygen in sea water was 6 ppm. The immersion period of these test specimens in sea water was as long as 1.8 years including 4 seasons and therefore, we could observe in detail the attachment behaviour of various spcies of marine life on these specimens. After the finish of this exposure test, all specimens were set off from the equipment and transfered immediately into the poly-ethylene vessels which were filled with 4% formaline. And in our laboratry, we examined the difference of the marine life attachments on each metal specimens by the detailed observation of their surface. (Photo.1) In addition, we tried to make clear the relation between the surface layer substances of metal specimens and marine life attachment. For this purpose, we picked up 3 kinds of metal specimens, pure Cu, Cu-Ni-Mn and Cu-Ni-Mn-Fe including 1% Fe which showed the great difference in marine life attachments and -growth and removed marine life as well as possible in the wet state. And then we separated the surface layers adherent to metal mechanically in the wet state and they were dried at room temperature and their crystal structures were analysed by X-ray diffraction method. (Fig.2)
The amounts of bearing metal to pure Cu is shown in Table.1

3. Results and Consideration

Comparison of the marine life attachments

From Photo.1, Non ferrous metals shown in Table.1 are classified as follows.
(1) Metal specimens whose surface was covered with numerous marine animals such as barnacles and mussels : Cu-Ni alloy bearing simultaneously Mn and Fe, Cu-Ni alloy bearing Ti, Cu-Ni alloy bearing Al and Cu-Ni alloy bearing simultaneously Mn

and Nb.
(2) Metal specimens whose surface was covered with tube worms, in addition to barnacles and mussels.: Cu-Ni alloy bearing simultaneously Mn and Si
(3) Metal specimens whose surface was covered with very few barnacle and no mussels. Metal specimens almost free from marine fouliing : Pure Cu and Cu-Ni-Mn alloy bearing appropriate amounts of Mn.
From Fig.2, the following results were obtained.
The main components of surface layer of Pure Cu are Cu_2O, $Cu_2(OH)_3Cl$.
The main components of surface layer of Cu-Ni alloy bearing appropriate amounts of Mn are Cu_2O, $Cu_2(OH)_3Cl$, γMn_2O_3, $3Cu(OH)_2CuCl_2$
The main component of surface layer of Cu-Ni alloy bearing simultaneously Mn andFe is FeS and tyny components are Fe_2O_3 and Mn_2O_3. The above mentioned Cu compouonds can not be detected in this surface layer.
The obtained results reveal that for the purpose of anti-fouling, the existence of $Cu_2(OH)_3Cl$ is inevitable as shown in the literature[7] and the co-existence of Cu_2O which can supply Cu^+ion steadily below the $Cu_2(OH)_3Cl$.
In addition, their stabilized precipitation at the metal surface for long term might be due to the existence of appropriate amounts of γMn_2O_3. This is in good agreement with the supposition of literatlure[7].
From the experimental results[3], semi-conductor theory[4] and ligand field theory[5], I showed the anti-fouling mechanism of Cu-Ni-Mn metal alloy in Fig.3. From this Fig, it is suppos ed that in this alloy, the control of the amounts of Mn is significant for anti-fouling effect. In fact, the 90%Cu-3%Ni-7%Mn was not effective and the 90%Cu-8%Ni-2%Mn was effective for this purpose. In addition, this anti-fouling model shown in Fig.3 suggests that in order to maintain this effect for long term, it is necessary to keep balance between appropriate thickness of Cu_2O including Ni^{2+} and appropriate oxydation reaction of Cu^+ to Cu^{2+}. It is well known that Cu^{2+} ion tends to create complex compouns with ligand such as Cl^-, H_2O, OH^-, and therefore the formation of such compounds as already mentioned can be easily explained.
In addition, it is said that the first step of marine fouling is the breeding of sulfate reducing bacteria in the

food chain in ocean environment. Consideration from this view point is as follows.
In order to guarantee the active breeding anaerobic bacteria sulfate reducing bacteria which is necessary for the first step of attachments of organic substance, it is inevitable that SO_4^{--} can approach to the substance surface which they can breed. But, the Cu^{2+} ion of 90%Cu-8%Ni-2%Mn seems to disturb such approach of SO_4^{--} and even if SO_4^{--} approaches to the surface of this alloy, Cu^{2+} tends to create $CuSO_4 5H_2O$[6] and therefore disturb the presence of SO_4^{--}. In general, it is said that this complex compound tend to change to Cu^{2+}-Organic ligand in case stronger organic ligand approaches. In any way, this characteristic of Cu^{2+}-ligand complex compound might be key substance which cause such a remarkable antifouling effect as above mentioned.
Cu-Ni alloy metal bearing as many as 1% Fe tends to promote the reduction of SO_4^{--} to S^{--} and the creation of FeS[6] by combination of dissolved Fe^{2+} ion and S^{--} and for this reason, the surface of such Cu-Ni alloy may be covered with the above sulfides and therefore, the above mentioned Cu compounds whcih are disagreable for mussels could not be created. In this case, the presence of Cu^+ ion with extreme high reduction force in the surface layer guarantees the stability of Fe^{2+} through the Oxydation-Reduction reaction of Cu^+ to Cu^{2+} and Fe^{3+} to Fe^{2+}[6]. In addition, the ability of oxydation reaction of Al, Si and Ti is much higher than Cu and therefore it is supposed that the surface of the Cu-alloy including these elements tends to be covered at least partly with these oxides and to decrease or disturb the formation of the effective Cu^{2+}-ligand complex.
At the surface of Titanium metal specimens, many amounts of mussels tended to attach and grow exceedingly. this might be due to the phenomena that the dissolved or floating organic substances, Detritus[7] in sea water tend to attach and accumulate in the Titanium Oxide layers adherent to Titanium metal, and therefore mushells larvas tend to attach to such nutrient-rich layers and to grow.
Comparison of corrosion rate of various kinds of Cu-alloys
For the purpose of applying this 90%Cu-8%Ni-2%Mn alloy to the marine structure aiming at anti-fouling, we have to offer the information on its thickness which maintains the anti-fouling effect during the period of practical use.

Therefore, we tried to determine the corrosion rate of this alloy. In order to avoid such mechanical damage as scratch in natural sea water, we immersed the polished and degreased test samples in Table.1 in artificial sea water for 1 year, by using beaker in laboratory. To avoid the accumulation of dissolved Copper ion, the artificial sea water was exchanged every 7 days. After 1 year, we could remove surface precipitates according to ISO/DIS 8407.3 standard. The surface of these test specimens were covered with very thin Cu film after this experiment. The blank test value of the orignal Cu-alloy metals showed that the weight loss is as low as 0.0001g which is negligible small.
The results obtained in this way are shown in table.2. In addition, we determined also the corrosion rate of these alloys in natural sea water during 1.8 years obtained in the same way as above mentioned although mechanical scratching damage in a part of test specimens wered observed. The obtained results are shown i Table.2. From Table 2,it was found that the corrosion rate of 90%Cu-8%Ni-2%Mn is 0.02 to 0.03 mm/year. Therefore, we can calculate the necesarry thickness from this data.
Now, we set the foil of this 90%Cu-8%Ni-2%Mn with thickness of 0.08 mm to the submerged pipe of oil platform by laminating organic materials at the surface of pipe. This experiment for practical use is shown in Photo.2 which is aiming the prevention of marine animals for the purpose of decreasing waving force. From Photo.2, it is clear that this alloy is effective for antifouling.

4. Summary

(1) The anti-fouling effect of 90%Cu-8%Ni-2%Mn which is economical than normal Cupronickel alloy was similar to that of pure Cu
(2) It was supposed that γMn_2O_3 plays the role of the control of Cu^+ to Cu^{2+} and keeping Cu^{2+} through its strong absoption
(3) Anti-fouling effect of Cu^{2+} may be explained by the supposition that Cu^{2+}-ligand complex compounds disturbing the approach of SO_4^{2-} hold the key for the first step of anti-fouling.

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Table.1 Test specimens melted in the induction furnace and hot rolled to 3mm in thickness.

Pure Cu
90% Cu- 8% Ni- 2% Mn
90% Cu- 6% Ni- 3% Mn- 1% Fe
90% Cu-7.5%Ni-0.5%Mn- 2% Si
90% Cu- 5% Ni- 4% Mn- 1% Al
90% Cu- 5% Ni- 5% Ti
90% Cu- 8% Ni- 2% Al
90% Cu- 3% Ni- 7% Mn
90% Cu- 7% Ni- 2% Mn- 1% Nb
90% Cu- 5% Ni- 2% Mn- 3% Nb

Ti prepared for this study is commercial pure Ti

Table.2 Corrosion rate of various Cu-alloys and Pure Cu

Spacimens	corrosion rate in artificial sea water (mm/year)	corrosion rate in natural sea water (mm/year)
pure Cu	0.029	0.036
90Cu-8Ni-2Mn	0.019	0.034
90Cu-6Ni-3Mn- 1Fe	0.009	0.007
90Cu-7.5Ni-0.5Mn-2Si	0.035	0.020
90Cu-5Ni-4Mn-1Al	0.004	0.019
90Cu-5Ni-5Ti	0.043	0.025
90Cu-8Ni-2Al	0.001	0.013
90Cu-3Ni-7Mn	0.034	0.038
90Cu-10Ni	0.016	
90Cu-7Ni-2Mn-1Nb	0.026	0.025
90Cu-5Ni-2Mn-3Nb	0.026	0.023
Ti	0	0

Corrosion rate was calculated from the weight loss of test specimens and their specific weight, for example the weight loss of pure Cu in artificial sea water(26.2mg/cm²/year)

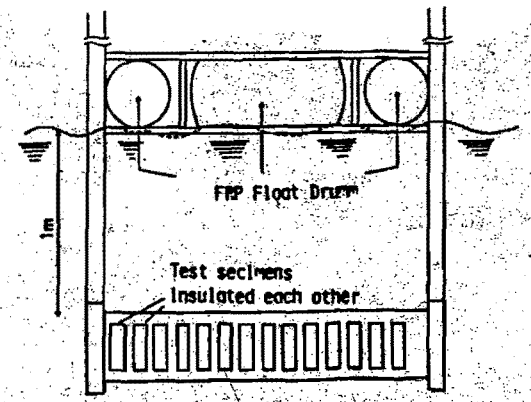


Fig.1 Exposure test of non ferrous metal in natural sea water

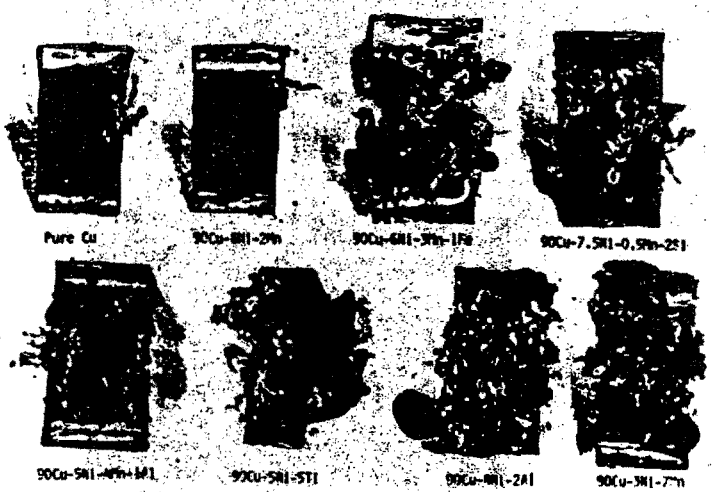


Photo. 1 Attachments behaviour of marine animals on the test specimens after exposure in natural sea water for 1.8 years

Photo. 1 [illegible] behaviour of marine animals [illegible] specimens [illegible]

Foils of 200 mm in width and 0.05 mm in thickness installed to
[illegible]

The repellent action of this foils against marine animals is clearly confirmed

Photo. 2 Field test of 90Cu-8Al-2Mn alloy for marine practical use

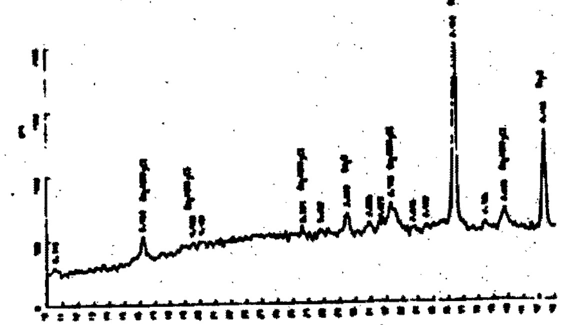


Fig. 2a X-ray diffraction pattern of the surface layer of Pure Cu immersed in natural sea water after removing marine animals

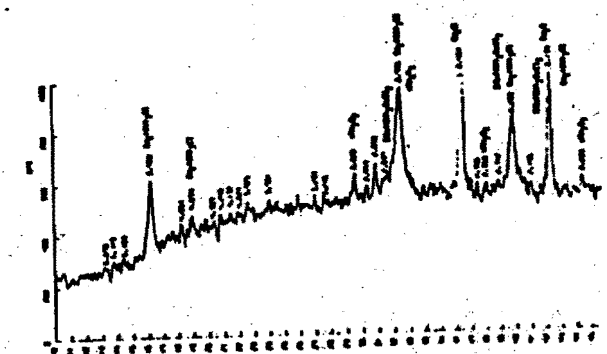
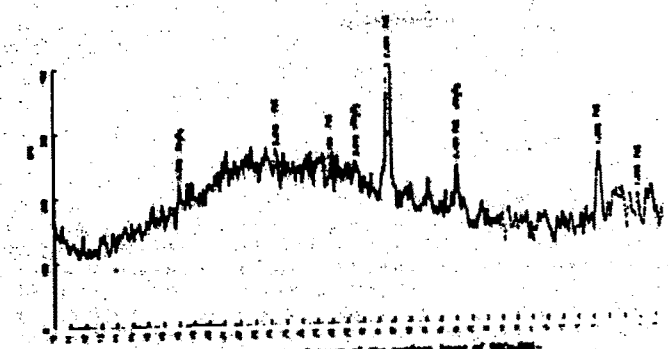
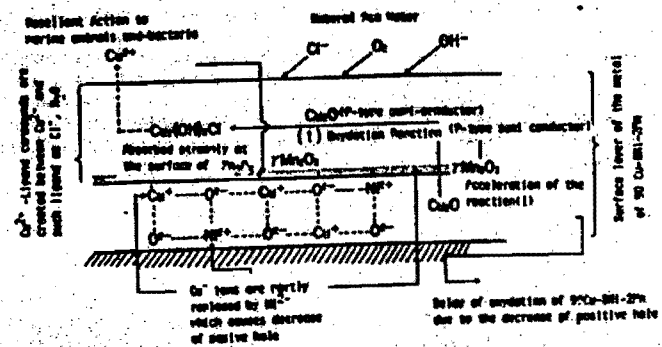


Fig. 2b X-ray diffraction pattern of the surface layer of 90Cu-8Al-2Mn immersed in natural sea water after removing marine animals




Cu²⁺
Cl⁻
O₂
OH⁻
Cu₂(OH)₃Cl
Cu₂O(P-type semi-conductor)
(1) Oxidation Reaction
(P-type semi conductor)
γ-Mn₂O₃
γ-Mn₂O₃
Acceleration of the reaction(1)
Cu₂O
Cu²⁺
O²⁻
Ni²⁺

Diversity and Seasonality of Microbial Aggregation-Adhesion Enhancing (AAE) Biofouling Macromolecules in Coastal Seawater

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Running Head: Surface Active Micro-algal Polysaccharides

ABSTRACT

Marine micro-algal and heterotrophic bacterial cells grown in laboratory culture produce a variety of macromolecules that mediate, and at elevated concentration, enhance their aggregation and adhesion to artificial surfaces. Aggregation-adhesion enhancing (AAE) materials are amino-nitrogen containing polysaccharide polymers. AAE macromolecules from different microbial sources show distinct specificities for cell surface receptors and artificial, metallic surfaces. Marine micro-algae and heterotrophic bacteria produce two major categories of AAE macromolecules, distinguished by the presence of immunogenic sites containing the simple sugar galactose. Concentrations of these AAE antigens varied seasonally in samples of ambient seawater taken along the southwest coast of Puerto Rico. This variability was related to seasonal fluctuations in microbial biomass, brought about by periods of increased rainfall and nutrient input (runoff), and coupled with increased production and release of microbial macromolecules in situ.

Marine micro-algal polysaccharides represent a class or group of surface interactive, polymeric macromolecules. These substances, with their broad range of potential immunogenic and carbohydrate receptor sites, may provide an appropriate framework for the design and construction of surfaces with defined interactive properties. A more precise understanding of these substances, and the living and artificial surfaces with which they interact, will aid development of needed biotechnologies to adequately modify and control the intensely competitive and rapid interactions in the marine environment between artificial surfaces, adsorbed soluble organic constituents and colonizing microbial cells.

I. Introduction:

Organic matter in the marine environment is composed of three components; dissolved organic matter (DOM), particulate organic matter (POM) and organic material found in living organisms (LOM). DOM is considered as that material which passes through a filter of pore size 0.45 micron and thus is comprised of organic substances in solution and organic colloidal material. POM consists of particulates, coated with organic substances and micro-organisms, and LOM is constituted by the organic contents of marine macro-biota (1). The relative concentrations of DOM, POM and LOM in the sea are found in proportions of 100 to 10 to 2, respectively.

Dissolved organic matter (DOM) adsorbs onto particulate, macro-biotic and artificial surfaces in the sea. Sufficient concentrations of DOM are found in surface waters to produce a layer of nitrogen containing material on all available surfaces (2). Carbonate minerals in suspension are coated by dissolved organic compounds (3). A measurable proportion of DOM found in surface waters is in the form of high molecular weight (HMW) substances (4). Concentrations of HMW constituents vary in ambient seawater, ranging from ug/ml in coastal areas to ug/l in offshore waters (5). These materials, frequently polymeric in nature, containing polysaccharide as well as peptide components, are not easily quantitated by conventional chemical techniques. Surface active HMW organic substances have a much greater tendency to adsorb and be held on particulate surfaces than small molecules (6). Particles suspended in seawater are selective with respect to the organic substances they adsorb (7). There is considerable evidence that HMW components found in ambient seawater adsorb to surfaces in the sea, forming an organic film that subsequently plays an important role in regulating microbial adhesions to these "conditioned' surfaces (8).

Marine bacteria adhere to glass slides, sand grains, chitin particles, and crude oil globules (9). Immobilized water in bacterial films formed on metallic surfaces exposed to flowing seawater sharply reduces their thermal conductivity. Changes in the thermal resistance were correlated with increased thickness of the wet biofilm on these surfaces (10). The attachment and growth of marine bacteria to surfaces exposed to ambient seawater constitute a necessary preliminary event in the subsequent colonization of these surfaces by macro-biota (11). Thus, surface associated marine bacteria play an important role in the settlement of "fouling organisms" on ship hulls and underwater structures (12). Marine bacteria attached on submerged surfaces release substances important in the "conditioning" of surfaces for subsequent attachment and growth of other forms. Both living and artificial surfaces display a considerable selectivity with respect to colonizing bacteria (13). Attachment and growth of cells on surfaces appears to occur in phases, involving an initial, "reversible" physical-chemical conditioning process, followed by a relatively "irreversible" cell attachment phase (14). Bacterial attachment to both animate and inanimate surfaces is mediated by polymeric materials exuded on the outside surfaces of these cells, and similar macromolecules found in solution in seawater. A film of these high molecular weight substances, imbedded with microbes, forms on surfaces immersed for relatively short periods in ambient seawater (15). The growth of attached cell populations is the final phase in this process.

II Microbial Aggregation-Adhesion Enhancement Activity

Surface interactions of marine microorganisms and their attachment to detrital particles and artificial surfaces in the marine environment are mediated by macromolecules found on these cell surfaces (16). Aggregation enhancing macromolecules released from microbial cell surfaces are found in cell free samples of both coastal and off shore seawater (17). These multivalent macromolecules are specific in their interactions with various artificial and microbial cell surfaces. Figure 1 illustrates the complexity of the processes that may take place between artificial surfaces (left hand side of Figure 1) "conditioned" by the adsorption of surface active macromolecular constituents found in the soluble pool of high molecular weight (HMW) materials in seawater (center portion of the Figure), and the complex outer layers of the microbial cell surface (right hand side of Figure 1). Little is known of the equilibrium between either of these interacting surfaces and the pool of HMW materials that mediate their attachment in the marine envirorment. The precise changes in configuration (and consequently surface specificity) that take place during the adsorption of HMW, adhesion mediators to artificial and/or living surfaces are not clear. In the work reported here the system employed to detect these macromolecules was based on the fact that their interactions with test cell surfaces promotes (enhances) cell aggregation and subsequently enhances or increases the number of cells adhered to artificial surfaces. This phenomenon is illutrated in Figure 2. The left hand side of the test surface shows a suspension of partially aggregated cells in equilibrium with those adhered to the surface at two loci. The right hand portion of Figure 2 shows a suspension in the presence of aggregation-adhesion enhancing (AAE) macromolecules, promoting the formation of aggregates and larger clusters of cells. While the number of surface loci at which cells are adhered remains the same, the actual number of cells remaining adhered to the test surface when it is removed from the suspension on the right hand side of the Figure, is significantly greater than that found in the untreated suspension (left hand side of Figure 2). Enhancement of aggregation leads to enhanced adhesion. Aggregation takes place in minutes following the exposure of microbial cells to these macromolecules, and may be independently assessed employing low angle light scattering measurements, whereas enhancement of their adhesion to glass takes three to five hours (16). Inhibition of aggregation enhancement with simple sugars prevents adhesion enhancement (17). Agents that promote aggregation (adrenalin), also enhance the adhesion of microbial cells to glass surfaces (18). AAE substances recovered from the culture media of marine micro-organisms are acidic polyanions, containing carbohydrate and amino-nitrogen moieties (19). Both micro-algal aggregation, assessed by light scattering, and adhesion, assessed by counting the number of cells adhered to test surfaces, are sensitive indicators of the presence of AAE macromolecules from divers sources, detecting the presence of these substances at concentrations of femtograms/ml (10^{-15} gm/ml).

III Immunological Isolation of AAE Macromolecules.

Macromolecular AAE materials found in solution in ambient seawater, on biofouled surfaces exposed to seawater and in the laboratory culture media of marine microorganisms are present at very low concentrations. Immunological methods have greatly enhanced our ability to detect and isolate AAE substances from both seawater and microbial media (20). These techniques are summarized in Figures 3 and 4. Crude macromolecular constituents were initially isolated by dialysis and size exclusion chromatography, from cell (particulate) free samples of microbial media, seawater and aqueous extracts of detrital matter adhered to biofouled metallic surfaces in ambient coastal seawater. AAE active components

in these preparations were selectively adsorbed on hydroxylapatite (HTP, BioRad) and subsequently recovered (Figure 3). Crude AAE polyanions from these individual sources were each used to make antibodies in the toad (Bufo marinus) and chicken. Respective antibodies were then immobilized to solid supports (aryl-amine glass beads and "affi-gels"; BioRad). Figure 4 illustrates the manner in which the immobilized immunoabsorbants are employed to isolate their corresponding AAE antigens directly from microbial media, seawater samples and detrital extracts. Particle free (filtered) media samples are exposed to an immobilized antibody preparation for a brief period, following which the immunoabsorbants are removed from the sample and washed in buffered artificial media. AAE antigens absorbed to their specific immobilized antibodies are eluted by exposing the immunoabsorbants to simple sugars (galactose) and/or low pH buffered media (ph = 2.2). Eluted AAE antigens are purified by dialysis and recovered by freeze drying. These techniques resulted in a significant purification of AAE activity. Antibodies were originally prepared to marine algal carbohydrates four decades ago, and since then have proven to be useful in basic and practical studies (21).

IV Specificity of AAE Macromolecules

A. Sugar Specificity.

AAE antigens recovered from divers cell free media of laboratory grown cultures of marine bacteria and micro-algal cells were found to be immunologically similar, however distinct in their interactions with receptors on test micro-algal (Chlorella vulgaris) cell surfaces (20). Table 1 illustrates the nature of this specificity and the distinction between the AAE active products of Chlorella and two bacterial strains, ASBl and NAS/OTEC. AAE antigens recovered from each source were tested for their aggregation-adhesion enhancement activity on Chlorella cells at concentrations of 0.1 pg/ml. AAE activities were determined in the presence of selected simple sugars (0.1 mg/ml) in order to assess differences in nature of the sites on the Chlorella cell surface with which each antigen reacted. Simple sugars employed included sorbose (S), mannose (M), galactose (GA), glucosamine (GO), n-acetyl glucosamine (AGO) and galactosamine (GaO). Table 1 summarizes which sugars inhibited the AAE antigen activity recovered from each of the microbial sources listed. Sorbose inhibited the activity of the AAE antigens recovered from Chlorella culture media, while the AAE activity of antigens recovered from the ASBl bacterial strain were inhibited by sorbose, galactose and glucosamine, and those recovered from NAS/OTEC bacterial cultures by mannose, glucosamine, n-acetyl glucosamine and galctosamine. AAE activity of each of the antigens was inhibited by a different set of simple sugars, indicating that these macromolecules interacted with different sites on the Chlorella cell surface.

B. Metallic Surface Specificity.

AAE biofouling polymers recovered from different artificial surfaces have been shown to be immunologically distinct (20). Titanium and aluminum surfaces exposed to ambient seawater rapidly acquire bacterial films and microbial populations. While certain strains of biofouling microbial cells are found on both, each type of surface is characterized by the presence of specific bacterial strains. Thus, in the coastal waters of Puerto Rico, a Pseudomnas sp. was found on biofouled titanium surfaces and not on aluminum, while Vibrio vulnificus was found on biofouled aluminum and not titanium surfaces. Immunoabsorbants were prepared to solubilized AAE macromolecules recovered from each of these metallic surfaces. The bacterial strains unique to each surface were grown in pure laboratory culture and their cell free media exposed to the prepared titanium surface (Surface T) and aluminum surface (Surface A) immunoabsorbants. Results of these analyses are illustrated in Figures 5 and 6. Figure 5 shows the analyses of the macromolecular products of the bacterial strain found only on biofouled aluminum surfaces (Strain A, V. vulnificus) and those of the strain found only on titanium (Strain T, Pseudomonas sp.) using the AAE immunoabsorbants recovered from the titanium surface. The molecular sizes (given in kilodaltons, 10^3 daltons) of the crude macromolecular products found in the cell free culture media of both species were determined using SDS gel elactrophoresis, as were the molecular sizes of the antigens recovered from these mixtures by the Surface T Immunosbsorbants. Thus, Surface T Immunoabsorbants recovered three (109, 90 and 40 kilodaltons) of the four (106, 86, 59 and 36 kilodaltons) macromolecular components produced by the bacterial strain not found on titanium surfaces (left hand side of Figure 5). The recovered antigens did not show AAE activity. Surface T Immunoabsorbants absorbed only one (157 kilodaltons) of the three (36, 84 and 169 kilodaltons) macromolecular components produced by the bacterial strain found on biofouled titanium surfaces, and this antigen had AAE activity (right hand side of Figure 5). Figure 6 summarizes similar studies of the macromolecular products of these two bacterial species, employing the immunoaborbants made to AAE macromolecules recovered from biofouled aluminum surfaces (Surface A Immunosbsorbants). One AAE active antigen was recovered from the macromolecular products of the bacterial strain found only on aluminum (Figure 6, left hand side), whereas the antigen recovered from the products of the strain not found on aluminum (Strain T) was inactive (Figure 6, right hand side). Artificial surface specificities of bacterial strains reside in part in the specificties of the macromolecules they produce to mediate interactions with that particular surface.

C. Microbial Source Specificity.

AAE active macromolecules are found in measurable quantity in particulate free (fitered) samples of seawater taken in coastal and offshore waters of the Eastern Caribbean Sea. The concentration of dissolved organic matter (DOM) in seawater is greater than particulate organic matter (POM), which in tropical environments in general is greater in concentration than suspended living organic matter (LOM). The steady state balance of DOM/POM/LOM in tropical seawater is approximately 100 to 10 to 2. Concentrations of dissolved high molecular weight organic materials (HMW) in the coastal waters of southwest Puerto Rico vary from 56 (fall and spring) to 7 (late winter and summer) mg/liter, with lower values in off shore eastern Caribbean waters. Approximately 20% of HMW materials are composed of AAE macromolecules (20). The presence of this activity in both seawater samples and laboratory culture media of heterotrophic marine bacteria and micro-algal cells suggests that AAE substances found in seawater are in part produced and released by marine microbial cells in situ. The role of microbial cells in this process has recently been directly demonstrated (22). Immunoabsorbants made to AAE active macromolecules recovered from coastal seawater samples selectively absorb two categories of AAE antigens. Antigenic sites on a portion of these AAE antigens are characterized by the presence of specific carbohydrate (galactose) residues, and hence may be eluted from their immobilized antibody components by low concentrations (micro gm/ml) of this simple sugar (20). The fraction of AAE antigens remaining absorbed to their antibodies in the presence of galactose can subsequently be eluted by low pH buffer (LPH antigens).

Microbial populations employed in laboratory culture to produce AAE active antigens are bacteria of the kingdom Monera and micro-algae of the kingdom Protoctiata (23). The major categories of antigens recovered from these two sources by immunoabsorbants made to AAE active macromolecules found in coastal seawater appear to be immunologically distinct. Immunoabsorbants made to seawater AAE macromolecules were employed to analyze macromolecules produced by laboratory cultures of bacteria as compared to micro-algal sources. Equally, macromolecular constituents found in coastal seawater samples taken in areas where microbial biomass was dominated by heterotrophic bacterial populations were analyzed and compared to similar constituents in samples taken in areas of constant, intense micro-algal blooms (coastal embayments of restricted water turnover). Results of these studies are illustrated in Figure 6. A bulk of the antigens recovered from the products of bacteria in laboratory culture were eluted by galactose. A similar quantitative pattern was seen in the antigens recovered from Seawater Sample I, a sample taken in an area where microbial biomass was dominated by heterotrophic bacteria populations. While a bulk of the bacterial antigens were eluted by galactose, AAE activity was only found in the antigens eluted at low pH (left hand side of Figure 7). In contrast to this, a bulk of the antigens recovered from products of micro-algae grown in laboratory culture, and those recovered from Seawater Sample II, taken in an area of intense microalgal productivity, were eluted from the immunoabsorbants at low pH. Among the micro-algal antigens only that fraction eluted by galactose had AAE activity (right hand side, Figure 7). Thus, the Chlorella, test cell surface distinguished between AAE antigens produced by heterotrophic marine bacteria and those elaborated by micro-algal cells. AAE macromolecules from bacterial sources appear to be immunologically distinct from those produced by micro-algae.

V AAE Macromolecules: Diversity, Production and Seasonality.

Table 2 summarizes the diversity in molecular size of AAE antigens isolated from microbial sources grown in laboratory culture, and those macromolecules recovered from samples of ambient seawater samples. The distribution of molecular sizes among AAE antigens suggests that they are polymeric in nature, with a monomeric unit of approximately 30 kilodaltons. The range of molecular weights recovered in the AAE antigens produced by bacteria and microalgae grown in laboratory culture were present in the crude, high molecular weight (HMW) components recovered from seawater samples.

The average production of crude HMW materials by laboratory grown cultures of marine bacteria and micro-algae, and the fractions of these materials recovered by AAE immunoabsorbants are summarized in Table 3, top two rows. Production of crude HMW materials, assessed after 24 to 48 hours of culture growth, ranged from 160 to 740 femtograms/cell. Active AAE antigens, those eluted by low pH (LPH antigens) buffer in the case of bacteria, and galactose (GAL antigens) in the case of the micro-algae were from 3 to 4% of these crude products. The production of crude HMW materials by bacterial populations adhered to aluminum and titanium surfaces exposed to ambient seawater was approximately 10x that seen in bacteria and micro-alge grown in suspension culture in the laboratory (Table 3, third row). The recovery of crude HMW components, and corresponding AAE active antigens, from particulate free coastal seawater samples taken in areas where microbial biomass was dominated by heterotrophic bacterial populations (SW I), as compared to micro-algal dominated sites (SW II), are shown in rows 5 and 6 of Table 3. Between 3 and 8% of these crude HMW materials (micro grams/ml seawater sample) were recovered in active AAE antigen fractions. Off shore seawater samples in the Caribbean contain lower concentrations of crude HMW materials, due largely to the relatively lower densities of productive microbial populations found in these waters (Table 3, bottom row).

Seasonal changes in particulates (detritus and microbial cells), macromolecular and AAE antigen contents of coastal seawater in southwest Puerto Rico are illustrated in a generalized form in Figure 8. This pattern was characterized by periods of energy input followed at some interval by cyclic fluctuations in particulate microbial biomass, soluble high molecular weight constituents and AAE antigens. Average concentrations of total antigens (those eluted by galactose plus glycine) varied in coastal seawater samples from 15.1 (SUMMER I) to 0.2 ug/ml (SUMMER II). Excluding SUMMER I values, peaks in antigen concentrarions were seen in the FALL and SPRING with minima in WINTER and SUMMER II. The fall season was the period with the greatest amount of total rainfall and, consquent increased nutrient input ("run off") into coastal waters. Thus, the fall was a season characterized by elevated particulate energy contents (PEC, ATP/filterable particulates), increased physiological activity and exudate production, resulting in relatively high concentrations of macromolecules and antigens. The winter season was characterized by low PEC levels and the highest sample particulate concentrations measured during the period in question (June 1983 through July 1983). Reduced levels of soluble macromolecules and antigens during winter may be the result of the uptake of these substances by micro-organisms and detrital particles. Similar to the events of the fall and winter, the second, major yearly rainfall in spring resulted in elevated PEC levels, and subsequent increased particulate populations in summer II. This sequence of events was similar to that of the fall. The more immediate response of particulate populations in spring to nutrient "run off" may be due to increased temperatures and light intensities over that of the fall-winter period.

Optical analyses indicated seasonal differences in the physical nature of the recovered antigens. The specific refractive increments (SRI, change in refractive index/mg test material) of antigens recovered from fall, spring and winter-summer II periods were significantly different from one another (Figure 9). Thus in addition to seasonal fluctuations in the biophysical characteristics of seawater samples, there were qualitative differences in antigens recovered during different seasons in coastal seawater.

VI Summary Comment.

Artificial surfaces introduced into the sea create complex microcosms. Marine micro-algal and bacterial surface interactions provide a useful model of this complexity, and may provide the means to control microbial surface colonization. Microbial competition for surface space is expressed through the surface active macromolecules they themselves produce. These multivalent mediators regulate interections among living, microbial surface receptors, as well as with artificial, man made surfaces. Adsorption of specific macromolecules determines the initial interactive natures of artificial surfaces, and ultimately the changes in specificities they will undergo in time. Structural modification of these macromolecules and/or introduction of chemical groups to alter either their initial adsorption or their specificities when adsorbed, may be an efffective means to regulate surface intersctions. A clearer understanding of the molecular structure of micro-algal,

aggregation-adhesion enhancing (AAE) polysaccharides, and design of the chemical and immunological means to modify their specificity, may represent a new focus and productive approach to the control of surface integrity in the marine environment.

High molecular weight (HMW) constituents of ambient seawater play an important role in the regulation of microbial surface interactions. Aggregation and adhesion enhancing substances have been recovered from biofouled surfaces exposed to ambient seawater. There are significant seasonal differences in the concentration and in the adhesion enhancing activity of the high molecular weight substances recovered from surfaces exposed to running coastal seawater. These macromolecules appear to be relatively stable in the marine environment for they accumulate on biofouled surfaces in the sea and in marine microbial culture media, and are found in samples of offshore surface water, as well as the Subtropical Underwater in the Caribbean (depth 130-140 meters), which is comprised of waters that flow into the Caribbean from areas as far away as the Sargasso Sea.

In the ocean, both living and artificial surfaces are sites of elevated microbial activity. Macromolecular adhesion enhancing materials and fractions immunologically isolated from high molecular weight (HMW) components of coastal seawater promote microbial-detrital particle clustering in these waters. Macromolecules that mediate surface recognition and specificity among these aggregates may play a fundamental role in their adsorption onto artificial surfaces. Tropical coastal marine environments are "patch works" of microbial clusters, embedded in a macromolecular fabric. The nature of these fabrics differ seasonally. The precise specificities of the macromolecules that shaped these surface micro-environments depended on both the nature and quantity of microbial producers and adsorbtive surfaces with which they interacted.

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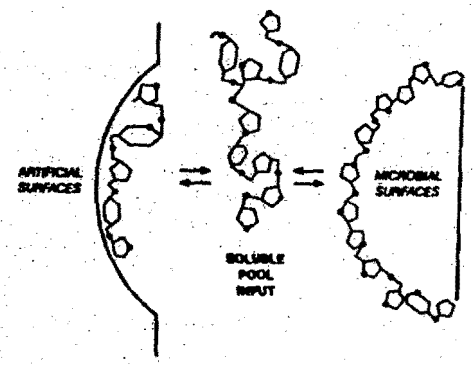

Figure 1: Macromolecular Adsorption and Surface Conditioning. Artificial surfaces with adsorbed macromolecules (left side of Figure), soluble pool of "conditioning" substances in seawater (center of Figure) and outer layer of microbial surfaces (right side of figure), connected by arrows indicating the unknown equilibria of macromolecular adhesion mediators. Changes in surface adsorbed, macromolecular configurations suggest alterations in "conditioned" surface specificity.

FIG. 1

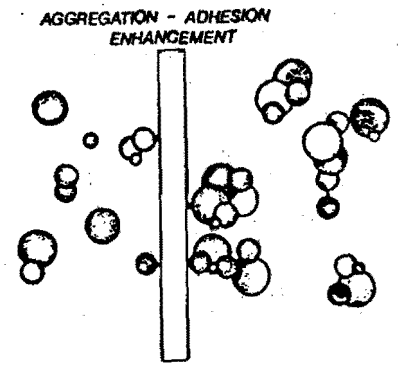

Figure 2: Aggregation-Adhesion Enhancement Activity. An artificial surface exposed to microbial cell suspensions in the absence (left side of Figure) and presence (right side of Figure) AAE macromolecules. Number of adhered cells increased in presence of AAE macromolecules due to cell aggregation.

FIG. 2

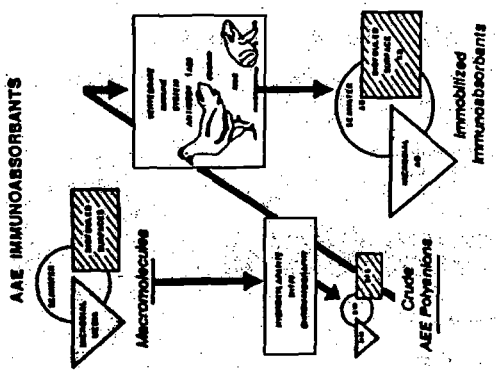

Figure 3: Production of AAE Immunoabsorbants. High molecular weight materials were recovered from cell free samples of seawater, marine microbial laboratory culture media and solubilized materials from biofouled surfaces. AAE macromolecules isolated from these preparations by hydroxylapatite chromatography were employed to make antibodies. Specific antibodies, isolated by reverse immunoaffinity chromatography, were immobilized on solid micro-bead supports for use as AAE immunoabsorbants.

FIG. 3

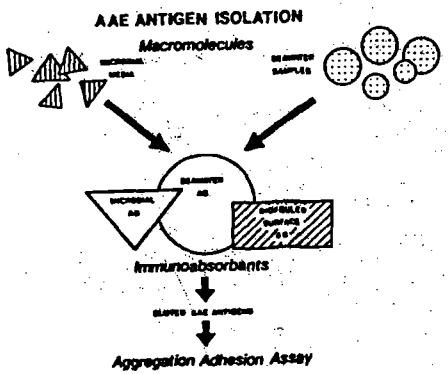

Figure 4: Recovery of AAE Antigens by Immunoaffinity Chromatography. Immobilized AAE immunoabsorbants were employed to recover their corresponding antigens from samples of cell free microbial laboratory culture media and seawater. Absorbed AAE antigens, eluted from their specific immobilized antibodies by acidic buffer (ph = 2.2), were tested for their aggregation and/or adhesion enhancement activities.

FIG. 4

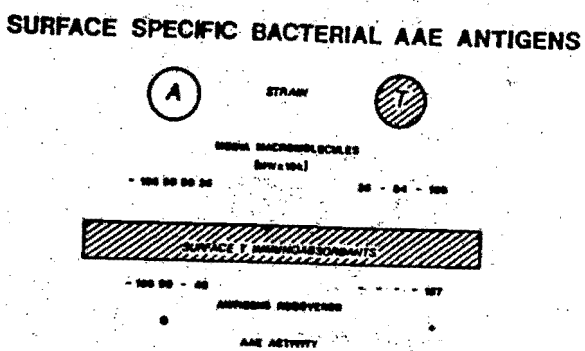

Figure 5: Recovery of Titanium Surface Specific AAE Antigens. Immunosbsorbants made to titanium surface AAE macromolecules, recovered AAE antigens from laboratory culture media of a bacterial strain found on biofouled titanium, but not aluminum surfaces.

FIG. 5

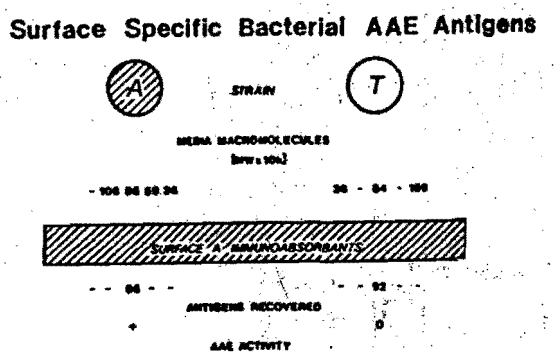

Figure 6: Recovery of Aluminum Surface Specific AAE Antigens. Immunosbaorbants made to aluminum surface AAE macromolecules recovered AAE antigens from laboratory culture media of a bacterial strain found on biofouled aluminum, but not titanium surfaces.

FIG. 6

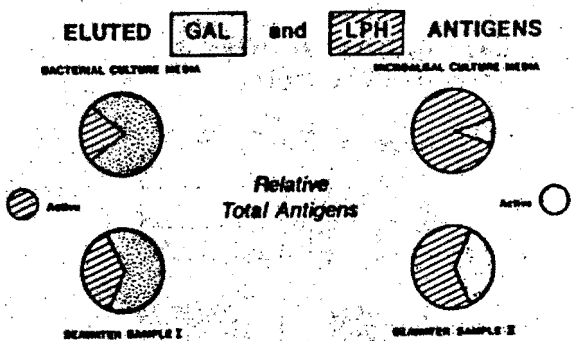

Figure 7: Microbial Source Specificity of AAE Antigens. Comparison of the relative quantities and AAE activities of galactose (GAL) and low ph (LPH) eluted antigens recovered from cell free bacterial and micro-algal laboratory growth media by immunoabsorbants made to AAE macromolecules recovered from samples of ambient seawater. Ocean AAE immunoabsorbants recovered similar relative quantities and AAE active fractions from bacterial laboratory culture media and seawater samples taken in areas dominated by bacterial biomass. The relative quantities of GAL and LPH antigens, and AAE activities, recovered from micro-algal culture media and seawater samples taken in areas dominated by micro-algal biomass were different from those found in the bacterial system.

FIG. 7

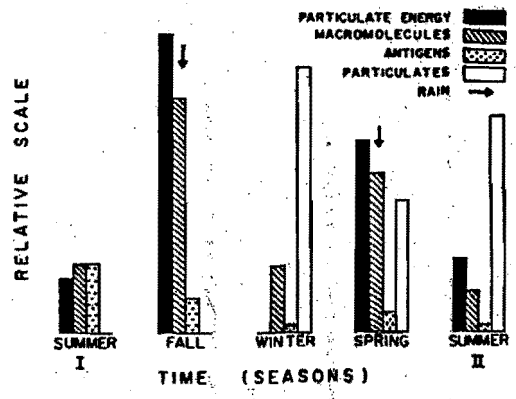

Figure 8: Seasonal Changes in Coastal Seawater Particulates, Macromolecules and AAE Antigens. Comparison of relative quantities of particulate energy (ATP/particle), concentrations of macromolecules, AAE antigens and particulates in seawater samples taken along the southwest coast of Puerto Rico during the seasons of the year. Arrows indicate peak rainfall periods, during FALL (42 cm) and SPRING (26 cm).

FIG. 8

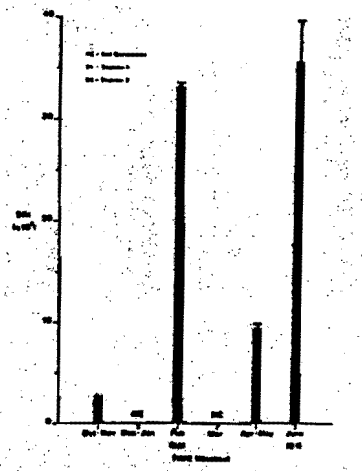

Figure 9: Specific Refractive Increment of AAE Antigens. Comparison of the specific refractive increment (SRI) of antigens recovered during the fall, winter, spring and summer months (SUMMER II) of the year. SRI values given with their standard errors.

FIG. 9

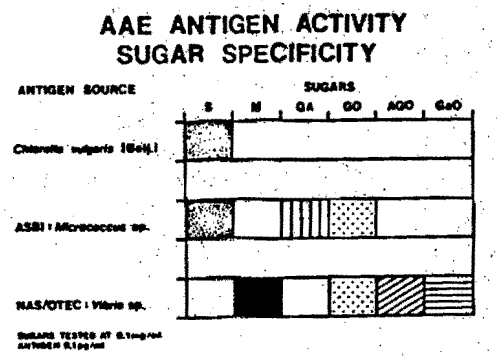

Table 1: Sugar Specificity of AAE Antigen Activity. Inhibition of AAE activity in antigens derived from micro-algal and bacerial sources in the presence of simple sugars; sorbose (S), mannose (M), galactose (GA), glucosamine (GO), n-acetyl glucosamine (AGO) and galactosamine (GaO).

TABLE /1

AAE ANTIGENS: ESTIMATED MW

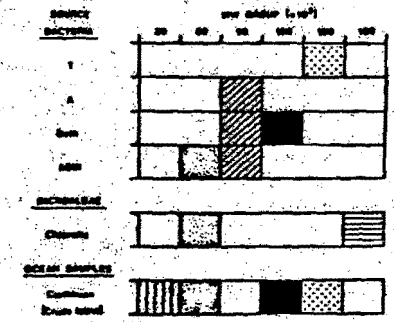

Table 2: Estimated Molecular Weights of AAE Antigens. The molecular weights of AAE antigens from bacterial and micro-algal sources, and those macromolecular components found in Caribbean seawater samples.

TABLE /2

AAE ANTIGENS: PRODUCTION AND RECOVERY

LABORATORY CULTURE *	CRUDE HMW	ANTIGEN	%
Bacteria (2)	740	22 (LPH)	3
Microalgae (2)	160	6 (GAL)	4
Coastal Surfaces	1800	-	-
OCEAN RECOVERY **			
Coastal SW I	32	1.8 (LPH)	6
SW II	35	1.2 (GAL)	3
Offshore SW	14	-	-

* 10^{-10} gm/ml
** 10^{-6} gm/ml

Table 3: AAE Antigen Production and Recovery. The quantities of crude high molecular weight (HMW) materials and AAE antigens recovered from bacterial and micro-algal laboratory culture media, compared to samples of coastal and offshore Caribbean seawater.

TABLE /3

An account of Frictional Resistance Incurred on Ocean going Research Vessel ORV Sagar Kanya due to Fouling

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Abstract

The fouling settlement of ORV Sagar Kanya one year after the previous drydocking showed Balanus tintinabulum as the dominant species with maximum biomass of 47.7 kg/m^2 at the bar keel aft and minimum of 0.54 kg/m^2 at the propeller. The frictional resistance due to fouling was calculated as 74% over the clean hull and enhanced the fuel consumption by 41% just before drydocking. The regression analysis showed a significant relation, r = 0.94, α =) 0.001 with the rate of settlement and fuel consumption.

Introduction

Marine biofouling causes deleterious effects to all man made structures in the marine environment. Considerable work has been carried out on fouling from different parts of the world (Paul, 1942; Woods Oceanographic Institute Publ. 1952; Wagh, 1965; Loeb et al., 1984; Crisp, 1984; Cleger and Preiser, 1984). Several reports regarding the important preventive methods of fouling had come from different parts of the world (Woods Hole Oceanographic Institute Publ. 1952, Fisher et al., 1984). Apparently, a great deal of money is being spent to check the effects caused by this growth. The significance of hull fouling and economics involved in the issue are well depicted in a small review by Haderlie (1984), according to it U.S Navy is spending over $ 15 million/anum towards the the only application of antifouling paint.

The fouling of hulls of fishing crafts in Indian waters was studied earlier by Erlansen (1936). Recently, many people have reported on ship hull fouling from different parts of the world (Cleger and Preiser, 1984; Gucinski et al., 1984, Lewthwaite,1982, Nair et al., 1984). In India, the maintenance cost of small fishing vessels due to fouling amounts to Rs. 30 million/anum approximately (Nair, et al 1984). Inspite off all these reports, work regarding the characterization of settlement is sparse (Igic, 1968) and attracted only less attention (Loeb et al., 1984). In this context attempts were made to study the extention of biofouling on ORV Sagar Kanya.

The samples were collected from the ship in May 1985, after a period of one year since the previous drydocking (May 1984). The impact imposed by this settlement during these period on the performance of the ship in terms of incremental frictional resistance and increased fuel consumption is reported in this paper.

Methods

Collection of sample and analysis

The ORV Sagar Kanya has a total wetted area of about 1350 m^2 with a draught of 5.6 m. The settlement was scrapped out from 1 m^2 area from various points along port side, starboard side, bow and stern at three different depths viz. 0.25 m, 3.0 m and 5.50 m, (Fig. 1). The fouling biomass was estimated by taking the total wet weight of the settlement in the sampled area (Anil, 1986). The samples so collected were sorted out and identified up to the species level. Morphometric measurements were made (rostro-carinal apex and base, latero-lateral apex and base, hight and thickness of the shell) for the calculation of the average thickness. The representatives of different size groups were also subjected to the gonadial index measurements (Karande, 1967) and this data is being published separately.

From the above measurements average roughness height of the fouling settlement was determined and this value was used in the calculation of frictional resistance.

Frictional resistance

The frictional resistance, which all moving objects in water have to overcome has been defined by Harvald (1983) as "the component obtained by integrating the tangential stresses over the wetted surface of the ship in the direction of the motion". Accordingly frictional resistance can be calculated by the formula

$$F = (\tfrac{1}{2} \rho U^2 A) \times C \qquad \text{------- (1)}$$

where ρ is the density of sea water, U is the speed of the ship, A is a suitably defined wetted area of the ship and C the coefficient of friction. Details of calculation are given in the following section.

Results

Quantification and morphometry of fouling settlement

The biomass values at different areas are given in Table 1. The lowest biomass value (0.54 kg/m^2) was recorded from the propeller. The edges of the blades were sparsely fouled while the shaft and central portion harboured moderate settlement. The highest biomass was recorded from the bar keel (aft) at 3m depth (47.7 Kg/m^2). It has been estimated that this vessel with an approximate wetted area of 1350 m^2 had about 16 tones of fouling biomass with an average of 12 Kg/m^2. The general trend of biomass increased towards the middle of draught but fell as it approached bottom.

The species composition revealed the absolute dominance of Balanus tintinabulum (94% by wet wt. of total settlement) throughout the hull. The average size of the barnacles varied between 35 - 40 mm (rostro-carinal base) and 25 - 35 mm height. The gonadial index study has shown that, most of the forms had their ovaries either in the developing stage or the stage just before hatching. It indicates that if the drydocking had been delayed by a few days the effects due to the fouling growth could have been more severe.

Calculation of frictional resistance

From the morphometric analysis, the maximum thickness of the settlement was found to be 5.7 cm at stern and minimum was 1.7 cm at the bow of the ship. The average thickness of the fouling growth was also calculated and was found to be 4.02 cm. As a result, the total coefficient of friction C amounts to

$$C = C' + C_A \qquad \text{——— (2)}$$

where C' is the coefficient of friction of a clean ship and C_A the incremental frictional coefficient due to the fouling community. For our calculation we use the formula for C_A suggested at ITTC (International Towing Tank Conference, 1939). It may be pointed out here that this empirical relationship is based on observations of roughness heights ranging from 0 to 1mm. For the calculated heights in the present study, we have assumed that the relationship continues to be valid in this range(Fig. 2).

$$C_A = \left[100\left(\frac{k}{L}\right)^{1/3} - 0.64\right] 10^{-3} \qquad \text{——— (3)}$$

where k is roughness height of the fouled hull due to fouling and L the length of the ship. An incremental frictional coefficient has been obtained using relation (3) of C_A

$$C_A = 6.9 \times 10^{-3}$$

The coefficient of friction of a clean (unfouled) hull for the dimensions of this ship is estimated to be (Woods Hole Oceanographic Publ., 1952) Hence,

$$C = (6.9 \times 10^{-3}) + (2.4 \times 10^{-3})$$

$$= 9.3 \times 10^{-3}$$

From the values of density of sea water, wetted area of ship, coefficient of friction and velocity (10 knots/hr.), by using the formula (1), The frictional resistance can be estimated as follows

$$F = 15.69 \times 10^6 \text{ N}$$

But the F for unfouled hull = 4.05×10^6 N

(as calculated above)

Hence, F due to fouling settlement =

$$(15.69 \times 10^6) - (4.05 \times 10^6) = 11.64 \times 10^6 \text{ N}$$

We can see that about 74% (11.64×10^6) of the total frictional resistance (15.69×10^6) is due to fouling.

Discussion

In marine environment the commencement of microfouling on submerged surface will always be followed by the macrofouling depending on the nature and extend of materials exposed. A great deal of work has been carried out regarding the drag imposed by this settlement on ship hulls (Loeb et al., 1984; Characklis et al., 1984; Bengough and Shepard, 1943, Characklis, 1973, Van London, 1972). Barnes (1970) and Crisp & Rayland (1960) opined that the microfilm layer attracts the fouling larvae and this increases the frictional resistance of the surface (Van London, 1972). Recently, Loeb et al., (1984) had shown that the microfilm composed

of bacteria enhances the frictional resistance by about 10 - 20% over the clean hull. As reported by Bres (1987) calcareous shells of fouling organisms attracts further their own larvae to settle and grow nearby. Thus this shelled and the non shelled (filamenteus) growth enhances the drag on the ship by enhancing frictional resistance (Trulear and Characklis, 1982).

The present observation has shown that the complete dominance of calcarious shelled organisms acted so adversely on the performance of ship like its fuel consumption, speed, power of engine etc. As revealed by the test of frictional resistance on steel plates after fouled by barnacles, resistance has been estimated to be increased by 4 times over a clean surface in 12 months (Bengough and Shepard, 1943). This result, although a laboratory test, agrees well with our present observation for same duration with an increment of 3.64 times over the clean hull (Total frictional resistance after fouling calculated earlier as 15.69×10^6 and that of clean hull 4.05×10^6). The incremental fricrional resistance due to this fouling settlement is calculated to be 74%. This is slightly higher than that suggested by Woods Hole Oceanography Institute Publ. (1952).

The effect of fouling on propellers of the ship is well described in Woods Hole Oceanography Publ. (1952). Any disturbance as roughness due to fouling is likely to cause adverse effects on the efficacy of the system. It is very well seen in present case, as the same quantum of power applied could only generate less propulsive force on the fouled ship than the one which is not fouled (just after the drydocking). The vessel just before drydocking recorded an rpm of 150 with 3 generators and 175 with 4 generators and attained a speed of about 10 knots/hr. But, immediatly after the drydocking 3 generators themselves could account for 175 rpm. and a speed of 12 knots/hr. (Ship log book, Sagar Kanya, 1984-85).

The rate of growth of fouling organisms has an important role to play in the fuel consumption rate. Their growth and year around settlement changes the topographical nature of the substrata (Bres, 1987). Moreover, these shells may attract or budout some other soft forms (Bres, 1987; Jernakeff, 1986) by making the surface more rough and complex. During this study the number of days completed by the vessel after the previous drydocking (May, 1984) and rate of fuel consumption showed a very possitive correlation in a leniar regression analysis ($r = 0.94, p => 0.001$, $Y = 7.07 + 0.11 X$., Fig. 3, Table 2). Though this factor depends on number of parameters such as wind direction, speed, sea state, current etc. the linear mode of incremental tendency of fuel consumption with time can be attributed to some extent to the increase in fouling biomass.

Efforts also have been made in this investigation

to calculate the economical impact of this on shipping operation. The fuel consumption data indicated an increase of about 41% of fuel just before 1985 drydocking. The overall year (1984 May - 1985 May) the average increase was calculated as 28.28% (Table 2). The approximate quantity of fuel consumed by the ship during this period was worth about Rs 9 millions, of which the enhanced consumption due to fouling alone estimated to be Rs 3 millions. So the countries which owe a large shipping fleet will have to spend a great amount of money so as to maintain it free fouling.

Acknowledgements

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Legends to figures

1. Representative diagram of sampling areas
2. Graph showing the relation between frictional resistance and surface roughness (source: Harvald, 1983)
3. Regression analysis of fuel consumption and the number of days completed by the vessel.

Table 1. The distribution of fouling biomass in different areas of the hull of ORV Sagar Kanya. (Expressed in kg/m^2)

Depth	Starboard side			Port side		
(m)	Bow	Middle	Stern	Bow	Middle	Stern
0	2.15	4.07	14.56	5.05	8.47	25.51
3	11.92	4.97	20.19	10.08	6.61	15.85
5.6	7.44	5.91	—	6.29	12.29	—

Propeller = 0.54; Rudder = 19.59; Bar keel (aft) = 47.70

Table 2. The increase in fuel consumption with period (1984 May to 1985 May) after the previous drydocking (1984 May).

Cruise No.	Period, area & position	Nautical miles/im.ton	% increase in fuel consumption
8	23 May 1984 to 6 July 1984 Central Indian Ocean	19.18	17
9	15 July 1984 to 27 August 1984 Bay of Bengal	19.80	13
10	1 September 1984 to 10 October 1984 Bay of Bengal	19.80	25
11	27 October 1984 to 10 December 1984 Central Indian Ocean	17.94	17
12	21 december 1984 to 3 February 1985 W.Continental Margin of India	19.26	37
13	8 February 1985 to 21 March 1985 Arabian Sea	15.15	48
14	25 March 1985 to 29 April 1985 Indian Ocean	15.92	41
	From Bombay to Goa 19 May 1985 to 21 May 1985. (The above calculations were made with respect to this reading)	22.5	0

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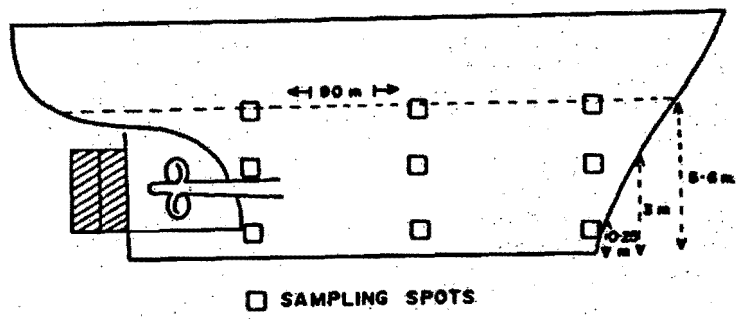
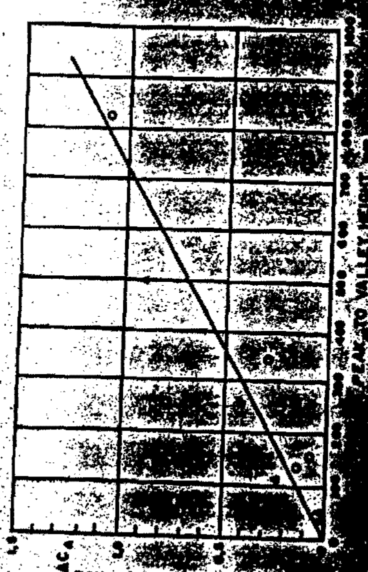
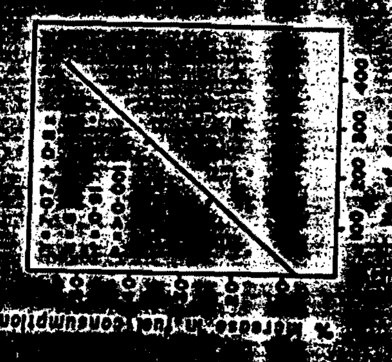


Fig. 1


FRICTIONAL RESISTANCE
Fig. 2


No. of days
% Increase in fuel consumption
Fig. 3