Chapter 7

DIATOM COMMUNITIES ON NON-TOXIC SUBSTRATA AND TWO CONVENTIONAL ANTIFOULING SURFACES IMMERSED IN LANGSTONE HARBOUR, SOUTH COAST OF ENGLAND.

S. PYNE[1], R.L. FLETCHER[1] and E.B.G. JONES[2]

[1] Department of Biological Sciences, Portsmouth Polytechnic, Marine Laboratory, Ferry Road, Hayling Island, Hampshire, U.K. PO11 ODG.

[2] Department of Biological Sciences, Portsmouth Polytechnic, King Henry 1 Street, Portsmouth, U.K. PO1 2DY.

7.1 INTRODUCTION

There have been a number of reports of diatom fouling on both nontoxic (Bastida et al., 1974; Bacon and Taylor, 1976; Karentz and McIntire, 1977) and toxic test panels (Hendey, 1951; Kingcome, 1959; Callow, 1984) on a world wide basis. In general a diverse and rich diatom flora has been described on the non-toxic test panels. For example Bacon and Taylor (1976) examined diatom communities on non-toxic surfaces and recorded a total of 183 species in 50 genera, the dominant species being Synedra fasciculata, Paralia sulcata and Achnanthes longipes. In comparison to this the antifouled/toxic test panels have been reported to support a much more restricted fouling community with fewer numbers of genera and species identified. Probably the most detailed investigation of fouling diatoms on toxic panels was that of Hendey (1951) who recorded 97 species distributed in 27 genera on copper based antifouling coatings immersed in Chichester Harbour, South Coast of England. The dominant genera recorded by Hendey were Amphora, Achnanthes, Navicula and Stauroneis, which are often referred to as 'true slime' formers as they lie closely adpressed to the surface and form a sheet or film over the substratum.

The present paper is concerned with an investigation of the marine fouling diatom communities on both non-toxic and toxic test panels suspended from a raft in Langstone Harbour, South Coast of England. A general comparison is made between the floristic composition of these two surface types, which is further related to aspects such as the quality and quantity of incorporated antifouling biocide and various environmental parameters, such as depth of immersion of the panels and water temperature. Using a weighted diversity index a semi-quantitative analysis of the fouling diatoms is also carried out.

7.2 Materials and Methods

7.2.1. Panel Exposure Site - Langstone Harbour

The test panels were immersed from test rafts moored in Langstone Harbour, north-east solent region on the south coast of England (grid ref. SU 615015). It is a fully marine harbour system being the central one of three interconnecting harbours (see fig. 7.1). The harbour is almost completely land locked with two narrow channels connecting it to Portsmouth Harbour in the west and Chichester Harbour in the east. It is shallow (does not exceed 10m in depth) and has an area of approximately 19.4km^2. Tidal range varies between 1.5m (neaps) and 4.5m (springs), with a tidal stream current reversing with the tide ranging from 2.5km/hr (neaps) to 4.54km/hr (springs) (Houghton, 1959). The surface water temperatures ranged from a minimum of 2.1°C (January) to a maximum of 22.1°C (August) for 1982 whilst salinities show slight variations from 30.7% to 34% . Water clarity is generally poor, usually ranging between 1-3m, and industrial pollution is light although the harbour does receive about 40000 litres of treated sewage per day (Anon, 1976).

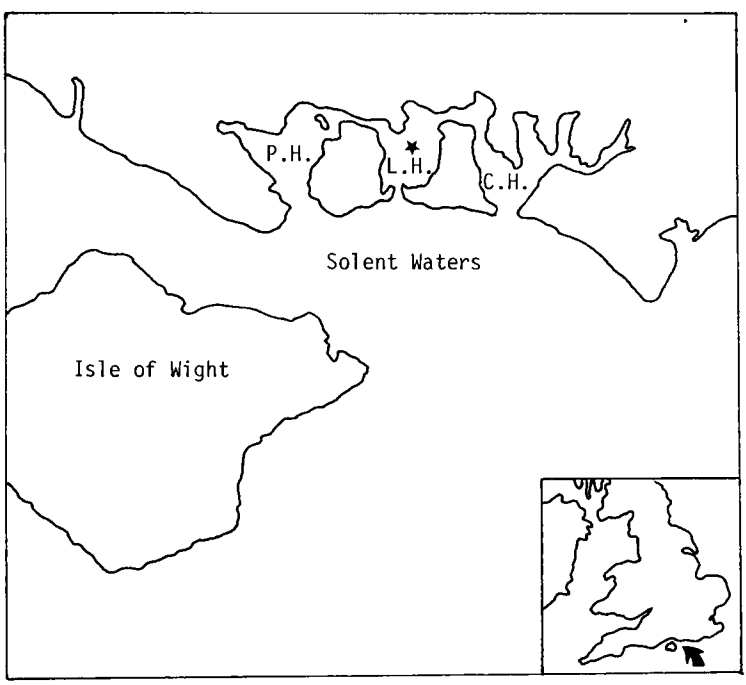

Fig.7.1 Map to show location of Langstone Harbour and raft site. (P.H. Portsmouth Harbour; L.H. Langstone Harbour; C.H. Chichester Harbour; ✱ Test Rafts)

7.2.2 Non toxic panel exposure

The test panels were made of polypropylene and measured 15cm x 8cm. They were attached to the frame by means of nylon nuts and bolts at depths of 0, 75 and 180 cms below the water-line. Duplicate panels were set up for each observation. The frame was immersed in the harbour in January 1982 and the panels examined at weekly intervals for a 12 month period. Panels were removed from the 0 and 75cm level every week and from the 180cm level every two weeks and replaced with cleaned panels.

7.2.3. Toxic panel exposure

A series of toxic panels comprising two commonly used biocides (copper oxide and organo-tin) were also immersed from the test raft from March 1982 onwards. Each panel, measuring 15cm x 8cm, was constructed of polyethylene onto which was sprayed two coats (approximately 100 m and 150 m thick respectively) of the relevant anti-fouling formulation. Three different loadings were used for both toxin types (see table 7.1).

TABLE 7.1. Loading value for both the copper and organo-tin formulations

(i)	Low loadings	-	10% weight/volume
(ii)	Medium loadings	-	30% weight/volume
(iii)	High loadings	-	50% weight/volume

A total of 12 panels were immersed (three loadings of both copper and organo-tin in duplicate) and examined each month over a 2 year period. All panels were immersed at the water line level.

7.2.4 Sampling procedure

The non-toxic panels were brushed with a hard-haired brush to remove all attached diatom cells. These samples were then transferred to a vial containing a 4% formal saline solution. The toxic panels were, however sampled lightly by brushing and removing all attached diatoms with a fine haired brush in order not to remove any excess toxin from the surface. These diatom cells were then placed into a 4% formal saline solution.

7.2.5 Preparation of permanant diatom slides from sampled material

The diatom supernatant, in formal saline, was decanted and 20ml of concentrated hydrochloric acid added. The mixture was heated for about 15 minutes until effervescence ceased and the solution then cooled. This was diluted in distilled water and left to settle. The clear liquid was decanted

and the diatoms repeatedly washed with distilled water to remove any acid. After the third wash the supernatant was decanted and 20mls of concentrated sulphuric acid was added and the mixture boiled for 15 minutes. The solution was left to stand for approximately 60 minutes after which time saturated potassium permanganate ($KMnO_4$) was added drop by drop until effervescence ceased (all organic matter has been oxidised). The solution was left to stand and cool after which oxalic acid was added to decolourise the liquid. The cleaned frustules were washed in distilled water several times and then dehydrated in 100% ethanol to allow easy attachment to cover slips. The material was mounted in a medium of high refractive index in order to increase clarity of the frustules (Naphrax: R.I. = 1.62, Fleming, 1954). Permanent diatom slides were produced for each sampling time, in duplicate and for all depths. Identification was carried out using the taxonomic nomenclature of van Heurck (1896) and Hendey (1951).

7.2.6 Semi-Quantitative analysis of biofouling (numerical diversity index)

For each permanent slide, twenty fields of view were examined, using a Wild M20 microscope with xl5 eye piece and x40 objective. Dominant species were identified and given a certain numerical weighting, depending on their estimated abundance; these were:

Percentage occurrence in population		Diversity Index
(i)	greater than 50% (dominant)	3
(ii)	25-50% (sub-dominant)	2
(iii)	12-25% (common)	1

The analysis was repeated for all types of toxic loading formulations and non-toxic panels.

7.3 RESULTS

7.3.1. Non-toxic panel analysis

A total of 187 species distributed in 37 genera were identified on all the non toxic panels. Table 7.2 shows the number of genera and species recorded on the panels at the three depths of immersion. There was generally a decrease in number of both genera and species with depth, ranging from 146 species in 34 genera at the water-line to 99 species in 20 genera at 180 cms immersion depth. The most abundant species recorded on the non-toxic panels were Achnanthes longipes, Amphora coffeaeformis, Biddulphia aurita, Cocconeis scutellum, Grammatophora oceanica, Navicula cincta and Synedra fasciculata. All of these species possess a distinct mode of attachment which allows the cell to adhere directly to the surface. The majority of diatom species recorded

on the test panels in Langstone Harbour, however were more obviously motile on the surface and within the biofilm.

TABLE 7.2. Number of species and genera on non-toxic panels

	Number of species	Number of genera
1. Total	187	37
2. Water-line	146	34
3. 75 cms below water-line	140	34
4. 180 cms below water-line	99	20

(i) Water-line panel

The genera with the greatest number of species at this depth were Navicula (25 species), Nitzschia (18 species), Amphora (10 species), Diploneis (9 species) and Cocconeis (6 species).

(ii) 75cms below waterline

The genera with most species at this depth were Navicula (29 species) and Nitzschia (14 species) while other genera prevalent within the fouling population were Amphora (10 species), Cocconeis (10 species) and Synedra (5 species).

(iii) 180cms below water-line

The genera with the highest number of species at this depth were Navicula (19 species) and Nitzschia (10 species), slightly lower figures than for the data for the other depths. Other genera which were common within the population were Cocconeis (6 species), Diploneis (4 species) and Synedra (4 species).

Using the weighted values of the diversity index (3, 2 and 1) and summing these for individual species, the dominant diatom species at the three immersion depths was estimated. The most common diatom species at the water-line and 180cms depth was Cocconeis scutellum (has the highest diversity index) while at 75cms Amphora coffeaeformis was the dominant species. Other species which had a relatively high diversity index were Synedra fasciculta (180 depth) and Grammatophora oceanica (all three depths).

Table 7.3 summarises the results for all three depths of immersion:

7.3.2. Copper and organo-tin anti-fouling panels

The dominant genus on both anti-fouling formulations was Amphora, comprising A. coffeaeformis var. coffeaeformis and A. coffeaeformis var.

perpusilla. In a general comparison with the non-toxic panels the numbers of species and genera were 50% less on the copper formulation and approximately 40% less on the organo-tin matrix. A summary of the numbers of species and genera recorded on both the copper and organo-tin anti-fouling panels is given in Table 7.4.

TABLE 7.3. Dominant diatom species on non-toxic panels

	Depth	Species	Diversity Index
(i)	Water-line	Cocconeis scutellum	75
		Amphora coffeaeformis	66
		Grammatophora oceanica	63
(ii)	75cms	Amphora coffeaeformis	71
		Cocconeis scutellum	65
		Grammatophora oceanica	60
(iii)	180cms	Cocconeis scutellum	30
		Syndera fasciculata	21
		Grammatophora oceanica	20

The monthly average number of genera and species (over a 12 month immersion period) on both anti-fouling compositions was estimated. It was found that copper panels had a lower species diversity throughout the year, having 21 species in 14 genera while the organo-tin formulation supported on average, 26 species in 15 genera. The dominant genus, on both compositions, throughout the immersion period, was Amphora, although other genera such as Cocconeis and Navicula were also present. The dominant species, estimated using the diversity index, are shown in Table 7.5.

The distribution of the monthly mean number of genera and species on both formulations from October 1982 to September 1983 are shown in figs. 7.2 and 7.3. Both graphs indicate that there was a fluctuation in diversity throughout the year. Particular attention was given to examining the temporal distribution of the two most common genera, Amphora and Navicula. Calculating the cumulative diversity index for both these genera it was shown that there was a 'cyclical' pattern in their appearance on the panels (Fig. 7.4). Amphora spp. were dominant during November and December 1983 while

TABLE 7.4 Total number of species and genera on copper and organo-tin panels

A. Copper treated panels

Toxin loading	Number of species	Number of genera
Total	91	35
low	66	28
Medium	73	28
High	71	30

B. Organo-tin treated panels

Toxin loading	Number of species	Number of genera
Total	111	35
low	85	32
Medium	78	30
High	81	28

TABLE 7.5. Prevalant diatom species on the biocide compositions

Species	Diversity Index
Amphora coffeaeformis var perpusilla	106
A. coffeaeformis var coffeaeformis	32
Navicula biskanteri	24
Cocconeis scutellum	11
Navicula grevilleana	11
Navicula ramosissima	9
Cocconeis speciosa	8

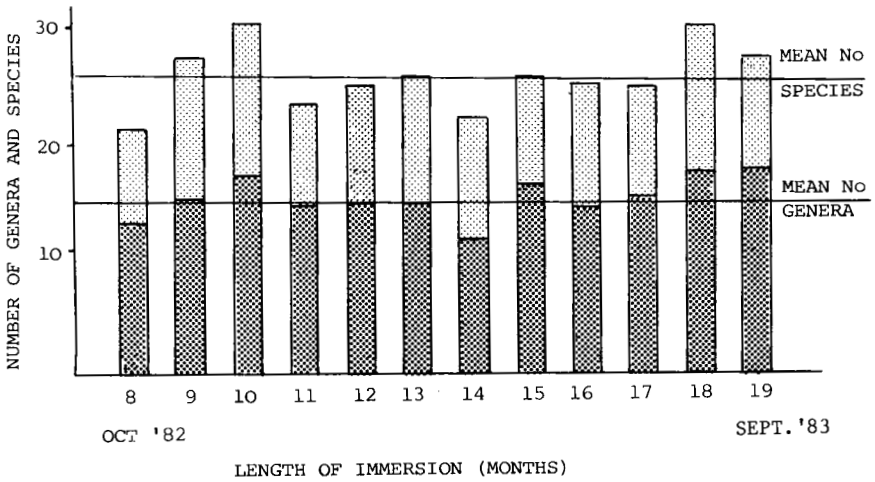

Fig. 7.2. Distribution of mean number of genera and species for organo-tin-based antifouled panels over a 12 month period.

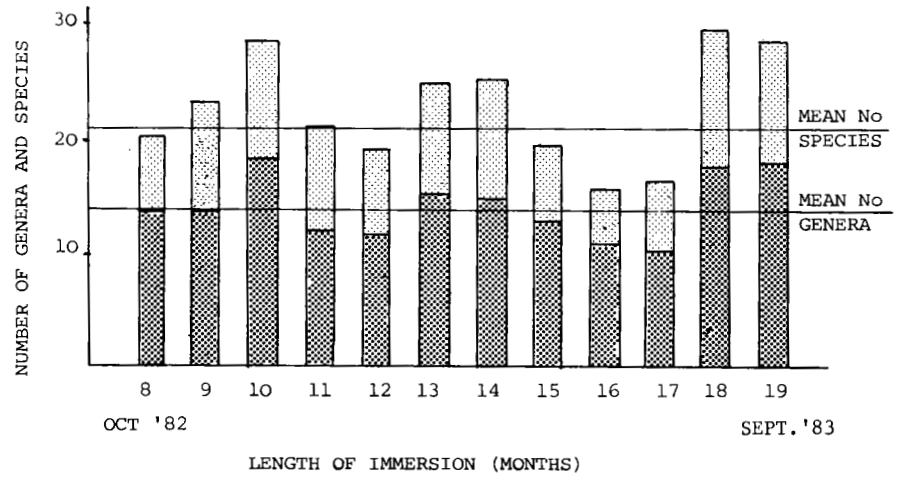

Fig. 7.3. Distribution of mean number of genera and species for copper-based antifouled panels over a 12 month period.

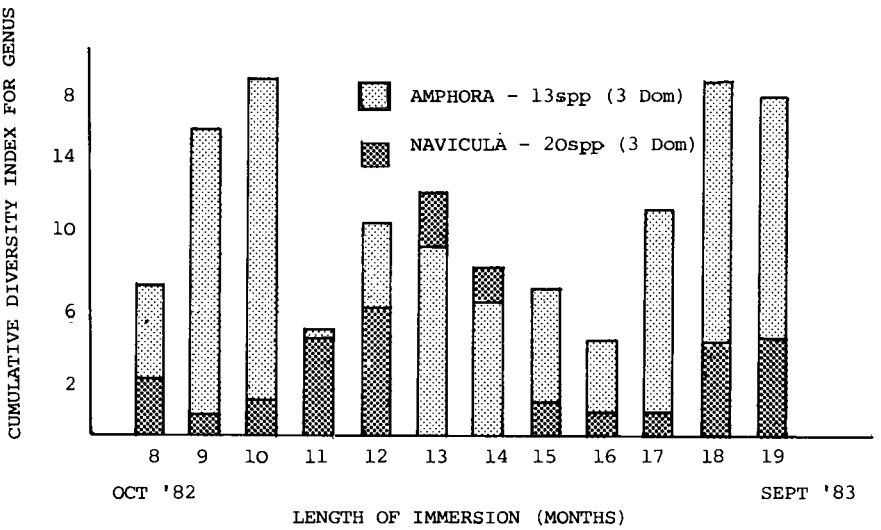

Fig. 7.4. Graph to show total cumulative diversity indices for two common genera present on all toxic panels.

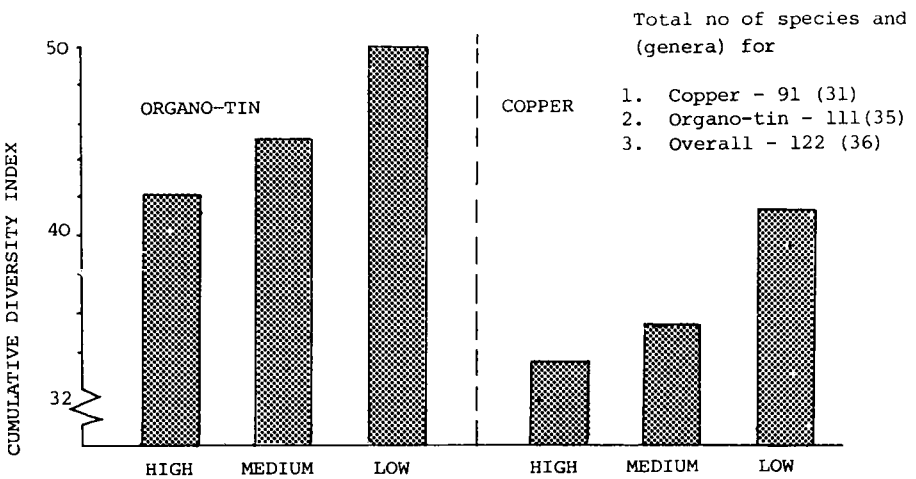

Fig. 7.5. Total cumulative indices for individual panels with various toxin loadings.

Navicula spp. became abundant later during March/April 1983; finally Amphora was again prevalent within the population during August/ September 1983.

The species diversity on both the copper and organo-tin formulations was also examined in relation to the toxin loadings and the results presented in Figure 7.5. The paints with the high loadings of both toxins had the lowest cumulative index (copper - 33; organo-tin - 42) while, in comparison, the low loading paints had the highest cumulative index (copper 41; organotin - 50).

7.4 DISCUSSION

The formation of a primary film and biofouling layers on a surface is influenced by a number of factors including sea-water chemistry, turbulence, temperature, light and incorporated antifouling biocides. In this discussion several physical factors will be highlighted and how they are thought to affect diatom distribution.

Several reports have drawn attention to the effect of depth on the development of benthic diatom communities (Bacon and Taylor, 1976; Stupak et al., 1976; McClean et al., 1981; Hundon and Bourget, 1983). With an increase in depth there is a corresponding decrease in light availability mainly due to water turbulence. In this study there are a number of common diatoms which were found to survive adequately on the deeper panels while other species could dominate the population only on the more shallow substrata. The former group are classed as 'light tolerant' diatoms and the latter group classed as 'light sensitive' species.

Bacon and Taylor (1976) and Stupak et al. (1976) found that with an increase in depth (below 75cms from the water-line) the number of Achnanthes cells attached to the test substratum was greatly reduced and this genus was virtually absent below 2 metres. In Langstone Harbour Achnanthes longipes was recorded on the 180 cms panels only seven times in twenty five sampling periods, suggesting that light penetration has an important influence on the vertical distribution of this species. A common chain forming genus, Melosira, although not considered a fouling species occurs quite abundantly in the biofilm. During the summer months when light intensity (penetration) was high, Melosira moniliformis occurs on the surface panels while M. nummuloides was found abundantly on the deeper panels (180 cms). McClean et al. (1981) suggested that these two species had different light requirements. They reported M. nummuloides was able to survive in deep water (high growth rate at low light intensities) while M. moniliformis could only survive on the shallower substrata (low growth rate in low light intensities). These observations are similar to those of Castenholz (1964) and Hudon and Bourget (1983) who found that M. nummuloides occurred on the deeper panels while M. moniliformis was present on

the shallower panels. These findings strongly suggest that the vertical distribution of the genus, and in particular these two species, is governed by light availability.

One physical factor that has a profound effect on fouling and the build up of the fouling community is temperature. Bacon and Taylor (1976) noted that Synedra fasciculata appeared on test panels, usually as the dominant species, throughout May and June. However, when the temperature exceeded 10°C there was a dramatic reduction in numbers. Similarly Synedra fasciculata was recorded in the present study at the start of the immersion periods frequently sub-dominant, when the temperature was approximately 7°C; above this temperature the species continues to be present but not in such high numbers, suggesting that 7-8°C is the optimum temperature for growth of this species in Langstone harbour. There will be a great deal of variability in temperature related growth within a genus but Reisen and Spencer (1970) noted that another Synedra sp. had an optimum growth rate at 6.5°C in laboratory culture, which is similar to that reported here for S. fasciculata.

Several workers have noted that Achnanthes spp. show a marked reaction to variation in temperature. Stupak et al. (1976) recorded that between 8-15°C A. longipes was dominant on most test panels from 0.5 - 2 metres depth. However, at temperatures greater than 15°C this species was merely present and rarely constituted a large percentage of the population. Similar trends were recorded by Bacon and Taylor (1976) who noted that A. longipes occurred on test panels more frequently at temperatures below 15°C than at values higher than this. The results presented in this paper from Langstone Harbour do not show any trends in distribution in Achnanthes spp. when related to temperature fluctuations.

One of the few chain-forming diatoms commonly found on the test panels was Biddulphia aurita (distribution shown in fig. 3). Stupak et al. (1976) continuously recorded this species at temperatures between 8-11°C; above these temperatures, species frequency was intermittent. Similarly, Bacon and Taylor (1976) noted B. aurita on test panels when the temperature was between 5-13°C; at higher temperatures it was no longer present. These results are similar to those obtained in the present study. B. aurita was recorded on panels in Langstone Harbour when the temperature range was 4-12°C, above which it had a 'patchy' appearance. It is evident that temperature plays an important role in the frequency of occurrence of this species.

One of the most important parameters which will affect diatom communities on a test surface is the presence of an anti-fouling biocide within the substratum matrix. There have been many reports on the colonisation of copper based paint formulations (Harris, 1946; Hendey, 1951; Lu et al., 1979; Callow,

1984) but only a few papers on the diatoms colonising organo-tin compounds (Blunn, 1982; Callow, 1984). The results of Hendey's (1951) study of the biofilms on copper based paints, in which 97 species (represented in 29 genera) were reported, is supported by the present investigations in Langstone Harbour (91 species in 35 genera). Also in agreement with Hendey, Amphora was by far the most abundant genus to be identified.

Callow (1984) examined the biofouling on copper based and organotin based formulations on a world-wide basis. Amphora spp. were the major constituents of the slime along with Achnanthes. The results agree with those presented in figs. 3 and 4 in which Amphora is the dominant genus although Achnanthes was identified on only a few occasions. Daniel and Chamberlain (1981) also examined a number of commercially used paint formulations and noted on some that Amphora formed a uni-algal slime adhering closely to the surface. The numerical diversity index adopted in this paper also revealed Amphora to be an important fouling genus; Amphora coffeaeformis var. perpusilla had an index of 106 and A. coffeaeformis var. coffeaeformis an index of 32.

In conclusion, it is evident that there are a large number of diatoms which are associated with the biofilm on non-toxic surfaces. However, only a few of these attach directly to the surface and can be classed as true 'slime-formers'. Amphora was by far the most abundant genus within the biofouling layer, other prominent genera being Navicula, Cocconeis and Synedra. The diversity index method allowed comparison of diatom communities from month to month and it was evident that Amphora coffeaeformis var. perpusilla was the dominant diatom species in most samples. In contrast to the report of Blunn (1982), it was shown that Achnanthes had no preference for the organo-tin based formulations and rarely occurred within the fouling population.

7.5 ACKNOWLEDGEMENTS

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