

Chapter 4
Spatial and temporal variations in diatom–bacterial
interactions

4.1. Introduction

The ‘antibiotics’ approach is an excellent tool to analyze diatom-bacterial interactions at the community level. In experiments carried out in the preceding chapters, sediment samples were treated with antibiotics, individually and in combination, to suppress bacteria. Antibiotics with different modes of action (penicillin, streptomycin and chloramphenicol) were used. This helped to circumvent the problem of inhibiting a particular set of bacteria in natural samples. The consequent effects on diatoms were analyzed. Through such an approach, the changes in diatom communities when specific types of bacteria are inhibited, could be investigated. The results of the earlier chapters unequivocally support the hypothesis that there is an effect of bacteria on diatom community structure. These effects are mediated by the quantitative extent of the bacteria inhibited, and are distinct from the direct effects of antibiotics (chloramphenicol), the combination of potentially direct and indirect effects (streptomycin) and the effect of all three antibiotics combined.

There are two aspects that must be considered here. Firstly, the experiments detailed in the earlier chapters were based on a single sampling in a monsoon-influenced environment. In view of the stochastic nature of the monsoons and the resulting effects on diatom and bacterial communities, it is necessary to study the seasonal variations in the interactions observed. Secondly, it is also important to evaluate whether these interactions differ with habitat. Therefore, the same experiments were replicated in 3 study areas during the same period - an intertidal sandflat, a mangrove area, and an anthropogenically-influenced port environment.

The following questions were addressed. (1) How does the effect of bacteria on diatom community structure (the penicillin treatment) vary across an annual seasonal cycle? (2) Are these seasonal effects distinct from the chloramphenicol, streptomycin and antibiotic combination (PSC) effects? and (3) Do these interactions vary across spatial scales?

4.2. Materials and methods

4.2.1. Study areas and sampling strategy

Sediment and water samples were collected from 3 locations: an intertidal sandflat (Dias Beach), a mangrove environment (Chorao), and a busy port environment (Mormugao port) (Fig. 4.1).

The intertidal sandflat (Dias Beach) (Fig. 4.1 [1]) is surrounded by the Mandovi and Zuari estuaries (15°27' N; 73°48' E). It is about 200 m long, sheltered on both sides by rocky cliffs and has a semi-diurnal tidal cycle. The Chorao island (Fig. 4.1 [2]) is located in the backwaters of the Mandovi estuary, between 15°25' - 15°30' N and 73°45' - 73°59' E, and has extensive mangrove cover (comprising 15 species) towards the western low-lying tip. The dominant mangroves in this region are *Rhizophora* sp., *Avicennia* sp., *Sonneratia* sp. and *Excoecaria* sp. This mangrove region is also the site of the Dr. Salim Ali bird sanctuary, spread over 1.8 sq. km, and housing around 400 species of birds, both local and migratory. The Mormugao port (Fig. 4.1 [3]), is situated at the mouth of the river Zuari (15° 25' N; 73° 47' E), and accounts for about 33% of India's iron ore export.

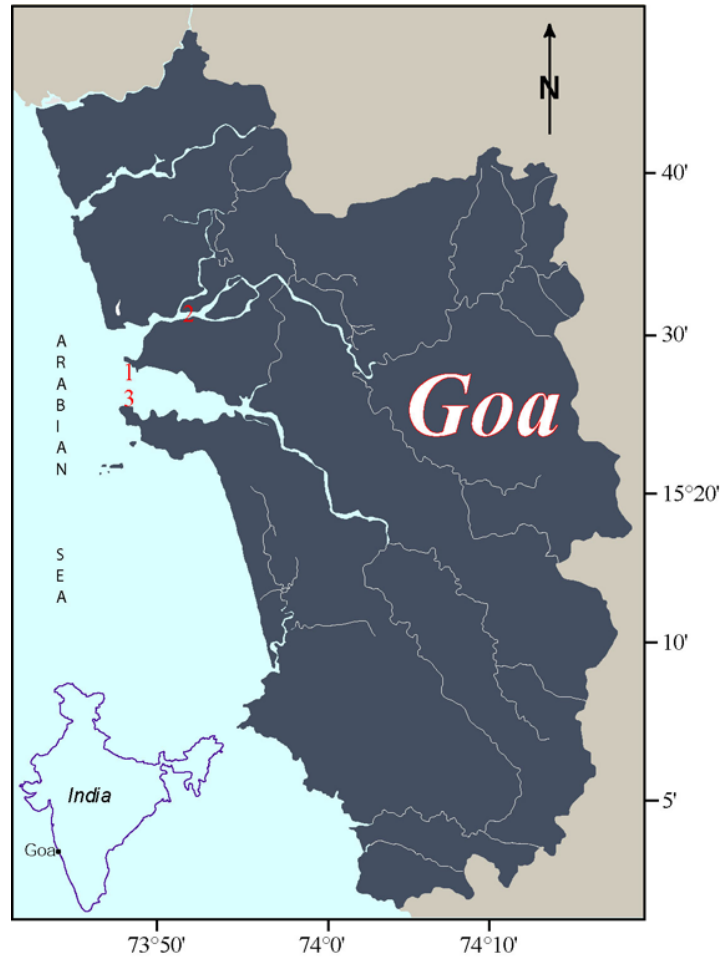


Fig. 4.1. Location of the study sites along the west coast of India. [1] the intertidal sandflat (Dias Beach), [2] the mangrove site (Chorao), and [3] the port site (Mormugao Port Trust).

The South West (SW) monsoon is the main source of climatic variation. A considerable amount of freshwater is added to the surrounding estuaries, due to precipitation during the SW monsoon. Different seasons with characteristic environmental characteristics can be discerned. The annual cycle can be broadly classified into pre-monsoon (February-May), monsoon (June-September) and post-monsoon (October-January) periods. Therefore, the study areas were sampled in January 2005 (post-monsoon I), May 2005 (pre-monsoon), September 2005

(monsoon) and January 2006 (post-monsoon II), to include an entire seasonal cycle and a repetition of one season.

4.2.2. Sediment sampling and analysis

At the intertidal sandflat and mangrove site, surface sediment (0-5 cm) was collected in triplicate from a fixed point using PVC cores with an inner diameter of 2.6 cm. Separate sediment cores of the same dimensions were collected for analysis of specific gravity. At the port site, sediment samples were collected using a modified van Veen grab with a grabbing area of 0.04 m². The grab was equipped with flaps on the top side, from which sediment cores could be collected. However, due to regular dredging in the area, the accumulated loose sediment was hardly 2 cm thick, and therefore, undisturbed cores could not be collected. The sediment was scooped out from the van Veen grab and stored in plastic packets at 5°C after wrapping in aluminium foil to keep out light.

Sediment texture was analyzed following the methods described in Buchanan (1984). For quantification of bacteria, sediment samples were suspended in autoclaved seawater, mixed well and fixed with 4% formalin. Bacteria were then counted using 4',6-diamidino-2-phenylindole (DAPI) and epifluorescence microscopy, and counts expressed as bacteria g⁻¹ wet sediment.

4.2.3. Water sampling and analysis

Surface water was sampled for the analysis of environmental variables. Interstitial water was also sampled at the intertidal sandflat, by digging the sediment with a shovel and allowing the interstitial water to accumulate. Temperature, pH, dissolved oxygen (Winklers method), salinity (Mohr-Knudsen titration method) (Strickland and

Parsons 1965), nutrients such as nitrate ($\text{NO}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), phosphate ($\text{PO}_4\text{-P}$) and silicate (Si) (SKALAR autoanalyzer), Suspended Particulate Matter (SPM) and chlorophyll *a* were analyzed using standard procedures (Parsons et al. 1984).

For analysis of phytoplankton, water samples (500 mL) were preserved with Lugol's iodine solution, decanted after a settling period of 48 hours, and then observed using an inverted microscope, at 100X and 200X magnification. Phytoplankton were identified based on the available literature (Subrahmanyam 1946, Desikachary et al. 1987a and b, Tomas 1997, Horner 2002) and expressed as cells L^{-1} .

For quantification of bacteria, water samples were fixed with 4% formalin and counted using DAPI staining. These counts are expressed as bacteria mL^{-1} .

4.2.4. Stability of antibiotics in f/2(MBL) and f/2(ASW) in presence of sediment

Antibiotics were dissolved in sterile f/2(MBL) and f/2(ASW) containing autoclaved sediment from the mangrove site. The stability of antibiotics in presence and absence of sandy sediment (from the intertidal sandflat) has already been presented in Chapter 3. The flasks were incubated at 12 h: 12 h L: D conditions at 25 ± 1 °C for a period of 7 days. Aliquots (0.1 mL) were withdrawn on days 0 and 7 and assayed against the test culture using the well diffusion method (Elyakov et al. 1996). Wells of 1 cm diameter were bored on agar plates that were seeded with the exponentially growing test bacterial isolate. The wells were subsequently filled with 0.1 mL aliquots of antibiotic-containing f/2(MBL) and f/2(ASW). Controls were represented by sterile distilled water. Assays were carried out in triplicates. The diameter of the inhibition zone (total diameter of inhibition zone minus diameter of well) was measured after 24 h incubation at room temperature, that ranged from 26-

28°C. Results are presented as zone of inhibition (percentage compared to day 0 values).

4.2.5. Analysis of microphytobenthic diatoms

Microphytobenthic diatoms were analyzed using the extinction-dilution method (most-probable-number method, MPN) (Thronsdén 1978). Sediment samples were incubated in appropriate media amended with antibiotics for 7 days. Subsequently, diatoms were identified and quantified with the aid of a statistical table (Thronsdén 1978).

Since this study involved 4 samplings spread out over 3 seasons, it was necessary to use a basal diluent with a defined chemical composition, rather than Aged Seawater (ASW) that tends to vary seasonally. Artificial seawater (MBL) (Cavanaugh 1975), enriched with f/2 level of nutrients (Guillard and Ryther 1962), was used as the basal diluent. f/2(ASW) was also used for comparison.

Penicillin, streptomycin and chloramphenicol were added to f/2(MBL), individually and in combination, at 0.2 mg mL⁻¹, 0.1 mg mL⁻¹ and 0.02 mg mL⁻¹ concentrations respectively. Penicillin inhibits cell wall peptidoglycan synthesis and is effective mainly against gram-positive bacteria. Streptomycin and chloramphenicol inhibit protein synthesis, streptomycin by binding to the 30S ribosomal subunit and chloramphenicol by binding to the 50S ribosomal subunit (Josephson et al. 2000). Both have the potential to affect diatoms directly by inhibiting protein synthesis in diatom organelles (70S) (Stanier et al. 1971, Cullen and Lesser 1991). Chloramphenicol also inhibits photosystem II in diatoms (Ohad et al. 1984).

Sediment was suspended in the different diluents (0.1 g mL^{-1}) and diluted ten-fold up to 10^{-5} . The diluted suspensions were inoculated into five replicate culture wells (1 mL each) and incubated for $25 \pm 1 \text{ }^{\circ}\text{C}$ with a 12:12 h light : dark cycle for 7 days. Diatoms were analyzed by light microscopy and confirmed as viable by observation of diatom autofluorescence. The wells in which viable diatoms occurred were marked as positive. Diatoms were identified based on Desikachary (1977), Desikachary et al. (1987a), Horner (2002), Subrahmanyam (1946) and Tomas (1997). The MPN of diatoms in the sediment sample (MPN g^{-1} wet sediment) was calculated by selecting a set of three consecutive dilutions and referring to a statistical table (Thronsen 1978). The relative diatom density (cm^{-3} wet sediment) was obtained by multiplying the MPN with the apparent specific gravity of the wet sediment (Imai and Itakura 1999), which was determined separately.

4.2.6. Quantification and identification of bacteria

Bacteria were enumerated by spread plating 0.1 mL aliquots of diluted sediment on Zobell Marine Agar (ZMA) 2216 containing respective antibiotics. Morphologically distinct bacterial colonies were purified by repeated streaking on ZMA 2216 plates and maintained on ZMA 2216 slants. Bacterial isolates were identified to genus level based on morphological and biochemical tests following Bergey's Manual of Systematic Bacteriology (Holt et al. 1994).

4.2.7. Data analyses

Canonical Correspondence Analysis (CCA) (ter Braak 1995) was performed to evaluate the temporal variations in the effect of the environmental characteristics of the overlying water column on the benthic diatom communities in the three study sites, using the Multi-Variate Statistical Package program version 3.1 (Kovach 1998).

Normality and homogeneity of variances were determined using Shapiro's and Levene's tests respectively. Majority of the data (diatom and bacterial abundance, absolute values and percentage values compared to control) failed the requirements for parametric analysis. Thus, these data were analyzed for seasonal variation using the non-parametric Kruskal-Wallis test using the STATISTICA 6 program.

The univariate measures of the diatom community (generic richness, evenness and diversity) were analyzed using PRIMER (version 5). Clustering analyses of the fourth root-transformed diatom data was carried out through Bray-Curtis coefficients (Bray and Curtis 1957) and the group average method (Pielou 1984) using PRIMER (version 5). The contribution of individual genera to the diatom community in the different antibiotic treatments (in terms of inhibition/enhancement compared to the control conditions) is presented as SURFER plots using SURFER 7 software. The ratio of the number of genera inhibited to the number of genera enhanced (I:E ratio) was also calculated.

4.3. Results

4.3.1. Diatoms and bacteria in the water column

At the intertidal sandflat, periods of high diatom abundance coincided with low bacterial abundance and vice-versa in surface waters (Fig. 4.2a). In interstitial waters, diatom and bacterial abundance showed a similar trend (Fig. 4.2b). At the mangrove and port sites, diatom abundance in the water column was highest in the pre-monsoon period (Fig. 4.2c,d). A simultaneous increase in bacterial abundance in the pre-monsoon period was recorded at the mangrove site (Fig. 4.2c).

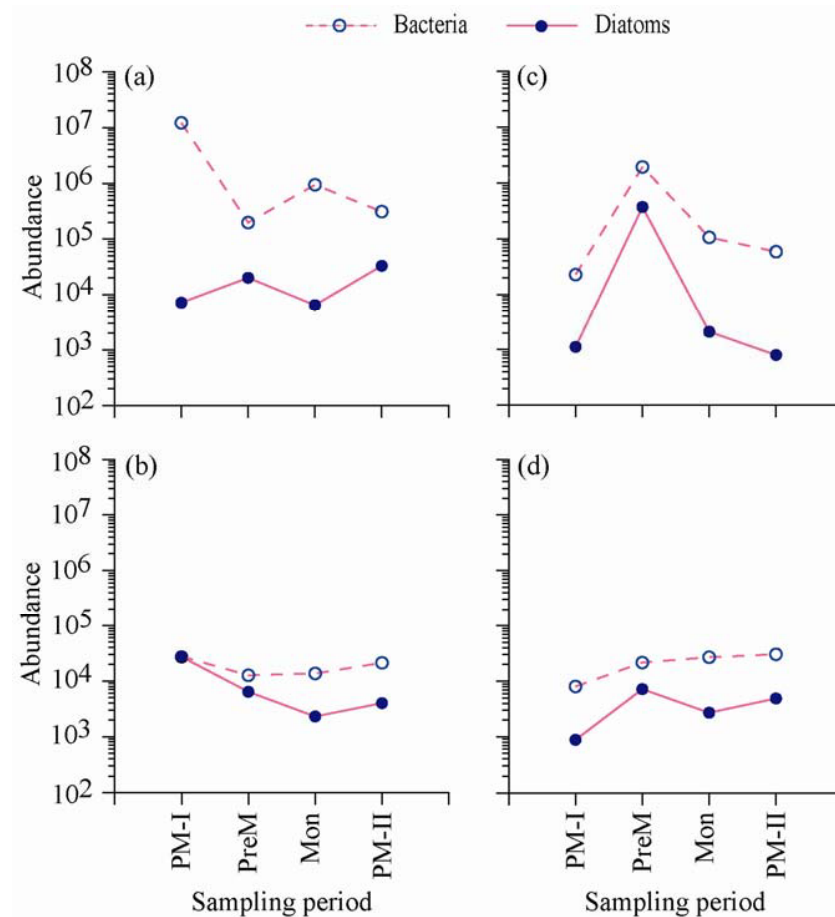


Figure 4.2. Temporal variation in diatom and bacterial abundance at the (a) intertidal sandflat (surface waters), (b) intertidal sandflat (interstitial waters), (c) mangrove site, and (d) port site. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

4.3.2. Effect of environmental variables on benthic diatom communities

The intertidal sandflat had a sandy sediment texture (Table 4.1) whereas the mangrove site had a silty sediment texture (Table 4.1). The port site had a rocky-sandy sediment texture (personal observation). Details of the other environmental variables are provided in Figs. 4.3-4.5.

Table 4.1. Sediment texture characteristics at the intertidal sandflat and the mangrove site. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

Study area	Percentage (%)	PM-I	PreM	Mon	PM-II
Intertidal sandflat	Sand	67.8	83.28	69.60	54.52
	Silt	32.12	16.6	30.36	45.44
	Clay	0.08	0.12	0.04	0.04
Mangrove site	Sand	28.44	26.56	19.2	23.72
	Silt	71.44	73.08	80.4	76.16
	Clay	0.12	0.36	0.4	0.12

In the CCA sampling biplot (Fig. 4.6a), 3 axes were extracted that explained 77% of the relationship between the microphytobenthic communities emerging under control conditions and the environmental variables. Dissolved oxygen, chlorophyll *a* and salinity were the most important variables. The different samplings showed a site-specific clustering pattern (Fig. 4.6a). The intertidal sandflat was the most dissimilar as it had high dissolved oxygen, chlorophyll *a*, salinity and low nutrient concentrations. The mangrove and port sites had comparatively high nutrient concentrations and low dissolved oxygen, chlorophyll *a* and salinity (Fig. 4.6a).

The site-specific clustering patterns of the different samplings were reflected in the accompanying genus biplot (Fig. 4.6b). *Cocconeis* and *Fragilariopsis* were recorded in only the intertidal sandflat, resulting from their inclination towards high dissolved oxygen, chlorophyll *a* and salinity and low nutrient concentrations,

conditions characteristic of the intertidal sandflat. *Asterionellopsis* was observed only at the port site whereas *Chaetoceros* and *Paralia* were recorded at the mangrove and port sites. These 3 genera showed preference for elevated concentrations of nitrate. *Amphiprora*, *Gyrosigma* and *Pleurosigma* were recorded only at the mangrove site, and were most abundant during the PM-I and Mon samplings probably due to their preference for elevated phosphate and silicate concentrations (Fig. 4.6b).

4.3.3. The microphytobenthic diatom communities under control conditions

All the 3 sites supported characteristically different benthic diatom communities. At the intertidal sandflat, 26 diatom species belonging to 12 genera emerged under control conditions (Table 4.2).

Diatom abundance ranged from 178 diatoms cm^{-3} wet sediment (monsoon) to 3,620 diatoms cm^{-3} wet sediment (post-monsoon II) (Fig. 4.7a) and showed no significant variation across the sampling periods (Kruskal-Wallis test, ns). Pennate diatoms dominated the community (Fig. 4.7b). Overall, the post-monsoon I period had the highest generic number and richness (Fig. 4.7c-d) and was dominated by *Navicula* and *Amphora* (Fig. 4.8a). The pre-monsoon period had the highest evenness and diversity (Fig. 4.7e,f) and was dominated by *Navicula*, *Amphora*, *Thalassiosira* and *Cocconeis* (Fig. 4.8a). The monsoon and ensuing post-monsoon II periods were dominated by *Navicula* (Fig. 4.8a). However, *Odontella*, *Grammatophora* and *Thalassionema* which occurred in the post-monsoon and pre-monsoon periods, were conspicuously absent in monsoon (Fig. 4.8a).

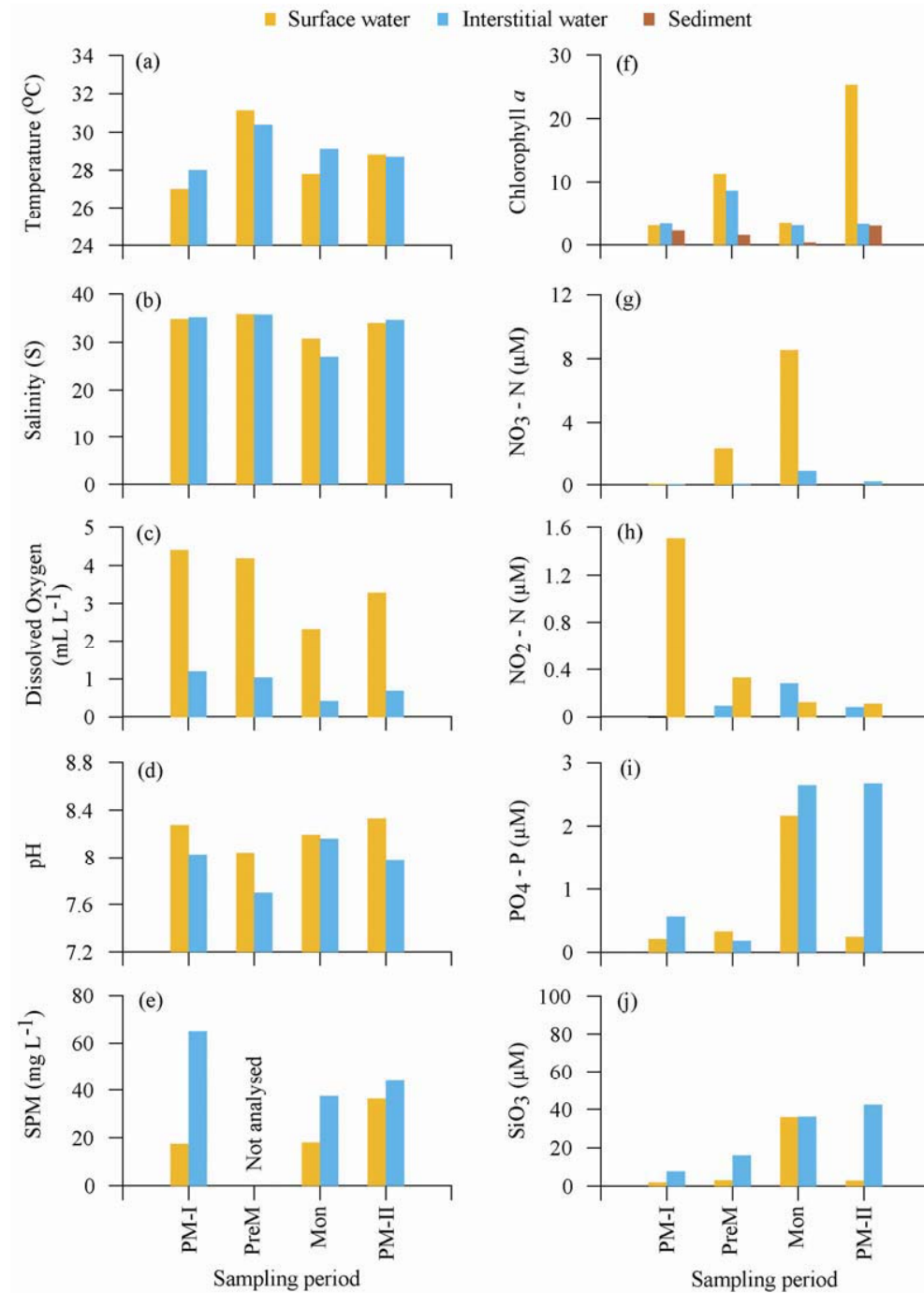


Fig. 4.3. Variation in physico-chemical variables at the intertidal sandflat. (a) temperature (°C), (b) salinity (S), (c) Dissolved Oxygen (mL L⁻¹), (d) pH, (e) Suspended Particulate Matter/SPM (mg L⁻¹), (f) chlorophyll *a* (µg L⁻¹ or µg g⁻¹), and concentrations of (g) nitrate (µmol L⁻¹), (h) nitrite (µmol L⁻¹), (i) phosphate (µmol L⁻¹), and (j) silicate. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

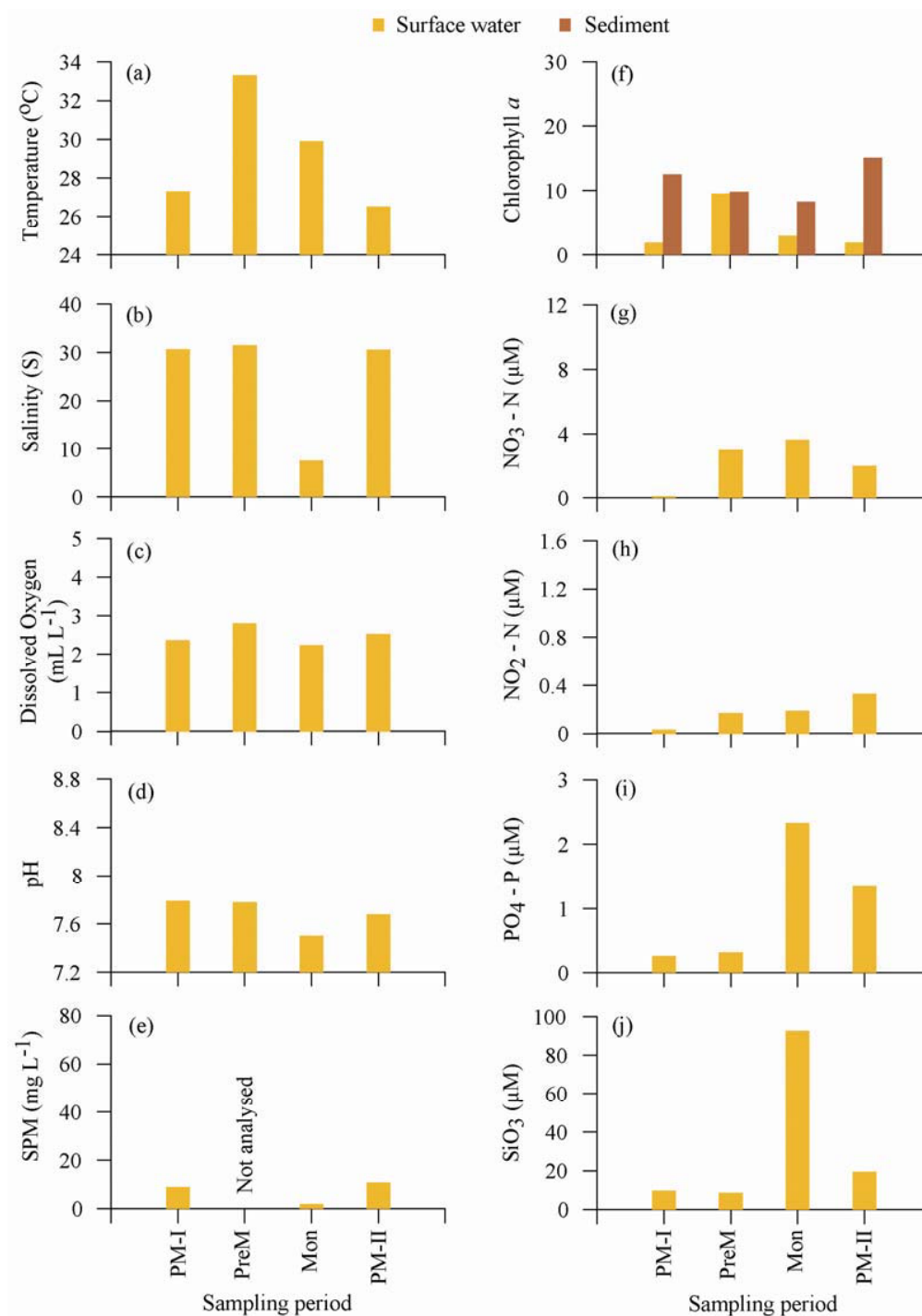


Fig. 4.4. Variation in physico-chemical variables at the mangrove site. (a) temperature (°C), (b) salinity (S), (c) Dissolved Oxygen (mL L⁻¹), (d) pH, (e) Suspended Particulate Matter/SPM (mg L⁻¹), (f) chlorophyll *a* (µg L⁻¹ or µg g⁻¹), and concentrations of (g) nitrate (µmol L⁻¹), (h) nitrite (µmol L⁻¹), (i) phosphate (µmol L⁻¹), and (j) silicate. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

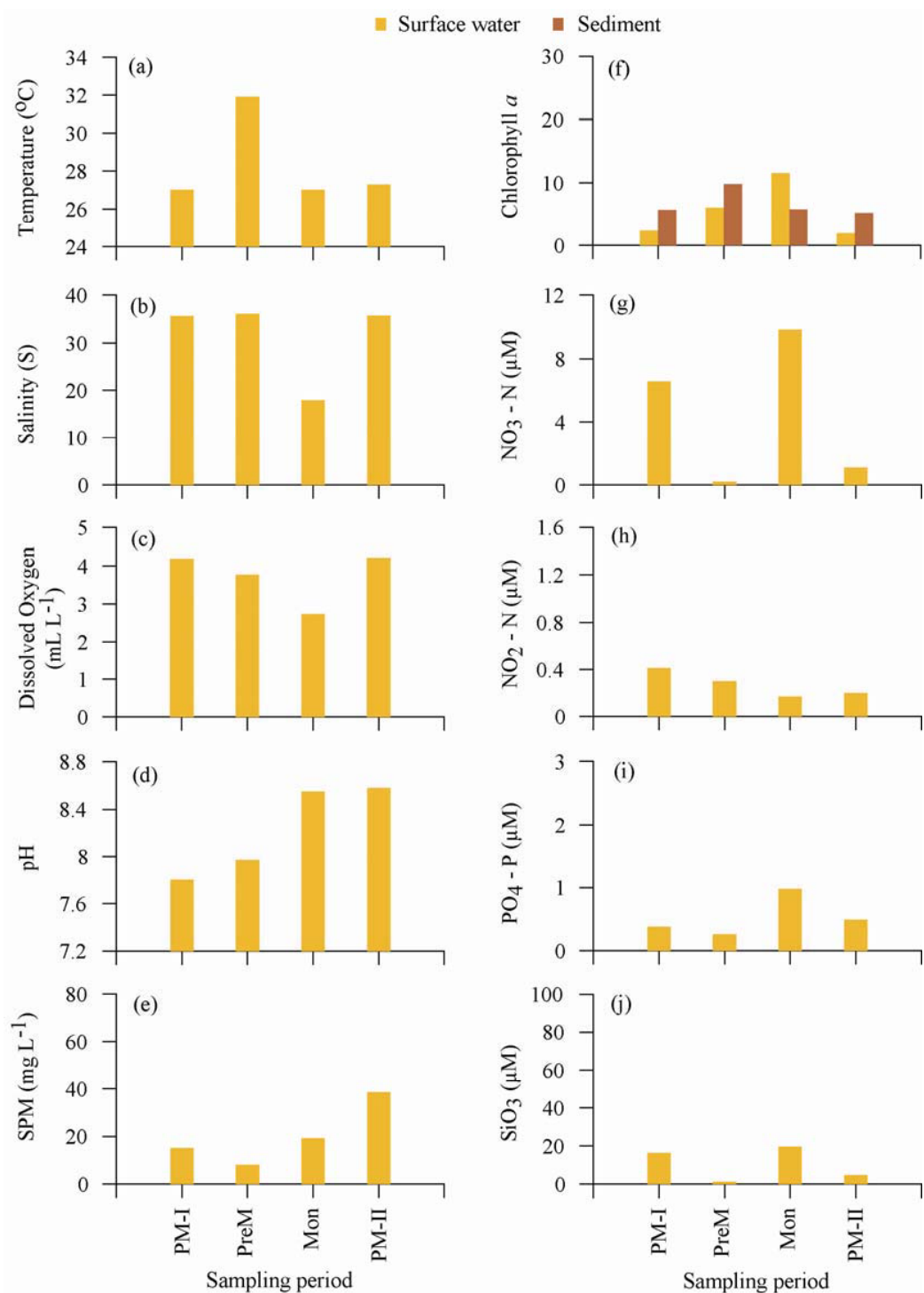


Fig. 4.5. Variation in physico-chemical variables at the port site. (a) temperature (°C), (b) salinity (S), (c) Dissolved Oxygen (mL L⁻¹), (d) pH, (e) Suspended Particulate Matter/SPM (mg L⁻¹), (f) chlorophyll *a* (µg L⁻¹ or µg g⁻¹), and concentrations of (g) nitrate (µmol L⁻¹), (h) nitrite (µmol L⁻¹), (i) phosphate (µmol L⁻¹), and (j) silicate. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

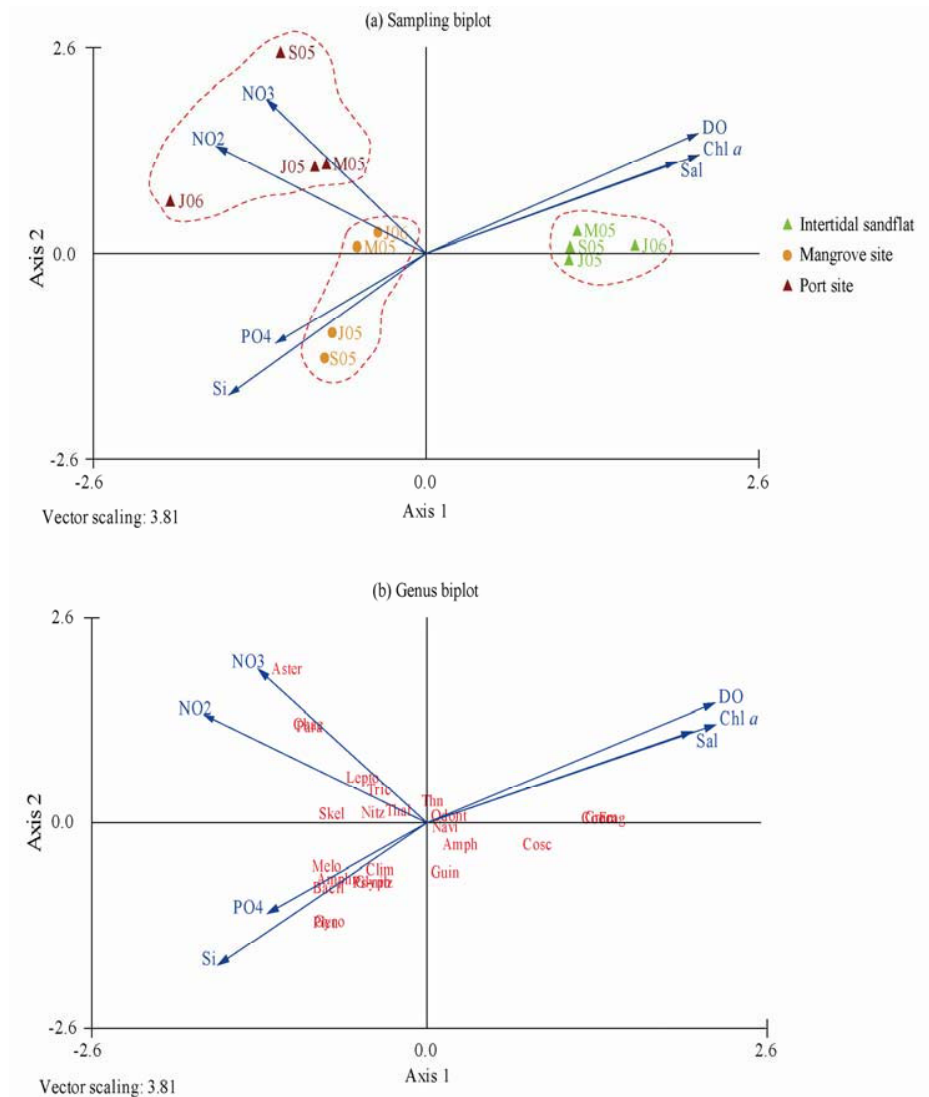


Fig. 4.6. Ordination diagrams for (a) sampling periods and (b) genera based on Canonical Correspondence Analysis (CCA) of the diatom community. The environmental variables (temperature, salinity, dissolved oxygen, chlorophyll *a*, nitrate, nitrite, phosphate and silicate) are indicated by arrows, labeled Temp, Sal, DO, Chl *a*, NO₃, NO₂, PO₄ and Si respectively. PM-I, PreM, Mon and PM-II represent the post-monsoon I, pre-monsoon, monsoon and post-monsoon II sampling periods respectively. *Chaetoceros* (**Chae**), *Coscinodiscus* (**Cosc**), *Guinardia* (**Guin**), *Leptocylindrus* (**Lepto**), *Melosira* (**Melo**), *Odontella* (**Odont**), *Paralia* (**Para**), *Skeletonema* (**Skel**), *Thalassiosira* (**Thal**), *Triceratium* (**Tric**), *Amphiprora* (**Amphi**), *Amphora* (**Amph**), *Asterionellopsis* (**Aster**), *Bacillaria* (**Bacil**), *Climacosphaenia* (**Clim**), *Cocconeis* (**Cocc**), *Fragilariopsis* (**Frag**), *Glyphodesmis* (**Glyph**), *Grammatophora* (**Gram**), *Gyrosigma* (**Gyro**), *Navicula* (**Navi**), *Nitzschia* (**Nitz**), *Pleurosigma* (**Pleu**), *Pseudo-nitzschia* (**Ps-nitz**), *Thalassiosionema* (**Thn**) General grouping of samplings are indicated. See text for interpretation of ordination diagrams.

Table 4.2. Diatoms observed in the different treatments throughout the study period at the intertidal sandflat. CONT: f/2(MBL), P: penicillin, S: streptomycin, C: chloramphenicol, PSC: antibiotic combination.

Taxon	f/2(ASW)	CONT	P	S	C	PSC
Centrics						
<i>Biddulphia rhombus</i> (Ehrenberg) Wm. Smith			+			
<i>Cerataulina bicornis</i> (Ehrenberg) Hasle					+	
<i>Coscinodiscus lineatus</i>	+	+	+	+	+	+
<i>Guinardia flaccida</i>		+				
<i>Melosira nummuloides</i> C.A. Agardh	+		+		+	
<i>Odontella aurita</i> (Lyngbye) C.A. Agardh	+	+		+	+	
<i>Odontella mobiliensis</i> (Bailey) Grunow	+	+		+		
<i>Odontella</i> sp.1	+	+	+	+	+	+
<i>Odontella</i> sp.	+	+	+	+	+	+
<i>Thalassiosira</i> sp.	+	+	+	+	+	+
Pennates						
<i>Achnanthes subsessilis</i>				+		
<i>Amphiprora</i> sp.				+		
<i>Amphora coffeaeformis</i> (Agardh) Kutzing	+	+	+	+	+	+
<i>Amphora costata</i> Wm. Smith	+	+	+	+		
<i>Amphora rostrata</i> Wm. Smith	+	+	+	+	+	+
<i>Amphora turgida</i> Gregory	+	+	+	+	+	+
<i>Amphora</i> sp.	+	+	+	+	+	+
<i>Cocconeis</i> sp.	+	+	+	+	+	+
<i>Fragilariopsis</i> sp.	+	+	+	+	+	+
<i>Grammatophora marina</i>	+	+	+	+	+	
<i>Mastogloia rostrata</i>			+		+	
<i>Navicula crucicula</i> (Wm. Smith) Donkin	+	+	+	+	+	
<i>Navicula directa</i> (W. Smith) Ralfs in Pritchard	+	+			+	+
<i>Navicula granii</i> (Jorgensen) Gran		+				
<i>Navicula pseudopalpebralis</i> spec. nov.					+	
<i>Navicula subinflata</i> Grunow					+	
<i>Navicula transitans</i> var. <i>derasa</i> (Grunow, in Cleve and Grunow) Cleve	+	+	+	+	+	+
<i>Navicula transitans</i> var. <i>derasa</i> f. <i>delicatula</i> Heimdal	+	+	+	+	+	+
<i>Navicula vanhoeffenii</i> Gran		+				
<i>Navicula</i> sp.	+	+	+	+	+	+
<i>Nitzschia panduriformis</i> Gregory				+		
<i>Nitzschia seriata</i> Cleve	+	+		+		
<i>Nitzschia</i> sp.	+	+	+	+	+	
<i>Pseudo-nitzschia</i> sp.	+	+		+		
<i>Thalassionema nitzschioides</i> (Grunow)	+	+	+	+	+	
Mereschkowsky						

At the mangrove site, 41 species of diatoms belonging to 19 genera were recorded (Table 4.3). Diatom abundance ranged from 538 diatoms cm^{-3} wet sediment (post-monsoon II) - 7,959 diatoms cm^{-3} wet sediment (post-monsoon I) (Fig. 4.7a) and did not vary significantly across the sampling periods (Kruskal-Wallis test, ns). Pennate diatoms dominated in the sediment (Fig. 4.7b). The pre-monsoon period supported the highest generic evenness and diversity whereas the monsoon period had the highest generic number and richness (Fig. 4.7c-f). *Navicula*, *Amphora* and *Thalassiosira* were the dominant diatoms, irrespective of the sampling period (Fig. 4.8b).

At the port site, 25 species belonging to 15 genera were recorded (Table 4.4). Diatom abundance ranged from 5 - 293 diatoms cm^{-3} wet sediment during post-monsoon II and pre-monsoon respectively (Fig. 4.7a). Significant variation was observed across the sampling periods (one-way ANOVA, $p=0.0017$). Centric diatoms were the dominant components (Fig. 4.7b). *Thalassiosira* dominated in the post-monsoon I (with the highest generic richness, Fig. 4.7d) and pre-monsoon periods (Fig. 4.8c); *Skeletonema* was the only diatom that emerged in the post-monsoon II period (Fig. 4.8c). The monsoon period had the highest generic evenness and diversity (Fig. 4.7e,f) and was dominated by multiple genera (Fig. 4.8c).

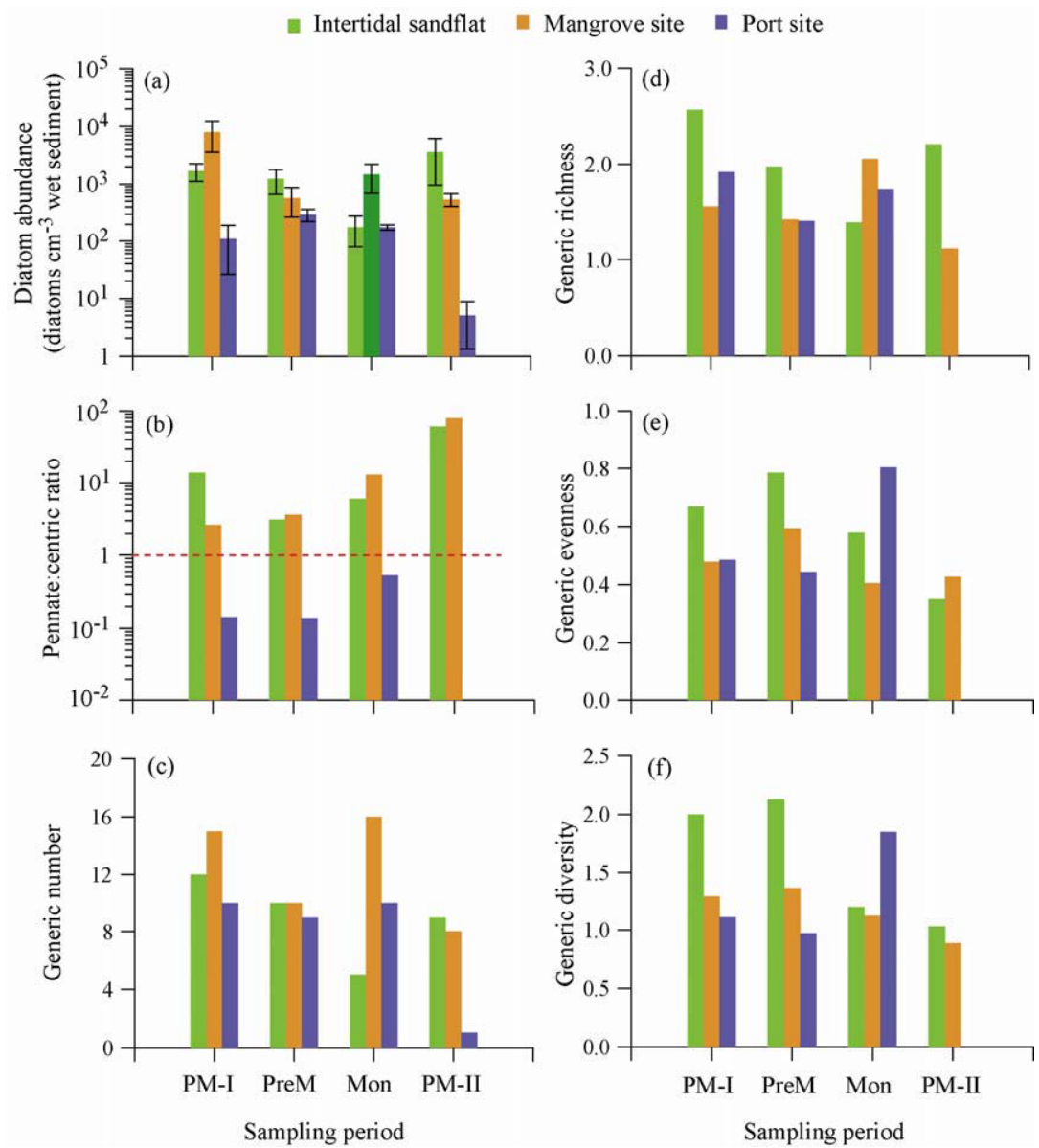


Fig. 4.7. Temporal variation in (a) diatom abundance cm⁻³ wet sediment, (b) pennate:centric ratio, (c) generic number, (d) generic richness, (e) generic evenness and (f) generic diversity of the diatom community under control conditions at the intertidal sandflat, mangrove and port sites. CONT: f/2 medium prepared in artificial seawater (MBL). PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II. The dotted line distinguishes between pennate and centric dominance. Values >1 indicate pennate dominance.

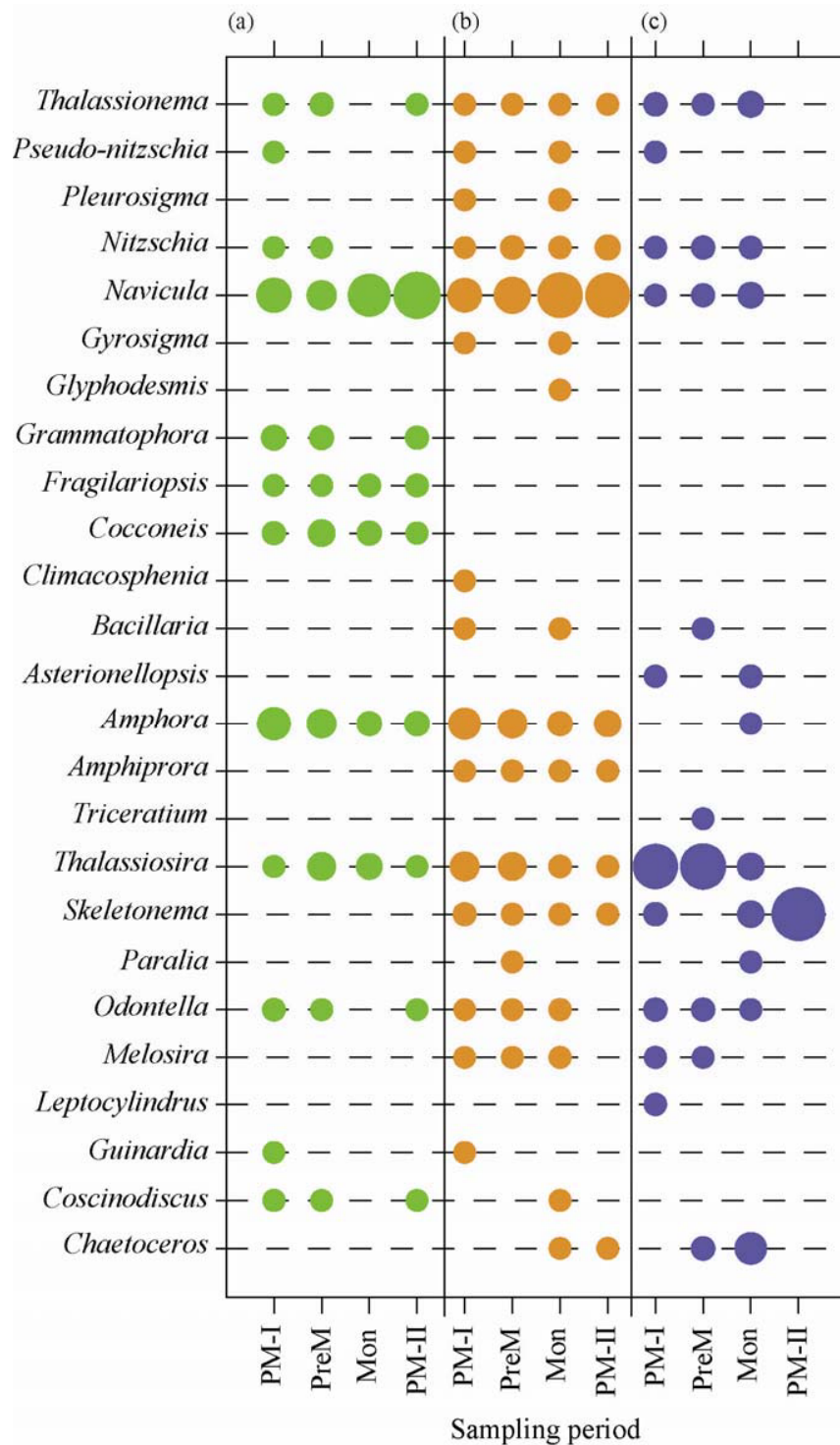


Fig. 4.8. Temporal variation in the contribution of individual diatom genera (%) to the microphytobenthic diatom community under control conditions at the (a) intertidal sandflat, (b) mangrove site and (c) port site. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II. Maximum symbol diameter corresponds to 100%.

Table 4.3. Diatoms observed in the different treatments throughout the study period at the mangrove site. CONT: f/2(MBL), P: penicillin, S: streptomycin, C: chloramphenicol, PSC: antibiotic combination.

Taxon	f/2(ASW)	CONT	P	S	C	PSC
Centrics						
<i>Biddulphia biddulphiana</i>				+		
<i>Biddulphia rhombus</i> (Ehrenberg) Wm. Smith		+	+	+		
<i>Chaetoceros didymus</i>	+	+		+		
<i>Chaetoceros subtilis</i>				+		
<i>Coscinodiscus radiatus</i> Ehrenberg	+	+				
<i>Guinardia flaccida</i> (Castracane) H. Peragallo		+				
<i>Melosira moniliformis</i> C.A. Agardh	+	+	+	+		+
<i>Melosira nummuloides</i> C.A. Agardh	+	+	+	+	+	+
<i>Melosira</i> sp.		+				
<i>Odontella aurita</i> (Lyngbye) C.A. Agardh	+	+		+	+	
<i>Odontella mobiliensis</i> (Bailey) Grunow	+					
<i>Odontella sinensis</i> (Greville) Grunow	+	+				
<i>Odontella</i> sp.1	+					
<i>Odontella</i> sp.	+	+	+	+	+	+
<i>Paralia sulcata</i> (Ehrenberg) Cleve		+	+			
<i>Skeletonema costatum</i> (Greville) Cleve	+	+	+	+	+	+
<i>Thalassiosira</i> sp.	+	+	+	+	+	+
Pennates						
<i>Achnanthes longipes</i> Agardh				+	+	+
<i>Achnanthes subsessilis</i> Kutzing					+	
<i>Amphiprora angustata</i> spec. nov.	+	+	+	+	+	+
<i>Amphiprora gigantea</i>	+	+		+	+	
<i>Amphora coffeaeformis</i> (Agardh) Kutzing	+	+	+	+	+	+
<i>Amphora costata</i>		+				
<i>Amphora rostrata</i> Wm. Smith	+	+	+	+	+	+
<i>Amphora turgida</i> Gregory	+	+		+	+	+
<i>Amphora</i> sp.	+	+	+	+	+	+
<i>Bacillaria paxillifera</i>	+	+	+	+	+	
<i>Bacillaria</i> sp.		+				
<i>Campylodiscus</i> sp.	+					
<i>Climacosphenia</i> sp.	+	+				
<i>Cocconeis scutellum</i> Ehrenberg	+		+	+	+	
<i>Cylindrotheca closterium</i> (Ehrenberg) Lewin & Reimann				+		
<i>Diploneis crabro</i> Ehrenberg	+				+	
<i>Glyphodesmis</i> sp?		+	+			
<i>Grammatophora marina</i> Kutzing	+					
<i>Gyrosigma balticum</i> (Ehrenberg) Cleve	+	+	+	+	+	+
<i>Gyrosigma wansbeckii</i> (Donkin) Cleve	+	+	+	+	+	+
<i>Gyrosigma</i> sp.	+	+	+	+	+	+

Continued...

Table 4.3. ...Continued

Taxon	f/2(ASW)	CONT	P	S	C	PSC
<i>Navicula directa</i> (W. Smith) Ralfs in Pritchard	+	+		+		
<i>Navicula pseudopalpebralis?</i>				+		
<i>Navicula subinflata</i> Grunow			+	+	+	
<i>Navicula transitans</i> var. <i>derasa</i> (Grunow, in Cleve and Grunow) Cleve	+	+	+	+	+	
<i>Navicula transitans</i> var. <i>derasa</i> f. <i>delicatula</i> Heimdal	+	+	+	+	+	
<i>Navicula</i> sp.	+	+	+	+	+	+
<i>Nitzschia granii</i>	+					
<i>Nitzschia longissima</i> (Brebisson, in Kutzing) Ralfs in Pritchard	+					
<i>Nitzschia panduriformis</i> Gregory	+	+		+	+	
<i>Nitzschia seriata</i> Cleve					+	
<i>Nitzschia sigma</i> (Kutzing) Wm. Smith	+	+				
<i>Nitzschia</i> sp.	+	+	+	+	+	+
<i>Pleurosigma angulatum</i> sensu W. Smith emend. Sterrenburg	+			+	+	
<i>Pleurosigma directum</i> Grunow in Cleve & Grunow						+
<i>Pleurosigma elongatum</i> Wm. Smith	+	+	+	+	+	+
<i>Pleurosigma fasciola</i>	+	+	+	+	+	
<i>Pleurosigma macrum</i>	+					
<i>Pleurosigma parkeri</i>	+	+				
<i>Pleurosigma</i> sp.	+	+	+	+	+	+
<i>Pseudo-nitzschia inflatula?</i>					+	
<i>Pseudo-nitzschia sigma</i>	+					
<i>Pseudo-nitzschia</i> sp.	+	+	+	+	+	+
<i>Surirella ovata</i>					+	
<i>Thalassionema bacillare</i> (Heiden in Heiden & Kolbe) Kolbe	+					
<i>Thalassionema frauenfeldii</i> (Grunow) Hallegraeff	+	+		+		
<i>Thalassionema nitzschioides</i> (Grunow) Mereschkowsky	+	+	+	+		

4.3.4. The microphytobenthic diatom communities emerging in f/2(ASW)

At the intertidal sandflat, 24 diatom species belonging to 12 genera emerged in f/2(ASW) (Table 4.2). Diatom abundance ranged from 217 diatoms cm⁻³ wet sediment (monsoon) to 2,641 diatoms cm⁻³ wet sediment (post-monsoon II) (Fig. 4.9a) and showed no significant variation across the sampling periods (Kruskal-

Wallis test, ns). Pennate diatoms dominated the community (Fig. 4.9b). The pre-monsoon period had the highest generic number, richness and diversity and along with the monsoon period had the highest generic evenness (Fig. 4.9c-f). Similar to the trends observed in f/2(MBL) (control conditions), the post-monsoon I period was dominated by *Navicula* and *Amphora* (Fig. 4.10a). The pre-monsoon period was dominated by *Navicula*, *Amphora* and *Thalassiosira*. The monsoon and ensuing post-monsoon II periods were dominated by *Navicula*. However, *Coscinodiscus*, *Odontella*, *Nitzschia* and *Thalassionema* which occurred in the post-monsoon and pre-monsoon periods, were conspicuously absent in monsoon (Fig. 4.10a).

Table 4.4. Diatoms observed in the different treatments throughout the study period at the port site. CONT: f/2(MBL), P: penicillin, S: streptomycin, C: chloramphenicol, PSC: antibiotic combination.

Taxon	f/2(ASW)	CONT	P	S	C	PSC
Centrics						
<i>Biddulphia rhombus</i> (Ehrenberg) W. SM.	+	+		+	+	+
<i>Cerataulina bicornis</i> (Ehrenberg) Hasle	+					
<i>Chaetoceros curvisetus</i> Ehrenberg	+	+	+	+		
<i>Chaetoceros didymus</i>	+					
<i>Chaetoceros subtilis</i>	+	+	+	+	+	+
<i>Coscinodiscus lineatus</i> Ehrenberg	+					
<i>Coscinodiscus radiatus</i> Ehrenberg	+					
<i>Ditylum sol</i> Grunow in Van Heurck			+			
<i>Helicotheca tamesis</i> (Shrubsole) Ricard			+			
<i>Leptocylindrus danicus</i> Cleve	+	+				
<i>Melosira moniliformis</i> C.A. Agardh		+		+	+	
<i>Melosira nummuloides</i> (Muller) Agardh	+	+				
<i>Melosira</i> sp.	+					
<i>Odontella aurita</i> (Lyngbye) C.A. Agardh		+		+		
<i>Odontella mobiliensis</i> (Bailey) Grunow	+	+	+	+		
<i>Odontella sinensis</i> (Greville) Grunow	+	+			+	+
<i>Odontella</i> sp.1	+	+	+	+	+	
<i>Paralia sulcata</i> (Ehrenberg) Cleve	+	+	+	+	+	+
<i>Skeletonema costatum</i> (Greville) Cleve	+	+	+	+	+	+
<i>Thalassiosira</i> sp.	+	+	+	+	+	+
<i>Triceratium favus</i>		+				

Continued...

Table 4.4. ...Continued

Taxon	f/2(ASW)	CONT	P	S	C	PSC
Pennates						
<i>Achnanthes subsessilis</i> Kutzing					+	
<i>Amphora coffeaeformis</i> (Agardh) Kutzing	+		+		+	
<i>Amphora turgida</i>	+		+			
<i>Amphora</i> sp.	+	+				
<i>Asterionellopsis glacialis</i>	+	+	+	+	+	
<i>Bacillaria paxillifera</i>		+				
<i>Cylindrotheca closterium</i> (Ehrenberg) Lewin & Reimann	+					
<i>Navicula crucicula</i>				+		
<i>Navicula directa</i> (W. Smith) Ralfs in Pritchard	+					
<i>Navicula distans</i> (W. Smith) Ralfs in Pritchard	+			+		
<i>Navicula subinflata</i> Grunow				+	+	
<i>Navicula transitans</i> var. <i>derasa</i> (Grunow, in Cleve and Grunow) Cleve	+	+	+	+		
<i>Navicula</i> sp.	+	+	+	+	+	+
<i>Nitzschia panduriformis</i> Gregory	+	+				
<i>Nitzschia seriata</i> Cleve	+	+				
<i>Nitzschia sigma</i> (Kutzing) Wm. Smith	+	+	+	+		
<i>Pleurosigma elongatum</i> Wm. Smith	+					
<i>Pseudo-nitzschia lineola</i> (Cleve) Hasle		+				
<i>Thalassionema bacillare</i> (Heiden in Heiden & Kolbe) Kolbe	+	+	+	+		
<i>Thalassionema nitzschioides</i> (Grunow) Mereschkowsky	+	+	+	+	+	

At the port site, 31 species of diatoms belonging to 16 genera were recorded (Table 4.4). Diatom abundance ranged from 8 - 1254 diatoms cm⁻³ wet sediment during post-monsoon II and monsoon respectively (Fig. 4.9a). Significant variation was observed across the sampling periods (Kruskal-Wallis test, p=0.0444). Centric diatoms were the dominant components (Fig. 4.9b). The post-monsoon I period had the highest generic evenness and diversity, whereas the monsoon period had the highest generic number and richness (Fig. 4.9c-f). *Thalassiosira* was the most dominant diatom throughout the sampling period, with the exception of the monsoon period that was dominated by *Chaetoceros* (Fig. 4.10c).

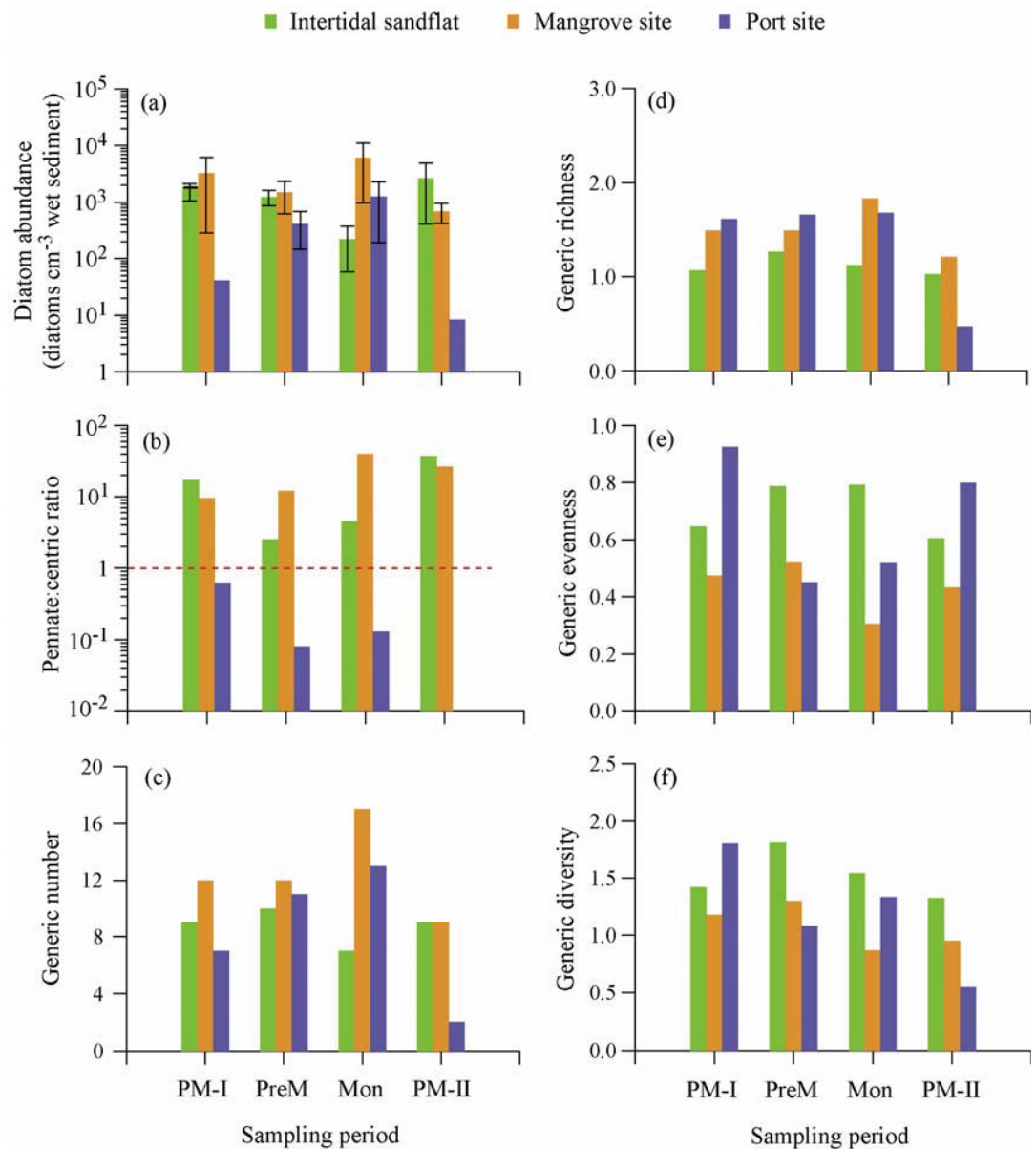


Fig. 4.9. Temporal variation in (a) diatom abundance cm⁻³ wet sediment, (b) pennate:centric ratio, (c) generic number, (d) generic richness, (e) generic evenness and (f) generic diversity of the diatom community emerging in $f/2$ (ASW) at the intertidal sandflat, mangrove and port sites. $f/2$ (ASW): $f/2$ medium prepared in aged seawater (ASW). PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II. The dotted line distinguishes between pennate and centric dominance. Values >1 indicate pennate dominance.

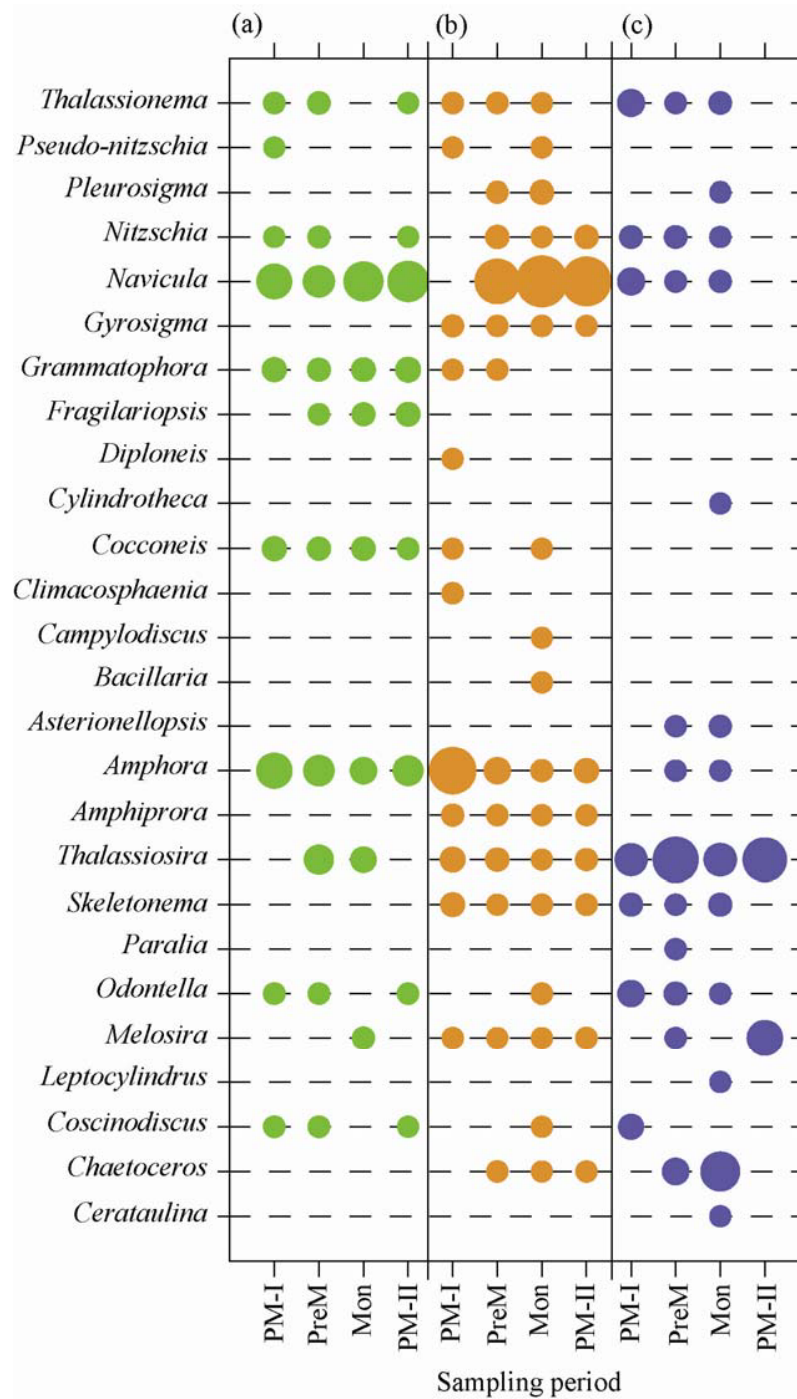


Fig. 4.10. Temporal variation in the contribution of individual diatom genera (%) to the diatom community emerging in f/2(ASW) at the (a) intertidal sandflat, (b) mangrove site and (c) port site. f/2(ASW): f/2 medium prepared in aged seawater (ASW). PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II. Maximum symbol diameter corresponds to 81.47%.

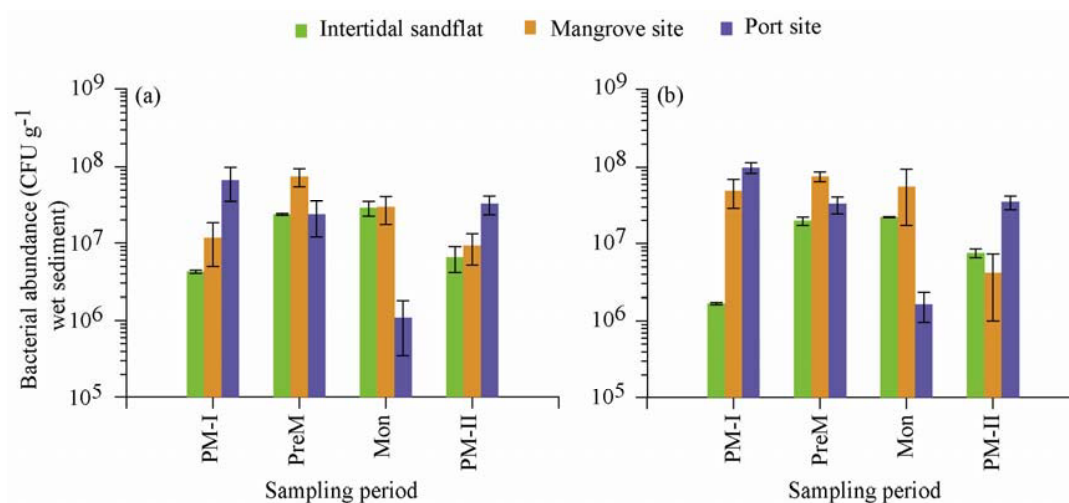


Fig. 4.11. Temporal variation in bacterial abundance (CFU g⁻¹ wet sediment) under (a) control conditions, and (b) f/2(ASW), at the intertidal sandflat, mangrove and port sites. CONT: f/2 medium prepared in artificial seawater (MBL). f/2(ASW): f/2 medium prepared in aged seawater (ASW). PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

4.3.5. The bacterial communities under control conditions and in f/2(ASW)

Under control conditions, at the intertidal sandflat, bacterial abundance ranged from 4.24×10^6 - 2.86×10^7 CFU g⁻¹ wet sediment (Fig. 4.11a), and varied significantly across the sampling periods (one-way ANOVA, $p=0.004$). *Alcaligenes* and *Pseudomonas* were the most dominant culturable bacteria throughout the study period; *Bacillus* and *Enterobacteriaceae* showed season-specific dominance trends (Table 4.5).

At the mangrove and port sites, bacterial abundance varied significantly from 9.23×10^6 - 7.46×10^7 CFU g⁻¹ wet sediment (Kruskal-Wallis test, $p=0.0307$) and from 1.08×10^6 - 6.67×10^7 CFU g⁻¹ wet sediment (one-way ANOVA, $p=0.0113$) respectively (Fig. 4.11a). Seasonal variations in the dominant culturable bacteria

Table 4.5. Culturable bacteria in the different treatments at the intertidal sandflat. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II; CONT: f/2(MBL), P: penicillin, S: streptomycin, C: chloramphenicol, PSC: antibiotic combination.

Treatments	PM-I	PreM	Mon	PM-II
CONT				
<i>Alcaligenes</i>	+	+	+	+
<i>Bacillus</i>			+	+
<i>Enterobacteriaceae</i>	+			
<i>Pseudomonas</i>	+	+	+	+
Penicillin				
<i>Alcaligenes</i>		+		+
<i>Pseudomonas</i>	+	+	+	
<i>Vibrio</i>	+		+	+
Streptomycin				
<i>Alcaligenes</i>	+	+	+	+
<i>Pseudomonas</i>	+	+	+	+
Chloramphenicol				
<i>Bacillus</i>		+		+
<i>Pseudomonas</i>	+		+	+
<i>Vibrio</i>		+	+	

were observed (Tables 4.6, 4.7). Similar variations in the seasonal bacterial abundance were observed in f/2(ASW) (Fig. 4.11b). These variations were significant at the intertidal sandflat (Kruskal-Wallis test, $p=0.0216$) and the port site (one-way ANOVA, $p=0.00001$), and were not significant at the mangrove site (Kruskal-Wallis test, $p=0.0572$).

Table 4.6. Culturable bacteria in the different treatments at the mangrove site. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II; CONT: f/2(MBL), P: penicillin, S: streptomycin, C: chloramphenicol, PSC: antibiotic combination.

Treatments	PM-I	PreM	Mon	PM-II
CONT				
<i>Alcaligenes</i>		+		+
<i>Bacillus</i>	+		+	
<i>Corynebacterium</i>	+	+	+	
<i>Micrococcus</i>		+		
<i>Pseudomonas</i>				+
Penicillin				
<i>Alcaligenes</i>	+	+	+	
<i>Bacillus</i>			+	+
<i>Pseudomonas</i>	+	+		+
Streptomycin				
<i>Alcaligenes</i>	+	+		
<i>Corynebacterium</i>	+		+	+
<i>Enterobacteriaceae</i>		+	+	
<i>Micrococcus</i>				+
Chloramphenicol				
<i>Alcaligenes</i>		+	+	+
<i>Pseudomonas</i>	+			+

Table 4.7. Culturable bacteria observed in the different treatments at the port site. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II; CONT: f/2(MBL), P: penicillin, S: streptomycin, C: chloramphenicol, PSC: antibiotic combination.

Treatments	PM-I	PreM	Mon	PM-II
CONT				
<i>Alcaligenes</i>	+	+		+
<i>Bacillus</i>		+	+	
<i>Corynebacterium</i>			+	+
<i>Micrococcus</i>	+			+
Penicillin				
<i>Alcaligenes</i>	+	+		+
<i>Corynebacterium</i>		+	+	
<i>Pseudomonas</i>			+	+
Streptomycin				
<i>Alcaligenes</i>	+		+	+
<i>Enterobacteriaceae</i>		+		+
<i>Pseudomonas</i>	+	+	+	
Chloramphenicol				
<i>Alcaligenes</i>		+	+	+
<i>Bacillus</i>	+			

4.3.6. Stability of antibiotics in f/2(MBL) and f/2(ASW) in presence of sediment

In presence of sediment from the mangrove site, antibiotic stability ranged from 26-59% in f/2(MBL) (Fig. 4.12a) and from 35-44% in f/2(ASW) (Fig. 4.12b). In f/2(MBL), the antibiotic combination was the most stable, followed by chloramphenicol (Fig. 4.12a). In f/2(ASW), chloramphenicol was the most stable (Fig. 4.12b).

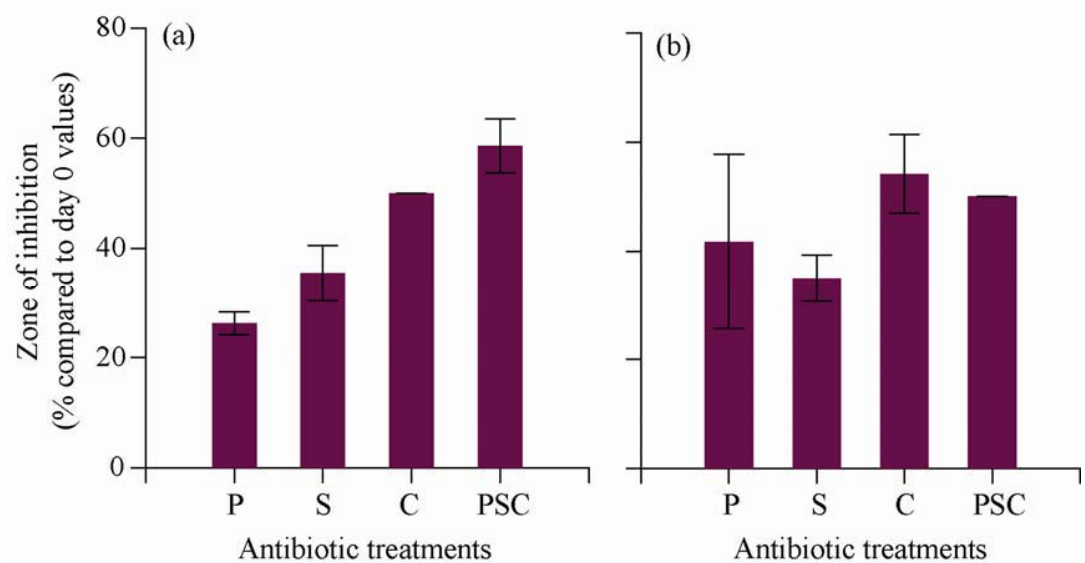


Fig. 4.12. Stability of the antibiotic treatments (a) under control conditions and (b) in f/2(ASW), in presence of silty sediment from the mangrove site, expressed in terms of zone of inhibition (percentage compared to day 0 values). Antibiotic treatment details and concentrations are provided in the text.

4.3.7. Effects on diatoms through bacteria - penicillin

Penicillin-resistant bacteria (in terms of percentage compared to control) showed significant seasonal variation in the intertidal sandflat and mangrove site (Table 4.8a). However, diatom survival (compared to control) varied significantly across seasons only in the port site (Table 4.8a).

Table 4.8. Significance values of the Kruskal-Wallis tests to evaluate the seasonal variation in diatom and bacterial abundance (in terms of percentage compared to control) in the antibiotic treatments, at the intertidal sandflat, mangrove and port sites. Values in bold indicate significant variation.

Treatments	p-value	
	Bacteria	Diatoms
Penicillin		
Intertidal sandflat	0.0249	0.5360
Mangrove site	0.0249	0.1540
Port site	0.2228	0.0279
Streptomycin		
Intertidal sandflat	0.1609	0.5839
Mangrove site	0.0307	0.8086
Port site	0.0378	0.1319
Chloramphenicol		
Intertidal sandflat	0.0423	0.9104
Mangrove site	0.0546	0.4668
Port site	0.0509	0.0284
Antibiotic combination		
Intertidal sandflat	-	0.2001
Mangrove site	-	0.0994
Port site	-	0.0254

At the intertidal sandflat, treatment with penicillin suppressed bacteria by 99-100% (Table 4.9). *Alcaligenes*, *Pseudomonas* and *Vibrio* were the most dominant culturable bacteria (Table 4.5). The effects of these changes in bacteria on diatom community structure, were clearly evident in terms of reduced diatom abundance (22-58% compared to control values) (Fig. 4.13a) and the consistent inhibition of *Coscinodiscus*, *Cocconeis*, *Grammatophora* and *Thalassionema*, throughout the study period (Fig. 4.14a). The least reduction in diatom abundance in the penicillin treatment was observed in the pre-monsoon period (Fig. 4.13a).

Table 4.9. Bacterial suppression (in terms of percentage compared to control), in the antibiotic treatments at the intertidal sandflat, mangrove and port sites.

Treatment	Bacterial suppression (%)		
	Intertidal sandflat	Mangrove	Port
Penicillin	99 -100	95 ~ 100	99 ~ 100
Streptomycin	>99	71 ~ 100	> 99
Chloramphenicol	>99	>99	~ 100
Antibiotic combination	100	100	100

Another intriguing feature is the dominance of the centric diatom, *Thalassiosira*, in the pre-monsoon period, resulting in a <1 pennate:centric ratio (Fig. 4.15a). This was coupled with the inhibition of *Amphora*, *Cocconeis* and *Navicula* (Fig. 4.14a), that dominated under control conditions (Fig. 4.8a). The I:E ratio was maximum in post-monsoon periods and minimum during the monsoon period (Fig. 4.16a).

At the mangrove site, penicillin treatment suppressed bacteria by 95~100% (Table 4.8). *Alcaligenes*, *Pseudomonas* and *Vibrio* were the most dominant culturable bacteria (Table 4.6). Diatom abundance ranged from 5-32% (Fig. 4.13a); *Amphora* was consistently enhanced whereas *Navicula* was consistently inhibited (Fig. 4.17a). The diatom community was pennate-dominated throughout the sampling period (Fig. 4.15a). The I:E ratio was >1 throughout the sampling period except monsoon (Fig. 4.16a).

At the port site, penicillin treatment suppressed bacteria by 99~100% (Table 4.9). *Alcaligenes*, *Pseudomonas* and *Vibrio* were the most dominant culturable bacteria (Table 4.7). Diatom abundance ranged from total suppression to enhancement (149%) (Fig. 4.13a). *Nitzschia* and *Thalassionema* were consistently inhibited (Fig. 4.18a). The diatom community was centric-dominated during the pre-monsoon and

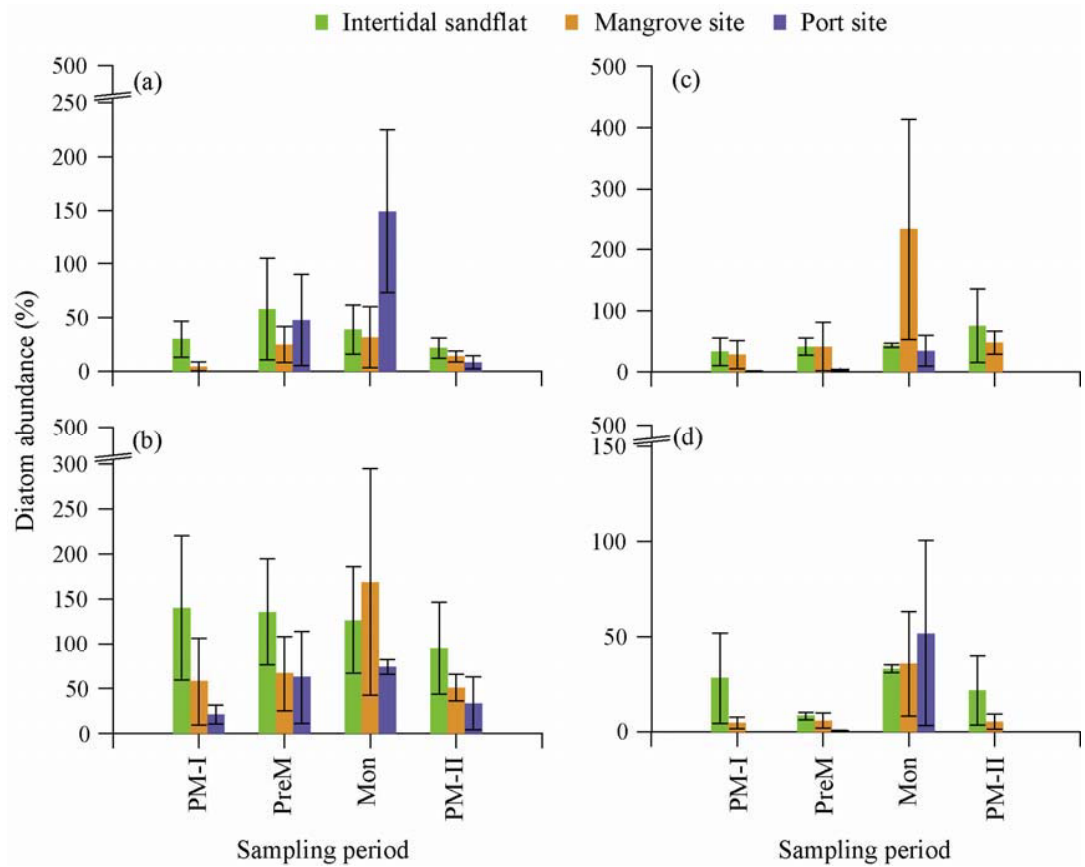


Fig. 4.13. Temporal variation in diatom abundance (expressed as percentage of control values) in the (a) penicillin, (b) streptomycin, (c) chloramphenicol and (d) antibiotic combination treatments, at the intertidal sandflat, mangrove and port sites. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

monsoon periods (Fig. 4.15a). The I:E ratio was ≥ 1 throughout the sampling period except post-monsoon I (Fig. 4.16a) where diatoms were totally inhibited (Fig. 4.18a).

4.3.8. Combination of potentially direct and indirect effects on diatoms - streptomycin

Streptomycin-resistant bacteria (in terms of percentage compared to control) showed significant seasonal variation in the mangrove and port sites (Table 4.8b). Correspondingly, diatom survival was consistent across seasons in all the 3 sites (Table 4.8b).

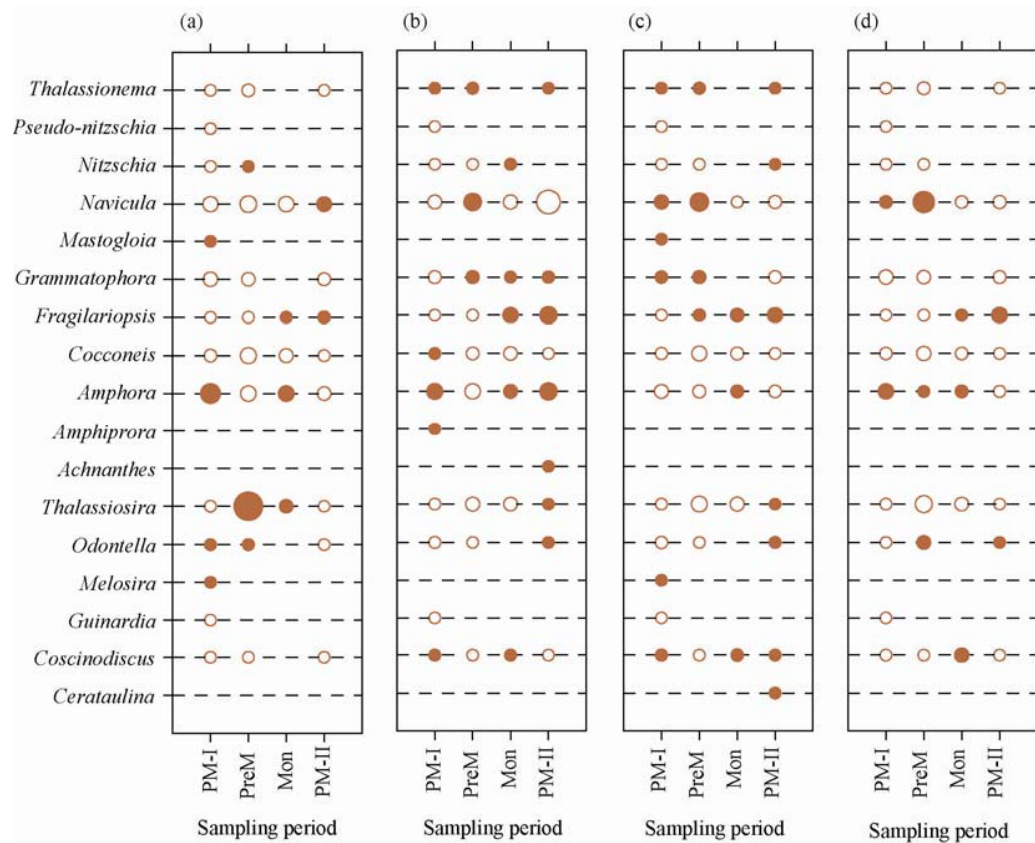


Fig. 4.14. Temporal variation in the contribution of individual diatom genera to the diatom community (in terms of inhibition/enhancement compared to control values) at the intertidal sandflat. (a) penicillin, (b) streptomycin, (c) chloramphenicol and (d) antibiotic combination treatments. Empty circles indicate inhibited genera; filled circles indicate enhanced genera. Maximum symbol diameter corresponds to 61.91%. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

At the intertidal sandflat, streptomycin treatment suppressed bacteria by >99% (Table 4.9). *Alcaligenes* and *Pseudomonas* were the most dominant culturable bacteria (Table 4.5). Streptomycin enhanced diatom growth (126-140% compared to control values) with one exception (96% in post-monsoon II) (Fig. 4.13b). *Thalassionema* was consistently enhanced (Fig. 4.14b). The diatom community was pennate- dominated throughout the sampling period (Fig. 4.15b). The I:E ratio was <1 in the monsoon and post-monsoon II periods (Fig. 4.16b).

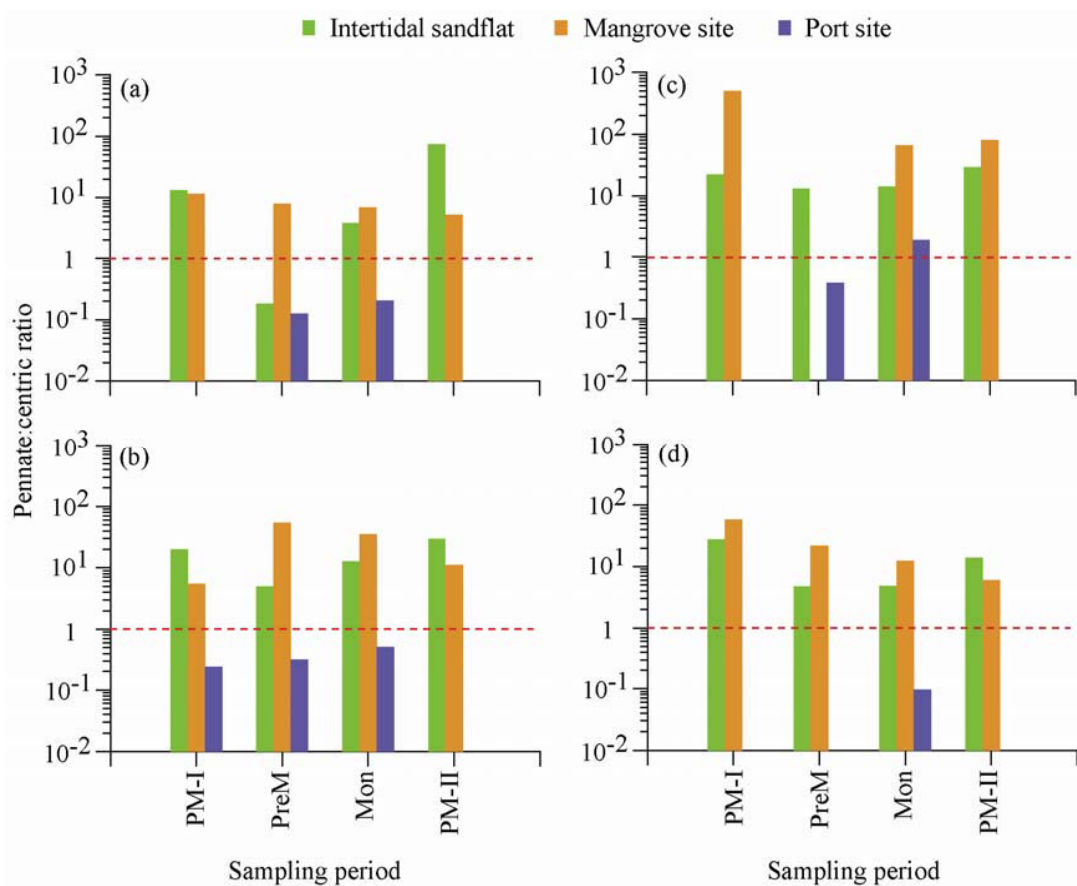


Fig. 4.15. Temporal variation in the pennate:centric ratios of the diatom community in the (a) penicillin, (b) streptomycin, (c) chloramphenicol and (d) antibiotic combination treatments, at the intertidal sandflat, mangrove and port sites. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II. The dotted line distinguishes between pennate and centric dominance. Values >1 indicate pennate dominance.

At the mangrove site, streptomycin treatment suppressed bacteria by 71~100% (Table 4.9). *Alcaligenes*, *Corynebacterium*, *Enterobacteriaceae* and *Micrococcus* were the most dominant culturable bacteria (Table 4.6). Diatom abundance varied from 51% to enhancement (169%) (Fig. 4.13b). *Amphora* was enhanced throughout the sampling period except post-monsoon II whereas *Thalassiosira* and *Navicula* were inhibited consistently (Fig. 4.17b). The diatom community was pennate-

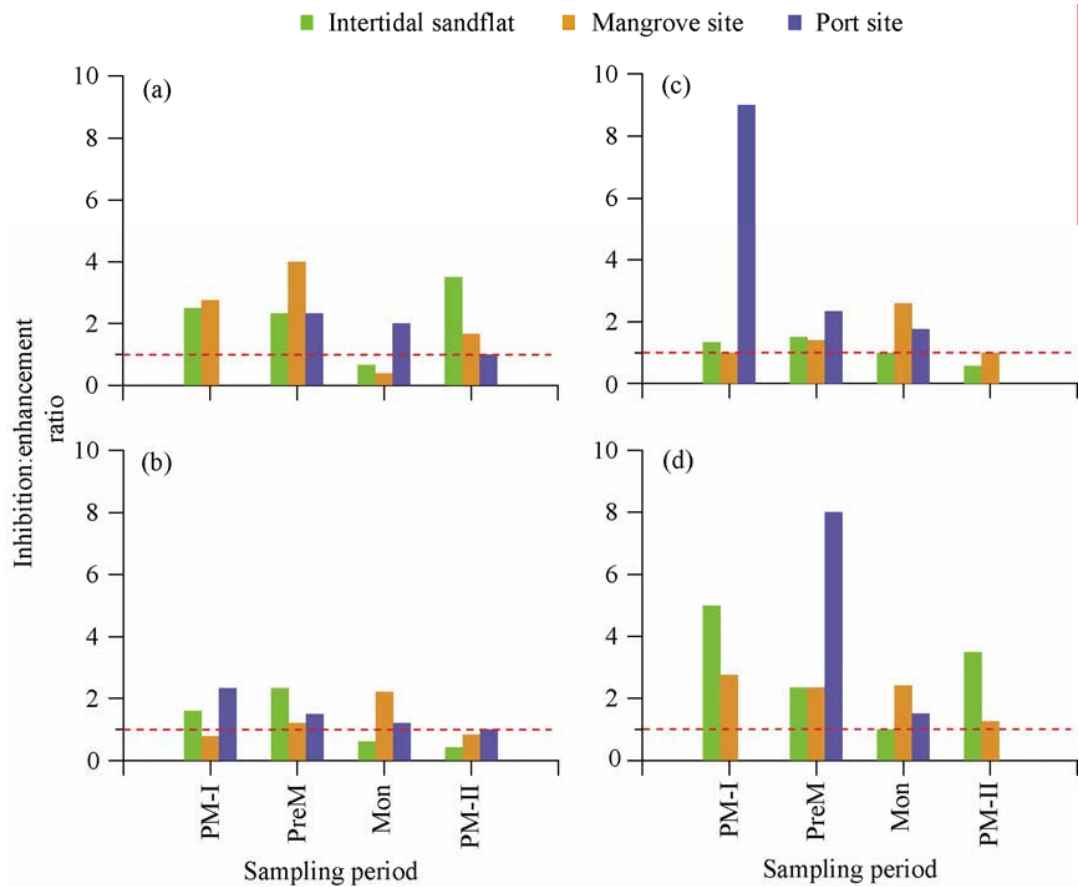


Fig. 4.16. Temporal variation in the inhibition:enhancement ratios of the diatom community in the (a) penicillin, (b) streptomycin, (c) chloramphenicol and (d) antibiotic combination treatments, at the intertidal sandflat, mangrove and port sites. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II. The dotted line distinguishes between inhibition and enhancement potential pennate and centric dominance. Values >1 indicate inhibition potential.

dominated throughout the sampling period (Fig. 4.15b). The I:E ratio was <1 in both post-monsoon periods (Fig. 4.16b).

At the port site, streptomycin treatment suppressed bacteria by >99% (Table 4.9). *Alcaligenes*, *Enterobacteriaceae* and *Pseudomonas* were the most dominant culturable bacteria (Table 4.7). Diatom abundance varied from 21-74% (Fig. 4.13b). *Odontella* and *Navicula* were enhanced whereas *Nitzschia* was inhibited consistently

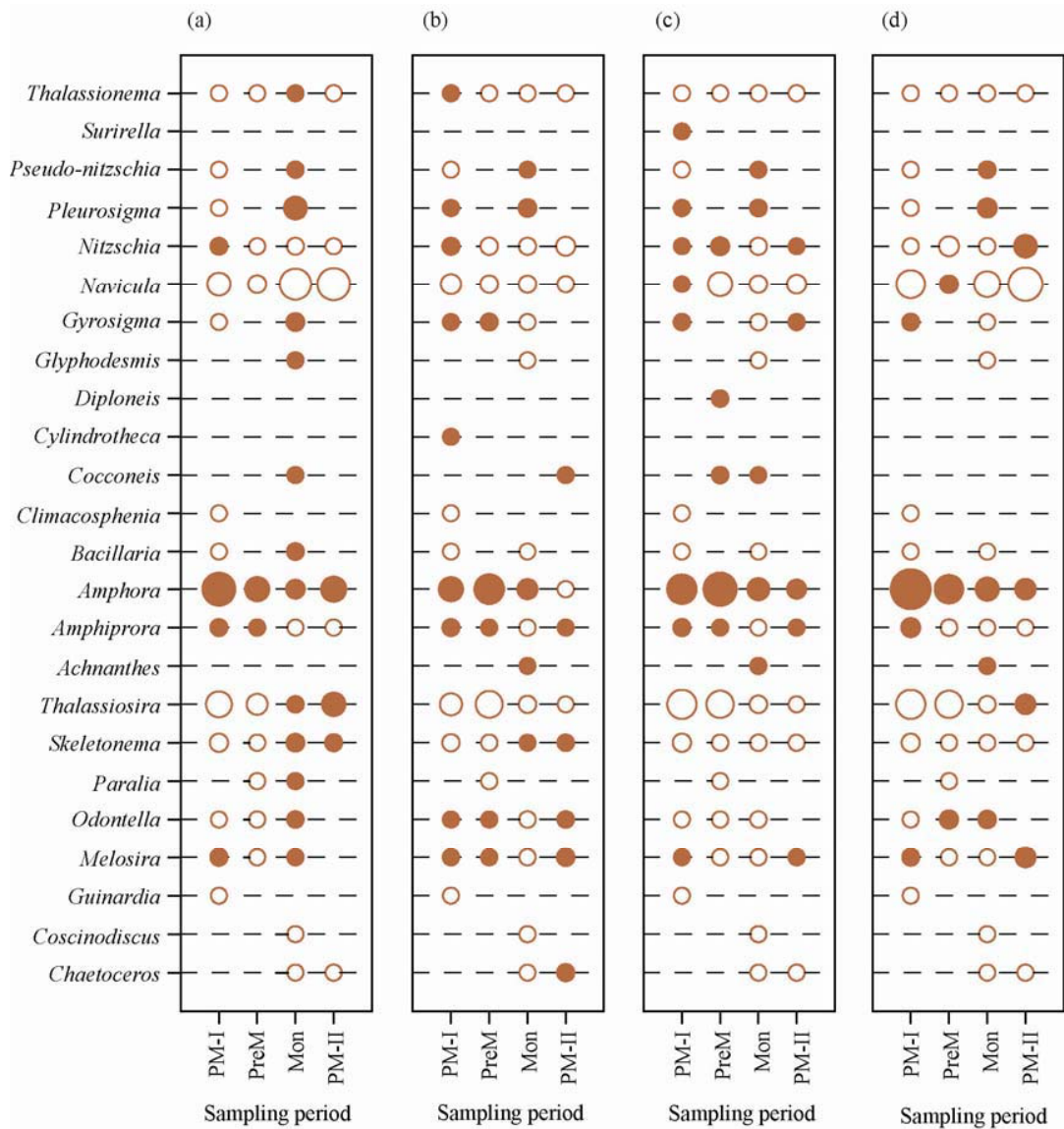


Fig. 4.17. Temporal variation in the contribution of individual diatom genera to the diatom community (in terms of inhibition/enhancement compared to control values) at the mangrove site. (a) penicillin, (b) streptomycin, (c) chloramphenicol and (d) antibiotic combination treatments. Empty circles indicate inhibited genera; filled circles indicate enhanced genera. Maximum symbol diameter corresponds to 42.49%. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

(Fig. 4.18b). The diatom community was centric-dominated throughout the sampling period (Fig. 4.15b). However, the pennate:centric ratio could not be calculated for

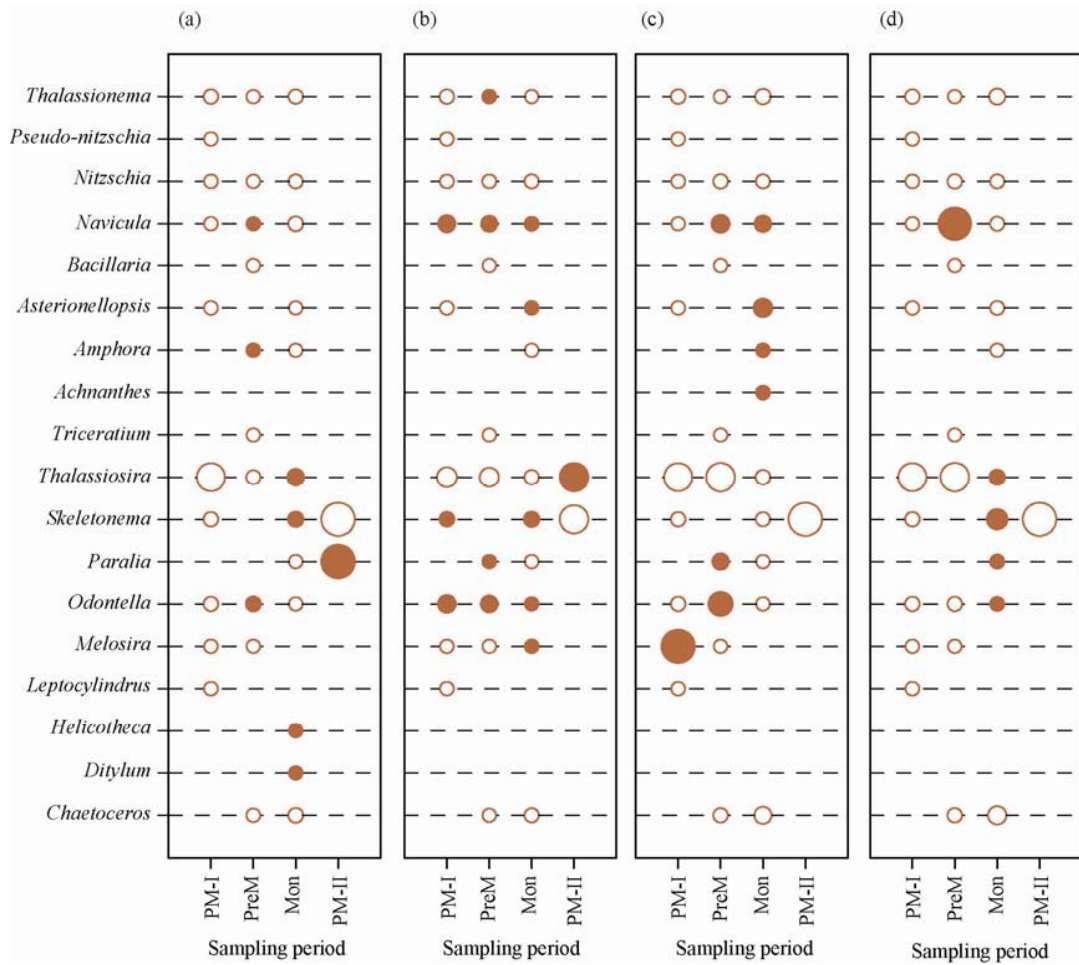


Fig. 4.18. Temporal variation in the contribution of individual diatom genera to the diatom community (in terms of inhibition/enhancement compared to the control treatment) at the port site. (a) penicillin, (b) streptomycin, (c) chloramphenicol and (d) antibiotic combination treatments. Empty circles indicate inhibited genera; filled circles indicate enhanced genera. Maximum symbol diameter corresponds to 100%. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

the post-monsoon II period, as only a single centric diatom (*Thalassiosira*) emerged in the streptomycin treatment (Fig. 4.15b). The I:E ratio was ≥ 1 throughout the sampling period (Fig. 4.16b).

4.3.9. Direct effects on diatoms - chloramphenicol

Chloramphenicol-resistant bacteria (in terms of percentage compared to control) varied significantly across seasons only in the intertidal sandflat whereas diatom survival showed significant seasonal variation only in the port site (Table 4.8c).

At the intertidal sandflat, chloramphenicol treatment suppressed bacteria by >99% (Table 4.9). *Bacillus*, *Pseudomonas* and *Vibrio* were the most dominant culturable bacteria (Table 4.5). Chloramphenicol inhibited diatoms consistently, ranging from 33-75% of control values (Fig. 4.13c). *Cocconeis* appeared to be the most sensitive; it was consistently inhibited, whereas *Thalassionema* occurred at enhanced levels compared to control conditions throughout the sampling period (Fig. 4.14c). The diatom community was pennate-dominated throughout the sampling period (Fig. 4.15c). The I:E ratio was ≥ 1 throughout the sampling period, except post-monsoon II; it was highest in pre-monsoon (Fig. 4.16c).

Similar to the observations at the intertidal sandflat, chloramphenicol suppressed bacteria by >99% at the mangrove site (Table 4.9). *Alcaligenes* and *Pseudomonas* were the most dominant culturable bacteria (Table 4.6d). Diatom abundance ranged from 28% of control values to enhancement (233%) (Fig. 4.13c). *Amphora* was enhanced and *Skeletonema*, *Thalassiosira* and *Thalassionema* were inhibited throughout the sampling period (Fig. 4.17c). The diatom community was pennate-dominated throughout the sampling period (Fig. 4.15c). The I:E ratio was ≥ 1 consistently (Fig. 4.16c).

Similar to the observations at both the sites mentioned above, chloramphenicol suppressed bacteria by ~100% at the port site (Table 4.9). *Alcaligenes* and *Bacillus* were the most dominant culturable bacteria (Table 4.7e). Diatom abundance varied

from 35% to total inhibition (Fig. 4.13c). *Skeletonema*, *Thalassiosira*, *Nitzschia* and *Thalassionema* were consistently inhibited (Fig. 4.18c). The diatom community was centric-dominated in the pre-monsoon period and pennate-dominated in the monsoon period (Fig. 4.15c). The pennate:centric ratio could not be calculated for both post-monsoon periods due to the emergence of only a single centric diatom (*Melosira*) in the chloramphenicol treatment during post-monsoon I (Fig. 4.18c), and total inhibition of diatoms in post-monsoon II (Fig. 4.13c). The I:E ratio was ≥ 1 throughout the sampling period, except post-monsoon II (Fig. 4.16c) where diatoms were totally inhibited (Fig. 4.13c). The I:E ratio was highest in post-monsoon I (Figs. 4.16c), due to inhibition of several diatom genera and simultaneous enhancement of a single genus (*Melosira*) (Fig. 4.18c).

4.3.10. The antibiotic combination treatment

The antibiotic combination treatment suppressed bacteria below detectable levels at all the sites, throughout the sampling period (Table 4.9). Diatom survival (compared to control) varied significantly across seasons only at the port site (Table 4.8d).

At the intertidal sandflat, diatom abundance varied from 8-33% (Fig. 4.13d). The diatom community was pennate-dominated (Fig. 4.15d) and had an I:E ratio of 1-5 (Fig. 4.16d). Having a wider spectrum of action, it inhibited *Cocconeis*, *Grammatophora* and *Thalassionema* throughout the sampling period (similar to the trend in the penicillin treatment) in addition to *Thalassiosira* (Fig. 4.14d).

At the mangrove site, diatom abundance varied from 4-36% (Fig. 4.13d). *Amphora* was enhanced whereas *Skeletonema* and *Thalassionema* were inhibited

consistently (Fig. 4.17d). The diatom community was pennate-dominated (Fig. 4.15d). The I:E ratio was >1 throughout the sampling period (Fig. 4.16d).

At the port site, diatoms were totally inhibited in all the sampling periods except pre-monsoon (0.40%) and monsoon (52%) (Fig. 4.13d). *Nitzschia* and *Thalassionema* were inhibited throughout the sampling period (Fig. 4.18d). The diatom community was centric-dominated in the monsoon period (Fig. 4.15d). The pennate:centric ratio could not be calculated for the pre-monsoon period due to inhibition of all the centric diatoms (Fig. 4.18d). The I:E ratio was ≥ 1 and was highest in pre-monsoon (Fig. 4.16d), due to inhibition of several diatom genera and simultaneous enhancement of a single pennate genus (*Navicula*) (Fig. 4.18d).

4.3.11. Monsoonal influences and comparison of all the treatments with respect to diatoms – temporal variation

At the intertidal sandflat, the monsoon period was characterized by a ≤ 1 I:E ratio in all the antibiotic treatments (Fig. 4.16). At the mangrove and port sites, the mangrove period was associated with enhancement of diatoms in the individual antibiotic treatments (Fig. 4.13a-c). This ‘uniqueness’ of the monsoon period was mirrored in the grouping of all the monsoon period treatments, irrespective of the nature of the antibiotics, in the temporal sampling cluster, at all the 3 sites (Fig. 4.19a-c). At the intertidal sandflat, 3 groups and 1 dissimilar entity was observed at 60% Bray-Curtis similarity (Fig. 4.19a). Group I consisted of all the monsoon period treatments, with an average diatom abundance of 97 diatoms cm^{-3} wet sediment (data not shown). Group II consisted of the rest of the antibiotic combination treatments

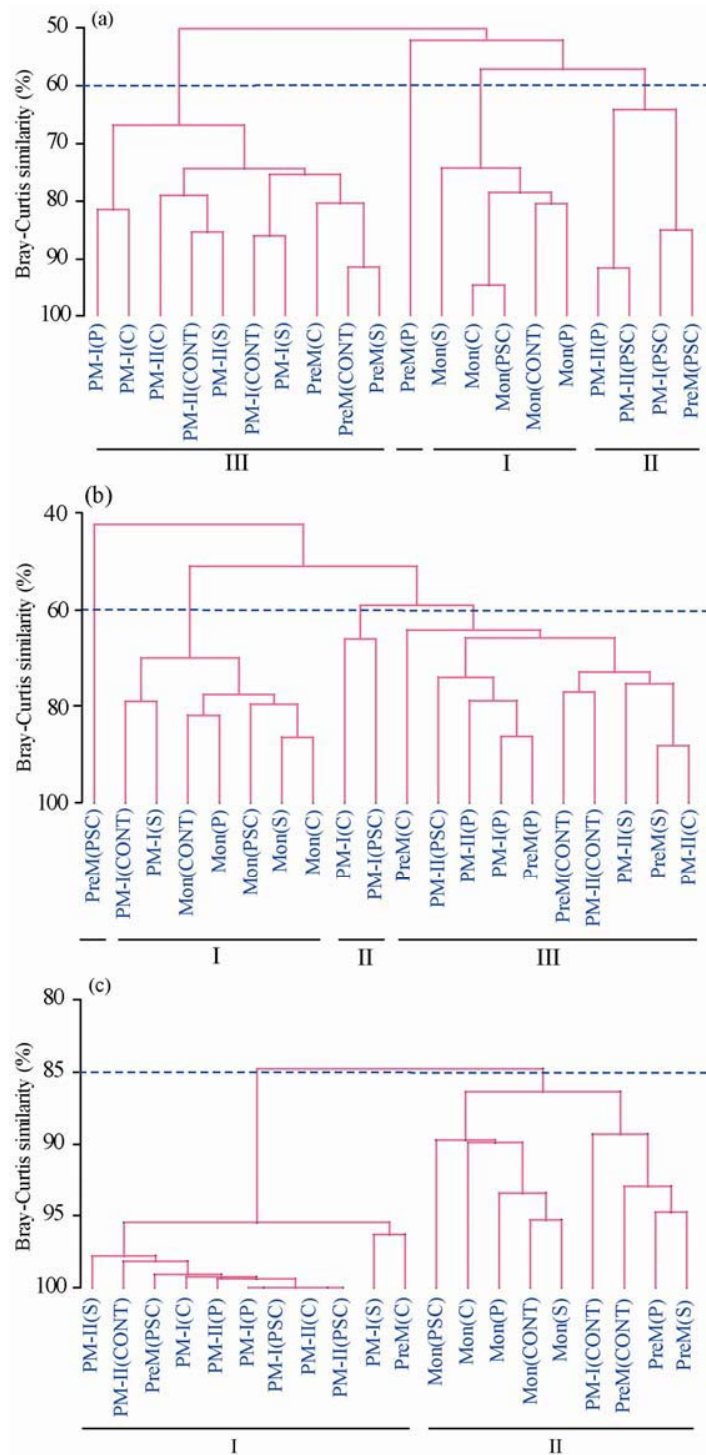


Fig. 4.19. Cluster dendrogram of the diatom community in the different treatments throughout the sampling period at the (a) intertidal sandflat, (b) mangrove site and the (c) port site, using the Bray-Curtis similarity coefficient and group average method. Grouping done at 60% Bray-Curtis similarity level in (a) and (b) and at 85% Bray-Curtis similarity level in (c).

(with minimum diatom abundance), and the PM-II(P) treatment (with lowest generic evenness and diversity; Fig. 4.20e,i). All the other treatments, except the PreM(P), clustered together (Group III). The PreM(P) treatment had the lowest generic richness among all the treatments (Fig. 4.20a) and was the sole ungrouped entity (Fig. 4.19a).

A cluster analysis of all the mangrove site treatments at 60% Bray-Curtis similarity revealed 3 groups and one ungrouped entity[PreM(PSC)] (Fig. 4.19b). All the monsoon treatments clustered together (Fig. 4.19b). They had the highest generic richness (Fig. 4.20a-d) and an average diatom abundance of 1697 diatoms cm^{-3} wet sediment (data not shown). The remaining penicillin treatments, with the lowest diatom abundance among all the individual antibiotic treatments (94 diatoms cm^{-3} wet sediment; data not shown), grouped together (Fig. 4.19b).

A cluster analysis of all the port site treatments indicated that all the treatments were similar at 60% Bray-Curtis similarity (Fig. 4.19c). Two groups were observed at 85% Bray-Curtis similarity, one of which consisted of all the monsoon treatments (Fig. 4.19c), with the highest generic diversity (Fig. 4.20i-l) and an average diatom abundance of 145 diatoms cm^{-3} wet sediment (data not shown).

4.3.12. Sampling-wise clusters

At the intertidal sandflat and mangrove site, a cluster analysis of the treatments for each sampling period, revealed that the control and streptomycin treatments supported similar diatom communities throughout the sampling period except the

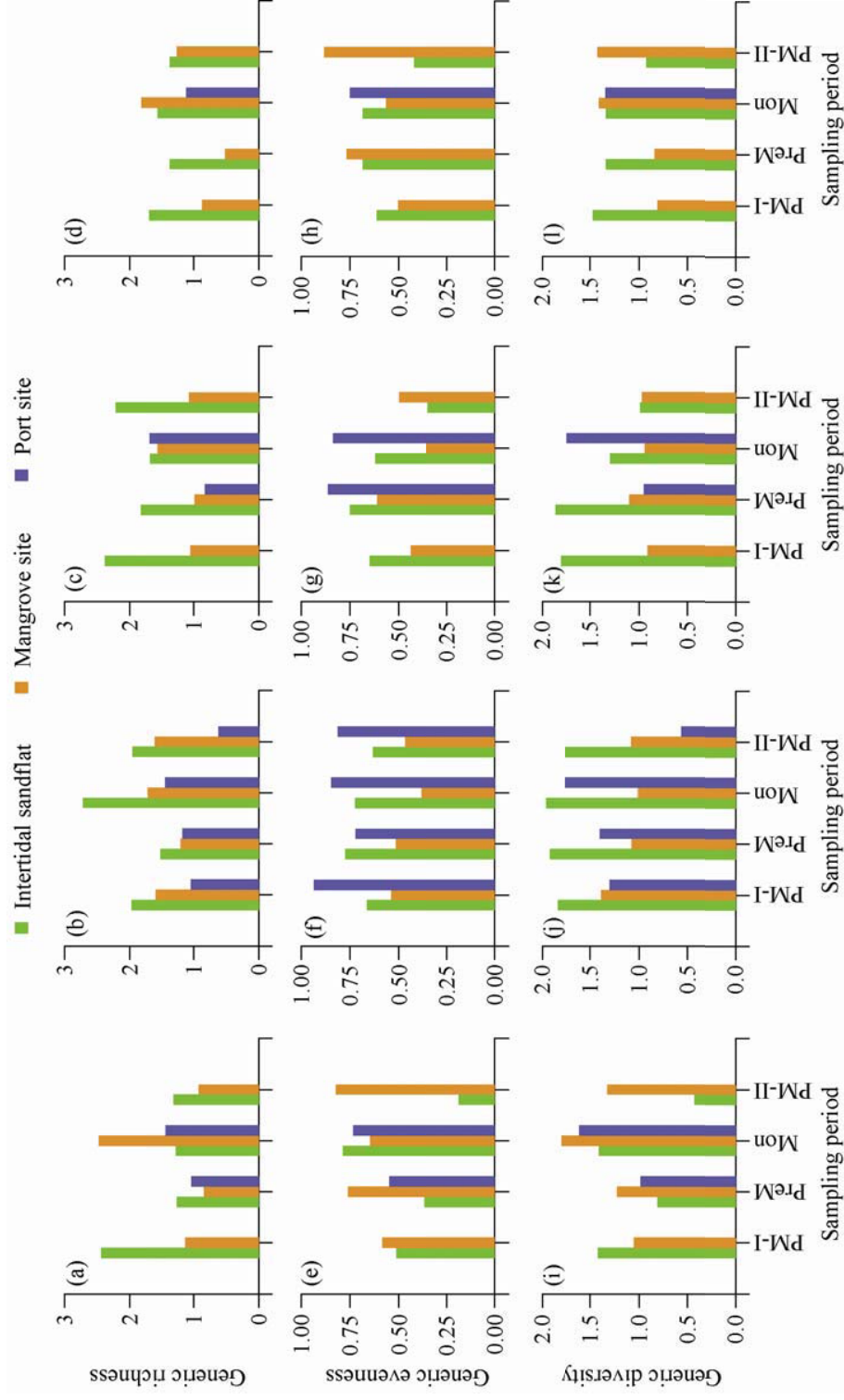


Fig. 4.20. Temporal variation in the (a-d) generic richness, (e-h) generic evenness and (i-l) generic diversity of the diatom community in the (a,e,i) penicillin, (b,f,j) streptomycin, (c,g,k) chloramphenicol, and (d,h,l) antibiotic combination treatments at the intertidal sandflat, mangrove and port sites. PM-I: post-monsoon I; PreM: pre-monsoon; Mon: monsoon; PM-II: post-monsoon II.

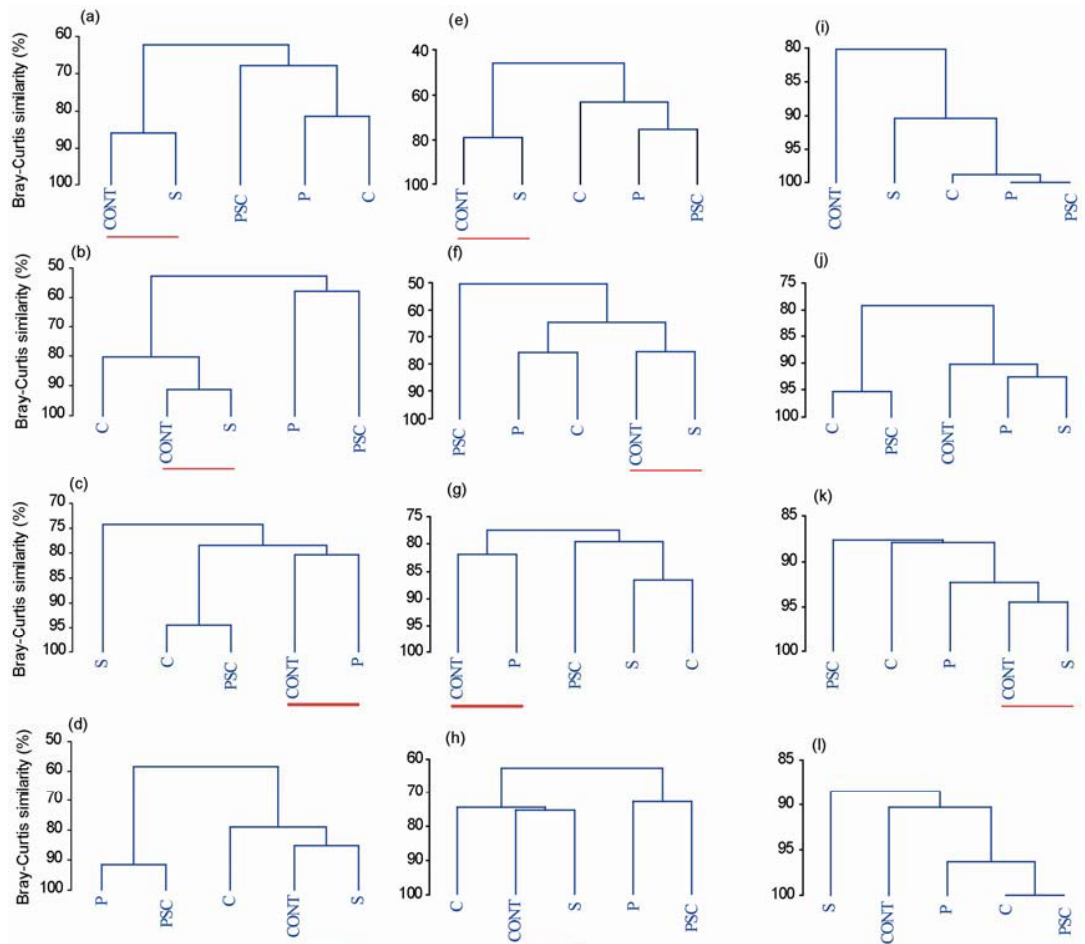


Fig. 4.21. Cluster dendrogram of the diatom community in the different treatments using the Bray-Curtis similarity coefficient and group average method at the (a-d) intertidal sandflat, (e-h) mangrove site, and (i-l) port site, during the (a,e,i) post-monsoon I, (b,f,j) pre-monsoon, (c,g,k) monsoon and (d,h,l) post-monsoon II.

monsoon (Fig. 4.21a-e). During the monsoon period, the control treatment was most similar to the penicillin treatment (Fig. 4.21c,g). At the intertidal sandflat, streptomycin supported maximum diatom abundance (Fig. 4.13b), generic richness and diversity in the monsoon period (Fig. 4.20b,j) and was the most dissimilar treatment (Fig. 4.21c). At the port site, the benthic diatom communities in the control

and antibiotic treatments showed a higher degree of similarity (Fig. 4.21i-l). The control and streptomycin treatments were similar throughout the sampling period, except the pre-monsoon (Fig. 4.21i-l). During the pre-monsoon period, the control treatment was similar to both penicillin and streptomycin treatments (Fig. 4.21j).

4.4. Discussion

Both f/2(MBL) and f/2(ASW) supported similar diatom abundance at the intertidal sandflat and mangrove site (Figs. 4.8, 4.10). At the port site, diatom abundance was higher in f/2(ASW) (Fig. 4.10c) compared to f/2(MBL) (Fig. 4.8c). Similarly, at the mangrove and port sites, f/2(ASW) supported a higher number of diatom species compared to f/2(MBL) (Tables 4.3, 4.4). However, considering the seasonal variation in the composition of the Aged Sea Water (ASW), the use of artificial seawater (MBL) is a better option.

As mentioned earlier, the antibiotics used have different modes of action. Penicillin inhibits cell wall synthesis in mainly Gram positive bacteria (Josephson et al. 2000). Therefore, diatoms, with a different cellular structure are not directly affected by penicillin. Chloramphenicol is a known inhibitor of PS-II in diatoms (Ohad et al. 1984), whereas streptomycin, which inhibits protein synthesis by binding to the 30S ribosomal subunit (Josephson et al. 2000), has the potential to inhibit diatoms directly.

Overall, the stability of these antibiotics in f/2(MBL) and f/2(ASW) was lower in presence of silty sediment from the mangrove site compared to in presence of sandy sediment from the intertidal sandflat (presented in Chapter 3). Even then, the residual antibiotic activity was sufficient to suppress bacteria by 71-100% at the mangrove

site, compared to 99-100% at the intertidal sandflat and port site (Table 4.9). The effectiveness of the antibiotics used was also obvious in the shift in dominant culturable bacteria in the different antibiotic treatments, compared to control conditions (Tables 4.5 - 4.7).

Among all the antibiotics used, the shift in diatom community structure in the penicillin treatment compared to control conditions, clearly indicates the role of bacteria in influencing diatom community dynamics. Penicillin G inhibits mainly Gram-positive bacteria (Josephson et al. 2000), which are surprisingly diverse in coastal ecosystems (Holt et al. 1994). Gram-positive bacteria were originally thought to comprise a minor fraction (5%) of bacterial communities in the ocean (Zobell 1946). However, recent research involving the use of low-nutrient media (Stevenson et al. 2004, Gontang et al. 2007, Stevens et al. 2007), has revealed that the abundance and diversity of Gram-positive bacteria in sediments may be considerably greater. They play prominent roles in coastal ecosystems. They have the ability to degrade various biopolymers (Gontang et al. 2007, Stevens et al. 2007) and are prolific producers of bioactive metabolites (Berdy 2005). In view of the results from this study, it is possible that this group of bacteria play a significant role.

At the intertidal sandflat, the most striking change in diatom community structure associated with suppression of bacteria by penicillin was observed in the pre-monsoon period; the diatom community was dominated by *Thalassiosira*, a centric diatom (Fig. 4.14a). Apparently, most of the pennate diatoms were inhibited (Fig. 4.14a), leading to favourable conditions for *Thalassiosira* to dominate the community. *Thalassiosira* is an important member of the microphytobenthic community in the study area (Mitbavkar and Anil 2002). It has a tychopelagic origin,

and has been reported to be viable upto 15 cm depth in the study area (Mitbavkar and Anil 2006). In temperate environments, *Thalassiosira* dominates spring blooms (Durbin 1974, Muylaert and Sabbe 1996, Popovich and Gayoso 1999). In tropical environments, *Thalassiosira* blooms were observed in non-monsoon periods, especially following inputs of nitrate (Patil and Anil 2008). The results of this study add a new dimension to studies on diatom-bacterial interactions, i.e., the dynamics of this important blooming diatom is also mediated by interactions with bacteria.

However, the restriction of *Thalassiosira* dominance to only the pre-monsoon period points to the important role of the interacting diatom and bacterial communities, mediated by seasonal environmental variations. In the pre-monsoon period, the microphytobenthic community had the highest generic evenness and diversity (Fig. 4.20d,e). The interactions of the constituent diatoms, influenced by the accompanying changes in the bacterial community, resulted in conditions favourable for the dominance of *Thalassiosira*.

At the mangrove site, the penicillin treatments in all the sampling periods except the monsoon, clustered together in the temporal cluster (Fig. 4.19b). However, *Navicula* was enhanced throughout the study period whereas *Amphora* was consistently suppressed (Fig. 4.17a). Comparatively, the port site showed a large variation in the response of the diatom community to bacterial suppression by penicillin, from total inhibition to enhancement (Fig. 4.13a).

Compared to the results obtained with penicillin, the direct effects of antibiotics (chloramphenicol) and the combination of potentially direct and indirect effects (streptomycin) elicited distinctly different responses from the diatom communities. Streptomycin inhibited diatoms in the mangrove and port sites throughout the study

period (with the exception of the monsoon period at the mangrove site) (Fig. 4.13b). At the intertidal sandflat, diatoms were enhanced (compared to control conditions) on most occasions (Fig. 4.13b). Similar results were obtained in an additional study (Chapter 3) at the intertidal sandflat, wherein erratic enhancement/inhibition of diatoms was observed in the streptomycin treatments at different concentrations. Additionally, in the present study, bacteria were suppressed from 71 ~ 100% (Table 4.9), and *Alcaligenes* and *Pseudomonas* consistently dominated in the streptomycin treatments at the intertidal sandflat (Table 4.5). From this, it can be inferred that either (1) streptomycin may not have affected diatoms directly at the concentration used, or (2) the bacteria suppressed by streptomycin may not be important for diatom growth; in fact, they may be repressing diatom growth, when present. These results were also corroborated by the observation of the highest diversity of diatoms in the streptomycin treatments compared to the other antibiotic treatments (Fig. 4.20j), and the similarity of the diatom communities in the streptomycin and control treatments on cluster analysis, except in the monsoon period (Fig. 4.21a-d).

Chloramphenicol, that represented the direct effect of antibiotics, inhibited diatoms as expected, in all the three sites (Fig. 4.13c). At the mangrove site, all the centric diatoms, except *Melosira*, were inhibited (Fig. 4.17c). No such difference in the sensitivity of centric and pennate diatoms to chloramphenicol was observed at the intertidal sandflat and port site (Figs. 4.14c, 4.18c).

It is apparent that the resident diatom communities at the 3 sites showed site-specific responses to not only the suppression of bacteria by penicillin, but also to the other antibiotic treatments. These variations in the emergence characteristics of diatoms in the different antibiotic treatments across the sites could be due to the

differences in the initial diatom and bacterial communities, which depends on the habitat characteristics of the sites (diatom trends clear in the CCA plots - Fig. 4.6).

The sites differ in several characteristics. The intertidal sandflat had a sandy sediment texture (Table 4.1), lower nutrient concentrations (Figs. 4.3 – 4.5), and showed a characteristic reverse trend between diatom and bacterial dynamics in surface waters (Fig. 4.2a). Sandflats are generally more oligotrophic than silt-rich sediments (Heip et al. 1995), characteristic of the mangrove site. They experience turbulent wave action, which reduces the silt and clay fractions of sediment through erosion processes. Diatoms are eroded easily due to the occurrence of migratory ripples (Grant et al. 1986) and due to collision of sand grains (Delgado et al. 1991).

The mangrove site has a silt-rich sediment texture (Table 4.1), which due to its small size fraction (2-63 μm), tends to have low loss of diatoms resulting from abrasion. They have a high specific surface area, and thus diatoms have greater access to the organic-rich layer surrounding the individual silt particles. These factors are responsible for the high growth rate of diatoms in silt-rich sediments compared to sandy sediments (van de Koppel et al. 2001). This is reflected in the high abundance and generic number of the microphytobenthic diatom community at the mangrove site (Fig. 4.7a,c). In fact, a positive feedback may exist, in which diatoms decrease erosion of silt through EPS production, which in turn, leads to high diatom growth and survival (van de Koppel et al. 2001).

The port site had higher dissolved oxygen, salinity, pH and suspended particulate matter compared to the mangrove site (Figs. 4.4 – 4.5). It had a rocky-sandy texture, and was regularly dredged. In fact, when a van Veen grab was deployed, it was difficult to get even a 2 inch intact sediment sample. The absence of a suitable,

undisturbed substratum could be the reason for the occurrence of pennates in low numbers, and the resulting dominance by transient, tychopelagic centric diatoms. The range of responses to the different antibiotics varied, from total inhibition to enhancement, and differed in terms of effects on community composition, compared to the species-stable intertidal sandflat and mangrove site. Centric diatoms dominated on several occasions at the port site, whereas pennate diatoms were the key players at the intertidal sandflat and mangrove site (Figs. 4.15).

How do these site-specific differences reflect in the results of this study? And especially with regard to the effect of penicillin on the dynamics of *Amphora* and *Navicula* reported at all the 3 sites? *Navicula* dominated consistently under control conditions at the intertidal sandflat and mangrove site (Fig. 4.8a,b). But when treated with penicillin, the intertidal sandflat showed fluctuating dominance patterns (Fig. 4.14a) whereas the mangrove site showed consistent dominance of *Amphora* (Figs. 4.17a). The port site, which was largely centric-dominated, showed fluctuations in *Amphora* and *Navicula* populations in both the control and penicillin treatments (Fig. 4.18a). In fact, seasonal variations in total diatom abundance (in terms of percentage compared to control) in the penicillin treatments, were significant only at the port site (Table 4.8). A reverse trend was observed for percentage bacterial abundance (Table 4.8).

The intertidal sandflat and mangrove site are subjected to lesser anthropogenic pressures compared to the port site. Both have stable, pennate-dominated diatom communities, and a hidden repository of diatoms that can emerge when faced with a perturbation, in this case, the suppression of bacteria by penicillin and the effects of the other antibiotic treatments. These diatoms, which emerged in only the antibiotic

treatments, but not under the control conditions, were *Cerataulina*, *Melosira*, *Achnanthes*, *Amphiprora* and *Mastogloia* at the intertidal sandflat (Figs. 4.8a, 4.14), *Achnanthes*, *Cocconeis*, *Cylindrotheca*, *Diploneis* and *Surirella* at the mangrove site (Figs. 4.8b, 4.17), and *Ditylum*, *Helicotheca* and *Achnanthes* at the port site (Figs. 4.8c, 4.18). From this, it is clear that the diatom communities at the intertidal sandflat and mangrove site have a higher capacity to respond to perturbations (in this case, suppression of bacteria and the other antibiotic treatments) compared to that at the anthropogenically influenced port site. In other words, a stable diatom community is relevant in influencing diatom-bacterial interactions as well as the changes in diatom communities in response to the different antibiotics. However, it should be noted that the high generic number of the benthic diatom community in the mangrove site was not reflected in the generic richness values (Fig. 4.7c-d), calculated on the basis of the formula provided by Margalef (1958), as this formula takes into account the number of individuals of each genus.

In spite of all these differences between the sites, there were some common aspects to be considered. Firstly, the response of the diatom communities to the different antibiotics was apparently influenced by the habitat characteristics of the diatoms. The case of *Thalassiosira*, a centric, tythropelagic diatom, has already been discussed. Some other diatoms are considered here. *Achnanthes*, a monoraphid diatom, did not emerge under control conditions at all the 3 sites (Fig.4.8), but was observed in the streptomycin treatment (intertidal sandflat) (Fig. 4.14), streptomycin, chloramphenicol and the antibiotic combination treatment (mangrove site) (Fig. 4.17) and the chloramphenicol treatment (port site) (Fig. 4.18). What is notable here is that *Achnanthes* never occurred in the penicillin treatment at all the 3 sites. *Achnanthes* is

a common fouling diatom (Molino and Wetherbee 2008), that through the process of gliding, stalk formation and rafting, adheres strongly to surfaces (Molino and Wetherbee 2008, Wang et al. 2000). Its emergence in response to streptomycin, chloramphenicol and the antibiotic combination treatment, suggests that it may be present in the benthic community but appears only when the dominant diatoms are suppressed by antibiotics. However, its absence in the penicillin treatment suggests that bacteria (mainly the penicillin-sensitive fraction) may have a role in the attachment and growth of this diatom.

Another monoraphid diatom, *Cocconeis*, appeared to be sensitive to not only the suppression of bacteria by penicillin, but also to the other antibiotic treatments. However, this trend seemed to be site-specific and was restricted to the intertidal sandflat. It differed from *Achnanthes* in that it emerged under control conditions throughout the study period. *Cocconeis* has a cosmopolitan distribution, and is a widely reported epiphyte (Romero and Navarro 1999, Buric et al. 2004). Since *Cocconeis* grows tightly appressed to the substratum, it has been described as a 'tightly attached alga' (Cattaneo 1990), and is typically resistant to changes in currents (Silvester and Sleigh 1985). Even in the present study, *Cocconeis* has been observed adhered to sediment grains. Since *Cocconeis* needs to attach to a substratum before growth and multiplication, and since most surfaces will already have been 'conditioned' by bacteria, it can be assumed that the antibiotics interfere with this particular process required for *Cocconeis* attachment.

The requirement for bacteria was also apparent in the sensitivity of *Grammatophora* and *Thalassionema* to the penicillin and antibiotic combination treatments. *Grammatophora* was recorded only at the intertidal sandflat (Figs. 4.8a,

4.14). The only exception for *Thalassionema* was that it occurred in the penicillin treatment only during the monsoon period at the mangrove site (Fig. 4.17a). Both these diatoms are araphid pennates, and together with *Achnanthes* and *Cocconeis*, constitute the epipsammic fraction of the microphytobenthic diatom community. Epipsammic diatoms are usually attached to sand grains, and capable of limited movement, if not totally immobile (Round 1965, Round 1979). These diatoms appear to be the most sensitive to suppression of bacteria and also, streptomycin and chloramphenicol. The other group of diatoms, the epipelon, is represented by the biraphid diatoms, namely *Amphora* and *Navicula*. Epipellic diatoms can move actively in the surface layers of the sediment (Round 1965, Round 1979), and contribute to sediment stabilization through the production of mucopolysaccharides (Paterson 1989). They have a higher chance of escaping unfavourable conditions/gradients. These characteristics could explain the comparatively lower sensitivity of these genera to the antibiotics used. Thus, the epipsammic diatoms appear to be more sensitive to suppression of bacteria, as well as antibiotics, compared to the epipellic diatoms.

Secondly, the diatom community in the monsoon period was resistant to not only the suppression of bacteria by penicillin, but also to all the antibiotic treatments. For e.g., at the intertidal sandflat, all the treatments had a ≤ 1 I:E ratio in the monsoon period (Fig. 4.16). Additionally, all the monsoon period treatments grouped together in the temporal cluster (Fig. 4.19a). The grouping of all the monsoon period treatments was also observed in the mangrove and port sites (Fig. 4.19b,c). At the mangrove site, the I:E ratio was ≤ 1 in the penicillin treatment during the monsoon period (Fig. 4.16a). However, the monsoon period was also associated with

enhancement of diatom growth (>100%) in the individual antibiotic treatments at the mangrove and port sites (Fig.4.13a-c).

During the monsoon period, a sizeable level of nutrients is injected into coastal water bodies, due to precipitation and run-off from terrestrial sources. Due to these factors, the monsoon period was characterized by turbulence and resuspension events, and also by lowered salinity and high nutrient concentrations. Under such conditions, erosion of the intertidal sandflat is enhanced due to the energy increase caused by wave action and currents (Rodrigues 1984). Such conditions favour resuspension of benthic diatoms into the water column, and could be responsible for the lowered abundance and generic richness of the microphytobenthic diatom community, and the increase in bacterial abundance at the intertidal sandflat. *Thalassiosira*, *Amphora*, *Cocconeis*, *Fragilariopsis* and *Navicula* were the only diatoms that survived the monsoon perturbation conditions (Fig. 4.8a). These particular 'robust' genera could possibly be responsible for the resilience of the monsoon diatom community to the different antibiotics (clear from the ≤ 1 I:E ratios) at the intertidal sandflat.

The monsoon conditions favoured the dominance of multiple genera at the mangrove and port sites (Fig. 4.8b,c). Such multispecific communities are most likely to have individuals that are able to survive and maybe, even thrive, under environmental perturbations (Duarte 2000). This could explain the resistance of the monsoon diatom community to the penicillin and other antibiotic treatments at these 2 sites. Overall, the resistance of the monsoon diatom community to the antibiotic treatments appears to be a robust trend, consistent across 3 distinctly different habitats.

Given that the strength of trophic linkages determines the response of a system to perturbations (Harrington et al. 1999), monsoonal effects are particularly relevant. In this context, it is imperative to consider the concept of environmental perturbations put forth by Bakun and Broad (2003) and extended by Irigoien et al. (2005). They suggest that environmental perturbations loosen some trophic links, resulting in the dominance of opportunistic species in the system. This has been already noticed for dinoflagellates (in Chapter 2), where changes in dinoflagellate groups were observed following two consecutive but dissimilar monsoon events. This occurred through the SW monsoon mediating changes in biological control mechanisms such as bacterial and copepod communities, which were ultimately reflected in the composition of the dinoflagellate community. Applying this to the present study, it can be inferred that the monsoon-driven changes in the bacterial community (Tables 4.5 – 4.7), modulate the diatom community that emerges, resulting in the dominance of the typical ‘robust’ diatom genera, in the monsoon period.

Summarizing the results, the effect of bacteria on diatom community structure, analyzed through the effects of penicillin, showed characteristic temporal variations at both sites. These patterns were distinct from the effects observed with the other antibiotics, mainly in terms of changes in species composition. The response of the diatom communities to suppression of bacteria by penicillin and the other antibiotic treatments, appeared to be influenced by the habitat characteristics of the constituent members, with the epipsammic diatoms (*Achnanthes*, *Cocconeis*, *Grammatophora* and *Thalassionema*) being the most sensitive. The temporal variations observed appeared to be site-specific. The mangrove site, with a stable, generically rich,

pennate-dominated community, showed consistency in dominance patterns in the different antibiotic treatments, compared to the intertidal sandflat and port site. Anthropogenic influences appeared to influence the responses of the diatom communities to the antibiotic treatments, as observed in the port site. These observations reveal the intricate dynamics between bacterial and benthic diatom communities, and highlight the effects of anthropogenic influences on them. The implications are wide-ranging, more so because firstly, bacterial communities are adaptive to perturbations in their surroundings. For example, bacterial assemblages in sediments respond extremely rapidly to an input of organic matter (Goedkoop et al. 1997). Secondly, recent research (Hibbing et al. 2010) has highlighted the profound impact of intra- and inter-species interactions between bacteria on competitive interactions in the environment. In fact, bacteria can confound allelopathic interactions between phytoplankton species through degradation of toxins (Hulot and Huisman 2004). These interactions are influenced by turbulence and mixing intensity (Hulot and Huisman 2004), conditions that can be related to the monsoon cycle in the present study. In a similar vein, the results of this study clearly demonstrate the potential for similar interactions on the community-scale in benthic environments and the modulations of these interactions by the monsoons, a consistent pattern observed across all the 3 study sites. In view of this, it is necessary to include interactions with bacteria in future studies on diatom communities, and their responses to monsoonal perturbations.