

Chapter 3

Diatoms of the fouling community

3.1 Introduction

A layer of microorganisms forming a microfilm quickly covers any surface submerged in water. These microfilms are observed in most marine waters including polar (Ford et al. 1989; Maki et al. 1990), temperate (Berk et al. 1981) and tropical ecosystems (Hofmann et al. 1978) as well as freshwater environments (Jones and Lock 1993). Biofouling and biofilm formation are of great concern to many modern industries including marine, food, water, mining and medical industries. 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. The economic consequence of biofouling is thus significant. Predicting the type, rate and extent of microfilm formation is useful in environmental studies, pollution abatement and design and operation of industrial equipment, which is prone to biofouling. 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 and Wilcox 1971). However, according to Horbund and Freiburger (1970) and Cooksey et al. (1980), although the presence of the bacterial film may facilitate the attachment of diatoms, it is not a pre-requisite. 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; 1987). Although microbiological investigations of the primary fouling film have consistently revealed the presence of diatoms in large numbers, few studies have focused on these organisms in the marine environment and their role in subsequent colonization of macrofoulers in marine environment (Cooksey et al. 1984; Edyvean et al. 1985; Karande 1987, 1989; Satyanarayan Rao and Balaji 1988; Bhosle et al. 1989;

Kelkar 1989; Shantakumaran 1989; Rao 1990; Raveendran et al. 1991; Bhosle 1993; Pangu 1993; Venugopalan et al. 1994; Prabhadevi 1995a; 1995b; Redekar 1997; Redekar and Wagh 2000b).

The structure and composition of the fouling community exhibit wide temporal and regional variations. 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.

Zuari estuary, the site of this investigation, is influenced by the south - west monsoon. 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 and 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). In this investigation, an effort has been made to evaluate its influence on the microfouling diatom community structure to answer the question, “How do different substratum’s and submersion periods influence the fouling diatom community structure?”

3.2 MATERIALS AND METHODS

3.2.1 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, ($15^{\circ} 27' N$, $73^{\circ} 48' E$), on the west coast of India (Fig. 3.1). 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 season. Based on this, a year has been classified into three seasons viz. monsoon season (June – September), followed by a recovery period during post – monsoon (October – January) and thereafter a stable period of the pre-

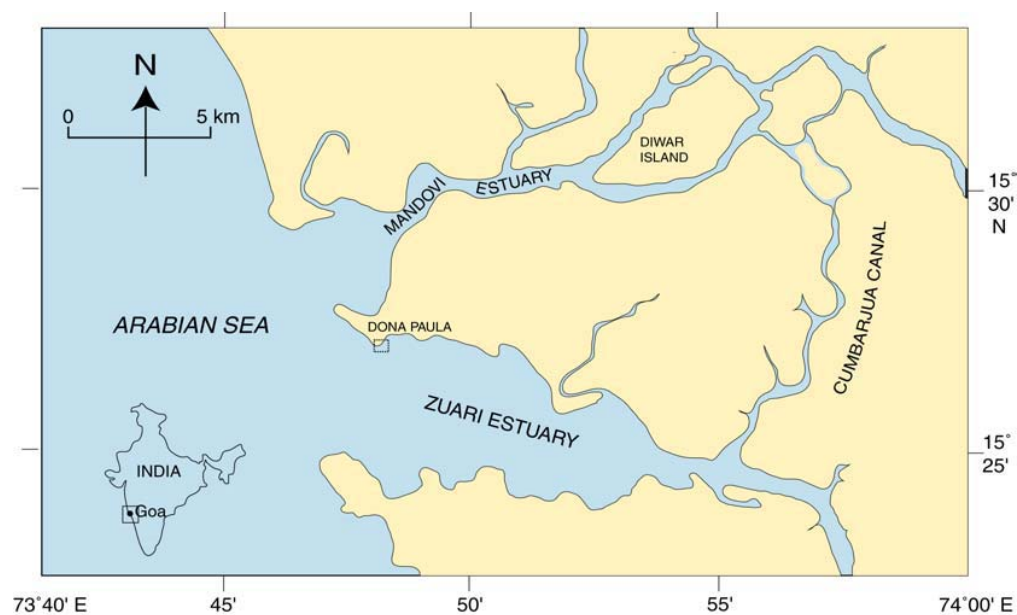


Fig. 3.1 Geographical location of the study area

monsoon (February – May).

3.2.2 Environmental parameters

Surface water samples (in triplicates) were collected every month for 5 consecutive days from the study site, during the period of panel submersion. Analysis of temperature, salinity (Mohr-Knudsen titration method) (Strickland and Parsons 1965), dissolved oxygen and nutrients ($\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and SiO_3) were carried out by standard procedures (Parsons et al. 1984). A known volume of the water sample was preserved with Lugol's iodine solution for estimating the diatom population by the sedimentation method (Hasle 1978).

3.2.3 Type of substrata

Stainless steel panels ($15 \times 10 \times 2\text{cm}$) and polystyrene (8.5 cm diameter) petridishes were used in this study.

3.2.4 Panel submersion

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 (~ 1m below lowest low tide level). Earlier reports (Smita et al. 1997; Smita and Anil 2000) and personal observations of the present study area have revealed the dominance of microorganisms on substrates for one to four days, followed by the arrival of macro organisms. 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).

3.2.5 Assessment of settlement

Panels (in triplicates) 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 0.45 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. 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 dm².

3.2.6 Chlorophyll a analysis

Simultaneously, another set of scraped material from the panels was used for chlorophyll *a* analyses (Parsons et al. 1984).

3.2.7 Data analysis

The water column and fouling diatom community were subjected to univariate and multivariate analyses.

Univariate analyses: Univariate techniques included the calculation of Shannon-Wiener's diversity index (H'), species richness ($H'_{\max} = \log_2 S$ 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 was subjected to Multiple Analysis of Variance (MANOVA) (Sokal and Rohlf 1981) to evaluate temporal (days and months) and substratum variance.

The water column and fouling film diatom community (which averaged to more than 5% of the total diatom community) were subjected to cluster analysis with respect to species for 1, 2, 3 and 4 days of submersion period. Clustering was performed through Bray-Curtis coefficients (Bray and Curtis 1957) and group average method (Pielou 1984). Data was subjected to square root transformation prior to analysis. Univariate and multivariate analyses were made using PRIMER software version 5.

Linear regression analysis was performed on the log-transformed values of diatom abundance and chlorophyll *a* concentrations of the fouling diatom community.

3.3 RESULTS

3.3.1 Environmental parameters

Temperature ranged from 25 °C (February) to 30 °C (November) and salinity ranged from 15 psu to 35 psu. Dissolved oxygen ranged from 3.0 to 6.1 ml L⁻¹. Nitrate (NO₃-N) peaks were observed in April, July and December. Nitrite (NO₂-N) peaked in March and phosphate (PO₄-P) in May. Silicate (SiO₃) peaked in July (Fig. 3.2).

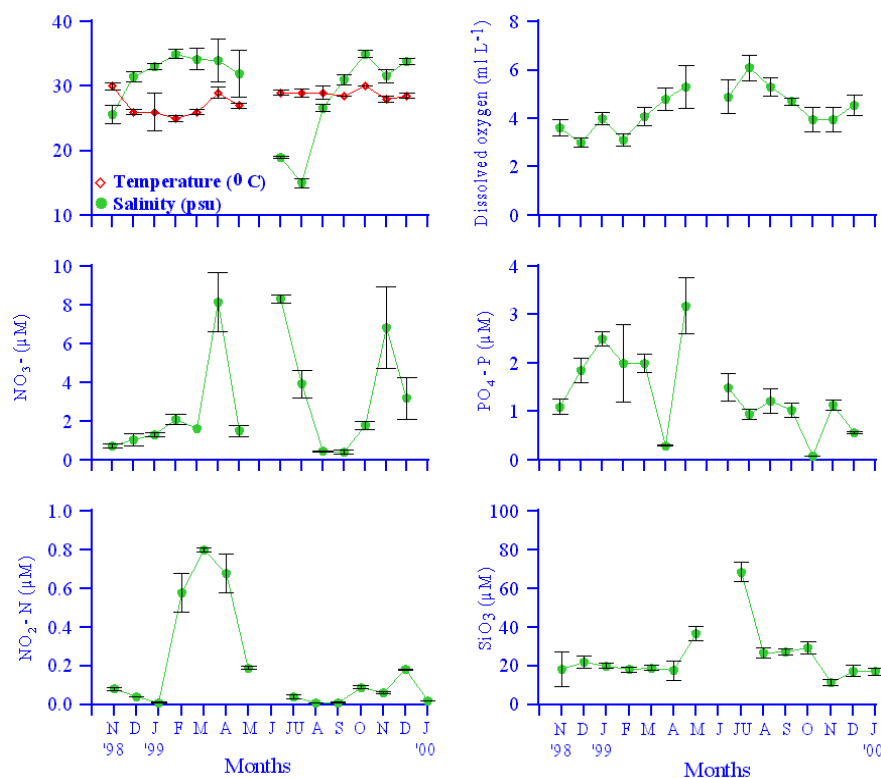


Fig. 3.2 Temporal variation in temperature, salinity, dissolved oxygen and nutrient concentrations of the ambient waters

3.3.2 Diatoms of the water column

In the surrounding waters, 50 species (27 centrics, 23 pennates) belonging to 34 genera (19 centrics, 15 pennates) were encountered (Table 3.1). The average variations in the diatom cell numbers ranged from 1 to 33×10^3 cells L⁻¹ (Fig. 3.3a). In September, blooms of *Skeletonema costatum*, *Fragilariopsis oceanica* and *Thalassionema nitzschioides* were observed. Blooms of *Leptocylindrus danicus* and *Cerataulina pelagica* were encountered in post-monsoon. The percentage ratio of pennate to centric diatoms remained below one during most of the months, indicating the dominance of centric diatoms (Fig. 3.3b).

Diatom species diversity was comparatively higher during the post monsoon. The lowest species diversity was encountered in September (0.8) due to blooms of *S. costatum*, *F. oceanica* and *T. nitzschioides* (Fig. 3.3c).

Table 3.1. List of diatom species encountered in the surrounding waters, on stainless steel (SS) and polystyrene (PS) substrata during the one to four day submersion period

PENNATES		Abbreviation	WC-0	WC-1	WC-2	WC-3	WC-4	SS-1	SS-2	SS-3
<i>Achnanthes brevipes</i> Agardh		<i>Ach bre</i>						+	+	+
<i>Achnanthes longipes</i> Agardh		<i>Ach lon</i>						+	+	+
<i>Achnanthes subsessilis</i> Kutzing		<i>Ach sub</i>						+	+	+
<i>Achnanthes taeniata</i> Grunow		<i>Ach tae</i>								
<i>Amphora coffeaeformis</i> (Agardh) Kutzing		<i>Amp cof</i>	+	+	+	+	+	+	+	+
<i>Amphora hyalina</i> Kutzing		<i>Amp hya</i>							+	+
<i>Amphora ovalis</i> (Kutzing) Kutzing		<i>Amp ova</i>						+	+	
<i>Amphora rostrata</i> Wm. Smith		<i>Amp ros</i>						+	+	+
<i>Amphora turgida</i> Gregory		<i>Amp tur</i>						+	+	+
<i>Asterionella japonica</i> Cleve & Moller ex Gran		<i>Ast jap</i>		+	+	+	+			
<i>Bacillaria paxillifera</i> (O.F. Muller)		<i>Bac pax</i>						+		+
<i>Climacosphaenia</i> sp.		<i>Cli sp.</i>								
<i>Cocconeis scutellum</i> Ehrenberg		<i>Coc scu</i>	+	+	+	+	+	+	+	+
<i>Cocconeis</i> sp.		<i>Coc sp.</i>						+		
<i>Cymbella gastroides</i> Kutzing		<i>Cym gas</i>	+	+	+		+		+	+
<i>Cymbella</i> sp.		<i>Cym sp.</i>								
<i>Diploneis smithii</i> (de Brebisson) Wm. Smith		<i>Dip smi</i>						+	+	+
<i>Fragilariopsis oceanica</i> (Cleve) Haslea		<i>Fra oce</i>	+	+	+	+	+	+	+	+
<i>Grammatophora marina</i> Kutzing		<i>Gra mar</i>	+	+	+	+	+	+	+	+
<i>Grammatophora serpentina</i> Ehrenberg		<i>Gra ser</i>						+	+	+
<i>Licmophora ehrenbergii</i> (Kutzing) Grunow		<i>Lic ehr</i>							+	+
<i>Licmophora flabellata</i> (Greville) Agardh		<i>Lic fla</i>						+	+	+
<i>Licmophora gracilis</i> (Ehrenberg) Grunow		<i>Lic gra</i>						+	+	+
<i>Licmophora juergensii</i> Agardh		<i>Lic jue</i>						+	+	+
<i>Licmophora paradoxa</i> (Lyngbye) Agardh		<i>Lic par</i>	+	+		+	+	+	+	+
<i>Meuniera membranaceae</i> (Cleve) P.C.Silva comb. nov.		<i>Meu mem</i>								+
<i>Navicula crucicula</i> (Wm. Smith) Donkin		<i>Nav cru</i>						+	+	+
<i>Navicula transitans</i> var. <i>derasa</i> f. <i>delicatula</i> Heimdal		<i>Nav del</i>	+	+	+	+	+	+	+	+
<i>Navicula subinflata</i> Grunow		<i>Nav sub</i>	+	+	+			+	+	+

<i>Navicula</i> sp.	<i>Nav</i> sp.	+	+	+	+	+	+	+	+
<i>Nitzschia angularis</i> Wm. Smith	<i>Nit ang</i>	+	+	+	+	+	+	+	+
<i>Nitzschia bilobata</i> Wm. Smith	<i>Nit bil</i>	+	+	+					+
<i>Nitzschia closterium</i> (Ehrenberg)	<i>Nit clo</i>	+	+	+	+	+	+	+	+
<i>Nitzschia longissima</i> (Brebisson, in Kutzing)	<i>Nit lon</i>	+	+	+	+	+	+		+
<i>Nitzschia panduriformis</i> Gregory	<i>Nit pan</i>		+	+	+	+	+	+	+
<i>Nitzschia sigma</i> (Kutzing) Wm. Smith	<i>Nit sig</i>	+	+					+	+
<i>Pinnularia rectangulata</i> (Gregory) Rabenhorst	<i>Pin rec</i>								+
<i>Pleurosigma angulatum</i> Sensu W. Smith emend. Sterrenburg	<i>Ple ang</i>	+	+	+	+	+	+	+	+
<i>Pleurosigma elongatum</i> Wm. Smith	<i>Ple elo</i>	+	+					+	+
<i>Pleurosigma</i> sp.	<i>Ple sp.</i>							+	+
<i>Pseudonitzschia seriata</i> Cleve	<i>Pse ser</i>	+	+	+	+	+	+		+
<i>Stauroneis septrionalis</i> Grunow	<i>Sta sep</i>							+	+
<i>Surirella</i> sp1.	<i>Sur sp1</i>	+	+		+	+	+	+	+
<i>Surirella</i> sp2.	<i>Sur sp2</i>	+	+						
<i>Synedropsis affinis</i> Kutzing	<i>Syn aff</i>							+	+
<i>Synedropsis gaillonii</i> Grunow	<i>Syn gai</i>	+	+	+	+	+	+	+	+
<i>Synedropsis hyperborea</i> (Grunow) Hasle, Medlin & Syvertsen	<i>Syn hyp</i>							+	+
<i>Thalassionema frauenfeldii</i> Grunow Hallegraeff	<i>Tha fra</i>							+	+
<i>Thalassionema nitzschioides</i> (Grunow) Mereschkowsky	<i>Tha nit</i>	+	+	+	+	+		+	+
<i>Thalassiothrix longissima</i> Cleve & Grunow	<i>Tha lon</i>							+	+

CENTRICS

<i>Bacteriastrum hyalinum</i> Lauder	<i>Bac hya</i>		+	+	+	+			
<i>Biddulphia mobiliensis</i> (Bailey) Grunow	<i>Bid mob</i>								+
<i>Biddulphia pulchella</i> Gray	<i>Bid pul</i>							+	+
<i>Biddulphia regia</i> (M. Schultze) Ostenfeld	<i>Bid reg</i>		+		+	+			
<i>Biddulphia rhombus</i> (Ehrenberg) Smith, W.	<i>Bid rho</i>							+	+
<i>Biddulphia sinensis</i> Greville	<i>Bid sin</i>		+	+	+	+			
<i>Cerataulina pelagica</i> (Cleve) Hendy	<i>Cer pel</i>		+	+	+	+			
<i>Chaetoceros curvisetus</i> Ehrenberg	<i>Cha cur</i>	+	+	+	+	+	+	+	
<i>Chaetoceros diversus</i> Cleve	<i>Cha div</i>		+	+	+	+			

<i>Chaetoceros lorenzianus</i> Grunow	<i>Cha lor</i>	+	+	+	+			
<i>Climacodium frauenfeldianum</i> Grunow	<i>Cli fra</i>	+	+					
<i>Corethron criophilum</i> Castracane	<i>Cor cri</i>		+	+	+			
<i>Coscinodiscus marginatus</i> Ehrenberg	<i>Cos mar</i>	+	+	+	+	+	+	+
<i>Coscinodiscus radiatus</i> Ehrenberg	<i>Cos rad</i>							
<i>Coscinodiscus</i> sp.	<i>Cos sp.</i>		+	+	+	+		
<i>Ditylum brightwellii</i> (T. West) Grunow ex Van Heurck	<i>Dit bri</i>		+	+	+	+		
<i>Eucampia zodiacus</i> Ehrenberg	<i>Euc zod</i>		+	+	+	+		
<i>Guinardia flaccida</i> (Castracane) Peragallo	<i>Gui fla</i>		+	+	+	+		
<i>Guinardia striata</i> (Stolterfoth) Hasle com. nov.	<i>Gui str</i>	+	+	+	+	+		
<i>Helicotheca tamesis</i> (Shrubsole) Ricard	<i>Hel tam</i>		+	+	+			
<i>Hemiaulus sinensis</i> Greville	<i>Hem sin</i>		+		+			
<i>Hyalodiscus stelliger</i> (Bailey) Mann	<i>Hya ste</i>						+	+
<i>Leptocylindrus danicus</i> Cleve	<i>Lep dan</i>		+	+	+	+		
<i>Melosira nummuloides</i> C. A. Agardh	<i>Mel num</i>	+	+	+	+	+	+	+
<i>Planktoniella sol</i> (Wallich) Schutt	<i>Pla sol</i>	+	+	+	+	+	+	+
<i>Rhizosolenia alata</i> Brightwell	<i>Rhi ala</i>	+	+	+	+	+	+	
<i>Rhizosolenia stotiforthii</i> H. Peragallo	<i>Rhi sto</i>		+	+	+	+		
<i>Skeletonema costatum</i> (Greville) Cleve	<i>Ske cos</i>	+	+	+	+	+		+
<i>Striatella</i> sp.	<i>Str sp.</i>						+	+
<i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve	<i>Tha ecc</i>	+	+	+	+	+	+	+
<i>Thalassiosira subtilis</i> (Ostenfled) Gran.	<i>Tha sub</i>		+		+	+		
<i>Thalassiosira</i> sp.	<i>Tha sp.</i>		+	+	+	+		

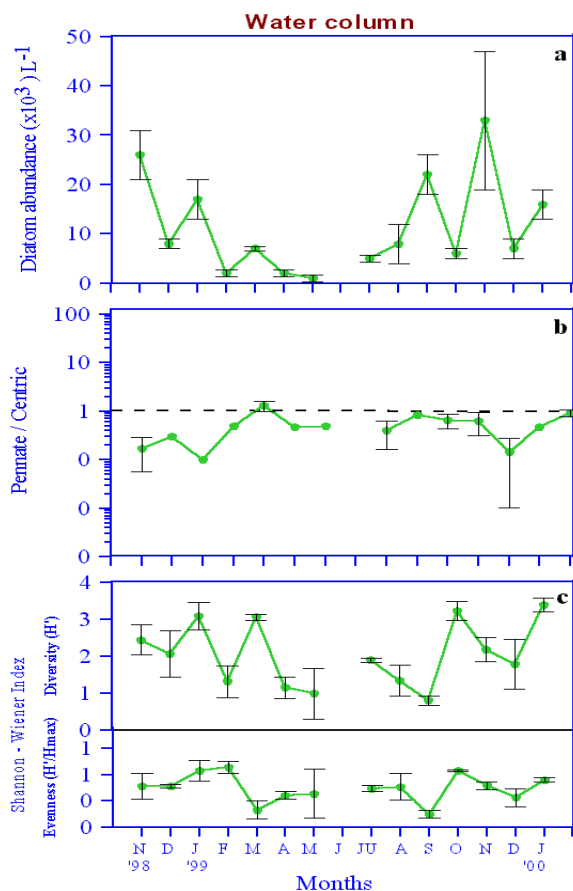


Fig. 3.3 Temporal variations in (a) diatom abundance; (b) ratio of pennate / centric diatoms; (c) diatom diversity and evenness in the ambient waters

Month wise clustering of the diatom population resulted in three groups (Fig. 3.4a). The first group comprised pre-monsoon months (February - May). The post-monsoon months (October - January) formed the second group while the third group comprised the monsoon months (July - September).

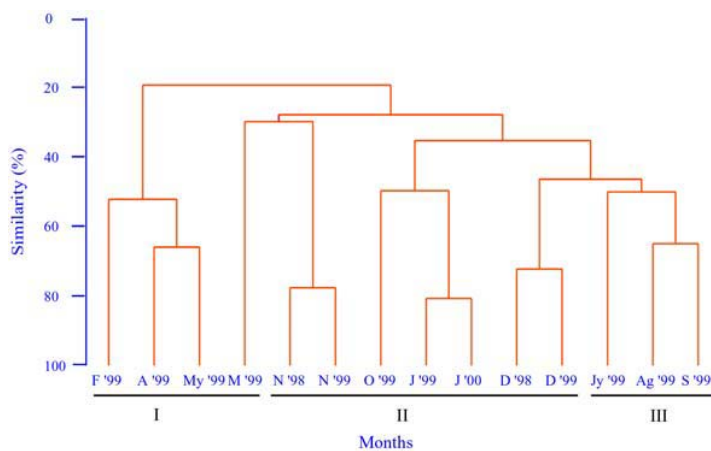


Fig. 3.4a Dendrogram of the diatom community from the ambient waters with respect to months

Cluster dendrograms with respect to diatom species revealed four major groups (Fig. 3.4b). Group I comprised of *N. delicatula* and *Thalassiosira eccentrica*. Groups II to IV comprised *S. costatum*, *T. nitzschoides*, *Cerataulina pelagica*, *L. danicus*, *F. oceanica*, *Pseudonitzschia seriata* and *Chaetoceros curvisetus*, which resulted in occasional blooms in the ambient waters. Rest of the minor forms comprised groups V to XIII.

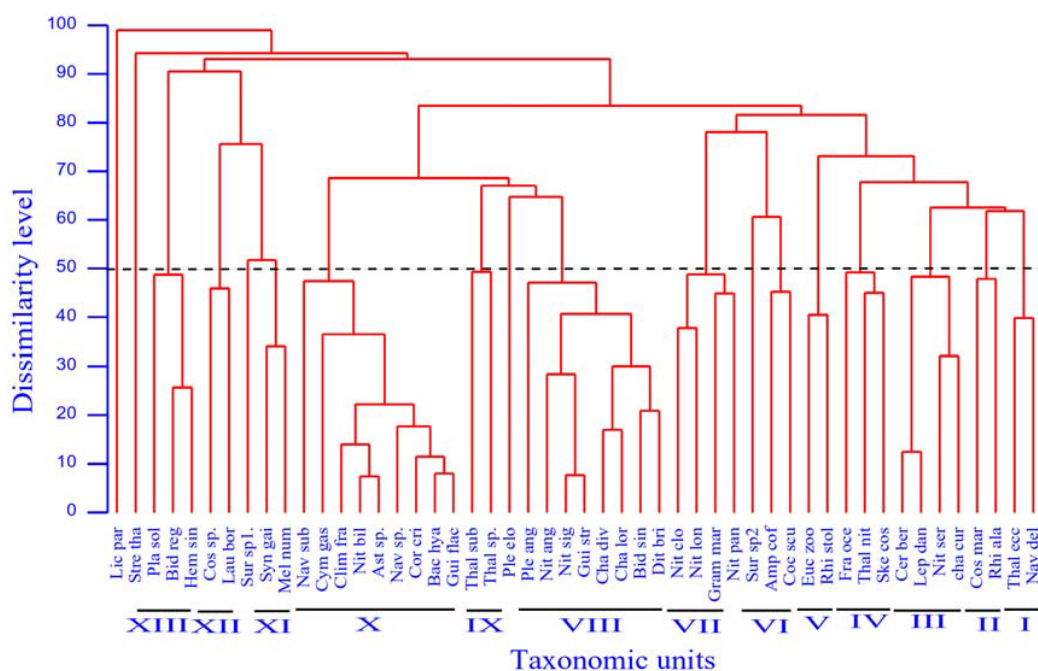


Fig. 3.4b Dendrogram of the diatom community from the ambient waters with respect to species

Diatoms of the fouling film

Stainless steel: Diatoms encountered on stainless steel substrata are presented in Table 3.1. The fouling population included 60 diatom species (46 pennates, 14 centric) belonging to 31 genera (20 pennates, 11 centric). The pennate / centric diatom percentage ratio on this substrata remained above 1 on all occasions except in May '99 indicating the dominance of pennate diatoms (Fig. 3.5). In May, the centric diatom, *Melosira nummuloides* dominated the two to four day old microfilm. The

total diatom abundance was lower in the pre-monsoon and beginning of monsoon season (Fig. 3.6).

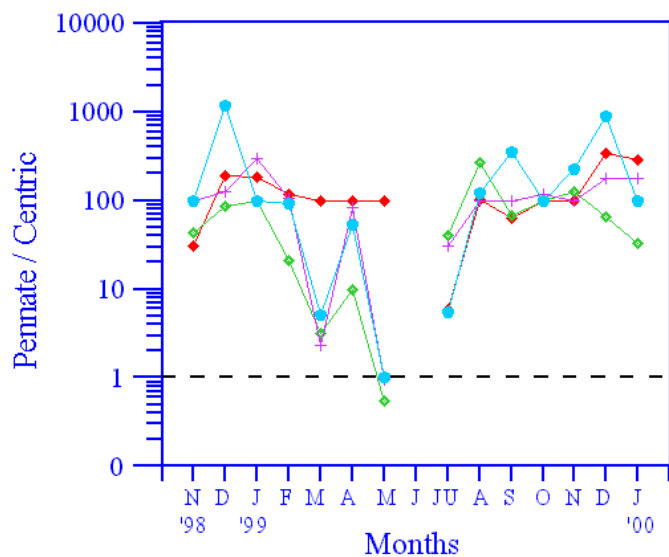


Fig. 3.5 The ratio of pennate / centric diatoms in the microfilm developed over stainless steel substrata (◇ Day 1; ◆ Day 2; + Day 3; ● Day 4)

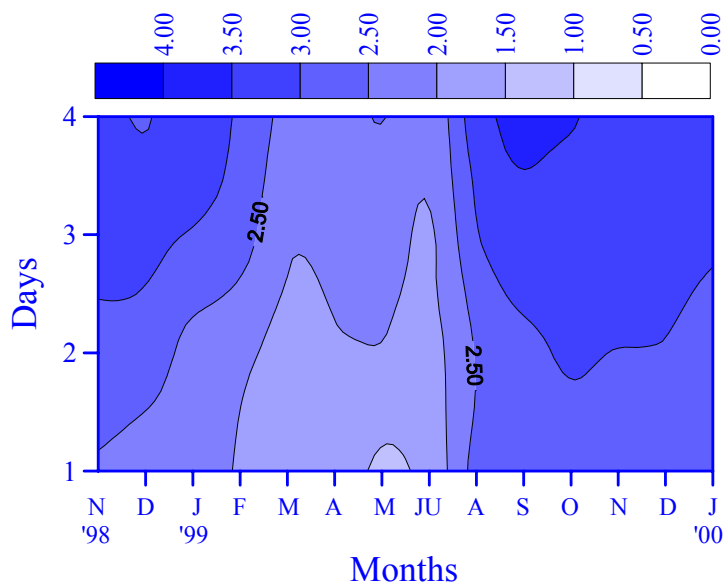


Fig. 3.6 Temporal variation in the fouling diatom abundance (cells dm^{-2}) on stainless steel substrata for different submersion periods (Day 1; Day 2; Day 3 and Day 4)

Distribution of the most dominant species, *N. delicatula* governed the diversity of the diatom community during different submersion days. Species diversity of one-day-old film peaked during December and January with a drop during February and May. In February, dominance by *N. delicatula* and *Grammatophora marina* resulted in lowered species diversity whereas in May comparatively less number of species were

encountered. Peaks were observed in December and April in case of the two day old community. In the three-day-old community, peak in species diversity was observed in April. In the four-day-old community, drop in species diversity was observed in October due to the dominance of *T. nitzschioides* (Table 3.2).

Table 3.2 Values of species diversity (H'), species richness (H' max) and evenness (H' / H' max) of the fouling diatom community over different exposure periods with reference to stainless steel substratum (Shannon-Wieners diversity index)

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'
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'98	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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

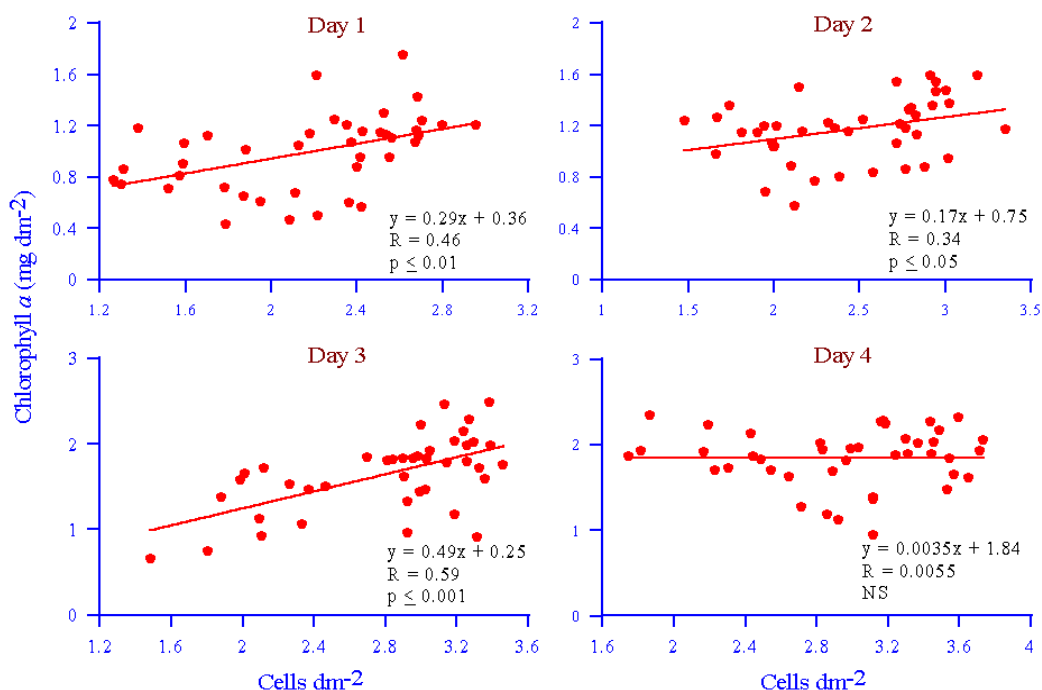


Fig. 3.7 Linear regression analysis between diatom abundance and chlorophyll a concentrations on stainless steel substratum for different submersion periods

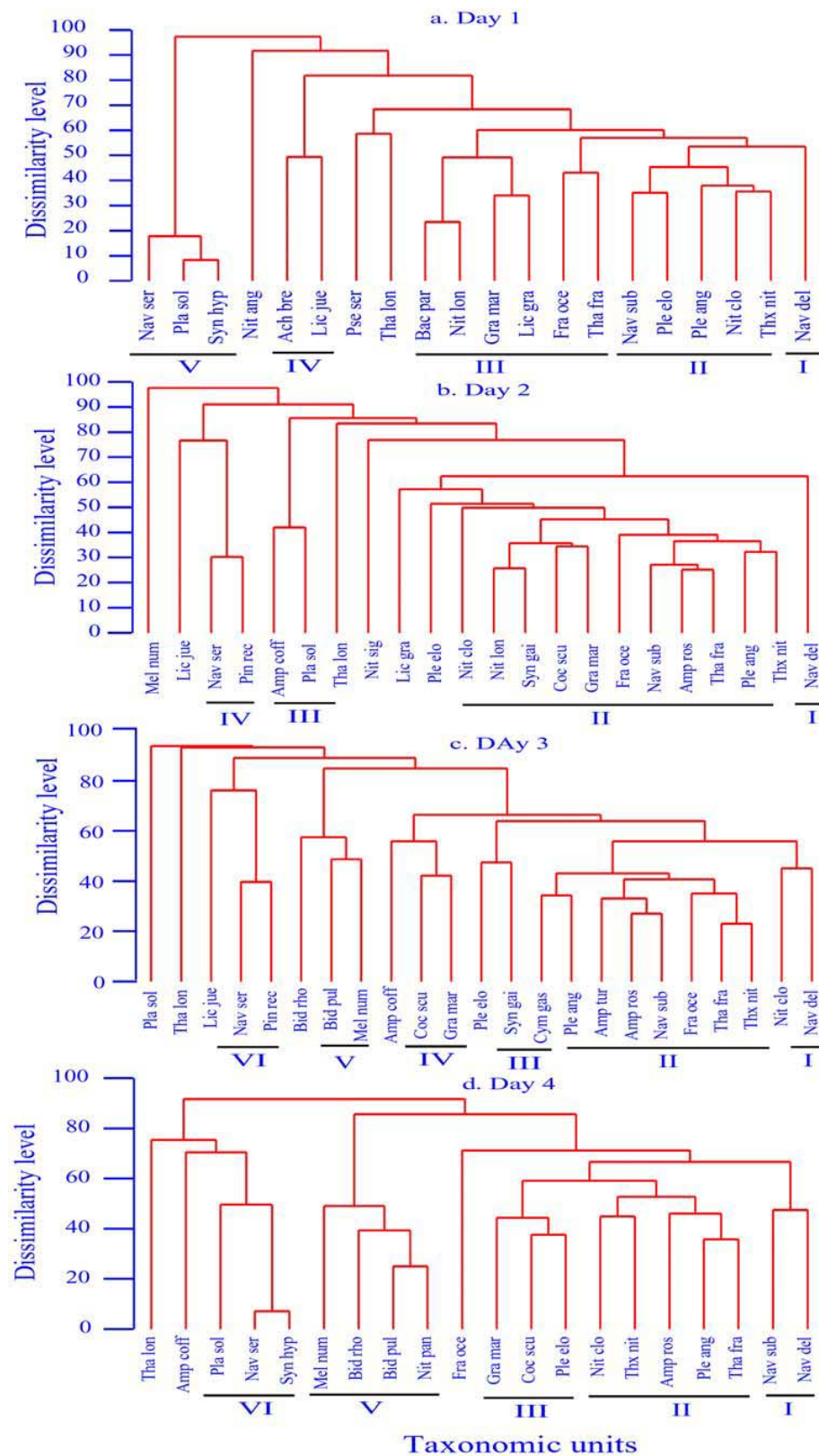


Fig 3.8 Dendrograms of the fouling diatom community developed on stainless steel with respect to species for different submersion periods (Day 1 (a), Day 2 (b), Day 3 (c), Day 4 (d))

Linear regression analyses between the total diatom abundance and chlorophyll *a* concentrations showed a significant relationship in case of the first three-days-old diatom communities (Fig. 3.7).

Clustering of the one to four-day-old diatom community with respect to the species is shown in Fig. 3.8. *N. delicatula* exhibited a dissimilar distribution from rest of the diatom members in the one and two day old communities. Whereas on the third and fourth day of submersion period, *N. delicatula* showed a similar distribution as that of *N. closterium* and *N. subinflata* respectively. Rest of the groups comprised the minor forms, which changed with increase in submersion period.

Polystyrene: Fouling diatoms encountered on polystyrene substrata belonged to 64 diatom species (49 pennates, 15 centrales) and 33 genera (21 pennates, 12 centrales) (Table 3.1). The fouling film was dominated by pennates throughout the submersion period, except in May (2 day old microfilm) (Fig. 3.9). In May, the community was dominated by *Biddulphia rhombus*. A comparatively lower total diatom abundance was observed in the pre-monsoon and beginning of monsoon season (Fig. 3.10).

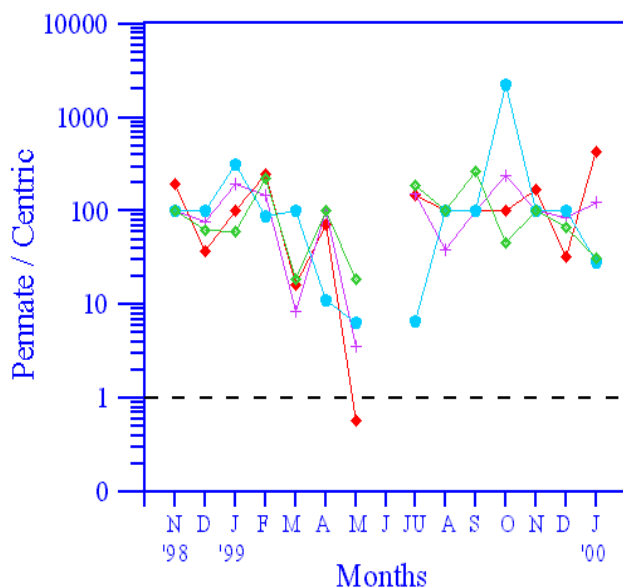


Fig. 3.9 The ratio of pennate / centric diatoms in the microfilm developed over polystyrene substrata (◆ Day 1; ◆ Day 2; + Day 3; ● Day 4)

Diversity of the diatom community during different submersion days was controlled by the distribution of the most dominant species, *N. delicatula*. Species diversity of one-day-old film peaked during December, January and March with a drop during November and February due to the dominance of the community by *N. delicatula*. In the two-day-old community, drop in species diversity was observed in February. In

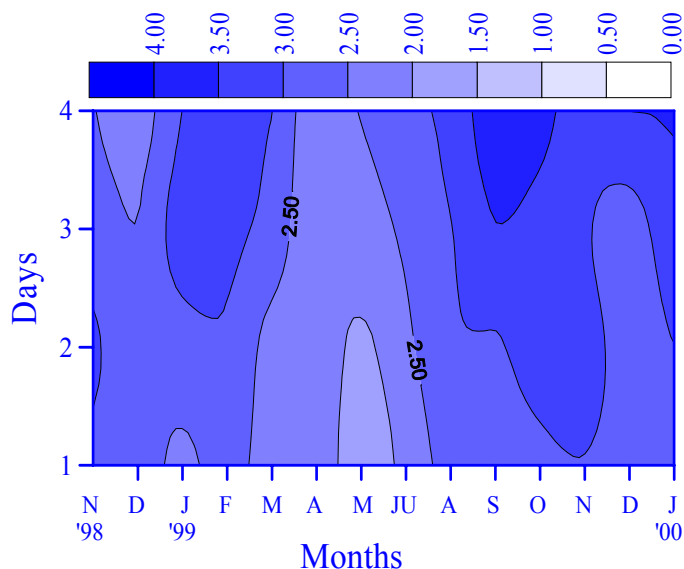


Fig. 3.10 Temporal variation in the fouling diatom abundance (cells dm^{-2}) on polystyrene substrata for different submersion periods (Day 1; Day 2; Day 3 and Day 4)

the three-day-old community, peak in species diversity was observed in January. In case of the four-day-old community, peak in species diversity was observed in December (Table 3.3).

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 (Fig. 3.11).

Table 3.3 Values of species diversity (H'), species richness ($H' \text{ max}$) and evenness ($H' / H' \text{ max}$) of the fouling diatom community over different exposure periods with reference to polystyrene substratum (Shannon-Wieners diversity index)

MONTHS	DAY 1			DAY 2			DAY 3			DAY 4		
	H'	$H' \text{ max}$	J'	H'	$H' \text{ max}$	J'	H'	$H' \text{ max}$	J'	H'	$H' \text{ max}$	J'
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'98	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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'99	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

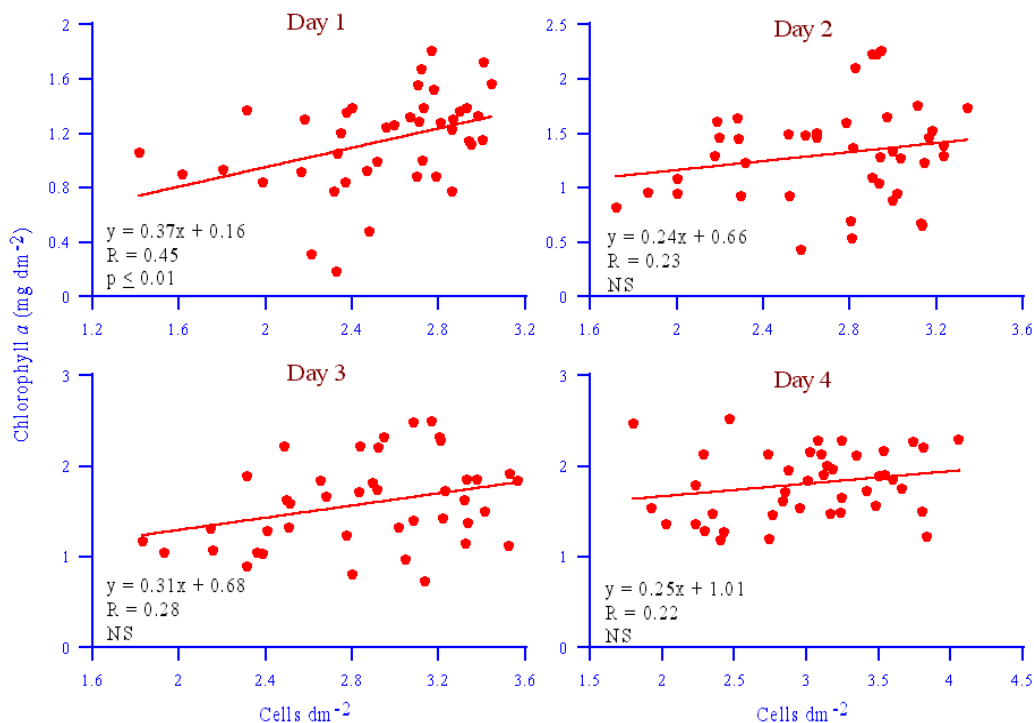


Fig. 3.11 Linear regression between diatom abundance and chlorophyll *a* concentrations on polystyrene substratum for different exposure periods

Clustering of the one to four-day-old diatom community with respect to the species is shown in Fig. 3.12. *N. delicatula* showed a dissimilar distribution throughout the submersion period. The forms which, comprised rest of the groups changed with increase in submersion period.

3.3.4 Comparison

Generally the total number of diatom species (Table 3.1) as well as total diatom abundance was higher on polystyrene substrata than on stainless steel (Fig. 3.6 and 3.10).

Distribution of the diatoms encountered on stainless steel and polystyrene substrata are given in Table 3.4 and Fig. 3.13. *N. delicatula* was the most dominant diatom 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 of the year, *B. rhombus* (one and two day old community) showed preference towards polystyrene, along with 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* 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 *F. oceanica* 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.

MANOVA of total diatom abundance, species diversity, species richness, evenness and chlorophyll *a*, for the two substrata and water column is shown in Table 3.5. 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.

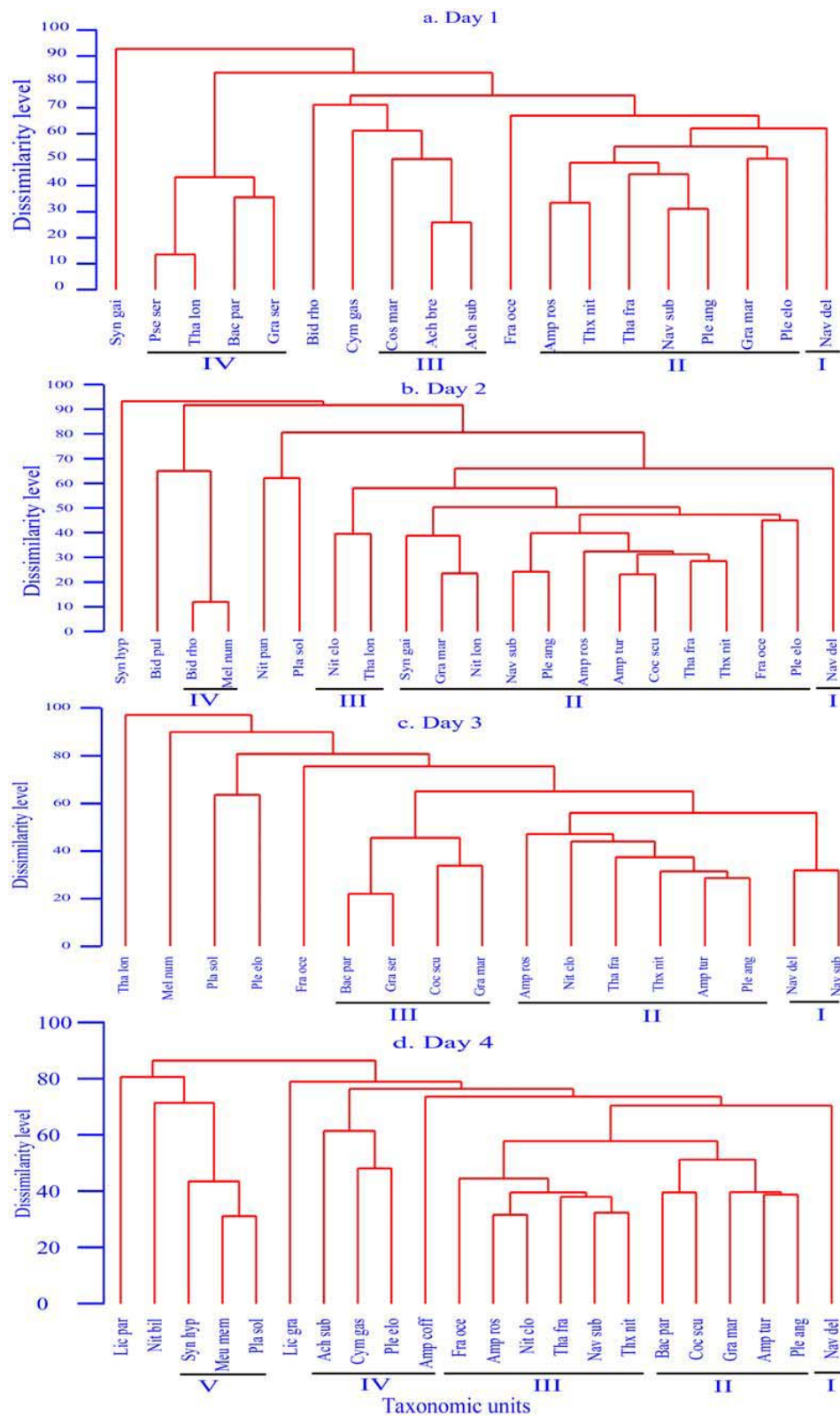


Fig 3.12 Dendrograms of the fouling diatom community developed on polystyrene with respect to species for different submersion periods (Day 1 (a), Day 2 (b), Day 3 (c), Day 4 (d))

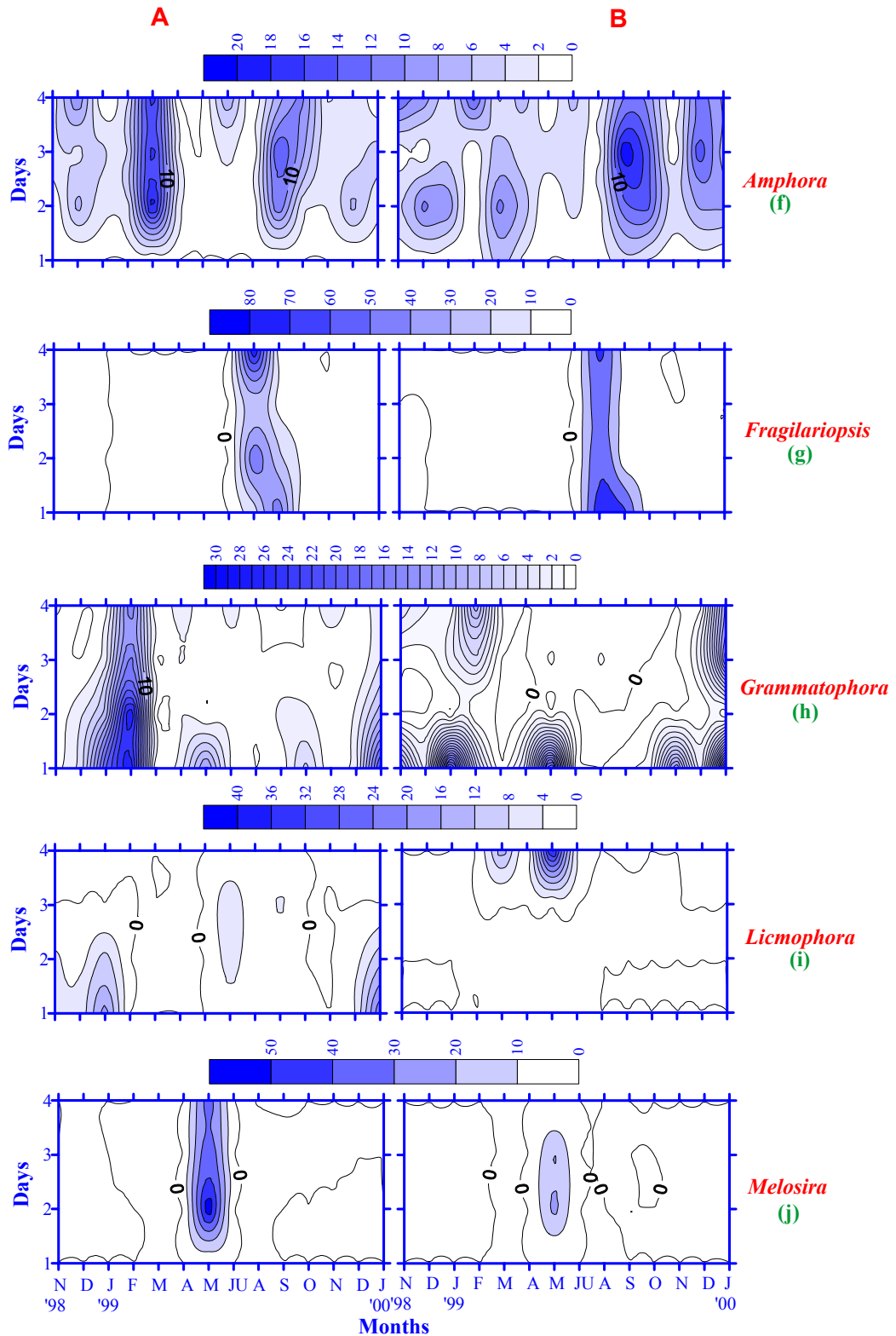


Fig. 3.13 Temporal distribution of *Amphora* (f); *Fragilariopsis* (g); *Grammatophora* (h); *Licmophora* (i); and *Melosira* (j) in the fouling diatom community developed over stainless steel (A) and polystyrene (B)

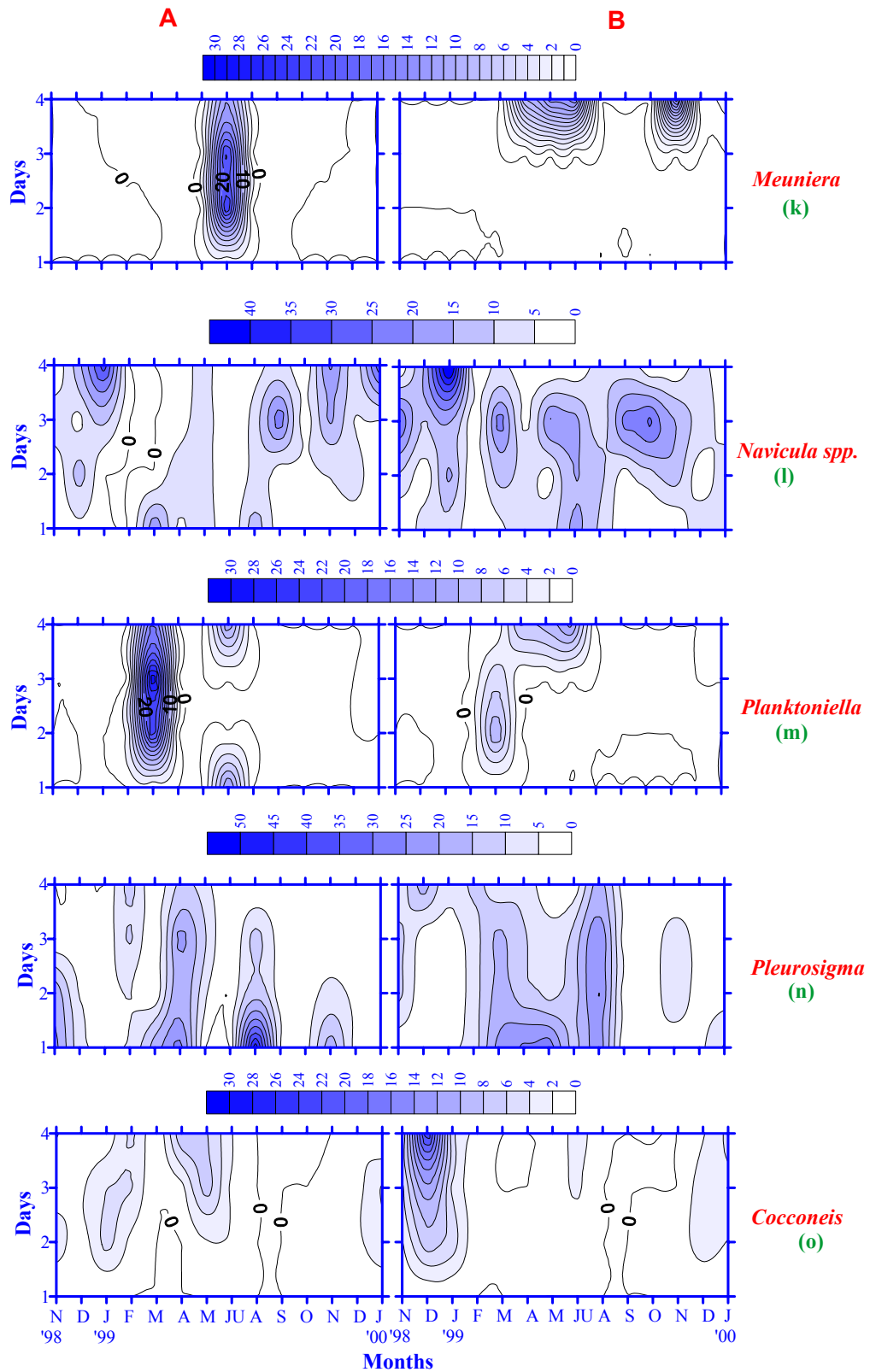


Fig. 3.13 Temporal distribution of *Meuniera* (k); *Navicula* spp. (l); *Planktoniella* (m); *Pleurosigma* (n); and *Cocconeis* (o) in the fouling diatom community developed over stainless steel (A) and polystyrene (B)

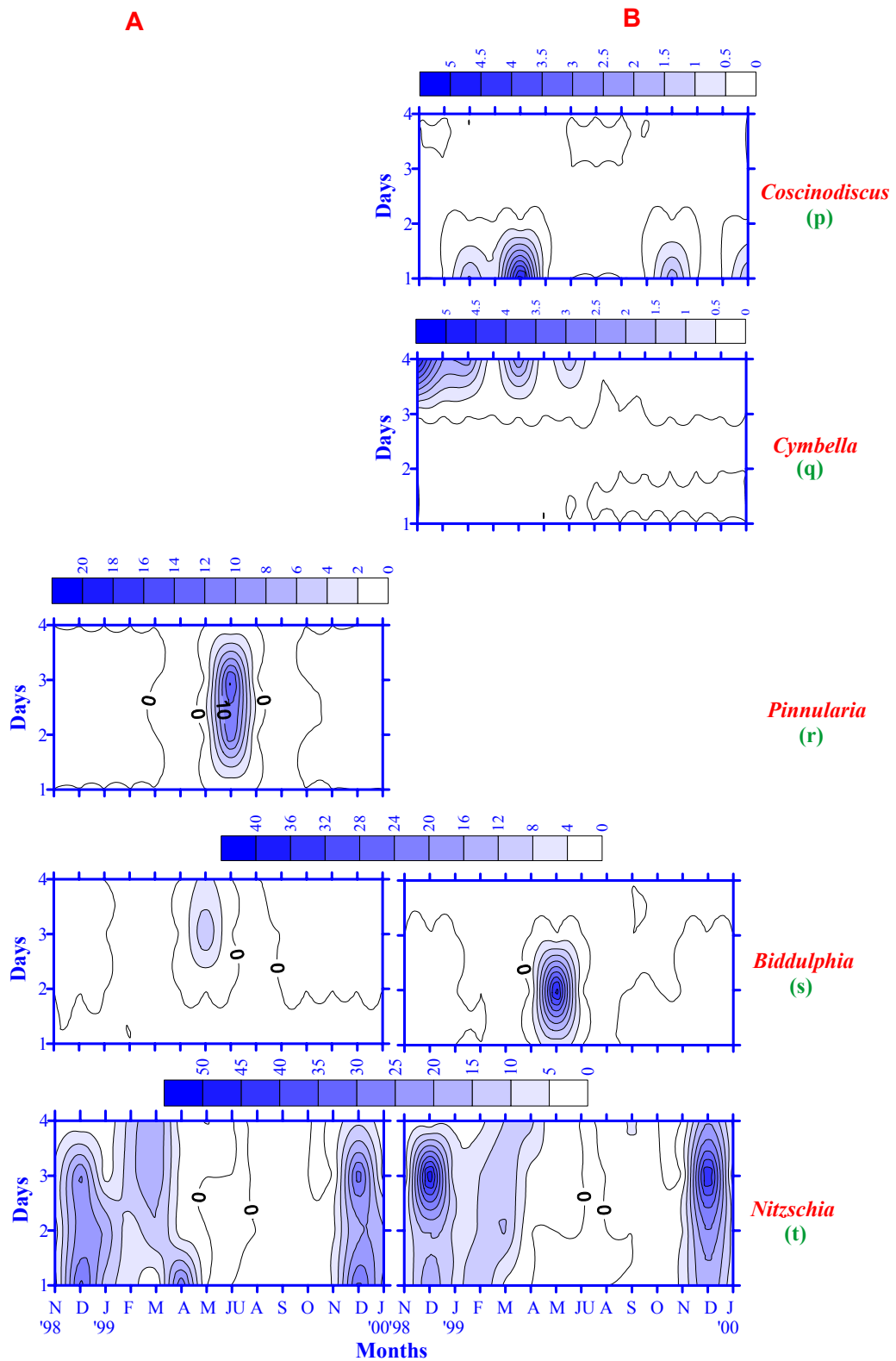


Fig. 3.13 Temporal distribution of *Coscinodiscus* (p); *Cymbella* (q); *Pinnularia* (r); *Biddulphia* (s); and *Nitzschia* (t) in the fouling diatom community developed over stainless steel (A) and polystyrene (B)

Table 3.4 Temporal distribution of diatoms (next in abundance to *N. delicatula* above 5%) over stainless steel and polystyrene substrata
(Day 1 = ▲; Day 2 = ★; Day 3 = ■; Day 4 = ●)

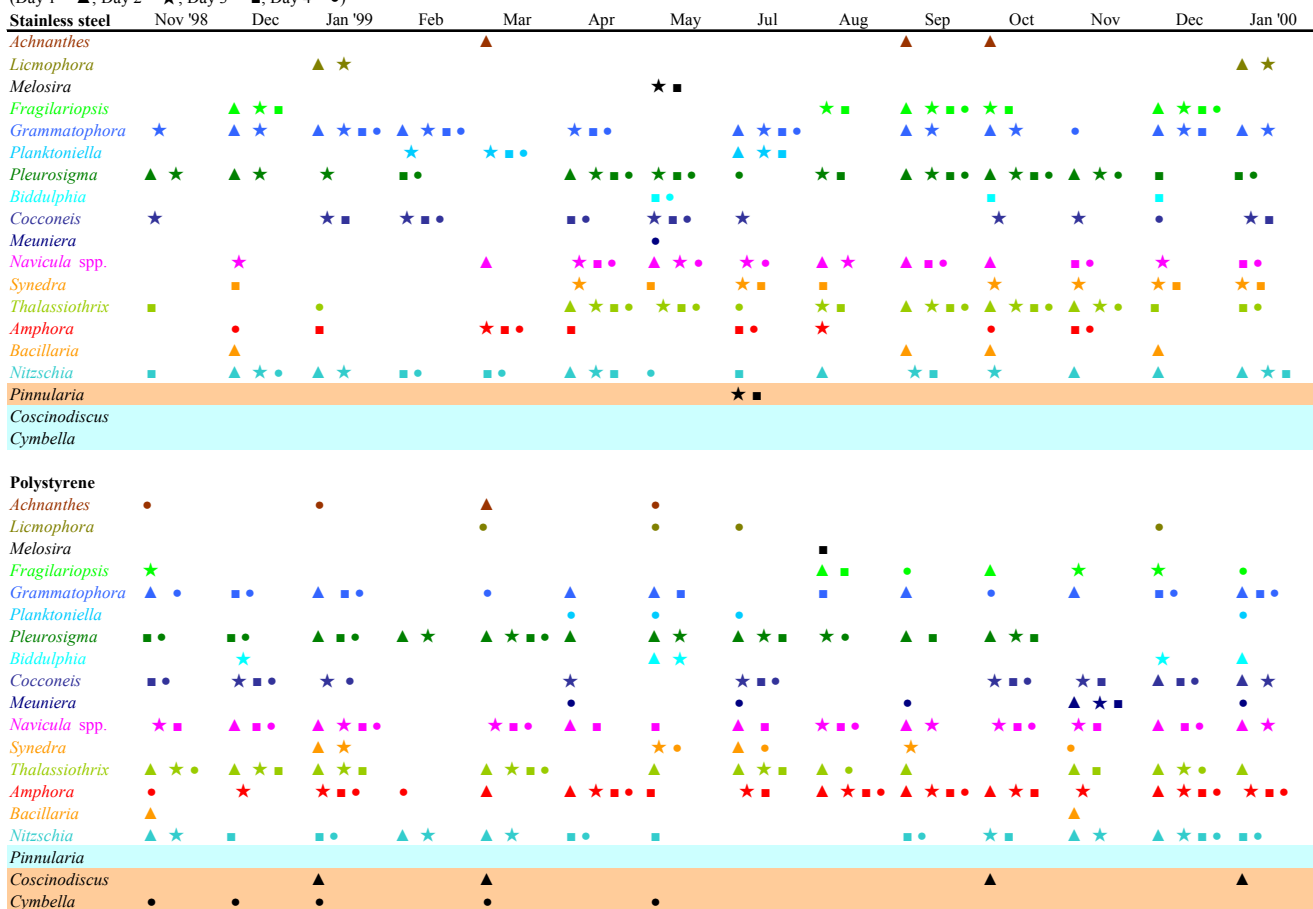


Table 3.5a. MANOVA (Without replication) for the temporal variation in the diatom abundance on stainless steel and polystyrene substrata with respect to submersion days (* $p \leq 0.05$; ** $p \leq 0.025$; *** $p \leq 0.001$; NS not significant)

Source of variation	<i>df</i>	SS	MS	F
SST+PS	1.00	1.206	1.210	
MONTHS	13.00	17.279	1.330	
DAYS	3.00	6.973	2.320	
(SST+PS)*MONTHS	13.00	2.020	0.160	3.61***
(SST+PS)*DAYS	3.00	0.370	0.120	2.87*
MONTHS*DAYS	39.00	2.870	0.070	1.7 ^{NS}
(SST+PS)*MONTHS*DAYS	39.00	1.677	0.040	

Table 3.5b. MANOVA (Without replication) for the temporal variation in the diatom diversity on stainless steel and polystyrene substrata with respect to submersion days (* $p \leq 0.05$; ** $p \leq 0.025$; *** $p \leq 0.001$; NS not significant)

Source of variation	<i>df</i>	SS	MS	F
SST+PS	1.00	0.000	0.000	
MONTHS	13.00	0.227	0.017	
DAYS	3.00	0.079	0.026	
(SST+PS)*MONTHS	13.00	0.120	0.009	2.3**
(SST+PS)*DAYS	3.00	0.035	0.012	2.91*
MONTHS*DAYS	39.00	0.241	0.006	1.54 ^{NS}
(SST+PS)*MONTHS*DAYS	39.00	0.156	0.004	

Table 3.5c. MANOVA (Without replication) for the temporal variation in the diatom richness on stainless steel and polystyrene substrata with respect to submersion days (* $p \leq 0.05$; ** $p \leq 0.025$; *** $p \leq 0.001$; NS not significant)

Source of variation	<i>df</i>	SS	MS	F
SST+PS	1.00	0.000	0.000	
MONTHS	13.00	0.063	0.005	
DAYS	3.00	0.013	0.004	
(SST+PS)*MONTHS	13.00	0.019	0.001	2.08*
(SST+PS)*DAYS	3.00	0.005	0.002	2.44 ^{NS}
MONTHS*DAYS	39.00	0.042	0.001	1.58 ^{NS}
(SST+PS)*MONTHS*DAYS	39.00	0.027	0.001	

Table 3.5d. MANOVA (Without replication) for the temporal variation in the evenness in the water column, stainless steel and polystyrene substrata with respect to submersion days (* $p \leq 0.05$; ** $p \leq 0.025$; *** $p \leq 0.001$; NS not significant)

Source of variation	<i>df</i>	SS	MS	F
SST+PS	1.00	0.035	0.035	
MONTHS	13.00	0.744	0.057	
DAYS	3.00	0.140	0.047	
(SST+PS)*MONTHS	13.00	0.243	0.019	1.98*
(SST+PS)*DAYS	3.00	0.014	0.005	0.49 ^{NS}
MONTHS*DAYS	39.00	0.382	0.010	1.04 ^{NS}
(SST+PS)*MONTHS*DAYS	39.00	0.368	0.009	

Table 3.5e. MANOVA (Without replication) for the temporal variation in the chlorophyll *a* in the water column, stainless steel and polystyrene substrata with respect to submersion days (* $p \leq 0.05$; ** $p \leq 0.025$; *** $p \leq 0.001$; NS not significant)

Source of variation	<i>df</i>	SS	MS	F
SST+PS	1.00	0.052	0.052	
MONTHS	13.00	7.114	0.547	
DAYS	3.00	8.849	2.950	
(SST+PS)*MONTHS	13.00	1.361	0.105	2.02*
(SST+PS)*DAYS	3.00	0.450	0.150	2.90*
MONTHS*DAYS	39.00	3.436	0.088	1.70 ^{NS}
(SST+PS)*MONTHS*DAYS	39.00	2.019	0.052	

3.4 Discussion

The Zuari estuary is a tide dominated coastal plain estuary (Qasim and Sengupta 1981), influenced by a southwest monsoon that is active during June to September. During this investigation, salinity dropped to a level of 15 psu. The peak in nitrate and silicate concentration in July followed by a decline during August and September (Fig. 3.2) coincided with blooms of *S. costatum*, *F. oceanica* and *T. nitzschoides* in the ambient waters suggesting nutrient uptake by the diatom cells. A multispecies bloom in March was followed by another nitrate peak in April. This could be due to a rise in herbivorous population during this period, which feeds on the bloom and the resulting fecal pellets being responsible for the increase in nitrate concentration.

The diatom population structure in the water column differed considerably from that of the microfilm. Obviously such differences arise, as the requirements for attachment and adhesion are not found in all the forms. The pennate type (Characklis and Cooksey 1983; Cooksey et al. 1984) often dominates the diatom fouling population. Similar results were encountered in this study, with pennate diatoms being more abundant in the fouling film than centric diatoms while the reverse was observed in case of the water column. The dominance of pennate diatoms has also been reported in the biofouling developed on test panels exposed in the Arabian Sea (Bhosle et al.

1989; Kelkar 1989; Bhosle 1993; Raveendran et al. 1991; Pangu 1993; Venugopalan et al. 1994; Prabhadevi 1995a; 1995b; Redekar 1997; Redekar and Wagh 2000b). The most abundant diatom in both, the water column as well as in the microfilm, was a pennate motile diatom, *N. delicatula*. Dominance of this pennate diatom species in the microfilm can be attributed to its dominance in the surrounding waters resulting in a higher surface encountering probability.

However, in the monsoon (August-September) the dominance shifted from *N. delicatula* to forms such as *F. oceanica*, *S. costatum* and *T. nitzschioides* in the ambient waters. The monsoon season characterized by stratification with nutrient rich and low saline waters at the surface, favours these species, which turned out to be better competitors than the otherwise dominant *N. delicatula*. Also, in the pre-monsoon period (May) the comparatively higher turbulence caused by the water movement, resulted in the dislodgement from bottom substrates of forms such as *M. nummuloides* and *B. rhombus* into the surface waters. Both, *M. nummuloides* and *B. rhombus*, although centric are known to lead an attached mode of life (Aleem 1973; Rendall et al. 1983; Ferreira 1985; Raveendran et al. 1991). When brought into the water column, these tychopelagic forms, which require a space for attachment, will tend to settle on a suitable substratum. The same holds true for *F. oceanica* and *T. nitzschioides*, which being pennate diatoms will prefer to settle on a suitable substratum.

Thus, changes in the surrounding diatom community composition during the monsoon and pre-monsoon influenced the fouling diatom community. However, some of these species exhibited substratum preferences. While *F. oceanica*, *T. nitzschioides* and *Pleurosigma* were found to dominate on both, stainless steel and polystyrene, *B.*

rhombus, *Achnanthes*, *Licmophora*, *Grammatophora* and *Synedra* dominated only on polystyrene and *M. nummuloides*, *Pinnularia* and *M. membranacea* dominated only on stainless steel. Blooms of the other centric forms such as *S. costatum*, *L. danicus* and *Cerataulina* sp. did not influence the fouling community, as these are free floating planktonic forms. Such substratum preferences indicate either an active choice by some diatom species or that the physical and chemical conditions of the substrate encourage or discourage settlement of certain species (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, was also responsible for the significant relationship in diatom abundance and chlorophyll *a* concentrations, which was restricted to only one-day-old community for polystyrene as compared to three day-old community in case of stainless steel. Once an initial colonisation 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.

It is pertinent to note that the microfilm community consists of different types of organisms. A study on thraustochytrid protists as a component of marine microbial films has shown that they readily colonize on aluminium (hydrophilic) than on fiberglass (Raghukumar et al. 2000). It was suggested that cell walls of thraustochytrids are highly adhesive and play direct role in attachment, which does not require an elaborate formation of attaching polysaccharides. Such different preferences by different types of organisms can also be an important factor in determining the community structure of microbial films and need careful consideration.