

Seasonal variations in the fouling diatom community structure from a monsoon influenced tropical estuary

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Abstract

Seasonal variations in the fouling diatom community from a monsoon influenced tropical estuary were investigated. Community composition did not differ significantly between stainless steel and polystyrene substrates due to dominance by *Navicula* spp. Experimental evidence suggests that *Amphora*, which is a dominant fouling diatom in temperate waters, ultimately dominates the community in tropical regions, under favourable conditions. These observations reveal that faster onset of macrofouling interferes with the microfouling community wherein the faster recruiters due to higher abundance in ambient waters dominate the community. Seasonal variations were observed in the fouling diatom community. *N. delicatula* dominated during the post-monsoon and tythropelagic diatoms (*Melosira* and *Odontella*) were observed in the monsoon. Low diatom abundance was recorded during pre-monsoon. Results indicate that although the fouling diatom community composition does not vary between substrata, with seasons the community changes depending on the physical, chemical and biological interactions.

Keywords: Fouling diatoms; tropical; stainless steel; polystyrene

Introduction

Biofouling and biofilm formation are of great concern to many modern industries including marine, food, water, mining and medical. The shipping industry has serious problems with biofouling on most surfaces submerged in seawater since it causes increase in water resistance, fuel consumption and microbial corrosion of metal surfaces (Schultz 2007). The economic consequence of biofouling is thus significant. In the process of fouling, a layer of microorganisms quickly covers any surface submerged in water forming a microfilm. Some of the earlier studies have reported bacteria to be the initial colonizers (Corpe, 1970; Sieburth, 1979; Bhosle et al. 1989) whereas others are of the opinion that diatoms colonize prior to bacteria (Skerman, 1956; O' Neil & Wilcox, 1971). However, some studies have shown that although the presence of the bacterial film may facilitate the attachment of diatoms, it is not a pre-requisite (Horbund & Freiburger, 1970; Cooksey et al. 1980). Further, diatom metabolic activities have been shown to provide the sole carbon and energy source for heterotrophic bacteria in films where both types of organisms are present (Murray et al. 1986). These microfilms are responsible for attracting the algal zoospores and larvae of higher organisms such as barnacles and polychaetes that result in macrofouling (Joint et al. 2002; Patil & Anil, 2005c; Dobretsov et al. 2006).

Temporal and spatial variations have been observed in the fouling diatom community structure (Cooksey et al. 1984; Edyvean et al. 1985; Kelkar, 1989; Pangu, 1993; Prabhadevi, 1995; Redekar, 1997; Patil & Anil, 2005a). Such a change in the community structure is influenced by various biotic and abiotic factors. These changes through time play a very important role in the dynamics of microfouling and macrofouling. Hence, it is important to understand the contribution of diatoms to the microfilm formation which will be useful in environmental studies, pollution abatement and design and operation of industrial equipment, which are prone to biofouling.

The site of this investigation, the Zuari estuary is a tide dominated coastal plain estuary (Qasim & Sengupta, 1981) influenced by the southwest monsoon that is active during June to September. During this period, a large quantity of fresh water is added to the estuary, resulting in low salinity conditions, very high turbidity and greatly reduced light levels (Devassy & Goes, 1988). The changes associated with the onset of the monsoon (June to September) affect phytoplankton dynamics, community structure and primary production of this estuary (Devassy, 1983; Bhattathiri et al. 1976). The objective of this study was to

evaluate the seasonal influence on the fouling diatom community structure on two artificial substrata, stainless steel and polystyrene, in a tropical estuary experiencing the southwest monsoon. A simultaneous study (Patil & Anil, 2005a) in the same bay has documented the fouling diatom community structure on fibreglass and glass substrata. We also examined the influence of salinity on the two most common fouling diatom genera, *Navicula* and *Amphora*, under controlled conditions.

Materials and methods

Study area

This study was carried out from November 1998 to January 2000 at Dona Paula Bay located at the mouth of the Zuari estuary, Goa, ($15^{\circ} 27.5' N$, $73^{\circ} 48' E$), on the west coast of India. This estuary is classified as a tide dominated coastal plain estuary. The river Zuari has its source in the Western Ghats, and it extends up to 70 Km before meeting the Arabian Sea. This river is influenced by seawater inflow up to a considerable distance inland, and it receives a large quantity of fresh water during the southwest monsoon. Based on this, a year has been classified into three seasons viz. monsoon (June-September), followed by a dry period referred to as the post-monsoon season (October-January) and thereafter a stable period known as the pre-monsoon season (February-May). During the southwest monsoon, this area receives most of the annual precipitation, which leads to addition of fresh water to the estuaries thereby reducing the salinity. During this season, precipitation is accompanied by stormy weather while quieter conditions prevail during rest of the year.

Environmental parameters, type of substrata and submersion

Details of the methodology employed for sampling and analyses of environmental parameters such as temperature, salinity and nutrients during the study period have been given elsewhere (Patil & Anil, 2005a).

Stainless steel ($15 \times 10 \times 2$ cm) and polystyrene (8.5 cm diameter) substrata were used in this study. The panels, with 8mm holes drilled at one end, were bolted to fibreglass flats with PVC nuts and bolts and suspended at the sub-surface level (~ 1 m below lowest low tide level). Earlier reports (Mitbavkar et al. 1997; Mitbavkar & Anil, 2000) have revealed the dominance of microorganisms on substrates immersed in the Dona Paula Bay for one to four days, followed by the arrival of macroorganisms. So the submersions were carried out for

four consecutive days, once every month in order to obtain 1, 2, 3 and 4 day old microfilm over a period of 14 months (November '98 to January 2000).

Assessment of diatom settlement on substrata

Three replicate panels were removed every consecutive day till the fourth day. They were then scraped with a nylon brush (Sharma et al. 1990) into known quantities (~ 100 ml) of 0.2 μm membrane filtered seawater. The scraped material was fixed with Lugol's iodine. This preserved material was subjected to sedimentation in a settling chamber (Hasle, 1978) and the diatom cells were enumerated both qualitatively and quantitatively under a compound microscope (Patil & Anil, 2005b). Diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyam (1946), Desikachary (1977, 1987) and Tomas (1997). The results are presented in terms of diatom abundance per dm^2 . Simultaneously, another set of scraped material from the panels was used for chlorophyll *a* analyses (Parsons et al. 1984).

Influence of salinity on two diatom species in culture

Experimental protocol

Isolation and culturing of diatom species are described in Mitbavkar & Anil (2007). The f/2 culture medium was prepared in filtered autoclaved seawater with salinities of 5, 15, 30, and 40. The culture medium with each of these salinities (5 ml) was transferred to polystyrene multiwells (Corning 430343; in triplicates). To these multiwells, cells of *Navicula transitans* var. *derasa* f. *delicatula* and *Amphora coffeaeformis* were inoculated separately, at an initial density of 1000 cells ml^{-1} from an exponentially growing parent culture. These multiwells were incubated at 27°C and were illuminated at an incident irradiance of 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, produced by fluorescent tubes over a 12h light: 12h dark photoperiod. These wells were examined microscopically for a period of 8 days for monitoring the cell counts. Growth rates of the two species were calculated using the equation $\mu = (\ln N_t - \ln N_0)/t$,

where N_0 = the initial cell concentration, N_t = the final cell concentration and t = incubation period.

Data analyses

Univariate analyses: Univariate techniques included the calculation of Shannon-Wiener's diversity index (H'), species richness ($H'_{\text{max}} = \log_2 S$ where 'S' is the number of taxa) and

evenness ($J' = H' / H'_{\max}$) of the diatom community, both in water column and the fouling film (Pielou, 1984).

Multivariate analyses: The log (x + 1) transformed data on the fouling diatom abundance data was subjected to Analysis of Variance (ANOVA) (Sokal & Rohlf, 1981) to evaluate temporal (days and months) and substratum variations.

The fouling diatom abundance data (which averaged to more than 5% of the total diatom abundance) were subjected to cluster analysis with respect to sampling months. Clustering was performed through Bray-Curtis coefficients (Bray & Curtis, 1957) and group average method (Pielou, 1984) using PRIMER software version 5. Data was subjected to square root transformation prior to analysis. Linear regression analysis was performed on the log (x + 1) transformed values of fouling diatom abundance and chlorophyll *a* from the substrata.

Results

Environmental parameters and diatoms in the ambient water column

During the observation period, temperature ranged from 25⁰C to 30⁰C. Low salinity ranging from 15 to 19 was recorded during the monsoon. During the post and pre-monsoon months the salinity ranged from 25 to 35. Nitrate concentration ranged between 0.4 μM and 8 μM with peak values in April, July and December 1999. Nitrite ranged between 0.01 μM and 0.68 μM being highest in the pre-monsoon (March). During May 1999, a rise was detected in the phosphate concentration. The silicate concentrations were highest in the monsoon (July 1999). The observed low salinity, high nitrate and silicate concentrations were due to considerable land run off during the monsoon season.

In the ambient water column, 51 species of diatoms (28 centric and 23 pennate) belonging to 36 genera (21 centric, 15 pennate) were observed during the study period. The diatom community was dominated by centric species on most occasions. Blooms of *Skeletonema costatum*, *Fragilariopsis* sp. and *Thalassionema nitzschioides* occurred in the monsoon (September) during which lowest species diversity was recorded. During the post-monsoon (November 1999), blooms of *Leptocylindrus danicus* and *Cerataulina* sp. were observed and species diversity was comparatively higher. In the pre-monsoon, lowered diatom population was recorded. Details of the diatom composition in the ambient water column during the study period have been described in Patil & Anil (2005a).

Diatoms in the fouling film

Stainless steel substrata

On this substrata, the fouling diatom population was represented by 57 diatom species (45 pennates, 12 centrales) belonging to 31 genera (21 pennates, 10 centrales) (Table I). The total diatom abundance was lower in the pre-monsoon and beginning of monsoon season (Figure 1a). Pennate diatoms dominated the community throughout the sampling period except in May '99 wherein the centric diatom, *Melosira nummuloides* dominated the two to four day old microfilm community. During the post-monsoon season, the fouling diatom community was mostly represented by *Grammatophora marina*, *Pleurosigma angulatum*, *N. delicatula*, *Navicula subinflata*, *Thalassionema frauenfeldii* and *Amphora rostrata*. The pre-monsoon fouling diatom community was represented by *N. delicatula*, *Pleurosigma elongatum*, *Cocconeis scutellum*, *M. nummuloides* and *A. coffeaeformis*. Diatoms such as *N. delicatula*, *T. frauenfeldii*, *T. nitzschioides*, *P. angulatum* and *Fragilariopsis* sp. were most frequently observed in the fouling film during monsoon (Figure 2).

During the post-monsoon, species diversity, species richness and evenness were highest in the one-day old biofilm and subsequently showed a decline. Such a distribution was observed when the diatom community was dominated by *N. delicatula*. During monsoon, species diversity declined after day three whereas species richness was high in the one day old community and evenness was low in the four day old community. In spite of the loss of species from the community after first day of incubation, species diversity was high up to day three because of the evenly distributed community. Subsequently, the diversity declined because of low species richness and low evenness due to the dominance of the community by *Fragilariopsis* sp. during August and *N. delicatula* in September. During the pre-monsoon, species diversity and species richness was high up to day three and then reduced on day four. But the community was evenly distributed throughout the four days submersion period which shows that there was loss of species with increase in exposure period (Table IIa).

Cluster analyses of the one to four day old fouling diatom community divided the sampling months into two groups at 50% similarity level. Group I comprised of months with total cell density ranging from 300 to 500, 180 to 1300, 950 to 2358 and 2290 to 4500 cells dm⁻² for day one, two, three and four respectively. Group II included rest of the months with total cell

density ranging from 20 to 300, 50 to 130, 70 to 600 and 90 to 800 cells dm^{-2} for day one, two, three and four respectively (Figure 4a).

Linear regression analyses between the total diatom abundance and chlorophyll *a* concentrations showed a significant relationship in case of the one to three-days-old diatom communities (Figure 5a).

Polystyrene substrata

Altogether 61 diatom species (48 pennates, 13 centrales) were recorded on the polystyrene substrata belonging to 33 genera (22 pennates, 11 centrales) (Table I). A comparatively lower total diatom abundance was observed in the pre-monsoon and beginning of monsoon season (Figure 1b). The fouling film was dominated by pennates throughout the submersion period except in May (2 day old microfilm) wherein the community was dominated by the centric diatom species, *Odontella rhombus*. During the post-monsoon season, the fouling diatom community was mostly represented by *P. angulatum*, *N. delicatula*, *N. subinflata*, *T. frauenfeldii*, *A. rostrata*, *Amphora turgida*, *C. scutellum* and *Bacillaria paradoxa*. The pre-monsoon fouling diatom community was represented by *N. delicatula*, *P. angulatum*, *G. marina* and *O. rhombus*. Diatoms such as *N. delicatula*, *N. subinflata*, *A. rostrata*, *T. frauenfeldii*, *T. nitzschioides*, *P. angulatum* and *Fragilariopsis* sp. were most frequently observed in the fouling film during monsoon (Figure 3).

During the post-monsoon, species diversity, species richness and evenness were highest on day one and subsequently showed a decline up to day 4. During this period, the diatom community was dominated by *N. delicatula*. During monsoon, the species diversity and species richness values were high and the community was evenly distributed. During the pre-monsoon, the species diversity increased on day four whereas the species richness reduced from day one to day four and the community was evenly distributed throughout the exposure period (Table IIb).

Cluster analyses of the one to four day old fouling diatom community divided the sampling months into three groups at 50% similarity level. Group I comprised of months with total cell density ranging from 480 to 970, 450 to 1680, 1433 to 3145 and 1046 to 7710 cells dm^{-2} for day one, two, three and four respectively. Group II included rest of the months with total cell density ranging from 200 to 780, 450 to 1680, 296 to 1108 and 140 to 1350 cells dm^{-2} for day one, two, three and four respectively. Group III included rest of the months with total cell

density ranging from 40 to 140, 74 to 210, 116 to 185 and 118 to 1340 cells dm^{-2} for day one, two, three and four respectively (Figure 4b).

Linear regression analyses between the total diatom abundance and chlorophyll *a* concentrations showed a significant relationship only in case of the one-day-old diatom community (Figure 5b).

Comparison of the fouling diatom community on stainless steel and polystyrene substrata

Generally, the total number of diatom species as well as total diatom abundance was higher on polystyrene substrata than on stainless steel (Figures 2 & 3). *N. delicatula* was the most dominant species observed on both the substrata. However its percentage composition varied depending on the season and distribution of the other co-existing species. In May, *M. nummuloides* (two and three day old community) was comparatively higher on stainless steel substrata than polystyrene. During this period, another centric diatom species, *O. rhombus* (one and two day old community) showed preference towards polystyrene, along with certain pennate diatom species belonging to other genera such as *Achnanthes* (four day old community), *Licmophora* (four day old community), *Grammatophora* (one day old community) and *Synedra* (one and two day old community). In July, *Pinnularia* sp. was encountered only on stainless steel substratum. During the August-September period, *Thalassiothrix* and *Pleurosigma* were found to be abundant on both the substrata while *Fragilariopsis* sp. was abundant on stainless steel substratum. During the post-monsoon period, while *Licmophora* was abundant on stainless steel, *Cocconeis*, *Cymbella* and *Thalassiothrix* were abundant on polystyrene. *Nitzschia* and *Bacillaria* were encountered on both the substratum. During the pre-monsoon period, *Planktoniella* and *Pleurosigma* showed higher preference for stainless steel substrata while *Coscinodiscus* and *Cymbella* were found to be abundant only on polystyrene. *Navicula* spp. and *Amphora* were found to be higher on polystyrene than stainless steel during most part of the year.

ANOVA of total diatom abundance, species diversity, species richness, evenness and chlorophyll *a*, for the two substrata and water column is shown in Table III. A significant variation between the two substrata was observed with respect to months. With respect to days, the total diatom abundance, species diversity and chlorophyll *a* revealed significant variation.

Influence of salinity on two diatom species in culture

During the incubation period, *N. delicatula* and *A. coffeaeformis* cells divided at all exposed salinities, but the population growth rate was slower at lower salinities. Comparatively, the growth rate of *A. coffeaeformis* was higher than that of *N. delicatula* (Table IV).

Discussion

Seasonal variation in the fouling diatom community structure

The source of the diatoms that have a tendency to settle on the substrata immersed in seawater can be the surrounding waters, neighbouring artificial or natural surfaces eg. inshore seaweeds, rock surfaces, sand grains/mud in intertidal areas or the sea bottom, from where the cells get dislodged due to physical forcing such as waves and tides and resuspend into the overlying waters. This community which is regarded as tycho pelagic comprises both pennate and centric diatoms.

Although the centric diatom species were dominant in the ambient water column, it was not reflected in the biofilm where the community was dominated by pennate diatoms. The pennate type often dominates the diatom fouling population (Characklis & Cooksey, 1983; Cooksey et al. 1984). Similar observations were made in another investigation carried out in the same study area (Mitbavkar & Anil, 2000; Patil & Anil, 2005a) and also in the Arabian Sea (Kelkar, 1989; Raveendran et al. 1991; Pangu, 1993; Redekar, 1997; Redekar & Wagh, 2000). Such differences could arise as the requirements for attachment and adhesion are not found in all diatoms. Unlike the centric diatoms, pennates possess a structure called a raphe that enables them to attach and glide over the substratum (Molino & Wetherbee, 2008).

Seasonal variations were observed in the fouling diatom community structure on both stainless steel and polystyrene substrata. The tropics do not experience much seasonal variation in temperature. However, the salinity ranges from a high of 34 during the post-monsoon and pre-monsoon to a low of 15 during the monsoon season due to precipitation and freshwater runoff. During the post-monsoon season, *N. delicatula*, a pennate motile diatom was the dominant species in the water column and a pioneer diatom dominating the fouling community throughout the four days of exposure, on both the substrata. Although a pennate diatom, the dominance of this species in the water column may be attributed to its resuspension from the bottom sediments or surrounding intertidal areas. Hence, its dominance in the surrounding waters could have resulted in a higher surface encountering probability for this species.

On the other hand, another pennate diatom, *A. coffeaeformis*, which is also a dominant representative of the microphytobenthic diatom community of the surrounding intertidal sand flat throughout the year (Mitbavkar & Anil, 2002; Mitbavkar & Anil, 2006) was less abundant in the water column and the fouling community as compared to *N. delicatula*. This genus was found to be abundant elsewhere in the fouling community on non-toxic substrata (polypropylene) as well as toxic substrata coated with a copper antifouling composition (Pyne et al. 1986; Daniel et al., 1980). So, this species was probably unable to adapt to a planktonic lifestyle as opposed to *N. delicatula* resulting in its lowered representation in the fouling community or another reason could be that lower number of cells get displaced into the water column due to its stronger attachment capabilities. *Amphora* has two raphes which may allow it to adhere more strongly and efficiently (Harper & Harper, 1967).

During monsoon, the diatom abundance in the ambient waters and the fouling film reduced drastically. The diatom community was represented by a mixture of chain forming pennate and centric diatoms such as *T. nitzschioides*, *Fragilariopsis* sp. and *S. costatum*. This was reflected in the fouling community where *N. delicatula* population declined and species belonging to *T. nitzschioides* and *Fragilariopsis* sp. were observed. It has been reported earlier that blooms of diatom species in surrounding waters leads to their entanglement into the fouling film (Patil & Anil, 2005a). The monsoon season characterized by stratification with nutrient rich and low saline waters at the surface, favoured these species, which turned out to be better competitors than the otherwise dominant *N. delicatula*. However, with the rise in salinity in September, increased numbers of *N. delicatula* were observed in the fouling film. Laboratory experiments carried out to observe the effect of salinity on the pure cultures of *N. delicatula* revealed that this species switched over its mode of reproduction from the regular vegetative to sexual type at lower salinities. This switching over resulted in a decline in the growth rate at lower salinities thereby resulting in reduced cell density as compared to that at higher salinities. Thus in nature, salinity probably played a role in structuring the population dynamics of this species which ultimately resulted in its lowered representation in the fouling film.

The ability of this benthic species to maintain rapid division over a broad range of salinities from 30 to 40 suggests that salinity levels within the range measured in the intertidal area during the post and pre-monsoon are not particularly detrimental to the diatom population, and thus may be of little importance in controlling the division rate. However,

lower salinities are not favourable for this species. Seasonal observations made in the same study area from where the species was isolated, showed that the population exhibited variations in abundance during the monsoon, post-monsoon and pre-monsoon seasons (Mitbavkar & Anil, 2002) wherein a lowered abundance was encountered in the monsoon, a period when precipitation and freshwater run off reduces the salinity to as low as 10. Comparatively, *A. coffeaeformis* was found to better tolerate lower salinities. This showed that this species is euryhaline capable of tolerating a wide range of salinities ranging from 5 to 40. However, its lowered representation could be because of reasons mentioned above.

In addition to salinity, after initial colonization, the relative abundance of the species present will depend on other growth conditions of the surrounding environment such as nutrient availability and biological interactions e.g., competition. While benthic microalgae inhabiting sediments may receive nutrients from the water column and from the sediment pore water, the benthic microflora on hard substrates depends upon water column nutrients and the internal recycling of nutrients (Riber & Wetzel, 1987; Hillebrand & Sommer, 1997). In an experimental study, it was observed that when biofilms developed in nature, with low initial numbers of *A. coffeaeformis* and high numbers of *N. delicatula*, were exposed to excess nutrient concentrations, the growth rate of the former increased and surpassed the *N. delicatula* population irrespective of the initial inoculum (Mitbavkar & Anil, 2007). However, in the unenriched conditions both the species were found to increase at the same growth rate. The slow movement of *A. coffeaeformis* (Hudon & Bourget, 1983), which may limit their colonizing capabilities, is offset by their strong adhesion (Harper & Harper, 1967) and their ability to tolerate darkness and anoxia for several days (Moss, 1977). Since in this study the panels were exposed only for four days because of the subsequent macrofouling, it could be possible that *A. coffeaeformis* would develop in later stages of the microfilm development if nutrients supply is adequate. So these factors could be responsible for the lower representation of this genus in the fouling film as compared to *Navicula* in this area. Laboratory studies suggest that *Amphora* and *Navicula* are likely to be the most abundant types of diatom on non-toxic and fouling release coatings (Cooksey et al. 1999; Finlay et al 2002).

In the pre-monsoon, common chain forming genus such as *Melosira* and *Odontella*, which are bottom living centric forms (Smyth, 1955; Aleem, 1973; Rendall & Wilkinson, 1983; Raveendran et al. 1991) although not considered as fouling species occurred quite abundantly

in the biofilm. *M. nummuloides* was able to survive in deep water exhibiting high growth rate at low light intensities (Pyne et al. 1986; Hudon & Bourget, 1983). The rough weather during the pre-monsoon period results in strong mixing due to which the bottom sediments get disturbed and get suspended into the overlying waters along with the resident flora. Hence these diatom species are found on the substrates usually in the presence of mud. Although observed in the initial stages of fouling, due to the lack of strong attachment capabilities, they are subsequently detached from the biofouling community (Mitbavkar & Anil, 2007).

Influence of substrata on the fouling diatom community structure

The diatom community composition did not differ much between the substrata due largely to the dominance of *Navicula* spp. *Navicula* spp. has been reported as the dominant fouling diatom on various non-toxic substrata in the Arabian Sea (Edyvean & Moss, 1986; Tuchman & Blinn, 1979; Blinn et al. 1980; Sullivan, 1975). Earlier studies have reported that there can be large differences in the numbers of organisms on different substrata, but species composition is often similar (Wetzel & Westlake, 1969; Neushul et al. 1976). Higher cell densities were recorded on polystyrene (hydrophobic) substrata than on stainless steel (hydrophilic). A simultaneous study with fibreglass and glass substrata revealed highest number of diatom species and total abundance on the former which is a hydrophobic surface (Patil & Anil, 2005a). However, some of the species exhibited substratum preferences. While *Fragilariopsis* sp., *T. nitzschioides* and *Pleurosigma* were found to dominate on both stainless steel and polystyrene, *O. rhombus*, *Achnanthes*, *Licmophora*, *Grammatophora* and *Synedra* dominated only on polystyrene and *M. nummuloides*, *Pinnularia* and *M. membranacea* dominated only on stainless steel. While selective community development on toxic surfaces is well known (Daniel & Chamberlain, 1981), any substratum preference shown by individual species on non-toxic surfaces indicate either an active selection by some diatom species or are due to more subtle environmental influences, such as topography, surface charge, surface tension or nutrient availability (Edyvean, 1986). Polystyrene is a non-metal and being hydrophobic it accumulates maximum diatom numbers initially (Fletcher, 1988; Pedersen, 1990). The slime film, which develops on any solid surface along with microorganisms attach to substrata and exude high molecular weight polysaccharides. These polysaccharides adsorb water and have a tendency to maintain a water layer even on objects, which are initially non-wetting such as polystyrene (Kenis et al. 1974). Stainless steel being an alloy is hydrophilic and showed low diatom numbers initially. This is because alloys are

known to be chemically active and each provides a unique physical and chemical environment to the colonizing microorganisms. Stainless steel has a more electropolished surface and is known to be more resistant to microbial attack (Dunsmore et al. 1981; Zoltai et al. 1981).

These substratum variations, which resulted in initial higher diatom colonisation on polystyrene compared to stainless steel, were also responsible for the significant relationship in diatom abundance and chlorophyll *a* concentrations, which was restricted to only one-day-old communities for polystyrene as compared to three day-old communities in the case of stainless steel. Once an initial colony has established, new species will settle and develop on top of it (Bishop et al. 1974; Paul et al. 1977). This layering process will rapidly diminish any differences between the substrata, and produce a surface more physically and chemically suitable for colonization by other organisms (Neushul et al. 1976; Paul et al. 1977; Blinn et al. 1980). Detrital material and some other photosynthetic algae may have contributed to the increasing chlorophyll *a* concentrations much earlier on polystyrene than stainless steel substrata.

Summary

In the fouling community, recruitment and reproduction are the two modes contributing to increase in cell numbers whereas cell death, cell loss due to sloughing and grazing are the factors responsible for decrease in cell numbers. Once recruitment takes place, the subsequent increase in cells can be through further recruitment as well as division of the existing cells in the biofilm. The rate of recruitment of a particular species will depend on its cell density in the ambient waters, their rate of attachment, and once colonized, their ability to proliferate under the existing physical, chemical and biological environmental conditions. This study showed that due to its higher abundance in the ambient waters, *N. delicatula* dominated the fouling community on most occasions. However, another genus, *Amphora* which is usually a dominant representative in the fouling film in temperate regions, was not as dominant as *N. delicatula*, which could be due to its lower numbers in the ambient waters in spite of its seed availability in the surrounding intertidal areas. However, experimental evidence has shown that this species can increase in numbers later on and form a well developed community and if conditions are favourable, probably overtake the coexisting species and ultimately dominate the community (Mitbavkar and Anil, 2007). So, in tropical regions, the faster onset of macrofouling probably interferes with the development of the fouling diatom community.

This study also suggests that although the fouling diatom community composition does not vary between the types of substrata, with seasons the community changes depending on the physical, chemical and biological interactions such as competition.

Acknowledgements

We are grateful to the Director of National Institute of Oceanography for his support and encouragement. The first author acknowledges CSIR for providing the Senior Research Fellowship. We are thankful to the anonymous reviewers for their valuable suggestions. This work has been supported by funding from the ONR Grant No: N000114-94-1-0423 and is a NIO contribution (.....#).

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Table captions

Table I. Diatom species observed on stainless steel (SS) and polystyrene (PS) substrata during the one to four day submersion period.

Table IIa. Values of species diversity (H'), species richness (H'_{max}) and evenness (J') of the fouling diatom community over different exposure periods with reference to stainless steel substratum (Shannon-Wieners diversity index).

Table IIb. Values of species diversity (H'), species richness (H'_{max}) and evenness (J') of the fouling diatom community over different exposure periods with reference to polystyrene substratum (Shannon-Wieners diversity index).

Table III. ANOVA to evaluate the temporal variation in diatom abundance, diversity, richness, evenness and chlorophyll *a* on stainless steel and polystyrene substrata with respect to submersion days.

Table IV. Growth rates (d^{-1}) of *N. delicatula* and *A. coffeaeformis* when incubated separately at different salinities.

Legend to figures

Figure 1. Temporal variation in the total diatom abundance (cells dm^{-2}) recorded on stainless steel (a) and polystyrene (b) substrata for different submersion periods (Day 1; Day 2; Day 3 and Day 4).

Figure 2. Temporal distribution of fouling diatoms (cells dm^{-2} ; $\log(x + 1)$ transformed) recorded from stainless steel substrata.

Figure 3. Temporal distribution of fouling diatoms (cells dm^{-2} ; $\log(x + 1)$ transformed) recorded from polystyrene substrata.

Figure 4. Cluster dendrogram of the one to four day old fouling diatom abundance on stainless steel (a) and polystyrene (b) using the Bray-Curtis similarity coefficient and group average method.

Figure 5. Linear regression analyses between the total diatom abundance and chlorophyll *a* concentrations from stainless steel (a) and polystyrene (b) substrata.

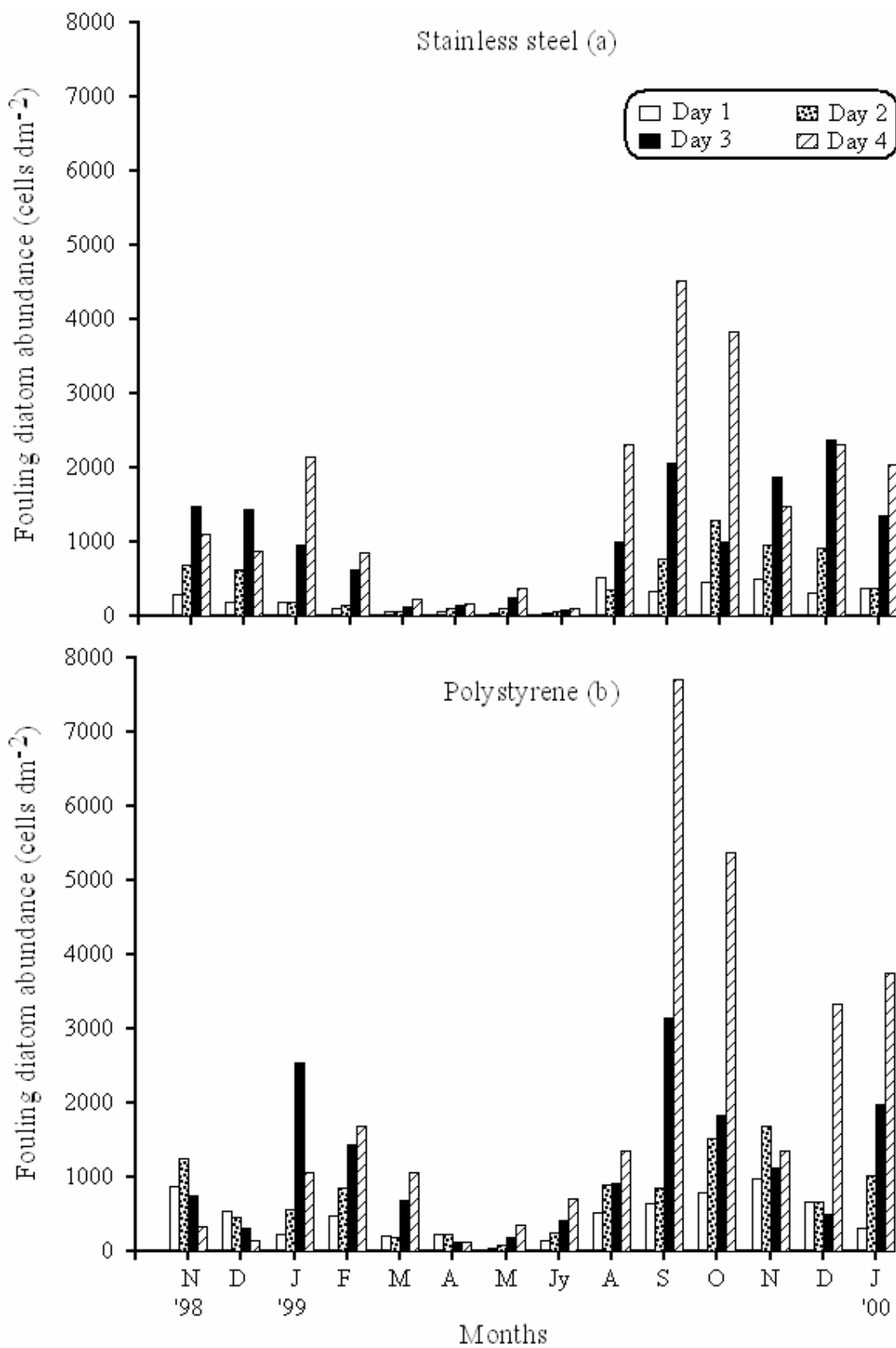


Figure 1.

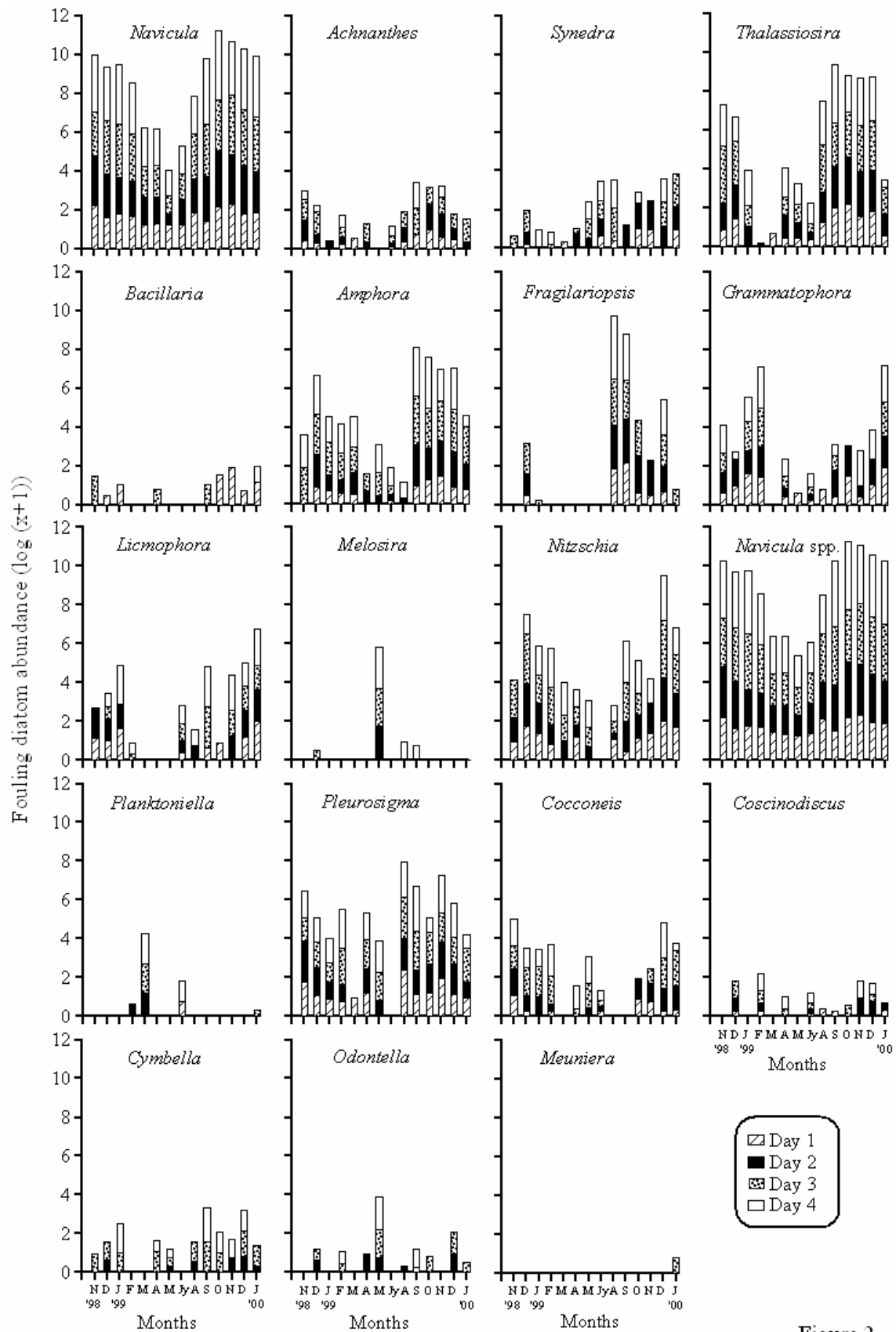


Figure 2.

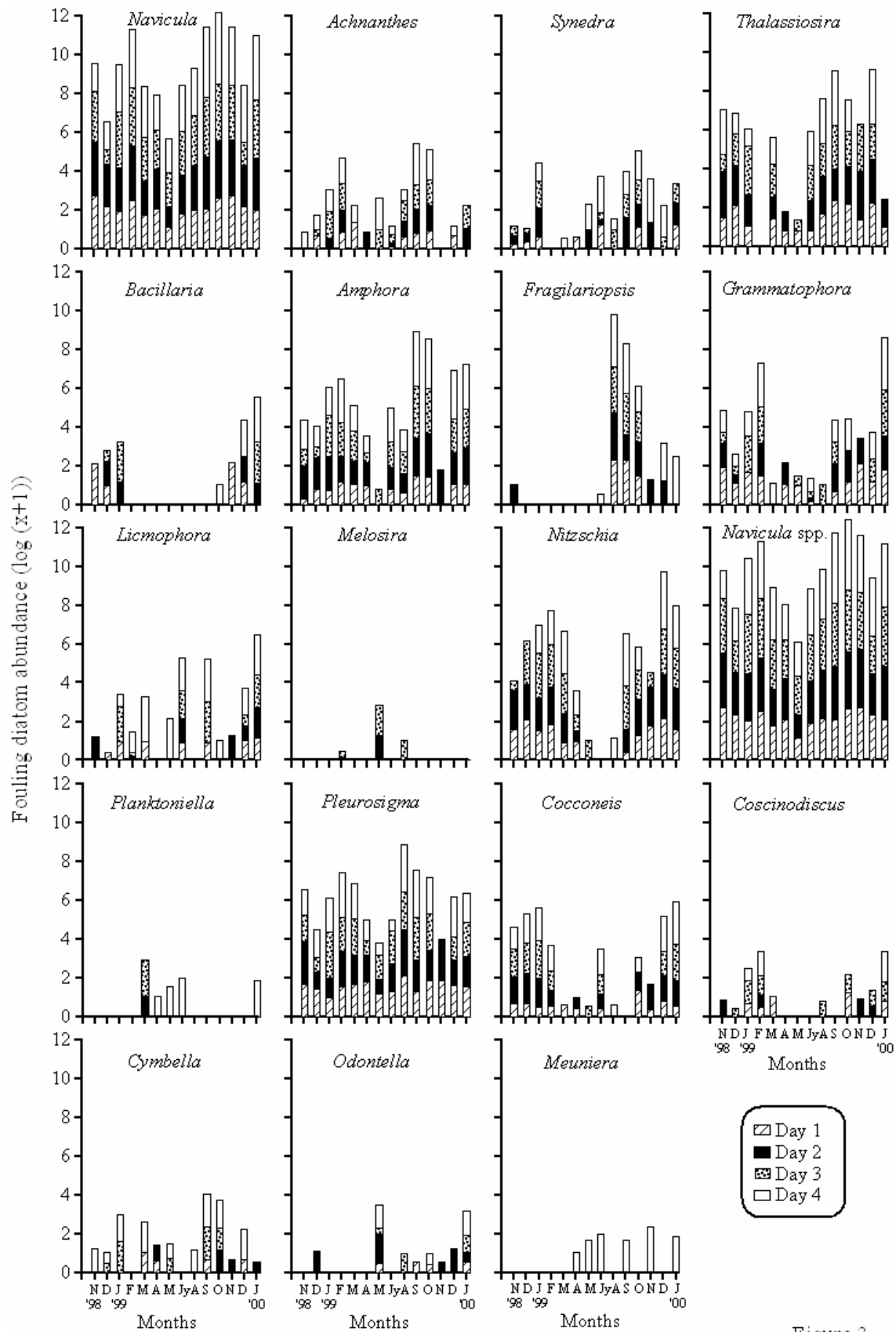


Figure 3.

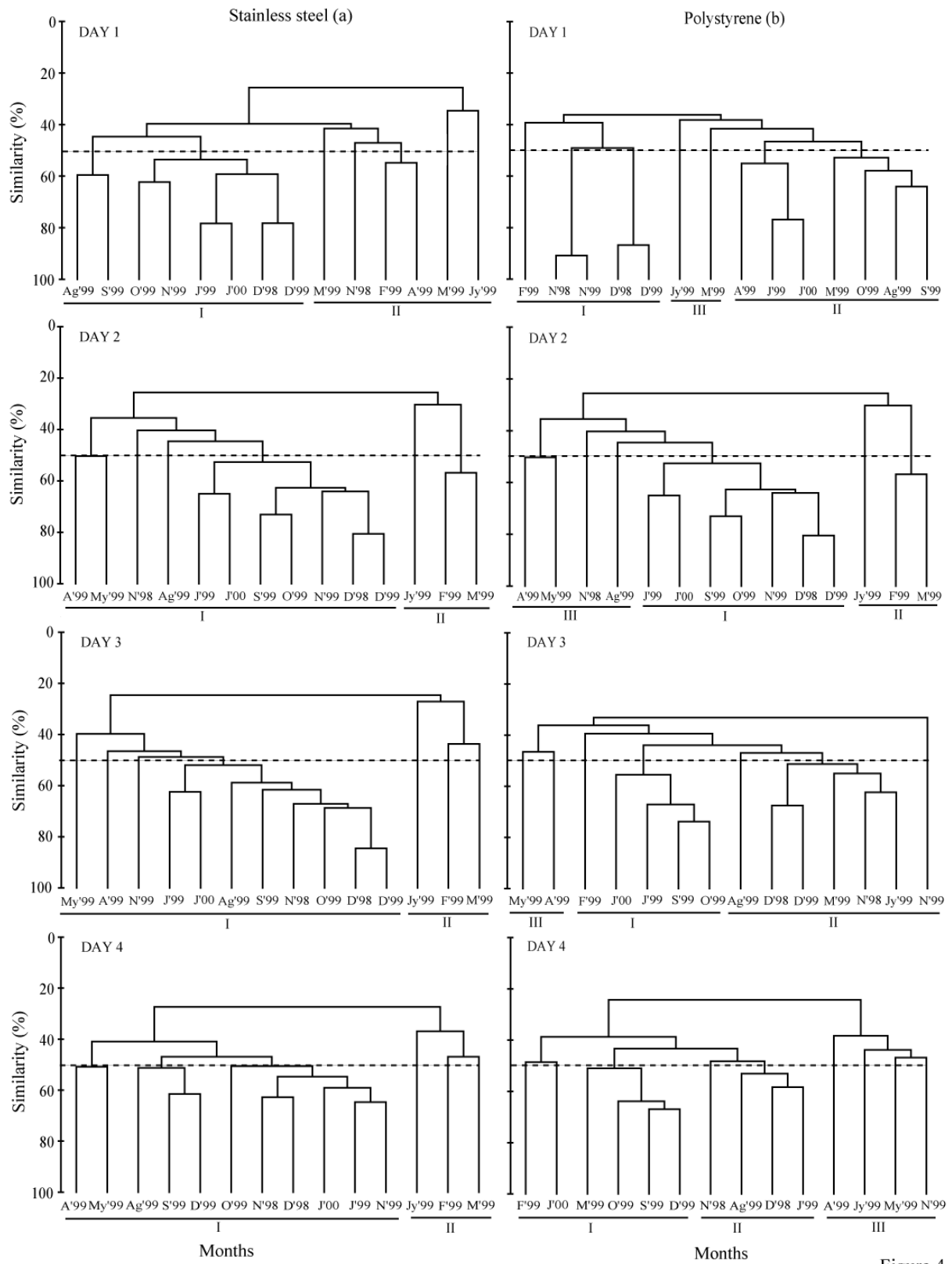


Figure 4.

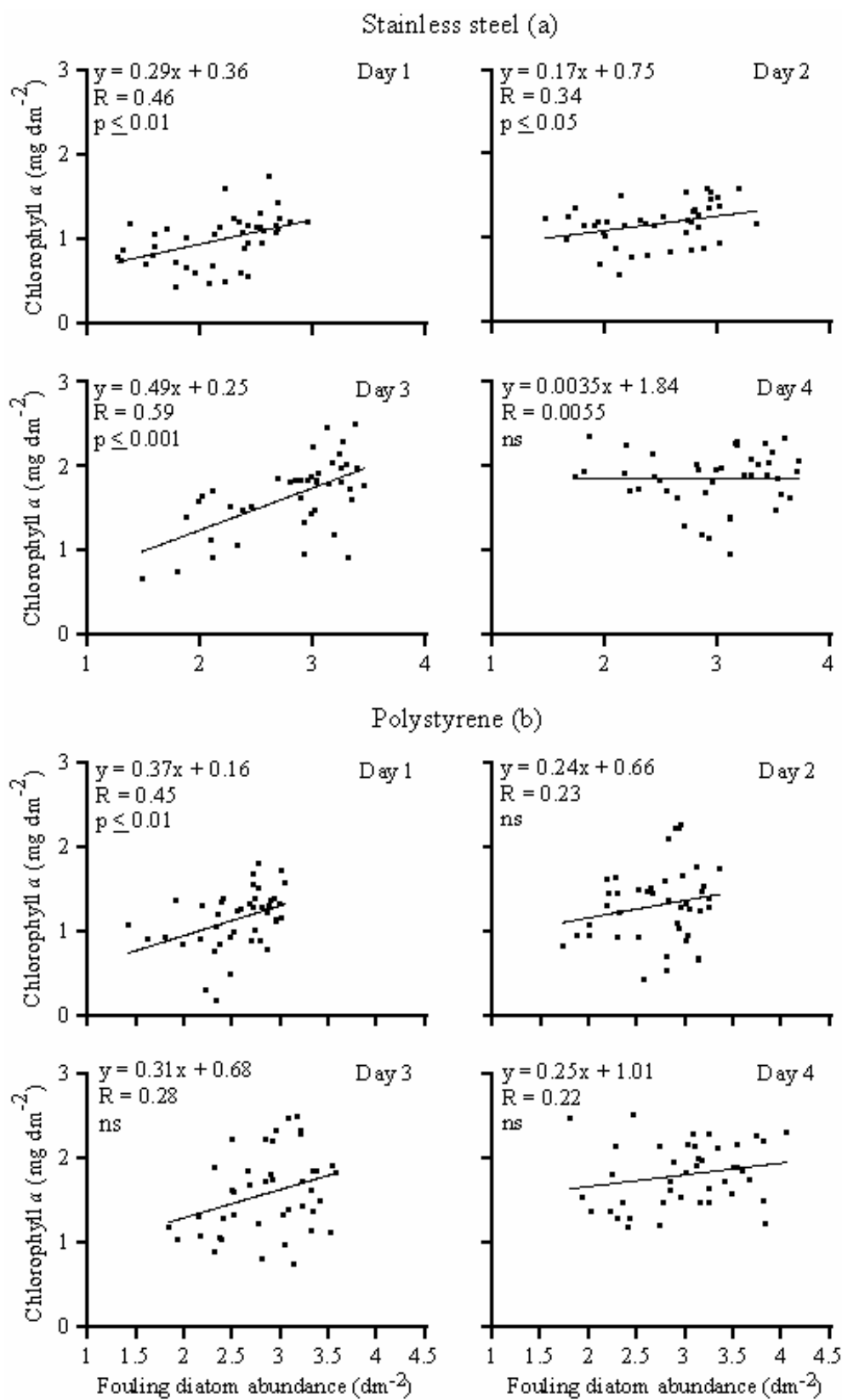


Figure 5.

Table 2. Values of species diversity (H'), species richness (H' max) and evenness (J') of the fouling diatom community over different exposure periods

Months	Day 1			Day 2			Day 3			Day 4		
	H'	H' max	J'	H'	H' max	J'	H'	H' max	J'	H'	H' max	J'
With reference to a SS substratum (Shannon - Wieners diversity index)												
Nov '98	1.92	1.61	0.58	1.88	1.38	0.57	2.48	2.61	0.57	1.32	1.14	0.42
Dec	3.58	4.67	0.77	3.24	3.89	0.69	2.70	2.61	0.62	1.65	2.21	0.41
Jan '99	2.93	3.07	0.72	2.84	2.50	0.75	2.04	2.63	0.48	1.77	1.43	0.49
Feb	1.95	2.45	0.54	2.18	2.27	0.61	2.20	1.56	0.64	2.22	1.49	0.64
Mar	2.66	2.37	0.80	1.90	0.73	0.95	1.94	0.64	0.97	1.85	0.56	0.92
Apr	2.70	2.01	0.85	3.38	3.80	0.81	3.55	4.68	0.77	2.52	1.97	0.73
May	1.33	0.96	0.67	2.45	3.06	0.63	3.16	2.57	0.81	3.22	2.37	0.82
Jul	2.58	3.29	0.70	2.71	3.22	0.71	2.92	3.04	0.77	3.27	3.31	0.82
Aug	2.33	2.25	0.60	2.42	2.58	0.60	2.82	1.45	0.81	1.20	1.55	0.32
Sep	2.33	3.47	0.53	3.07	2.26	0.77	3.25	3.28	0.69	2.63	2.73	0.57
Oct	2.87	2.46	0.72	2.44	2.10	0.61	2.65	2.32	0.65	0.84	1.33	0.23
Nov	3.04	3.39	0.68	3.10	3.21	0.69	1.98	1.86	0.51	2.72	2.74	0.62
Dec	3.62	4.37	0.77	3.51	4.85	0.69	2.71	2.70	0.61	2.52	3.75	0.51
Jan '00	3.08	3.06	0.73	3.54	4.56	0.74	3.02	5.42	0.57	1.45	1.44	0.41
With reference to a PS substratum (Shannon - Wieners diversity index)												
Nov '98	1.98	1.92	0.52	2.89	2.38	0.69	1.47	1.36	0.44	2.03	1.38	0.64
Dec	3.32	3.34	0.75	2.76	2.12	0.72	2.61	3.34	0.60	3.05	2.23	0.85
Jan '99	2.97	2.77	0.74	3.06	2.06	0.80	3.72	3.19	0.79	2.33	2.88	0.53
Feb	1.62	1.46	0.49	1.50	1.63	0.42	1.48	1.51	0.41	1.92	1.08	0.60
Mar	3.33	2.26	0.90	2.89	1.55	0.91	2.84	1.23	0.89	2.99	2.73	0.69
Apr	2.72	2.95	0.67	2.72	3.56	0.63	0.89	1.26	0.32	2.16	1.26	0.77
May	2.65	1.87	0.88	2.54	1.39	0.90	3.19	3.45	0.75	2.58	1.36	0.81
Jul	2.55	2.64	0.67	2.76	2.72	0.69	2.75	2.34	0.70	2.95	2.14	0.75
Aug	2.50	1.28	0.79	2.61	1.18	0.82	2.46	2.05	0.63	2.69	2.22	0.66
Sep	2.48	3.10	0.56	2.31	2.23	0.58	3.09	2.98	0.66	2.57	2.35	0.58
Oct	2.57	2.70	0.60	2.63	2.87	0.59	2.35	2.13	0.58	0.99	2.56	0.22
Nov	2.10	1.16	0.66	2.88	2.83	0.65	1.58	0.57	0.68	1.16	0.42	0.58
Dec	3.56	3.55	0.78	3.21	3.24	0.72	2.84	2.58	0.70	3.45	3.33	0.72
Jan '00	3.45	3.48	0.78	3.04	4.33	0.61	2.66	2.50	0.62	2.68	2.07	0.64

Table 3. ANOVA to evaluate the temporal variation in diatom abundance, diversity, richness, evenness and chlorophyll *a* on stainless steel and polystyrene substrata with respect to submersion days

Source of variation	df	Abundance			Diversity			Species richness			Evenness			Chlorophyll <i>a</i>		
		SS	MS	<i>F_s</i>	SS	MS	<i>F_s</i>	SS	MS	<i>F_s</i>	SS	MS	<i>F_s</i>	SS	MS	<i>F_s</i>
A: Substrata	1	1.21	1.21		0.00	0.00		0.04	0.04		0.000	0.000		0.05	0.05	
B: Months	13	17.28	1.33		0.23	0.02		0.74	0.06		0.063	0.005		7.11	0.55	
C: Days	3	6.97	2.32		0.08	0.03		0.14	0.05		0.013	0.004		8.85	2.95	
A x B	13	2.02	0.16	3.61***	0.12	0.01	2.3**	0.24	0.02	1.98*	0.019	0.001	2.08*	1.36	0.10	2.02*
A x C	3	0.35	0.12	2.87 ^{ns}	0.04	0.01	2.91*	0.01	0.00	0.49 ^{ns}	0.005	0.002	2.44 ^{ns}	0.45	0.15	2.90*
B x C	39	2.87	0.07	1.7 ^{ns}	0.24	0.01	1.54 ^{ns}	0.38	0.01	1.04 ^{ns}	0.042	0.001	1.58 ^{ns}	3.44	0.09	1.70 ^{ns}
A x B x C	39	1.68	0.04		0.16	0.00		0.37	0.01		0.027	0.001		2.02	0.05	
Total	111	32.37			0.85			1.93			0.170			23.16		

(*p < 0.05; **p < 0.025; ***p < 0.001; ns = not significant)

Table 4. Growth rates (day^{-1}) of *N. delicatula* and *A. coffeaeformis* when incubated separately at different salinities

Incubation period (days)	Salinity				Salinity			
	5	15	30	40	5	15	30	40
	<i>N. delicatula</i>				<i>A. coffeaeformis</i>			
0-4	0.09	0.09	0.03	0.07	0.2	0.11	0.16	0.18
4-6	-0.02	-0.02	0.05	0.04	0.03	0.01	0.06	0.11
6-8	0.04	0.03	0.06	0.09	0.04	0.13	0.15	0.12