Eco-biology of marine diatoms with emphasis on the influence of physico-chemical parameters

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Dedicated to my parents

Contents

Statement

As required under the University ordinance 0.19.8 (vi), I state that the present thesis entitled "Eco-biology of marine diatoms with emphasis on the influence of physico-chemical parameters" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

Smita

Smita S. Mitbavkar

Certificate

This is to certify that the thesis entitled "Eco-biology of marine diatoms with emphasis on the influence of physico-chemical parameters", submitted by Ms. Smita S. Mitbavkar for the award of the degree of Doctor of Philosophy in Marine Science is based on her original studies carried out by her under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any Universities or Institutions.

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All the Corrections Suggested by the Referee is incorporated.

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Chapter 1
General Introduction

General Introduction

Diatoms (Greek – cut in two) belonging to the class Bacillariophyceae of the phylum Bacillariophyta are eukaryotic, autotrophic microorganisms with a cosmopolitan distribution. They are either solitary or colonial (occurring in chains). Size of individual diatom ranges from ~ 2 μm to 2 mm, but some chains can be several millimeters in length. Diatoms are ubiquitous phytoplankton widespread in both marine and freshwater, plankton and sediments. The most distinctive feature of this unicellular organism is its extracellular coat or frustule, which comprises of two overlapping valves, composed of silica (SiO_2) fitting into each other just like a soapbox. The upper half is known as the epitheca and the lower half as hypotheca. Each theca is further divided into two parts – the main surface and its incurved margins termed valve and connecting bands respectively. The two connecting bands represent incurved sides of the lid and the main body whereas the valve relates to the top or bottom of the box. When fitted together, the connecting band of epitheca overlaps that of hypotheca and the two bands remain united in the overlapping region called girdle, by a cementing organic substance present between them. Accordingly, a cell can be seen from two different aspects, the valve view and the girdle view. Most diatoms appear rectangular in girdle view whereas in valve view their shape is variable. The line connecting the middle of the two valves constitutes the pervalvar axis and the place along which the cell divides (at right angles to pervalvar axis) is called the valvar plane. The frustule is usually sculptured into patterns of spines, pores, channels, and / or ribs, which are distinctive to individual species. The ornamentations are confined to the valve position of the silica wall. The vast structural diversity of the frustule leads to a remarkable number of morphologically distinctive varieties of diatoms and forms a base for their identification. Depending on the shape

and symmetry, two types of diatoms are recognized: the pennate and centric forms. Pennate diatoms have elongated or "boat-shaped" valves with a bilateral symmetry. They are mostly benthic, but few planktonic genera may be abundant in the plankton. Centric diatoms have a radial symmetry and are much more common in the plankton. Along the length of its frustule, pennate diatoms have a long slit known as the raphe, which helps in their motility and attachment to substrata. Reproduction is usually by means of vegetative cell division i.e., binary fission (two new individuals formed within the parent frustule). Repeated cell division results in diminution of cell size. In order to regain the maximal size, the vegetative cell division is interrupted by sexual reproduction through auxospore formation.

Diatoms constitute the major part of the phytoplankton of the sea; they also exist as sessile communities or attached to debris, sand grains and mud. Their importance lies in the fact that they are primary producers and serve as a vital link in the aquatic food webs, either directly or indirectly and are thought to be responsible for up to 25% of the world's net primary productivity (Jeffrey and Hallegraeff 1990). To the fishing industry they are of paramount importance as at some stage of their life cycle, all fish, mollusks, bivalves and crustaceans are diatom feeders.

The term microphytobenthos refers to the microscopic, photosynthetic, eukaryotic organisms living in intertidal areas. This includes cyanobacteria and diatoms. Generally, diatoms are the major representatives of this community (Meadows and Anderson 1968; Round 1979b). Over the last few decades, there has been increased interest regarding the benthic diatoms that inhabit shallow coastal areas and their functional importance in benthic communities. They contribute significantly to the total primary production of the estuarine and other shallow water ecosystems. These diatoms are consequently an important carbon source for benthic heterotrophs and can

significantly affect the exchange of oxygen and nutrients across the sediment-water interface (Risgaard-Petersen et al. 1994).

Benthic epipelic diatoms are the most important group of primary producers in intertidal sand and mudflats (Meadows and Anderson 1968; Sullivan 1975; Round 1979a, b; Taasen and Hoisaeter 1981; McClatchier et al. 1982; Admiraal 1984). They are known to produce copious amounts of extracellular polymeric substances (EPS) mainly consisting of carbohydrates (Hoagland et al. 1993). By doing so, diatoms form a biofilm that serves to produce their own microenvironment, which protects them from the rapidly changing conditions in intertidal sediments (Decho 1994). The excretion of EPS plays a role in the movement of epipelic benthic diatoms (Edgar and Pickette Heaps 1983) and allows the organisms to adhere to sediment surfaces (Wang et al. 1997). The presence of biofilms helps in stabilization of the sediment against resuspension (Kornman and de Deckere 1998; Paterson 1989). Through the excretion of EPS, diatoms are responsible for a considerable input of high quality organic carbon into the sediment that may be utilized as a food source of heterotrophic consumers (King 1986; Van Duyl et al. 1999).

Intertidal sandflats are dynamic environments, where the tidally generated water movement and the associated processes of deposition and resuspension of sediment affect the composition and distribution of diatoms. In addition, hydrodynamic processes carry planktonic diatoms present in the ambient water to the intertidal sediment. In many studies, diatoms were only investigated in the top few centimeters of the sediment (Riznyk et al. 1978; Colijn and Dijkema 1981; Varela and Penas 1985; Lukatelich and McComb 1986). However, the presence of diatoms at a depth of 20 cm has also been reported (Steele and Baird 1968; Colijn and Dijkema 1981; de Jonge and Colijn 1994), based on chlorophyll *a* estimations.

Many free living diatom cells in intertidal sediments have diel rhythms of vertical migration, moving to the surface when the sediment is exposed at low tide and descending before it is flooded (Palmer and Round 1965; Round 1979a, b). This may serve to avoid transport due to resuspension by advancing tides (Faure-Fremeit 1951; Ganapati et al. 1959; Heckman 1985) and escape predation (Connor and Edgar 1982). Consequently, migration can have important consequences for measurements of both diatom abundance and photosynthesis (Pinckney and Zingmark 1993). However, these migrating cells are likely to dominate biomass and productivity in intertidal sediments. Several researchers have reported large differences in benthic diatom productivity in exposed vs. immersed sediment and have attributed these differences to vertical migration below the sediment photic zone during immersion (Pomeroy 1959; Darley et al. 1976; Holmes and Mahall 1982).

Much of the literature concerning vertical migration of benthic microalgae has centered on species inhabiting mud flats (Aleem 1950; Hopkins 1963; Round and Happey 1965; Round and Eaton 1966; Round and Palmer 1966; Palmer and Round 1967; Round 1978; Paterson 1986; Pinckney et al. 1994). In case of the sand flat community, diatom migration has been recorded by Meadows and Anderson (1968), Harper (1969), Riznyk and Phinney (1972), Round (1979a, b), Joint et al. (1982), and Kingston (1999).

The upper surface sediment layers are, in fact, characterized by strong physico-chemical gradients and the sediment perturbation caused by water movements may resuspend the benthic cells in the water column. When resuspended into the water column by wind mixing (Lukatelic and McComb 1986; Pejrup 1986; Demers et al. 1987) or tidal currents (Baillie and Welsh 1980; Shaffer and Sullivan 1988), benthic diatoms may be an important source of food for both micro and macroheterotrophs

(Roman and Tenore 1978; Wainright 1990; de Jonge and Van Beusekom 1992). The microphytobenthic diatoms are also an important source of food for surface deposit feeders (Pace et al. 1979), some of which are able to switch between surface deposit feeding and suspension feeding depending on the water flow velocity (Miller et al. 1992). The presence and importance of benthic diatoms as temporary members of the phytoplankton is a well-known phenomenon (Cadee and Hegeman 1974; Baillie and Welsh 1980; Demers et al. 1987; de Jonge and Van Beusekom 1992, 1995).

Once introduced into the water column, as a basic requirement these diatoms will be in search of a substratum for attachment. As a rule, a layer of microorganisms forming a biofilm quickly covers any surface submerged in water. Zobell (1943) observed that the initial phase in the biofilm development involves the adsorption of organic compounds over the solid surfaces exposed to marine environment. These surfaces, which act as nutrient sinks, enable diverse microbial communities to develop and maintain themselves at high population diversities (Marshall 1972). Diatoms are the earliest autotrophic colonizers and constitute much of the microbial biomass accumulated on an illuminated surface (Caron and Sieburth 1981; Marzalek et al. 1979). In Indian waters, work on fouling with special reference to diatoms has been done by Daniel (1955); Mathew and Nair (1981); Kelkar (1989); Bhosle et al. (1990a, b); Pangu (1993); Prabha devi (1995a) and Redekar (1997). Such communities termed as periphytic, can serve as food for the planktonic herbivores thus playing an essential role in food web dynamics. The attached diatom community also constitutes a system rich in information for environmental monitoring which can be exploited through analysis of communities' structural characteristics (Gold et al. 2002). In such communities, where space becomes a limiting resource, close interactions among the species inevitably results in competition. Competition occurs when two species

require a resource that is in short supply, so that the availability of that resource to one species is negatively influenced by the presence of the other species (Valiela 1984). Role of each species and their interaction with the other members will decide the fate of the climax community. In a homogenous habitat, species differ in their competitive abilities and are limited by a resource and compete for the same single resource. According to Tilman (1982, 1999) in such a habitat at equilibrium, the best competitor among the species present would win.

An important aspect of all the above studies is the microscopic identification of individual diatom species, depending on their morphological characteristics. However, it is subject to changes depending on the environmental and culture conditions. In the recent years, identification is being made simpler through molecular approaches. In this regard, immunofluorescence technique through the use of antibodies developed against the cell surface antigens of a target species is being employed extensively (Bates et al. 1993). In the dynamic marine environment, factors such as temperature and salinity also influence survival and dispersal thereby controlling phytoplankton communities.

Taking the above into consideration, this thesis presents work carried out from a tropical marine environment and covers different aspects of eco-biology of diatoms. The study includes

- **Diatoms of the benthic community**
- **Diatoms of the fouling community**
- **Modulations in the periphytic diatom diversity**
- **Morphological changes and detection of diatoms**

Chapter 2

Diatoms of the benthic community

2A.1 Introduction

Diatoms constitute an important part of the microphytobenthic community at intertidal sand flats. The diatom population here is usually composed of pennate diatoms, which are either epipsammic (attached to sand grains) or epipelic (motile forms within sediments). Intertidal sand flats are dynamic environments, where the tidal generated water movement and the associated processes of deposition and resuspension of sediment affect the composition and distribution of diatoms. In addition, hydrodynamic processes carry planktonic diatoms present in the ambient water to the intertidal sediment. These planktonic forms can be in either their vegetative or their resting stage, and can contribute to population dynamics. So far, studies on diatom populations have been restricted to the epipelic pennate diatoms, and those forms which reside, although temporarily, on or within the sediment grains have not received due attention. Diatom populations could, however, be better understood by taking into consideration both permanent residents and temporary visitors.

In many studies, diatoms were only investigated in the top few centimeters of the sediment (Riznyk et al. 1978; Colijn and Dijkema 1981; Varela and Penas 1985; Lukatelich and McComb 1986). However, the presence of diatoms at a depth of 20 cm has also been reported (Steele and Baird 1968; Colijn and Dijkema 1981; de Jonge and Colijn 1994), based on chlorophyll *a* estimations. In intertidal sand flats, a number of factors may be responsible for displacing the diatoms from the surface sediment layers to the deeper layers. Diatoms can retain photosynthetic capacity in the dark, deeper sediments and thus form an important pool of potential primary producers, which can resume photosynthesis if resurfaced (Fielding et al. 1988).

In the present study, sediment at the low, mid and high tide zones was investigated for the temporal variation in diatom abundance up to a depth of 15 cm. Chlorophyll *a* content and physico-chemical parameters such as wind speed, sediment characteristics, nutrients and suspended load were correlated with diatom abundance. Such a study will help gain insight into the dynamics of diatom populations, which form an important component of the microphytobenthic community, responsible for the littoral production and thus forming a basic link in intertidal food webs.

2A.2 Materials and methods

2A.2.1 Study area and sampling strategy

Sediment sampling was carried out on a sand flat at Dias Beach (15^0 27' N; 73^0 48' E), located near Dona Paula Bay and surrounded by the Mandovi and Zuari estuaries (Fig. 2A.1). This beach is about 200 m in length. The tides are semi-diurnal with an average

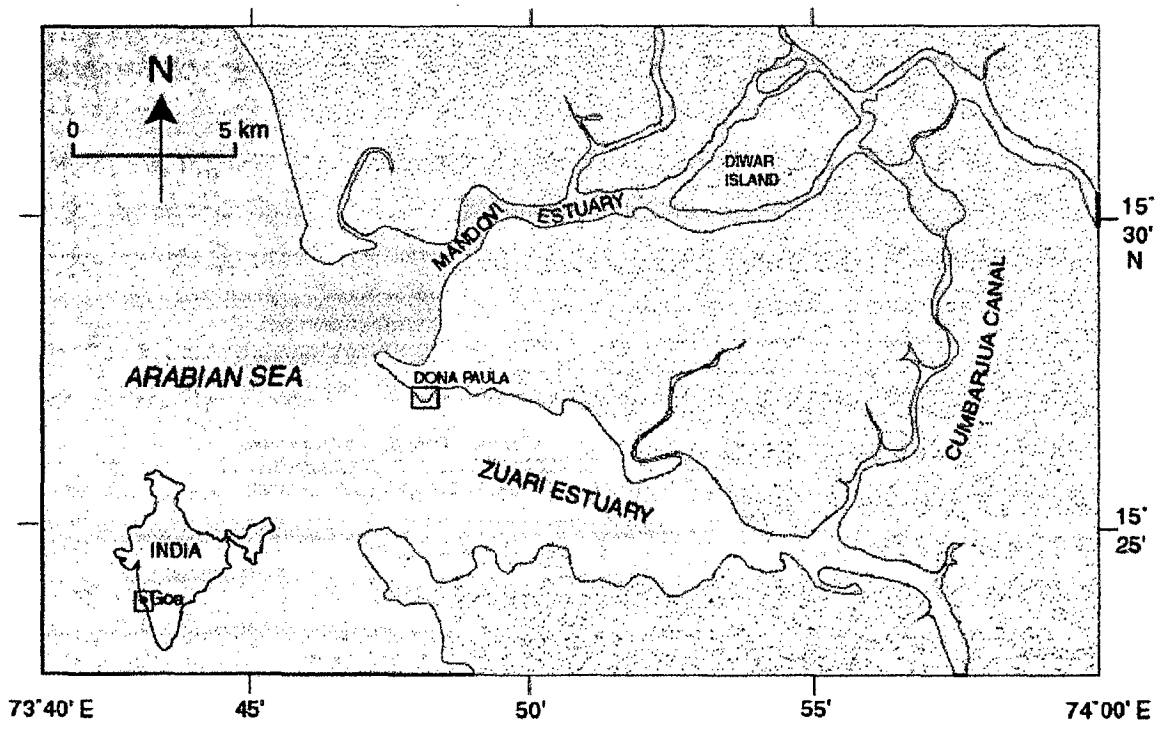


Fig. 2A.1 Geographical location of study

spring tide range of ~ 2 m and neap tide range of ~ 0.7 m. In this area, the wave heights are > 1.5 m during June-August and low (< 0.7 m) during October-April (Chandramohan et al. 1997). Dias Beach is sheltered and protected on both sides by rocky cliffs. This locality experiences three seasons: the pre-monsoon (February-May), the southwest monsoon (June-September) and the post-monsoon (October-January). The sediment sampling at the low tide zone was carried out on a monthly basis for 17 months (May 1998-September 1999) whereas at the mid and high tide zones was carried out every alternate month. Sediment samples were collected in triplicates during the lowest low tide, using a hand-corer with an inner diameter of 4.5 cm. The core length collected was 15 cm. Each core was divided into three sections of 5 cm intervals (0-5, 5-10 and 10-15 cm). Simultaneously, a separate sediment core of the same dimensions was collected for chlorophyll *a* and grain size analysis.

Interstitial water samples were collected by digging the sediment (~ 15 cm) with a shovel and allowing the water to collect. The water samples were allowed to stand for a few seconds while sand particles settled. Temperature of the surface sediment was recorded at the study site, and analysis of salinity (Mohr-Knudsen titration method) (Strickland and Parsons 1965) and nutrients such as nitrate (NO_3-N), nitrite (NO_2-N), phosphate (PO_4-P) and silicate (SiO_3) along with chlorophyll *a* (corrected for phaeopigments) were carried out by standard procedures (Parsons et al. 1984). Salinity and nutrient values were obtained for the entire 15 cm core, whereas chlorophyll *a* was estimated for each of the three core sections (0-5, 5-10 and 10-15 cm). Simultaneously, surf water samples were also collected from the surface at about 3 m from the low tide zone for the analysis of water parameters as mentioned above. A known volume of surf water (500 ml) was transferred to PVC bottles (triplicates) and

preserved with Lugol's iodine solution to estimate the diatom population following the method described by Hasle (1978).

2A.2.2 Enumeration of diatoms in the sediment

Since this study was carried out on a sandy beach and included the observation of attached forms, direct observation and quantification of samples posed a problem. Even if diatoms in their attached state could be observed directly, there was always a chance of missing cells attached to the other side of the grain. The ultrasonication technique was not of much help either, since some forms did not detach from the substrata and fragile forms were at risk of being ruptured, thus leading to false quantification results. Therefore, the extinction dilution method (most-probable-number method, MPN) (Imai et al. 1984; Yamochi 1989; Imai et al. 1990; Ishikawa and Tamaguchi 1994; Itakura et al. 1997) was employed for the quantification of the diatom flora in each section of the sediment core at the low, mid and high tide zones. Subsequent to incubation of the sediment samples, the appearance of centric and pennate diatoms was observed. The appearance of both these forms may have been from two sources; either they were the products of multiplication of vegetative cells or germination of resting stages.

An appropriate amount (1 to 2 g wet wt) of sediment sample was suspended in f/2 medium (Guillard and Ryther 1962) at a concentration of 0.1 g wet wt ml^{-1}. This stock was then subjected to a serial ten-fold dilution (10^{-1} - 10^{-5}) with the culture medium, and then 1 ml aliquots of diluted suspensions were inoculated into five replicate culture wells. Incubation was carried out at 27 ± 1 ^{0}C with a 12 h light: 12 h dark photocycle. The growth of diatoms in each culture well was examined microscopically after an incubation period of 6-8 days. The wells in which growth was observed were scored as positive. The MPN (for a series of 3 ten fold dilutions) of diatoms in the sediment

sample (MPN g^{-1} wet sediment) was then calculated according to a statistical table (Throndsen 1978). This table covers a range of five dilution steps, and a set of three dilutions have to be chosen out of the five cultured to get the MPN number. The diatom density per cubic centimeter wet sediment was obtained by multiplying the MPN value with the apparent specific gravity of the wet sediment (Imai and Itakura 1999). The diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyan (1946), Desikachary (1977,1987) and Tomas (1997).

2A.2.3 Grain size analysis

Sediment grain size at the low, mid and high tide zones was analyzed by dry sieving (Folk 1968). Categories of grain size included: >1,000 μm (very coarse sand); 500-999 μm (coarse sand), 250-499 μm (medium sand), 125-249 μm (fine sand), 63-124 μm (very fine sand) and < 63 μm (silt and clay).

2A.2.4 Data analysis

Univariate statistical methods

Univariate techniques included the calculation of the Shannon-Wiener diversity index (*H'*), richness (*H' max.* = $\log_2 S$, where S is the number of taxa) and evenness (*J'* = *H'/H' max.*) of the total diatom population (both benthic and planktonic) (Pielou 1969) at the low, mid and high tide zones.

Multivariate statistical methods

The log (X + 1)- transformed data on the relative abundance of diatoms in the intertidal sediment at the low, mid and high tide zones was used for the construction of a lower triangular dissimilarity matrix using Bray-Curtis coefficients (Bray and Curtis 1957). This dissimilarity matrix was then subjected to clustering and ordination analysis. Clustering was performed using the weighted pair group average method (Pielou 1984). Ordination was by non-metric multidimensional scaling (NMDS)

(Kruskal and Wish 1978). There are advantages in applying more than one method, since each is based on different assumptions and may give different insights (Gray et al. 1988). In case of the cluster analysis: (1) the individual, once placed in a group, loses its identity (2) the sequence of individuals is arbitrary and (3) only the inter-group relationships are shown. In view of these disadvantages, it is advisable to employ an additional method of presentation to show individual relationships such as NMDS (ordination).

The log (X + 1)- transformed data on diatom abundance in the water column was subjected to clustering by squared Euclidean distance and the group average method (Pielou 1984).

A two-way analysis of variance (ANOVA) (Sokal and Rohlf 1981) was conducted on the log (X +1) transformed relative abundance data of each diatom, at the low, mid and high tide zones, in order to test the significance of month and depth. The diversity, richness and evenness values of the diatom assemblages were also subjected to two-way ANOVA to evaluate the temporal (month) and spatial (depth) variations.

Multiway ANOVA (MANOVA) without replication (Sokal and Rohlf 1981) was performed on the arcsine-transformed grain size values (sediment composition) at the low, mid and high tide zones, in order to assess whether any significant variance existed with respect to different sampling periods (months) and depths.

Diatom relative abundance and the arcsine-transformed grain size data for the different depths and tidal zones as well as the log (X + 1)- transformed data on water parameters (temperature, salinity and nutrients) were subjected to multiple regression analysis.

Regression was performed on the log (x+1)- transformed data on sediment chlorophyll *a* (linear function), wind speed and suspended load (power function) versus diatom abundance.

2A.3 Results

2A.3.1 Environment

Surf water temperature ranged between 20 ^{0}C and 31 ^{0}C whereas that of sediment surface ranged between 21 ^{0}C and 30 ^{0}C respectively. Salinity was as low as 10 psu in the monsoon, while during the other seasons it ranged between 28 and 35 psu. The nutrient concentrations in the surface and interstitial waters showed a similar trend. Nitrate concentration ranged between 0.08 and 12 µM and was maximum in monsoon

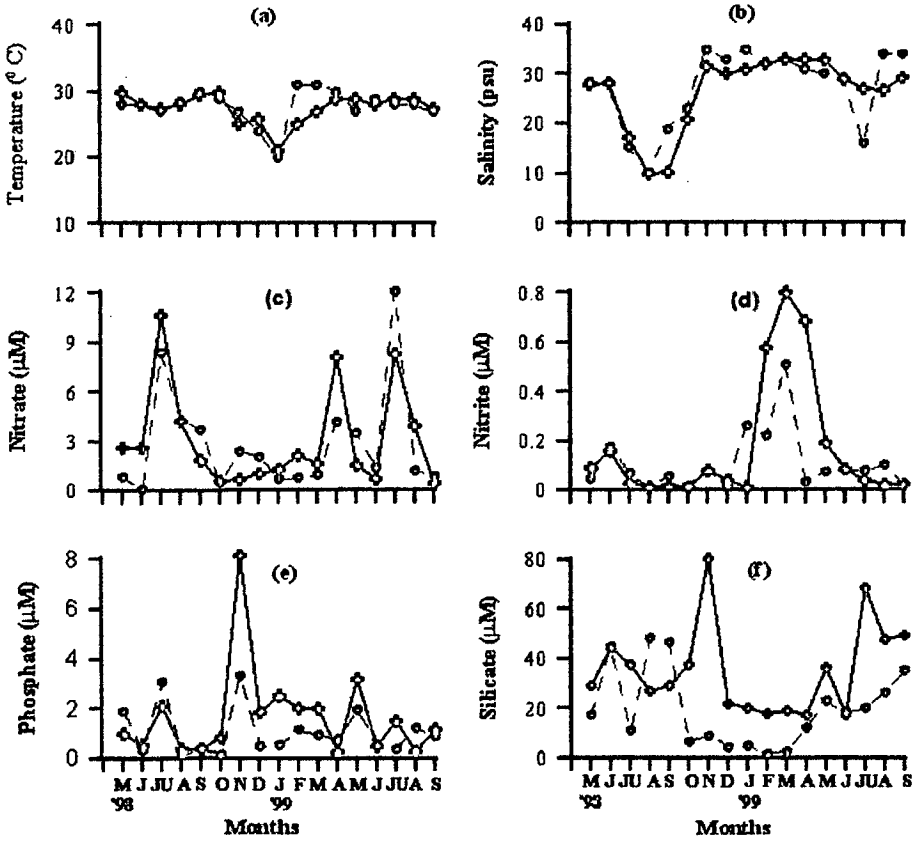


Fig. 2A.2 Temporal variations in environmental parameters of surf waters and interstitial waters
(a) Temperature; (b) Salinity; (c) Nitrate (NO_3-N); (d) Nitrite (NO_2-N); (e) Phosphate (PO_4-P) and (f) Silicate (SiO_3)
Interstitial waters — — Surf waters

with a peak in July, which was followed by a bloom of *Skeletonema costatum* in the ambient waters. Nitrite ranged between 0.013 and 0.8 µM being highest in premonsoon (March). Premonsoon and postmonsoon seasons experienced a rise in the phosphate concentration in the surf and interstitial waters. The surf water silicate concentrations were generally highest during the monsoon whereas in interstitial waters it was found to be above 17 µM throughout the investigation period (Fig 2A.2). The observed low salinity, high nitrate and silicate concentrations were due to high land runoff during the monsoon season.

2A.3.2 Diatoms in surf waters

In the water column, 34 species of diatoms belonging to 27 genera were encountered (Table 2A.1a). Of these, 19 were centric and 15 pennate. *Navicula delicatula* was found to be the most abundant diatom species.

Clustering of this diatom community at 50% dissimilarity level revealed *Navicula delicatula* to be the most dissimilar diatom in terms of distribution.

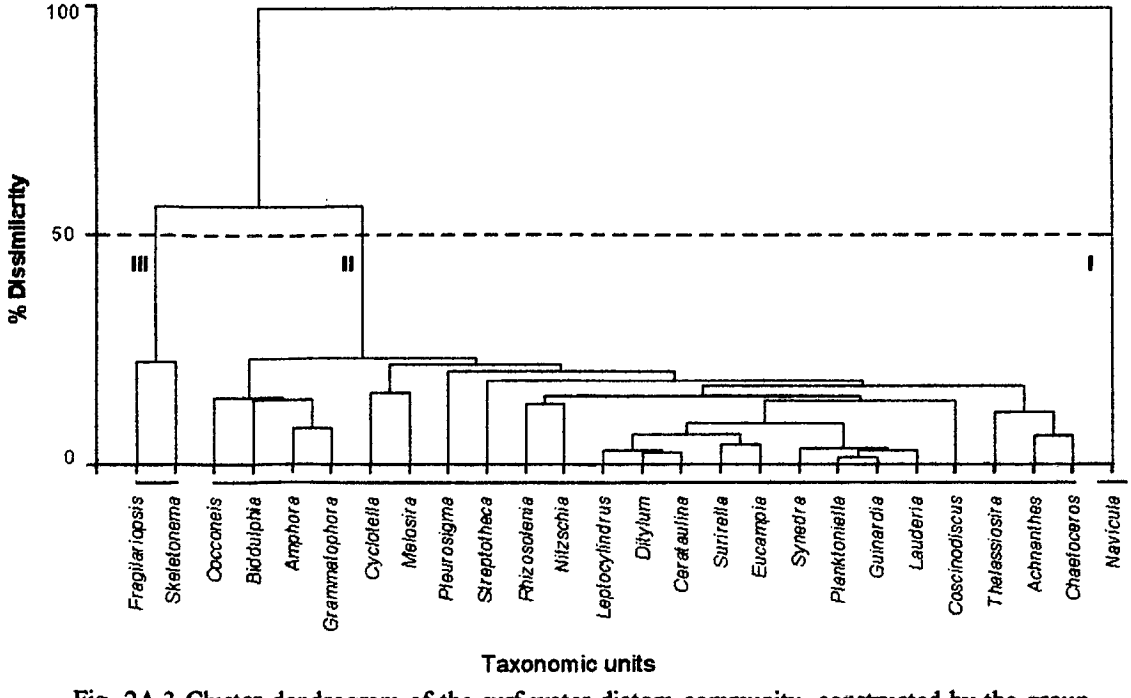


Fig. 2A.3 Cluster dendrogram of the surf water diatom community, constructed by the group average method

Table 2A.1. Diatoms recorded in (a) surf waters, (b) low tide zone, (c) mid tide zone, and (d) high tide zone of intertidal sediment (0-15 cm) during the study period

	a	b	c	d
Centric diatoms				
Bellerochea malleus (Brightwell) Van Heurck		+	+	+
Biddulphia mobiliensis (Bailey) Grunow	+	+	+	+
Biddulphia sinensis (Greville) Grunow	+	+	+	+
Cerataulina pelagica H. Peragallo	+			
Chaetoceros curvisetus Ehrenberg	+	+		
Chaetoceros diversus Ehrenberg	+	+		
Coscinodiscus concinnus Wm. Smith			+	
Coscinodiscus marginatus Ehrenberg	+	+	+	+
Cyclotella sp.	+		+	+
Ditylum brightwellii (West) Grunow	+			
Eucampia zodiacus Ehrenberg	+			
Guinardia flaccida (Castracane) Peragallo	+			
Lauderia borealis Cleve	+			
Leptocylindrus danicus Cleve	+			
Melosira nummuloides C.A. Agardh	+	+	+	+
Planktoniella sol (Wallich) Schutt Peragallo	+			
Rhizosolenia alata Brightwell	+			
Rhizosolenia sp.	+			
Skeletonema costatum Greville	+	+		
Helicotheca tamesis Shrubs	+	+	+	+
Thalassiosira eccentrica (Ehrenberg) Cleve	+	+	+	+
Thalassiosira sp1		+	+	+
Thalassiosira sp2		+	+	+
Pennate diatoms				
Achnanthes longipes Agardh	+			
Achnanthes subsessilis Kutzing			+	+
Amphora coffeaeformis (Agardh) Kutzing	+	+	+	+
Amphora rostrata Wm. Smith	+	+	+	+
Amphora sp.	+	+	+	+
Cocconeis scutellum Ehrenberg	+	+	+	+
Cymbella sp.			+	+
Fragilariopsis cylindrus (Grunow) Cleve	+	+	+	+
Grammatophora marina Kutzing	+	+	+	+
Navicula crucicula (Wm. Smith) Donkin			+	
Navicula transitans var. *derasa* f. *delicatula* Heimdal	+	+	+	+
Navicula subinflata Grunow	+	+	+	+
Navicula sp.	+	+	+	+
Nitzschia closterium (Ehrenberg)			+	+
Pinnularia sp.			+	+
Pleurosigma angulatum Sensu W. Smith	+	+	+	
Pseudonitzschia seriata Cleve	+	+	+	+
Raphoneis sp.			+	+
Surirella sp.	+			
Synedropsis gaillonii Grunow	+			
Tabellaria sp.			+	+
Thalassiothrix nitzschioides (Grunow) Mereschkowsky	+	+	+	+

Clustering revealed 3 main groups (Fig. 2A.3). The first group comprised of *Navicula*. The third group was represented by *Skeletonema costatum* and *Fragilariopsis cylindrus*, which dominated blooms in the ambient waters during August and

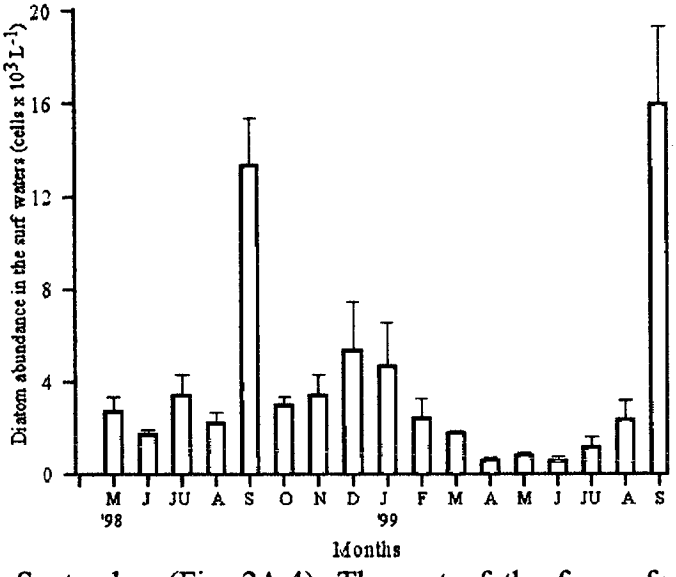


Fig. 2A.4 Temporal variations in total diatom abundance of the surf water

September (Fig. 2A.4). The rest of the forms formed the members of the second group.

2A.3.3 Low tide zone

Sediment characteristics: Very fine sand (63-124 μm - silt/clay) was found to be the most abundant grain size fraction of the sediment throughout the study period across the 15 cm core (Fig. 2A.5). The contribution of sediment particles > 250 μm was negligible.

MANOVA of different months versus grain size and depth versus grain size revealed

Table 2A.2. MANOVA (Without replication) for the temporal variation in the sediment characteristics along the vertical gradients at the low tide zone (0-5, 5-10 and 10-15 cm sediment core sections) (* $p \leq 0.025$; NS = not significant)

Source of variation	*df*	SS	MS	Fs
Months	16	27.76	1.73	
Core sections	2	106.72	53.35	
% Grain size fractions	5	70,930.88	14,186.18	
MonthsXCore sections	32	914.22	28.57	0.787^{NS}
Months X % Grain size fractions	80	5,361.15	67.01	1.845*
Core sections X Grain size fractions	10	749.1	74.91	2.063*
Months X Core sections X % Grain size fractions	160	5,810.90	36.32	

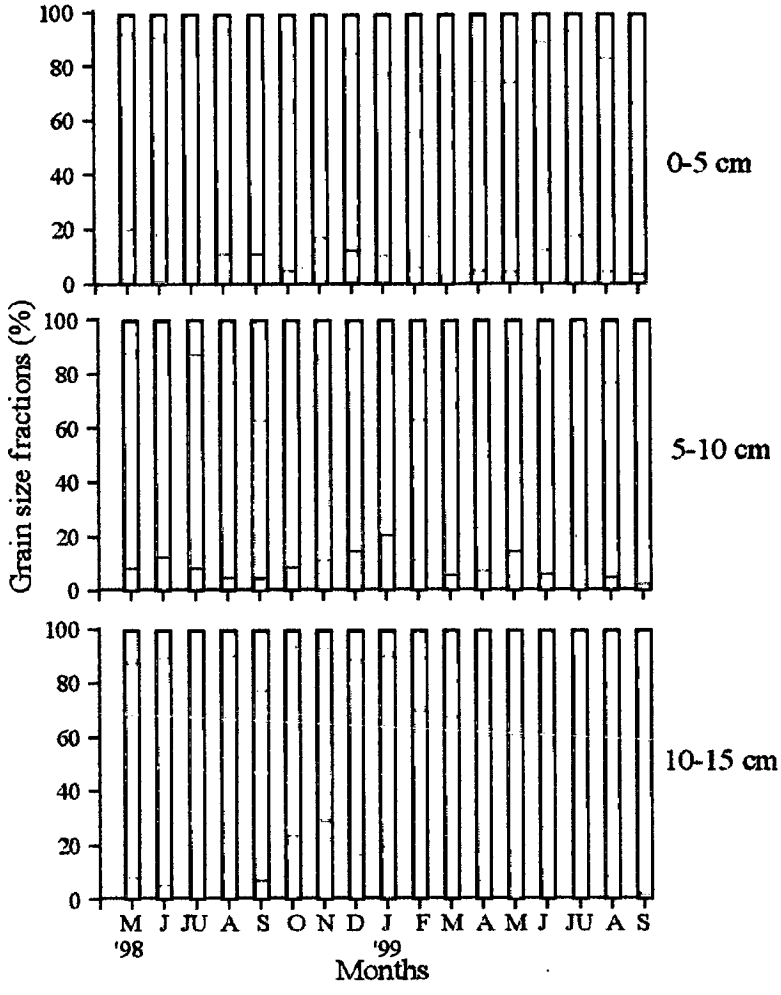


Fig. 2A.5 Temporal variations in grain size fractions (> 1000 □, 500-999 □, 250-499 □, 125-249 □, 63-124 □ and < 63 □ μm) across the vertical gradient of the core

a significant variation (Table 2A.2). Across the three sections, the percentage of < 63 μm grain size fraction peaked on two occasions. One peak was observed towards the end of monsoon (September to October) while the other was evident during February to April (Pre-monsoon). During these periods, a simultaneous decrease in the percentage of 63-124 μm grain size fractions was observed.

Diatoms in sediment: In the sediment, 24 species of diatoms (12 centric, 12 pennate) belonging to 16 (8 each of pennates and centrics) genera were recorded (Table 2A.1b). Pennate diatoms were dominant in terms of abundance (Fig. 2A.6). The most abundant pennate diatoms were *Amphora* and *Navicula*; *Thalassiosira* was the abundant centric diatom.

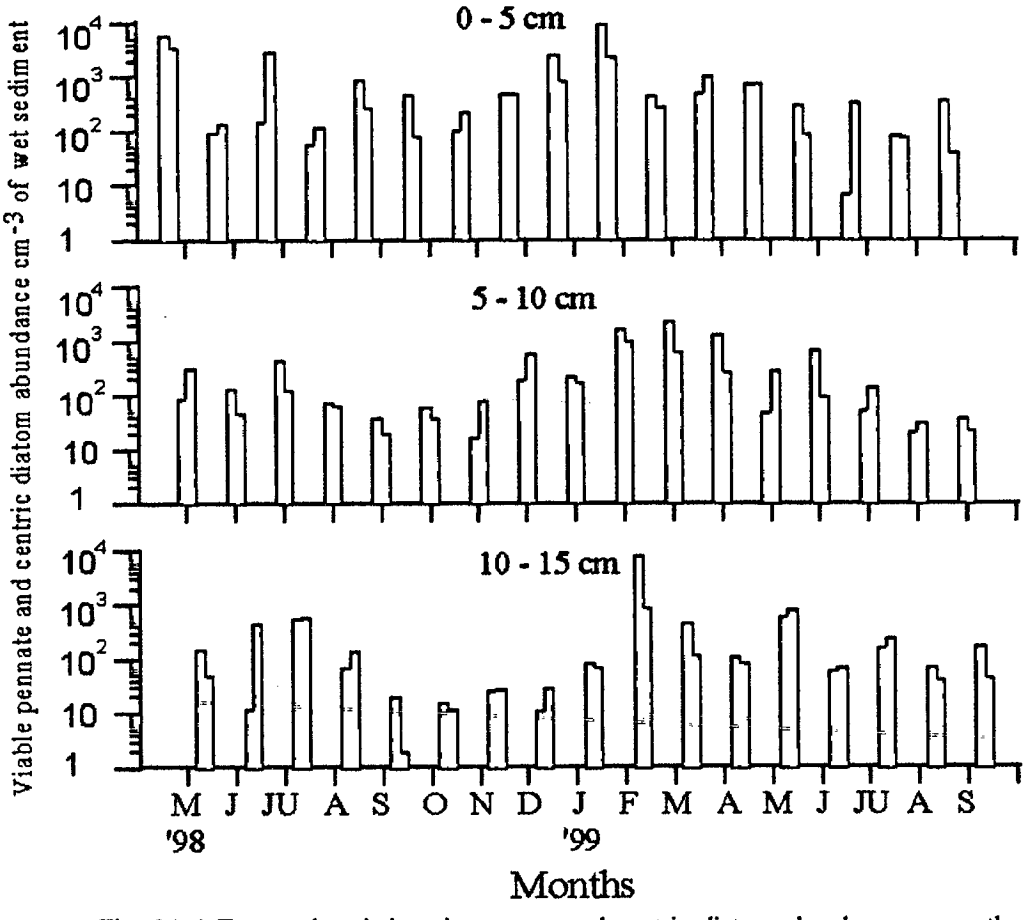


Fig. 2A.6 Temporal variations in pennate and centric diatom abundance across the vertical gradient of the core

□ Pennate diatoms; □ Centric diatoms

Diatoms encountered in this area can be divided into three groups based on their abundance; *Amphora*, *Navicula* and *Thalassiosira*, which were present throughout the period of investigation, formed the first group (Fig 2A.7a). The second group comprised forms that were frequently encountered in the sediment and included eight genera, of which four were centric diatoms (*Biddulphia*, *Melosira*, *Streptotheca* and *Coscinodiscus*) and four were pennates (*Grammatophora*, *Cocconeis*, *Nitzschia* and *Thalassiothrix*) (Fig. 2A.7b). The diatoms appearing rarely formed the third group and included *Pleurosigma*, *Fragilariopsis*, *Skeletonema*, *Chaetoceros and Bellerochea.*

Temporal and Vertical distribution of diatoms in the sediment: The total diatom abundance varied from one sampling period (month) to another, but was found to peak during the pre-monsoon season (Fig. 2A.8). Diatoms belonging to group I (*Amphora, Navicula* and *Thalassiosira*) and group II (*Biddulphia, Melosira, Coscinodiscus, Streptotheca, Cocconeis, Thalassiothrix, Nitzschia* and *Grammatophora*) were abundant in the pre-monsoon period, whereas diatoms of group III (*Pleurosigma, Fragilariopsis, Skeletonema, Chaetoceros and Bellerochea)* were abundant during the monsoon and post-monsoon seasons.

The temporal variation in abundance of the diatoms across the three individual core sections did not reveal any particular trend. The group I diatoms (*Amphora, Navicula* and *Thalassiosira*) were found to peak in February across the three core sections. The

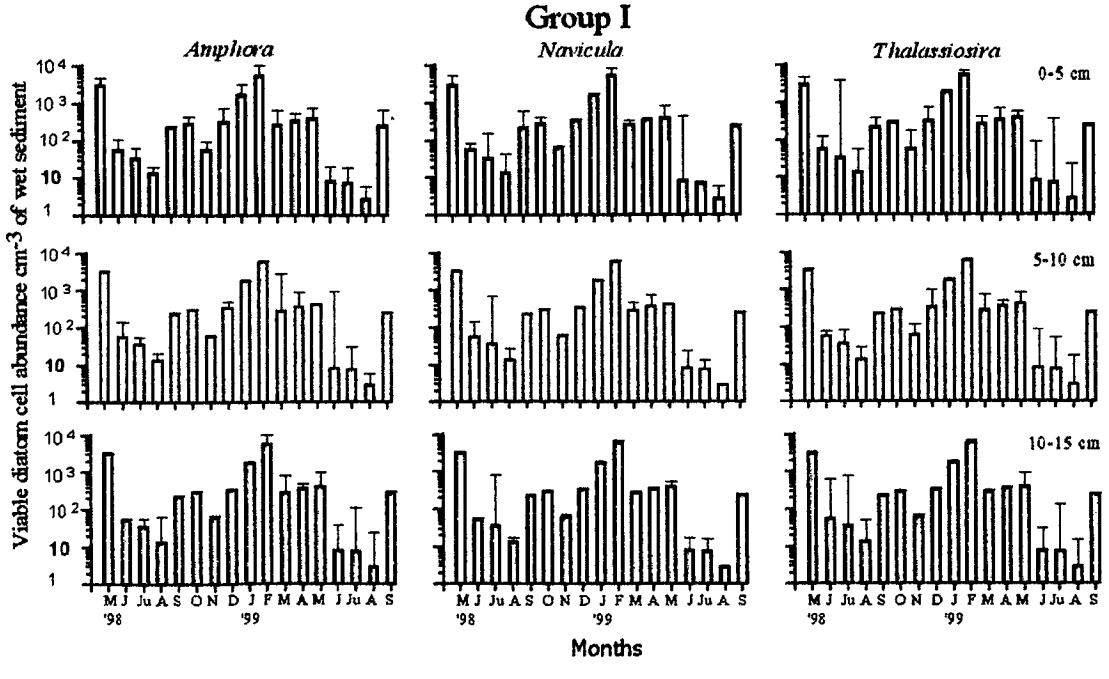


Fig. 2A.7a Temporal variation in diatom abundance across the vertical gradient of the core for group I

distribution of *Amphora* kept a lower profile during the monsoon period, while *Thalassiosira* formed a trough from August to October. *Biddulphia* (group II) peaked during February-March and was not encountered in September down to 15 cm depth.

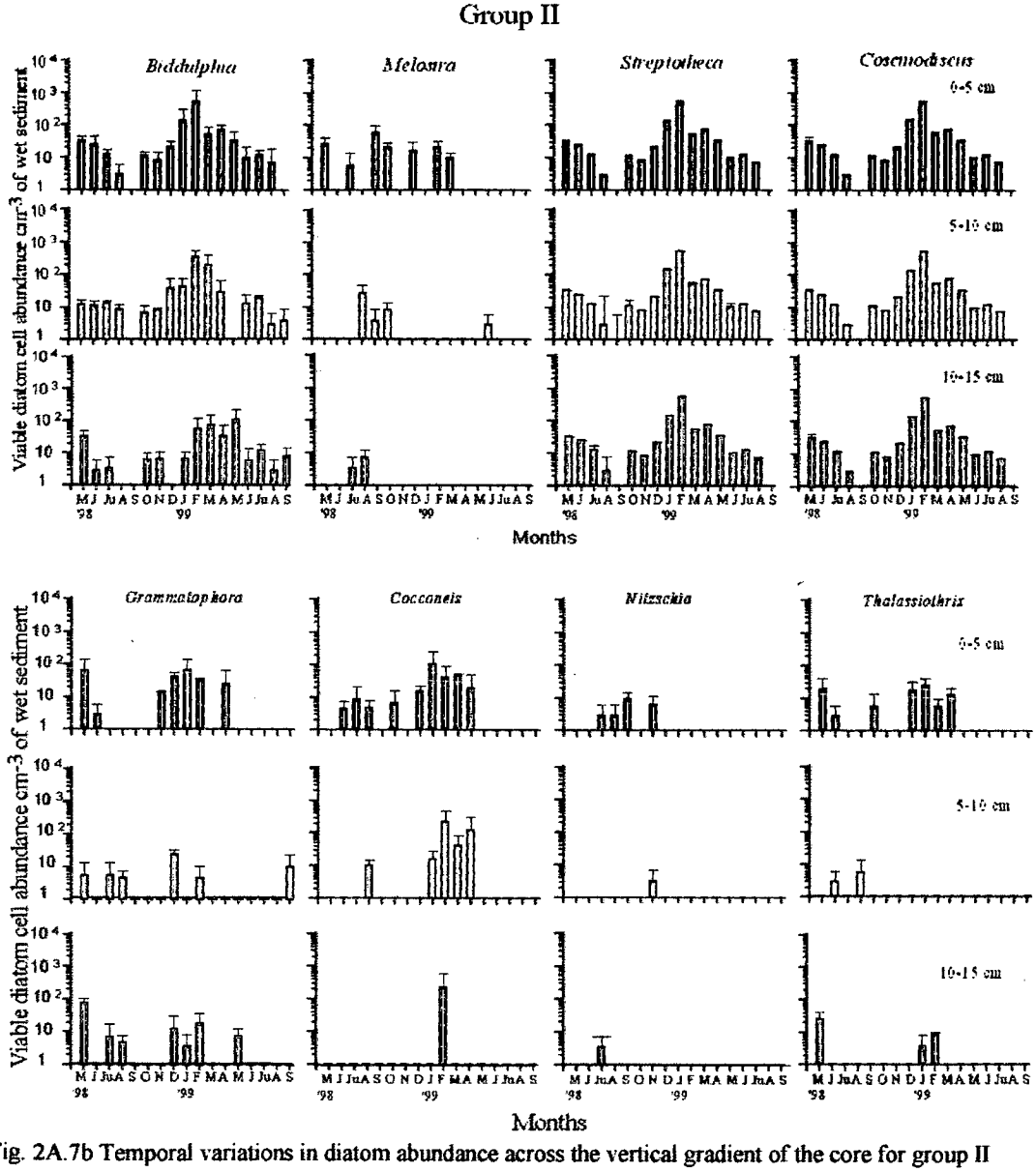


Fig. 2A.7b Temporal variations in diatom abundance across the vertical gradient of the core for group II

Amongst the group III diatoms, *Bellerochea* was present only in the 0-5 cm stratum (May and June), while *Chaetoceros* was found at 0-5 cm (May and August) and at 10-15 cm (August) depths. *Pleurosigma* was encountered on only one occasion (January) in the 0-5 cm stratum, while at 5-10 cm it was observed during January and August.

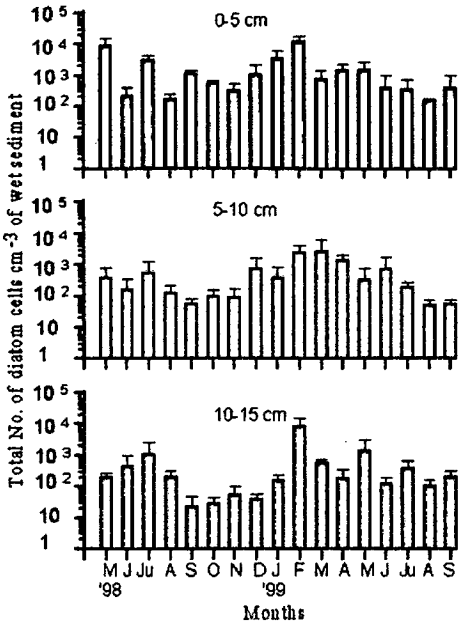


Fig. 2A.8 Temporal variations in the total diatom abundance across the vertical gradient of the core at low tide

Thus, the diatom data when subjected to two-way ANOVA revealed significant variation with respect to months ($P \leq 0.001$), depths (except for *Skeletonema* and *Streptotheca*) and also the month X depth interaction (except for *Skeletonema*) (Table 2A.3).

Table 2A.3 Significance values of the two way ANOVA evaluating the variations in diatom abundance with respect to months and core sections (0-15 cm) (NS = not significant)

Diatoms in the intertidal sediment	Month $p \leq$	Core section $p \leq$	Month x Core section $p \leq$
Navicula	0.001	0.005	0.05
Amphora	0.001	0.005	0.001
Grammatophora	0.001	0.005	0.001
Pleurosigma	0.001	0.025	0.001
Nitzschia	0.001	0.01	0.001
Thalassiothrix	0.001	0.001	0.001
Cocconeis	0.001	0.001	0.001
Fragilaria	0.001	0.005	0.001
Bellerochea	0.001	0.001	0.001
Thalassiosira	0.001	0.001	0.005
Biddulphia	0.001	0.001	0.001
Coscinodiscus	0.001	0.005	0.001
Melosira	0.001	0.001	0.001
Skeletonema	0.001	NS	NS
Streptotheca	0.001	NS	0.001
Chaetoceros	0.001	0.005	0.001

Diatom diversity was found to decrease drastically with a low evenness value in July at the 0-5 cm depth, as *Thalassiosira* dominated the community. During the other

Table 2A.4 Two way ANOVA for the diatom (a) diversity (b) generic richness and (c) evenness of the sediment with respect to months and core section (* = $p \leq 0.001$; ** = $p \leq 0.005$; *** = $p \leq 0.01$)

	df	SS	MS	Fs
(a)				
Months	16	23.58	1.47	5.28*
Core Section	2	3.29	1.64	5.88**
Months X Core Section	32	24.15	0.75	2.7*
Within subgroup error	102	28.5	0.28	
Total	152	79.52		
(b)				
Months	16	21.04	1.31	11.41*
Core Section	2	6.15	3.07	26.7*
Months X Core Section	32	24.6	0.77	6.67*
Within subgroup error	102	11.76	0.11	
Total	152	63.54		
(c)				
Months	16	1.39	0.087	2.81*
Core Section	2	0.3	0.15	4.81***
Months X Core Section	32	2.07	0.065	2.10**
Within subgroup error	102	3.15	0.03	
Total	152	6.9		

Table 2A. 5 Values of species diversity(*H'*), species richness (*H'* max.) and evenness (*J'*) of the diatom community at the low tide zone (Shannon-Wieners diversity index)

	0-5 cm			5-10 cm			10-15 cm		
MONTHS	*H'*	*H'*max	*J'*	*H'*	*H'*max	*J'*	*H'*	*H'*max	*J'*
May '98	1.71	3.32	0.52	1.15	2.32	0.49	2.4	2.81	0.85
June	2.12	3	0.71	1.85	2.32	0.8	0.24	2	0.12
July	0.41	3	0.14	1.24	2.58	0.48	1.29	2.81	0.46
August	2.22	3	0.74	3.03	3.32	0.91	2.49	3	0.83
September	1.76	2.81	0.62	1.87	2.32	0.8	1.07	1.58	0.67
October	1.7	2.81	0.6	2.29	2.58	0.88	1.89	2	0.94
November	1.51	2.58	0.58	1.26	2.32	0.55	1.85	2	0.92
December	2.1	3.17	0.66	1.31	2.32	0.56	0.85	1	0.85
January '99	2.02	3.17	0.64	2.7	2.81	0.96	2.1	3	0.71
February	1.74	3.32	0.52	2.37	3	0.79	1.31	3	0.44
March	2.3	3.17	0.72	1.33	2.81	0.47	1.09	2.32	0.47
April	1.78	2.81	0.63	2.07	3	0.7	1.39	1.58	0.88
May	1.75	2.32	0.75	0.7	1.58	0.44	2.11	2.58	0.82
June	1.1	2.32	0.48	0.8	2.58	0.31	1.9	2.32	0.82
July	0.35	1.58	0.22	1.76	2.32	0.76	1.7	2.32	0.73
August	1.54	2.58	0.6	1.73	2.32	0.74	1.82	2.32	0.78
September	1.51	2.58	0.58	2.41	2.81	0.86	1.93	2.8	0.69

months the diversity in the 0-5 cm stratum ranged between 1.5 and 2.3, while at 5-10 and 10-15 cm depths it fluctuated between 0.2 and 2.5. Thus, diatom diversity (*H'*), richness (*S* = *H' max.*) and evenness (*J'* = *H'/H' max.*) revealed significant variation

richness ($S = H'$ *max.*) and evenness ($J' = H'/H'$ *max.*) revealed significant variation with respect to months ($P \leq 0.001$), depths, as well as months X depths (Table 2A.4 and Table 2A.5).

The dendrogram at 50% dissimilarity level revealed 5 groups of diatoms in the three

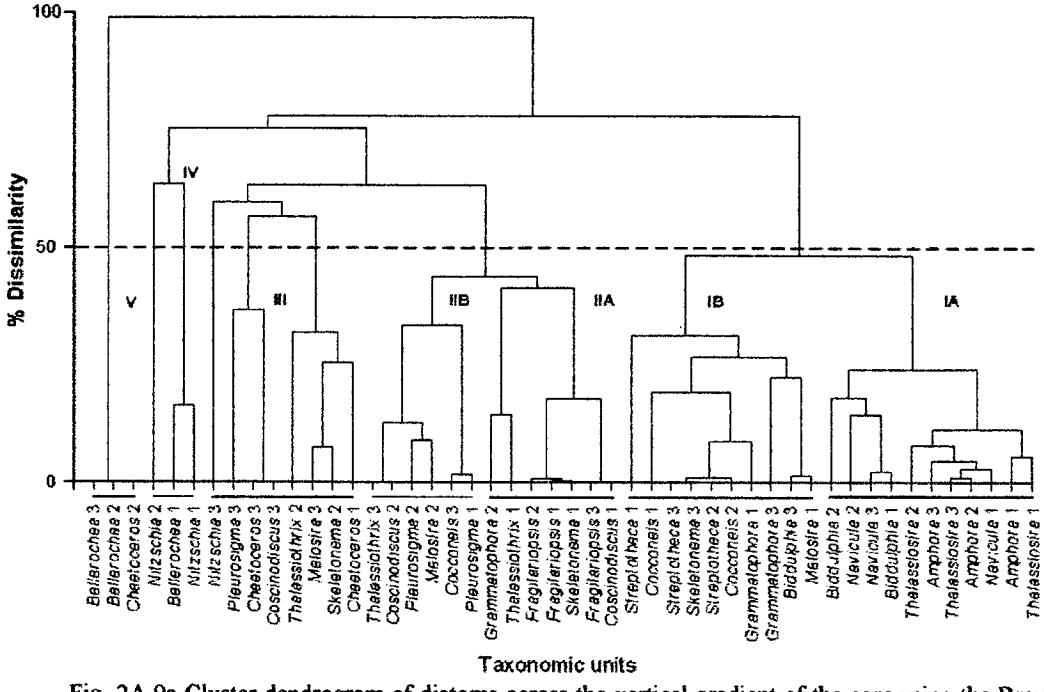


Fig. 2A.9a Cluster dendrogram of diatoms across the vertical gradient of the core using the Bray-Curtis coefficient and group average method.

sediment core sections (Fig. 2A.9a). Group I comprised two sub-groups, IA and IB. Sub-group IA was represented by the most abundant diatoms, i.e. *Thalassiosira*, *Amphora* and *Navicula* for the entire core. *Biddulphia* from the 0-5 and 10-15 cm core sections also formed a member of this sub-group. The abundance of these diatoms ranged up to 10^4 cells cm^{-3} wet sediment. The second sub-group (IB) included those forms present at particular depths up to an abundance of 10^2 cells cm^{-3} wet sediment. Group IIA comprised forms occurring rarely and with abundance below 10^2 cells cm^{-3} wet sediment at particular depths during the study period, and group IIB included forms such as *Cocconeis* (10-15 cm) and *Pleurosigma* (0-5 cm) with

abundance ranging between 10^2 and 10^3 cells cm^{-3} wet sediment. The abundance of rest of the forms belonging to this group was below 10^2 cells cm^{-3} wet sediment.

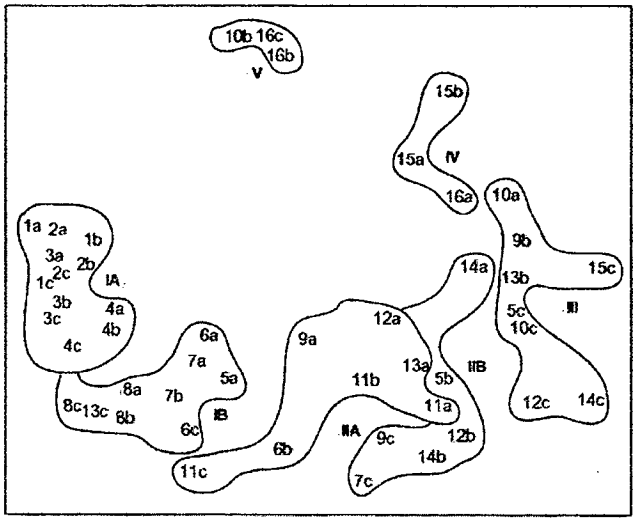


Fig. 2A.9b Non-metric Multidimensional Scaling ordination of diatoms across the vertical gradient of the core using the Bray-Curtis coefficient and group average method.

1. *Thalassiosira*. 2. *Amphora*; 3. *Navicula*. 4. *Biddulphia*. 5. *Melosira*; 6. *Grammatophora*. 7. *Cocconeis*. 8. *Streptotheca*; 9. *Thalassiothrix*; 10. *Chaetoceros*. 11 *Fragilaria*. 12 *Coscinodiscus*; 13. *Skeletonema*. 14 *Pleurosigma*. 15. *Nitzschia*; 16. *Bellerochea*

Group III included the depths at which the respective forms occurred only once or twice during the study period. Forms with an abundance ranging between 1 and 10 cells cm^{-3} wet sediment clustered to form group IV. Group V represented the forms, at particular depths, that were absent throughout the study period. The diatom distribution portrayed a similar pattern as that of the cluster analysis when subjected to two-dimensional NMDS ordinations (Fig. 2A.9b).

Relationship with observed interstitial water parameters: Multiple regressions were performed on the nutrient (nitrogen, phosphorus, silicate), temperature and salinity

Table 2A.6 Partial regression coefficients (Beta values) for the dependent variable (viable diatoms cm^{-3} wet sediment) and independent variable (water parameters)

Diatoms	Intercept	R	Temperature(^{0}C)	Salinity(psu)	Nitrate (NO_3-N)	Nitrite(NO_2-N)	Phosphate(PO_4-P)	Silicate(SiO_3)
Pennate	1.12	0.8	0.186	0.135	0.086	0.057	0.41	-0.84*
Centric	-2.55	0.87	0.307	0.212	0.69*	-0.18	0.83*	-0.8*

values (obtained for the entire 15 cm core) versus the total count of pennate and centric diatoms (0-15 cm). It was observed that, for pennates, which mainly reside in the sediments, nutrients did not play an important role, whereas, for centric diatoms, nitrate and phosphate positively influenced their abundance (Table 2A.6).

Relationship with grain size: Distribution of different grain size fractions was found to differ with the different depths, as well as the different months (Fig. 2A.5).

Table 2A.7 Partial regression coefficients (Beta values) for the dependent variable (viable diatoms cm^{-3} wet sediment) and independent variable (grain size) (I = 0-5; II = 5-10; III = 10-15 cm)

Depth	Diatoms	Intercept	R	1000	500	250	125	63	<63
I	*Navicula*	-13.7	0.84	-1.02 *	0.45	0.38	1.71	1.71	2.97
	Amphora	9.74	0.88	-0.29	-0.28	0.68 *	-0.29	-0.99	-0.43
	Grammatophora	-4.48	0.71	-0.36	-0.21	0.96 *	0.42	0.49	1.11
II	*Pleurosigma*	-0.025	0.74	0.222	-0.42	-0.154	0.87*	-0.456	0.681 *
	Thalassiothrix	-0.004	0.87	-0.028	-0.23	0.8 *	-0.39	0.28	-0.19
III	*Fragilaria*	2.07	0.85	-0.11	0.074	-0.253	-0.235	-0.668*	-0.082

However, subjection of this data to multiple regression analysis indicated that the grain size could serve as a predictor of only some of the pennate diatoms, e.g. *Amphora*, *Grammatophora*, *Pleurosigma* and *Thalassiothrix* (Table 2A.7). Amongst these, the 250μm grain size fraction served as a predictor of the abundance of *Amphora* and *Grammatophora* at 0-5 cm depth. *Pleurosigma* was influenced by 125 and < 63 μm grain size at 5-10 cm, whereas, for *Thalassiothrix*, the choice of substratum was the 250 μm grain size at the same depth. However, a similar substratum preference of these diatoms at the other depths was not evident. *Navicula*, which was abundant throughout the year, was negatively influenced by the 1000 μm grain size fraction at 0-5 cm. However, the percentage composition of this grain size was negligible. *Fragilariopsis* was negatively influenced by 63μm grain size at the lowermost depth (10-15 cm).

Wind speed and suspended load: Wind speed in the study area ranged from 2.0 to

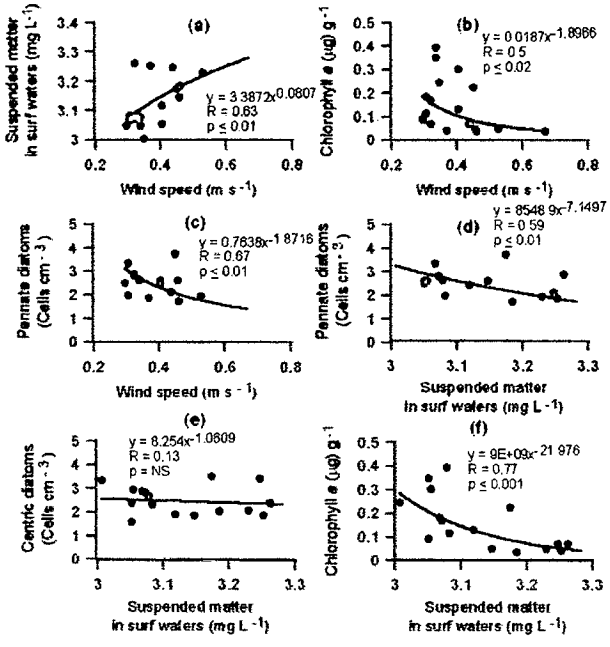


Fig. 2A.10 Polynomial regression between wind speed and suspended matter (a), chlorophyll *a* (b), and pennate diatom abundance (c); and between suspended matter and pennate diatom abundance (d), centric diatom abundance (e) and chlorophyll *a* (f) (NS = not significant)

4.7 m s^{-1}. Regression analysis between suspended matter and wind speed was statistically significant. Chlorophyll *a* concentrations and pennate diatom abundance in the 0-5 cm stratum revealed a significant negative correlation between wind speed and suspended load (Fig. 2A.10).

Chlorophyll a and diatoms: During monsoons, chlorophyll *a* concentrations were lower irrespective of depth; the highest concentration in this season was observed in the 10-15 cm stratum. During late post-monsoon and pre-monsoon seasons, the 0-5 cm stratum had higher concentrations of chlorophyll *a*; these peaked during December and March (Fig. 2A.11). Regression analysis revealed that the chlorophyll *a* concentrations at 0-5 and 5-10 cm depths were good predictors of pennate as well as centric diatoms, whereas, for the third depth (10-15 cm), this was not the case (Fig.

2A.12). Although the regression analysis showed a negative correlation of diatom abundance with chlorophyll *a* at 10-15 cm depth, the appearance of diatoms in the culture wells provided with growth media and light (prerequisite factors for photosynthesis) confirms the presence of functional chlorophyll *a* at this depth.

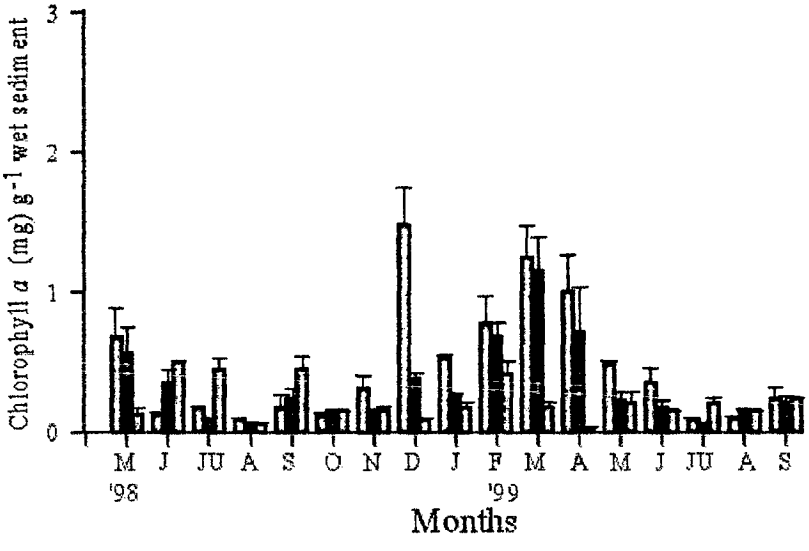


Fig. 2A.11 Concentrations of chlorophyll *a* across the three vertical sections of the sediment core at low tide zone

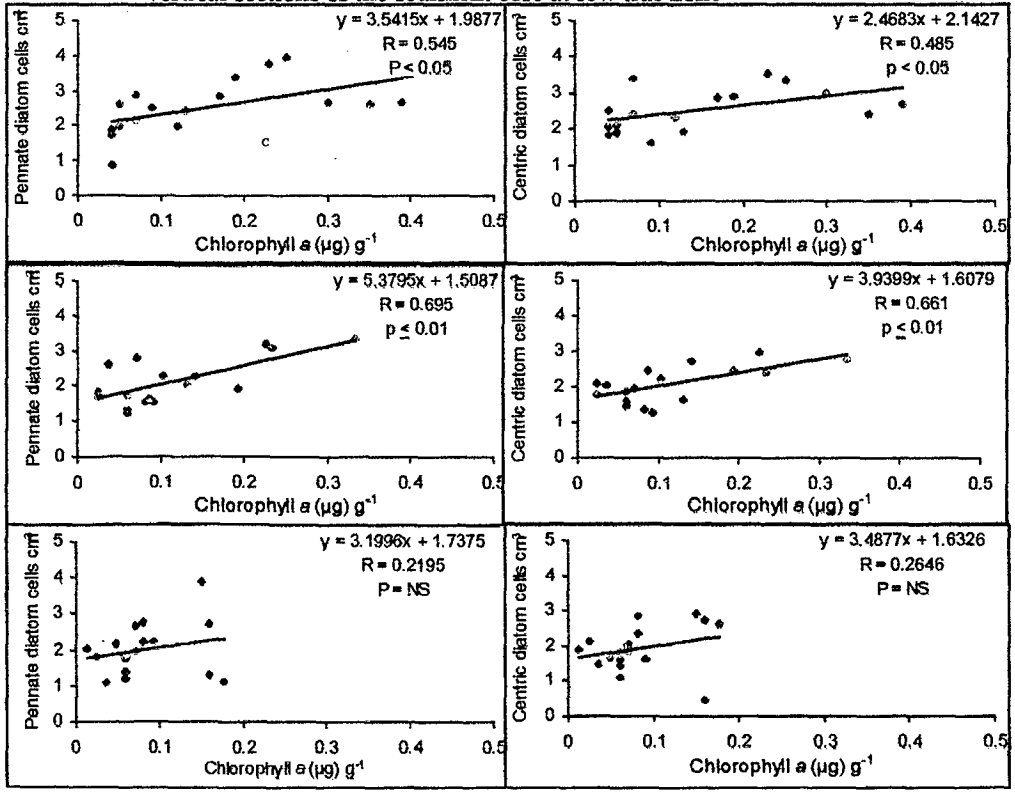


Fig. 2A.12 Linear regression between chlorophyll *a* and pennate and centric diatom abundance across the three vertical sections of the core (NS = not significant)

2A.3.4 Mid tide zone

Sediment characteristics: Fine sand (125-249 μm) was found to be the most abundant grain size fraction of the sediment throughout the study period across the 15 cm core (Fig. 2A.13b). The contribution of sediment particles > 250 μm was negligible. MANOVA of different depth versus grain size revealed a significant variation (Table 2A.8b).

Diatoms in sediment: The sediment harbored 30 species of diatoms (11 centric, 19 pennate) belonging to 20 (7 centric, 13 pennate) genera (Table 2A.1c). Pennate diatoms were dominant in terms of abundance (Fig. 2A.14b). The most abundant pennate diatoms were *Amphora* and *Navicula*, whereas, *Thalassiosira* was the abundant centric diatom. Centric diatoms such as *Skeletonema* and *Chaetoceros*, recorded at the low tide zone were not observed at this zone.

Based on their abundance diatoms encountered in this area can be divided into three groups as given in Table 2A.9 and Figs. 2A.15a-c.

Temporal and Vertical distribution of diatoms in the sediment: At the 0-5 cm stratum the total diatom abundance peaked in November, January and March whereas at the 5-15 cm stratum not much variation was observed. Amongst the group I diatoms, *Navicula* peaked from November to March across the three core sections; *Amphora* and *Thalassiosira* peaked only in the 0-5 cm depth, during the same period. Their distribution in the 5-15 cm depth did not show any particular trend. Amongst the Group II diatoms, in the 0-5 cm, *Melosira* was most abundant from July to March, *Biddulphia* and *Nitzschia* from September to March and *Pleurosigma*, *Thalassiothrix* and *Cyclotella* from November to March. In the group III diatoms, *Bellerochea* peaked from November to March, *Fragilariopsis* in September, *Grammatophora* and *Coscinodiscus* in July and *Streptotheca* in September.

Thus, all but *Bellerochea, Raphoneis* (depth and month x depth) and *Thalassiothrix* (depth) showed significant variation when subjected to two-way ANOVA (Table 2A.10b).

Diatom diversity peaked in July with a high evenness due to an evenly distributed population (Table 2A.11a). Significant variation with respect to month ($P \leq 0.001$), depth as well as month x depth was observed for diatom diversity (*H'*), richness (*S = H' max.*; except with respect to depth) and evenness (*J' = H'/H' max.*) (Table 2A.12b).

The dendrogram at 50% dissimilarity level revealed 7 groups of diatoms (Fig. 2A.16b). The first group comprised two pennate diatoms, *Amphora* and *Navicula,* and a centric diatom *Thalassiosira,* which were the most abundant forms. Abundance of these diatoms ranged up to 10^4 cells cm^{-3} wet sediment. Group II was represented by forms occurring 4-7 times, with an abundance ranging between 10^1 and 10^2 cells cm^{-3} wet sediment. Group III comprised forms with an abundance ranging between 10 and 10^3 cells cm^{-3} wet sediment and which mostly occurred 3 times during the sampling period. The forms belonging to group IV occurred once during the sampling period with an abundance ranging between 10^1 and 10^2 cells cm^{-3} wet sediment. Forms, which occurred twice and thrice during the sampling period comprised group V and group VI respectively. Group VII was represented by forms, which had an abundance ranging between 10 and 10^2 cells cm^{-3} wet sediment.

The diatom distribution portrayed a similar pattern as that of the cluster analysis when subjected to 2D NMDS ordinations (Fig. 2A.17b).

Relationship with grain size: Distribution of different grain size fractions was found to differ with the different depths (Fig. 2A.13b). Multiple regression analysis indicated that the grain size could serve as a predictor of diatoms such as

Grammatophora, Coscinodiscus, Biddulphia, Bellerochea, Navicula, Cyclotella, Thalassiothrix, Raphoneis, Streptotheca and *Pleurosigma* (Table 2A.13a). Amongst these, the < 63 μm grain size fraction served as a predictor of the abundance of *Grammatophora* and *Coscinodiscus* at 0-5 cm depth. *Biddulphia* and *Bellerochea* were influenced by 500 and < 63 μm grain size at 5-10 cm. At the 10-15 cm depth for *Navicula*, the choice of substratum was the 125, 63 and < 63 μm grain size whereas for *Cyclotella, Thalassiothrix, Bellerochea, Raphoneis* and *Pleurosigma* 500 μm grain size served as a predictor.

Wind speed: Regression analysis between wind speed and parameters such as chlorophyll *a*, pennate and centric diatom abundance did not reveal any significant relation.

Chlorophyll a and diatoms: Chlorophyll *a* concentrations were higher at the 0-5 cm stratum with peaks from September through March (Fig. 2A.18b). Regression analysis revealed that the chlorophyll *a* concentrations only at 0-5 cm depth were good predictors of pennate as well as centric diatoms (Fig. 2A.19b).

2A.3.5 High tide zone

Sediment characteristics: Fine sand (125-249 μm) was found to be the most abundant grain size fraction of the sediment throughout the study period across the 15 cm core (Fig. 2A.13c). The contribution of sediment particles > 250 μm was negligible. MANOVA of different months versus grain size revealed a significant variation (Table 2A.8c).

Diatoms in sediment: 27 species of diatoms (10 centric, 17 pennate) belonging to 19 (7 centric, 12 pennate) genera were recorded in the sediment (Table 2A.1d). Pennate diatoms were dominant in terms of abundance (Fig. 2A.14c). The most abundant pennate diatoms were *Amphora* and *Navicula*, whereas, *Thalassiosira* was the

abundant centric diatom. Centric diatoms such as *Skeletonema* and *Chaetoceros*, recorded at the low tide zone were not observed at this location.

Based on their abundance, diatoms encountered in this area can be divided into three groups as given in Table 2A.9 and Figs. 2A.15a-c.

Temporal and Vertical distribution of diatoms in the sediment: The total diatom abundance peaked in September, November, January and March at the 0-5 cm stratum whereas the 5-10 cm stratum did not exhibit much variation. Amongst the Group I diatoms, *Amphora* and *Thalassiosira* peaked from September through March whereas *Navicula* peaked from November through March in the 0-5 cm depth. Amongst the Group II diatoms, peaks were observed for *Melosira* from July through September, *Cocconeis* and *Thalassiothrix* from November through March and *Streptotheca* from September through March. Group III diatoms such as *Nitzschia, Grammatophora* and *Achnanthes* peaked in September, January and July respectively.

Thus, the diatom data when subjected to two-way ANOVA revealed a significant variation with respect to months ($p \leq 0.001$) and depths for all forms except *Pinnularia*, which did not reveal significant relationship with respect to month x depth (Table 2A.10c).

The diatom diversity was lower as compared to the low and mid tide zones (Table 2A.11b). Significant variation with respect to months ($p \leq 0.001$), depths as well as months x depths was observed for diatom diversity (H'), richness ($S = H'$ max.) and evenness ($J' = H'/H'$ max.) (Table 2A.12c).

The dendrogram at 50% dissimilarity level revealed 5 groups of diatoms respectively (Fig. 2A.16c). The first group comprised two pennate diatoms, *Amphora* and *Navicula*, and a centric diatom *Thalassiosira*, which were the most abundant forms, except for *Thalassiosira* in the 0-5 cm section. Abundance of these diatoms ranged up

to 10^3 cells cm^{-3} wet sediment. Group II comprised forms, which occurred 3-7 times during the sampling period. Forms, which occurred once represented group III whereas those which, occurred 1-3 times during the sampling period, represented group IV. Group V comprised forms occurring 1-2 times during the sampling period with an abundance ranging between 10 and 10^2 cells cm^{-3} wet sediment.

The diatom distribution portrayed a similar pattern as that of the cluster analysis when subjected to 2D NMDS ordinations (Fig. 2A.17c).

Relationship with grain size: Distribution of different grain size fractions was found to differ with the different months (Fig. 2A.13c). Subjection of this data to multiple regression analysis indicated that the grain size could serve as a predictor of *Grammatophora* (500µm grain size fraction) at 0-5 cm, and *Achnanthes, Biddulphia, Melosira* and *Streptotheca* (250 µm grain size fraction), *Cyclotella* (500 and 125µm grain size fraction), *Pinnularia* (63 µm grain size fraction) and *Coscinodiscus* (500, 125 and 63 µm grain size fraction) at the 10-15 cm (Table 2A.13b).

Wind speed: Regression analysis between wind speed and parameters such as chlorophyll *a*, pennate and centric diatom abundance did not reveal any significant relation.

Chlorophyll a and diatoms: Chlorophyll *a* concentrations generally followed a low profile except in the 10-15 cm stratum during July (Fig.2A.18c). Regression analysis did not reveal significant relationship between chlorophyll *a* concentrations and pennate and centric diatoms (Fig. 2A.19c).

2A.3.6 Comparison between the low, mid and high tide zones

Sediment characteristics: While very fine sand (63-124 µm - silt/clay) was found to be the most abundant grain size fraction of the sediment at the low tide zone, it was fine sand (125-249 µm) which dominated at both, the mid and high tide zones throughout the study period across the 15 cm core. Exception was in July at the mid tide zone at 10-15 cm where 63-124 µm grain size fraction dominated and at high tide zone during May at 5-15 cm depth (Table 2A.8a-c and Fig. 2A.13a-c).

Table 2A.8a. MANOVA (Without replication) for the temporal variation in the sediment characteristics along the vertical gradients at the low tide mark (0-5, 5-10 and 10-15 cm sediment core sections) (* $p \leq 0.05$; NS = not significant)

Source of variation	*df*	SS	MS	Fs
Months	8	7.62	0.95	-
Core sections	2	90.3	45.15	-
% Grain size fractions	5	34989.15	6997.83	-
Months X Core sections	16	684.8	42.8	0.83^{NS}
Months X % Grain size fractions	40	3062.23	76.5	1.5*
Core sections X % Grain size fractions	10	833.97	83.39	1.63^{NS}
Months X Core sections X % Grain size fractions	80	4085.62	51.07	-

Table 2A.8b. MANOVA (Without replication) for the temporal variation in the sediment characteristics along the vertical gradients at the mid tide mark (0-5, 5-10 and 10-15 cm sediment core sections) (* $p \leq 0.05$; NS = not significant)

Source of variation	*df*	SS	MS	Fs
Months	8	4.68	0.58	-
Core sections	2	5.56	2.78	-
% Grain size fractions	5	71,107.24	14,221.45	-
Months X Core sections	16	22.97	1.44	0.061^{NS}
Months X % Grain size fractions	40	745.02	18.63	0.79^{NS}
Core sections X % Grain size fractions	10	458.84	45.88	1.94*
Months X Core sections X % Grain size fractions	80	1,891.74	23.65	-

Table 2A.8c MANOVA (Without replication) for the temporal variation in the sediment characteristics along the vertical gradients at the high tide mark (0-5, 5-10 and 10-15 cm sediment core sections) (*** $p \leq 0.001$; NS = not significant)

Source of variation	*df*	SS	MS	Fs
Months	8	43.87	5.48	-
Core sections	2	0.65	0.32	-
% Grain size fractions	5	75,960.36	15,192.07	-
Months X Core sections	16	41.8	2.61	0.097^{NS}
Months X % Grain size fractions	40	2,773.48	69.33	2.56***
Core sections X % Grain size fractions	10	187.59	18.76	0.69^{NS}
Months X Core sections X % Grain size fractions	80	2164.14	27.05	-

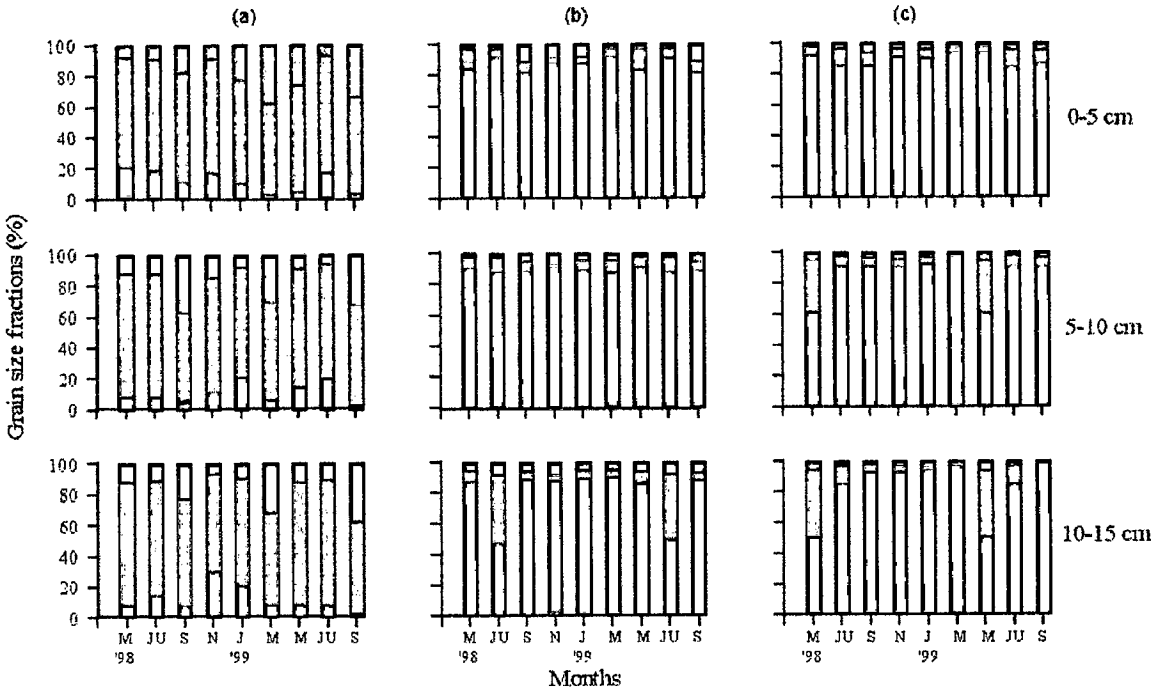


Fig. 2A.13 Temporal variations in grain size fractions (> 1000 ▨, 500-999 □, 250-499 ▨, 125-249 □, 63-124 □ and < 63 □ µm) across the vertical gradient of the core at the low tide zone (a); mid tide zone (b) and high tide zone (c)

Diatoms in intertidal sediments: Diatoms were encountered up to 15 cm depth throughout the intertidal zone. The pennates dominated over the centrics throughout

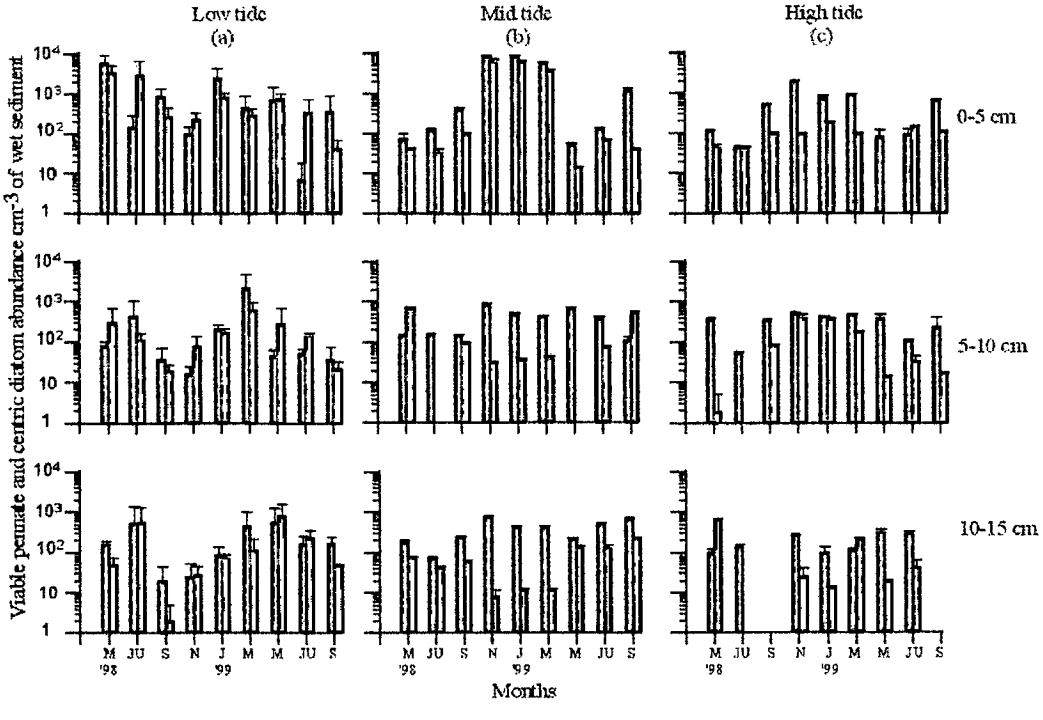


Fig. 2A.14 Temporal variations in pennate and centric diatom abundance across the vertical gradient of the core
□ Pennate diatoms: □ Centric diatoms

the intertidal zone (Fig. 2A.14a-c). The dominant diatoms which comprised group I were common to all the three tidal zones whereas those which comprised group II and III differed with the tidal zone. The highest number of species was encountered at the mid tide zone followed by the high and low tide zones (Figs. 2A.7a-b and 2A.15a-c). The abundance, diversity, richness and evenness of diatom cells showed spatial and

Table 2A.9 Diatom groups encountered at the low, mid and high tide zones (a = low tide, b =mid-tide and c =high tide)

Diatoms	Group I			Group II			Group III		
	a	b	c	a	b	c	a	b	c
Amphora	+	+	+						
Navicula	+	+	+						
Thalassiosira	+	+	+						
Biddulphia				+	+				+
Melosira				+	+	+			
Streptotheca				+		+			
Coscinodiscus				+				+	+
Cyclotella					+				+
Grammatophora				+				+	+
Cocconeis				+	+	+			
Nitzschia				+	+				+
Thalassiothrix				+	+	+			
Pleurosigma					+		+		
Fragilariopsis							+	+	+
Skeletonema							+		
Chaetoceros							+		
Bellerochea							+	+	+
Cymbella					+				+
Achnanthes								+	+
Streptotheca								+	
Raphoneis								+	+
Pinnularia									+
Tabellaria					+	+			

temporal variations throughout the intertidal zone (Tables 2A.10, 2A.11 and 2A.12). Grain size fractions, which served as predictors of some diatoms differed with the tidal zones and also depth (Table 2A.8a-c and Fig. 2A.13a-c). Wind stimulated the resuspension of the sediment, along with the pennate diatoms down to 5 cm depth only at the low tide zone (Fig. 2A.10). The depth up to which chlorophyll *a* concentrations could serve as predictors of diatom abundance differed with the tidal level, from 10 cm

depth at the low tide zone, to 5 cm depth at the mid tide zone to 0 cm at the high tide zone (Fig. 2A.19a-c).

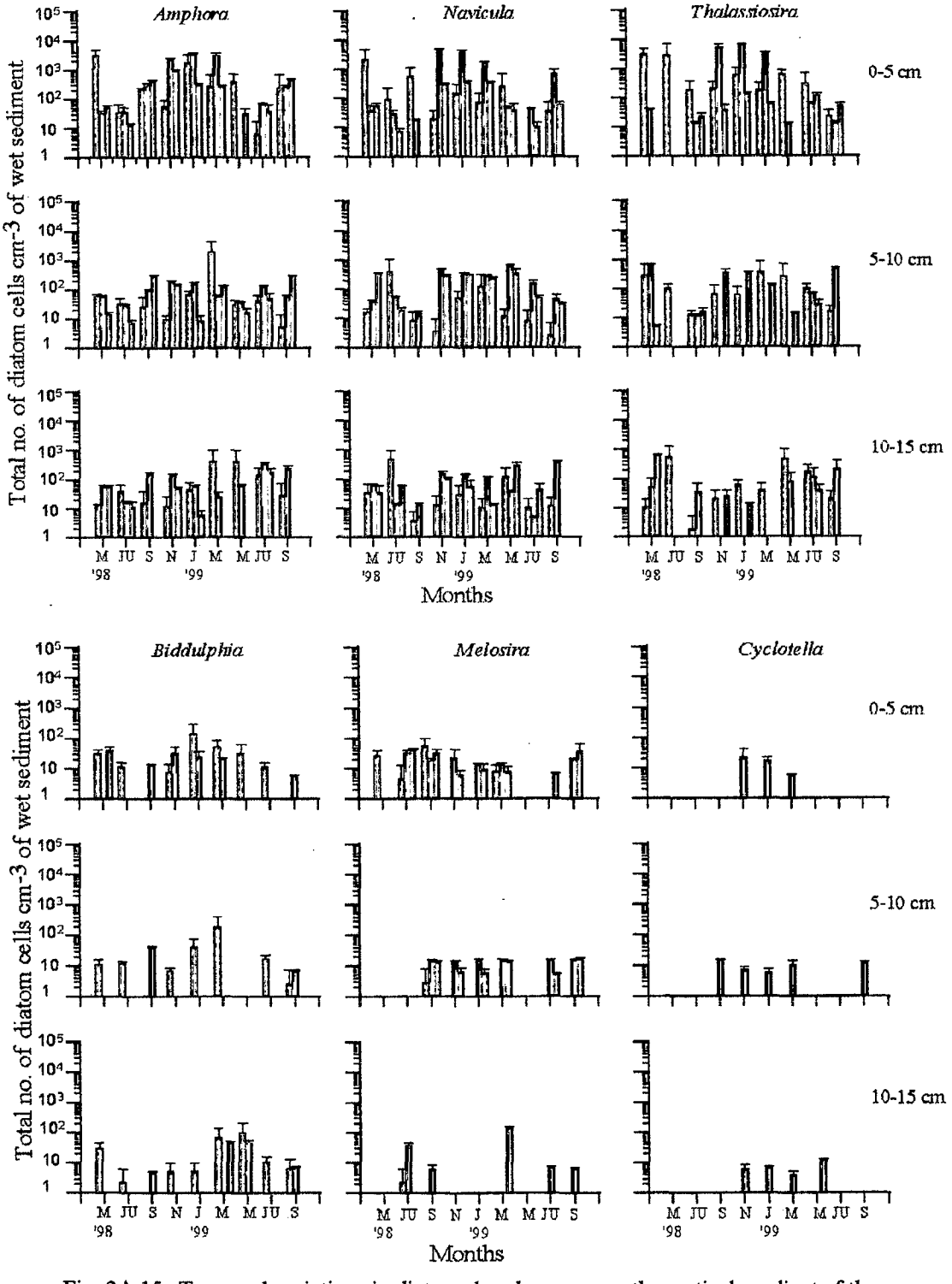


Fig. 2A.15a Temporal variations in diatom abundance across the vertical gradient of the core

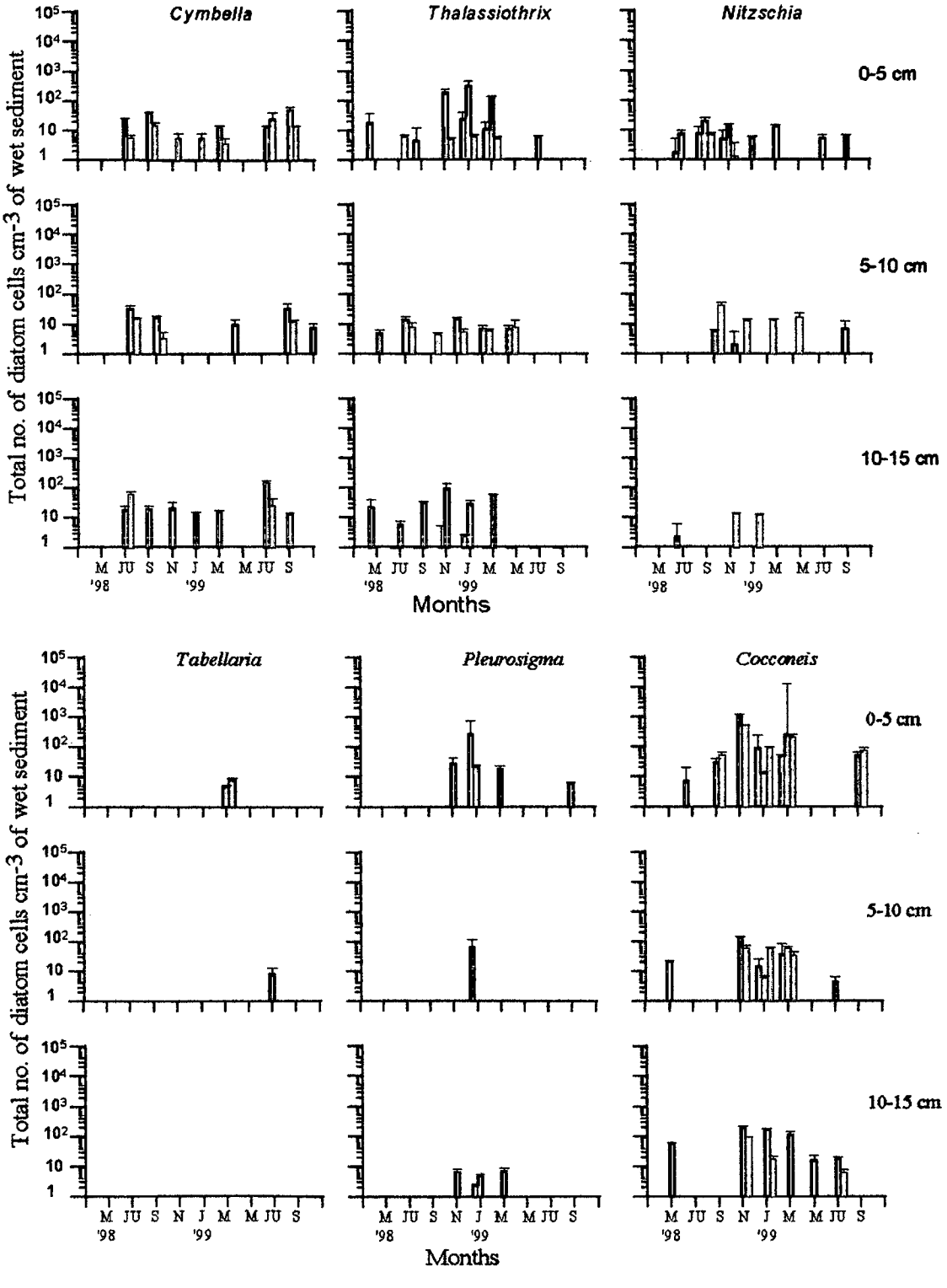


Fig. 2A.15b Temporal variation in diatom abundance across the vertical gradient of the core

□ Low tide zone; □ Mid tide zone; □ High tide zone

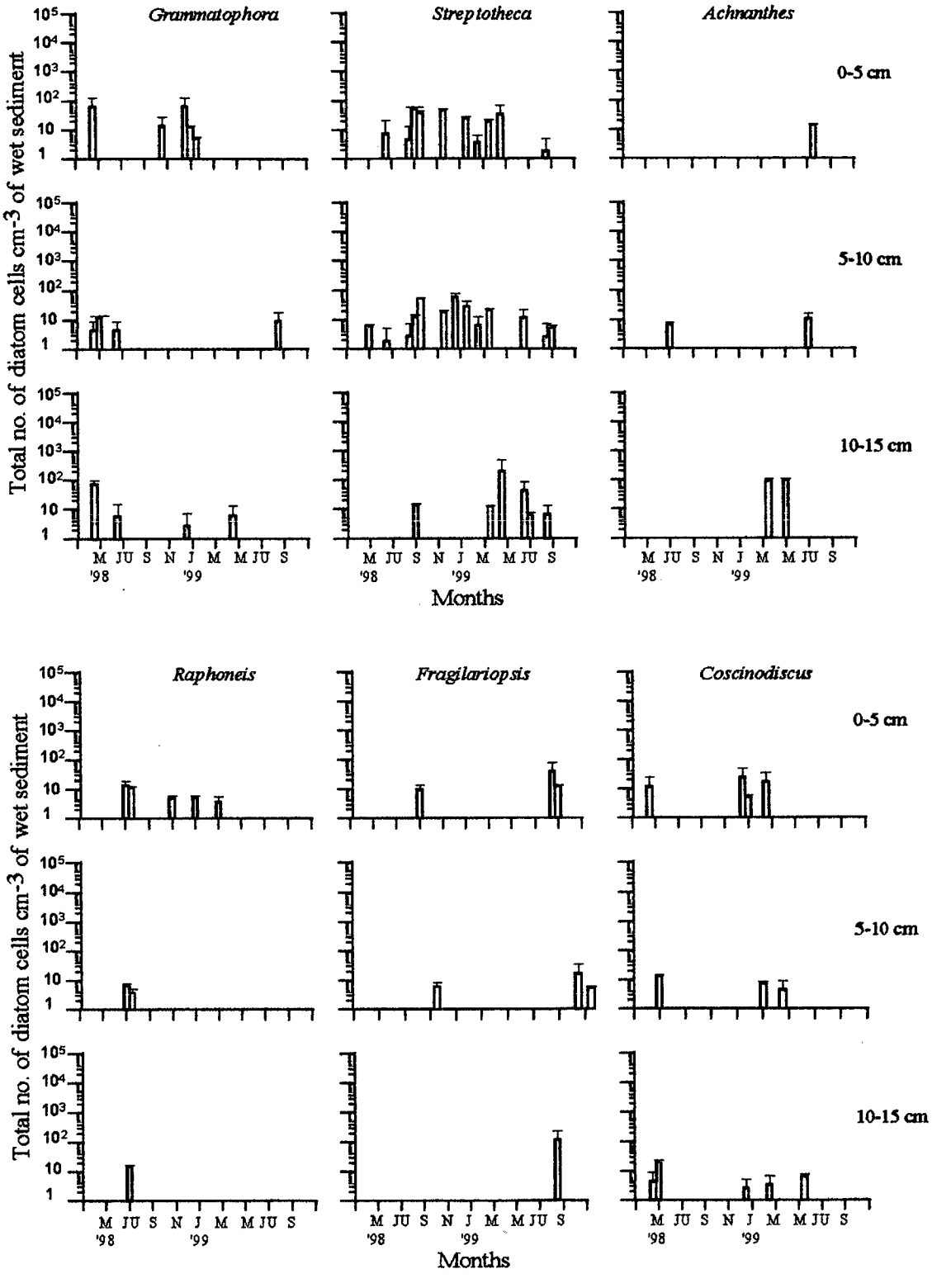


Fig. 2A.15c Temporal variation in diatom abundance across the vertical gradient of the core

□ Low tide zone; □ Mid tide zone; □ High tide zone

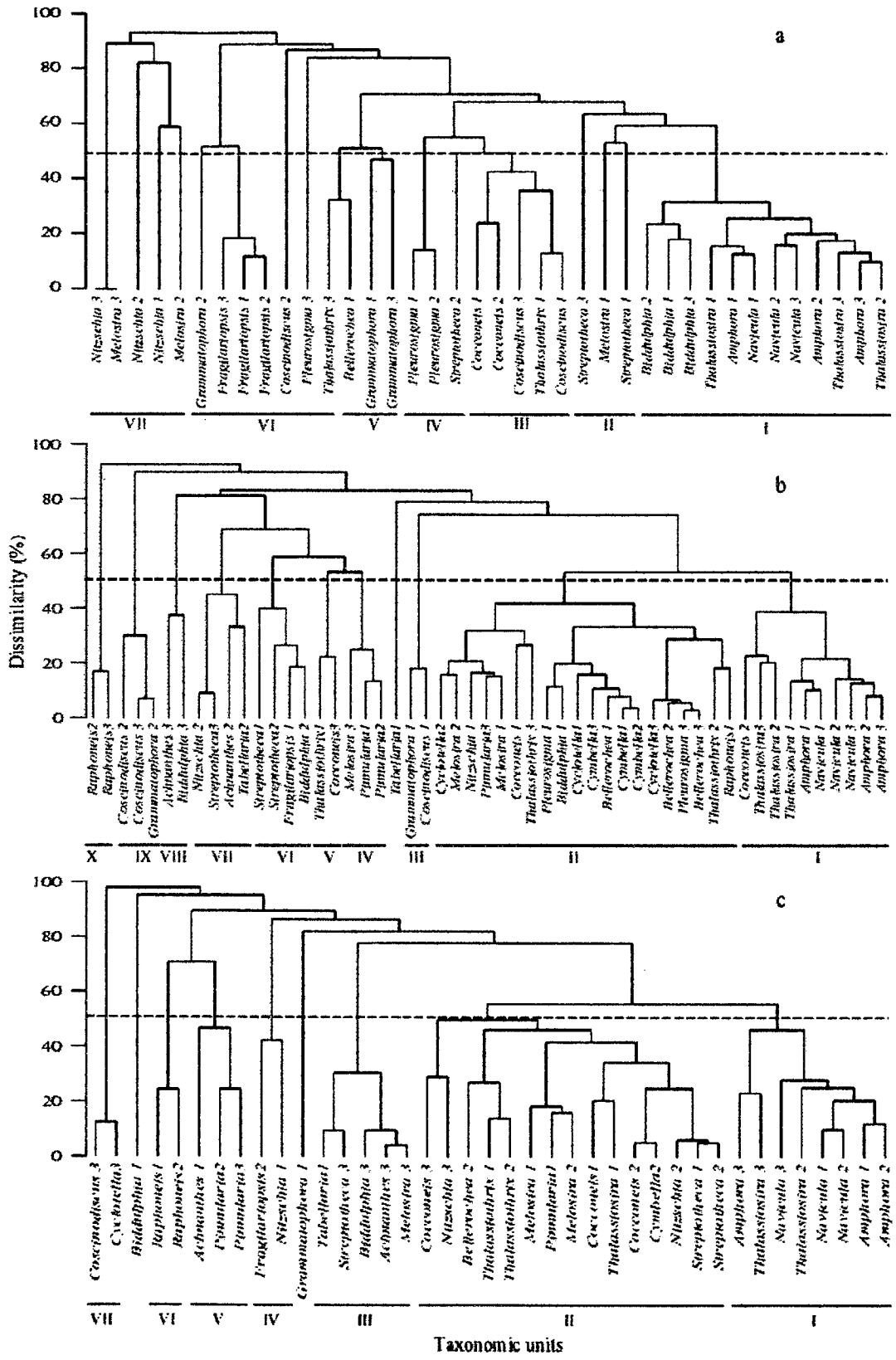
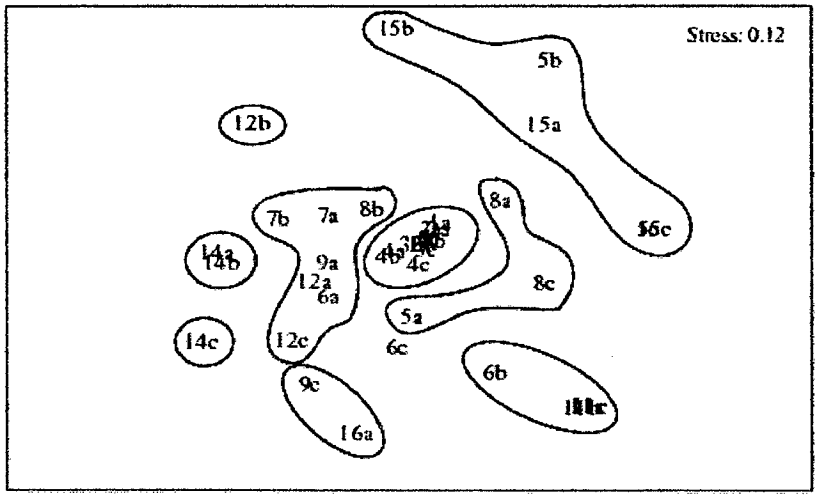
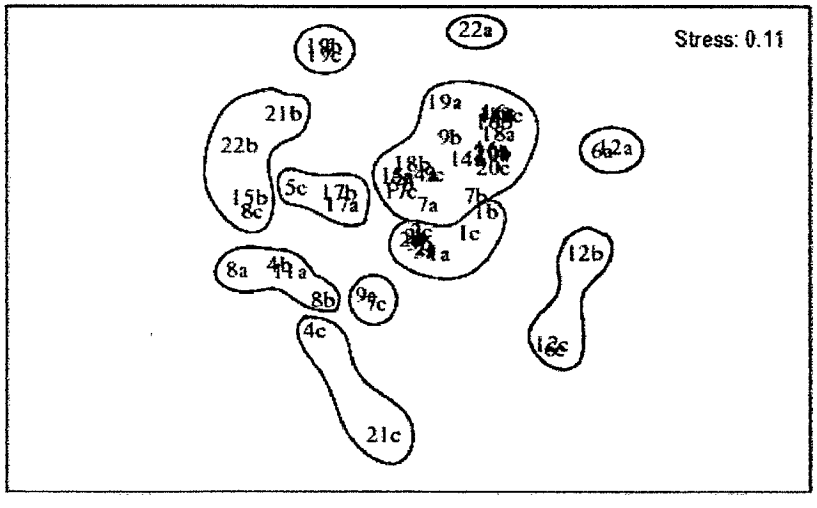


Fig. 2A.16 Cluster dendrogram of diatoms across the vertical gradient of the core using Bray Curtis coefficient and group average method a) low tide, b) mid tide and c) high tide

a. Low tide



b. Mid tide



c. High tide

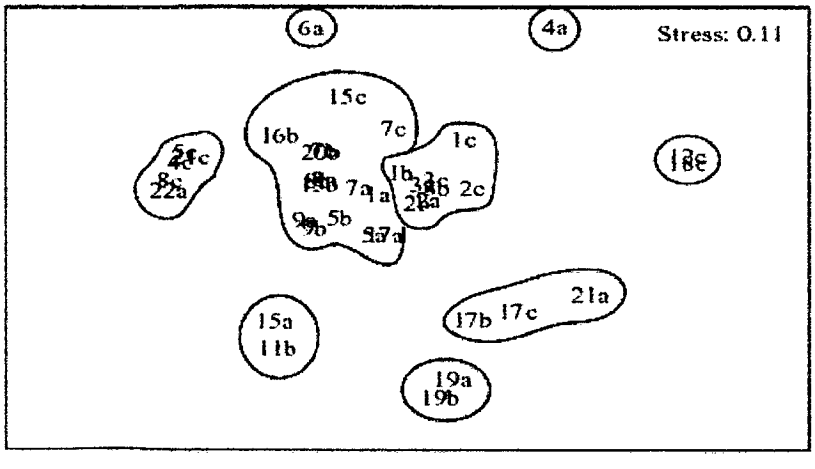


Fig. 2A.17 Multidimensional scaling ordination of diatoms across the vertical gradient based on the Bray Curtis dissimilarity coefficient

1. *Thalassiosira* 2. *Amphora* 3. *Navicula* 4. *Biddulphia* 5. *Melosira* 6. *Grammatophora* 7. *Cocconeis* 8. *Streptotheca* 9. *Thalassiothrix* 10. *Chaetoceros* 11. *Fragilariopsis* 12. *Coscinodiscus* 13. *Skeletonema* 14. *Pleurosigma* 15. *Nitzschia* 16. *Bellerochea* 17. *Pinnularia* 18. *Cyclotella* 19. *Raphoneis* 20. *Cymbella* 21. *Achnanthes* 22. *Tabellaria*

Table 2A.10. Significance values of the two-way ANOVA evaluating the variations in diatom abundance with respect to months and core sections (0-15 cm) at the low tide (a), mid tide (b) and high tide marks (c) (NS = not significant).

Diatoms in the intertidal sediment	Month p≤			Core section p≤			MonthXCore section p≤		
	a	b	c	a	b	c	a	b	c
Navicula	0.001	0.005	0.001	0.005	0.001	0.025	0.05	0.025	0.005
Amphora	0.001	0.005	0.005	0.005	0.05	0.001	0.001	0.001	0.001
Grammatophora	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.001
Pleurosigma	0.001	0.001	0.001	0.025	0.001	0.001	0.001	0.001	0.001
Nitzschia	0.001	0.001	0.001	0.01	0.001	0.001	0.001	0.001	0.001
Thalassiothrix	0.001	0.001	0.001	0.001	NS	0.001	0.001	0.001	0.001
Cocconeis	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Fragilariopsis	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.001
Bellerochea	0.001	0.001	0.001	0.001	NS	0.001	0.001	NS	0.001
Thalassiosira	0.001	0.001	0.001	0.001	0.001	0.001	0.005	0.001	0.001
Biddulphia	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Coscinodiscus	0.001	0.001	0.001	0.005	0.005	0.001	0.001	0.001	0.001
Melosira	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Skeletonema	0.001	-	-	NS	-	-	NS	-	-
Streptotheca	0.001	0.001	0.001	NS	0.005	0.001	0.001	0.001	0.001
Chaetoceros	0.001	-	-	0.005	-	-	0.001	-	-
Cyclotella	-	0.001	0.001	-	0.01	0.001	-	0.001	0.001
Tabellaria	-	0.001	0.001	-	0.001	0.001	-	0.001	0.001
Pinnularia	-	0.001	0.001	-	0.001	0.05	-	0.001	NS
Cymbella	-	0.001	0.001	-	0.001	0.001	-	0.001	0.001
Raphoneis	-	0.001	0.001	-	NS	0.001	-	NS	0.001
Achnanthes	-	0.001	0.001	-	0.001	0.001	-	0.001	0.001

Table 2A. 11a. Values of species diversity (*H'*), species richness (*H'* max.) and evenness (*J'*) of the diatom community at the mid tide zone (Shannon-Wieners diversity index)

	0-5 cm			5-10 cm			10-15 cm		
MONTHS	H'	H'max	J'	H'	H'max	J'	H'	H'max	J'
May '98	1.58	1.28	1.00	2.89	2.86	0.96	2.31	1.89	1.00
July	2.56	2.38	0.99	2.54	2.44	0.98	2.56	2.50	0.99
September	3.27	3.36	0.99	3.13	3.27	0.99	2.93	2.97	0.98
November	3.54	3.65	0.96	2.90	2.72	0.96	3.06	2.98	0.97
January '99	3.69	4.28	0.94	3.04	3.25	0.96	3.07	3.15	0.97
March	3.71	4.29	0.95	3.09	3.16	0.98	3.08	3.11	0.97
May	0.97	0.95	0.97	0.94	0.68	0.94	2.57	2.15	0.99
July	2.51	2.39	0.97	3.07	3.16	0.97	2.66	2.54	0.95
September	3.19	3.33	0.96	2.88	2.92	0.96	2.44	2.15	0.94

Table 2A. 11b. Values of species diversity (*H'*), species richness (*H'* max.) and evenness (*J'*) of the diatom community at the high tide zone (Shannon-Wieners diversity index)

	0-5 cm			5-10 cm			10-15 cm		
MONTHS	H'	H'max	J'	H'	H'max	J'	H'	H'max	J'
May '98	1.58	1.22	1.00	1.42	1.32	0.90	1.54	1.11	0.97
July	2.54	2.67	0.98	2.28	2.46	0.98	1.55	1.30	0.98
September	2.74	2.51	0.98	2.87	2.98	0.96	-	-	-
November	2.93	3.01	0.92	3.20	3.30	0.96	2.29	1.90	0.99
January '99	3.03	3.03	0.96	3.19	3.38	0.96	2.28	2.20	0.98
March	3.01	3.05	0.95	3.24	3.30	0.98	2.27	1.92	0.98
May	1.00	0.87	1.00	1.49	1.24	0.94	1.43	1.33	0.90
July	2.53	2.37	0.98	2.27	2.10	0.98	2.26	1.94	0.97
September	2.54	2.09	0.98	1.88	1.67	0.94	-	-	-

Table 2A.12. Two-way ANOVA for diatom diversity, richness and evenness with respect to months, and coresections at the (a) low tide, (b) mid tide and (c) high tide marks

a	*df*	SS	MS	Fs
Diversity				
Months	16	23.58	1.47	5.28
Core sections	2	3.29	1.64	5.88
MonthsXCore section	32	24.15	0.75	2.7
Within subgroup error	102	28.5	0.28	
Total	152	79.52		
Generic richness				
Months	16	21.04	1.31	11.41
Core section	2	6.15	3.07	26.7
MonthsXCore section	32	24.6	0.77	6.67
Within subgroup error	102	11.76	0.11	
Total	152	63.54		
Evenness				
Months	16	1.39	0.087	2.81
Core section	2	0.3	0.15	4.81
MonthsXCore section	32	2.07	0.065	2.1
Within subgroup error	102	3.15	0.03	
Total	152	6.9		

b	*df*	SS	MS	Fs
Diversity				
Months	8	10.66	1.33	12.17
Core sections	2	1.47	0.73	6.7
MonthsXCore section	16	10.75	0.67	6.14
Within subgroup error	54	5.91	0.11	
Total	80	28.78		
Generic richness				
Months	8	6.81	0.85	31.29
Core section	2	0.14	0.07	2.6
MonthsXCore section	16	2.18	0.14	5.01
Within subgroup error	54	1.47	0.03	
Total	80	10.61		
Evenness				
Months	8	0.84	0.11	8.57
Core section	2	0.3	0.15	12.34
MonthsXCore section	16	1	0.06	5.08
Within subgroup error	54	0.66	0.01	
Total	80	2.8		

c	*df*	SS	MS	Fs
Diversity				
Months	8	24.72	3.09	33.54
Core sections	2	7.36	3.68	39.93
MonthsXCore section	16	8.35	0.52	5.67
Within subgroup error	54	4.97	0.09	
Total	80	45.4		
Generic richness				
Months	8	7.09	0.89	23.2
Core section	2	3.64	1.82	47.61
MonthsXCore section	16	2.87	0.18	4.7
Within subgroup error	54	2.06	0.04	
Total	80	15.67		
Evenness				
Months	8	2.38	0.3	10.85
Core section	2	0.72	0.36	13.08
MonthsXCore section	16	2.62	0.16	5.96
Within subgroup error	54	1.48	0.03	
Total	80	7.2		

Table 2A.13a. Partial regression coefficients (Beta values) for the dependent variable (viable diatoms cm^{-3} wet sediment) and independent variable (grain size) (I = 0-5 cm; II = 5-10 cm; III = 10-15 cm) at mid tide zone (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$)

Depth	Diatoms	Intercept	R	1000	500	250	125	63	< 63
I	*Navicula*	-1.14	0.94	-	-0.99*	-1.29	0.43	1.23	0.11
	Biddulphia	2.87	0.99	-	-0.62**	0.43	-0.08	-0.98*	-0.007
	Pleurosigma	-2.42	0.98	-	-1.01**	-0.86	0.51	0.78	0.057
	Grammatophora	-2.73	0.98	-	0.09	0.76	0.48	-0.46	1.12***
	Coscinodiscus	-1.98	0.99	-	0.09	0.76	0.48	-0.46	1.12***
	Cocconeis	8.06	0.99	-	-0.92***	-0.58	-0.19	-0.1	-0.59**
	Streptotheca	8.56	0.92	-	0.63*	2.29	-1.07	-3.14*	-0.29
II	*Biddulphia*	-3.69	0.95	-	0.86*	-0.69	0.32	-0.17	0.29*
	Bellerochea	-3.69	0.95	-	0.86*	-0.69	0.32	-0.17	0.29*
	Raphoneis	-8.43	0.98	-	0.86*	-0.69*	0.38	-0.21	0.29
III	*Navicula*	-30.7	0.95	-	-0.21	0.56	1.27*	1.73*	2.19*
	Cyclotella	-3.68	0.95	-	0.86*	-0.69	0.32	-0.17	0.29
	Thalassiothrix	21.49	0.98	-	0.95**	-0.93**	-0.54	-1.39*	-0.65
	Bellerochea	-3.69	0.95	-	0.86*	-0.69	0.32	-0.17	0.29
	Raphoneis	-8.43	0.98	-	0.86*	-0.69*	0.39	-0.21*	0.29
	Pleurosigma	-3.69	0.95	-	0.86*	-0.69	0.32	-0.17	0.29

Table 2A.13b. Partial regression coefficients (Beta values) for the dependent variable (viable diatoms cm^{-3} wet sediment) and independent variable (grain size) (I = 0-5 cm; II = 5-10 cm; III = 10-15 cm) at high tide zone (* $p < 0.05$; ** $p < 0.005$; *** $p < 0.01$; **** $p < 0.001$)

Depth	Diatoms	Intercept	R	1,000	500	250	125	63	< 63
I	*Grammatophora*	-54.04	0.96		1.56*	-1.53*	11.95	9.48	2.95
III	*Achnanthes*	-0.97	0.99	-0.38**	0.26	0.72*	0.22	0.49	-0.35
	Biddulphia	-0.87	0.99	-0.38**	0.26	0.72*	0.22	0.48	-0.35
	Melosira	-1.08	0.99	-0.38**	0.26	0.72*	0.22	0.48	-0.35
	Cyclotella	-3.63	0.99	-1.02****	0.12*	-0.04***	2.16**	3.28	0.25
	Pinnularia	17	0.99	0.011	-1.01	1.01	-5.44*	0.54*	0.044
	Coscinodiscus	-2.74	0.99	-1.02****	0.12*	-0.044	2.16***	3.28****	0.25
	Streptotheca	-0.55	0.99	-0.38**	0.26	0.72*	0.22	0.49	-0.35

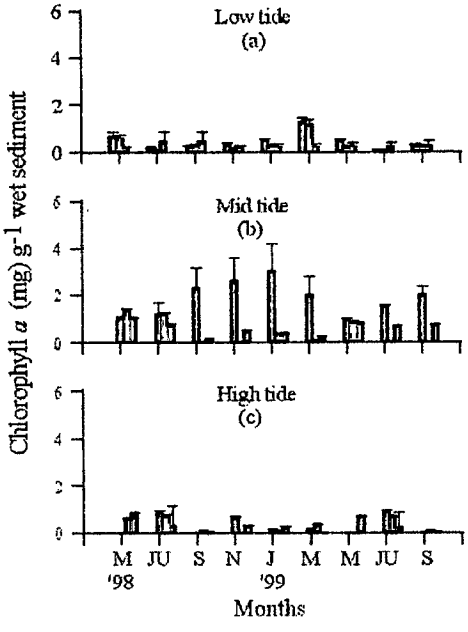


Fig. 2A.18 Concentrations of chlorophyll *a* across the three vertical sections of the sediment core at the low, mid and high tide zones

2A.4 Discussion

The sediment-dwelling diatoms form a major component of the microphytobenthic community in the intertidal region (Meadows and Anderson 1968; Round 1979a). This diatom community is generally classified as the epipsammon (particle attached diatoms) and the epipelon (free living diatoms). However, a third group of diatoms encountered in surf water, i.e. the phytoplankton, may also get incorporated into this diatom community (MacIntyre et al. 1996), some of whose members form a permanent component of this community throughout the year. So far, investigations on diatoms of the microphytobenthic community have been focused on epipsammic and epipelic pennate diatoms (Fenchel and Staarup 1971; Admiraal et al. 1982; de Jonge 1985). Steele and Baird (1968) have reported that the distribution of these epipsammic and epipelic autotrophic flora may extend to a depth of 20 cm. Observation under microscope usually reveals either quite clean-looking grains or grains with a film of mucilage in which bacteria and organic particles are prolific (Round 1981). Due to the difficulties of microscopic observation, the labor involved

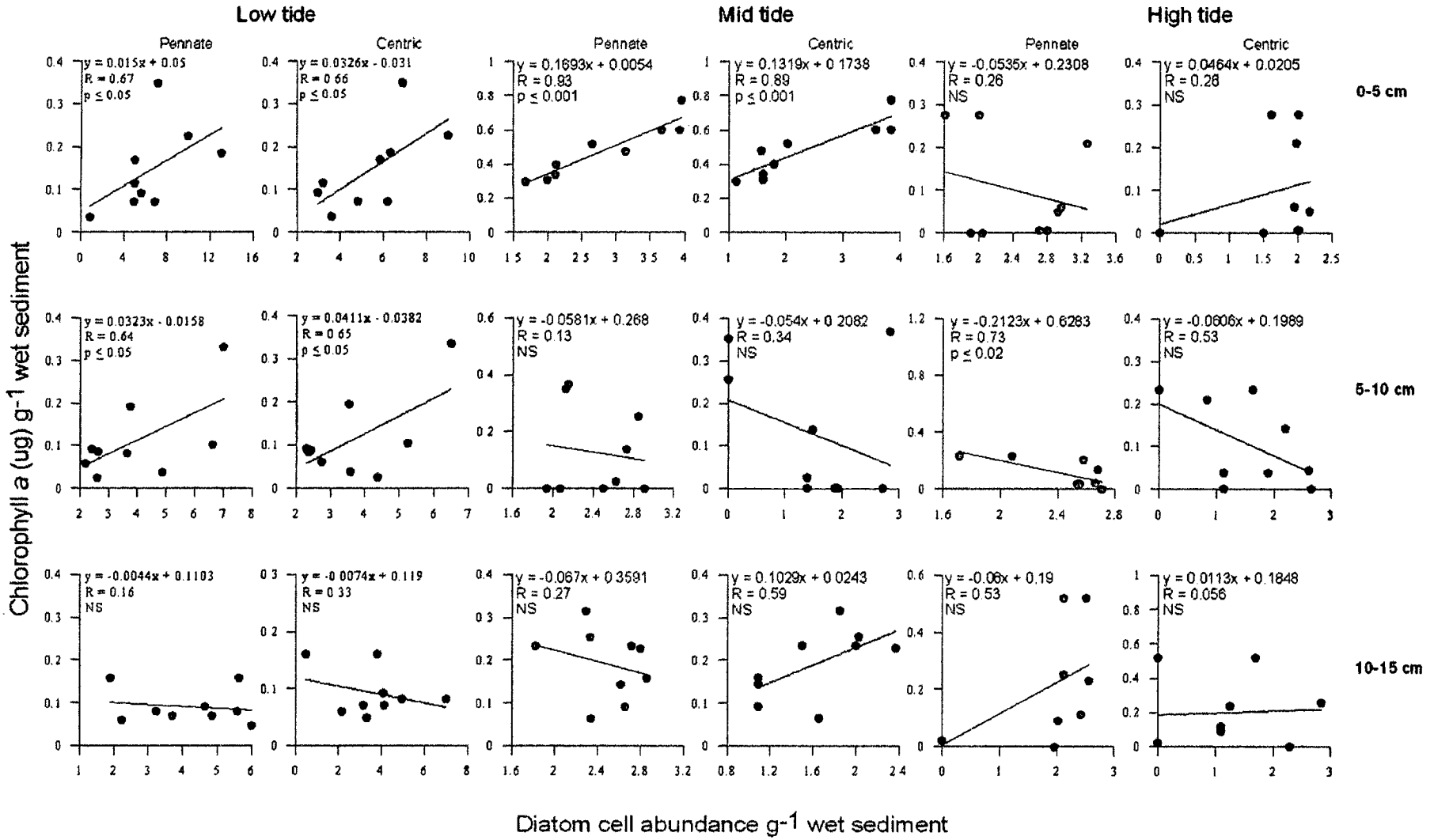


Fig. 2A.19 Linear regression analysis between chlorophyll *a* and pennate and centric diatom abundance across the three vertical sections of the core at the low (a), mid (b) and high tide (c) zones

and the time required, findings have been based on either chlorophyll estimation (Steele and Baird 1968) or epifluorescence microscopic techniques (Grontved 1960; Admiraal et al. 1982; Baillie 1986; Delgado 1989; Cheng et al. 1993). Another widely used method of harvesting diatom cells by using the lens tissue is not purposeful here, since it harvests only the motile pennate diatoms, without providing any information on the sub-surface diatom population (Round and Palmer 1966). The ludox-TM density separation method described by de Jonge (1979) gives quantitative results but is laborious. The MPN method employed in this investigation includes incubation of the sample, which allows the diatoms to grow and multiply and thus enables easy observation (Imai et al. 1984, 1990; Yamochi 1989; Ishikawa and Tamaguchi 1994; Itakura et al. 1997). Although this method does not give the exact number of diatom cells, since it is based on the presence or absence of the diatoms (both vegetative and resting), it qualitates the relative abundance of different taxa. In the present study, f/2 medium was employed for the growth of diatoms, as it has been extensively used in the culture of phytoplankton (French and Hargraves 1980; Eilertsen et al. 1995). However, differences in experimental conditions and the composition of the medium could result in variations that need to be evaluated in detail.

2A.4.1 Intertidal horizontal distribution

The presence of diatoms from the low to the high tide zone reveals their constant redistribution over intertidal areas by tidal action. The diatom population was abundant in the mid tide zone followed by the low and the high tide zone. This pattern of intertidal distribution is the result of one or a combination of several environmental factors. The high tide zone is the most terrestrial of the beach environments. Sand in the low tide zone dries out only at the surface during exposure, whilst in the high tide zone, sand dries for longer periods and to a greater depth. This will also be enhanced

due to the comparatively coarser grain size at this zone. Since, the high tide zone is exposed for a longer period of time, the effects of desiccation in this zone may keep the population low (Bush 1966; McIntire and Wulff 1969). A decreasing oxygen concentration from the waterline landwards (Brafield 1964) can also explain a lower abundance in the high tide zone. Sediment temperatures are more variable with increasing elevation on the beach coincident with a longer period of exposure to incident radiation (Johnson 1965). This greatly fluctuating environment may be a hindrance to the growth of diatoms at the high tide zone. In the low and mid tide zone, the diatom population is exposed to nutrient-laden seawater for longer periods of time than those of the high tide zone. These factors may also enhance the growth of diatoms of the low and mid tide zones.

2A.4.2 Intertidal Vertical distribution

The appearance of diatoms down to a depth of 15 cm from the low to the high tide zones revealed that their viability was not affected by the conditions prevailing at this depth. It was observed that this depth harbored, not only the pennate diatoms, some of which are permanent residents of this area, but also centric forms, which are usually found in the plankton. The occurrence of group I diatoms in the sediment throughout the year indicated that the two pennate forms were natives of this area. The centric diatom *Thalassiosira*, which was present in surf water but whose vegetative cells were not observed in the sediment, appeared after incubation; this must have occurred through the germination of resting stages, which are regularly brought in with coastal sediment and redeposited in the intertidal sediment. Most of the pennate diatoms such as *Navicula* and *Amphora*, which are primarily encountered on bottom sediments, are also often found in the water column (de Jonge and Van Beusekom 1992; Tomas 1997) through resuspension, and it seems reasonable to assume that their primary

production in the water column is as effective as it is on the tidal flats (de Jonge and Van Beusekom 1995). Their presence in the water column is also reflected in the microfouling population of different types of substrata immersed in the sub-surface estuarine waters of the present study area (Smita et al. 1997).

A number of physical, biological and chemical factors may be responsible for the temporal and spatial variation in the diatom abundance, diversity, richness and evenness.

2A.4.3 Physical factors

Wind speed and suspended load: Gabrielson and Lukatelich (1985) and de Jonge (1995) showed that chlorophyll *a* increase in the water column was related to resuspension. Resuspension is dependent on the sediment composition of the tidal flats and wind speed (de Jonge 1995). In a study on the resuspension of microphytobenthos and mud, a statistically significant linear relationship was found between wind speed and the fraction of suspended matter < 55 μm in the water above tidal flats in EMS estuary (de Jonge and Van Beusekom 1992). The wind speed ranged between 2 to 14 m s^{-1} in the EMS estuary (de Jonge 1995). This is much higher than the speeds encountered in the present study area, which range from 2.0 to 4.7 m s^{-1}. However, even at such a comparatively low wind speed, a significant relationship was observed between wind speed and suspended load at the low tide zone, suggesting that the wind speed contributes to resuspension of the sediment only at this zone. Also, the relation of both wind speed and suspended load with chlorophyll *a* and relative abundance of pennate diatoms at the low tide zone, in the 0-5 cm stratum was observed to be negative (Fig. 2A.10). This shows that wind stimulates resuspension of the sediment along with pennate diatoms and, thus, chlorophyll *a* in the 0-5 cm stratum, only at the low tide zone.

Grain size: The dynamic habitat of the sandy shore encompasses grains of different size groups, and their proportion was found to alter with month and depth, only depth and only months for the low, mid and high tide zone respectively (Table 2A.2 and Table 2A.8a-c; Fig. 2A.5 and Fig. 2A.13a-c). It was observed that although a particular grain size at one depth served as a predictor of some diatoms, a similar observation was not evident at other depths and tidal zones (Table 2A.7 and Table 2A.13). This indicates that factors other than grain size play a role in the temporal and vertical distribution of diatoms.

Suspension and deposition: Diatoms such as *Amphora, Navicula* and *Thalassiosira*, which were dominant in the surface sediment layers, were also the dominant diatoms throughout the 15 cm core of the low, mid and high tide zones; this included both pennate and centric diatoms. In sandy sediments, light irradiance can decrease to 1% at a depth of 2-3mm (Rasmussen et al. 1983). Therefore, the dominance of the above-mentioned species beyond the photic zone cannot be attributed to cell division. Moreover, since centric diatoms do not possess the ability to move on their own, a migratory mode of transport can also be ruled out for these forms. Hydrodynamic processes such as the waves are responsible for deposition and resuspension of the sediment (Baillie and Welsh 1980, Delgado et al. 1991; de Jonge and Van Beusekom 1992, 1995; de Jonge and Van den Bergs 1987, de Jonge 1992). The churning of water caused by these processes leads to the suspension of both the diatom-laden sediment particles and the free-living diatom cells in the surface waters. After redistribution, these suspended particles are again deposited on or within the sediment. During this process, an exchange of diatom cells can take place between the surface and deeper layers of the sediment.

Advection: Advective transport of the diatom cells can occur through the sediment column via percolation of surface water, when the sand is loosened during flood tide. The degree of permeability of the sediment depends on the grain size, being higher for sand than for silty sand (Rusch and Huettel 2000). This vector will be more effective in case of planktonic diatoms, present in the overlying waters or left behind on the sediment surface by the receding tides (Huettel and Rusch 2000). In the case of pennate diatoms, only those detached will be affected. Although, all the forms dominant in surface layers were found throughout the 15 cm core in this investigation, the centric diatom *Thalassiosira* dominated the entire community up to 15 cm (Fig. 2A.9a-b and Fig. 2A.15a). It can be assumed that in sandy sediments, oscillatory wave motions (Reid and Kajiura 1957) will cause water exchange to a sediment depth of at least 20 cm (Steele and Munro 1970). Thus, this vector may act as a selective carrier mostly for the loosely attached or the free-living diatom cells, especially the centric forms.

2A.4.4 Biological factors

Grazing: Since diatoms are the major autotrophic organisms of this region, they also form the major food for consumers, e.g. for meio- and macrobenthos. An earlier study revealed that macrobenthic organisms are higher in the food chain than meiobenthic and are represented by more detritus feeders than suspension feeders [amphipoda (40.73 %) and polychaetes (32.52 %] (Rodrigues 1984). It was also observed that the energy increase caused by the wave action and currents at Dona Paula beach is initiated during the pre-monsoon and peaks during the monsoon period, resulting in erosion of the beach (Rodrigues 1984). This results in an increase in the population of these grazers during the post-monsoon and a decrease during the monsoon and pre-

monsoon seasons, which may be one of the factors responsible for the higher abundance of group I and group II diatoms during the pre-monsoon period.

Vertical migration: In a dynamic environment, such as the intertidal region, permanent residents will be adapted to the changing environmental conditions and possess mechanisms to overcome difficult situations. The free-living epipelic pennate diatoms are known to exhibit migratory behavior, whereby they move in the sediment, away from or towards light, the former when the tide rises and the latter when the tide recedes. Migration is said to occur in order: (1) to escape the bright light conditions and reach a place where the conditions are feasible for primary production, (2) to reduce predation by surface feeders and (3) to avoid transport due to resuspension by the advancing tide (Faure-Fremiet 1951; Ganapati et al. 1959; Palmer and Round 1965; Round 1979a; Heckman 1985). In this investigation, two major pennate diatoms (*Navicula* and *Amphora*) were observed up to 15 cm sediment depth (Fig. 6a). Vertical migratory behavior in different sediment types has been related to physical factors such as tidal rhythms and light intensity (Hopkins 1966; Round and Palmer 1966). But many of these field studies were based on a millimeter scale (Hopkins 1963) except one, which reported four diatom species migrating down to 1 cm (Joint et al. 1982). However, work conducted in shallow, outdoor tidal tanks equipped with wave generators showed that vertical migration of a diatom, *Hantzschia virgata* extends beyond 8 cm during high tide (Kingston 1999). Hence, such migratory behavior of these diatoms can result in the transportation of the surface diatoms to deeper layers, much beyond the photic zone. However, this self-initiated mode of transportation is only possible for epipelic pennate diatoms, and hence this cannot be the only factor responsible for diatom transport.

Bioturbation: During the processes of feeding, defecation and locomotion, deposit feeders such as polychaetes, bivalves, amphipods, gastropods and crustaceans continuously rework the sediment. Burrowing forms such as polychaetes (e.g. *Scoloplus kerguelensis*) and amphipods (e.g. *Talorchestia*) dominate the macrofauna of this area (Rodrigues 1984). Many field and laboratory studies have demonstrated that continuous sediment reworking by burrowing animals generally stimulates sediment transportation, carrying along with it the attached and adhered microflora and thus acting as another biological factor in the regulation of species diversity and benthic community structure (Pamatmat 1968; Lukatelich and McComb 1986; de Jonge and Van den Bergs 1987). The fact that diatoms were observed up to 15 cm depth indicates that burrowing organisms play an active role even at this depth. However, in the present investigation, even though the diatom numbers did not reveal any particular trend in depth-wise distribution and were encountered up to 15 cm depth in appreciable numbers, the correlation between chlorophyll *a* and diatom numbers was found to be highest in the surface (0-5 cm) stratum, followed by the 5-10 cm core section and negligible at 10-15 cm depth at the low tide zone. This positive correlation reduced to a depth of 5 cm at the mid tide zone, to 0 cm at the high tide zone. Hence, this indicates that even if bioturbation activity reached beyond the 10 cm zone, its effects must have been overridden by dormancy or desiccation in case of the high tide zone.

2A.4.5 Chemical factors

Nutrients can play an important role in the distribution of some of the diatoms, especially during the late monsoon period, which is characterized by the renewal of nutrients to the surface waters by upwelling and land run off. *Fragilariopsis* and *Skeletonema* (group III) appeared in the intertidal sediment at the low tide zone during

August-September (monsoon). The centric diatom *Skeletonema* blooms during August-September in these waters (Smita et al. 1997) and is carried to the intertidal sediment by advective transport. *Fragilariopsis*, a pennate diatom occurring in chains, was found in high numbers during the same period, both in the water column and in the intertidal sediment. Since the subsiding bloom of *Skeletonema* undergoes resting stage formation and *Fragilariopsis* is a resident of the coastal sediment, carried by water masses to the intertidal sediment or the water column (Fig. 2A.4, 2A.7a-b and 2A.15c). The abundance of centric diatoms at the low tide zone was influenced by nitrate and phosphate, whereas in the case of pennates, which mainly reside in the sediments, nutrients did not play an important role throughout the intertidal zone (Table 2A.6).

2A.4.6 Survival strategies

Once the diatoms have reached a depth of 15 cm, either voluntarily or forcefully, they have to adapt to some life saving strategies, at least till they are brought back to the surface by some mechanism. One of the possibilities for survival is that they adapt to spending long periods in the dark, at low metabolic rates with negligible degradation of pigments (Steele and Baird 1968; French and Hargraves 1980) and uptake of dissolved organic compounds (Admiraal and Peletier 1979).

Laboratory observations have shown that cells of *Hantzschia virgata* exhibit migration up to 8 cm during high tide (Kingston 1999). Taking this fact into consideration, it is obvious that in an area at the lowest low tide zone, the cells cannot migrate up and down during the short period of exposure at every tidal interval. If these vegetative cells are subjected to high sedimentation rates, their presence at such depths without photosynthesizing, for a period beyond their tolerance limit will expose them to unfavorable conditions, such as darkness, under which photosynthesis will be hindered.

Thus, the cell will either undergo transformation from the vegetative to a resting stage, triggered by the surrounding conditions, in order to survive the unfavorable environmental condition(s), or face death and degradation. Experimental work has shown that permanent darkness and low nutrients may result in resting stage formation (Anderson 1975; Hargraves and French 1983).

Although chlorophyll *a* in itself is insufficient to describe fluctuations in benthic diatom standing stock (de Jonge 1980), this pigment provides a useful index for the photosynthesis potential of a population (McIntyre et al. 1996). A significant relation between diatom cell numbers and chlorophyll *a* to a depth of 10 cm and 5 cm at the low tide and mid tide zones respectively revealed that both pennate and centric diatoms contributed to the total pool of chlorophyll *a* down to this depth (Fig. 2A.12 and Fig. 2A.19). In the 0-5 cm stratum, the ability of pennate diatoms to remain attached to the substrate may play an important role in this contribution, whereas centric diatoms may be brought to the sediments by incoming tides. In the 5-10 cm stratum, the positive correlation with chlorophyll *a* revealed that resuspension was not effective down to this depth and that the stock was securely placed. Although viable cells did occur at 15 cm, the reason for the lack of relationship between chlorophyll *a* and diatom cell number at 10-15 cm depth at low tide zone and 5-15 cm at the mid tide zone could be either that there was no sufficient renewal of cells even when advection occurred or that the cells were in dormant stage. The negative correlation at the high tide zone throughout the 15 cm depth may be due to the effects of desiccation when exposed for longer durations. However, they would remain viable as primary producers throughout the intertidal zone down to 15 cm depth, and when they resurfaced could play an important role in the benthic community.

2B.1 Introduction

The intertidal areas are dynamic habitats, subjected to harsh hydrodynamic processes such as waves, currents and tidal exposure. These areas are inhabited by benthic diatoms, which have been divided into two categories. Some of the diatoms move actively in the surface layers and form a component of the epipelon, whilst others are relatively, although not completely immobile, being attached to sand grains contributing to the epipsammon component of the diatom community (Round 1965; 1979b). While both the assemblages are capable of movement, a necessity in such an unstable habitat, only the epipelic species move sufficiently fast (1 to 25 $\mu m\ s^{-1}$) to undergo diurnal rhythmic migrations (Round 1971). The epipsammic species move only very slowly from one sand grain to the other, although even here low-level diurnal rhythmic activity has been reported (Harper and Harper 1967; Harper 1969). Depending on the grain size and organic content, irradiance in sandy sediment decreases to 1% at a depth of 2 to 3 mm (Rasmussen et al. 1983) during low tide. So, only the diatoms in that sediment layer play a role in photosynthesis. But, during high tide turbulence and shear stresses generated by tidal currents or wind waves results in the resuspension of the sediment surface along with the associated diatoms, thus disturbing the microphytobenthic community at the sediment surface. However, as a result of this a substantial part of the biomass of these algae may be found in deeper layers of the sediment. In such conditions, the ecology of the residing microphytobenthos gets highly complex. In order to overcome these difficulties and survive in such an environment, certain mechanisms have been adopted. The survival strategies include adaptation to spending long periods in the dark at low metabolic rates and with negligible degradation of pigments (Steele and Baird 1968; French and Hargraves 1980), uptake of dissolved organic compounds (Admiraal and Peletier

1979) or to undergo transformation from vegetative to a resting stage (Anderson 1975; Hargraves and French 1983). On the other hand, the self-initiated vertical migration helps diatoms to move to the surface during low tides for photosynthesis and move down during high tides (Round and Palmer 1966; Round 1979b; Joint et al. 1982; Paterson 1989). There exists a controversy regarding the reason for the vertical migration and the factors affecting it. Light and tidal rhythm are the two variables, which seem to induce this migratory behavior (Round and Palmer 1966).

Much of the literature concerning vertical migration of benthic microalgae has centered on species inhabiting mud flats, where the amplitude of migration is generally 3mm for diatoms (Aleem 1950; Hopkins 1963; Round and Happey 1965; Round and Eaton 1966; Round and Palmer 1966; Palmer and Round 1967; Round 1978; Paterson 1986; Pinckney et al. 1994). In case of the sand flat community, Round (1979a) observed *Hantzschia virgata* migrate up to a depth of 3 mm whereas Joint et al. (1982) found three diatom genera (*Navicula, Diploneis and Nitzschia*) migrate down to 1cm depth. Several other studies confirmed that most of the living diatoms on sand flats occur in the uppermost centimeter of sand (Meadows and Anderson 1968; Harper 1969; Riznyk and Phinney 1972; Round 1979a, b). However, work conducted in shallow outdoor tidal tanks equipped with wave generators showed that vertical migration of *Hantzschia virgata* could extend beyond 8 cm during high tide (Kingston 1999).

Vertical migration can have important consequences for measurements of both diatom abundance and photosynthesis. Moreover, the effort and expense of counting diatoms and the difficulty of calculating biomass from cell abundance preclude the use of cell counts in most investigations. Instead, the photosynthetic pigment, chlorophyll *a* is used as an index of microphytobenthic biomass.

This investigation explored the behavior of diatoms with reference to tidal cycles in the field, as well as when removed from the effects of tides in the laboratory and incubated under 12 h light: 12 h dark, continuous dark, or continuous light.

2B.2 Materials and methods

2B.2.1 Sampling site

Sediment sampling was carried out on a sand flat at Dias Beach (15^0 27' N; 73^0 48' E), located near Dona Paula Bay and surrounded by the Mandovi and Zuari estuaries. This beach is 200 m long. The tides are semi-diurnal, with an average spring tide range of ~ 2 m and neap tide range of ~ 0.7 m.

2B.2.2 Sampling period

The first sampling was carried out on 30 - 31 March 2001 (Summer season; case I) and the second sampling on 14 - 15 January 2002 (Winter season; case II). The sampling program is given in Table 2B.1.

Table 2B.1. Details of the tidal time, tidal amplitude, temperature, day length, total sunshine and solar radiation of the sampling days

Sampling date	Sampling period	Tidal Time (H)	Tidal amplitude (m)	Temperature (^{0} C)	Day length (H)	Total Sunshine (H)	Solar radiation (mw cm^{-2})
30th March 2001	Morning Low Tide	08:02	0.33	27	12.17	8.2	10.4 - 27.4
	Noon High Tide	14:50	1.94				54.1 - 73.5
	Evening Low Tide	20:25	1.11				1.1 - 1.2
31st March 2001	Night High Tide	01:51	1.9		12.2	9.5	1 - 1.1
	Morning Low Tide	08:49	0.38				27.2 - 47.1
14th January 2002	Mid-Morning High Tide	10:48	1.83	21	11.04	9.9	33.4 - 61.4
	Evening Low Tide	17:25	0.27				4.5 - 20.7
	Night High Tide	00:32	2.27				1.0 - 1.1
15th January 2002	Early Morning Low Tide	06:27	1.09		11.05	9.5	1.1 - 1.4
	Mid Morning High Tide	11:25	1.82				60.7 - 70.2

2B.2.3 Sediment characteristics

Very fine sand (63 to 124 μm) is the most abundant grain size fraction of the sediment. The sediment was moderate to well sorted, with a sorting coefficient ranging from 0.32 to 0.58 (Mitbavkar and Anil 2002).

2B.2.4 Sediment core collection

Sediment cores (1 cm length) were collected in five replicates using small poly-vinyl chloride (PVC) corers with an inner diameter of 3 cm.

2B.2.5 Sampling program

During each of the morning and noon tides of case I and mid-morning and evening tides of case II (using a particular reference point), 10 cores were collected for immediate observation whereas for laboratory incubation 80 cores were collected in summer and 120 cores in winter (Fig. 2B.1). The cores were sealed at the lower ends with PVC caps and transferred to the laboratory. Of the total cores collected for laboratory incubation, 40 cores were incubated in 12 h light: 12 h dark, 40 cores in continuous dark, and the additional 40 cores collected during winter were incubated in continuous light. Incubation was carried out at 27 ± 1 ^{0}C under a light intensity of 32.3 mw cm $^{-2}$. Simultaneously, the set of cores collected for immediate observation were sectioned immediately and out of the 10, 5 were used for diatom enumeration and 5 for chlorophyll *a* analyses.

Out of the 40 cores from each of the incubated conditions, 10 were removed after every 6-hour. Five cores were used for chlorophyll *a* analyses and 5 were used for diatom enumeration. Simultaneously during the same period, cores were collected from field for immediate observation.

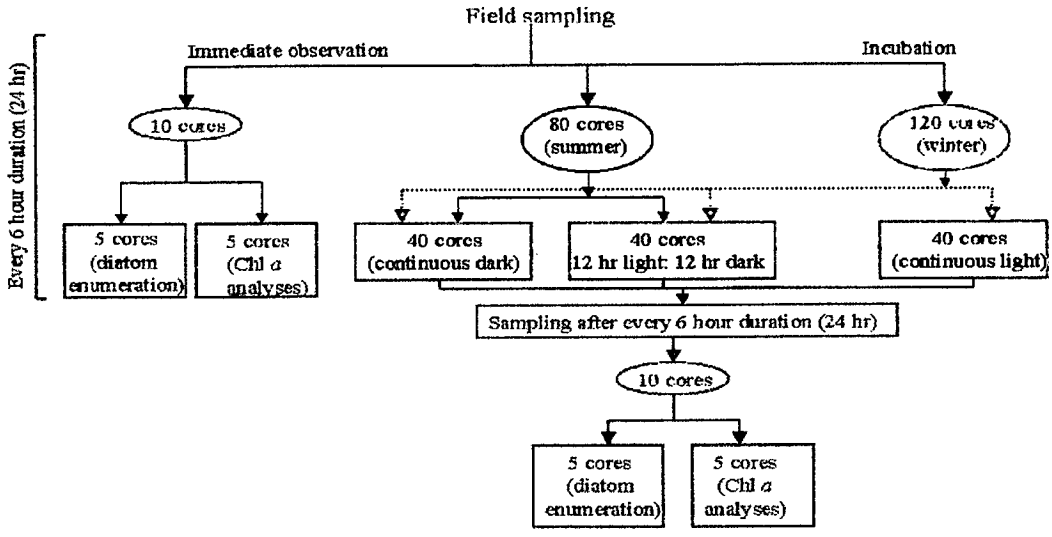


Fig. 2B.1 Schematic representation of the experimental protocol

2B.2.6 Core sectioning

All sediment cores were sectioned at 1mm intervals. Out of these, the 1st, 3rd, 5th and 7th mm sections were used for diatom enumeration and chlorophyll *a* analyses.

2B.2.7 Diatom enumeration

Each of the sediment core sections was suspended in autoclaved seawater. As epipelic diatoms dominated the summer samples, the sediment was washed thoroughly five times to suspend the free and loosely adhered epipelic diatom cells from sand grains. This supernatant was separated and used for epipelic diatom quantification and identification. In the winter samples, epipelic as well as epipsammic diatoms dominated the population, so the washed sediment, after the separation of epipelic diatoms was further subjected to ultrasonication to separate the attached epipsammic diatoms. The diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyan (1946), Desikachary (1987) and Tomas (1997).

2B.2.8 Chlorophyll a analyses

A known amount of the sediment sample from the respective depth interval was extracted in 90% acetone for 18 h (corrected for phaeopigments) (Parsons et al. 1984).

2B.2.9 Data analysis

The log (X+1) transformed data on diatom abundance of the low tide and high tide incubated cores under different conditions were subjected to multiple analyses of variance (MANOVA) (Sokal and Rohlf 1981).

2B.3 Results

2B.3.1 Case I

On this occasion, low tide occurred in the morning after sunrise. The values of solar radiation and tidal amplitude are given in Table 2B.1. During the morning exposure period, randomly distributed brownish patches were observed. Immediate microscopic observation of this sediment revealed dominance of two epipelic diatom species, *Navicula transitans* var. *derasa* f. *delicatula* Heimdal and *Amphora coffeaeformis* (Agardh) Kutzing. Hence, case I study was restricted only to these two dominant forms.

Temporal variation in the field

Cell abundance of epipelic diatoms and chlorophyll *a* concentrations were high in the surface 1mm only during the morning low tide (Fig. 2B.2a and 2B.3a). Of the two dominant diatoms, *A. coffeaeformis* was abundant (Fig. 2B.4a and 2B.5a). Cell density at the 3, 5 and 7mm sediment core depths was negligible as compared to that of the surface. The fact that the period of decline in surface population was not reflected as an increase at the lower depths sampled, suggests that either the cells have been suspended into the surface waters or migrated beyond the 7mm depth.

Observation of the laboratory incubated cores sampled during (a) Morning low tide

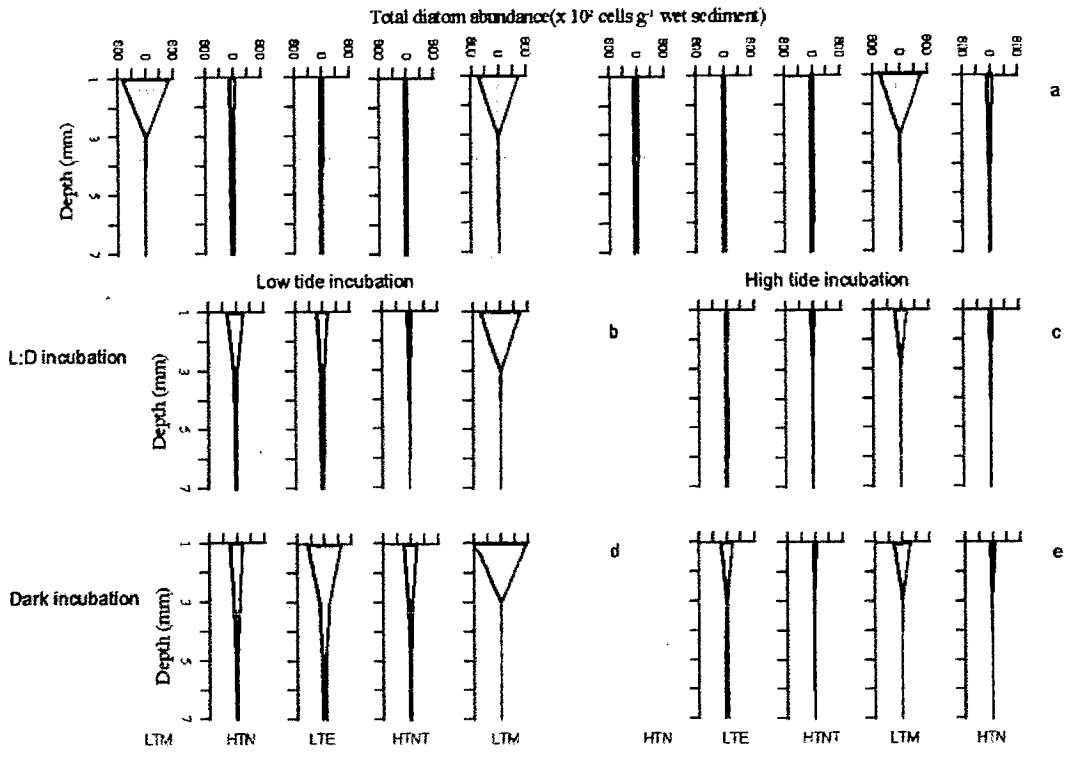


Fig. 2B.2 Depthwise distribution of mean total diatom (10^2 cells g^{-1} wet sediment) of case I sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e)]
LTM=Low tide morning; HTN=High tide noon; LTE=Low tide evening; HTNT=High tide night

12 h light: 12 h dark incubation: When incubated under this condition, the diatom population and chlorophyll *a* concentrations were found to follow a trend similar to that observed in the field (Fig. 2B.2b and 2B.3b).

Continuous dark incubation: When incubated in continuous darkness, the surface cell abundance and chlorophyll *a* concentrations revealed a tidal rhythm, wherein the period of field emersion and high tide cover coincided with surface maxima and subsequent fall respectively (Fig. 2B.2d and 2B.3d).

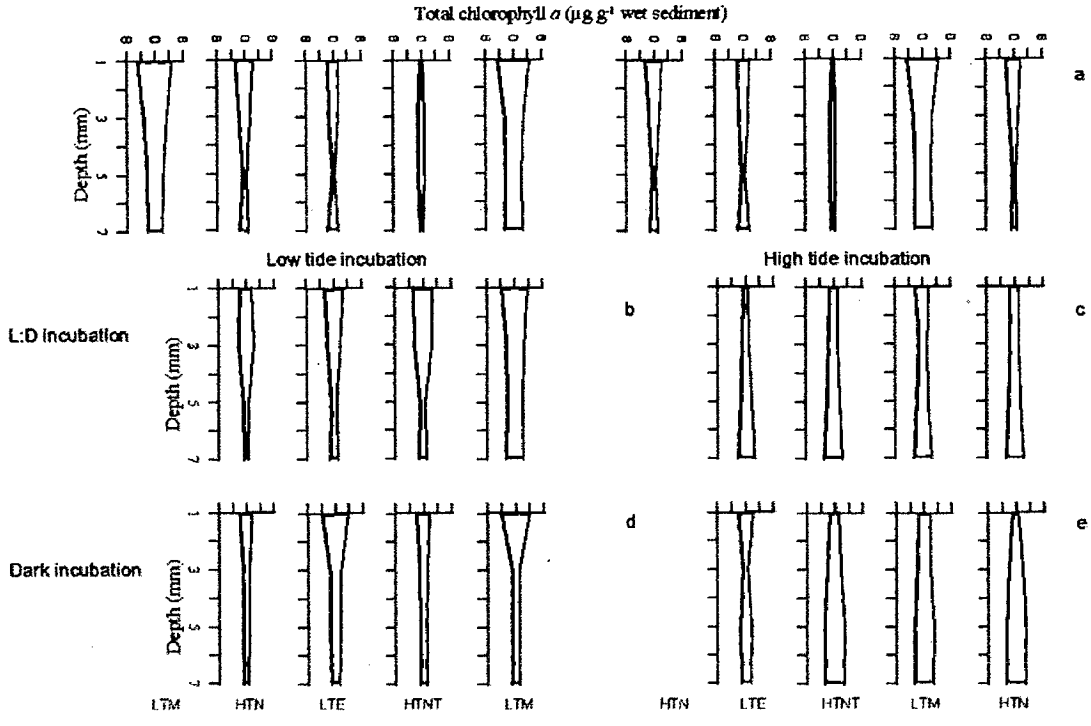


Fig. 2B.3 Depthwise distribution of chlorophyll *a* (µg g $^{-1}$ wet sediment) of case I sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e)]
LTM=Low tide morning; HTN=High tide noon; LTE=Low tide evening; HTNT=High tide night

(b) Noon high tide

As the cores were collected during high tide, the surface diatom population was comparatively lower than that collected during low tide. Resuspension into surface waters or migration beyond the sampled depth are the probable factors responsible for the low cell abundance. On incubation in the 12 h light: 12 h dark and continuous dark condition, this population exhibited the same rhythm as the cores collected during the morning low tide (Fig. 2B.2c and 2B.2e).

Comparison between low tide and high tide incubated cores

MANOVA did not reveal any significant variation between the total diatom abundance of the low tide and high tide incubated cores with incubation time and depth when subjected to 12 h light: 12 h dark incubation (Fig. 2B.2b and 2B.2c). *A.*

coffeaeformis and *N. delicatula* population behaved similarly (Fig. 2B.4b, 2B.4c, 2B.5b and 2B.5c). However, in the dark incubated cores, variation in diatom abundance between the tides with incubation time (Fig. 2B.2d and 2B.2e) is due to the

Table 2B.2 Significance values of the MANOVA evaluating the temporal variation in diatom abundance of case I along the vertical gradient (1, 3, 5 and 7mm sediment core sections) with respect to incubation conditions.
(NS- Not-Significant) (a=Tides; b=Incubation time; c=Depth)

	12 hr light: 12 hr dark incubation			Continuous dark incubation		
	axb	axc	bxc	axb	axc	bxc
		p ≤			p ≤	
Total diatom abundance	NS	NS	NS	0.025	NS	NS
Amphora coffeaeformis abundance	NS	NS	NS	NS	NS	NS
Navicula delicatula abundance	NS	NS	NS	0.025	NS	0.05
Chlorophyll *a*	0.005	0.001	NS	NS	0.025	NS

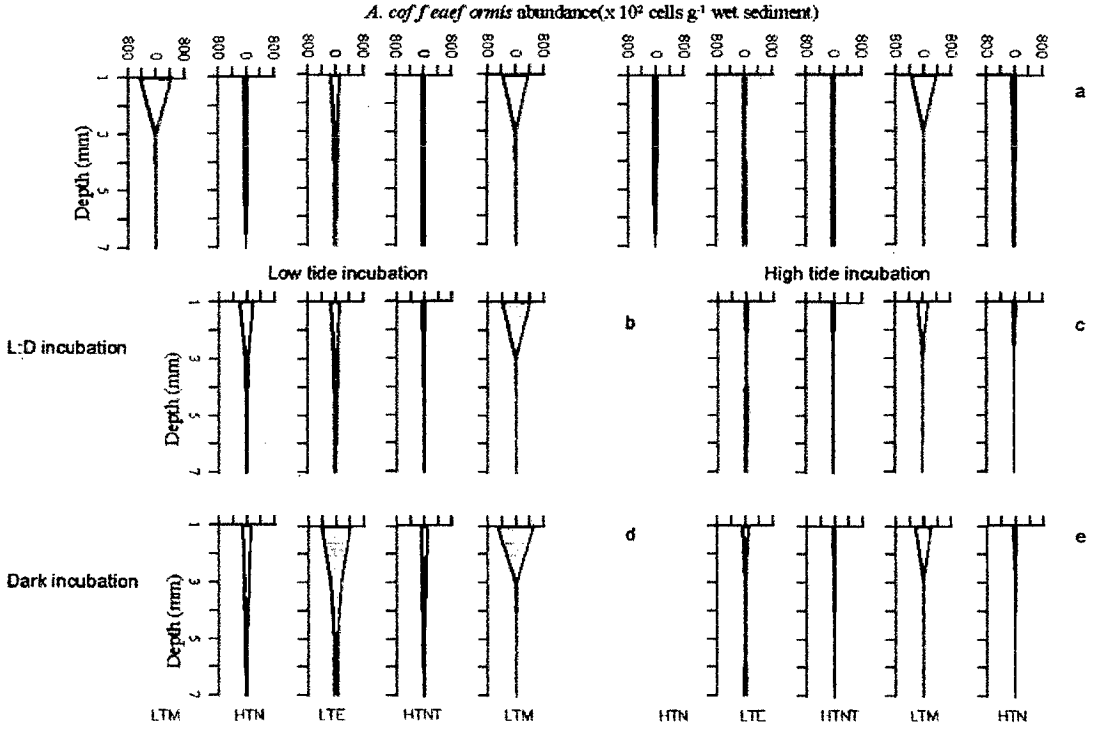


Fig. 2B.4 Depthwise distribution of *A. coffeaeformis* (10^2 cells g^{-1} wet sediment) of case I sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e)]
LTM=Low tide morning; HTN=High tide noon; LTE=Low tide evening; HTNT=High tide

comparatively lower initial number of cells in the high tide incubated cores which resulted in a lower abundance during the surface migration as compared to the low tide incubated core (Table 2B.2).

Comparison between 12 h light: 12 h dark and continuous dark incubations

Table 2B.3 Significance values of the MANOVA evaluating the temporal variation in diatom abundance of case I along the vertical gradient (1, 3, 5 and 7mm sediment core sections) with respect to low and high tide incubations. (NS- Not-Significant) (b=Incubation time; c=Depth; d=Conditions)

	Low tide incubation			High tide incubation		
	dxb	dxc	bxc	dxb	dxc	bxc
		p ≤			p ≤	
Total diatom abundance	0.005	NS	0.001	NS	NS	0.001
Amphora coffeaeformis abundance	0.05	0.025	0.05	NS	0.025	0.05
Navicula delicatula abundance	0.05	NS	0.001	0.001	NS	0.01
Chlorophyll *a*	NS	NS	NS	NS	NS	NS

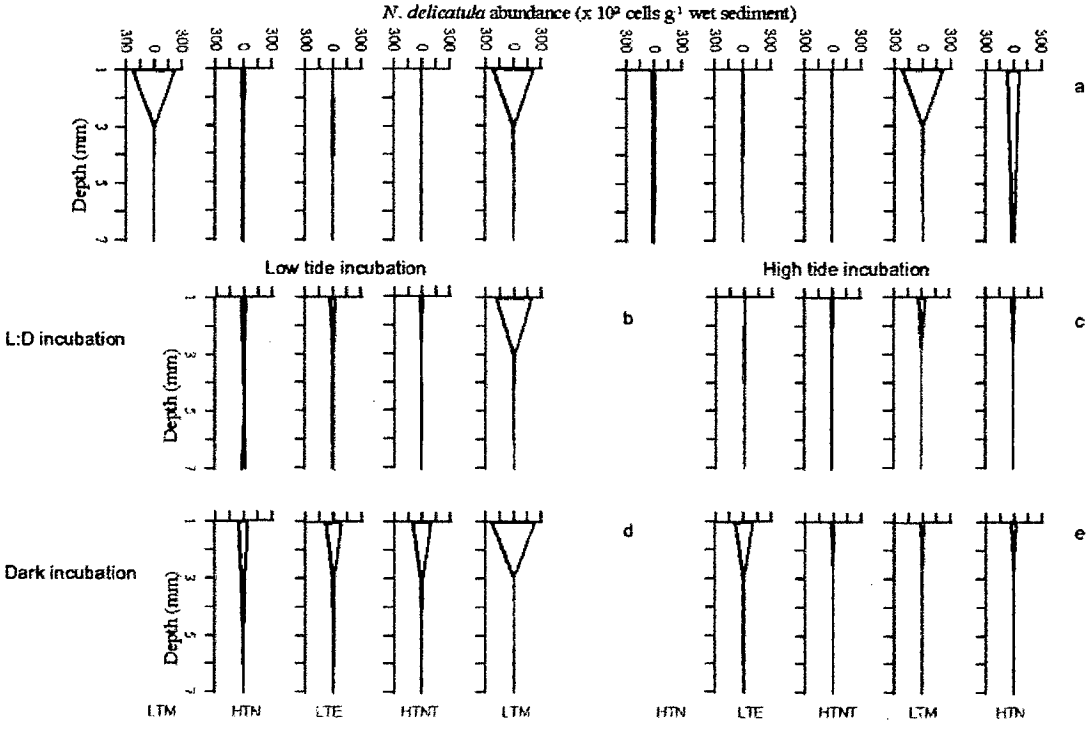


Fig. 2B.5 Depthwise distribution of *N. delicatula* (10^2 cells g^{-1} wet sediment) of case I sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e)]
LTM=Low tide morning; HTN=High tide noon; LTE=Low tide evening; HTNT=High tide

When the diatom abundance of the low tide incubated cores were subjected to MANOVA, the total epipelic diatom abundance revealed a significant variation between the conditions (12 h light: 12 h dark and continuous dark) with time (Fig. 2B.2b and 2B.2d). Both *A. coffeaeformis and N. delicatula* populations exhibited this variation (Fig. 2B.4b, 2B.4d, 2B.5b and 2B.5d). Each of these conditions also revealed depthwise variation in diatom abundance with incubation time. In the high

tide incubated cores, in each of the conditions the total diatom abundance revealed a significant depthwise variation with incubation time (Fig. 2B.2c, 2B.2e, 2B.4c, 2B.4e, 2B.5c and 2B.5e). Chlorophyll *a* concentrations did not show any significant variation between the two conditions with time as well as with depth (Table 2B.3).

2B.3.2 Case II

On this occasion, low tide occurred before sunrise. The values of solar radiation and tidal amplitude are given in Table 2B.1. First sampling was carried out during mid-

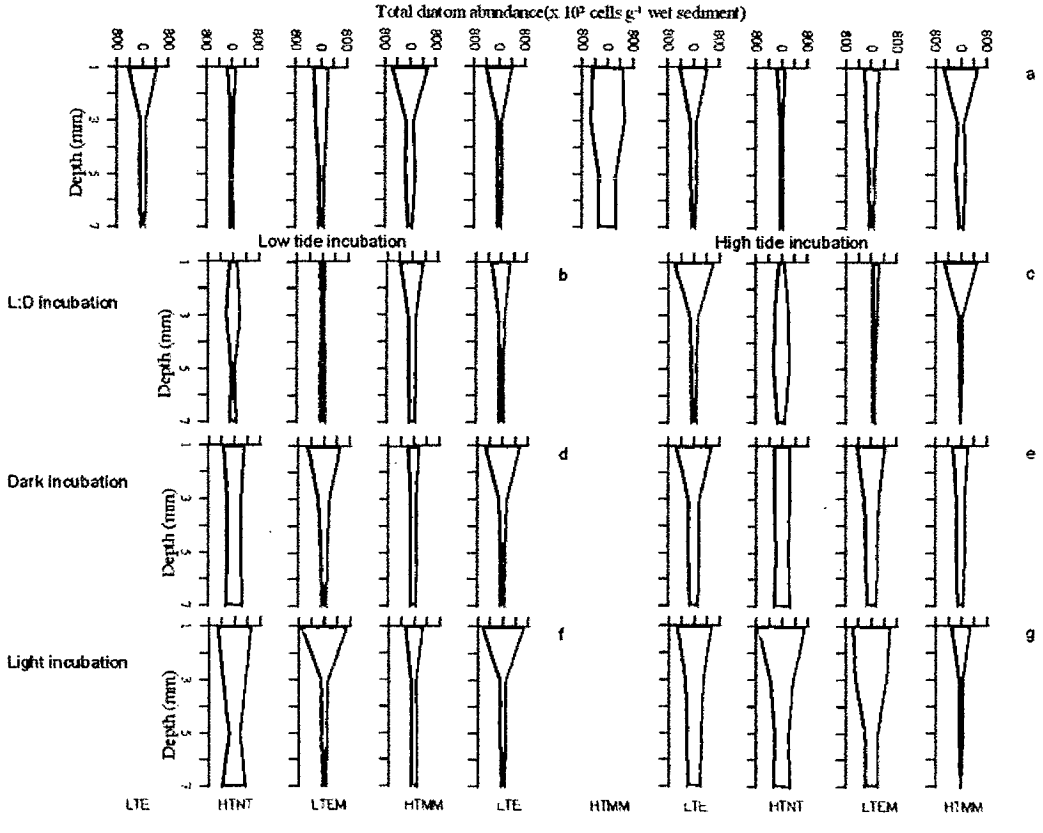


Fig. 2B.6 Depthwise distribution of mean total diatom (10^2 cells g^{-1} wet sediment) of case II sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d), continuous light (f)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e), continuous light (g)] LTE=Low tide evening; HTHT=High tide night; LTEM=Low tide early morning; HTMM=High tide mid-morning

morning high tide subsequent to the early morning low tide. Immediate observation of the field-collected cores revealed a mixture of epipelic (*N. delicatula* and *A. coffeaeformis*) and epipsammic diatoms (*Cocconeis scutellum* Ehrenberg, *Cymbella*

sp., *Amphora costata* Wm. Smith, *Rhaphoneis amphiceros* (Ehrenberg) Ehrenberg and *Grammatophora marina* (Lyngbye) Kutzing), which were found in almost equal abundance.

Temporal variation in the field

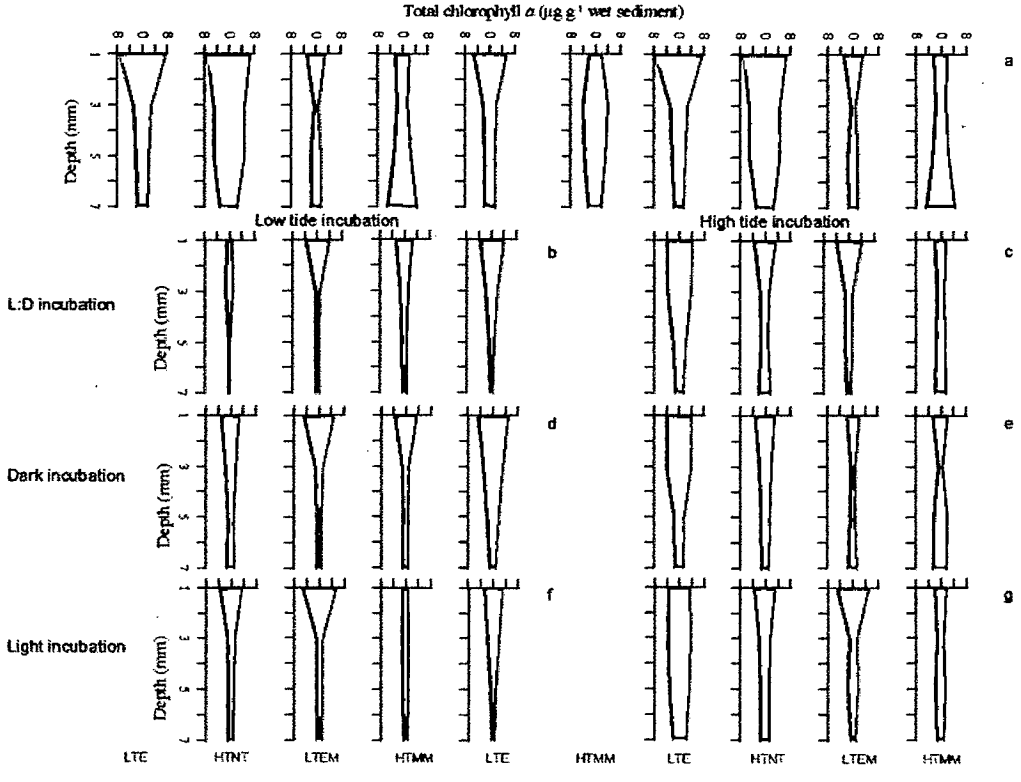


Fig. 2B.7 Depthwise distribution of chlorophyll *a* (µg g $^{-1}$ wet sediment) of case II sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d), continuous light (f)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e), continuous light (g)]
LTE=Low tide evening; HTHT=High tide night; LTEM=Low tide early morning; HTMM=High tide mid-morning

Cell abundance of epipelic diatoms was high in the surface 1mm during the mid-morning high tide. This maximum continued even during the subsequent evening low tide. The following night high tide and early morning low tide revealed a decrease in the surface epipelic cell abundance (Fig. 2B.6a). Thus in this winter season, diatom surface migration was observed from mid-morning to evening on a diel basis. The surface cell abundance of epipelic and epipsammic diatom population was not reflected in the chlorophyll *a* concentrations during the mid-morning high tide (Fig.

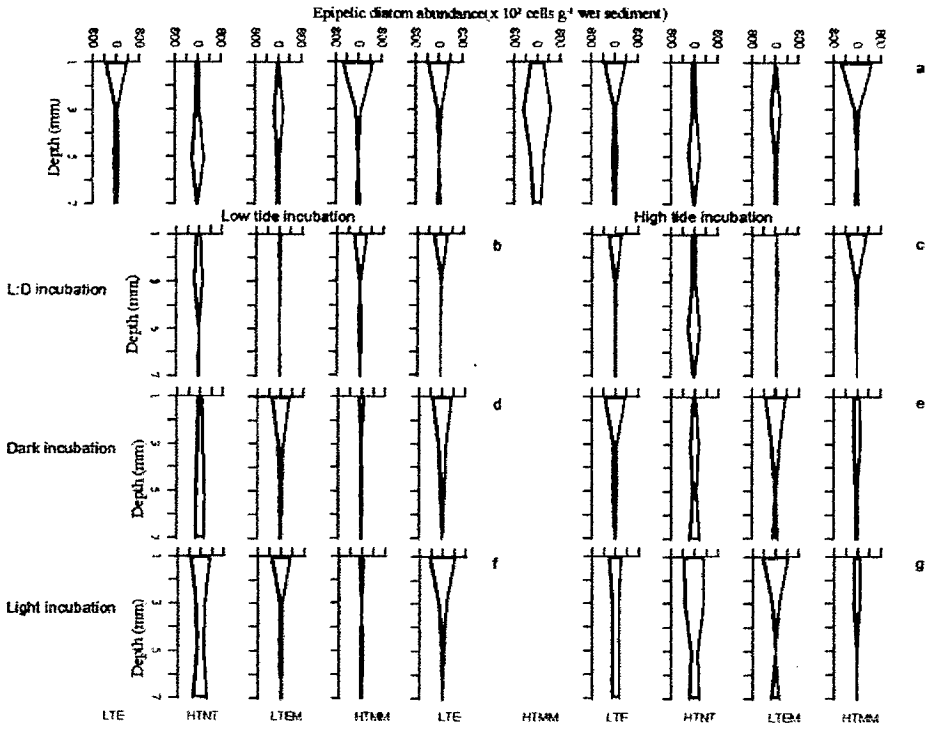


Fig. 2B.8 Depthwise distribution of mean epipelic diatom (10^2 cells g^{-1} wet sediment) of case II sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d), continuous light (f)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e), continuous light (g)]
LTE=Low tide evening; HTHT=High tide night; LTEM=Low tide early morning; HTMM=High tide mid-morning

2B.7a, 2B.8a and 2B.9a). However, in the evening low tide exposure, the surface abundance of epipelic diatoms was reflected in chlorophyll *a* concentrations (Fig. 2B.7a and 2B.8a).

Observation of the laboratory incubated cores sampled during (a) Mid-morning high tide

12 h light: 12 h dark incubation: When incubated under this condition, similar results were obtained as that during case I incubation wherein the surface epipelic diatom abundance (*N. delicatula* and *A. coffeaeformis*) was found to follow a trend similar to that observed in the field (Fig. 2B.8c, 2B.10c and 2B.11c). Such a behavior was not observed in the epipsammic diatom population (Fig. 2B.9c).

Continuous dark incubation: When incubated in continuous darkness, epipelic diatom population exhibited a tidal rhythm (Fig. 2B.8e, 2B.10e and 2B.11e).

Continuous light incubation: When incubated in continuous light, the epipelic diatom population was seen to remain at the surface for a period of 18 h, i.e., from the time of collection, mid-morning high tide to the corresponding evening low tide to night high tide to early morning low tide of field (Fig. 2B.8g). Chlorophyll *a* concentrations also revealed similar results (Fig. 2B.7g).

(b) Evening low tide

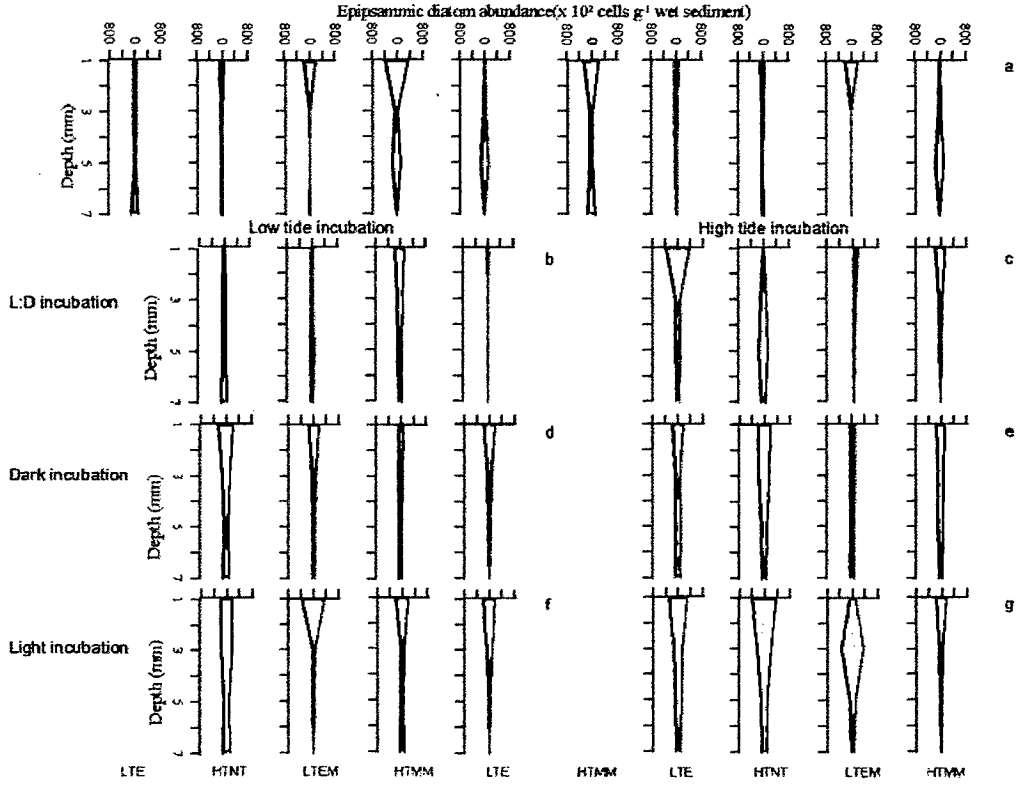


Fig. 2B.9 Depthwise distribution of mean epipsammic diatom (10^2 cells g^{-1} wet sediment) of case II sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b). continuous dark (d), continuous light (f)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e), continuous light (g)]
LTE=Low tide evening; HTHT=High tide night; LTEM=Low tide early morning: HTMM=High tide mid-morning

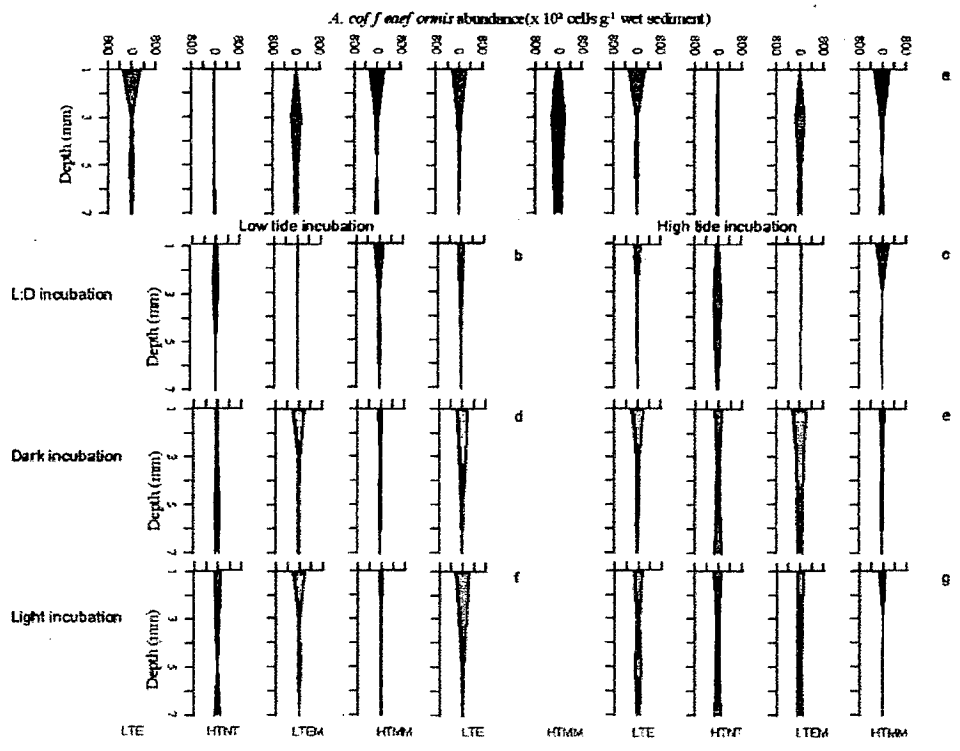


Fig. 2B.11 Depthwise distribution of *A. coffeaeformis* (10^2 cells g^{-1} wet sediment) of case II sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d), continuous light (f)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e), continuous light (g)]
LTE=Low tide evening; HTHT=High tide night; LTEM=Low tide early morning; HTMM=High tide mid-morning

In this case, the 12 h light: 12 h dark and continuous dark incubation trend was similar as that observed for the high tide incubated cores (Fig. 2B.8b and 2B.8d). Chlorophyll *a* concentrations did not reveal a similar trend (Fig. 2B.7b and 2B.7d).

Continuous light incubation: When incubated in continuous light, the diatom population remained at the surface for a period of 18 h, i.e., mid-morning high tide to the evening low tide field exposure, to the corresponding night high tide to early morning low tide of field (Fig. 2B.8f). Chlorophyll *a* concentrations were high up to 12 h, beyond which it showed a decline (Fig. 2B.7f).

Comparison between low and high tide incubated cores

Table 2B.4. Significance values of the MANOVA evaluating the temporal variation in diatom abundance of case II along the vertical gradient (1, 3, 5 and 7mm sediment core sections) with respect to incubation conditions.
(NS- Not-Significant) (a=Tides; b=Incubation time; c=Depth; d=Condition)

	12 hr light: 12 hr darkincubation			Continuous dark incubation			Continuous light incubation		
	axb	axc	bxc	axb	axc	bxc	axb	axc	bxc
		p ≤			p ≤			p ≤	
Total diatom abundance	NS	NS	NS	NS	NS	NS	NS	NS	NS
Epipelic diatom abundance	NS	NS	NS	0.01	NS	NS	0.005	NS	NS
Amphora coffeaeformis abundance	NS	NS	NS	0.025	NS	NS	0.025	NS	NS
Navicula delicatula abundance	NS	NS	NS	0.05	NS	NS	0.005	NS	NS
Epipsammic diatom abundance	NS	NS	NS	NS	NS	NS	NS	NS	NS
Chlorophyll *a*	NS	NS	0.05	NS	NS	0.025	NS	NS	0.025

MANOVA did not reveal any significant variation between the diatom abundance (for epipelic i.e., *N. delicatula* and *A. coffeaeformis* and epipsammic diatoms) of the low and high tide incubated cores when subjected to 12 h light: 12 h dark incubation (Fig.

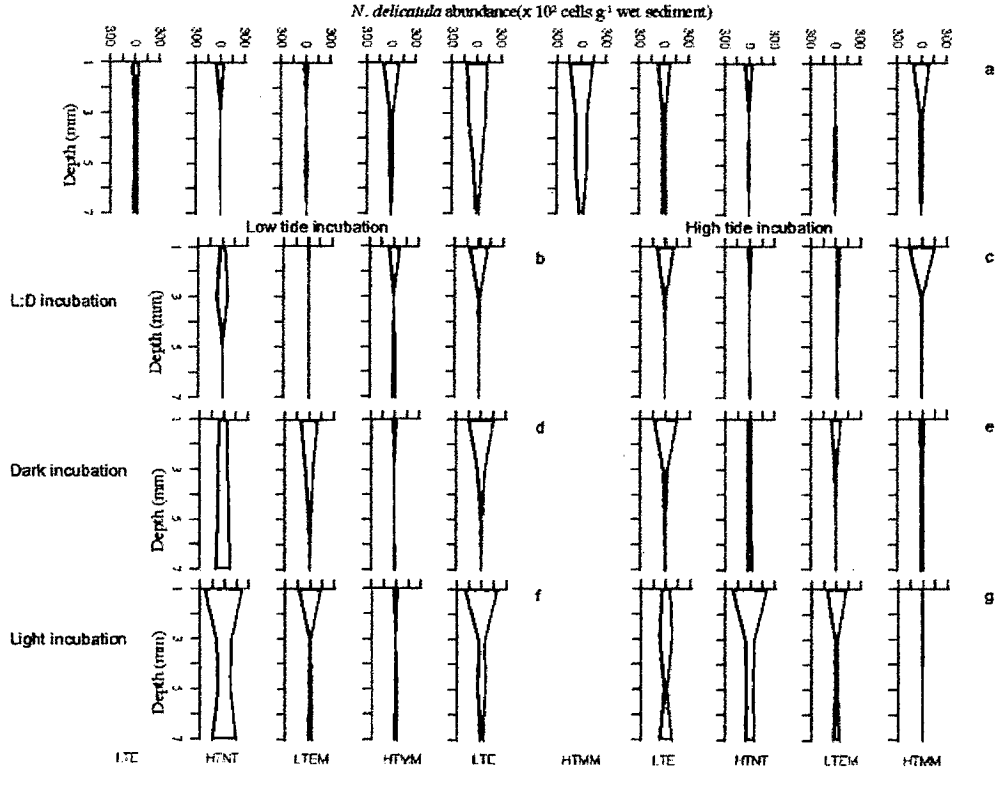


Fig. 2B.11 Depthwise distribution of *N. delicatula* (10^2 cells g^{-1} wet sediment) of case II sediment cores for the different tidal periods in the field (a), under controlled conditions for the "low tide" [12 h light: 12 h dark (b), continuous dark (d), continuous light (f)] and high tide incubated cores [12 h light: 12 h dark (c), continuous dark (e), continuous light (g)]
LTE=Low tide evening; HTHT=High tide night; LTEM=Low tide early morning; HTMM=High tide mid-morning

2B.8b, 2B.8c, 2B.9b, 2B.9c, 2B.10b, 2B.10c, 2B.11b and 2B.11c). In the continuous dark and continuous light condition, epipelic diatoms (*N. delicatula* and *A. coffeaeformis*) showed significant variation between the tides and incubation time (Fig. 2B.8d-g, 2B.10d-g, 2B.12d-g; Table 2B.4).

Comparison between 12 h light: 12 h dark, continuous dark and continuous light incubations

Table 2B.5. Significance values of the MANOVA evaluating the temporal variation in diatom abundance of case II along the vertical gradient (1, 3, 5 and 7mm sediment core sections) with respect to low and high tide incubations. (NS- Not-Significant) (a=Tides; b=Incubation time; c=Depth; d=Condition)

	Low tide incubation			High tide incubation		
	dxb	dxc	bxc	dxb	dxc	bxc
		p ≤			p ≤	
Total diatom abundance	NS	NS	0.05	NS	NS	0.001
Epipelic diatom abundance	NS	NS	0.01	NS	NS	0.005
Amphora coffeaeformis abundance	NS	NS	0.01	NS	NS	0.001
Navicula delicatula abundance	NS	NS	0.05	0.025	NS	0.05
Epipsammic diatom abundance	0.001	NS	NS	NS	NS	0.001
Chlorophyll *a*	NS	NS	NS	NS	NS	0.001

When the diatom abundance values of the low tide and high tide incubated cores were subjected to MANOVA, the epipelic diatoms (*N. delicatula* and *A. coffeaeformis*) revealed a significant variation between incubation time and depths (Fig. 2B.8, 2B.10 and 2B.11). This variation was not significant for the epipsammic diatoms (Fig. 2B.9; Table 2B.5).

2B.4 DISCUSSION

During the morning low tide exposure, comparatively higher irradiance and lower tidal amplitude triggered the upward migration of diatoms in case I, in contrast to case II. Subsequently, availability of sufficient light for photosynthesis during the morning exposure and also, increasing light intensity and tidal amplitude, triggered the diatoms to migrate downwards just prior to the noon high tide. Round (1979b) and Admiraal (1984) have proposed that benthic microalgae are adapted to respond to excessive

irradiance levels by migrating downwards into darker layers of sediment. Nielsen et al. (1995) and Nielsen and Ekelund (1995) has demonstrated that exposure to solar ultraviolet radiation significantly affects phytoplankton photosynthesis and cell motility. The ability of microphytobenthos to migrate vertically within the surface sediment may be considered as a form of behavioral photoacclimation, allowing cells to avoid potentially damaging irradiance and temperature conditions (Kromkamp et al. 1998; Perkins et al. 2001). There is also a possibility of some part of the population being suspended into the overlying waters due to the rising tides. Case II, where the diatoms exhibited upward migration during the high tide coverage reveals the overriding influence of light over tides. During the high tide, the overlying water and turbidity reduces the amount of light available for photosynthesis, as a result of which the diatoms continued to remain at the surface even during the evening low tide. Perkins (1960), Round and Palmer (1966), Happey-Wood and Jones (1988) and Janssen et al. (1999) have reported a similar behavior for diatoms inhabiting muddy sediments. So, migratory behavior of diatoms can persist at the sediment-water interface in the evening low tides, provided the light intensity and tidal amplitudes are favorable, as in case II. In case I, the lowered light intensity and higher tidal amplitude did not favor surface migration. The subsequent rise in tidal amplitude and absence of light triggered the diatoms towards positive geotrophy, in both case I and case II. Strickland (1958) has reported that optimal irradiance for photosynthesis varies with latitude and season, which may explain the behavioral differences between the two cases.

In this entire process of vertical migration, an important factor that needs consideration is the impact of physical forcing on the sediment caused by wind or tidal currents. Turbulence and shear stress generated by the incoming tide lead to

suspension of the diatom cells into the overlying waters (Baillie and Welsh 1980; Delgado et al. 1991; de Jonge and Van Beusekom 1992; 1995; de Jonge and Van den Bergs 1987). This may be an additional factor responsible for the lowering of the surface diatom population, other than their positive geotrophic movement during immersion. However, the entire suspended population will not be lost to water column, since a part of it will start resettling at the beginning of emersion. This process will in turn contribute to the rise in cell numbers at the sediment surface during subsequent exposure, along with the upward migration from the deeper sediment layers.

2B.4.1 Chlorophyll a

The high concentrations of chlorophyll *a* with a significant relation with diatom abundance (Table 2B.2) confirms the upward migration during the morning low tide in case I (Fig. 2B.2a). In case II, lack of sufficient amount of light for photosynthesis due to the water coverage during the mid-morning high tide resulted in reduced chlorophyll *a* concentrations (Fig. 2B.7a). This is evident from the non-significant relation between the chlorophyll *a* and diatom abundance (Table 2B.3). Variation in cellular chlorophyll *a* as a function of irradiance is well documented for diatoms (Durbin 1974; Paasche 1968; Brown and Richardson 1968; Beardall and Morris 1976). In the temporal conditions, Hitchcock (1980) has reported that *Skeletonema costatum* can potentially alter its chlorophyll *a* content on an hourly time scale, thereby maximizing its light harvesting ability during relatively "dark" periods. Probably, the benthic diatoms also adopt similar mechanisms to cope with the low light intensities, which are reflected in the increased chlorophyll *a* concentrations during the evening low tide (Fig. 2B.7a). Both these cases reveal the importance of

light in terms of intensity, depending on which the diatoms will decide their exposure duration.

2B.4.2 Laboratory incubations

The daily light:dark cycle is one of the strongest and most predictable environmental fluctuations that phytoplankton experience (Brand and Guillard 1981). Accordingly they must have evolved a variety of adaptations to this cycle as well as to the daily fluctuations of irradiance. Behavior of benthic diatom assemblages, similar to that observed in field, when incubated under the 12 h light: 12 h dark condition, with the tidal effects removed, confirmed the presence of an endogenous biological clock. Round and Palmer (1966) have observed such a behavior for the diatom population from muddy intertidal sediments. In the present study, the absence of suspension under controlled conditions in contrast to the natural environment was responsible for a higher density of diatom cells as compared to the field.

In continuous darkness, expression of a tidal rhythm may be attributed to an innate behavior. This behavior is otherwise superimposed by the diel rhythm in the presence of light. Odum (1970) stated that the biological clock not only couples internal and external rhythms, it enables individuals to anticipate changes. Thus, it is possible that when light is present, this tidal endogenous rhythm is manipulated according to the light intensity. Harper (1960) has reported daily migrations in continuous darkness for diatoms inhabiting muddy sediments. Round and Palmer (1966) observed that suppression of migratory cycle by pre-treatment in continuous dark had little or no effect on its subsequent expression on re-exposure to favorable conditions (i.e., when light intensity is sufficient). They suggested that the clock continued to run in the dark in spite of the fact that the overt rhythm was not displayed. Such tidal rhythms can also be an attempt to search for light, which is of prime requirement for

photosynthesis. In this search, the diatoms probably tend to produce more of chlorophyll *a*, which is a light harvesting pigment. Several workers have recorded increasing concentrations of chlorophyll *a* in the dark (or night) (Yentsch and Reichert 1963; Steeman Nielsen and Jφrgensen 1968; Taguchi 1976). In the present study, this is evident from the chlorophyll *a* concentrations in case I and case II, which is in par with that produced during the other incubation conditions. This is confirmed from the non-significant variation shown by chlorophyll *a* concentrations between incubation conditions and time as well as depth (Table 2B.2 and 2B.3).

On exposure to continuous light, capability of the diatom population to remain at the surface for a period of 18 h followed by a subsequent downward migration shows that there is an optimum duration up to which the diatoms can tolerate light exposure. In both the high tide and low tide incubated cores, the cells started migrating downwards after 18 h of light exposure. But in case of low tide incubation, reduction of chlorophyll *a* after 12 hours of continuous exposure to light (Fig. 2B.7f), followed by a subsequent downward migration of epipelic diatom cells (Fig. 2B.8f) reveals the impact of continuous light exposure on the physiology of the cells. However, this was not observed in the high tide incubation. The difference probably might be a result of the entire days exposure to natural light before the population was sampled. Whereas in case of the high tide incubated core, the population was exposed for a longer duration to a fixed irradiance in the laboratory. The physiological effect (i.e., degradation of chlorophyll *a*) might act as a trigger for the cells to become positively geotrophic. Nelson and Brand (1979) made the assumption regarding the temporal separation of metabolic processes, which has resulted in the evolution of one or more vital processes that can occur only during the dark phase and are unable to operate in continuous light.

In the natural environment, a high degree of hourly and fortnightly variability in benthic microalgal productivity has been reported by Pinckney and Zingmark (1991) and Serodio and Catarino (2000). These workers have attributed this variability to vertical migrations by epipelic diatoms within the upper layers of the intertidal sediments. The present study suggests that vertical migration in diatoms is not strictly a tidal or a diel behavior and differs with different seasons depending on the environmental conditions. Also, the migratory behavior of the epipelic diatoms is not related to the time of the day but to the duration of exposure to light, which is in turn dependent on the irradiance. This behavior has also been suggested by, Verduin (1957) in case of phytoplankton, which after being exposed to dim light during the day can carry on high rates of photosynthesis during late afternoon hours. It is also evident that for the migratory behavior investigations, chlorophyll *a* alone cannot be used as a predictor because it is subject to change depending on the physiology of the cells, which is controlled by the prevailing environmental conditions such as light availability. Light availability is in turn controlled by the season or temporal variations. For e.g., in case II, during the mid-morning high tide the cell numbers were high but not chlorophyll *a*, since sufficient light was not available. Thus, it can give erroneous results that upward migration has not occurred. Also, unlike cell abundance, chlorophyll *a* concentrations did not show significant variations with depth. By taking, both cell abundance as well as the chlorophyll *a*, physiology of the cells can be inferred and such information can be important for primary productivity studies.

Resurfacing of diatom population, after their homogenous distribution through tidal resuspension, is observed in the field as well as in controlled conditions when subjected to 12 h light: 12 h dark, continuous dark and continuous light conditions

even in the absence of tidal action. So these benthic diatoms via vertical migration are responsible for a considerable input of organic carbon into the sediment thereby supporting various communities of heterotrophic organisms in the intertidal areas.

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 Freiberger (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, (15^0 27' N, 73^0 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-

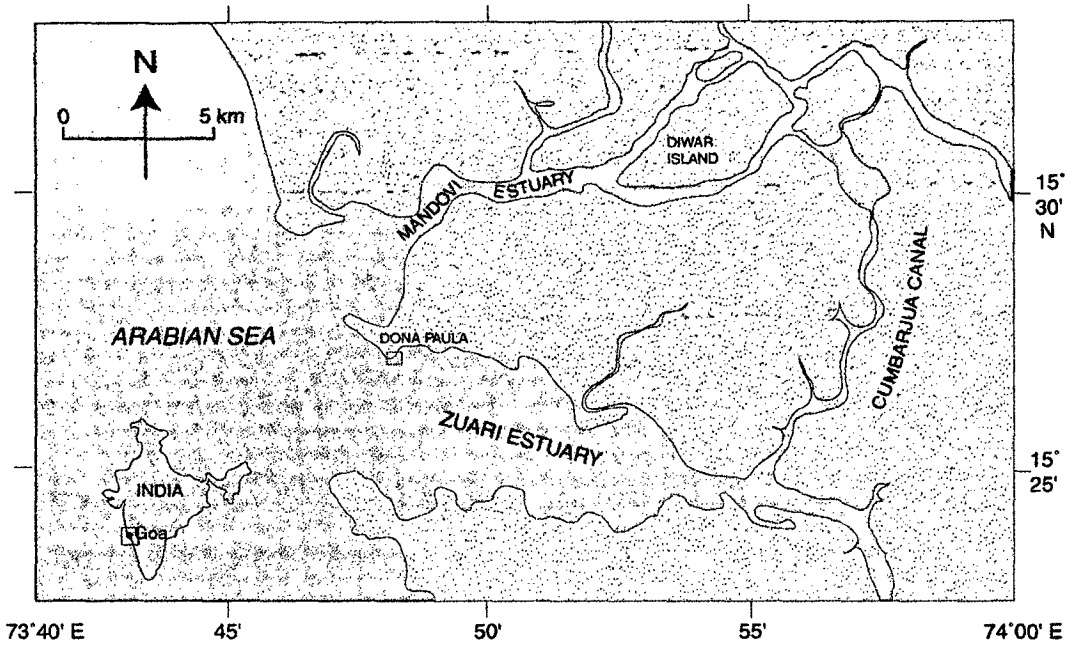


Fig. 3.1 Geographical location of the study area

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 (NO_2-N, NO_3-N, PO_4-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 × 10 × 2cm) 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), Subrahmanyan (1946), Desikachary (1977, 1987) and Tomas (1997). The results are presented in terms of diatom abundance per dm^2.

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 ^{0}C (February) to 30 ^{0}C (November) and salinity ranged from 15 psu to 35 psu. Dissolved oxygen ranged from 3.0 to 6.1 ml L^{-1}. Nitrate (NO_3-N) peaks were observed in April, July and December. Nitrite (NO_2-N) peaked in March and phosphate (PO_4-P) in May. Silicate (SiO_3) peaked in July (Fig. 3.2).

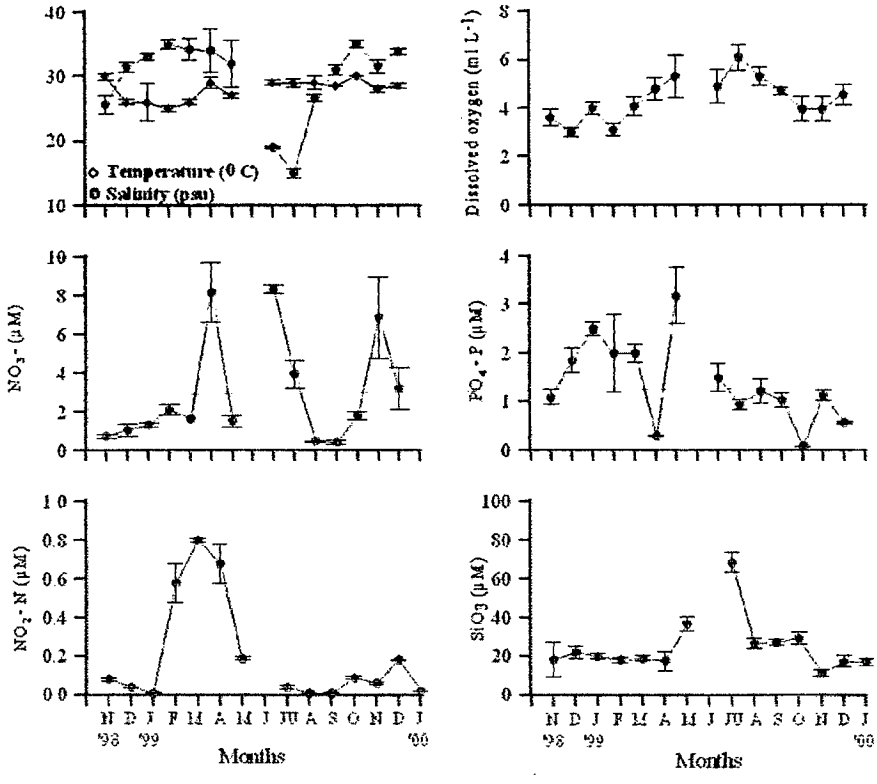


Fig. 3.2 Temporal variation in temperature, salinity, dissolved oxygen and nutrient concentrations of the ambient waters

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

Navicula sp.	*Nav* sp.	+	+	+
Nitzschia angularis Wm. Smith	*Nit ang*	+	+	+
Nitzschia bilobata Wm. Smith	*Nit bil*	+	+	+
Nitzschia closterium (Ehrenberg)	*Nit clo*	+	+	+
Nitzschia longissima (Brebisson, in Kutzing)	*Nit lon*	+	+	+
Nitzschia panduriformis Gregory	*Nit pan*		+	+
Nitzschia sigma (Kutzing) Wm. Smith	*Nit sig*	+	+	
Pinnularia rectangulata (Gregory) Rabenhorst	*Pin rec*			
Pleurosigma angulatum Sensu W. Smith emend. Sterrenburg	*Ple ang*	+	+	+
Pleurosigma elongatum Wm. Smith	*Ple elo*	+	+	
Pleurosigma sp.	*Ple* sp.			
Pseudonitzschia seriata Cleve	*Pse ser*	+	+	+
Stauroneis sepentrionalis Grunow	*Sta sep*			
Surirella sp1.	*Sur* sp1	+	+	
Surirella sp2.	*Sur* sp2	+	+	
Synedropsis affinis Kutzing	*Syn aff*			
Synedropsis gaillonii Grunow	*Syn gai*	+	+	+
Synedropsis hyperborea (Grunow) Hasle, Medlin & Syvertsen	*Syn hyp*			
Thalassionema frauenfeldii Grunow Hallegraeff	*Tha fra*			
Thalassionema nitzschioides (Grunow) Mereschkowsky	*Tha nit*	+	+	+
Thalasssiothrix longissima Cleve & Grunow	*Tha lon*			
CENTRICS				
Bacteriastrum hyalinum Lauder	*Bac hya*		+	+
Biddulphia mobiliensis (Bailey) Grunow	*Bid mob*			
Biddulphia pulchella Gray	*Bid pul*			
Biddulphia regia (M. Schultze) Ostenfeld	*Bid reg*		+	
*Biddulphia rhombus (*Ehrenberg) Smith, W.	*Bid rho*			
Biddulphia sinensis Greville	*Bid sin*		+	+
Cerataulina pelagica (Cleve) Hendy	*Cer pel*		+	+
Chaetoceros curvisetus Ehrenberg	*Cha cur*	+	+	+
Chaetoceros diversus Cleve	*Cha div*		+	+

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

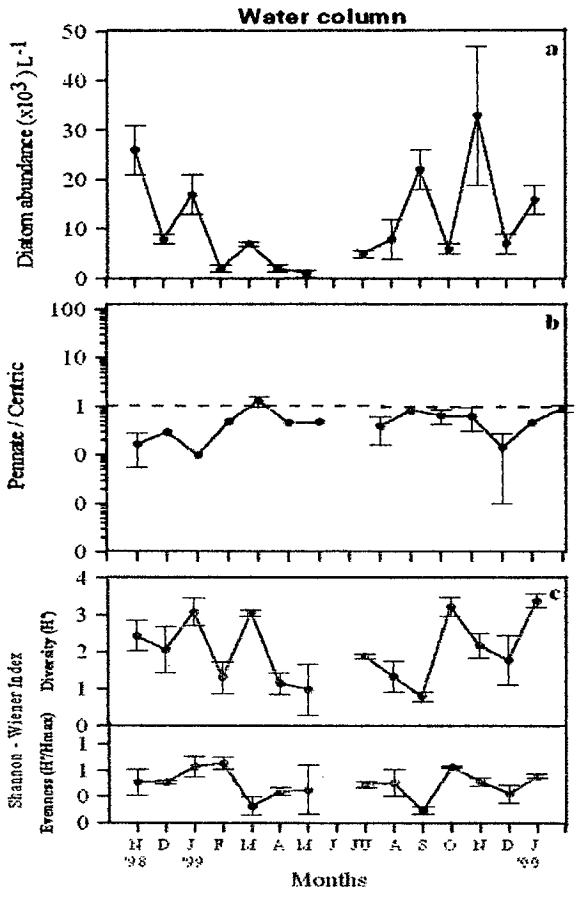


Fig. 3.3 Temporal variations in (a) diatom abundance; (b) ratio of pennate / centric diatoms; (c) diatom diversity and evenness in the ambient waters

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).

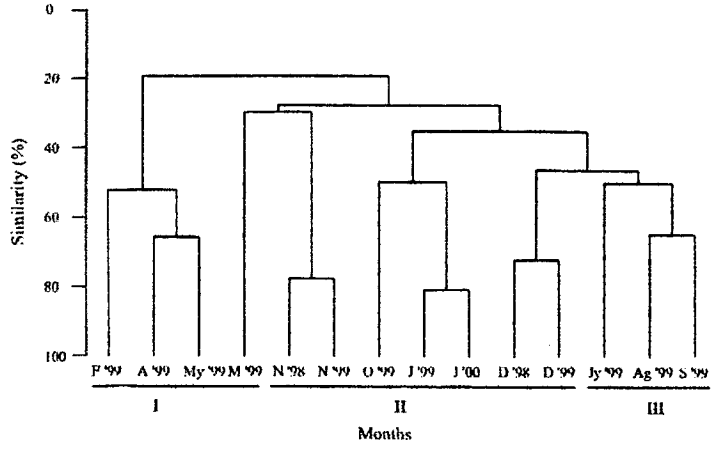


Fig. 3.4a Dendrogram of the diatom community from the ambient waters with respect to months

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. nitzschioides, 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.

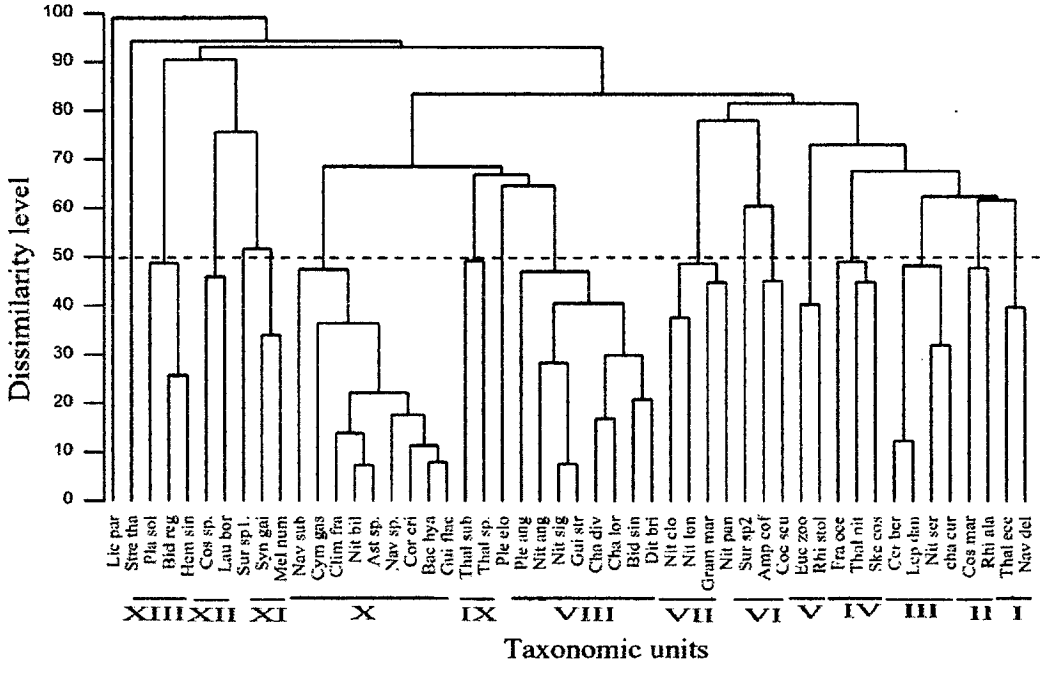


Fig. 3.4b Dendrogram of the diatom community from the ambient waters with respect to species

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 centrales) belonging to 31 genera (20 pennates, 11 centrales). 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).

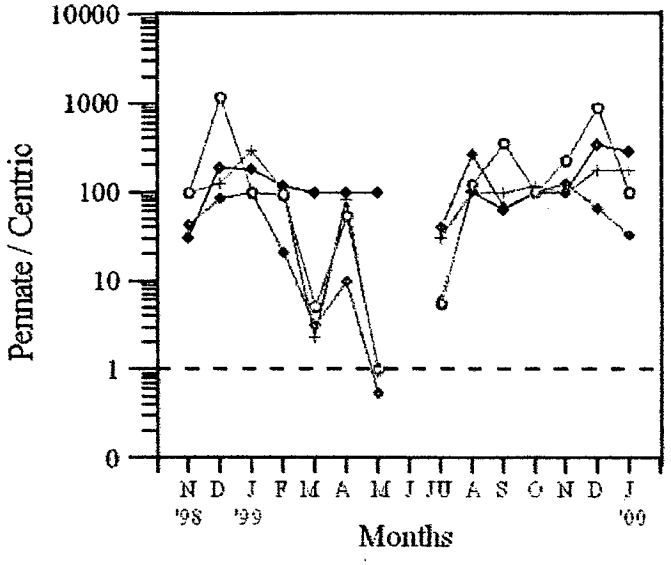


Fig. 3.5 The ratio of pennate / centric diatoms in the microfilm developed over stainless steel substrata (◈ Day 1; ◇ Day 2; + Day 3; ○ Day 4)

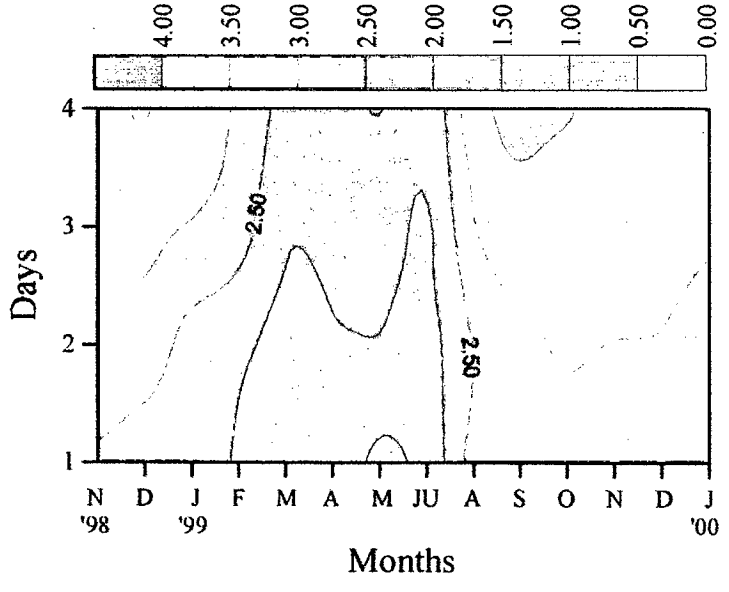


Fig. 3.6 Temporal variation in the fouling diatom abundance (cells dm^{-2}) on stainless steel substrata for different submersion periods (Day 1; Day 2; Day 3 and Day 4)

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)

	DAY 1			DAY 2			DAY 3			DAY 4		
MONTHS	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

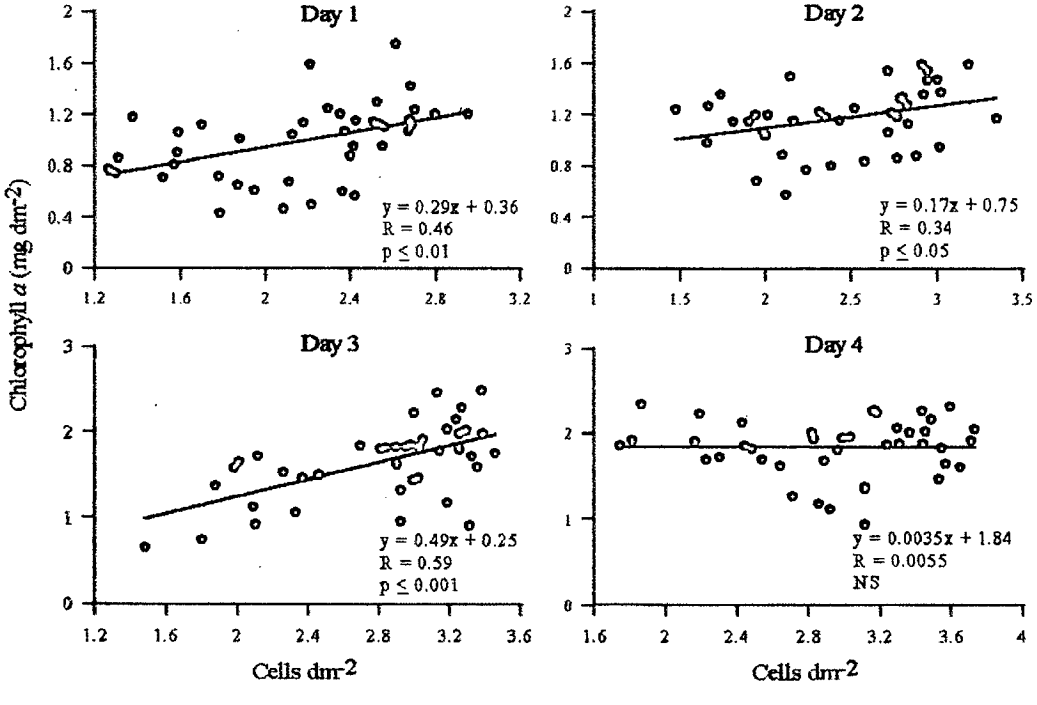


Fig. 3.7 Linear regression analysis between diatom abundance and chlorophyll *a* concentrations on stainless steel substratum for different submersion periods

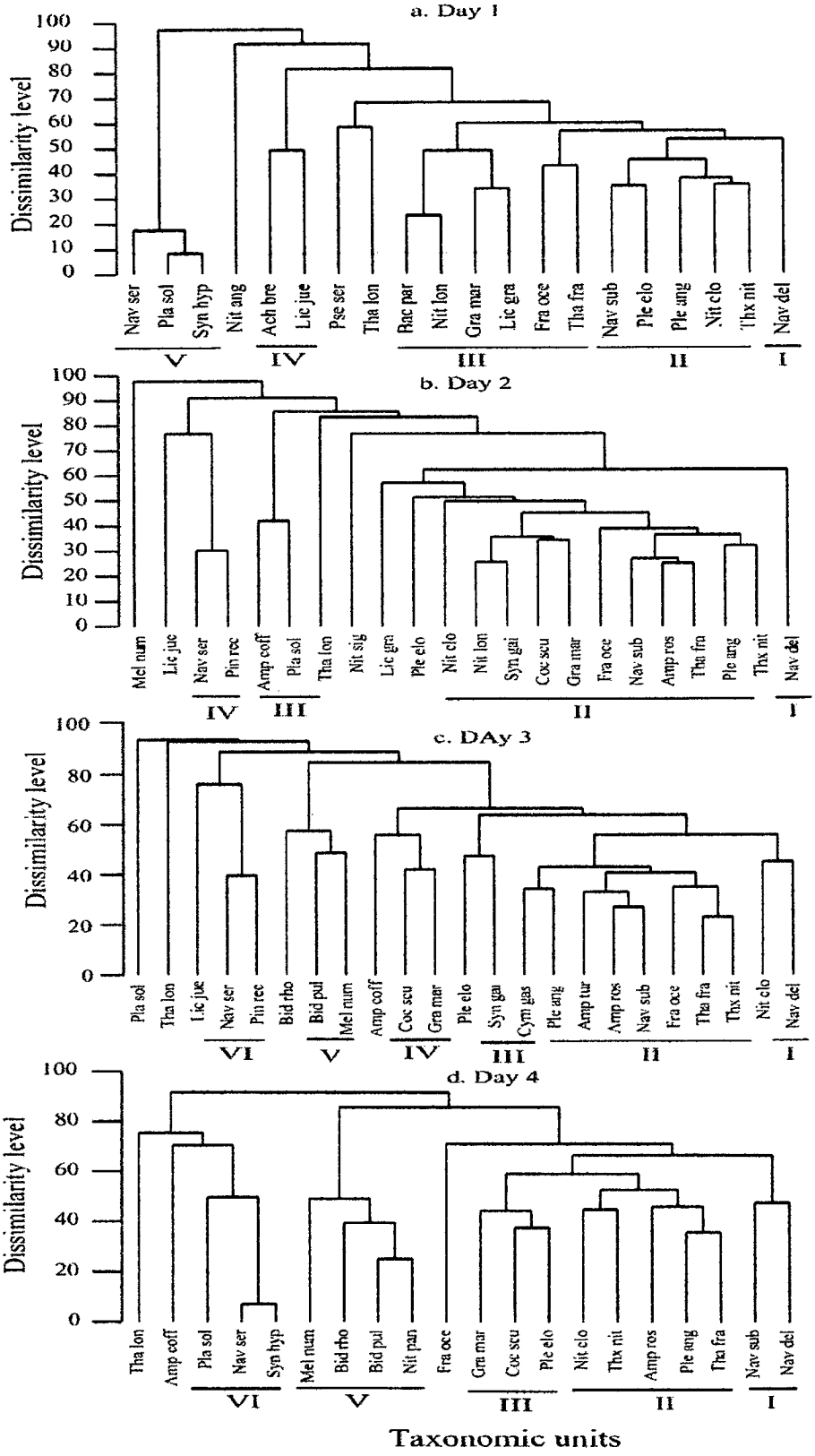


Fig 3.8 Dendograms of the fouling diatom community developed on stainless steel with respect to species for different submersion periods (Day 1 (a), Day 2 (b), Day 3 (c), Day 4 (d))

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).

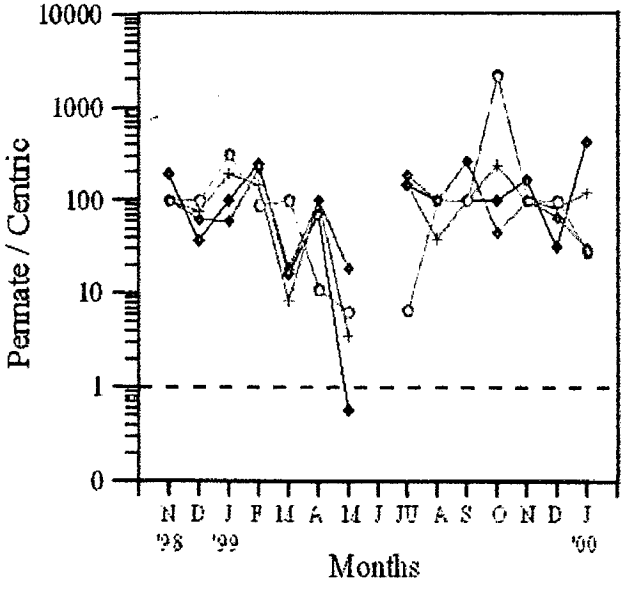


Fig. 3.9 The ratio of pennate / centric diatoms in the microfilm developed over polystyrene substrata (◇ Day 1; ◇ Day 2; + Day 3; O Day 4)

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

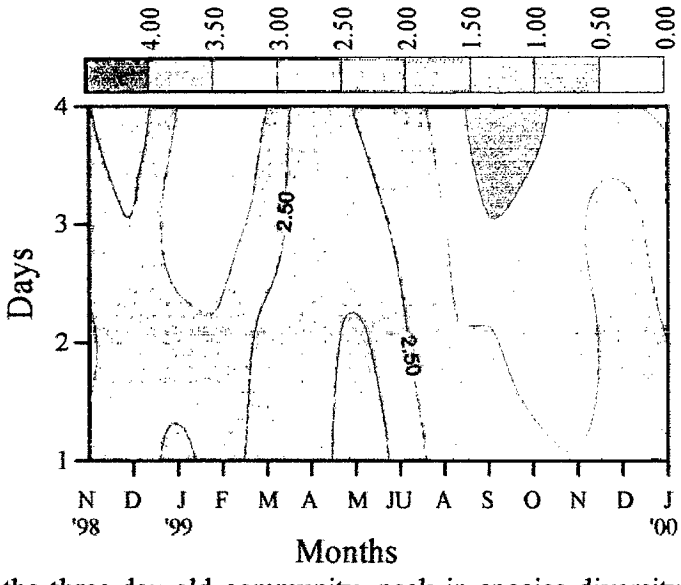


Fig. 3.10 Temporal variation in the fouling diatom abundance (cells dm^{-2}) on polystyrene substrata for different submersion periods (Day 1; Day 2; Day 3 and Day4)

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' max) and evenness (H' / H' max) of the fouling diatom community over different exposure periods with reference to polystyrene substratum (Shannon-Wieners diversity index)

	DAY 1			DAY 2			DAY 3			DAY 4		
MONTHS	H'	H'max	J'	H'	H'max	J'	H'	H'max	J'	H'	H'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

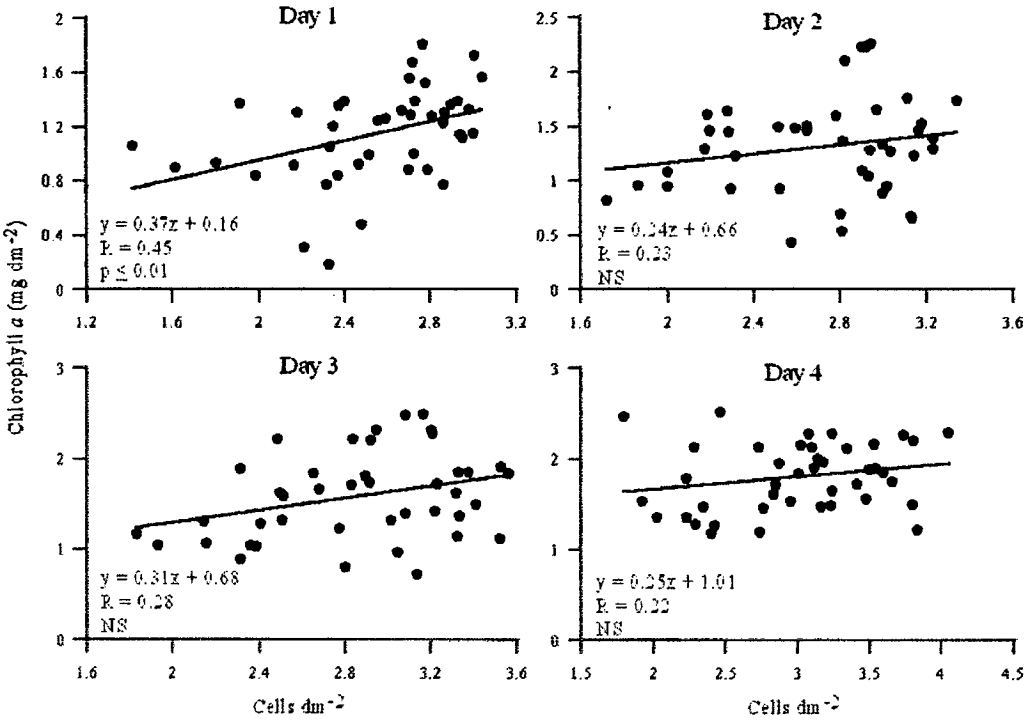


Fig. 3.11 Linear regression between diatom abundance and chlorophyll *a* concentrations on polystyrene substratum for different exposure periods

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.

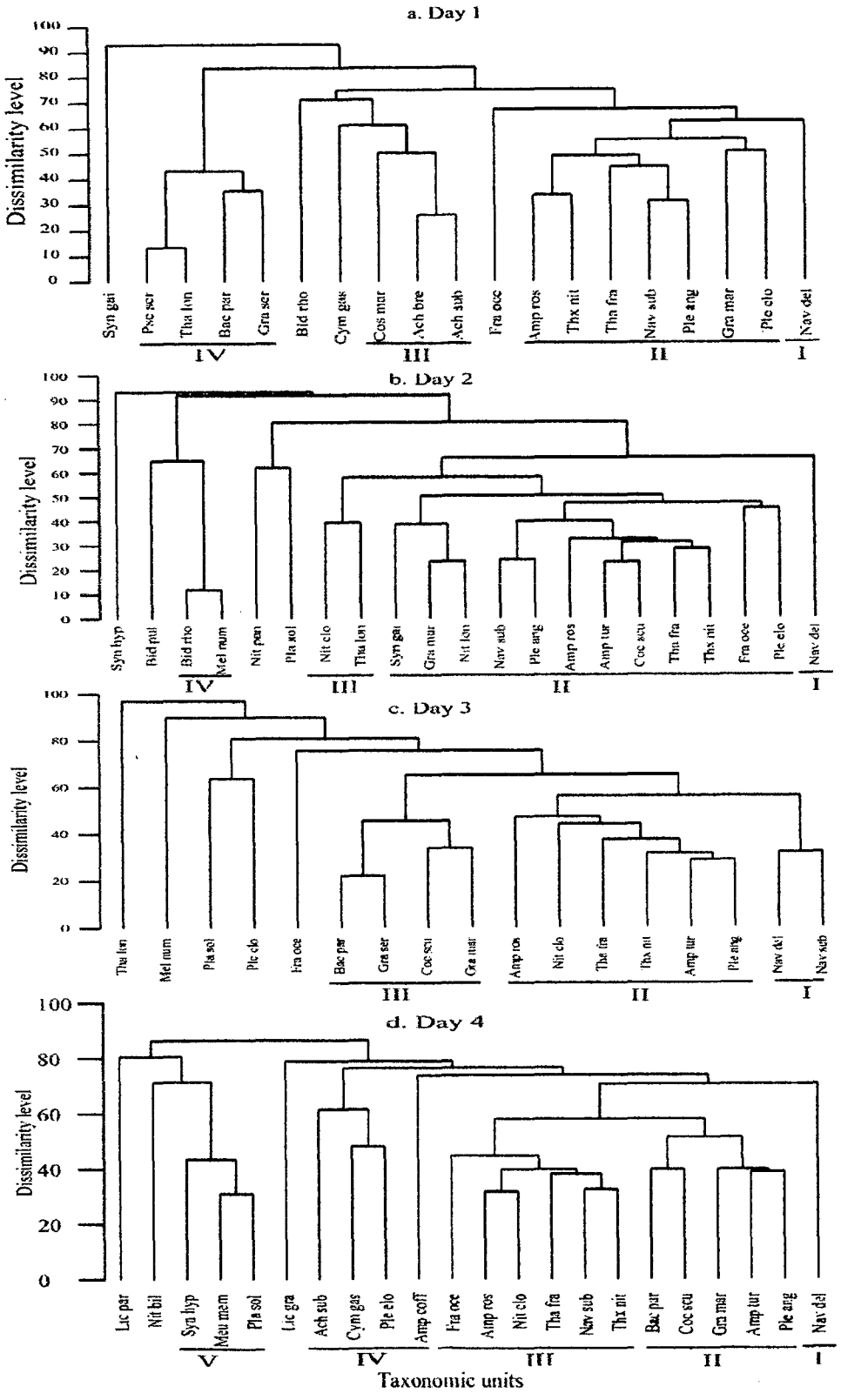


Fig 3.12 Dendograms of the fouling diatom community developed on polystyrene with respect to species for different submersion periods (Day 1 (a), Day 2 (b), Day 3 (c), Day 4 (d))

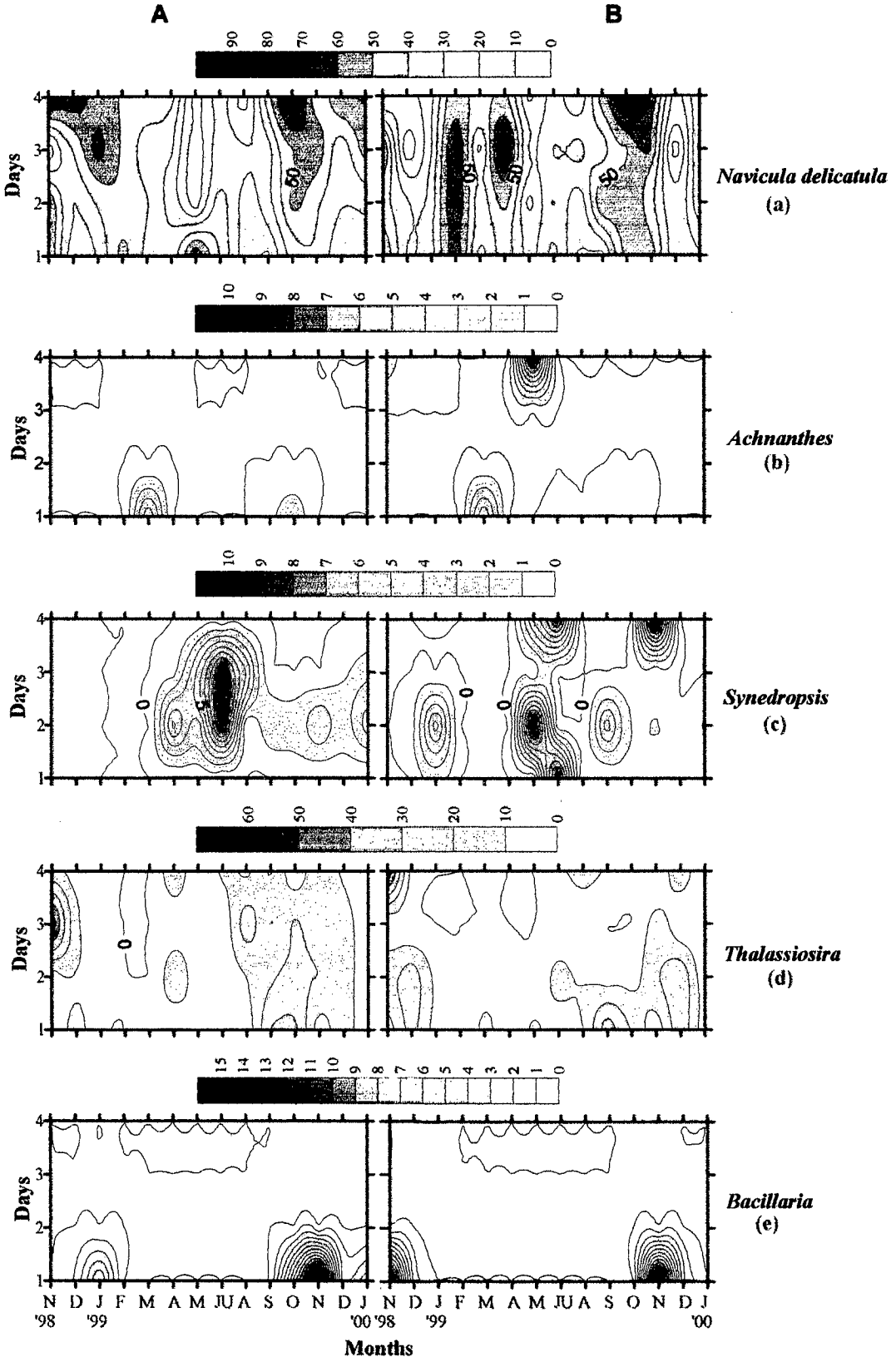


Fig. 3.13 Temporal distribution of *Navicula delicatula* (a); *Achnanthes* (b); *Synedropsis* (c); *Thalassiosira* (d); and *Bacillaria* (e) in the fouling diatom community developed over stainless steel (A) and polystyrene (B)

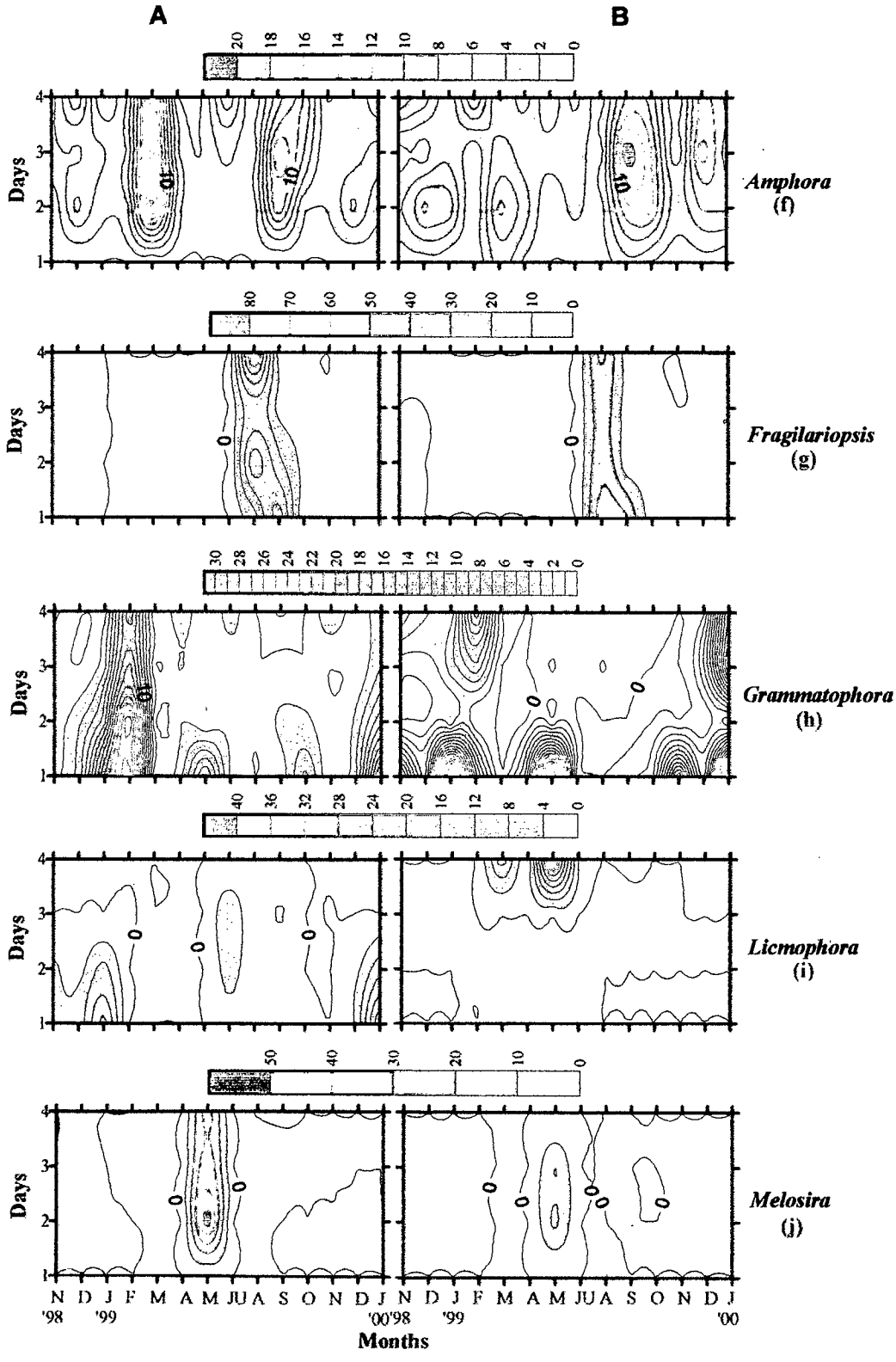


Fig. 3.13 Temporal distribution of *Amphora* (f); *Fragilariopsis* (g); *Grammatophora* (h); *Licmophora* (i); and *Melosira* (j) in the fouling diatom community developed over stainless steel (A) and polystyrene (B)

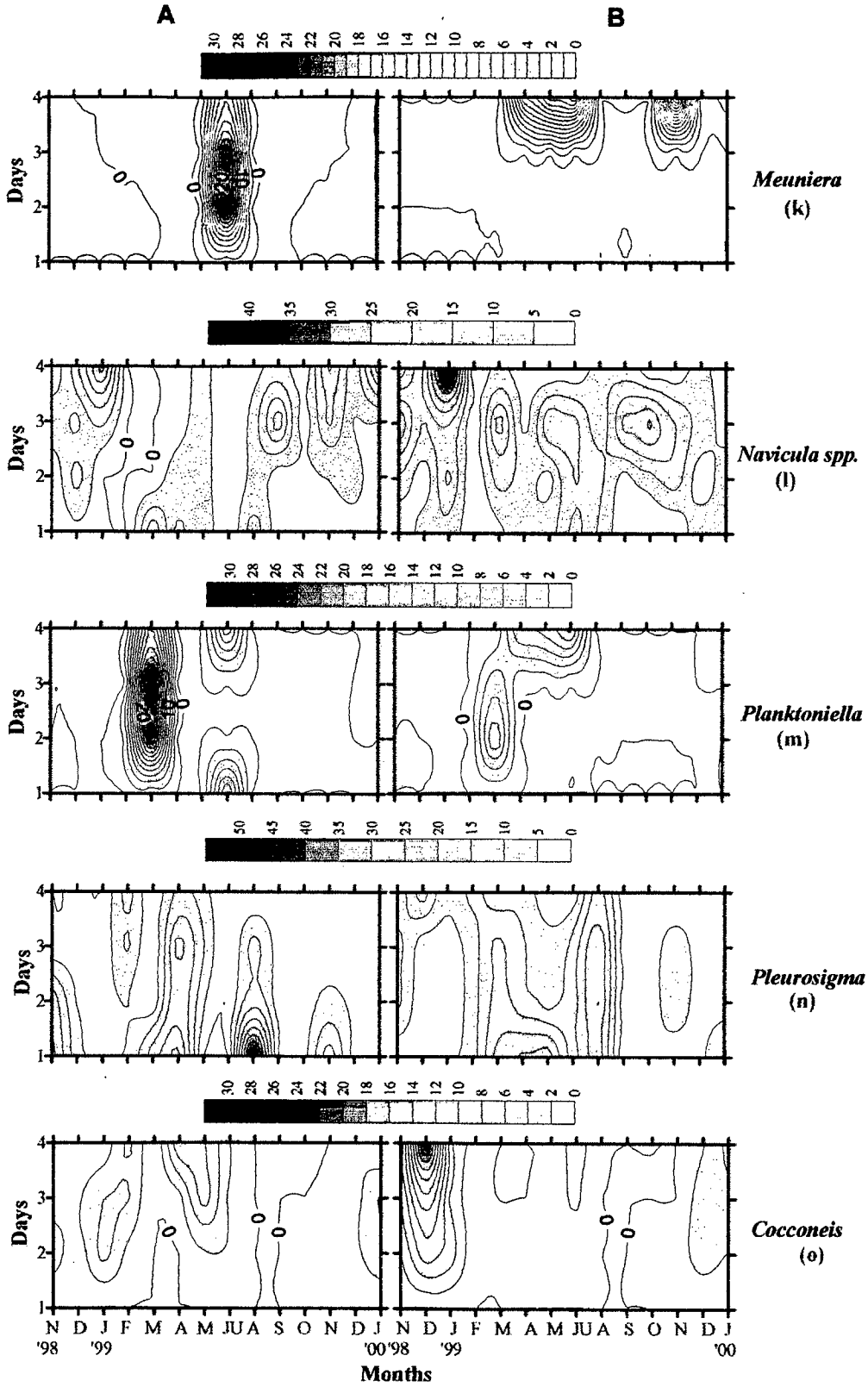


Fig. 3.13 Temporal distribution of *Meuniera* (k); *Navicula* spp. (l); *Planktoniella* (m); *Pleurosigma* (n); and *Cocconeis* (o) in the fouling diatom community developed over stainless steel (A) and polystyrene (B)

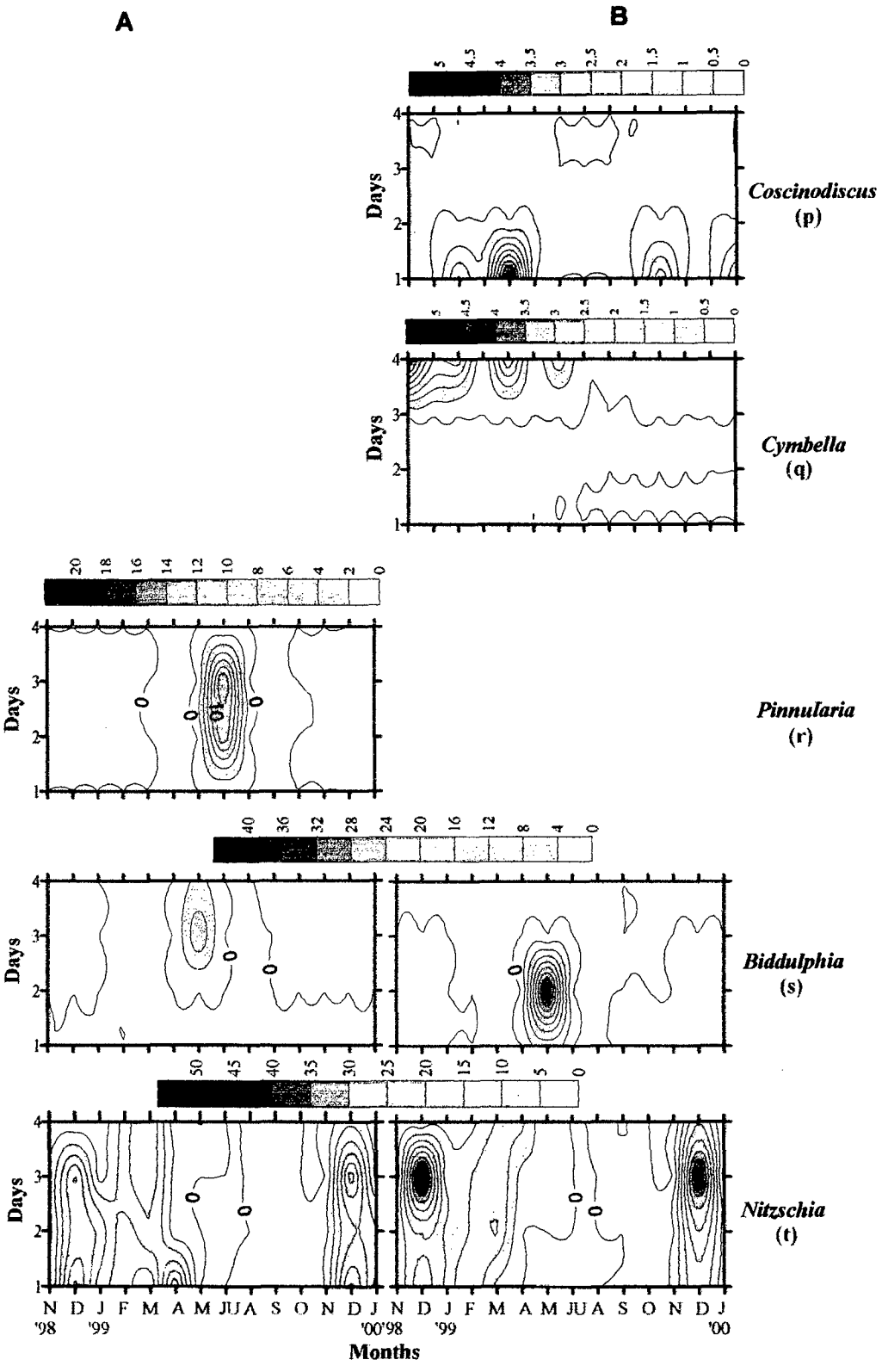


Fig. 3.13 Temporal distribution of *Coscinodiscus* (p); *Cymbella* (q); *Pinnularia* (r); *Biddulphia* (s); and *Nitzschia* (t) in the fouling diatom community developed over stainless steel (A) and polystyrene (B)

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 = ●)

Stainless steel	Nov '98	Dec	Jan '99	Feb	Mar	Apr	May	Jul	Aug	Sep	Oct	Nov	Dec	Jan '00
Achnanthes					▲					▲	▲			
Licmophora			▲ ★											▲ ★
Melosira							★ ■							
Fragilariopsis		▲ ★ ■							★ ■	▲ ★ ■ ●	★ ■		▲ ★ ■ ●	
Grammatophora	★	▲ ★	▲ ★ ■ ●	▲ ★ ■ ●		★ ■ ●		▲ ★ ■ ●		▲ ★	▲ ★	●	▲ ★ ■	▲ ★
Planktoniella				★	★ ■ ●			▲ ★ ■						
Pleurosigma	▲ ★	▲ ★	★	■ ●		▲ ★ ■ ●	★ ■ ●	●	★ ■	▲ ★ ■ ●	▲ ★ ■ ●	▲ ★ ●	■	■ ●
Biddulphia							■ ●				■		■	
Cocconeis	★		★ ■	★ ■ ●		■ ●	★ ■ ●	★			★	★	●	★ ■
Meuniera							●							
Navicula spp.		★			▲	★ ■ ●	▲ ★ ●	★ ●	▲ ★	▲ ■ ●	▲	■ ●	★	■ ●
Synedra		■				★	■	★ ■	■		★	★	★ ■	★ ■
Thalassiothrix	■		●			▲ ★ ■ ●	★ ■ ●	●	★ ■	▲ ★ ■ ●	▲ ★ ■ ●	▲ ★ ●	■	■ ●
Amphora		●	■		★ ■ ●	■		■ ●	★		●	■ ●		
Bacillaria		▲								▲	▲		▲	
Nitzschia	■	▲ ★ ●	▲ ★	■ ●	■ ●	▲ ★ ■	●	■	▲	★ ■	★	▲	▲	▲ ★ ■
Pinnularia								★ ■						
Coscinodiscus														
Cymbella														

Polystyrene														
Achnanthes	●		●		▲		●							
Licmophora					●		●	●					●	
Melosira									■					
Fragilariopsis	★								▲ ■	●	▲	★	★	●
Grammatophora	▲ ●	■ ●	▲ ■ ●		●	▲	▲ ■		■	▲	●	▲	■ ●	▲ ■ ●
Planktoniella						●	●	●						●
Pleurosigma	■ ●	■ ●	▲ ■ ●	▲ ★	▲ ★ ■ ●	▲	▲ ★	▲ ★ ■	★ ●	▲ ■	▲ ★ ■			
Biddulphia		★					▲ ★						★	▲
Cocconeis	■ ●	★ ■ ●	★ ●			★		★ ■ ●			★ ■ ●	★ ■	▲ ■ ●	▲ ★
Meuniera						●		●		●		▲ ★ ■		●
Navicula spp.	★ ■	▲ ■ ●	▲ ★ ■ ●		★ ■ ●	▲ ■	■	▲ ■	★ ■ ●	▲ ★	★ ■ ●	★ ■	▲ ■ ●	▲ ★
Synedra			▲ ★				★ ●	▲ ●		★		●		
Thalassiothrix	▲ ★ ●	▲ ★ ■	▲ ★ ■		▲ ★ ■ ●		▲	▲ ★ ■	▲ ●	▲		▲ ■	▲ ★ ●	▲
Amphora	●	★	★ ■ ●	●	▲	▲ ★ ■ ●	■	★ ■	▲ ★ ■ ●	▲ ★ ■ ●	▲ ★ ■	★	▲ ★ ■ ●	★ ■ ●
Bacillaria	▲											▲		
Nitzschia	▲ ★	■	■ ●	▲ ★	▲ ★	■ ●	■			■ ●	★ ■	▲ ★	▲ ★ ■ ●	■ ●
Pinnularia														
Coscinodiscus			▲		▲						▲			▲
Cymbella	●	●	●		●		●							

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 ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.001; NS not significant)

Source of variation	*df*	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 ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.001; NS not significant)

Source of variation	*df*	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 ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.001; NS not significant)

Source of variation	*df*	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 ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.001; NS not significant)

Source of variation	*df*	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	*df*	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. nitzschioides* 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-wetttting 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.

Chapter 4

Modulations in the periphytic diatom diversity

4.1 Introduction

Periphytic diatoms in the littoral zone originate from a number of sources, including the water column, adjacent surfaces and benthos. Once dislodged, any substratum in the pelagic zone serves as an "island" for immigration to these tychoplanktonic cells. Ecologically, the pennate diatoms are important from two perspectives: first and foremost, they are important components of the microphytobenthic community, in both sandy and muddy intertidal flats (Round and Palmer 1966; Round 1979a, b; Round 1981). In addition to being a source of energy and nutrients to the benthic food web, they supply organic carbon to the plankton system during resuspension by tidal currents and wind-induced turbulence (de Jonge 1980; de Jonge 1985; de Jonge and Van Beusekom 1992; de Jonge and Colijn 1994; de Jonge and Van Beusekom 1995; de Jonge 1995). Secondly, when displaced from their original habitat, into the water column, these forms on encountering any kind of substratum tend to settle and develop a community of their own and are responsible for a major input of energy in the form of reduced carbon to the surface (Cooksey et al. 1984). The attached diatom community also constitutes a system rich in information for environmental monitoring, which can be exploited through analysis of communities' structural characteristics (Gold et al. 2002). Periphytic diatom communities have been used as biological indicators of river acidification (Ter Braak and Van Dam 1989), organic pollution (Desey and Coste 1991) and eutrophication (Kelly and Whitton 1995).

Space is a resource of utmost importance for the settlement and growth of the marine periphytic diatom community. However, limited availability of space as compared to the vast diversity of species, leads to intense competition for this resource. Thus, diversity is controlled by the competitive strategies employed by each member of the community, whether a pioneer or a late arrival. In this struggle for existence, the

fittest species can carve a better niche for itself, by exhibiting competitive traits. Valiela (1984) stated that competition occurs when two species require a resource that is in short supply, so that availability of that resource to one species is negatively influenced by the presence of other species, wherein the more competitive species

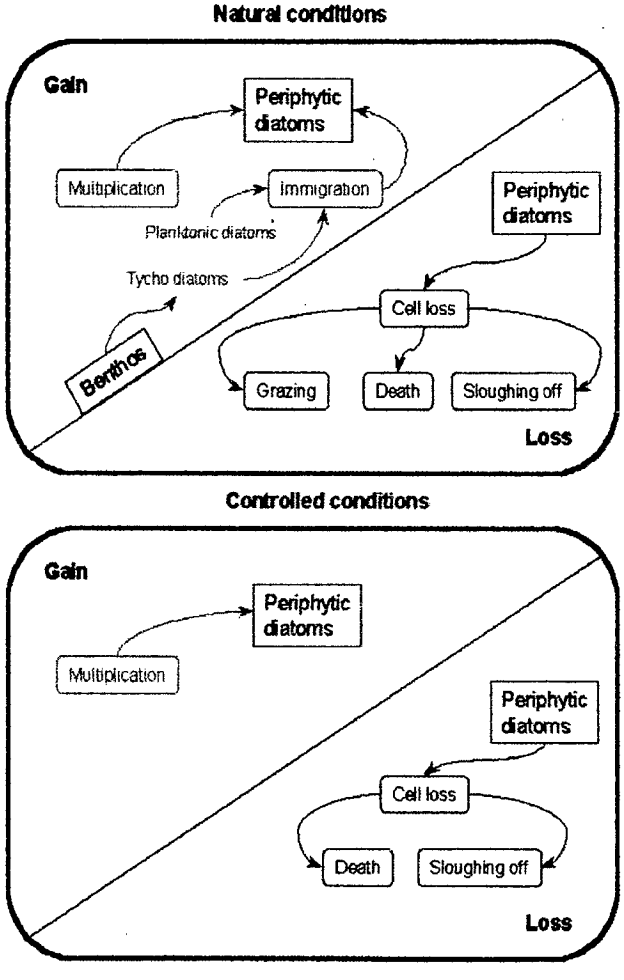


Fig. 4.1. Schematic representation of the processes governing the periphytic diatom community

proliferates. Ecosystem processes are governed by the biodiversity within its community and the gain or loss factors as illustrated in Fig. 4.1. Immigration and multiplication are the gain factors whereas grazing, sloughing off or death, are the loss factors influencing the periphytic diatom community in nature. Incubation of the

population from nature in the laboratory minimizes the gain factors only to multiplication and eliminates the grazing component from the loss factors.

In a homogenous habitat, species differ in their competitive abilities and are limited by and compete for the same single resource. According to Tilman (1982, 1999), in such a habitat at equilibrium, the best competitor, among the species present would win. In this effort, we subjected the diatom films developed on glass surface in the marine environment to incubation under controlled conditions. Through this, we illustrate the roles of seeding, nutrients and competitive strategies in the variations of marine periphytic diatom diversity.

4.2 Materials and methods

4.2.1 Experimental design

Glass slides (measuring 7.6 x 2.2 cm) were fixed on to a wooden frame, which was suspended at the sub-surface level (~ 1m below the low tide level so as to ensure continuous immersion in seawater) at the Dona Paula Bay (15^0 27' N, 73^0 48' E), Goa, west coast of India. A total of four glass slides were retrieved every consecutive day, for a period of four days in order to obtain 1, 2, 3 and 4 days old diatom community. 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. As our aim was to understand the progression of a periphytic diatom community in the very early stages in an undisturbed condition, this study restricted the field exposures to only four days. After recovering from field, the slides were subjected to quantification and characterization of the diatom community.

4.2.2 Laboratory incubation

Out of the four slides, two were incubated in autoclaved nutrient enriched seawater (ES) (f/2, Guillard and Ryther 1962) whereas the other two were incubated in autoclaved seawater (AS) separately. The salinity of seawater ranged between 32 to 35 psu. The molar ratios of N:P:Si (μM) in ES were 882:42:123 and in AS were 1.3:1.8:25, 1.5:3.2:37 and 0.45:1.2:50 for post-monsoon, pre-monsoon and monsoon respectively. The slides were later used for direct diatom counts every alternate day

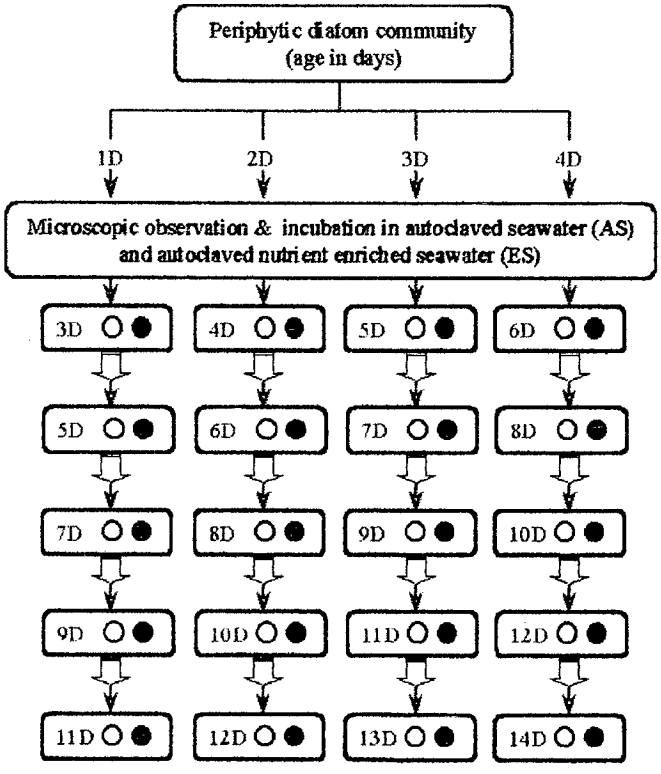


Fig. 4.2. Schematic representation of the experimental protocol (D = Days; O = Observation of glass slide; ● = Observation of sloughed off community; ⇪= Transfer of glass slide to fresh medium)

and transferred into fresh ES and AS respectively. Counts were also taken of the cells lost from the slides into the media (Fig. 4.2). Incubation was carried out at 27 ^{0}C, which was close to ambient temperatures. Slides were illuminated at an incident irradiance of 50 μmol photons m^{-2} s^{-1}, produced by fluorescent tubes over a 12 h light: 12 h dark photoperiod.

Earlier observations have shown a considerable variation in the diatom species diversity during the three seasons i.e., post-monsoon (January '99), pre-monsoon (May '99) and monsoon (September '99) experienced by this locality (Smita and Anil 2000). Hence this experiment was carried out thrice, once in each season.

4.2.3 Enumeration of diatoms

Diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyan (1946), Desikachary (1977,1987) and Tomas (1997). The diatom flora on either surface of the slide was analyzed and is expressed in terms of their variation per dm^2. For the purpose of linear regression analysis, specific growth rates (K) of the dominant periphytic diatom species were calculated according to Carreto and Catoggio (1976).

$$K = (\log_{10} N - \log_{10} N_0) * 2.3 / t$$

Where N = Cell concentration for every second day

N_0 = Cell concentration at t = 0

t = day

Area of the periphytic diatom species encountered on glass slides was calculated in terms of cm^2 (Hillebrand et al. 1999; Table 4.1).

4.2.4 Competition between N. delicatula and A. coffeaeformis in culture

***Isolation and culture of benthic diatoms*:** Glass slides were suspended for 5 days at the sub-surface level (approx. 1m below low tide level) at Dona Paula Bay. After retrieval, the slides were rinsed with filtered seawater and the biofilm was brushed off with sterile paintbrushes into 50 ml CORNING tubes containing filtered seawater. A dilution series of the biofilm suspension was inoculated onto 1% agar prepared with f/2 nutrient medium. Agar plates were incubated at 27 ± 1 ^{0}C under a 12 h light: 12 h dark photocycle. Distinguishable diatom colonies of *A. coffeaeformis* and *N.*

delicatula formed within 1 week of inoculation which were isolated and successively sub-cultured until monospecific diatoms were obtained. Pure cultures were then maintained in f/2 nutrient medium enriched seawater.

***Experimental protocol*:** Pure diatom suspensions of each of the two species were prepared by brushing the culture flasks with sterile paintbrushes. Subsequently, aliquots of the two diatom suspensions were transferred into polystyrene multiwells (Corning-430343; 3 replicates) containing sterile f/2 medium. The initial inoculum density ratios of *N. delicatula* and *A. coffeaeformis* were (a) 99:1 (b) 95:5 (c) 85:15 (d) 75:25 (e) 50:50 (f) 25:75 (g) 15:85 (h) 5:95 and (i) 1:99. The final concentration was 50, 000 cells ml^{-1}. These multivells were incubated at 27 ± 1 ^0C under 12h light: 12h dark condition and were observed microscopically at regular intervals for quantification. Results are presented in terms of cells mm^{-2}. Specific growth rates of the two diatom species were calculated according to Carreto and Catoggio (1976).

4.2.5 Data analysis

Univariate analysis: Univariate techniques included the calculation of Shannon-Wiener diversity index (*H'*) (Pielou 1969).

Multivariate analysis: A similarity matrix for the square root transformed diatom abundance data was constructed using the Bray-Curtis measure (Bray and Curtis 1957), which was then subjected to ordination analysis. Ordination was by non-metric multidimensional scaling (NMDS) (Kruskal and Wish 1978) using bubble plots. Univariate and multivariate analysis were made using PRIMER software version 5.

Linear regression analysis was performed on the specific growth rates of the dominant periphytic diatom species. Polynomial regression analysis was performed on the species diversity and percent coverage by the periphytic diatoms.

4.3 RESULTS

4.3.1 Water column diatom

Diatom composition during the three occasions is shown in Table 4.1 and figure 4.3. *Navicula delicatula* was the most abundant diatom in post-monsoon and pre-monsoon. In monsoon, the major forms were *Skeletonema costatum* (62%) and *Thalassionema frauenfeldii* (27%). Comparably high numbers of *Coscinodiscus marginatus* were present in post-monsoon, but this species decreased in abundance through pre-monsoon and monsoon. During post-monsoon and pre-monsoon, abundance of *Helicotheca tamesis* (8-13%) and *Pleurosigma angulatum* (5-17%) was greater than that in monsoon.

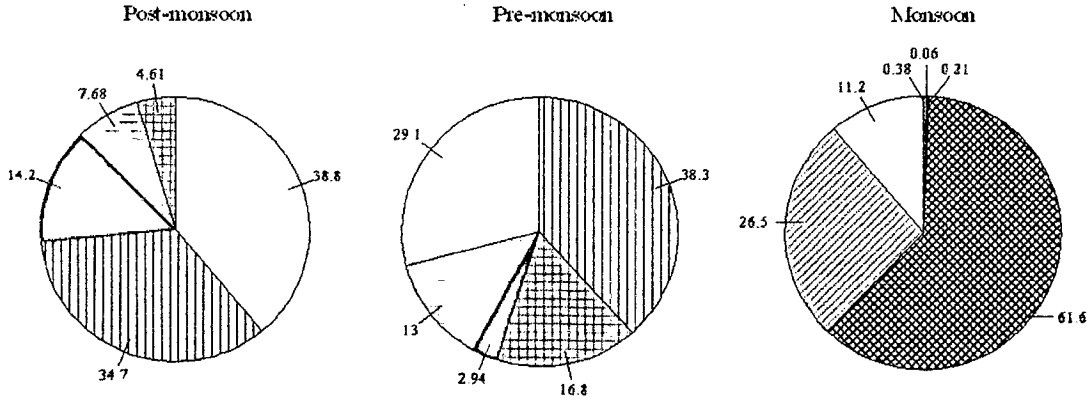


Fig. 4.3. Diatoms encountered in the ambient waters during post-monsoon, pre-monsoon and monsoon

Table 4.1 Diatoms encountered in case I, case II and case III and the area of periphytic diatoms

Pennate diatoms	Case I		Case II		Case III		Area of periphytic diatoms (x $10^3 \mu m^2$)
	WC	GL	WC	GL	WC	GL	
Achnanthes longipes Agardh	+			+		+	2.6
Achnanthes subsessilis Kutzing		+	+	+	+	+	0.8
Amphora coffeaeformis (Agardh) Kutzing	+	+		+		+	0.06
Amphora hyalina Kutzing		+	+	+		+	0.3
Amphora rostrata Wm. Smith		+				+	0.8
Amphora turgida Gregory		+	+	+		+	0.09
Cocconeis scutellum Ehrenberg	+	+	+	+		+	1.2
Diploneis smithii (de Brebisson) Wm. Smith					+	+	0.9
Fragilariopsis oceanica (Cleve) Hasle					+	+	0.1
Grammatophora marina Kutzing	+		+	+			1.4
Grammatophora serpentina Ehrenberg						+	1.9
Licmophora flabellata (Greville) Agardh						+	2.2
Licmophora juergensii Agardh	+	+	+			+	0.2
Licmophora lingbyei (Kutzing) Grunow		+					0.2
Licmophora paradoxa (Lyngbye) Agardh				+		+	0.26
Meuniera membranacea (Cleve) P. C. Silva comb. nov.						+	3.9
Navicula transitrans var. derasa f. delicatula Heimdal	+	+	+	+	+	+	0.7
Navicula septentrionalis (Grunow) Gran				+		+	0.16
Navicula subinflata Grunow		+		+	+	+	0.076
Nitzschia angularis Wm. Smith		+				+	3.6
Nitzschia closterium (Ehrenberg) W. Smith	+	+	+	+	+	+	0.24
Nitzschia longissima (Brebisson) Grunow		+			+	+	8.4
Nitzschia sigma (Kutzing) W. Smith						+	2.2
Pleurosigma angulatum Sensu Wm. Smith emend. Sterrunburg	+	+	+	+	+	+	2.1
Pleurosigma elongatum Wm. Smith	+		+		+		
Pseudo-nitzschia seriata (Cleve) H. Peragallo				+		+	0.69
Surirella sp.			+				
Synedropsis gaillonii Grunow					+	+	4.4
Thalassionema frauenfeldii (Grunow) Hallegraeff		+			+		0.76
Thalassionema nitzschioides (Grunow) Mereschkowsky		+				+	0.58
Centric diatoms							
Biddulphia mobiliensis (Bailey) Grunow	+				+		
Biddulphia obtusa (Kutzing) Ralfs in Pritchard		+		+			3.8
Biddulphia regia (M. Schultze) Ostenfeld					+		
Biddulphia rhombus (Ehrenberg) W. Smith	+		+	+		+	14.9
Cerataulina sp.	+						
Chaetoceros sp1	+				+		
Chaetoceros sp2	+				+		
Coscinodiscus marginatus Ehrenberg	+	+	+	+	+		4.3
Coscinodiscus concinnus Wm. Smith	+		+		+		
Eucampia zodiacus Ehrenberg	+						
Guinardia striata (Stolterfoth) Hasle com. nov.					+		
Helicotheca tamesis (Shrubsole) Ricard	+		+				
Hyalodiscus stelliger (Bailey) Mann				+			11.3
Leptocylindrus danicus Cleve					+		
Melosira nummuloides C. A. Agardh			+	+		+	0.47
Skeletonema costatum (Greville) Cleve					+		
Thalassiosira eccentrica (Ehrenberg) Cleve						+	6.1

.(WC: Water column, GL: Glass slide)

4.3.2 Field incubated slides prior to laboratory incubation

A progressive increase in periphytic diatom abundance on the field incubated slides from one to four days, was a common feature observed during the three occasions. The diatom cell abundance was highest in post-monsoon followed by monsoon and pre-monsoon (Appendix 4.1a-d to 4.6a-d). Periphytic diatoms encountered on the three occasions are presented in Table 4.1. Pennate genera were dominant throughout the study period. Amongst these, *N. delicatula* was the most dominant diatom.

4.3.3 Laboratory incubation of one to four days old periphytic diatom community after retrieval from field

Subsequent to laboratory incubation, the periphytic diatom abundance increased with time, the increase being higher in ES than AS. This trend was observed on all three occasions, except for the third and fourth day incubation in post-monsoon. A change in the total diatom cell abundance gradient from post-monsoon > monsoon > pre-monsoon (Appendix 4.1a-d to 4.6a-d) to monsoon > pre-monsoon > post-monsoon was observed subsequent to laboratory incubation (Appendix 4.1e-h to 4.6e-h).

The observations from post-monsoon, pre-monsoon and monsoon yielded three scenarios and these are presented as Case I (Intergeneric competition), Case II (Inter and Intrageneric competition) and Case III (Competitive exclusion or co-existence) respectively.

Case I (Intergeneric competition)

N. delicatula, Amphora coffeaeformis, T. frauenfeldii, P. angulatum and *Nitzschia closterium* were the dominant species of the one to four days old field periphytic diatom community (Appendix 4.1a-d, 4.2a-d, 4.7-10). *N. delicatula,* which was the most dominant diatom, continued to be so subsequent to laboratory incubation in ES even after a period of 11 days, in case of the one day old community (Fig. 4.5d,

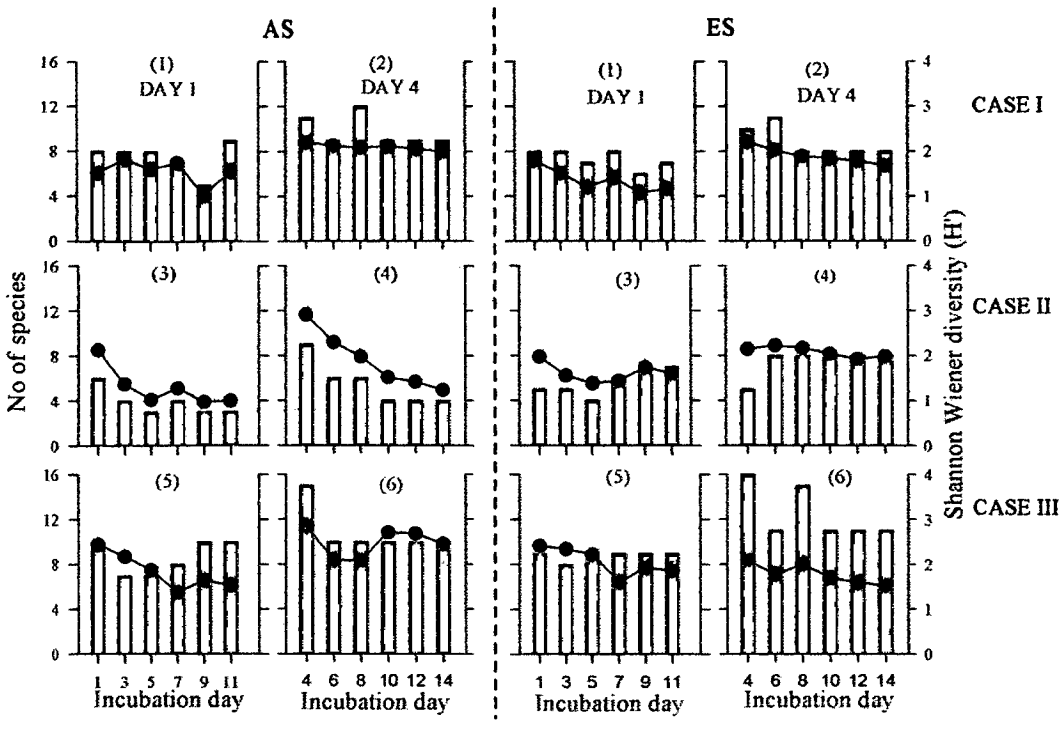


Fig. 4.4 Periphytic diatom species diversity in case I, case II and case III subsequent to laboratory incubation of one and four day old periphytic diatom community in AS and ES.
(Bars indicate number of species and • indicate species diversity)

rates in case of the incubation of two to three days old field communities in ES (Appendix 4.2f-g, 4.8g-l and 4.9g-l). In case of the four days old field community incubation in ES, the abundance of *A. coffeaeformis* increased at a faster rate, eventually surpassing *N. delicatula* with longer incubation period (Appendix 4.2h). This is reflected in the NMDS bubble plots (Fig. 4.5h, Appendix 4.10e-h) and resulted in a decreasing diversity profile (Fig. 4.4). Comparatively, in AS, the two major competitors were growing at almost the same pace (Fig. 4.5f and Appendix 4.1h, 4.7a-d to 4.10a-d). The community that had dislodged from the slide, in ES, had a meager population of *A. coffeaeformis* as compared to *N. delicatula* (Appendix 4.2i-l). Rest of the periphytic community which could not be in pace with the major competitors were also found to contribute to this sloughed off community. In AS, the two competitors, *N. delicatula* and *A. coffeaeformis* did not contribute much to this community while the other minor forms did (Appendix 4.1i-l). The *A. coffeaeformis* to *N. delicatula* abundance ratio in the initial field inoculum of the ES incubated four

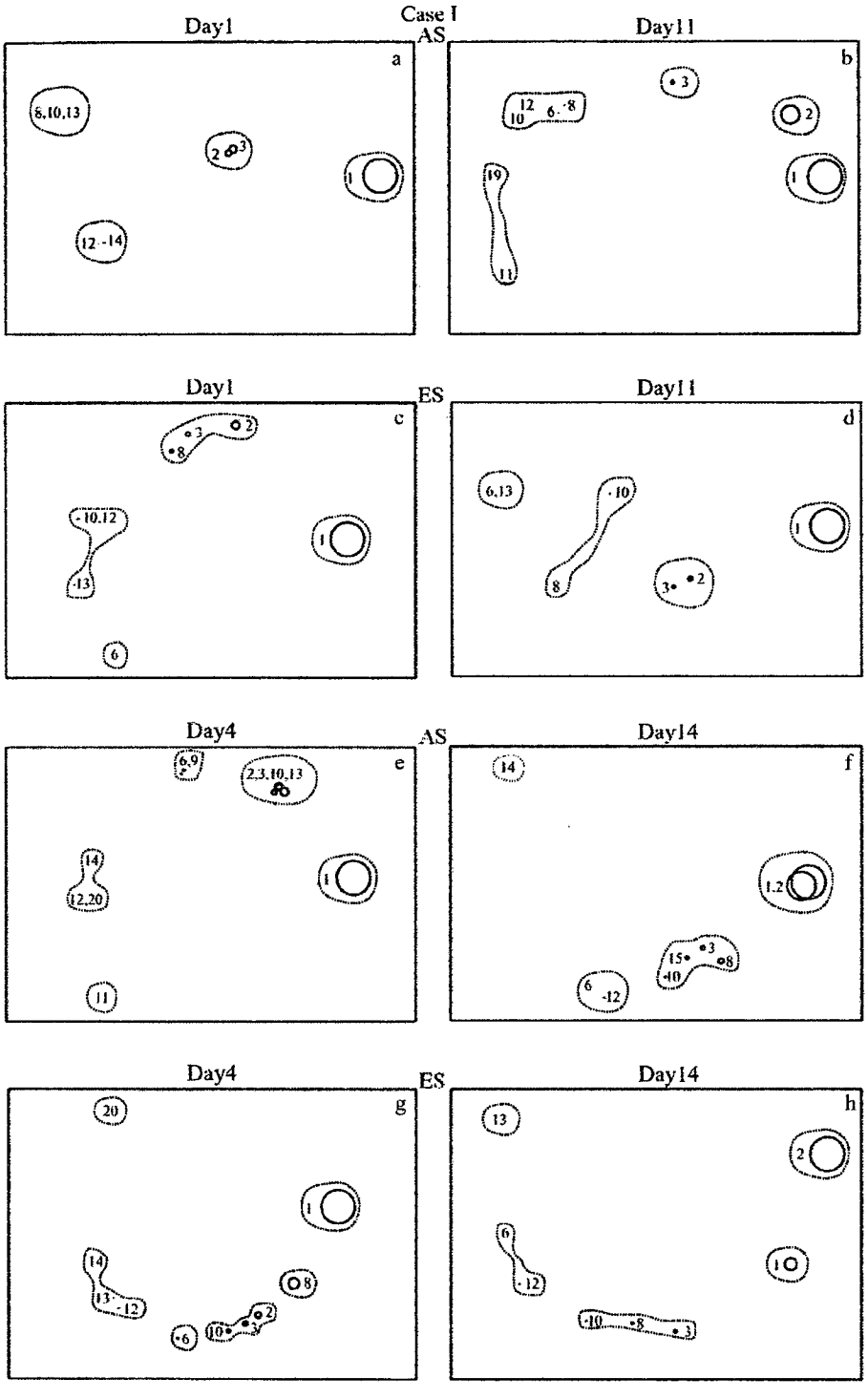


Fig. 4.5. Multidimensional scaling (MDS) ordinations for the case I periphytic diatom community incubated in AS and ES based on Bray-Curtis similarities. In the bubble plot, increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species. 4.5a, c, d, e and g. *N. delicatula* **(1)** is the dominant diatom. 4.5b. *N. delicatula* **(1)** and *A. coffeaeformis* **(2)** are the dominant diatoms. 4.5f. *N. delicatula* **(1)** and *A. coffeaeformis* **(2)** exhibiting similar distribution. 4.5h. *A. coffeaeformis* **(2)** is the dominant diatom. 1. *N. delicatula*; 2. *A. coffeaeformis*; 3. *T. frauenfeldii*; 6. *N. longissima*; 8. *P. angulatum*; 9. *N. subinflata*; 10. *N. closterium*; 11. *C. marginatus*; 12. *C. scutellum*; 13. *N. angularis*; 14. *L. juergensii*; 15. *B. obtusa*; 19. *A. subsessilis*; 20. *L. lingbyei*

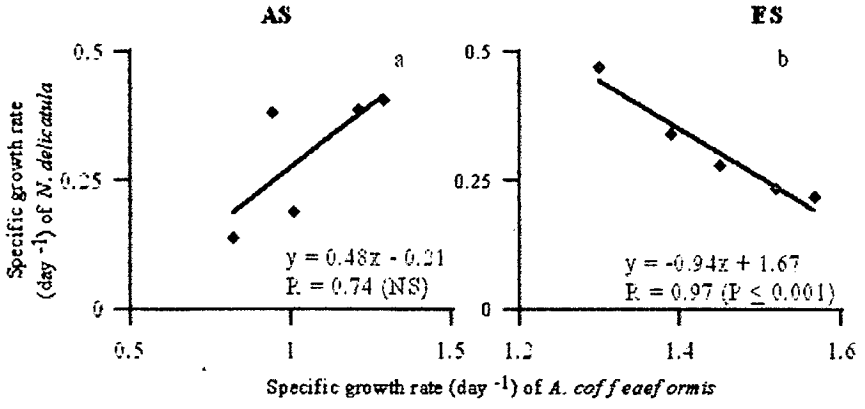


Fig. 4.6 Linear regression analysis of the specific growth rates of *A. coffeaeformis* and *N. delicatula* in AS and ES
NS=not significant

days old community was ~ 0.2:0.85 and linear regression analysis revealed a negative correlation between their growth rates with longer incubation period (Fig. 4.6). *A. coffeaeformis* occupied lesser area as compared to *N. delicatula* and even though the carrying capacity was not exceeded, *N. delicatula* cells were lost from the slide. Polynomial regression analysis between the percent coverage by the periphytic diatom community and species diversity in AS resulted in a trough, revealing a lower coverage at intermediate species diversity and an increase towards higher and lower species diversity (Fig. 4.7a) whereas in case of ES, the percent coverage and species diversity did not show any significant relationship (Fig. 4.7b).

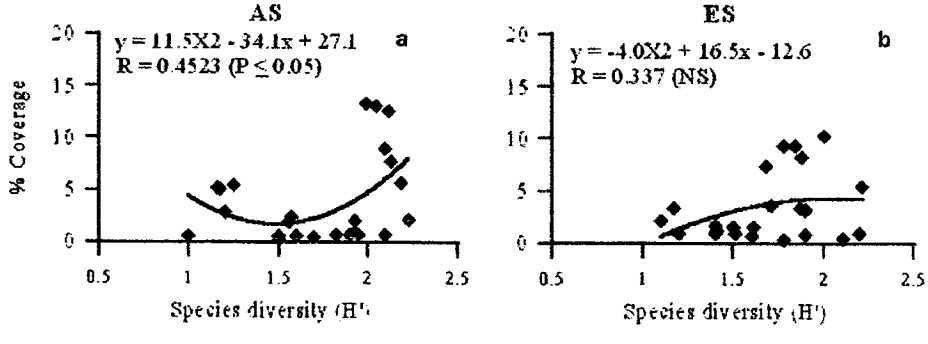


Fig. 4.7 Polynomial regression analysis between the periphytic diatom species diversity and percent coverage in AS and ES of case I
NS=not significant

Competition between N. delicatula and A. coffeaeformis in culture

Pure culture of *A. coffeaeformis* exhibited higher cell abundance as compared to *N. delicatula* with increase in incubation period (Fig. 4.8). The behavior of mixed diatom

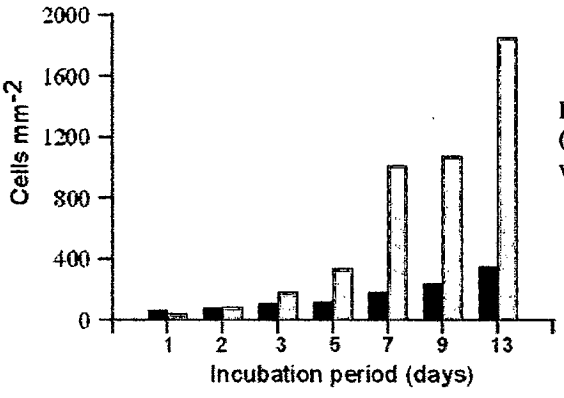


Fig. 4.8 Cell abundance (mm^{-2}) of *N. delicatula* (■) and *A. coffeaeformis* (□) in pure culture with reference to incubation period.

culture of *A. coffeaeformis* and *N. delicatula*, when grown at various initial density ratios is given in Fig. 4.9. At an initial density ratio of *N. delicatula* and *A. coffeaeformis* ranging from 99:1 to 1:99, *A. coffeaeformis* showed an increasing trend

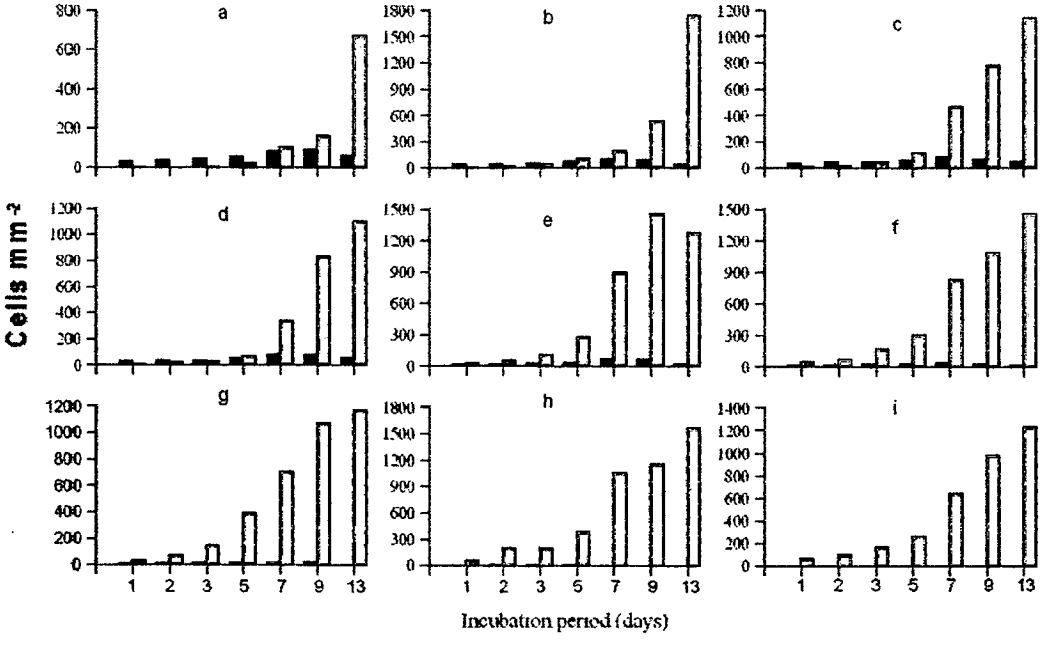


Fig. 4.9 Cell abundance (mm^{-2}) in mixed culture at different initial cell density ratios of *N. delicatula* (■) and *A. coffeaeformis* (□) (a) 99:1 (b) 95:5 (c) 85:15 (d) 75:25 (e) 50:50 (f) 25:75 (g) 15:85 (h) 5:95 and (i) 1:99.

with increase in incubation period (Fig. 4.9a-i). With increase in initial density from 1 to 50% of *A. coffeaeformis*, the time required to overtake *N. delicatula* reduced from 7 to 2 days (Fig. 4.9a-e). At densities ranging from 75% to 99%, the initial inoculum density of *A. coffeaeformis* being higher, dominated over *N. delicatula* throughout the incubation period (Fig. 4.9).

Case II (Inter and Intrageneric competition)

The periphytic diatom community from the environment differed substantially with exposure duration (Fig. 4.4, 4.10a, c, e & g, Appendix 4.3a-d, 4.4a-d, 4.12a & g, 4.13a & g). The dominant species were *N. delicatula, Navicula subinflata, Amphora turgida, Amphora hyalina, A. coffeaeformis* and *Biddulphia rhombus*. The changes in the community structure after incubation is shown in figures 10a, b, e and f and Appendix 4.3e-h in case of AS and figures 10c, d, g and h and Appendix 4.4e-h in case of ES. Incubation resulted in continued dominance of *N. delicatula* and *A. hyalina* irrespective of nutrient enrichment (e.g. the one day old community). Forms such as *B. rhombus* and *N. subinflata,* which also had a higher initial field inoculum, could not hold on to the substrata and were found in the sloughed off community (Appendix 4.3i-l and 4.4i-l). While *B. rhombus* was lost soon after incubation, high numbers of *N. subinflata* were encountered with longer incubation period. *N. delicatula* and *A. hyalina* were in less numbers in the sloughed off community. Incubation of the three days old community revealed that higher initial field inoculum of *A. turgida* resulted in its higher growth rate than *A. hyalina,* in ES (Appendix 4.4g, 4.13g-l). Similar pattern was observed in AS (Appendix 4.3g, 4.13a-f), even though the initial inoculum of *A. turgida* was low as compared to *A. hyalina*. In the four days

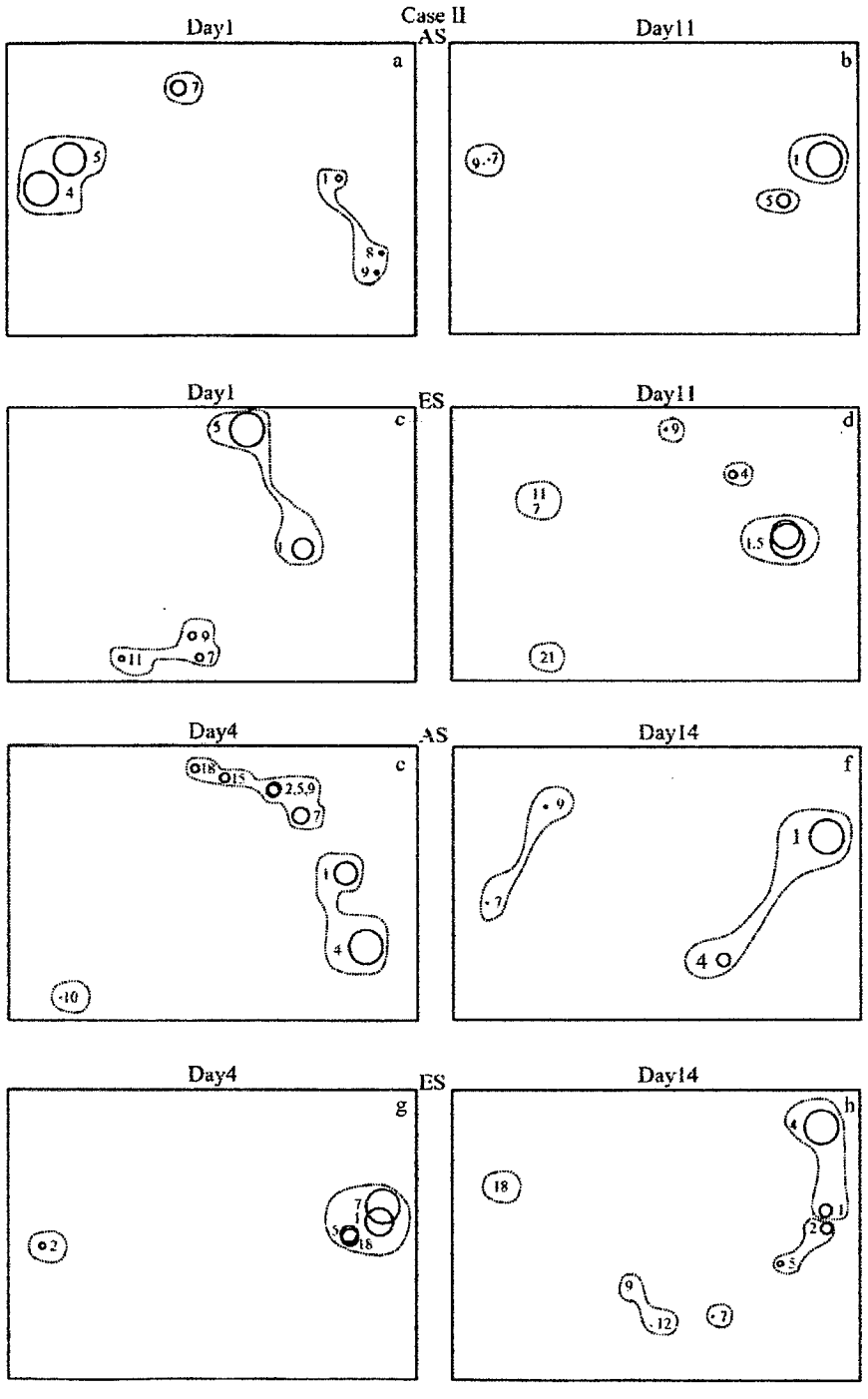


Fig. 4.10. Multidimensional scaling (MDS) ordinations for the case II periphytic diatom community incubated in AS and ES based on Bray-Curtis similarities. In the bubble plot, increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species. 4.10b. *N. delicatula* **(1)** is the dominant diatom. 4.10a. *N. delicatula* **(1),** *P. angulatum* **(8)** and *N. subinflata* **(9)** are the dominant diatoms. 4.10c. *N. delicatula* **(1)** and *A. hyalina* **(5)** are the dominant diatoms. 4.10d. *N. delicatula* **(1)** and *A. hyalina* **(5)** exhibiting similar distribution. 4.10e, f and h. *N. delicatula* **(1)** and *A. turgida* **(4)** are the dominant diatoms. 4.10g. *N. delicatula* **(1),** *A. hyalina* **(5),** *B. rhombus* **(7)** and *G. marina* **(18)** are the dominant diatoms 1. *N. delicatula*; 2. *A. coffeaeformis*; 4. *A. turgida*; 5. *A. hyalina*; 7. *B. rhombus*; 8. *P. angulatum*; 9. *N. subinflata*; 11. *C. marginatus*; 12. *C. scutellum*; 15. *B. obtusa*; 18. *G. marina*; 21. *A. longipes*

old community, introduction of *A. coffeaeformis* in ES was followed by an intrageneric competition between the three species of *Amphora* in ES (Appendix 4.4h, 4.14e-h). *A. turgida* turned out to be a comparatively stronger competitor (Fig. 4.10h). Simultaneously, an intergeneric competition was observed between the three species of *Amphora* and *N. delicatula*. In this competition, *A. turgida* and *N. delicatula* were found to be dominant. Comparatively, incubation of the four days old

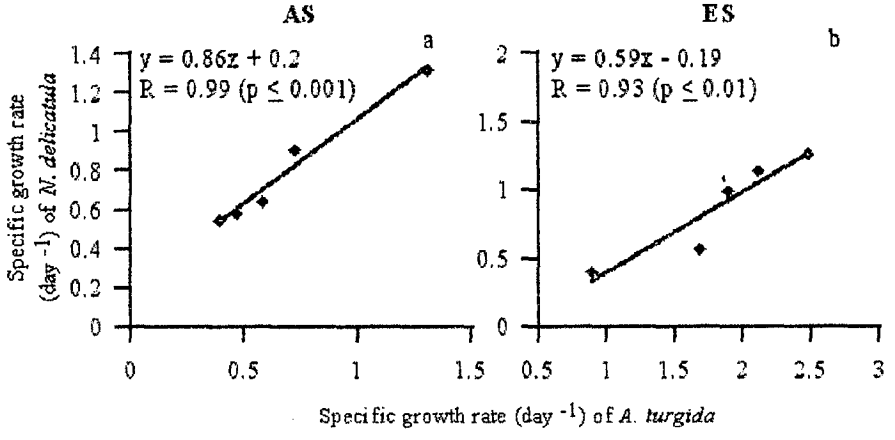


Fig. 4.11 Linear regression analysis of the specific growth rates of *A. turgida* and *N. delicatula* in AS and ES
NS=not significant

field community in AS, showed *A. turgida* and *N. delicatula* to be dominant (Fig. 4.10f, Appendix 4.3h, 4.14a-d). Linear regression analysis revealed a positive correlation between their growth rates with longer incubation period (Fig. 4.11a and 4.11b). The other species of *Amphora*, viz. *A. hyalina* and *A. coffeaeformis*, were observed in the sloughed off community along with *N. subinflata* and *B. rhombus*, which had a higher initial field inoculum (Appendix 4.3i-l). As in case I, it is observed that the area coverage of *A. turgida* was less and did not exceed the carrying capacity, and yet resulted in the loss of competitor species. Polynomial regression analysis revealed that the percent coverage by the periphytic diatom community decreased as the species diversity increased in AS (Fig. 4.12a) whereas in case of ES, species diversity and area coverage did not show any significant relationship (Fig. 4.12b).

Case III (Competitive exclusion or co-existence)

Dominance of periphytic diatoms in the differently aged slides differed (Fig. 4.4, Appendix 4.5a-d and 4.6a-d). In addition to *N. delicatula, N. subinflata, N. closterium A. turgida, A. coffeaeformis* and *A. hyalina*, encountered in case I and II, this community included other major forms such as *Thalassionema nitzschioides and Nitzschia longissima*. The incubation of one-day-old field community showed *N.*

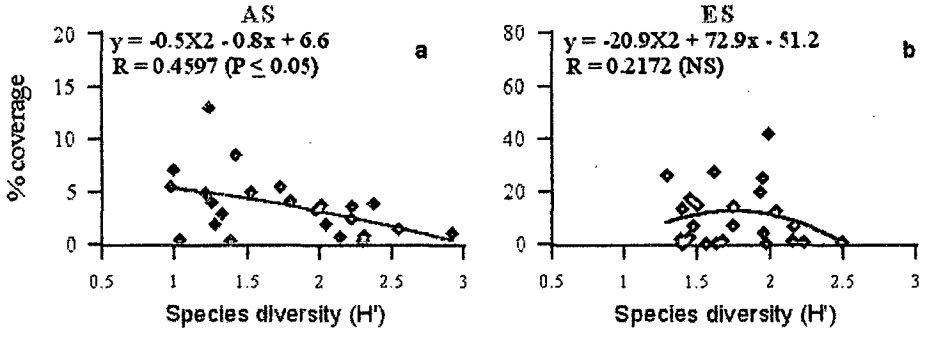


Fig. 4.12 Polynomial regression analysis between the periphytic diatom species diversity and percent coverage in AS and ES of case II
NS=not significant

delicatula and *N. subinflata* to continue their dominance irrespective of nutrient enrichment (Fig. 4.13a, b, c and d, Appendix 4.5e, 4.6e, 4.15a-h). In addition to these forms, the ES incubated community showed the presence of *N. closterium* whereas the AS incubated community showed the presence of *A. coffeaeformis*. Subsequent to incubation, the two days old-field community did not differ much with respect to nutrient enrichment (Appendix 4.5f, 4.6f, 4.16a-l). It comprised major forms of one-day field community and an addition of *A. turgida*. The initial inoculum of the *Amphora* species was low as compared to others. In case of the three days old community incubation in ES, *N. delicatula* was closely followed by *N. subinflata, N. longissima* and *A.coffeaeformis* (Appendix 4.6g, 4.17g-l). *N. closterium* and *T. frauenfeldii* with a high initial field inoculum were not found to establish on the original substrata and were found in the sloughed off community (Appendix 4.6i-l).

The AS incubated three days old field community had a high initial inoculum of *N.*

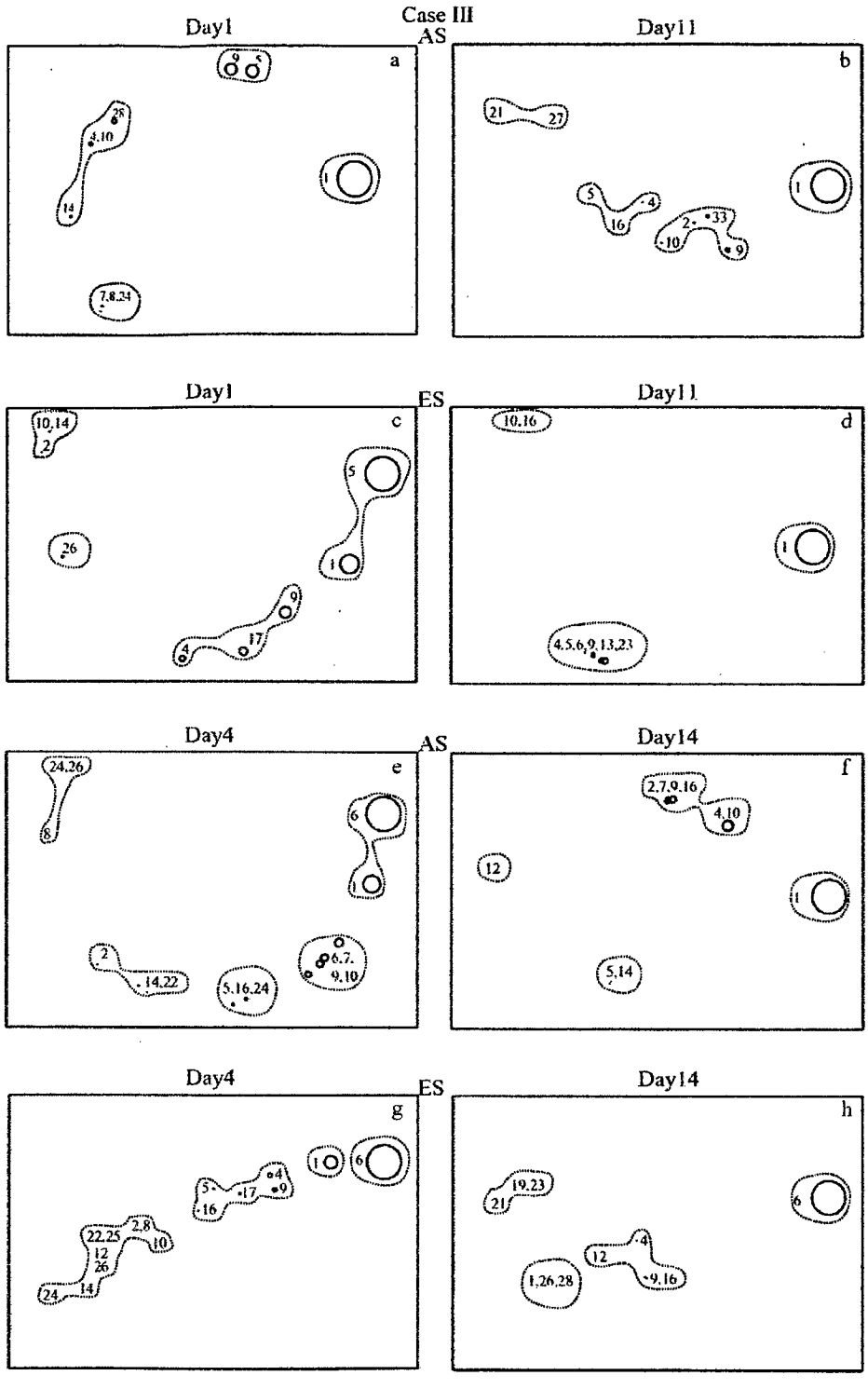


Fig. 4.13. Multidimensional scaling (MDS) ordinations for the case III periphytic diatom community incubated in AS and ES based on Bray-Curtis similarities. In the bubble plot, increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species. 4.13a, b, d, h. *N. delicatula* **(1)** is the dominant diatom. 4.13f. Co-existence of *N. delicatula* **(1),** *N. closterium* **(10)** *A. turgida* **(4)** and *A. rostrata* **(16).** 4.13e. *N. delicatula* **(1)** and *N. longissima* **(6)** are the dominant diatoms. 4.13c. *N. delicatula* **(1)** and *A. hyalina* **(5)** are the dominant diatoms. 4.13g, h. *N. longissima* **(6)** is the most dominant diatom 1. *N. delicatula*; 4. *A. turgida*; 5. *A. hyalina*; 6. *N. longissima*; 7. *B. rhombus*; 8. *P. angulatum*; 9. *N. subinflata*; 10. *N. closterium*; 12. *C. scutellum*; 13. *N. angularis*; 14. *L. juergensii*; 16. *A. rostrata*; 17. *T. nitzschioides*; 19. *A. subsessilis*; 21. *A. longipes*; 22. *L. paradoxa*; 23. *P. seriata*; 24. *T. eccentrica*; 25. *N. septentrionalis*; 26. *M. nummuloides*, 27. *G.serpentina*; 28. *M. membranacea*; 33. *N. sigma*

delicatula, *N. subinflata*, *T. nitzschioides*, *N. longissima* and *N. closterium* (Appendix 4.5g, 4.17a-f). However, out of these only *N. delicatula* and *N. subinflata* continued their dominance after incubation. Rest of the forms were found in the sloughed off

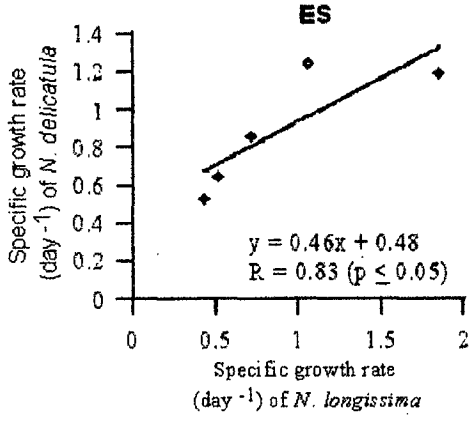


Fig. 4.14 Linear regression analysis of the specific growth rates of *N. longissima* and *N. delicatula* in ES
NS=not significant

community (Appendix 4.5i-l). *N. longissima* was a dominant component of the four days old field community (73%) and this species maintained its dominance over *N. delicatula*, in ES (Fig. 4.13h). Linear regression analysis revealed a negative correlation between their growth rates with longer incubation period (Fig. 4.14). In the sloughed off community, *N. delicatula* was seen, along with *N. subinflata* and *A. coffeaeformis*. Dominance of *N. longissima* was reflected in the area coverage wherein it exceeded the carrying capacity of the substrata. In case of AS, *N. longissima*, even though the initial inoculum was high, could not grow and gave way to other forms such as *N. delicatula*, *N. closterium*, *N. subinflata*, *A. turgida*, *A. hyalina* and *A. coffeaeformis* (Fig. 4.13f). In this case, difference between the original substrate (Appendix 4.5e-h) and sloughed off community was minimal (Appendix 4.5i-l). Polynomial regression analysis revealed that the percent coverage by the periphytic diatom community was higher at lower species diversity in ES (Fig. 4.15b) whereas in AS a trough was observed which showed lesser area coverage at intermediate species diversity (Fig. 4.15a). Area of the individual cell for each of the periphytic diatom species is given in Table 4.1.

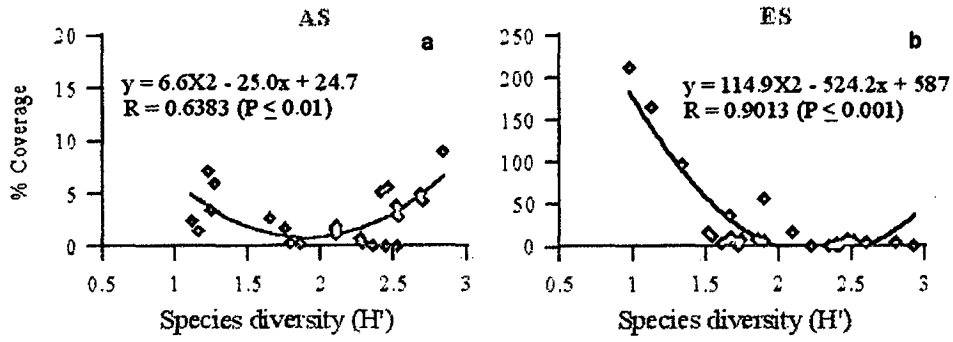


Fig. 4.15 Polynomial regression analysis between the periphytic diatom species diversity and percent coverage in AS and ES of case III
NS=not significant

4.4 DISCUSSION

An increase in periphytic diatom cell abundance in the field with longer submersion period reveals that the effect of cell immigration and cell reproduction overrides that of the other factors (Fig. 4.1a). Subsequent to incubation of the one to four days old-field diatom community in laboratory conditions, where physical disturbance and grazers were absent, increase in the periphytic diatom population, can be attributed to cell multiplication (Fig. 4.1b). However, these communities included a large number of rare species (Fig. 4.5, 4.10, and 4.13, Appendix 4.1e-h to 4.6e-h, Table 4.1), which did not contribute to the corresponding assemblage by building up a sizeable population.

In this study, three scenarios emerged from the interactions between forms of the periphytic diatom community (Fig. 4.16a-c). In case I (Fig. 4.16a), the major players, *A. coffeaeformis* and *N. delicatula* are the natives of the surrounding intertidal sand flat, where *A. coffeaeformis* is the abundant form (Mitbavkar and Anil 2002). However, *A. coffeaeformis* represents the ambient waters in low numbers, which limit their colonizing capabilities in the periphytic diatom community (e.g. substratum away from their natural habitat) in this area. Subsequent to incubation of the different

aged (one to four days old) field communities, the increasing trend of total diatom abundance observed for the one and two days old-field communities, being higher for

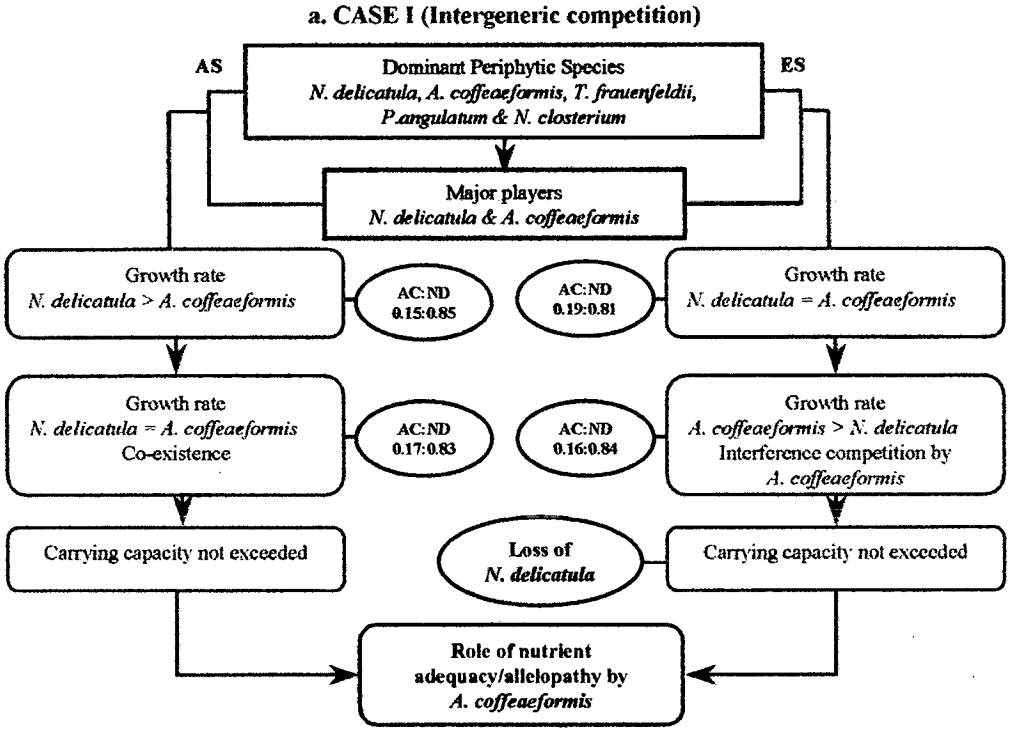


Fig. 4.16a Schematic representation of the periphytic diatom community interactions encountered during the laboratory incubations in case I

ES than AS, was reversed for the three and four days old-field communities. Reason for this reversal was the elimination of *N. delicatula* from the substrate by *A. coffeaeformis* in the presence of additional nutrients. *A. coffeaeformis* exhibited a faster growth rate and surpassed *N. delicatula* within four days of incubation, at an initial field inoculum ratio of 0.2:0.85. Though *A. coffeaeformis* was increasing in abundance thereby resulting in a higher composition, exceeding that of *N. delicatula*, it was not reflected in the area coverage, because of its smaller size compared to *N. delicatula* (Table 4.1). In this case, even though the substrate carrying capacity had not exceeded, cells of *N. delicatula* were being eliminated from the substratum. Conversely, both these species co-existed in AS. In a study carried out by Buzzelli et

al. (1997) on *A. coffeaeformis*, cultures showed the best growth rate at 900 µM nitrate and 36 µM orthophosphate, which is close to ES N: P ratios.

In another laboratory experiment carried out in order to study the competition between *A. coffeaeformis* and *N. delicatula* in ES, competitive influence of the population of *A. coffeaeformis* on *N. delicatula* was independent of the initial cell density ratio, wherein it could overtake its competitor species even at 1% initial inoculum. But, as the initial density increased the rate of competitive ability to overtake increased thus reducing the time, from 7 days in case of 1% to 2 days in case of 50%. This shows that even though the competitive abilities are independent of the initial density, it does have an influence over the time within which the superior competitor can overtake its counterpart. Growth limitation due to nutrient competition between two species may be explained by higher demands and higher uptake rates of nutrients by *A. coffeaeformis* when nutrients were available above a threshold level. According to the classical ecological competition theory, the one species that can best utilize the limiting nutrients should displace all its other competitors, under idealized conditions. However, coexistence of many species in a natural ecosystem puts such a situation at bay. This discrepancy between the nature and theoretical prediction was referred to as the 'paradox of the plankton' by Hutchinson (1961). Temporal and spatial variability of nature (Hutchinson 1961; Richerson et al. 1970), mortality rates from differential grazing (Paine 1966; O' Brien 1974) and differences in the ability of species to acquire nutrients (Stewart and Lewin 1973; Greeney et al. 1973; Petersen 1975) are the theories put forth to explain such a situation. A study by Tilman (1976) has shown that long-term co-existence of competing species can occur only when the growth rate of each species is limited by a different nutrient. It has been suggested

that the co-existence may be the result of the infinite number of possible ratios of resource concentrations that can occur in evolving a natural system (Tilman 1982).

Probably such exclusion is also aided by allelopathy wherein members of one species interferes with or directly harm members of a second species, which ultimately loses the competitive battle through chemical interactions (Valiela 1984). Production of such a compound would be regulated by nutrients. Such interaction has also been reported among marine phytoplankton species and is suggested to play an important role in ecology. Pratt (1966) found a temporary inhibition of *Skeletonema costatum* by filtrates taken from a culture of *Olithodiscus luteus* only when the *Olithodiscus* concentration exceeded 108 cells liter $^{-1}$. *A. coffeaeformis*, which is known for domoic acid production, a water-soluble toxin (Maranda et al.1989), is reported to have certain toxic effects (Buzzelli et al. 1997). Since the toxicity of these allelopathic compounds depends on the amount released and on the abundance of both, *A. coffeaeformis* and *N. delicatula* (target species), this effect was not observed in the absence of additional nutrients. This possibility has to be investigated in detail.

It is evident from this observation that the species composition will determine the outcome of a particular community interaction. Grazing cannot influence community structure, since for such an effect; selective grazing of a particular key species is necessary. Such a process at the micro scale level has to be investigated in detail. Area coverage was controlled by species diversity, which was in turn dependent on the nutrient supply. In the presence of additional nutrients, the elimination of *N. delicatula* by *A. coffeaeformis*, resulted in a non-significant relationship between the species diversity and percent coverage (Fig. 4.7b). However, in case of AS, similar growth rates of both these forms resulted in a significant relationship between the species diversity and percent coverage (Fig. 4.7a).

As compared to case I, where only one species, *A. coffeaeformis*, competed with *N. delicatula* for the dominant position, in case II (Fig. 4.16b), the competition was tougher involving three species, *A. hyalina*, *A. turgida* and *A. coffeaeformis*, which competed with *N. delicatula*, thus playing an important role in community structure. In the presence of nutrients, an intrageneric competition was brought about between the three species of *Amphora*, with a simultaneous intergeneric competition with *N. delicatula*. Ultimately, *A. turgida* turned out to be a better competitor in the intrageneric competition. In the intergeneric competition, both *A. turgida* and *N. delicatula* were dominant. However, in the absence of additional nutrients, the other congeneric competitor species were eliminated from the substrata and *A. turgida* proved to be a superior competitor, along with *N. delicatula*. The losers were seen in the sloughed off community. The sloughed off community showed the presence of *N. subinflata* (one day old community; ES) and *B. rhombus* (three days old community; AS). These forms were not recorded on slide, which indicates that their presence went unobserved due to their low numbers. Also, an increase in cell numbers after an initial slow loss was observed which shows that these cells were present and were multiplying on the slide, however at a slower rate than the other major forms. *A. turgida* and *N. delicatula* could grow in both, nutrient enriched as well as in conditions where additional nutrients were absent.

From these observations, it is evident that all three members of *Amphora* probably have similar nutrient requirements. Even then they co-exist in ES, where the common nutrient supply is sufficient, whereas in AS, where the nutrients could be limiting, according to the resource competition theory, only one of the three species of *Amphora*, i.e., *A. turgida*, which could possibly sequester the limiting nutrients at a faster rate thus making it unavailable to the other species of *Amphora* i.e., *A. hyalina*

and *A. coffeaeformis*, proved to be a successful competitor. This species was found to co-exist with *N. delicatula*, which could be due to a difference in nutrient requirements. Competition for nutrients has been reported by Mickelson (1979), wherein *Thalassiosira gravida* is rapidly displaced from ammonium limited chemostats by *Skeletonema costatum* and *Chaetoceros septentrionalis*.

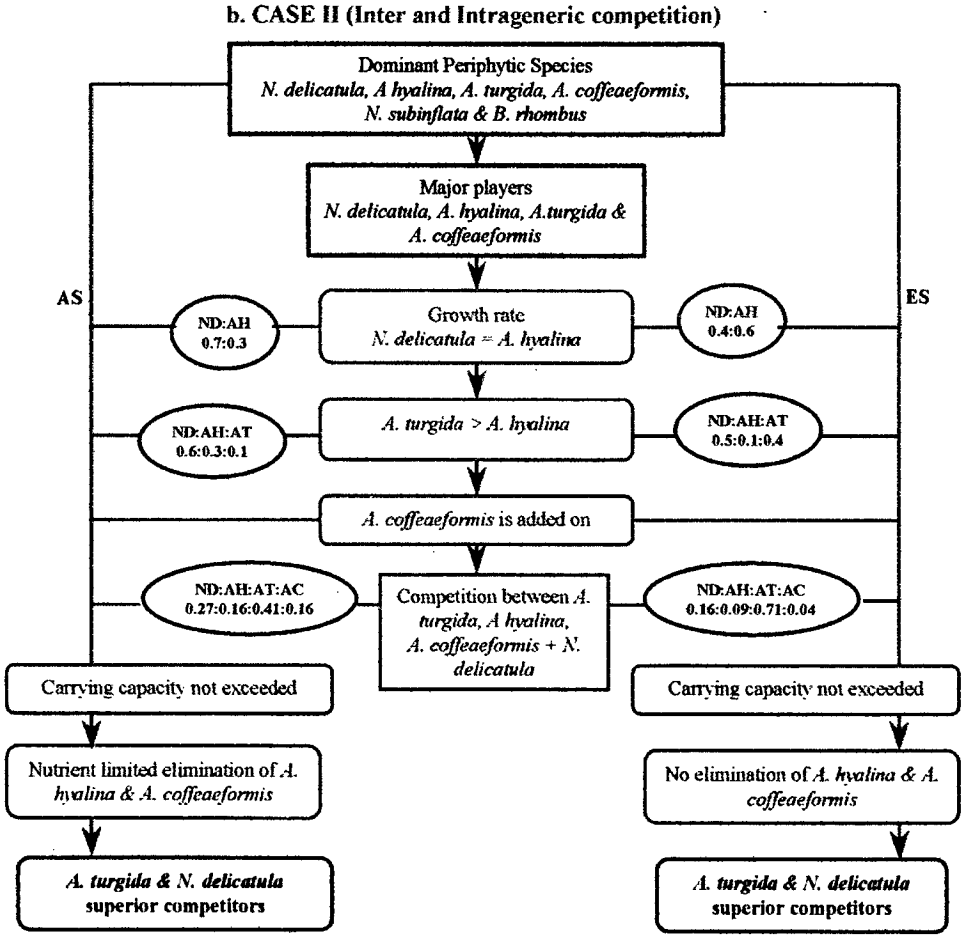


Fig. 4.16b Schematic representation of the periphytic diatom community interactions encountered during the laboratory incubations in case II

A. coffeaeformis, the key player of case I was not given an opportunity to exert its influence in this interaction, as it could not reach the optimal density for bringing about the competitive effect. Even though *A. turgida* played an important role in structuring the diatom community, it did not contribute much to the carrying capacity of the substrata. In case of AS, lowered species diversity due to the elimination of *A.*

hyalina and *A. coffeaeformis*, resulted in a significant relationship between the species diversity and percent coverage, with lowered diversity favoring higher area coverage (Fig. 4.12a).

In case III (Fig. 4.16c), *N. longissima* was the key controller of the community structure. In the presence of additional nutrients, substrate surface area available for attachment became increasingly important for *N. longissima*, thereby resulting in a direct interference competition for space. The probable allelopathic effect shown by *A. coffeaeformis* in case I was not experienced in the present scenario, wherein its initial field inoculum was low and it was displaced by *N. longissima* before it reached the appropriate numbers. *N. delicatula*, *N. subinflata* and *N. closterium* were likewise completely eliminated from the shared environment through competitive exclusion. These forms were seen in the sloughed off community. Such, competitive exclusion has been reported by Hambright and Zohary (2000) in their study in a subtropical area, where seasonal dominance of microcystis (blue-green algae) eliminates other species, light being the primary limiting resource. Sommer (1984) through multispecies competition experiments demonstrated that co-existence could occur when a key nutrient is supplied at regular intervals. The high densities achieved by the opportunistic species, *N. longissima* diminished the carrying capacity of the substrate and probably denied access to the key nutrients to its competitors, thereby suppressing their growth and resulting in lowered diversity. In spite of the lowered species diversity, the larger size of *N. longissima* (Table 4.1) resulted in higher percent coverage. This is evident from the significant relationship between species diversity and percent coverage, with the lowered diversity favoring higher percent coverage (Fig. 4.15b). In the absence of additional nutrients, *N. longissima* could not prosper, even though its initial field inoculum was high, probably because it could not

sequester the key nutrients present in low concentrations. This gave way to forms such as *N. subinflata*, *N. closterium* and *A. turgida* which could grow efficiently even

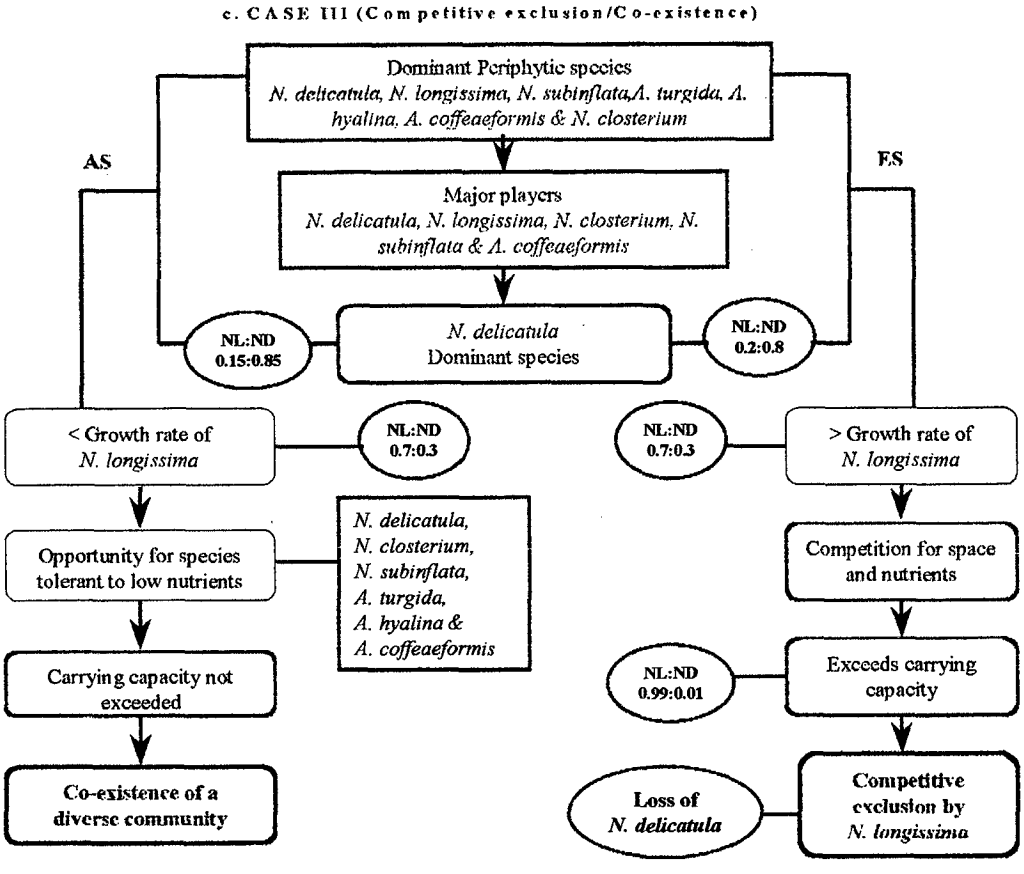


Fig. 4.16c Schematic representation of the periphytic diatom community interactions encountered during the laboratory incubations in case III

in the absence of additional nutrients, each probably having a different resource requirement, thereby resulting in a diverse community. Here, increased species diversity resulted in higher percent coverage (Fig. 4.15a). Another reason for the differences in community structure between AS and ES could be the half-saturation constant which is usually lower for the small-sized organisms as compared to the larger ones. Thus, in oligotrophic conditions small phytoplankton thrive better than larger ones. Shifts in diatom community structure have been reported by Chiba and Saino (2002) in the offshore areas of Japan Sea, wherein low concentrations of phosphate caused a change in the dominant diatom species from those adapted to high nutrient conditions to those adapted to low nutrient conditions.

Hillebrand and Sommer (2000) demonstrated that enhanced nutrient supply decreased micro algal diversity with increase in colonization time (12 weeks). Our study which was restricted to 4 days due to the appearance of macro organisms, revealed an increasing trend in species diversity. This difference could be due to the spatial variations. Subsequent incubation of these communities in controlled conditions revealed differences in species diversity depending on nutrient availability. While in ES, the species diversity decreased in case I and III and remained stable in case II; in AS, species diversity remained stable in case I and III and decreased in case II. Species interactions in case I (intergeneric competition), case II (inter and intrageneric competition) and case III (competitive exclusion and co-existence) might have resulted in the variations in species diversity (Fig. 4.4).

Since every substrate will have a particular carrying capacity, each of the species belonging to the diverse community will try its best, to own a territory for itself. The carrying capacity is mainly determined by the substrate area coverage by a particular species. However, a species might be increasing in abundance but the area covered will be less due its smaller size, as seen in case I and II (Fig. 4.16a and b). In such situations, faster uptake of nutrients or chemical interactions (allelopathy) between competing species is one of the possibilities to own space (case I). In another scenario of intrageneric competition, wherein members of the same genus might have similar resource requirements, possibility to own space is by displacing the competitors via resource competition (case II). The superior competitor utilizes nutrients at a faster rate thereby making it unavailable to its competitors. On the other hand, if a species is larger in size it can occupy space through physical exclusion (competitive exclusion) of the competitor species in the presence of sufficient nutrients (case III) (Fig. 4.16c). However, competitive trait of a particular species clicks on at an appropriate cell

density ratio of the competitive and target species. Nutrient adequacy is another factor, which can trigger the competitive expression.

Chapter 5

Morphological changes and detection of diatoms

5A. Study of life cycle of diatoms

5A.1 Introduction

Diatom life cycle includes vegetative and sexual stages. They also, but may not necessarily pass through a seed-like phase known as the resting stage. Resting stages are of two different types, resting spores and resting cells. While, the resting spores are heavy-walled stages that are morphologically distinct from vegetative cells, the resting cells are similar to vegetative cells, except for altered cytoplasmic characteristics (Anderson 1975; Hargraves and French 1975; Hoban et al. 1980; Sicko-Goad et al. 1986; Round et al. 1990). Normally, diatoms reproduce by vegetative division and for many species the vegetative stage is the only one commonly observed (Garrison 1984). According to the Mc Donald – Pfitzer hypothesis most diatoms decrease in valve diameter during the vegetative phase of the life cycle (Round 1972). This phenomenon is unique to diatoms and can be attributed to frustule structure and morphogenesis. During this "shrinking division" mode of asexual reproduction, the two valves of the test separate. Each forms the epivalve of a daughter cell, and a new hypovalve is secreted within each of the parent valves. The result is one cell that is the same size as the parent cell, and one cell that is slightly smaller. Due to the rigidity of the test material, growth of the cell is impossible once the test is secreted. Thus, the average diatom size gets progressively smaller with each round of replication. Sexuality or some similar size regenerative process is thus periodically necessary to keep a population from passing the lower limit of size viability (French and Hargraves 1985). The restoration of cell size by sexual reproduction is a unique feature of diatoms (Drebes 1977). The sexual reproduction mode allows for growth of the zygote to relatively large size. It is the escape hatch for diatoms from the ever-shrinking asexual mode (Drebes 1977; Round et al. 1990;

Mann and Stickle 1993). An alteration of physical or chemical factors has typically been used to initiate auxospore formation, including changes in light intensity, photoperiod, temperature and salinity (Drebes 1977).

In this study, an effort was made to observe the different stages in the life cycle of two pennate diatoms, *Amphora coffeaeformis* and *Navicula delicatula* by exposing them to different salinities. The study focused on these two species, as they are the major members representing the microphytobenthic community of intertidal sediments.

5A.2 Materials and Methods

5A.2.1 Isolation and maintenance of cultures

The two species of pennate diatoms, *A. coffeaeformis* and *N. delicatula* were isolated from the intertidal sediments of the Dias Beach, near Dona Paula Bay, west coast of India (15^0 27' N 73^0 48' E). Cultures of these two species were maintained in the exponential growth phase by weekly reinoculation into fresh culture medium, prepared with autoclaved filtered seawater (35 psu) enriched with nutrients (f/2 media; Guillard and Ryther 1962). Cells were grown in Erlenmeyer flasks (100 ml) containing 50 ml culture medium and were maintained at 27 ± 1 ^{0}C under a 12 h light: 12 h dark photocycle.

5A.2.2 Experimental protocol

The culture medium f/2 was prepared in filtered autoclaved seawater with salinities ranging from 5 to 40 psu. The culture medium with each of these salinities (5 ml) was transferred to polystyrene multiwells (Corning 430343; in triplicates). To these multiwells, cells of *A. coffeaeformis* and *N. delicatula* were inoculated at an initial density of 1000 cells ml $^{-1}$ from an exponentially growing parent culture. These wells were examined everyday to observe any morphological changes that the cells

undergo. Cultures were observed by inverted microscopy (Olympus IX71) and photomicrographs were taken using digital camera (Kodak DC 290).

5A.3 Results

Vegetative or asexual mode of reproduction by cell fission was observed as the predominant method of reproduction in both the species of diatoms at all the

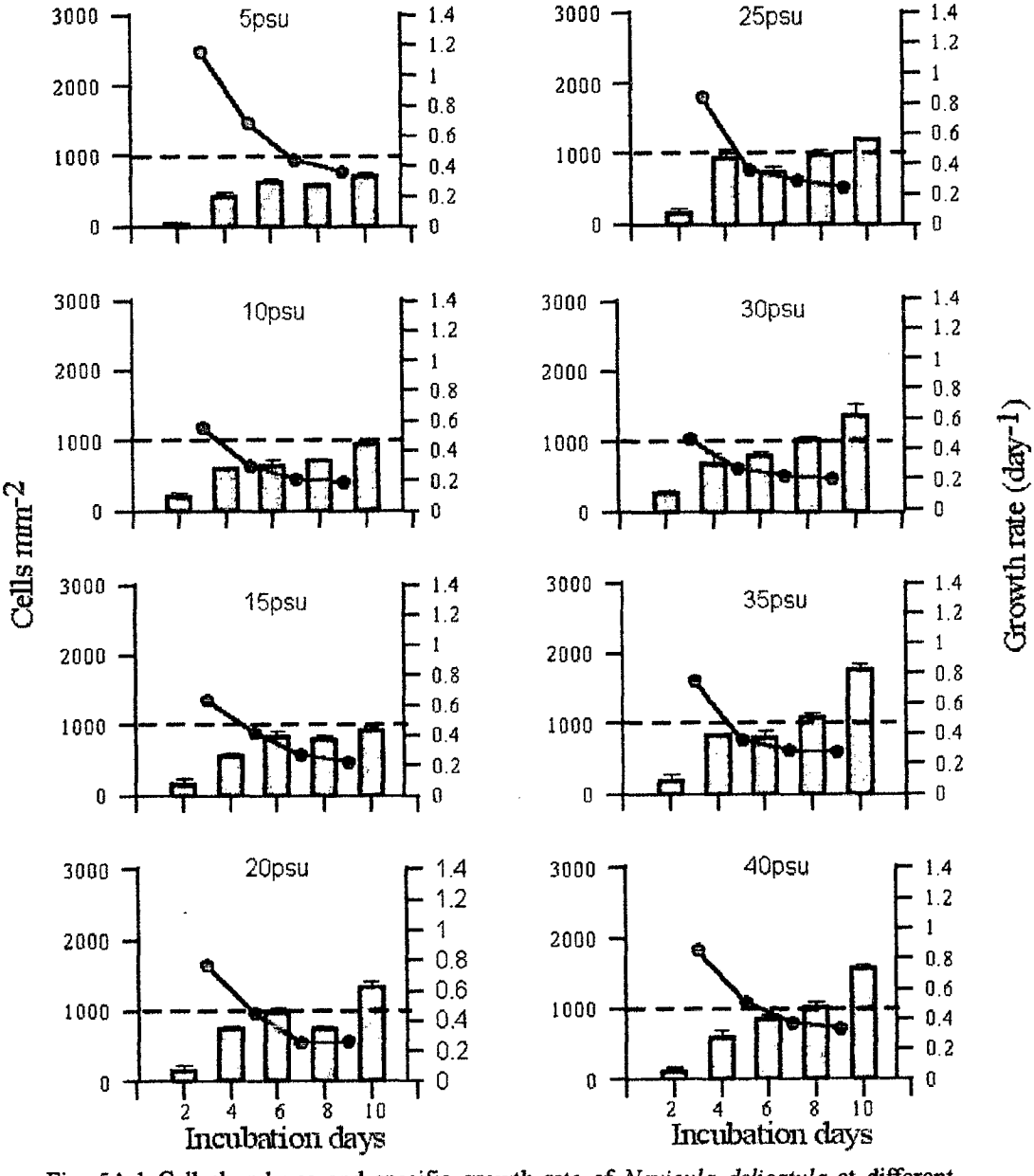


Fig. 5A.1 Cell abundance and specific growth rate of *Navicula delicatula* at different salinities. (Bars indicate cell abundance and line graph indicates specific growth rate)

experimental salinities. Cell abundance and growth rate of the two species at different salinities is given in Fig. 5A.1 and 5A.2. Both the species exhibited faster growth at higher salinities i.e., 30 to 40 psu compared to lower salinities.

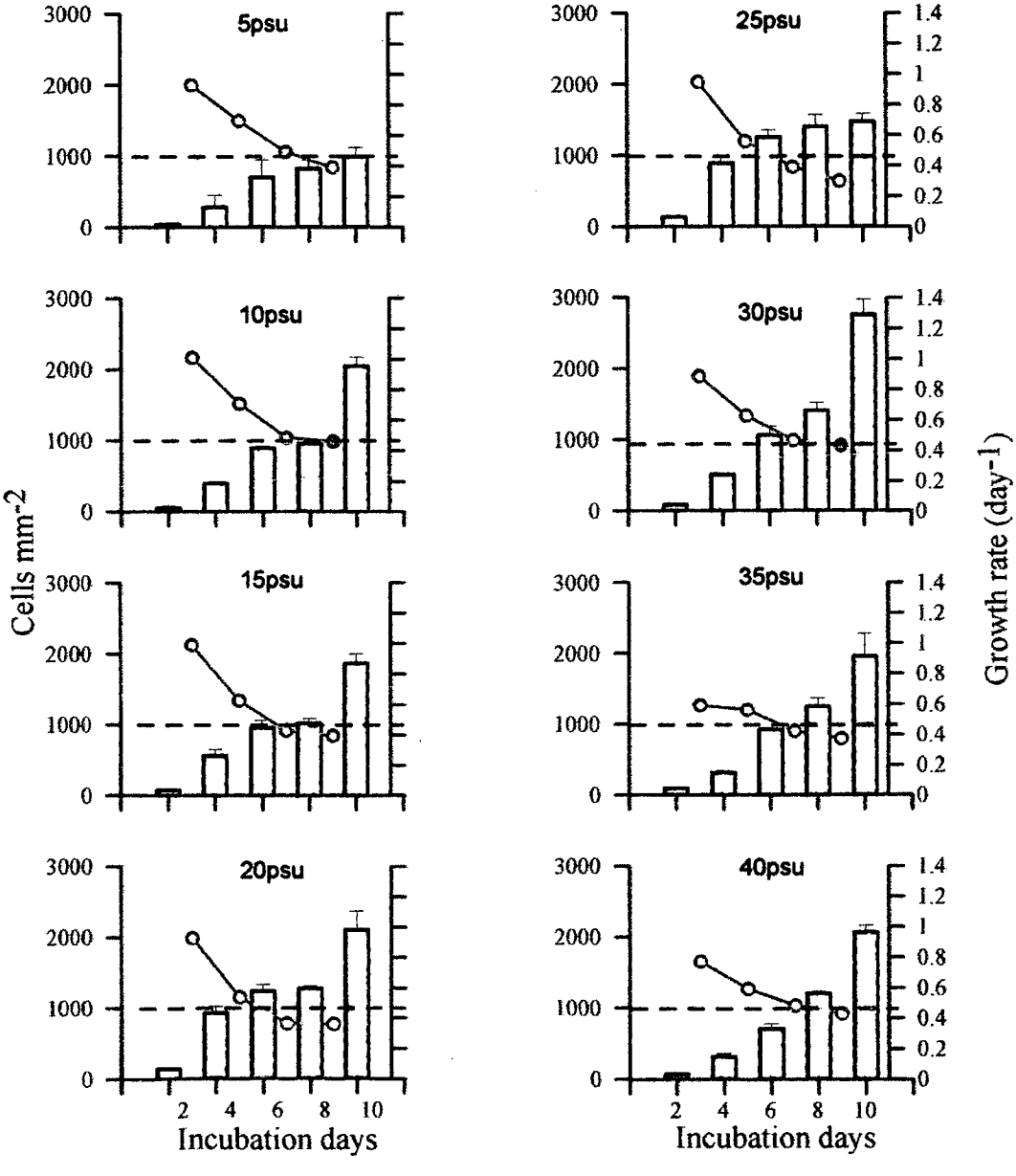


Fig. 5A.2 Cell abundance and specific growth rate of *Amphora coffeaeformis* at different salinities. (Bars indicate cell abundance and line graph indicates specific growth rate)

In both species, sexual reproduction occurred in low numbers as part of a normal life cycle process at all salinities exposed. However, in case of *N. delicatula* an abrupt change in salinity i.e., when parent clones growing at 35 psu were mixed directly into a medium of 5 psu and 10 psu, induced sexual reproduction. Hence, at lower salinities the growth rate of *N. delicatula* was slower than that of *A. coffeaeformis*.

Sexual reproduction showed the following steps in *N. delicatula* (Plate 5A.1a) and *A. coffeaeformis* (Plate 5A.1b).

First stage: Pairing of parent cells (gametangiogamy) wherein gametangia contacted each other valve-to-valve, lying parallel.

Second stage: Gametogenesis wherein each cell in a pair divides meiotically forming spherical gametes. Each gametangium produces two morphologically isogamous nonflagellated gametes.

Third stage: The frustules opened completely, permitting both gametes in one cell to move by amoeboid action toward the passive gametes in other gametangium.

Fourth stage: Plasmogamy wherein only one pair of gametes fused at a time to form a spherical zygote.

Fifth stage: Resulting zygote then started to expand to form auxospore although not synchronously. Sometimes one auxospore enlarged relatively rapidly to its maximal size, whereas the other was still only at the beginning of expansion. The two auxospores eventually attained a similar length. Most of the auxospore volume appeared to be occupied by a single vacuole.

Sixth stage: The restored initial cell was formed inside the auxospore.

5A.4 Discussion

Phytoplankton life cycle stages are of importance in taxonomy and systematics. The present study shows that low salinity stress induces sexual reproduction in the pennate diatom, *N. delicatula* but not in *A. coffeaeformis*.

Both the species acquired greater cell density at 30 to 40 psu but could successfully maintain themselves even at lower salinities (5-25 psu). Low salinity may be one of any number of triggers or stresses that induce an asexual population to resort to sexual reproduction as in case of *N. delicatula*. Comparatively, *A. coffeaeformis* was found to be better tolerant to lower salinities. This showed that this species is euryhaline capable of tolerating a wide range of salinities ranging from 5 psu to 40 psu. While *N. delicatula* is comparatively less tolerant to lower salinities (5-10 psu) wherein there is a reduction in the normal vegetative division with increase in sexual reproduction through auxospore formation. This reveals that sexual reproduction, other than a mode of regaining normal cell size, can be induced on sudden exposure to stress conditions such as salinity variations.

Ragothaman and Rao (1978) have reported auxospore formation in *A. coffeaeformis* at lower salinities. However, in their study any increase in cell length was assumed to have been derived by auxospore formation and there was no visual observation of the actual auxospores and the process of sexual reproduction. Although resting stages were not observed for either of the two species during this study, Anderson (1975; 1976) has reported resting cell formation in *A. coffeaeformis,* when placed in continuous dark. No such reports are available in case of *N. delicatula.*

Howell and Pfiester (1983) have studied the sexual life history of *Amphora veneta* from the natural population. However, much of their work focuses on the

morphological and ultrastructural features of the mature initial cell. In case of *Navicula*, sexual reproduction has been studied in *Navicula cuspidata* (Cohn et al. 1984; Schoeller et al.1984), *Navicula directa* (Mizuno 2000) and *Navicula oblonga* (Mann and Stickle 1989). As observed for other pennate diatoms (Mann 1982; Chepurnov and Mann 1997; Davidowich and Bates 1998), the gametes of *A. coffeaeformis* and *N. delicatula* observed were morphologically indistinguishable from each other and had no flagella.

The number of diatom species in which sexual reproduction has been observed is very small relative to the total number of species recognized (Round et al. 1990). This is due to the difficulty of observing sexually reproducing cells in the natural environment. First and foremost, the ratio of auxospores to vegetative cells is low because relatively few individuals may be involved in sexual reproduction at any one time (Jewson 1992). Also the association between pairs of gametangia is likely to be so weak as to be disturbed during normal sampling (Davidowich and Bates 1998). However, this study will help and simplify identifying their different stages of life cycle in nature.

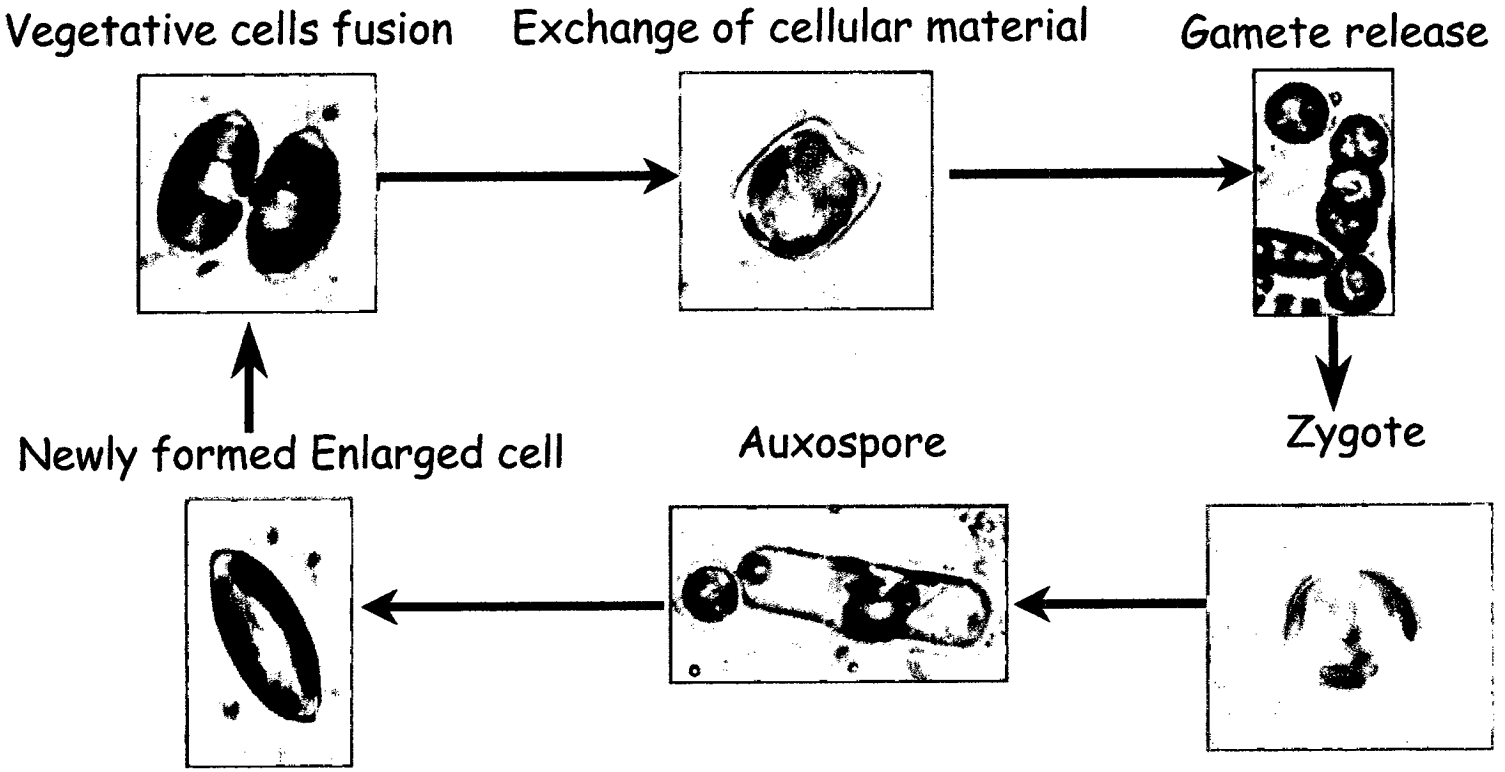


Plate 5A.1b Life cycle stages of *Navicula delicatula*

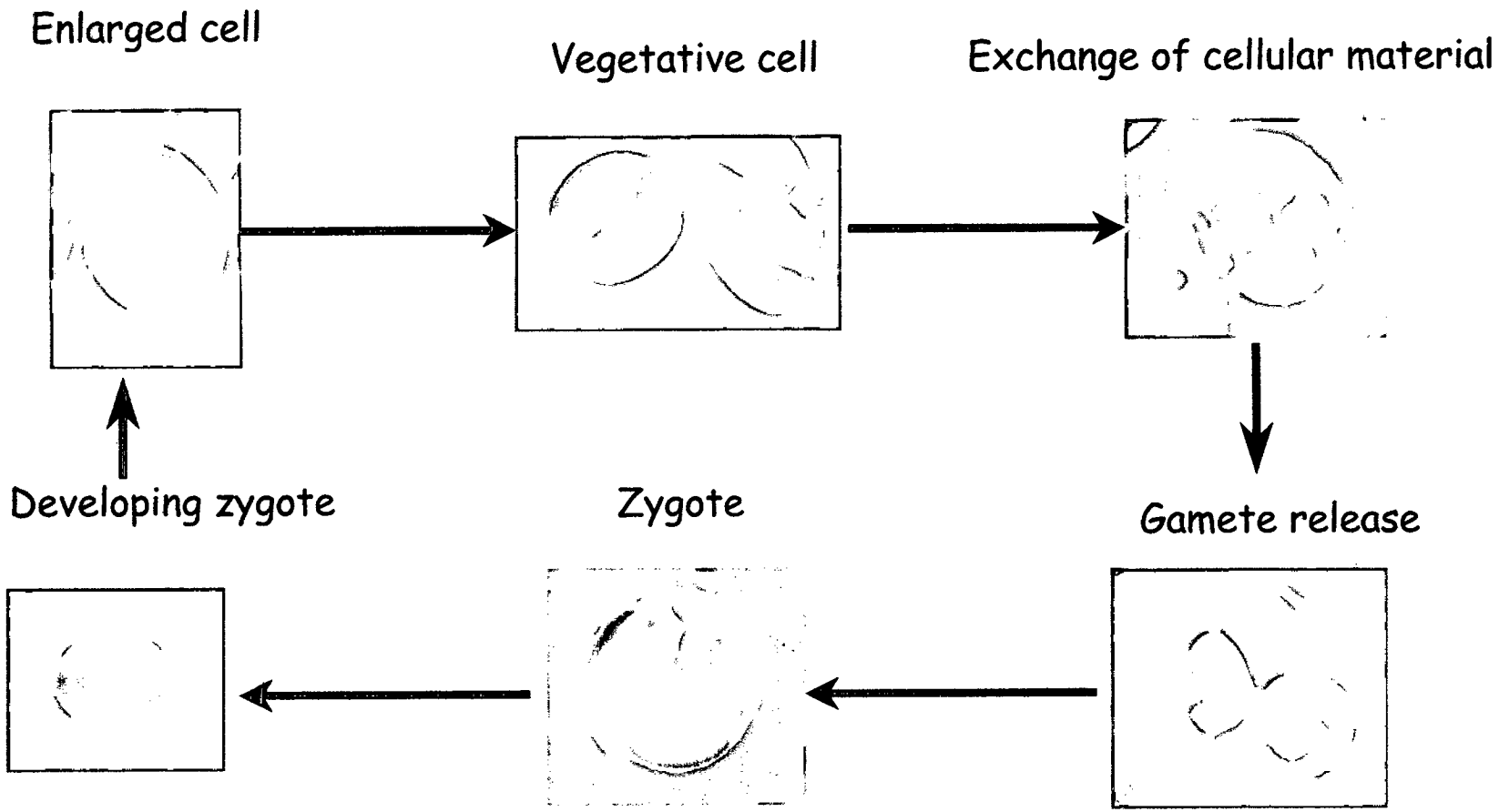


Plate 5A.2b Life cycle stages of *Amphora coffeaeformis*

5B. Effects of low temperature on diatoms

5B.1 Introduction

Freezing is lethal to most living systems, yet it can also preserve cells and their constituents. It slows or stops some biochemical reactions, but it accelerates others (Mazur 1970). At temperatures below –150 ^{0}C, biophysical processes do not affect organisms (Mazur 1964), and at the temperature of liquid nitrogen, temperature variability is independent of storage time (Morris 1981). By this means, the symptoms of aging such as reduced morphological competence and secondary product formation are avoided.

Cryopreservation has been widely used in medical and veterinary sciences, but its application in marine science is new and is only now being explored as an innovative adjunct in biotechnology. Cryopreservation studies include storage of spermatozoa of invertebrates and fish (Dunn and McLachlan 1973; Iwata et al. 1989; Kurokura et al. 1986; Kurokura et al. 1989), cryopreservation of rotifer (*Brachionus plicatilis*) (Toledo and Kurokura 1990), bivalve embryos (Renard 1991; Renard and Cochard 1989) and barnacle larvae (*Balanus amphitrite*) (Anil et al. 1997). Cryopreservation is an especially useful technique for the maintenance of phytoplankton culture collections (McLellan et al. 1991). Relatively few investigations have dealt with the effect of cryopreservation on algal cells (Hwang and Horneland 1965; Morris 1976a, b; McGrath and Daggett 1977) and more particularly on marine ones (Tsuru 1973; Saks 1978; Ben Amotz and Gilboa 1980; Meyer 1985; Fenwick and Day 1992; Levy and Zamir 1994; Bertrand et al. 1996). When algal cultures are maintained at an actively growing state, there is always the possibility of contamination or of genetic changes causing a gradual change in the hereditary makeup of the organism. For

these reasons, as well as because it offers a great saving in time, effort, and space, algal cultures are subjected to long-term preservation.

It is well known that the use of cryoprotectants is important to prevent cell damage during freezing. The absence of an effective cryoprotectant leads to the formation of intracellular ice crystals, which puncture cell organelles and cell membranes. Also, water within the medium freezes, cells become dehydrated, altering the salt concentration and causing morphological or biological changes after recovery. Grout and Morris (1987) reviewed the adverse effects of freezing on cells. They included, osmotic stress induced by freezing out of water. During formation of the ice lattice, dissolved solutes become highly concentrated (Pitt 1990), producing a hyperosmotic environment, which may lead to cell death. The present work evaluates the tolerance of two co-existing benthic diatom species, *Amphora coffeaeformis* and *Navicula delicatula* to low temperature and its effect on their internal morphology and viability. These two are the most abundant diatom species encountered in the intertidal sediments of the Dona Paula Bay (Mitbavkar and Anil 2002). Of these two species, *N. delicatula* also dominates the ambient waters and the microfilm population (Smita and Anil 2000). *A. coffeaeformis* is a species widely studied for its pioneer role in biofouling (Cooksey 1981; Cooksey and Cooksey 1980, 1984, 1986).

5B.2 Materials and Methods

5B.2.1 Culture

Flasks containing 100 ml of f/2 media (Guillard and Ryther 1962) were inoculated with cell culture (*N. delicatula* or *A. coffeaeformis*) obtained from pre-existing stock cultures in the exponential growth phase. These cultures were kept at room temperature in a 12 h light: 12 h dark photocycle for 10 days. Subsequently, the cells

in the exponential phase were collected by centrifugation at 12, 000 x g for 10 min and resuspended in culture medium in cryovials, at an initial concentration of 3×10^5 cells ml^{-1}.

5B.2.2 Experimental protocol

Three different protocols were employed to study the effects of low temperature (Fig.5B.1). In the first set, cryovials containing the cell culture were subjected to

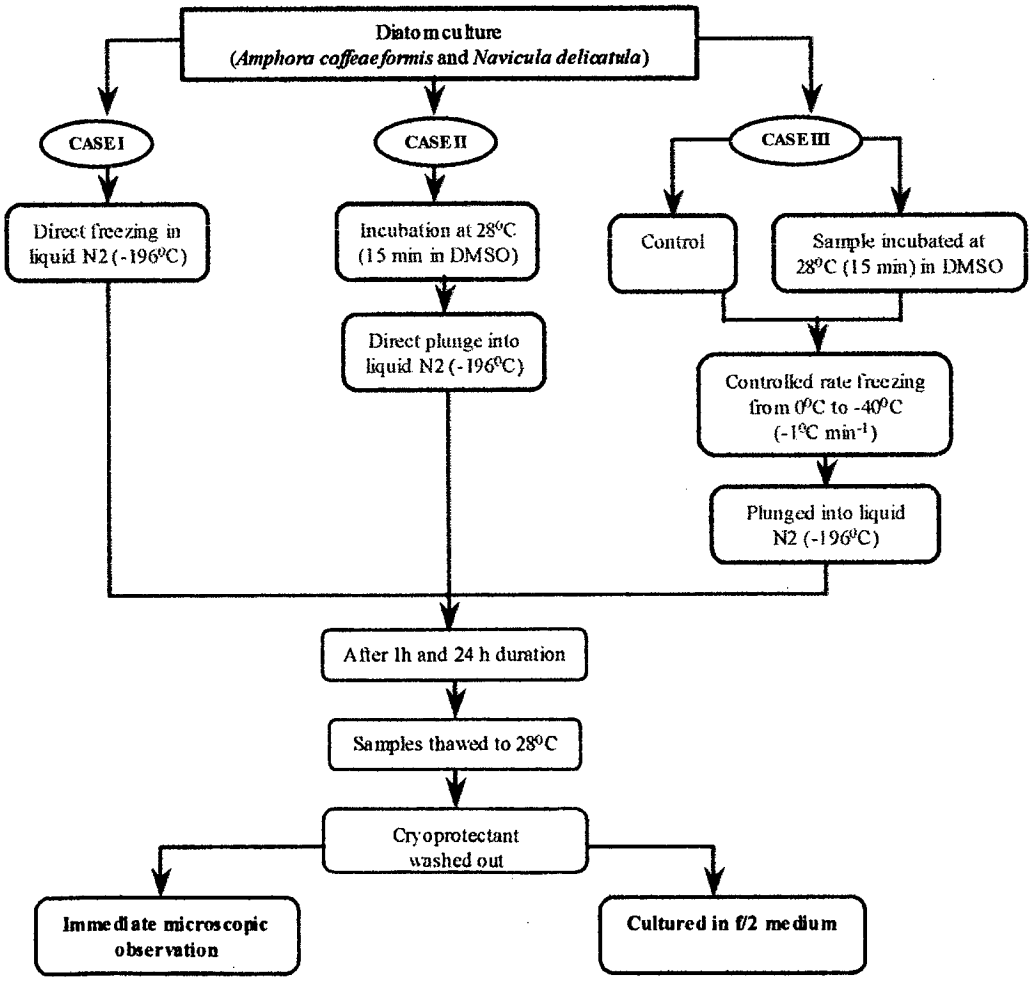


Fig. 5B.1 Schematic representation of the experimental protocol

uncontrolled direct freezing in liquid nitrogen (– 196 ^{0}C) and storage at the same temperature (Case I). The second set involved one-step cooling technique, in which the cryovials with cell culture were initially incubated at room temperature for 15 min

with the cryoprotectant, Dimethyl sulfoxide (DMSO). This was followed by fast cooling in liquid nitrogen (Case II). The third set involved a two-step cooling method, in which the cryovials containing a suspension of concentrated diatom cells were incubated with DMSO (2M) for 15 min at room temperature. These vials were then cooled under controlled conditions with the help of a controlled-rate freezer (Planer Model KRYO 10/16) from + 20 to 0 ^{0}C at – 5 ^{0}C / min and from 0 ^{0}C to – 40 ^{0}C at a rate of –1 ^{0}C / min. Subsequently, these cryovials were cooled rapidly by transfer into liquid nitrogen (Case III). Control was run simultaneously without the cryoprotectant (Case III control). One set of samples was retrieved after 1 hour and another after 24 hours. Each set had four replicates, which were immediately thawed to room temperature. The samples with cryoprotectant were washed thoroughly with f/2 media to remove the cryoprotectant. Later, all the samples were cultured in fresh f/2 media.

5B.2.3 Evaluation of viability

The cells immediately after retrieval were observed for any morphological or internal changes. The same was done for the cultured cells at regular intervals to observe the cells regain their viability. Simultaneously, a control sample was kept for comparison with the treated sample during the recovery and growth of the cells. Cell numbers were also enumerated every alternate day. Staining with neutral red dye was done in order to confirm the time of regaining viability (Songdong and Jixun 2000).

5B.2.4 Data analysis

The cell abundance on incubation of *A. coffeaeformis* and *N. delicatula* after one hour and 24 hours of retrieval were subjected to multiple analyses of variance (MANOVA)

(Sokal and Rohlf 1981). Specific growth rates were calculated for both, *A. coffeaeformis* and *N. delicatula* according to Carreto and Cattogio (1976).

5B.3 Results

5B.3.1 Effects of exposure to low temperatures on N. delicatula

Immediate observations revealed ruptured cell chloroplasts, in case I and case II (Plate 5B.1). In case III and its control, the cell chloroplast was contracted (Plate 5B.1). On incubation, observations at regular intervals showed only the cells from case III recover wherein the contracted chloroplast showed a gradual increase in size, finally regaining the normal size and structure (Plate 5B.1). However, the growth rate of the cryopreserved cells was slower than that of the normal cells (Fig. 5B.2 and 5B.3). The cells in the case III control (i.e., without cryoprotectant) did not show any recovery (Fig. 5B.2 and Plate 5B.1).

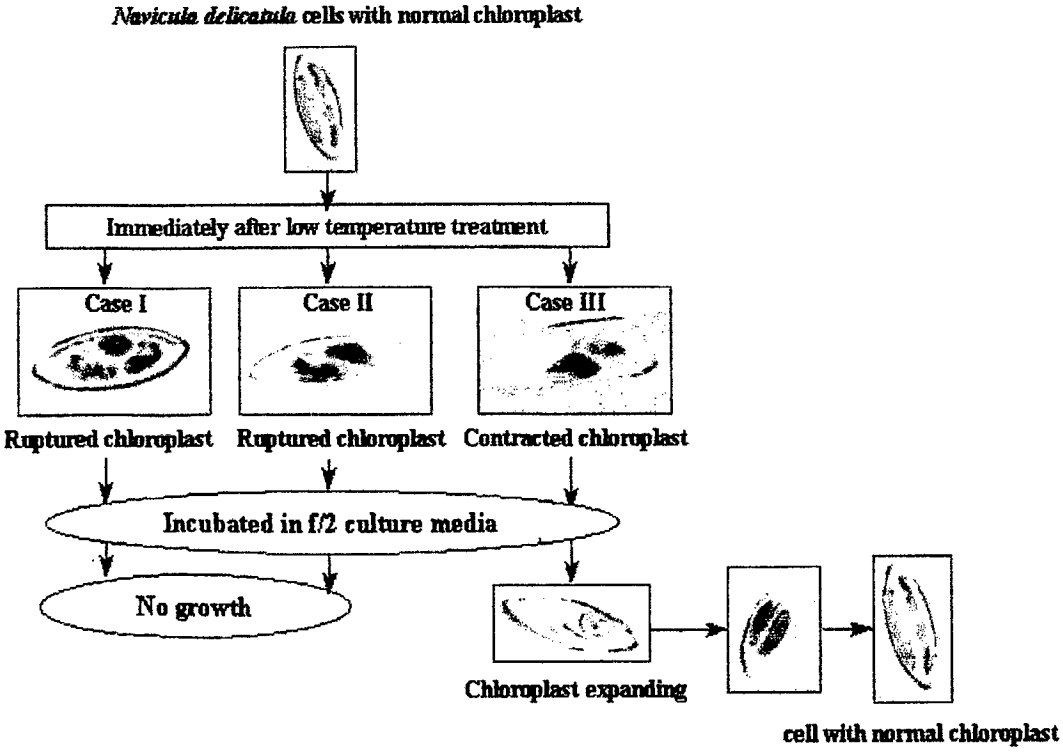


Plate 5B.1 Influence of low temperature on *Navicula delicatula*

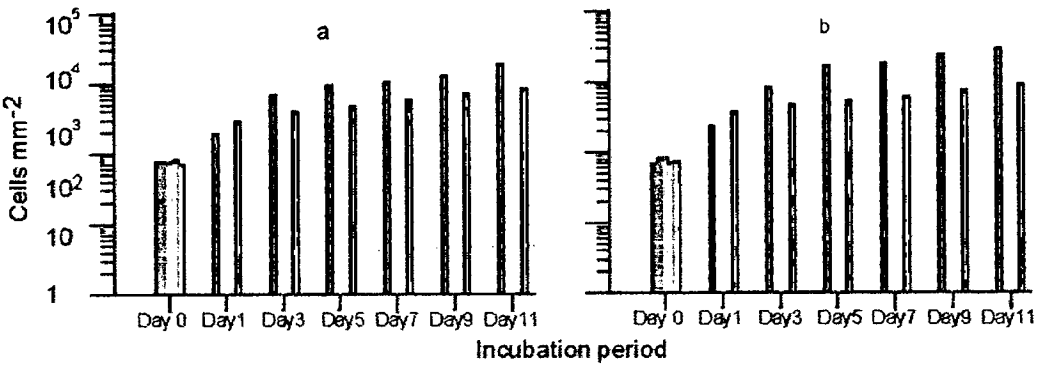


Fig. 5B.2 Cell abundance on incubation of *N. delicatula* after a period of 1h (a) and 24 h (b) of retrieval in case I, case II and case III

□ Control; ▨ Case I; □ Case II; □ Case III control; □ Case III

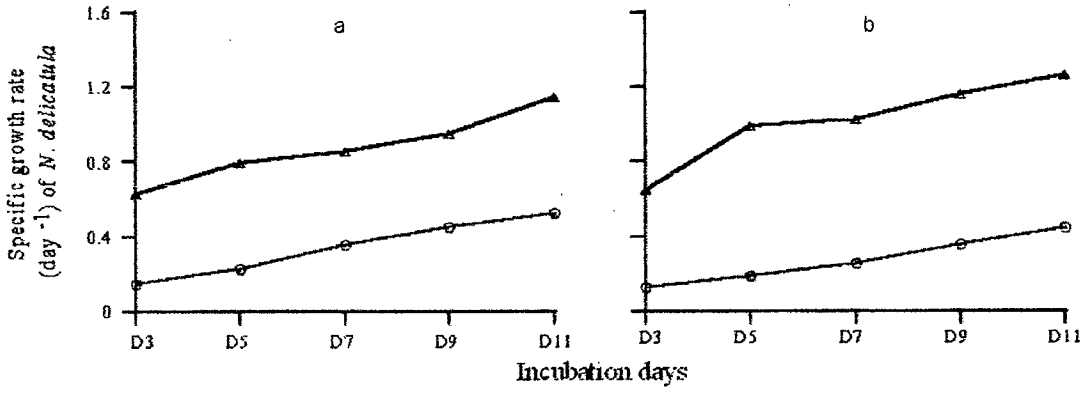


Fig. 5B.3 Specific growth rate of *N. delicatula* on incubation after a period of 1h (a) and 24 h (b) of retrieval in case I, case II and case III

△ Control; □ Case I; ◇ Case II; ○ Case III control; ○ Case III

5B.3.2 Effects of exposure to low temperatures on A. coffeaeformis

Immediate observations showed that the cell chloroplasts were intact in all the cases (Plate 5B.2). Similar to case III of *N. delicatula*, the chloroplasts were contracted, lying in the center of the cell. On incubation, the cells from case I, II and III regained viability, with the chloroplasts attaining the normal shape and size (Plate 5B.2 and Fig. 5B.4). This revealed the capability of cells to survive exposure to low temperatures without any cryoprotectants (Fig. 5B.4 and Fig. 5B.5).

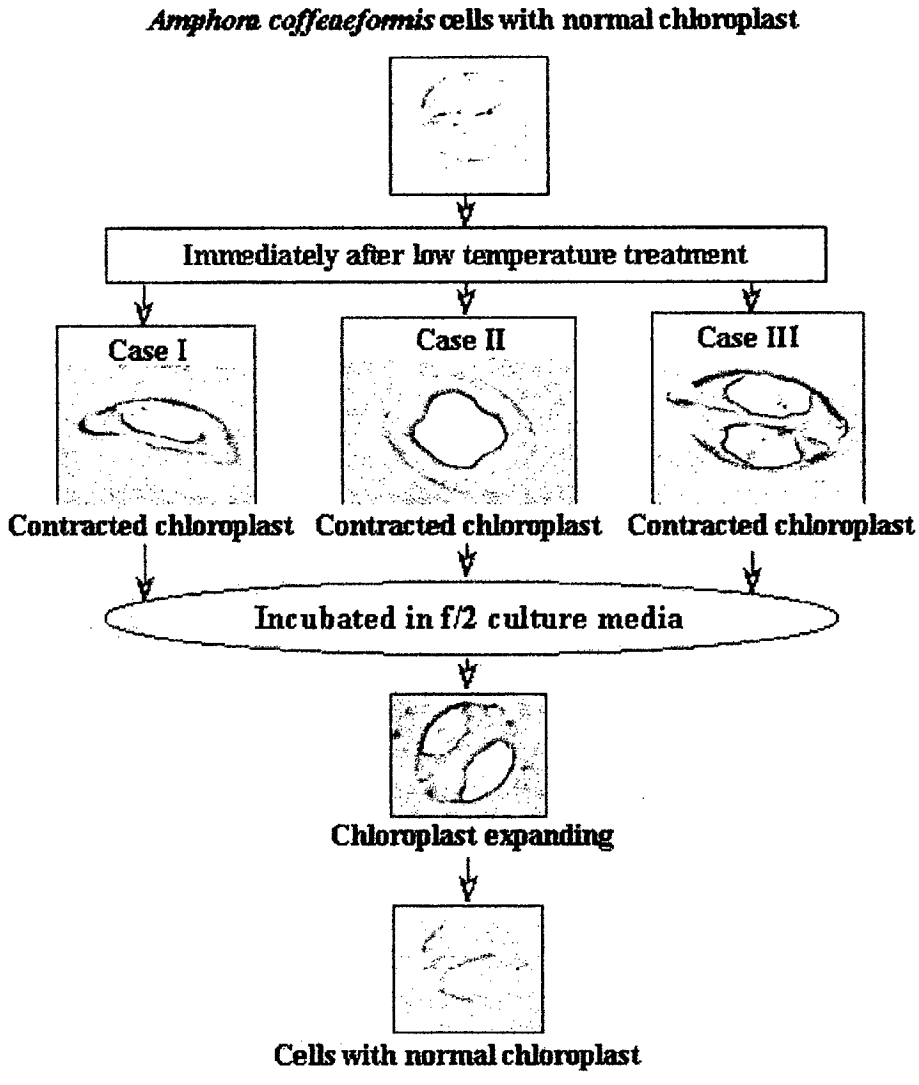


Plate 5B.2 Influence of low temperature on *Amphora coffeaeformis*

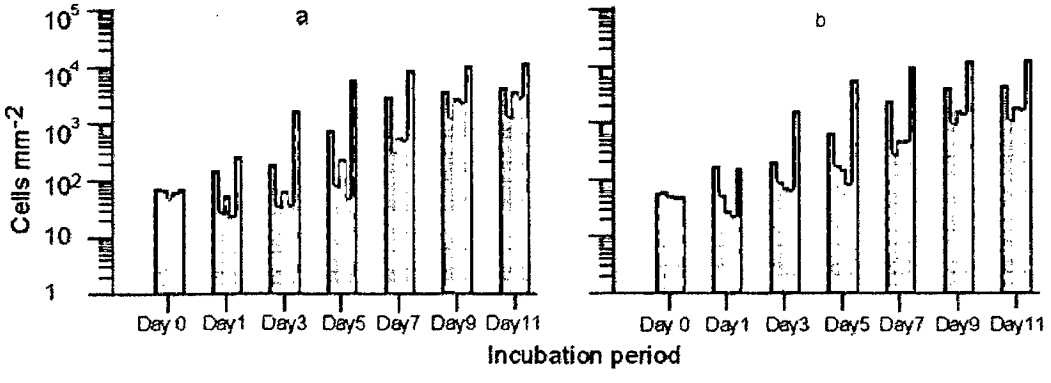


Fig. 5B.4 Cell abundance on incubation of *A. coffeaeformis* after a period of 1h (a) and 24 h (b) of retrieval in case I, case II and case III

☐ Control; ☐ Case I; ☐ Case II; ☐ Case III control; ☐ Case III

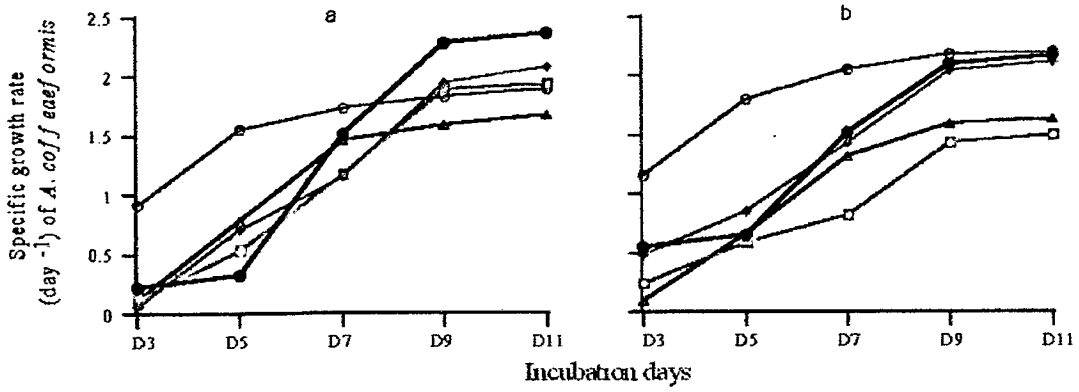


Fig. 5B.5 Specific growth rate of *A. coffeaeformis* on incubation after a period of 1h (a) and 24 h (b) of retrieval in case I, case II and case III
△ Control; □ Case I; ◇ Case II; ● Case III control; ○ Case III

Table 5B.1. MANOVA (Without replication) for the variation in cell abundance between species, incubation days and experimental conditions after a duration of one hour (* $p < 0.001$; NS - not significant)

Source of variation	*df*	SS	MS	F
Diatom species	1	39.38	39.38	
Experimental condition	4	49.52	12.38	
Incubation days	5	8.42	1.68	
Diatom speciesXExperimental condition	4	4.24	1.06	20.46*
Diatom speciesXIncubation days	5	5.01	1.00	19.28*
Experimental conditionXIncubation days	20	0.57	0.03	0.54^{NS}
Diatom speciesXExperimental condition xIncubation days	20	1.04	0.052	

MANOVA revealed a significant variation in cell abundance between the two species with respect to incubation days and experimental conditions for the samples retrieved after one hour as well as those retrieved after 24 hours (Table 5B.1 and 5B.2).

5B.4 Discussion

A. coffeaeformis exhibited better survival capabilities as compared to *N. delicatula*, when exposed to low temperature. As Levitt (1980) has reported for higher plants, chloroplasts were the organelles showing the most striking and easily detectable change for these two species. According to Farrant et al. (1977) the reasons for the

differences among cells exposed to freezing lie first in their particular sensitivity to shrinkage and swelling under hypertonic and hypotonic conditions respectively.

Initially, when cells are subjected to subzero temperatures they supercool. The manner in which they regain equilibrium depends mainly on the rate at which they are cooled and on their permeability to water. If cooled slowly or if their permeability to water is high, they will equilibrate by dehydration. But if they are cooled rapidly or if their permeability to water is low, they will equilibrate by intracellular freezing. Thus, rapid cooling not only produces intracellular crystals it also produces small crystals, which are likely to enlarge during warming because of their high surface free energies (Mazur 1970). Thus, direct freezing into liquid nitrogen resulted in rupture of *N. delicatula* chloroplast (Case I). But this was not the case with *A. coffeaeformis*, which was viable even after direct transfer to this low temperature. This species could probably equilibrate faster by dehydration thus resulting in contracted chloroplasts.

Table 5B.2. MANOVA (Without replication) for the variation in cell abundance between species, incubation days and experimental conditions after a duration of 24 hours (* $p < 0.001$; NS - Not significant)

Source of variation	*df*	SS	MS	F
Diatom species	1	36.67	36.67	
Experimental condition	4	52.26	13.06	
Incubation days	5	7.42	1.48	
Diatom speciesXExperimental condition	4	4.25	1.06	25.92*
Diatom speciesXIncubation days	5	4.01	0.80	19.57*
Experimental conditionXIncubation days	20	0.59	0.03	0.72^{NS}
Diatom speciesXExperimental condition xIncubation days	20	0.82	0.04	

But regardless of whether cells equilibrate by dehydration or by intracellular freezing, freezing exposes them to loss of liquid water and to increase in concentration of intra and extracellular solutes. These events, which the cell undergoes during freezing are referred to as "solution effects" by Mazur (1970). The cells can survive if a protective additive is present. Lovelock and Bishop (1959) discovered the protective effect of

DMSO. DMSO is a protective non-electrolyte that is able to penetrate readily into cells (Smith 1950; Farrant 1965). This permeable cryoprotectant can act as a water-binding substance (Taylor 1987), reducing the amount of water molecules forming ice and, by colligative effects may reduce the osmotic stress. Most cryoprotectants appear to protect against solution effects rather than against intracellulatr freezing. Thus, survival of *N. delicatula* increased when cooled slowly in the presence of DMSO (Case III) as compared to that when cooled rapidly (Case II). Comparatively *A. coffeaeformis*, which is smaller in size survived even when subjected to rapid cooling (case I) and slow cooling (case III) in the absence of DMSO. Cryopreservation of marine algae without cryoprotectants has been reported in very few instances e.g., *Tetraselmis chuii*, *Nannochloropsis gadiatana*, *Nannochloropsis atomus* (Canavate and Lubian 1995a) and *Chaetoceros gracilis* (Canavate and Lubian 1994, 1995b). The survival percentage was higher in the presence of DMSO when subjected to slow cooling (Case III) which reveals the protective effect of the cryoprotectant by cell penetration and reduction of the hypertonic osmotic effects of cooling. Because, permeant DMSO replaced a proportion of the impermeant extracellular solute, less shrinkage occurred and the damage to the cells of both species was minimal.

Ben-Amotz and Gilboa (1980) has reported cryopreservation of the diatom *Phaeodactylum tricornutum* using 0.5 M DMSO while Canavate and Lubian (1994, 1995) succeeded in cryopreserving *Chaetoceros gracilis* at 1.5 M DMSO. *N. subinflata* (Redekar and Wagh 2000a) and *Chaetoceros calcitrans* (Joseph et al. 2000) were cryopreserved using 4 M and 1.5 M DMSO respectively. In the present study, 2M DMSO could successfully cryopreserve *A. coffeaeformis* and *N. delicatula* by following the two-step cooling method.

The numerical value for the critical cooling rate that produces internal ice depends on the ratio of the volume of the cell to its surface area and on its permeability to water. The critical rate should be lower for larger spherical cells and for those less permeable to water than for smaller or more permeable cells (Mazur 1970). The better survival capabilities of *A. coffeaeformis* as compared to *N. delicatula* when cooled at a rate of $-1\ ^{0}C\ min^{-1}$ shows that this rate of cooling was appropriate for *A. coffeaeformis*.

Significant differences between the two species in cell viability following the two-step cooling technique indicates that membrane properties / or physico-osmotic causes may contribute to cold hardening. The above results are in agreement with the general assumption that the primary site of injury to cells following freezing and thawing is the cell membrane (Mazur 1970). Differences in freezing injury among the marine algae may therefore reflect different properties and compositions of diatom membranes.

A. coffeaeformis is known to be a very sturdy diatom species capable of surviving harsh, unfavorable environmental conditions (Buzzelli et al. 1997). A few alga possess a natural capability to survive freezing better than others. The nature of freezing tolerance is not yet clear, but the ability of certain alga to survive at frost temperatures suggests that these algae have developed a cellular mechanism of freezing protection. When subjected to cold stress, Antarctic algae are known to produce a tertiary sulfonium compound, dimethyl sulfoniopropionate (DMSP). DMSP is reported to have a role to play in cryopreservation of polar algae (Karsten et al. 1990).

Species-specific genetic makeup may be responsible for such tolerance to low temperature exposures. Study of these cryoprotective proteins, which are de novo, synthesized during low temperature stress, will be of utmost importance.

5C. Immunofluorescence technique

5C.1 Introduction

Considerable time and effort are invested to identify a particular species on the basis of morphological characteristics. Often it is difficult to distinguish many features using the light microscope. An alternative to microscopic identification is the use of molecular probes, which can bind to either internal or external sites on the target species and be visualized using fluorescence techniques (Anderson 1993). Recently, immunological techniques such as species-specific (labelled) antibodies are being increasingly in use for taxonomy of bacteria and protozoa. The specificity of the antigen-antibody reaction provides a powerful tool for the study of individual microorganisms in their natural environment (Shapiro et al. 1998).

The major advantage of an immunochemical probe is that it is a highly specific and sensitive tool for investigating known systems and characterizing and localizing known antigens. One of the primary drawbacks of the immunochemical approach, however, is that, the investigator must have the culture or antigen in hand to generate the antibody probes.

Diatom taxonomy is based primarily on frustule morphology. Frustule morphology, however, can change with environmental (Schmid 1979; Mizuno 1987) and culture conditions. Immunofluorescence assays may be especially useful for diatoms for an easier and quicker identification. In the present study, an attempt was made to distinguish a pennate diatom, *Navicula delicatula,* by developing antisera directed against its cell surface antigens. *N. delicatula* is the most dominant diatom encountered in biofilms.

5C.2 Materials and methods

5C.2.1 Immunization culture

Unialgal batch cultures of the diatom *N. delicatula* were established in f/2 enriched seawater medium (Guillard and Ryther 1962) at a temperature of 27 ^{0}C under a 12 h light: 12 h dark photocycle. Cells were harvested by centrifugation at the end of exponential growth phase (7 days). The pelleted cells were preserved in 0.6% (v/v, final concentration) paraformaldehyde prepared in seawater and divided into 3 duplicate 1ml aliquots in microcentrifuge tubes each containing ~ 5 x 10 ^{6} cells of *N. delicatula* and stored at 4 ^{0}C until used for immunization (Bates et al. 1993).

5C.2.2 Test culture

Biofilms were developed by exposing stainless steel and polystyrene substrata at the Dona Paula Bay (15^0 27' N 73^0 48' E). These films were scraped with a nylon brush into filtered seawater. This sample was used for the immunological tests along with laboratory maintained diatom cultures (Table 5C.1). Test cultures were generally harvested during the exponential phase and preserved in 2% (v/v, final concentration) paraformaldehyde-glutaraldehyde. In addition, *N. delicatula* cells were tested either fresh, frozen or preserved as indicated in Table 5C.2, to determine the best fixation procedure for use.

5C.2.3 Immunization of rabbits

Antibodies were raised commercially (Genei India Pvt. Ltd. Bangalore, India). According to the manufacturers protocol the preserved cells were washed with phosphate-buffered saline (PBS) in order to remove the preservative. Subsequently, these cells were mixed with Freund's complete adjuvant. This mixture (0.5 ml) was

subcutaneously injected into the rabbits. The antibody titre was monitored by Dot ELISA and yielded a titre value of 1:5000.

5C.2.4 Immunofluorescence protocol

The indirect immunofluorescence (IF) protocol by Bates et al. (1993) was followed in this study (Fig. 5C.1). For the IF assay, 1ml of the preserved culture of *N. delicatula*

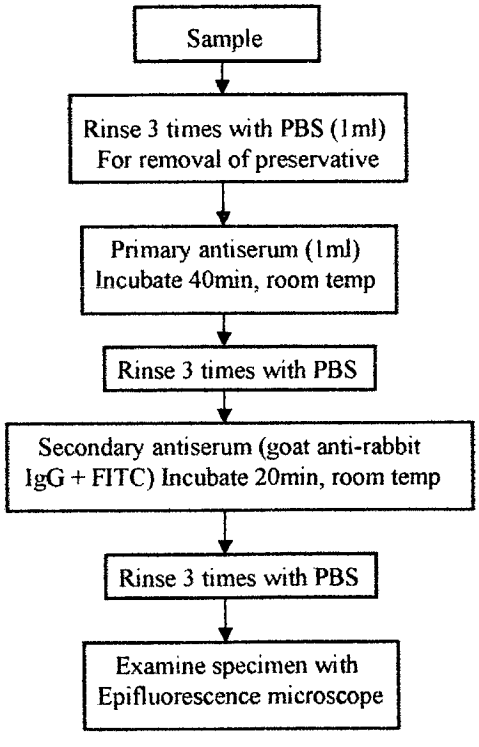


Fig. 5C.1 Experimental protocol of immunofluorescence assay

was first rinsed three times [Eppendorff microcentrifuge; 20 min at 4000 rpm) with PBS buffer (0.02 M Phosphate; 0.15 M NaCl; pH 7.45) to remove the seawater and preservative. Cells were then incubated for 40 min in the primary test antiserum, 10 times diluted with PBS. After three rinses with PBS, the cells were incubated for 20 min with 1 ml of FITC conjugated goat anti-rabbit antiserum, diluted 1:800 with PBS, as the secondary antibody and rinsed three more times with PBS to wash off the excess conjugate. One drop of this preparation was placed on a microscope slide and observed using an inverted epifluorescence microscope (Olympus IX-AN) at 100x,

using blue light excitation. To determine the composition of *N. delicatula* in the biofilm samples, first the total numbers of cells were counted through normal light microscope and then the counts of immunofluorescently labeled cells in the same field were noted.

5C.3 Results

Table 5C.1 Diatom species representing two orders of class Bacillariophyceae. Summary of results obtained by indirect immunofluorescence assay for unialgal cultures using antisera raised against *Navicula delicatula*. Cross reactivity is scaled from very strong (+++) to none (-)

S.No	Species	Family	Order	Isolated from	Cross reactivity
1	*Navicula subinflata*	Naviculaceae	Pennales	Biofilm	-
2	*Navicula crucicula*	Naviculaceae	Pennales	Biofilm	-
3	*Amphora coffeaeformis*	Amphoraceae	Pennales	Biofilm	-
4	*Amphora rostrata*	Amphoraceae	Pennales	Biofilm	-
5	*Skeletonema costatum*	Skeletonemataceae	Centrales	Water column	-
6	*Chaetoceros calcitrans*	Chaetocerotaceae	Centrales	Water column	-

In the IF assay, the antiserum successfully labelled 100% of *N. delicatula* cells from culture which was used to develop the antiserum. It also labelled those cells, which had changed their normal shape and become deformed and short due to lack of sexual reproduction. This antiserum when tested with unialgal cultures of the same genera and other species given in Table 5C.1 did not show any cross reactivity.

Table 5C.2 Comparison of various preservation techniques, including freezing on the immunofluorescent reaction between *Navicula delicatula*. Immunological cross reactivity is scaled from very strong (+++) to average (+) (Note: +++ very good, ++ Good, + Average, ab-absent

S.No	Preservative technique	*Navicula delicatula*
1	Formalin (2%)	++
2	Glutaraldehyde (0.5%)	++
3	Paraformaldehyde (2 %)	+++
4	Paraformaldehyde/Glutaraldehyde (2 % v/v)	+++
5	Lugols Iodine (4%)	+
6	2% Paraformaldehyde (frozen at -20^{0}C)	+++
7	0.5 % Glutaraldehyde (frozen at -20^{0}C)	++
8	Control A without fixative	+
9	Control B (frozen at -20^{0}C) without fixative	++

Table 5C.3 Cell count of *Navicula delicatula* present in the biofilm samples developed over polystyrene and stainless steel substrata, obtained through the indirect immunofluorescence assay (PS = Polystyrene ; SS= Stainless steel)

Biofilm (n)	Total cells ml^{-1} normal count	cells ml^{-1} Stained	Non Stained	Stained(%)	Non Stained(%)
PS-1	2802	1528	1274	55	45
2	1528	1274	255	83	17
3	764	255	509	33	67
4	1783	255	1528	14	86
5	1019	509	509	50	50
6	1019	509	509	50	50
7	3057	1783	1274	58	42
8	3057	764	2293	25	75
9	3312	2038	1274	62	38
10	1783	764	1019	43	57
		PS Average (%)		47	53
SS-11	1528	1019	509	67	33
12	1528	1019	509	67	33
13	1783	764	1019	43	57
14	1274	764	509	60	40
15	1019	1019	0	100	0
16	1783	764	1019	43	57
17	1274	509	764	40	60
18	1019	509	509	50	50
19	2547	1783	764	70	30
20	1528	1274	255	83	17
		SS Average (%)		62	38
		Total Average (%)		54	46

Table 5C.4 Summary of the cross reactivity results obtained for field samples by indirect immunofluorescence assay using antisera raised against *N. delicatula*

Diatom species	Cross reactivity
Amphora coffeaeformis	-
Pleurosigma angulatum	-
Licmophora paradoxa	-
Nitzschia closterium	+
Nitzschia longissima	-
Achnanthes longipes	-
Licmophora flabellata	-
Biddulphia pulchella	-
Nitzschia sigma	-
Surirella ovata	-
Cocconeis scutellum	-
Cymbella sp.	+

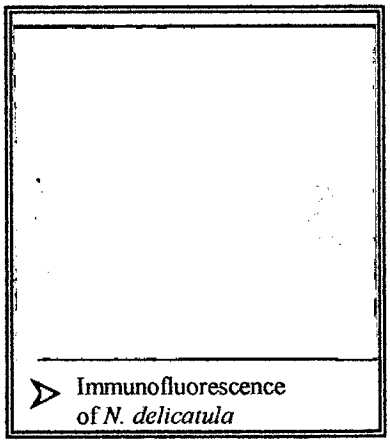
Several different preservation techniques were evaluated to determine which gave acceptable immunofluorescent results. Cells preserved with 2% (v/v, final concentration) paraformaldehyde-glutaraldehyde and 2% paraformaldehyde gave the

strongest fluorescence. *N. delicatula* cells frozen at –20 ^{0}C with 2% paraformaldehyde also gave a bright fluorescence. Unfixed samples and those fixed with lugol's iodine reacted most poorly (Table 5C.2).

Among the field populations observed, on average 50% of *N. delicatula* cells were labelled by the antisera. While on stainless steel substrata, the tagging ranged from 40% to 100%, on polystyrene substrata it ranged from 14% to 83% (Table 5C.3). It showed cross reactivity with species such as *Cymbella* sp. and *Nitzschia closterium* in the biofilm samples (Table 5C.4).

5C.4 Discussion

Generally, morphological characters are used to classify diatoms to species level but increasing in the sophistication of our knowledge of non-morphological features relating to the species problem now requires a new synthesis (Taylor 1993). On the other hand the role played by genetic variability within phytoplanktonic species is unclear (Wood and Leathan 1992). In this regard, immunological procedures are promising. In this study, the primary antibody used, was produced in a rabbit in response to immunization with intact diatom cells. The antibodies are produced against the antigens located on the surface of the diatom cell.



FITC labelled antibodies have proven convenient in IF techniques with chlorophyll containing phytoplankton because both, FITC and chlorophyll are excited by blue

light, but have different emissions, FITC emitting green and chlorophyll emitting red fluorescence (Shapiro et al. 1998).

For long-term storage of sample, paraformaldehyde was the best preservative for indirect IF microscopy as found by Campbell and Itturiaga (1988) and Bates et al. (1993). Glutaraldehyde fixation not only reduces the binding capacity, resulting in a decrease of fluorescence intensity but also induces as interfering yellow-greenish background fluorescence (Campbell and Itturiaga 1988).

All of the *N. delicatula* cells reacted with the appropriate antiserum, regardless of cell size or the presence of deformities. This observation is in agreement with the results obtained by Bates et al. (1993) for *Pseudonitzschia pungens.* The same was true for *Emiliana huxleyii* (Prymnesiophyceae) (Shapiro and Campbell 1989), *Gymnodinium* and *Alexandrium* (dinoflagellates) (Mendoza et al. 1995).

On an average only up to 50% of the *N. delicatula* in field population were identified. Apparently, natural populations contain more than one serogroup. This may indicate diversity among this species wherein there is a possibility of genetic differences among clones for surface antigens. This has been demonstrated among the heterotrophic bacterial populations in the ocean (Giovannoni et al. 1990). Campbell and Carpenter (1987) observed variations in the composition of field populations of *Synechococcus* spp. by immunofluorescence, wherein ~30% of the population was labelled. Although, it is possible that physiological state of cells may alter cell surface epitopes of this diatom, Campbell et al. (1994) have shown through culture studies on eukaryotic algae that IF labelling is not affected by growth rate of the culture or by light or nutrient limitation. Campbell (1988) has reported similar results for the cyanobacterium, *Synechococcus.* Also, the physiological stage of cells did not

influence the cells of marine dinoflagellates, *Gymnodinium* and *Alexandrium* (Mendoza et al. 1995). It is also possible that the culture does not represent the strains actually present in nature (Campbell and Iturriaga 1988). Thus, it is likely that the probe is clone specific.

In this assay, slight labelling was found with other diatoms such as *Cymbella* sp. and *N. closterium*. It would be interesting to study the genetic relationship by examining the ribosomal DNA or RNA sequences of the cross reacting species. This method has already been used to obtain markers for fine-scale analysis of populations and taxonomy of *Alexandrium* (Scholin et al. 1993).

Physiologically important differences do exist among representative clones of different serogroups (Campbell and Carpenter 1987). Therefore, understanding variations of serogroups among *N. delicatula* populations is of considerable importance. Using IF assay, it is possible to define serogroups (i.e., clusters of strains labelled by one antiserum) (Campbell et al. 1983; Campbell and Carpenter 1987). Immunological tests may advance the pace of biofouling studies. They are promising in the identification, separation and enumeration of cells, but biological problems such as cross reactivity and physiological variability and technical problems i.e., signal strength, autofluorescence and loss of cells during processing must be resolved.

Chapter 6

Summary

SUMMARY

Diatoms constitute an important part of the microphytobenthic community in the intertidal sand flat. Intertidal sandflats are dynamic environments, where the tidally generated water movement and the associated processes of deposition and resuspension of sediment affect the composition and distribution of diatoms. In many studies, diatoms were only investigated in the top few centimeters of the sediment (Rizynk et al. 1978; Colijn and Dijkema 1981; Varela and Penas 1985; Lukatelich and McComb 1986). However, the presence of diatoms at a depth of 20 cm has also been reported (Steele and Baird 1968; Colijn and Dijkema 1981; de Jonge and Colijn 1994), based on chlorophyll *a* estimations. In intertidal sandflats, a number of factors may be responsible for displacing the diatoms from the surface sediment layers to the deeper layers. Temporal and spatial variations in the viable diatom population of the microphytobenthic community revealed the presence of diatoms up to a depth of 15 cm all along the intertidal zone, from the low tide to the high tide zone. Their rejuvenation in culture revealed that their viability was not affected by the conditions prevailing at this depth. This depth harbored not only the pennate (epipsammic and epipelic) diatoms, some of which are permanent residents of this area, but also the centric diatoms of planktonic origin. The occurrence of diatoms such as *Amphora* and *Navicula* throughout the year in the sediments indicated that the two pennate forms were natives of this area. The appearance of the centric diatom, *Thalassiosira* only upon incubation indicated the presence of their resting stages. These resting stages must have been carried to the study area with coastal sediments and redeposited in intertidal sediments.

A number of physical, biological and chemical factors may be responsible for the temporal and spatial variation in the diatom abundance, diversity, diatom richness and

evenness. Grain size fractions which, served as predictors of some diatoms, differed with depths at the low, mid and high tide zones. This depicts that factors other than grain size have a role to play in the temporal and vertical distribution of diatoms.

Wind stimulated the resuspension of the sediment along with pennate diatoms up to 5 cm depth only at the low tide zone. The depth up to which chlorophyll *a* could act as indicators of both, the pennate and centric diatom abundance reduced from 10 cm depth at the low tide zone to 5 cm at the mid tide zone and 0 cm at the high tide zone.

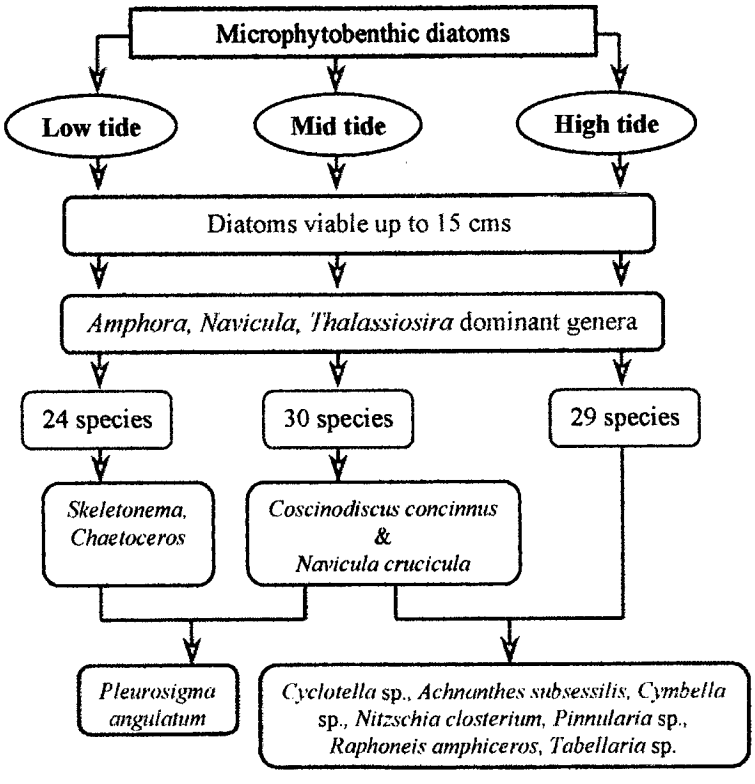


Fig. 6.1 Variations in the microphytobenthic diatoms across the intertidal zone

In the 0-5 cm depth, the ability of the pennate diatoms to remain attached to the substrate may play an important role in this contribution whereas in centric diatoms they may be the ones brought onto the sediments by the incoming tides. In the 5 to 10 cm depth at the low tide zone, the positive correlation with chlorophyll *a* revealed that resuspension was not effective up to this depth and the stock was securely placed.

However, a negative correlation of chlorophyll *a* with diatoms in the 10-15 cm depth and below 5 cm depth at the low and mid tide zones respectively, even when viable diatoms were found in appreciable numbers, suggests survival of these diatoms below physical disturbance level through the adoption of survival strategies such as heterotrophy or resting stage formation. The non-significant relationship at the high tide zone throughout the 15 cm depth may be due to the effects of desiccation when exposed for longer durations. However, they were viable as primary producers, when resurfaced and could play an important role in the benthic community (Fig. 6.1).

Vertical migratory behavior of benthic diatoms is one of the adaptive strategies employed for a life in intertidal habitats. This self-initiated migration helps diatoms to move to the surface during low tides for photosynthesis and move down during high tides (Round and Palmer 1966; Round 1979; Joint et al. 1982; Paterson 1989). There exists a controversy regarding the reason for the vertical migration and the factors affecting it. Irradiance and tidal rhythm are the two variables considered to be governing the vertical migration (Round and Palmer 1966). Experiments carried out to delineate the influence of these factors in a tropical intertidal sand flat revealed that rising to the sediment surface for fulfilment of their light requirements for photosynthesis was the first priority. If not fulfilled during the low tide exposure, diatoms could withstand the tidal effects and stay up at the surface even during the high tide coverage. In summer, the surface cell abundance of epipelic diatoms was high only during the morning low tide whereas in winter it was found to be the highest during the mid-morning high tide and continued to be so even during the following evening low tide. This ability of microphytobenthos to migrate vertically within the surface sediment when their requirements are fulfilled may be considered as a form of behavioral photoacclimation, allowing cells to avoid potentially

damaging irradiance and temperature conditions (Kromkamp et al. 1998; Perkins et al. 2001). In the laboratory experiments wherein the effects of tides were removed, the endogenous clock continued to operate in a similar fashion as that in the field when provided with 12 h light: 12 h dark condition whereas continuous darkness brought in a tidal rhythm. Expression of a tidal rhythm may be attributed to an innate behavior. This behavior is otherwise superimposed by the diel rhythm in the presence of light. In continuous light, diatoms preferred to stay up at the surface longer than that observed in field. This indicates that there is an optimum duration up to which the diatoms can remain exposed to light. The above observations reveal that irradiance has an overriding effect over tides. Temporal differences in the irradiance and the resulting changes in diatom migration can have implications in the littoral primary productivity.

In this entire process of vertical migration, an important factor that needs consideration is the impact of physical forcing on the sediment caused by wind or tidal currents. Turbulence and shear stress generated by the incoming tide lead to suspension of the diatom cells into the overlying waters (Baillie and Welsh 1980; Delgado et al. 1991; de Jonge and Van Beusekom 1992, 1995; de Jonge and Van den Bergs 1987). This may be an additional factor responsible for the lowering of the surface diatom population, other than their positive geotrophic movement during immersion. However, the entire suspended population will not be lost to water column, since a part of it will start resettling at the beginning of emersion. This process will in turn contribute to the rise in cell numbers at the sediment surface during subsequent exposure, along with the upward migration from the deeper sediment layers.

Presence of diatoms in the water column is also reflected in the microfouling population of different types of substrata immersed in the sub-surface estuarine waters of the present study area. Diatoms, the early autotrophic colonizers, are an important constituent of the biofouling community in the marine environment. The diatom populations in the surrounding environment and that in the fouling community revealed that the diversity is not evenly reflected. Pennate diatoms were abundant in the fouling film than centric diatoms while the reverse was evident in case of the water column. This difference is attributed to the capability of the pennate diatoms to attach to surfaces with the help of a raphe. The diatom populations, both in the water and the biofilm were dominated by the pennate diatom, *Navicula delicatula.* The distribution of *N. delicatula* in the water column and the biofilm was found to be independent of the distribution of the other diatoms. In the surrounding waters, 32 genera (20 centrics, 12 pennates) including 50 species (28 centrics, 22 pennates) were encountered. The abundance and diversity changed with the substratum. It was found to be higher on polystyrene than on stainless steel. Species such as *Coscinodiscus concinnus* and *cymbella* sp. were found exclusively on polystyrene whereas *Pinnularia* sp. was encountered only on stainless steel (Fig. 6.2). Such substratum influences can differ with organisms and would need careful consideration in determining the factors that govern the diversity of microbial films.

Although, a dominant pennate benthic diatom of the microphytobenthic community, *Navicula delicatula* representing the water column as the most abundant form indicates that it can extend its niche from the intertidal habitat to the ambient waters. Most of the pennate diatoms which are primarily encountered on the bottom sediments, are often found in the water column (de Jonge et al 1992; Tomas 1997)

through resuspension and it seems reasonable to assume that their primary production in the water column is as effective as it is on the tidal flats (de Jonge et al. 1995).

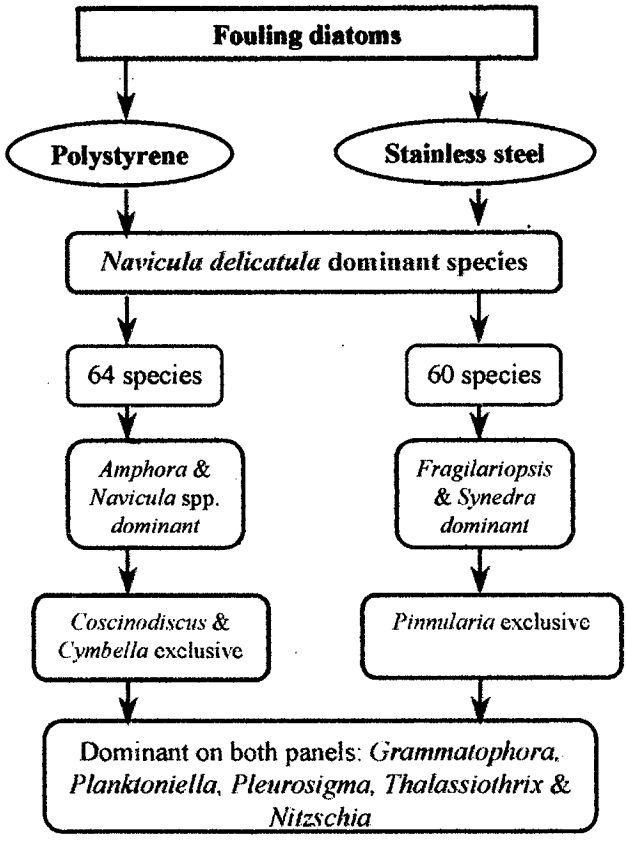


Fig. 6.2 Variations in the fouling diatom community over polystyrene and stainless steel

However, the other dominant diatom, *Amphora coffeaeformis* restricted its distribution to the intertidal sediments. Such a distribution reveals species-specific differences in habitat selection.

Space as a resource is important to periphytic diatoms. However, limited availability of space as compared to the vast diversity of species, leads to intense competition for this resource. Thus, diversity is controlled by the competitive strategies employed by each member of the community, whether a pioneer or a late arrival. In this struggle for existence, the fittest species can carve a better niche for itself, by exhibiting competitive traits. The community structure is influenced by gain and loss processes

and competitive ability of each member. In field, the gain factors are immigration of fresh recruit and their multiplication while the loss factors are grazing, death and sloughing off. The study carried out to evaluate variations in the marine periphytic diatom diversity by eliminating fresh recruitment and grazing, components of the gain and loss processes respectively revealed three cases. These cases illustrated the influences of intergeneric competition (case I), simultaneous inter and intrageneric competition (case II) and competitive exclusion or co-existence (case III) (Fig. 6.3).

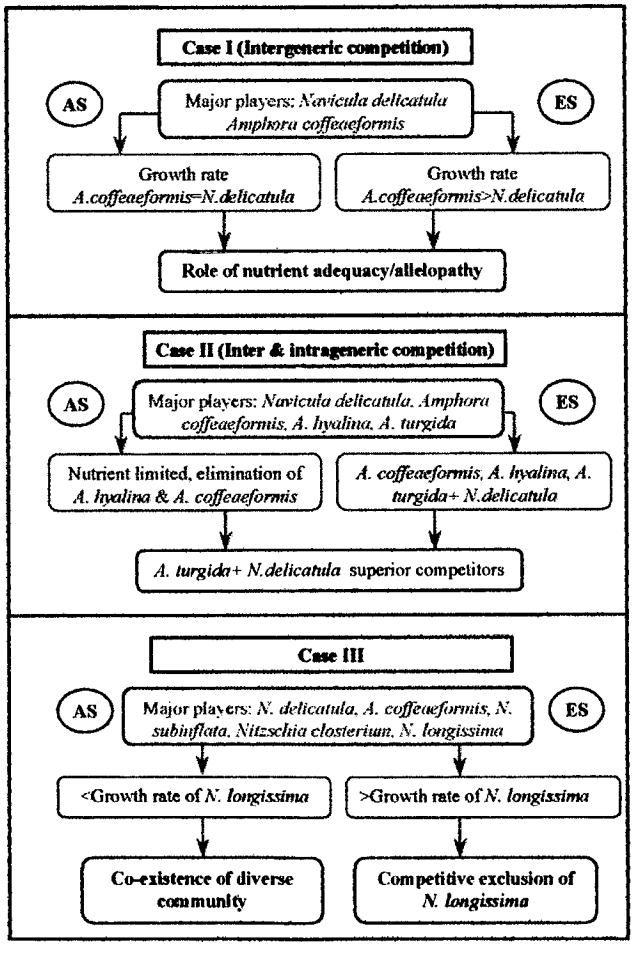


Fig. 6.3 Variations in the periphytic diatom community during case I, case II and case III

In case I, where *Navicula delicatula* and *Amphora coffeaeformis* were the major players, exclusion of *N. delicatula* was observed below the substratum carrying capacity levels, indicating a role of nutrient adequacy or allelopathy. Laboratory

experiments revealed that the competitive influence of the population of *A. coffeaeformis* over *N. delicatula* is independent of the initial cell density, with capability to overtake its competitor species even at 1% initial inoculum. Time required by *A. coffeaeformis* to overtake *N. delicatula* reduced from 7 days (1% initial inoculum) to 3 days (20% initial inoculum). In case II, intrageneric competition was observed among three species of *Amphora* i.e., *A. turgida, A. hyalina* and *A. coffeaeformis* wherein the success of *A. turgida* was not influenced by the nutrient availability whereas that of other two species was nutrient dependent. *A. turgida, A. hyalina* and *A. coffeaeformis* can co-exist in nutrient enriched conditions, where the common nutrient supply is sufficient, whereas in nutrient limiting conditions, according to the resource competition theory, only one of the three species of *Amphora*, i.e., *A. turgida* proved to be a successful competitor. This species could possibly sequester the limiting nutrients at a faster rate thus making it unavailable to the other species of *Amphora* i.e., *A. hyalina* and *A. coffeaeformis*. This species was found to co-exist with *N. delicatula*, which could be due to a difference in nutrient requirements. A simultaneous intergeneric competition between *N. delicatula* and three species of *Amphora* was not nutrient dependent. In case III, capability of *Nitzschia longissima* to eliminate the other components was positively influenced by nutrient availability. Paucity of nutrients supported richer diversity. These three cases also illustrated that, competitive traits of a periphytic diatom species is switched on at an appropriate cell density ratio of the competitor and target species. Such traits will determine the community variations in oligo, meso and eutrophic conditions.

Studies also showed that exposure to low temperature can result in morphological changes in diatoms. Low temperature influences the survival capabilities, which differed with species. *A. coffeaeformis* turned out to be a better survivor to low

temperature than *N. delicatula*. This study has implications in cryopreservation of these diatoms (Fig. 6.4).

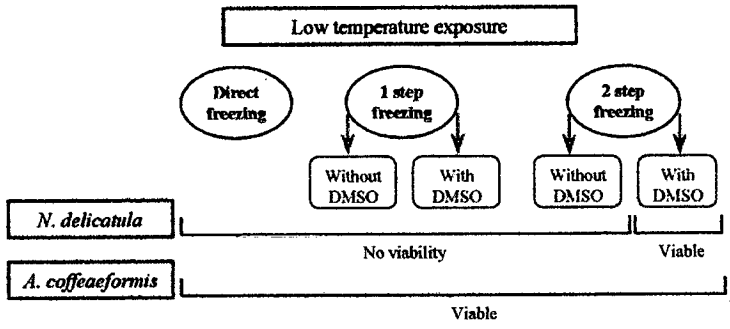


Fig. 6.4 Influence of low temperature on *N. delicatula* and *A. coffeaeformis*

Study of life cycle in *Amphora coffeaeformis* and *Navicula delicatula* revealed that sexual reproduction other than a mode of regaining normal cell size could be induced on sudden exposure to stress conditions such as salinity variations in pennate diatoms. This may be a type of survival strategy adopted to overcome stress conditions.

Generally morphological characters are used to classify diatoms to species level. Frustule morphology, however, can change with environmental and culture conditions. Also, considerable time and effort are required to identify a particular species when different morphological characteristics are difficult to distinguish under the light microscope. An alternative to microscopy identification is the use of molecular probes, which can bind to either internal or external sites on the target species and be visualized using fluorescence techniques. The specificity of the antigen-antibody reaction provides a powerful tool for the study of individual microorganisms in their natural environment. *Navicula delicatula* is a pioneer and most dominant diatom encountered in the biofilms. Antibodies directed against cell surface antigens of *N. delicatula* were developed for an easier and quicker identification and to trace the relative abundance of this pennate diatom.

The antiserum could successfully label 100% of *N. delicatula* cells from culture, which was used to develop antisera. No cross reactivity was observed with unialgal

cultures of the same genera. However, on an average 50% of *N. delicatula* cells were labelled by the antisera among the field populations observed (Fig. 6.5). It is evident

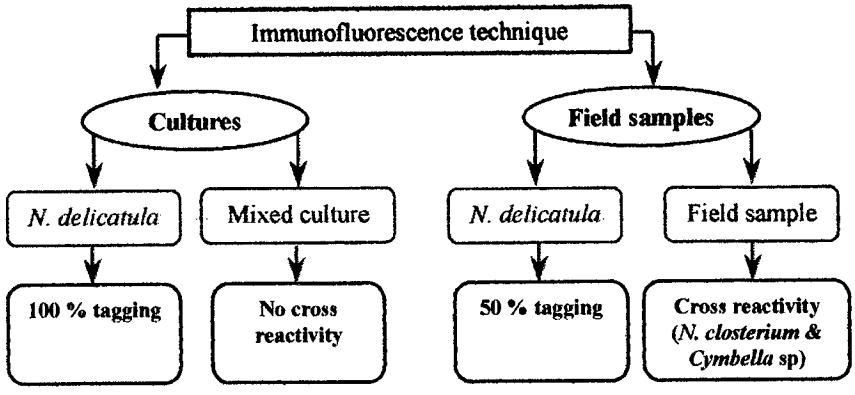


Fig. 6.5 Results of immunofluorescence technique used for identification of *N. delicatula* in culture and in field samples

that this method is not completely reliable for population studies and needs further validation. However, an important and interesting fact, which surfaced, is the occurrence of more than one 'serotype' of a particular species in the natural population. This indicates genetic diversity among the species and gives scope for population genetic studies and needs further attention.

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Chapter 2A
Appendix 1

Two-way ANOVA evaluating the variations in diatom abundance at the low tide zone with respect to months and core sections (0-15 cm) (* = P ≤ 0.001; ** = p ≤ 0.005; *** = p ≤ 0.025; **** = p ≤ 0.05; ***** = p ≤ 0.01; NS = not significant)

Navicula

	df	SS	MS	Fs
Month	16	48.7	3.05	5.7*
Depth	2	7.67	3.84	7.2**
Month X Depth	32	26.5	0.83	1.6****
Within subgroup error	102	54.5	0.53	
Total	152	137.5		

Amphora

	df	SS	MS	Fs
Month	16	49.6	3.11	10.97*
Depth	2	4.02	2.0	7.09**
Month X Depth	32	40.3	1.26	4.44*
Within subgroup error	102	28.9	0.28	
Total	152	123		

Grammatophora

	df	SS	MS	Fs
Month	16	21.8	1.37	9.36*
Depth	2	2.11	1.0	7.23**
Month X Depth	32	16.6	0.53	3.6*
Within subgroup error	102	14.9	0.14	
Total	152	55.7		

Pleurosigma

	df	SS	MS	Fs
Month	16	5.4	0.34	5.95*
Depth	2	0.44	0.23	3.91***
Month X Depth	32	9.65	0.31	5.42*
Within subgroup error	102	5.79	0.057	
Total	152	21.5		

Nitzschia

	df	SS	MS	Fs
Month	16	1.7	0.11	3.46*
Depth	2	0.34	0.17	5.5*****
Month X Depth	32	2.5	0.078	2.56*
Within subgroup error	102	3.13	0.03	
Total	152	7.68		

Thalassiothrix

	df	SS	MS	Fs
Month	16	8.37	0.53	8.9*
Depth	2	2.32	3.36	19.8*
Month X Depth	32	10.33	0.32	5.5*
Within subgroup error	102	6	0.058	
Total	152	27		

Cocconeis

	df	SS	MS	Fs
Month	16	27.7	1.73	13.8*
Depth	2	4	2	14.8*
Month X Depth	32	20.87	0.65	4.8*
Within subgroup error	102	13.8	0.13	
Total	152	66.5		

Fragilaria

	df	SS	MS	Fs
Month	16	23.6	1.47	36.17*
Depth	2	0.58	0.29	7.17**
Month X Depth	32	0.59	0.3	7.38*
Within subgroup error	102	4.16	0.04	
Total	152	37.9		

Bellerochea

	df	SS	MS	Fs
Month	16	0.43	0.026	3.6*
Depth	2	0.11	0.056	7.68*
Month X Depth	32	0.85	0.026	3.6*
Within subgroup error	102	0.74	0.007	
Total	152	2.12		

Thalassiosira

	df	SS	MS	Fs
Month	16	25.6	1.6	6.76*
Depth	2	12.2	6.11	25.8*
Month X Depth	32	15.98	0.5	2.1**
Within subgroup error	102	24.16	0.24	
Total	152	78		

Biddulphia

	df	SS	MS	Fs
Month	16	44.3	2.77	14.4*
Depth	2	4.2	2.1	11*
Month X Depth	32	15.4	0.48	2.5*
Within subgroup error	102	19.6	0.19	
Total	152	83.5		

Coscinodiscus

	df	SS	MS	Fs
Month	16	11.2	0.7	12.2*
Depth	2	0.8	0.41	7.1**
Month X Depth	32	7.98	0.25	4.3*
Within subgroup error	102	5.86	0.06	
Total	152	25.6		

Melosira

	df	SS	MS	Fs
Month	16	9.8	0.61	9.7*
Depth	2	4.48	2.24	35.6*
Month X Depth	32	15.7	0.49	7.78*
Within subgroup error	102	6.42	0.06	
Total	152	36.37		

Skeletonema

	df	SS	MS	Fs
Month	16	12.9	0.81	9.6*
Depth	2	0.29	0.15	1.75^{NS}
Month X Depth	32	2.07	0.06	0.77^{NS}
Within subgroup error	102	8.54	0.08	
Total	152	23.8		

Streptotheca

	df	SS	MS	Fs
Month	16	29.9	1.87	8.9*
Depth	2	0.48	0.24	3.16^{NS}
Month X Depth	32	22.25	0.7	3.32*
Within subgroup error	102	21.4	0.21	
Total	152	74.0		

Chaetoceros

	df	SS	MS	Fs
Month	16	3.44	0.22	20.1*
Depth	2	0.15	0.076	7.14**
Month X Depth	32	2.45	0.076	7.17*
Within subgroup error	102	1	0.011	
Total	152	7.13		

Chapter 2A
Appendix 2

Two-way ANOVA evaluating the variations in diatom abundance at mid tide zone with respect to months and core sections (0-15 cm) (* = $p \leq 0.001$; ** = $p \leq 0.005$; *** = $p \leq 0.025$; **** = $p \leq 0.05$; ***** = $p \leq 0.01$; NS = not significant)

Navicula

	df	SS	MS	Fs
Month	8	9.46	1.18	3.75****
Depth	2	6.14	3.07	9.72*****
Month X Depth	16	10.98	0.69	2.18**
Within subgroup error	54	17.04	0.32	
Total	80	43.62		

Amphora

	df	SS	MS	Fs
Month	8	8.5	1.06	4.15****
Depth	2	1.93	0.96	3.77*
Month X Depth	16	16.14	1.01	3.94*****
Within subgroup error	54	13.81	0.26	
Total	80	40.38		

Grammatophora

	df	SS	MS	Fs
Month	8	3.71	0.46	39.83*****
Depth	2	0.53	0.26	22.76*****
Month X Depth	16	9	0.56	48.37*****
Within subgroup error	54	0.63	0.01	
Total	80	13.86		

Pleurosigma

	df	SS	MS	Fs
Month	8	14.82	1.85	38.70*****
Depth	2	5.78	2.89	60.36*****
Month X Depth	16	9.61	0.60	12.55*****
Within subgroup error	54	2.58	0.05	
Total	80	32.79		

Nitzschia

	df	SS	MS	Fs
Month	8	7.61	0.95	13.10*****
Depth	2	17.08	8.54	117.55*****
Month X Depth	16	10.32	0.64	8.88*****
Within subgroup error	54	3.92	0.07	
Total	80	38.93		

Thalassiothrix

	df	SS	MS	Fs
Month	8	44.54	5.57	38.72*****
Depth	2	0.06	0.03	0.20 NS
Month X Depth	16	15.42	0.96	6.70*****
Within subgroup error	54	7.76	0.14	
Total	80	67.78		

Cocconeis

	df	SS	MS	Fs
Month	8	32.31	4.04	42.58*****
Depth	2	2.02	1.01	10.62*****
Month X Depth	16	28.45	1.78	18.75*****
Within subgroup error	54	5.12	0.09	
Total	80	67.90		

Fragilariopsis

	df	SS	MS	Fs
Month	8	2.57	0.32	37.96*****
Depth	2	1.45	0.72	85.52*****
Month X Depth	16	5.13	0.32	37.96*****
Within subgroup error	54	0.46	0.01	
Total	80	9.60		

Bellerochea

	df	SS	MS	Fs
Month	8	27.82	3.48	40.02*****
Depth	2	0.49	0.24	2.79 NS
Month X Depth	16	2.28	0.14	1.64 NS
Within subgroup error	54	4.69	0.09	
Total	80	35.28		

Thalassiosira

	df	SS	MS	Fs
Month	8	21.96	2.75	13.34*****
Depth	2	20.04	10.02	48.71*****
Month X Depth	16	44.44	2.78	13.50*****
Within subgroup error	54	11.11	0.21	
Total	80	97.55		

Biddulphia

	df	SS	MS	Fs
Month	8	13.77	1.72	16.97*****
Depth	2	6.19	3.09	30.50*****
Month X Depth	16	25.63	1.60	15.78*****
Within subgroup error	54	5.48	0.10	
Total	80	51.07		

Coscinodiscus

	df	SS	MS	Fs
Month	8	10.41	1.30	45.35*****
Depth	2	0.37	0.19	6.45****
Month X Depth	16	6.59	0.41	14.36*****
Within subgroup error	54	1.55	0.03	
Total	80	18.93		

Melosira

	df	SS	MS	Fs
Month	8	13.13	1.64	29.27*****
Depth	2	2.72	1.36	24.30*****
Month X Depth	16	16.92	1.06	18.86*****
Within subgroup error	54	3.03	0.06	
Total	80	35.79		

Achnanthes

	df	SS	MS	Fs
Month	8	4.31	0.54	66.39*****
Depth	2	1	0.50	61.48*****
Month X Depth	16	11.54	0.72	88.78*****
Within subgroup error	54	0.44	0.01	
Total	80	17.29		

Streptotheca

	df	SS	MS	Fs
Month	8	15.69	1.96	61.76*****
Depth	2	0.46	0.23	7.23****
Month X Depth	16	12.08	0.76	23.78*****
Within subgroup error	54	1.72	0.03	
Total	80	29.95		

Cyclotella

	df	SS	MS	Fs
Month	8	26.42	3.30	38.96*****
Depth	2	0.97	0.49	5.72***
Month X Depth	16	5.01	0.31	3.69*****
Within subgroup error	54	4.58	0.08	
Total	80	36.97		

Pinnularia

	df	SS	MS	Fs
Month	8	30.74	3.84	43.98*****
Depth	2	1.73	0.87	9.93*****
Month X Depth	16	6.78	0.42	4.85*****
Within subgroup error	54	4.72	0.09	
Total	80	43.98		

Cymbella

	df	SS	MS	Fs
Month	8	2.86	0.36	16.83*****
Depth	2	0.41	0.21	9.66*****
Month X Depth	16	6.94	0.43	20.42*****
Within subgroup error	54	1.15	0.02	
Total	80	11.35		

Tabellaria

	df	SS	MS	Fs
Month	8	13.70	1.71	41.45*****
Depth	2	3.57	1.79	43.19*****
Month X Depth	16	6.76	0.42	10.22*****
Within subgroup error	54	2.23	0.04	
Total	80	26.27		

Raphoneis

	df	SS	MS	Fs
Month	8	46.96	5.87	84.30*****
Depth	2	0.03	0.02	0.24 NS
Month X Depth	16	0.98	0.06	0.88 NS
Within subgroup error	54	3.76	0.07	
Total	80	51.74		

Chapter 2A
Appendix 3

Two-way ANOVA evaluating the variations in diatom abundance at high tide zone with respect to months and core sections (0-15 cm) (* = $p \leq 0.001$; ** = $p \leq 0.005$; *** = $p \leq 0.025$; **** = $p \leq 0.05$; ***** = $p \leq 0.01$; NS = not significant)

Navicula

	df	SS	MS	Fs
Month	8	31.57	3.95	24.44*****
Depth	2	1.41	0.71	4.37**
Month X Depth	16	8.29	0.52	3.21****
Within subgroup error	54	8.72	0.16	
Total	80	49.99		

Amphora

	df	SS	MS	Fs
Month	8	5.05	0.63	3.59****
Depth	2	18.03	9.02	51.27*****
Month X Depth	16	18.03	1.13	6.41*****
Within subgroup error	54	9.50	0.18	
Total	80	50.60		

Grammatophora

	df	SS	MS	Fs
Month	8	1.09	0.14	29.60*****
Depth	2	0.27	0.14	29.60*****
Month X Depth	16	2.18	0.14	29.60*****
Within subgroup error	54	0.25	0	
Total	80	3.79		

Pleurosigma

	df	SS	MS	Fs
Month	8			
Depth	2			
Month X Depth	16			
Within subgroup error	54			
Total	80			

Nitzschia

	df	SS	MS	Fs
Month	8	11.67	1.46	31.18*****
Depth	2	3.21	1.60	34.29*****
Month X Depth	16	10.82	0.68	14.45*****
Within subgroup error	54	2.53	0.05	
Total	80	28.23		

Thalassiothrix

	df	SS	MS	Fs
Month	8	8.18	1.02	43.62*****
Depth	2	4.80	2.40	102.44*****
Month X Depth	16	5.08	0.32	13.56*****
Within subgroup error	54	1.27	0.02	
Total	80	19.33		

Cocconeis

	df	SS	MS	Fs
Month	8	34.63	4.33	80.50*****
Depth	2	7.58	3.79	70.50*****
Month X Depth	16	16.88	1.06	19.63*****
Within subgroup error	54	2.90	0.05	
Total	80	62		

Fragilariopsis

	df	SS	MS	Fs
Month	8	0.94	0.12	30.78*****
Depth	2	0.54	0.27	70.32*****
Month X Depth	16	1.88	0.12	30.78*****
Within subgroup error	54	0.21	0	
Total	80	3.56		

Bellerochea

	df	SS	MS	Fs
Month	8	1.43	0.18	36.07*****
Depth	2	1.41	0.71	142.66*****
Month X Depth	16	2.86	0.18	36.07*****
Within subgroup error	54	0.27	0	
Total	80	5.98		

Thalassiosira

	df	SS	MS	Fs
Month	8	25.80	3.22	28.42*****
Depth	2	5.50	2.75	24.25*****
Month X Depth	16	21.76	1.36	11.99*****
Within subgroup error	54	6.13	0.11	
Total	80	59.18		

Biddulphia

	df	SS	MS	Fs
Month	8	5.02	0.63	190.21*****
Depth	2	0.72	0.36	108.91*****
Month X Depth	16	12.18	0.76	230.86*****
Within subgroup error	54	0.18	0	
Total	80	18.10		

Coscinodiscus

	df	SS	MS	Fs
Month	8	0.90	0.11	84.14*****
Depth	2	0.23	0.11	84.14*****
Month X Depth	16	1.80	0.11	84.14*****
Within subgroup error	54	0.07	0	
Total	80	3		

Melosira

	df	SS	MS	Fs
Month	8	15.84	1.98	22.93*****
Depth	2	9.35	4.68	54.18*****
Month X Depth	16	13.87	0.87	10.04*****
Within subgroup error	54	4.66	0.09	
Total	80	43.72		

Achnanthes

	df	SS	MS	Fs
Month	8	2.91	0.36	9.92*****
Depth	2	0.72	0.36	9.76*****
Month X Depth	16	7.42	0.46	12.62*****
Within subgroup error	54	1.98	0.04	
Total	80	13.03		

Streptotheca

	df	SS	MS	Fs
Month	8	27.35	3.42	91.21*****
Depth	2	5.29	2.65	70.58*****
Month X Depth	16	10.14	0.63	16.91*****
Within subgroup error	54	2.02	0.04	
Total	80	44.80		

Cyclotella

	df	SS	MS	Fs
Month	8	0.66	0.08	22.13*****
Depth	2	0.17	0.08	22.13*****
Month X Depth	16	1.33	0.08	22.13*****
Within subgroup error	54	0.20	0	
Total	80	2.36		

Pinnularia

	df	SS	MS	Fs
Month	8	17.66	2.21	50.90*****
Depth	2	5.30	2.65	61.07*****
Month X Depth	16	8.63	0.54	12.44*****
Within subgroup error	54	2.34	0.04	
Total	80	33.92		

Cymbella

	df	SS	MS	Fs
Month	8	20.26	2.53	6.82*****
Depth	2	2.59	1.29	3.48*
Month X Depth	16	5.44	0.34	0.91 NS
Within subgroup error	54	20.06	0.37	
Total	80	48.34		

Tabellaia

	df	SS	MS	Fs
Month	8	4.85	0.61	88.91*****
Depth	2	0.30	0.15	22.23*****
Month X Depth	16	2.43	0.15	22.23*****
Within subgroup error	54	0.37	0.01	
Total	80	7.95		

Raphoneis

	df	SS	MS	Fs
Month	8	5	0.62	22.29*****
Depth	2	4.99	2.49	88.97*****
Month X Depth	16	10	0.62	22.29*****
Within subgroup error	54	1.51	0.03	
Total	80	21.49		

Chapter 2B
Appendix 1

MANOVA for the temporal variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) in the low tide incubated cores of case I at different incubation conditions
(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Conditions	1	0.24	0.24	-
Incubation time	3	2.24	0.75	-
Depths	3	7.40	2.47	-
Conditions X Incubation time	3	0.41	0.14	13.81****
Conditions X Depths	3	0.07	0.02	2.27^{NS}
Incubation time X Depths	9	2.18	0.24	24.5*****
Conditions X Incubation time X Depths	9	0.09	0.01	-

(b) Source of variation	*df*	SS	MS	F
Conditions	1	2.63	2.63	
Incubation time	3	0.38	0.13	
Depths	3	7.70	2.57	
Conditions X Incubation time	3	1.07	0.36	4.79*
Conditions X Depths	3	1.42	0.47	6.35**
Incubation time X Depths	9	3.3	0.37	4.91*
Conditions X Incubation time X Depths	9	0.67	0.07	

(c) Source of variation	*df*	SS	MS	F
Conditions	1	0.09	0.09	-
Incubation time	3	0.57	0.19	-
Depths	3	8.53	2.84	-
Conditions X Incubation time	3	0.25	0.08	4*
Conditions X Depths	3	0.13	0.04	2.1^{NS}
Incubation time X Depths	9	2.02	0.22	10.73*****
Conditions X Incubation time X Depths	9	0.19	0.02	-

(d) Source of variation	*df*	SS	MS	F
Conditions	1	0.03	0.03	-
Incubation time	3	0.12	0.04	-
Depths	3	0.25	0.08	-
Conditions X Incubation time	3	0.04	0.01	3.19^{NS}
Conditions X Depths	3	0.04	0.01	3.08^{NS}
Incubation time X Depths	9	0.03	0.003	0.88^{NS}
Conditions X Incubation time X Depths	9	0.03	0.004	-

Chapter 2B
Appendix 2

MANOVA for the temporal variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) in the high tide incubated cores of case I at different incubation conditions
(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Conditions	1	0.003	0.003	-
Incubation time	3	1.82	0.61	-
Depths	3	5.59	1.86	-
Conditions X Incubation time	3	0.06	0.02	2.74^{NS}
Conditions X Depths	3	0.07	0.02	2.84^{NS}
Incubation time X Depths	9	1.88	0.21	26.69*****
Conditions X Incubation time X Depths	9	0.07	0.008	-

(b) Source of variation	*df*	SS	MS	F
Conditions	1	1.07	1.06	
Incubation time	3	1.2	0.4	
Depths	3	6.47	2.16	
Conditions X Incubation time	3	0.24	0.08	0.86^{NS}
Conditions X Depths	3	1.60	0.53	5.68**
Incubation time X Depths	9	0.49	0.45	4.89*
Conditions X Incubation time X Depths	9	0.84	0.09	

(c) Source of variation	*df*	SS	MS	F
Conditions	1	0.13	0.13	-
Incubation time	3	0.36	0.12	-
Depths	3	5.24	1.75	-
Conditions X Incubation time	3	0.7	0.23	17.98*****
Conditions X Depths	3	0.05	0.018	1.38^{NS}
Incubation time X Depths	9	0.63	0.07	5.41***
Conditions X Incubation time X Depths	9	0.12	0.013	-

(d) Source of variation	*df*	SS	MS	F
Conditions	1	0.016	0.016	-
Incubation time	3	0.032	0.011	-
Depths	3	0.13	0.05	-
Conditions X Incubation time	3	0.002	0.0008	0.15^{NS}
Conditions X Depths	3	0.02	0.006	1.11^{NS}
Incubation time X Depths	9	0.03	0.003	0.58^{NS}
Conditions X Incubation time X Depths	9	0.051	0.005	-

Chapter 2B
Appendix 3

MANOVA for the temporal and depthwise variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) of case I in 12 h light: 12 h dark incubation for low and high tide incubated cores
(*$p \leq 0.05$; **$p \leq 0.025$; ***$p \leq 0.01$; ****$p \leq 0.005$; *****$p \leq 0.001$; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Tides	1	0.78	0.78	-
Incubation time	3	1.57	0.52	-
Depths	3	5.32	1.77	-
Tides X Incubation time	3	0.33	0.11	1.24^{NS}
Tides X Depths	3	0.05	0.015	0.17^{NS}
Incubation time X Depths	9	1.15	0.13	1.44^{NS}
Tides X Incubation time X Depths	9	0.80	0.09	-

(b) Source of variation	*df*	SS	MS	F
Tides	1	2.37	2.36	
Incubation time	3	0.18	0.06	
Depths	3	2.09	0.69	
Tides X Incubation time	3	0.1	0.04	0.52^{NS}
Tides X Depths	3	0.03	0.01	0.16^{NS}
Incubation time X Depths	9	0.48	0.05	0.84^{NS}
Tides X Incubation time X Depths	9	0.57	0.06	

(c) Source of variation	*df*	SS	MS	F
Tides	1	1.06	1.06	-
Incubation time	3	0.46	0.15	-
Depths	3	5.43	1.81	-
Tides X Incubation time	3	0.15	0.05	0.67^{NS}
Tides X Depths	3	0.10	0.034	0.45^{NS}
Incubation time X Depths	9	0.97	0.11	1.45^{NS}
Tides X Incubation time X Depths	9	0.67	0.074	-

(d) Source of variation	*df*	SS	MS	F
Tides	1	0.0012	0.0013	-
Incubation time	3	0.082	0.027	-
Depths	3	0.03	0.01	-
Tides X Incubation time	3	0.045	0.015	8.82****
Tides X Depths	3	0.22	0.072	42.09*****
Incubation time X Depths	9	0.022	0.0024	1.42^{NS}
Tides X Incubation time X Depths	9	0.015	0.0017	-

Chapter 2B
Appendix 4

MANOVA for the temporal and depthwise variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) of case I in total dark incubation for low and high tide incubated cores

(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Tides	1	2.04	2.04	-
Incubation time	3	1.63	0.54	-
Depths	3	7.75	2.58	-
Tides X Incubation time	3	1	0.33	5.14**
Tides X Depths	3	0.03	0.01	0.16^{NS}
Incubation time X Depths	9	1.68	0.19	2.87^{NS}
Tides X Incubation time X Depths	9	0.58	0.06	-

(b) Source of variation	*df*	SS	MS	F
Tides	1	0.89	0.89	
Incubation time	3	0.92	0.31	
Depths	3	14.99	4.99	
Tides X Incubation time	3	0.32	0.1	0.77^{NS}
Tides X Depths	3	0.09	0.029	0.21^{NS}
Incubation time X Depths	9	0.4	0.044	0.33^{NS}
Tides X Incubation time X Depths	9	1.21	0.13	

(c) Source of variation	*df*	SS	MS	F
Tides	1	0.94	0.94	-
Incubation time	3	0.62	0.21	-
Depths	3	8.24	2.75	-
Tides X Incubation time	3	0.67	0.22	6.62**
Tides X Depths	3	0.19	0.062	1.85^{NS}
Incubation time X Depths	9	1	0.11	3.39*
Tides X Incubation time X Depths	9	0.3	0.033	-

(d) Source of variation	*df*	SS	MS	F
Tides	1	0.069	0.069	-
Incubation time	3	0.046	0.015	-
Depths	3	0.03	0.01	-
Tides X Incubation time	3	0.018	0.006	0.66^{NS}
Tides X Depths	3	0.16	0.055	5.92**
Incubation time X Depths	9	0.025	0.0028	0.3^{NS}
Tides X Incubation time X Depths	9	0.083	0.0092	-

Chapter 2B
Appendix 5

MANOVA for the temporal variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) in the low tide incubated cores of case II at different incubation conditions

(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Conditions	2	0.34	0.17	-
Incubation time	3	0.14	0.048	-
Depths	3	1.08	0.36	-
Conditions X Incubation time	6	0.95	0.16	1.35^{NS}
Conditions X Depths	6	0.61	0.1	0.87^{NS}
Incubation time X Depths	9	4.2	0.47	3.99***
Conditions X Incubation time X Depths	18	2.10	0.12	-

(b) Source of variation	*df*	SS	MS	F
Conditions	2	2.52	1.26	-
Incubation time	3	0.37	0.12	-
Depths	3	3.96	1.32	-
Conditions X Incubation time	6	1.5	0.25	1.92^{NS}
Conditions X Depths	6	0.43	0.07	0.55^{NS}
Incubation time X Depths	9	4.6	0.51	3.95***
Conditions X Incubation time X Depths	18	2.33	0.13	-

(c) Source of variation	*df*	SS	MS	F
Conditions	2	1.01	0.51	-
Incubation time	3	0.76	0.25	-
Depths	3	3.26	1.08	-
Conditions X Incubation time	6	0.95	0.16	1.55^{NS}
Conditions X Depths	6	0.33	0.05	0.54^{NS}
Incubation time X Depths	9	3.6	0.4	3.92***
Conditions X Incubation time X Depths	18	1.83	0.1	-

(d) Source of variation	*df*	SS	MS	F
Conditions	2	6.62	3.3	-
Incubation time	3	7.9	2.63	-
Depths	3	13.30	4.44	-
Conditions X Incubation time	6	7.9	1.32	1.23^{NS}
Conditions X Depths	6	2.40	0.4	0.37^{NS}
Incubation time X Depths	9	37.3	4.14	3.87***
Conditions X Incubation time X Depths	18	19.26	1.07	-

(e) Source of variation	*df*	SS	MS	F
Conditions	2	1.35	0.68	-
Incubation time	3	2.55	0.85	-
Depths	3	4.50	1.50	-
Conditions X Incubation time	6	7.17	1.19	7.89*****
Conditions X Depths	6	1.47	0.25	1.62^{NS}
Incubation time X Depths	9	1.42	0.16	1.04^{NS}
Conditions X Incubation time X Depths	18	2.73	0.15	-

Chapter 2B
Appendix 6

MANOVA for the temporal variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) in the high tide incubated cores of case II at different incubation conditions

(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Conditions	2	0.58	0.29	-
Incubation time	3	13.69	4.56	-
Depths	3	6.89	2.30	-
Conditions X Incubation time	6	0.51	0.08	2.23^{NS}
Conditions X Depths	6	0.18	0.03	0.82^{NS}
Incubation time X Depths	9	14.75	1.64	43.49*****
Conditions X Incubation time X Depths	18	0.68	0.04	-

(b) Source of variation	*df*	SS	MS	F
Conditions	2	0.81	0.4	-
Incubation time	3	20.15	6.72	-
Depths	3	6.92	2.30	-
Conditions X Incubation time	6	0.99	0.17	0.73^{NS}
Conditions X Depths	6	1.21	0.2	0.88^{NS}
Incubation time X Depths	9	8.86	0.98	4.31****
Conditions X Incubation time X Depths	18	4.11	0.23	-

(c) Source of variation	*df*	SS	MS	F
Conditions	2	0.43	0.22	-
Incubation time	3	30.45	10.15	-
Depths	3	11.85	3.95	-
Conditions X Incubation time	6	0.68	0.11	1.44^{NS}
Conditions X Depths	6	0.35	0.06	0.75^{NS}
Incubation time X Depths	9	19.73	2.19	28*****
Conditions X Incubation time X Depths	18	1.41	0.078	-

(c) Source of variation	*df*	SS	MS	F
Conditions	2	0.43	0.22	-
Incubation time	3	30.45	10.15	-
Depths	3	11.85	3.95	-
Conditions X Incubation time	6	0.68	0.11	1.44^{NS}
Conditions X Depths	6	0.35	0.06	0.75^{NS}
Incubation time X Depths	9	19.73	2.19	28*****
Conditions X Incubation time X Depths	18	1.41	0.078	-

(e) Source of variation	*df*	SS	MS	F
Conditions	2	0.65	0.32	-
Incubation time	3	9.43	3.14	-
Depths	3	12.30	4.00	-
Conditions X Incubation time	6	1.4	0.23	1.4^{NS}
Conditions X Depths	6	0.67	0.11	0.67^{NS}
Incubation time X Depths	9	10.95	1.22	7.28*****
Conditions X Incubation time X Depths	18	3	0.17	-

Chapter 2B
Appendix 7

MANOVA for the temporal and depthwise variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) of case II in 12 h light: 12 h dark incubation for low and high tide incubated cores

(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Tides	1	0.37	0.37	
Incubation time	3	3	1	
Depths	3	1.80	0.60	
Tides X Incubation time	3	2.9	0.97	3.8^{NS}
Tides X Depths	3	0.99	0.33	1.3^{NS}
Incubation time X Depths	9	2.25	0.25	0.98^{NS}
Tides X Incubation time X Depths	9	2.29	0.25	

(b) Source of variation	*df*	SS	MS	F
Tides	1	0.017	0.017	-
Incubation time	3	2.4	0.8	-
Depths	3	6.07	2.02	-
Tides X Incubation time	3	1.66	0.55	1.68^{NS}
Tides X Depths	3	0.27	0.092	0.28^{NS}
Incubation time X Depths	9	4.18	0.46	1.4^{NS}
Tides X Incubation time X Depths	9	2.97	0.33	-

(c) Source of variation	*df*	SS	MS	F
Tides	1	0.26	0.26	-
Incubation time	3	2.58	0.86	-
Depths	3	5.31	1.77	-
Tides X Incubation time	3	6.19	2.06	3.43^{NS}
Tides X Depths	3	1.16	0.39	0.64^{NS}
Incubation timeXDepths	9	4.48	0.5	0.83^{NS}
Tides X Incubation time X Depths	9	5.42	0.6	-

(d) Source of variation	*df*	SS	MS	F
Tides	1	1.62	1.62	
Incubation time	3	8.34	2.78	
Depths	3	23.58	7.86	
Tides X Incubation time	3	8.29	2.76	3.1^{NS}
Tides X Depths	3	4.38	1.46	1.64^{NS}
Incubation time X Depths	9	23.42	2.6	2.92^{NS}
Tides X Incubation time X Depths	9	8	0.89	

(e) Source of variation	*df*	SS	MS	F
Tides	1	0.14	0.14	-
Incubation time	3	3.68	1.23	-
Depths	3	2.09	0.70	-
Tides X Incubation time	3	0.15	0.05	0.2^{NS}
Tides X Depths	3	1.05	0.35	1.37^{NS}
Incubation time X Depths	9	3.92	0.44	1.7^{NS}
Tides X Incubation time X Depths	9	2.3	0.26	-

Chapter 2B
Appendix 8

MANOVA for the temporal and depthwise variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) of case II in total dark incubation for low and high tide incubated cores

(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Tides	1	0.09	0.09	
Incubation time	3	2.47	0.82	
Depths	3	2.00	0.67	
Tides X Incubation time	3	3.83	1.28	2.99^{NS}
Tides X Depths	3	1.26	0.42	0.98^{NS}
Incubation time X Depths	9	3.4	0.38	0.89^{NS}
Tides X Incubation time X Depths	9	3.84	0.43	

(b) Source of variation	*df*	SS	MS	F
Tides	1	1.36	1.36	-
Incubation time	3	3.82	1.27	-
Depths	3	1.87	0.62	-
Tides X ncubation time	3	6.17	2.05	7.3***
Tides X Depths	3	0.50	0.17	0.59^{NS}
Incubation time X Depths	9	1.96	0.22	0.78^{NS}
Tides X Incubation time X Depths	9	2.53	0.28	-

(c) Source of variation	*df*	SS	MS	F
Tides	1	0.64	0.64	-
Incubation time	3	4.35	1.45	-
Depths	3	3.58	1.19	-
Tides X Incubation time	3	7.26	2.42	6.99**
Tides X Depths	3	0.85	0.28	0.82^{NS}
Incubation time X Depths	9	2.31	0.26	0.74^{NS}
Tides X Incubation time X Depths	9	3.12	0.35	-

(d) Source of variation	*df*	SS	MS	F
Tides	1	0.82	0.83	
Incubation time	3	2.57	0.86	
Depths	3	9.41	3.14	
Tides X Incubation time	3	17.00	5.7	3.88*
Tides X Depths	3	2.28	0.76	0.52^{NS}
Incubation time X Depths	9	7.33	0.82	0.56^{NS}
Tides X Incubation time X Depths	9	13.15	1.46	

(e) Source of variation	*df*	SS	MS	F
Tides	1	0.006	0.006	-
Incubation time	3	4.07	1.36	-
Depths	3	8.66	2.89	-
Tides X Incubation time	3	9.08	3.02	6.36**
Tides X Depths	3	0.23	0.076	0.16^{NS}
Incubation time X Depths	9	2.16	0.24	0.5^{NS}
Tides X Incubation time X Depths	9	4.28	0.47	-

Chapter 2B
Appendix 9

MANOVA for the temporal and depthwise variation in total diatom abundance (a); *Amphora coffeaeformis* (b); *Navicula delicatula* (c); Chlorophyll *a* (d) of case II in total light incubation for low and high tide incubated cores

(*p ≤ 0.05; **p ≤ 0.025; ***p ≤ 0.01; ****p ≤ 0.005; *****p ≤ 0.001; NS – not significant)

(a) Source of variation	*df*	SS	MS	F
Tides	1	0.04	0.04	
Incubation time	3	3.14	1.04	
Depths	3	3.98	1.33	
Tides X Incubation time	3	2.68	0.89	3.45^{NS}
Tides X Depths	3	0.81	0.27	1.05^{NS}
Incubation time X Depths	9	3.33	0.37	1.43^{NS}
Tides X Incubation time X Depths	9	2.33	0.26	

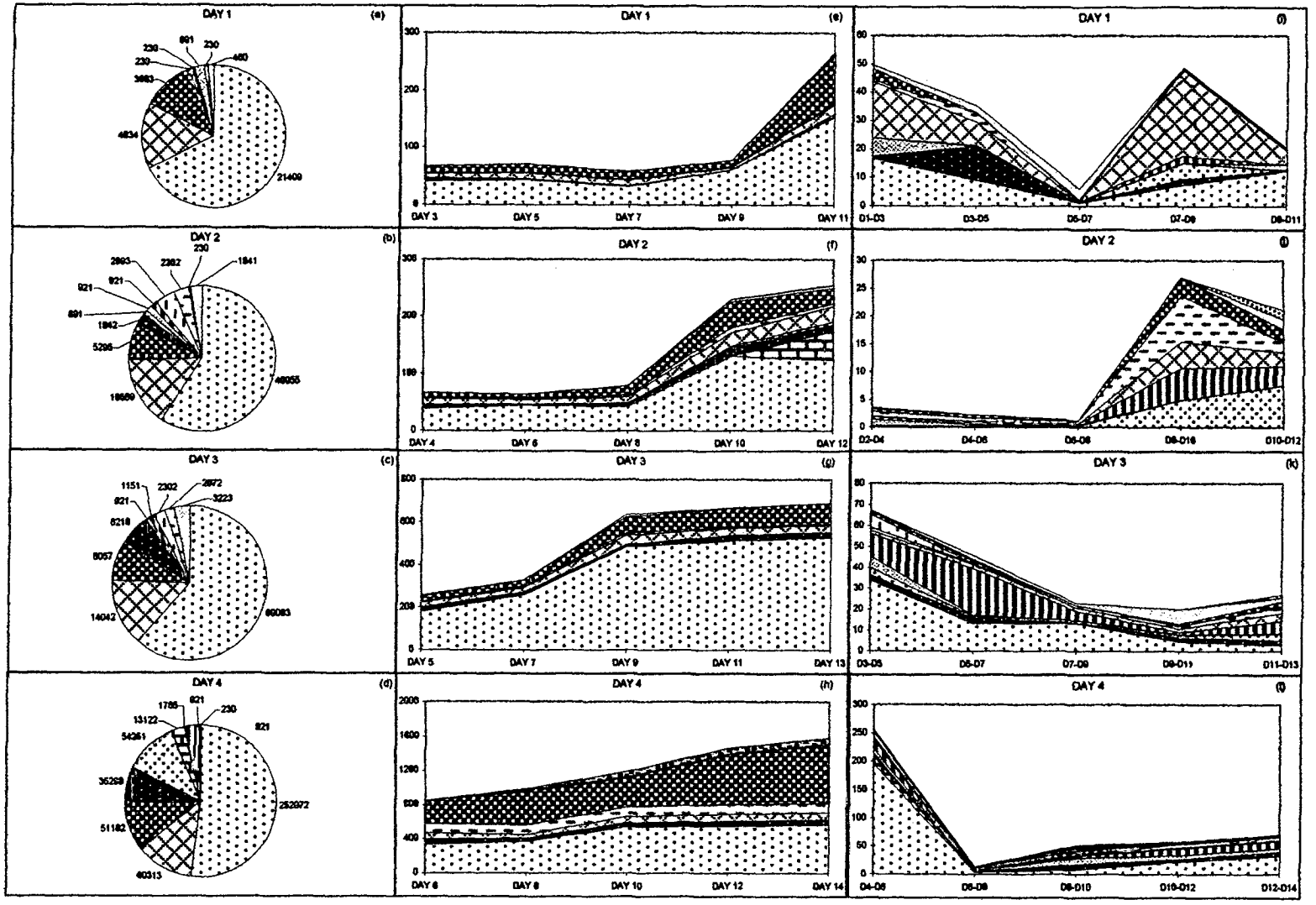
(b) Source of variation	*df*	SS	MS	F
Tides	1	0.23	0.23	-
Incubation time	3	4.03	1.34	-
Depths	3	3.57	1.19	-
Tides X Incubation time	3	4.92	1.64	9.81****
Tides X Depths	3	0.23	0.07	0.45^{NS}
Incubation time X Depths	9	3.36	0.37	$^{2.23NS}$
Tides X Incubation time X Depths	9	1.5	0.17	-

(c) Source of variation	*df*	SS	MS	F
Tides	1	0.68	0.68	-
Incubation time	3	4.74	1.58	-
Depths	3	3.82	1.27	-
Tides X Incubation time	3	7.7	2.57	5.26**
Tides X Depths	3	1.07	0.36	0.73^{NS}
Incubation time X Depths	9	3.89	0.43	0.89^{NS}
Tides X Incubation time X Depths	9	4.39	0.49	-

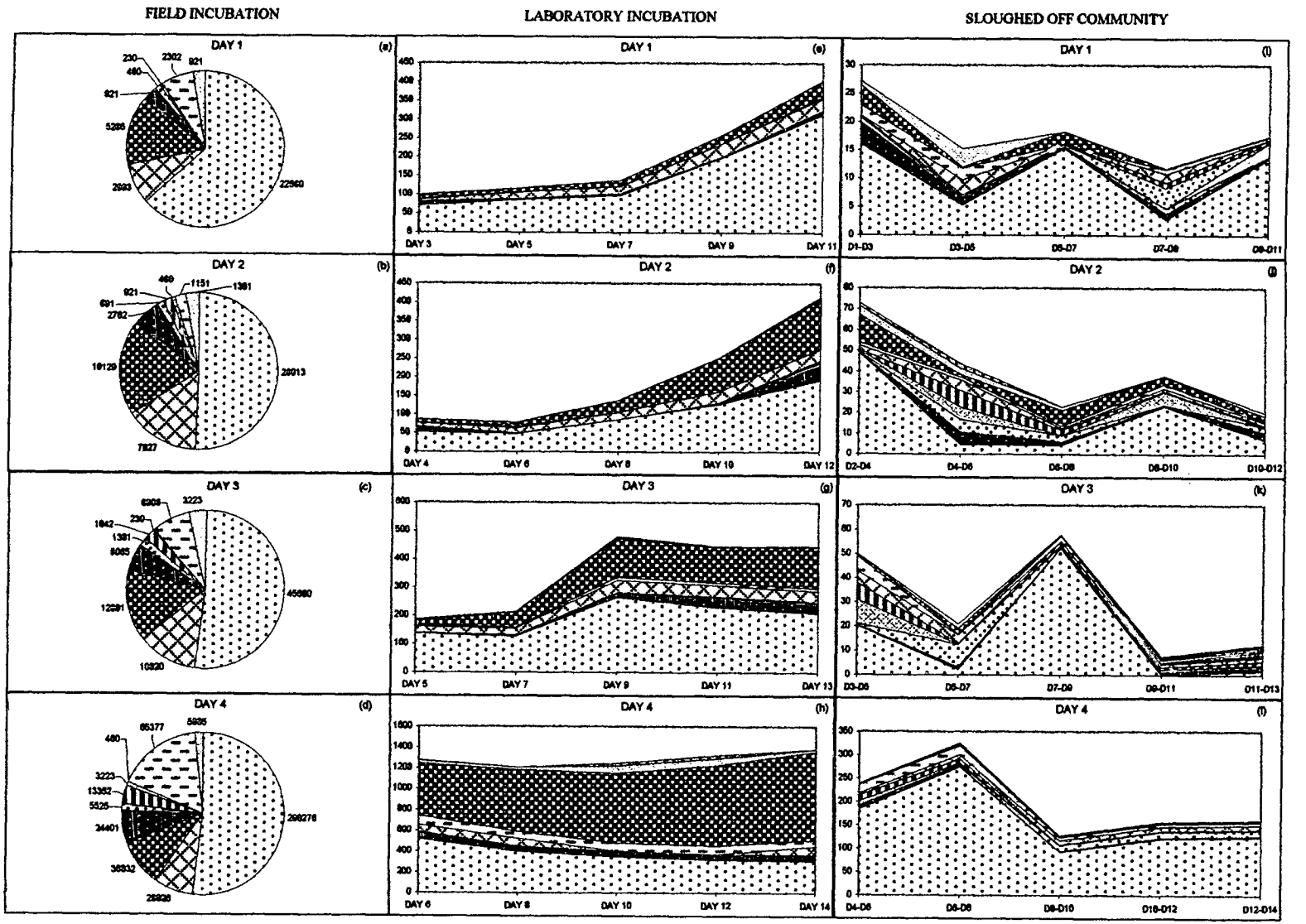
(d) Source of variation	*df*	SS	MS	F
Tides	1	1.58	1.58	
Incubation time	3	7.58	2.52	
Depths	3	5.59	1.86	
Tides X Incubation time	3	12.20	4.05	9.17****
Tides X Depths	3	4.27	1.42	3.22^{NS}
Incubation time X Depths	9	8.08	0.89	2.03^{NS}
Tides X Incubation time X Depths	9	3.97	0.44	

(e) Source of variation	*df*	SS	MS	F
Tides	1	0.33	0.33	-
Incubation time	3	1.91	0.64	-
Depths	3	5.58	1.86	-
Tides X Incubation time	3	1.55	0.52	2.48^{NS}
Tides X Depths	3	1.55	0.52	2.48^{NS}
Incubation time X Depths	9	3.42	0.38	1.83^{NS}
Tides X Incubation time X Depths	9	1.87	0.21	-

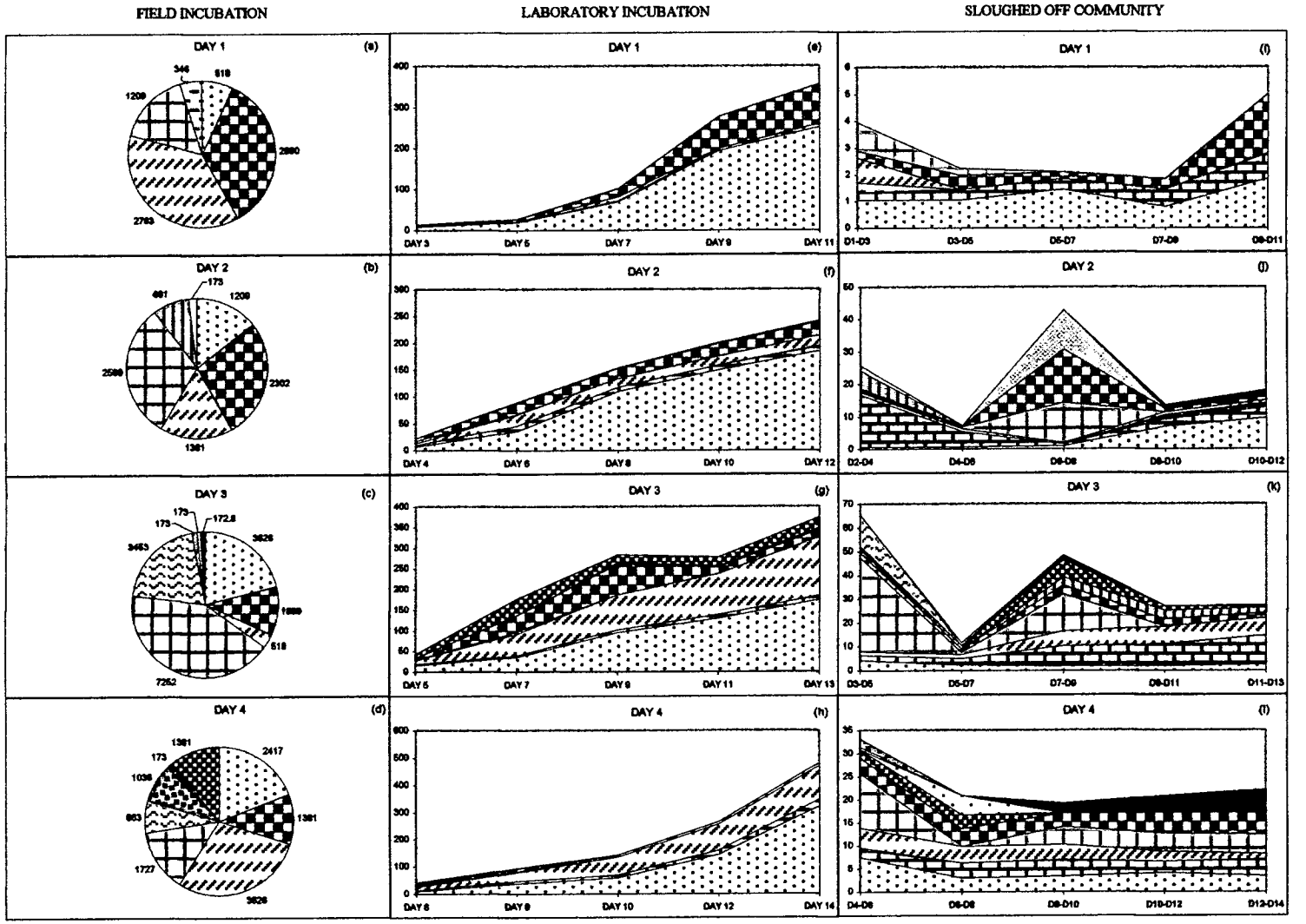
FIELD INCUBATION | LABORATORY INCUBATION | SLOUGHED OFF COMMUNITY



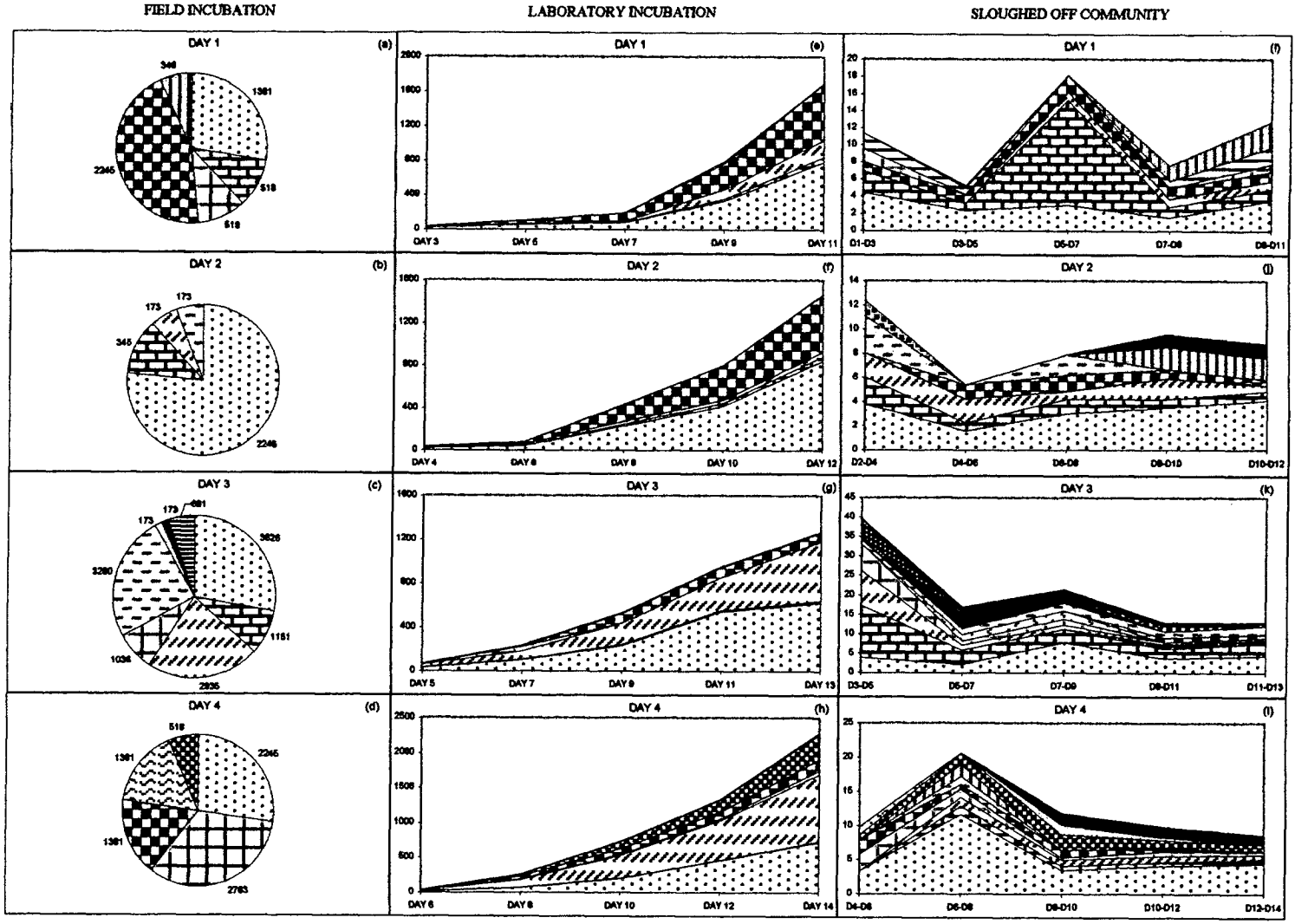
Appendix 1 Case I: Periphytic diatom abundance on the glass slides incubated in the field (a-d) (cells dm^{-2}), AS (e-h) and in the sloughed off community (I-l)



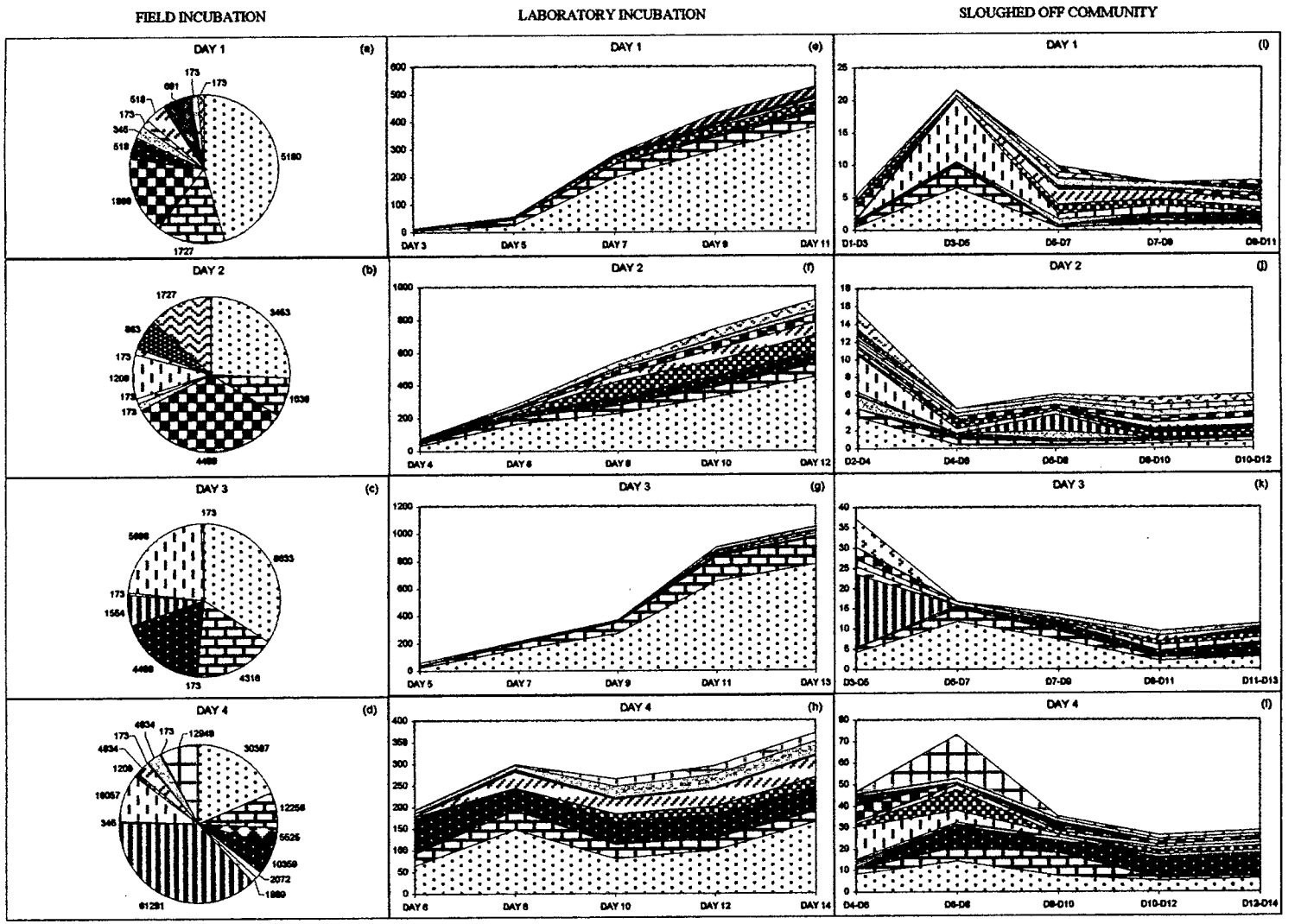
Appendix 2 Case I: Periphytic diatom abundance on the glass slides incubated in the field (a-d) (cells dm^{-2}), ES (e-h) and in the sloughed off community (I-l)



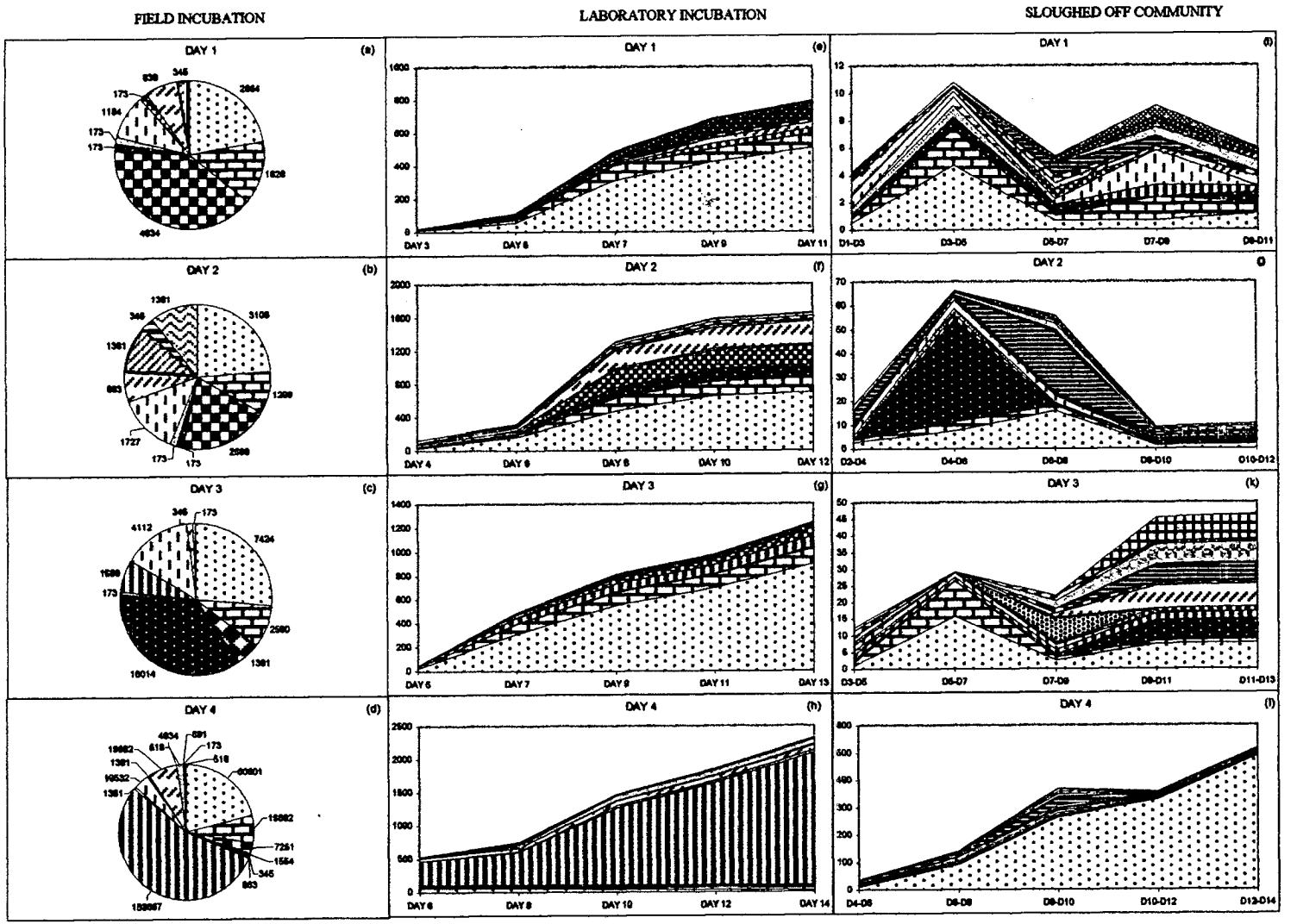
Appendix 3 Case II: Periphytic diatom abundance on the glass slides incubated in the field (a-d) (cells dm^{-2}), ES (e-h) and in the sloughed off community (i-l)



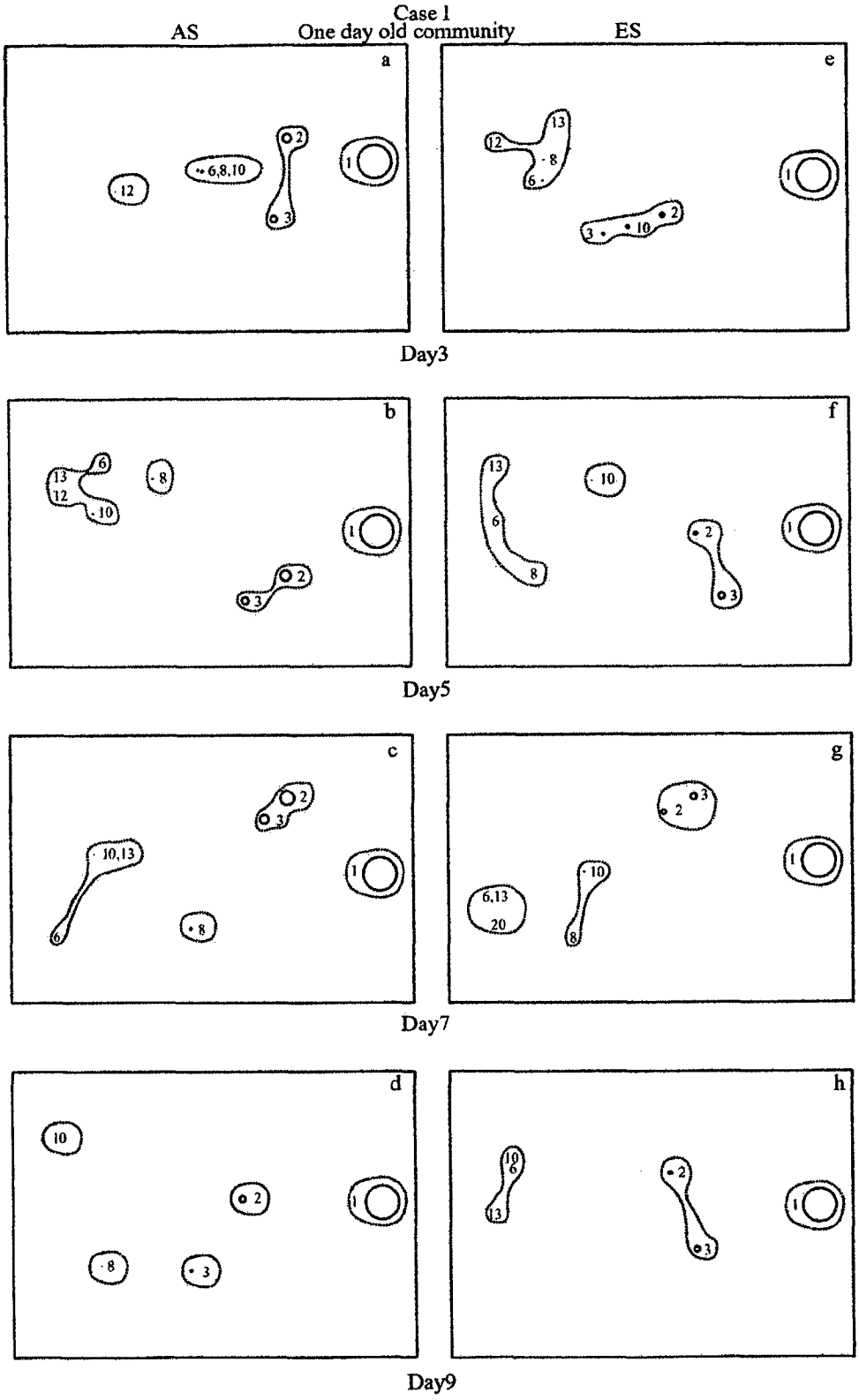
Appendix 4 Case II: Periphytic diatom abundance on the glass slides incubated in the field (a-d) (cells dm^{-2}), ES (e-h) and in the sloughed off community (I-l)



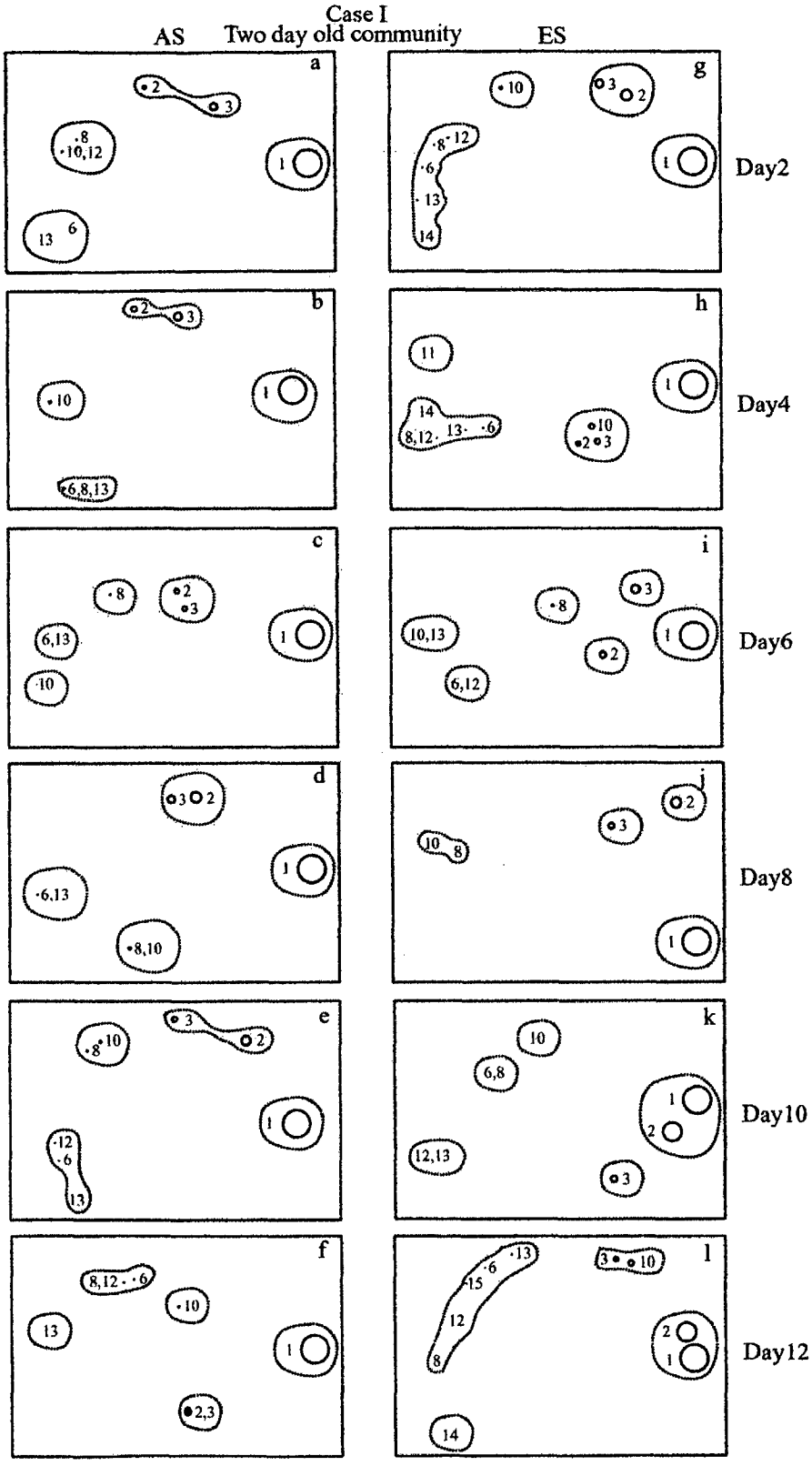
Appendix 5 Case III: Periphytic diatom abundance on the glass slides incubated in the field (a-d) (cells dm^{-2}), ES (e-h) and in the sloughed off community (I-l)



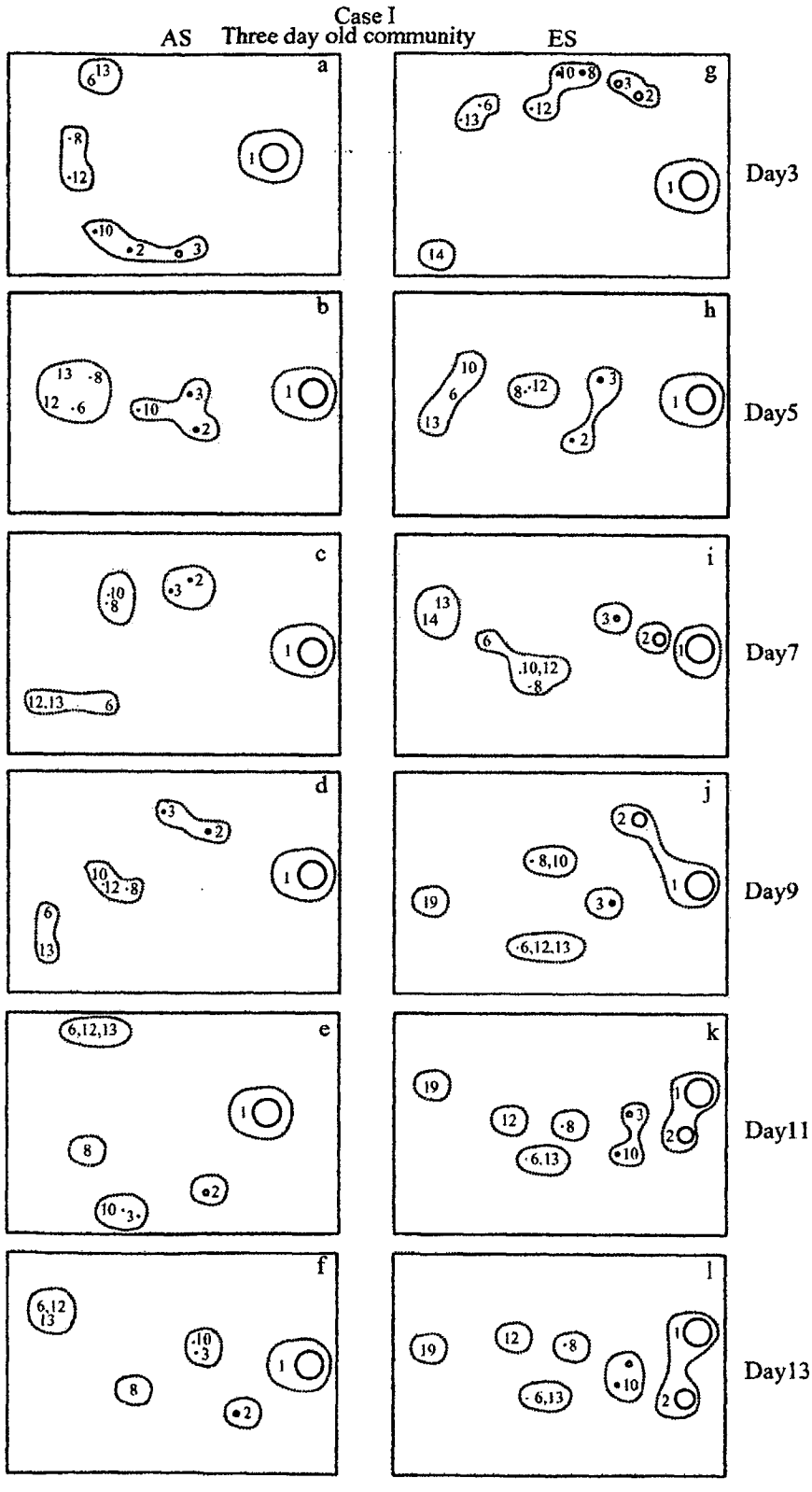
Appendix 6 Case III: Periphytic diatom abundance on the glass slides incubated in the field (a-d) (cells dm^{-2}), ES (e-h) and in the sloughed off community (I-l)



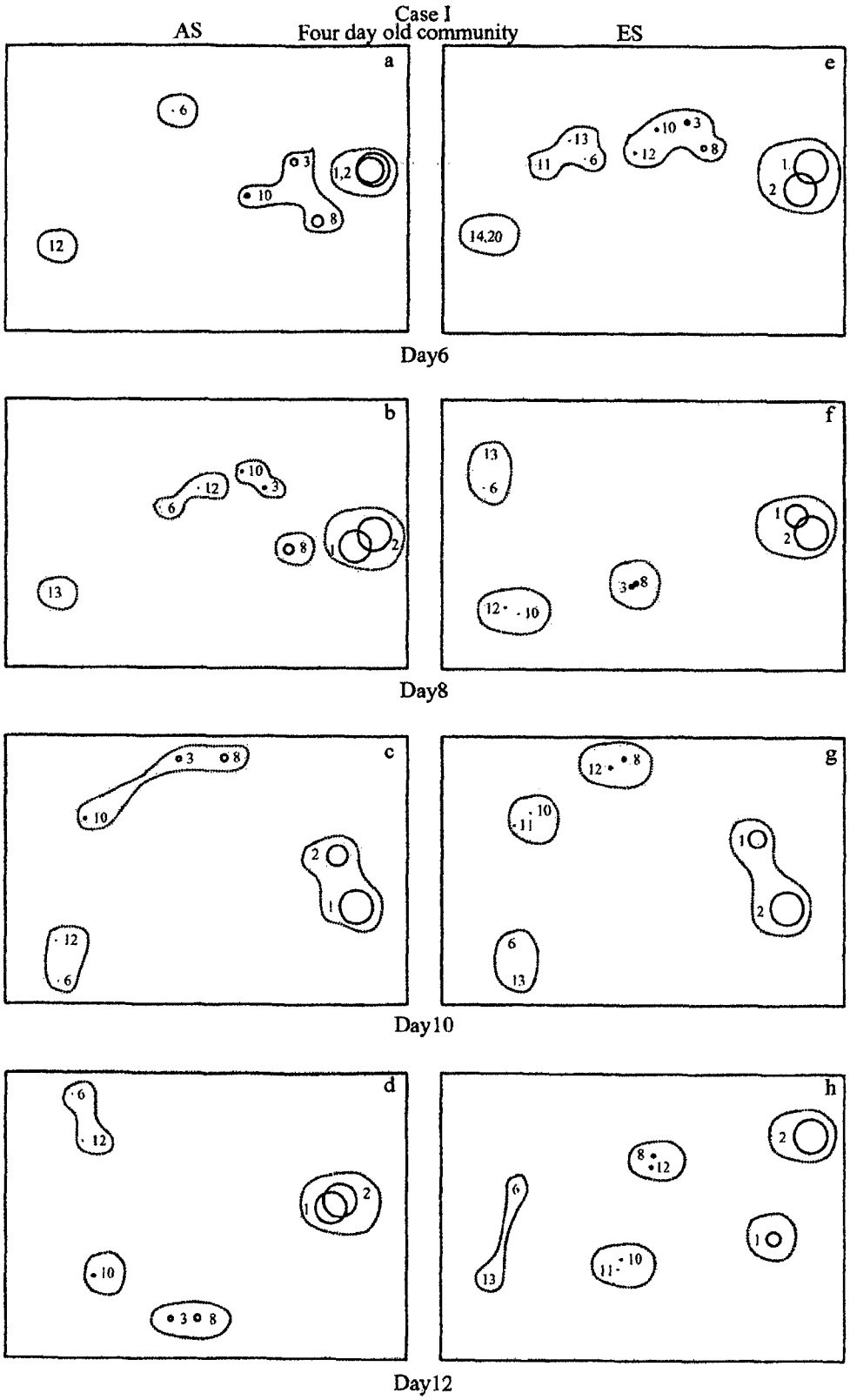
Appendix 7. Multidimensional scaling (MDS) ordinations for the one day old, case I periphytic diatom community incubated in AS (a-d) and ES (e-h) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



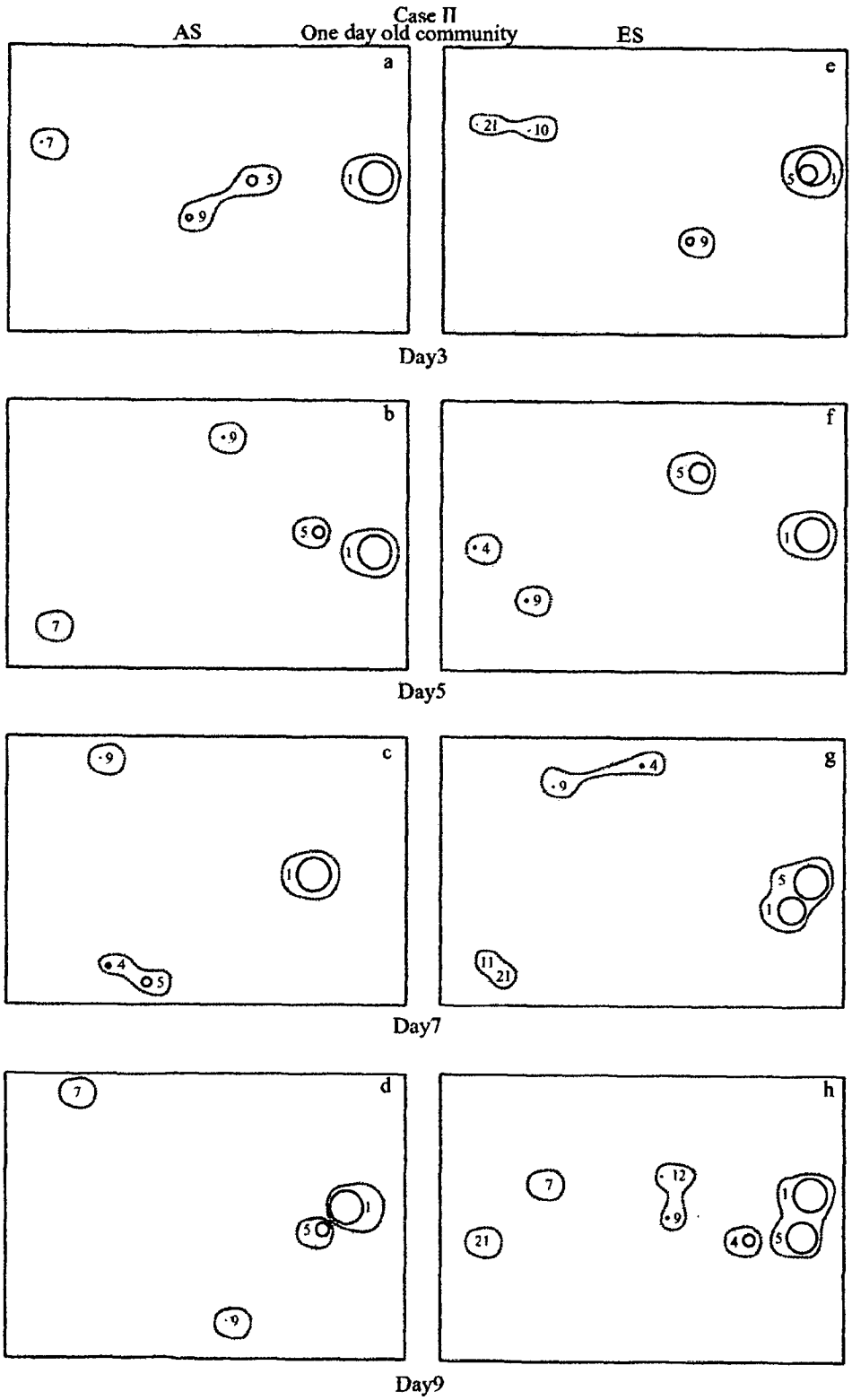
Appendix 8. Multidimensional scaling (MDS) ordinations for the two day old, case I periphytic diatom community incubated in AS (a-f) and ES (g–l) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



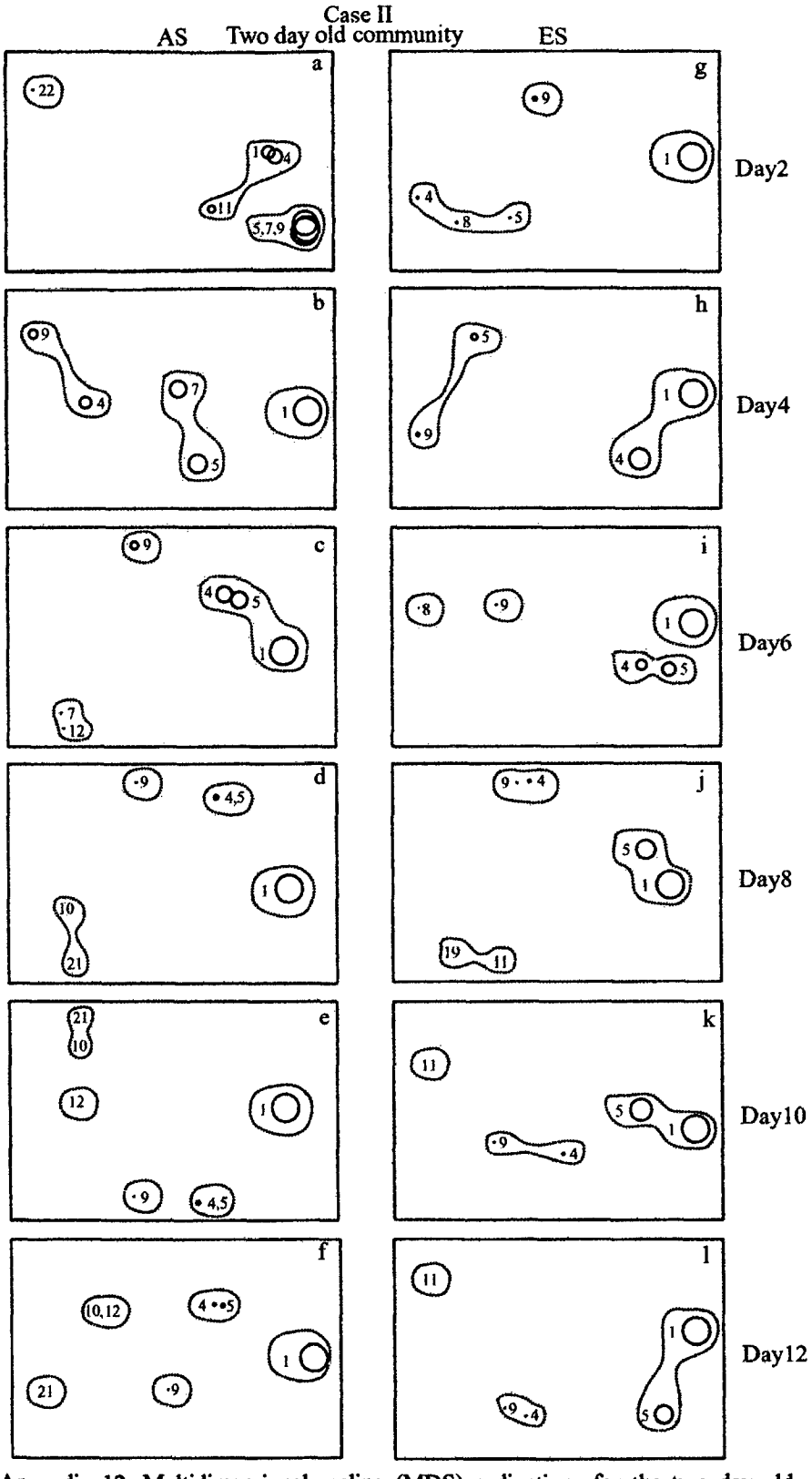
Appendix 9. Multidimensional scaling (MDS) ordinations for the three day old, case I periphytic diatom community incubated in AS (a-f) and ES (g–l) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



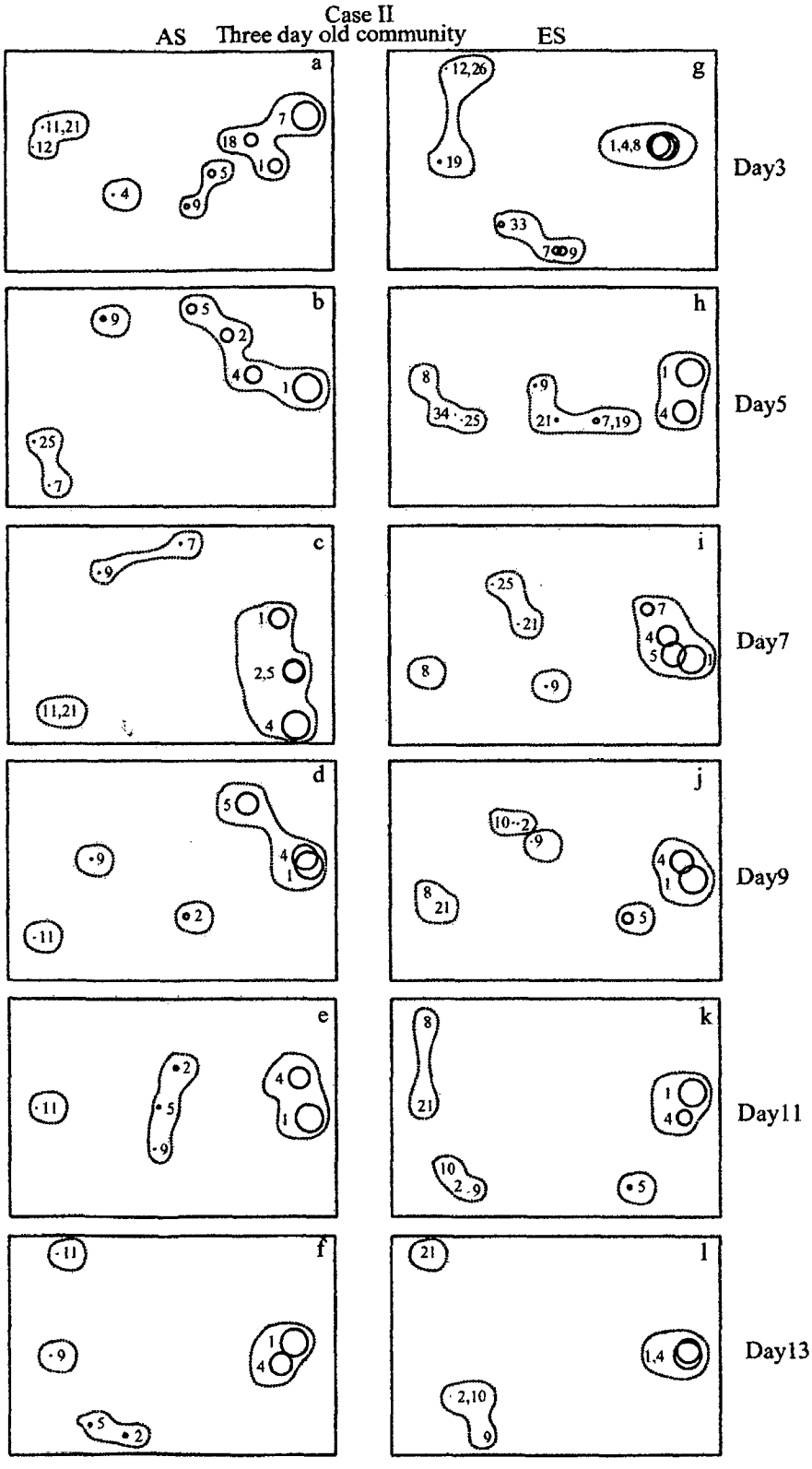
Appendix 10. Multidimensional scaling (MDS) ordinations for the four day old, case I periphytic diatom community incubated in AS (a-d) and ES (e–h) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



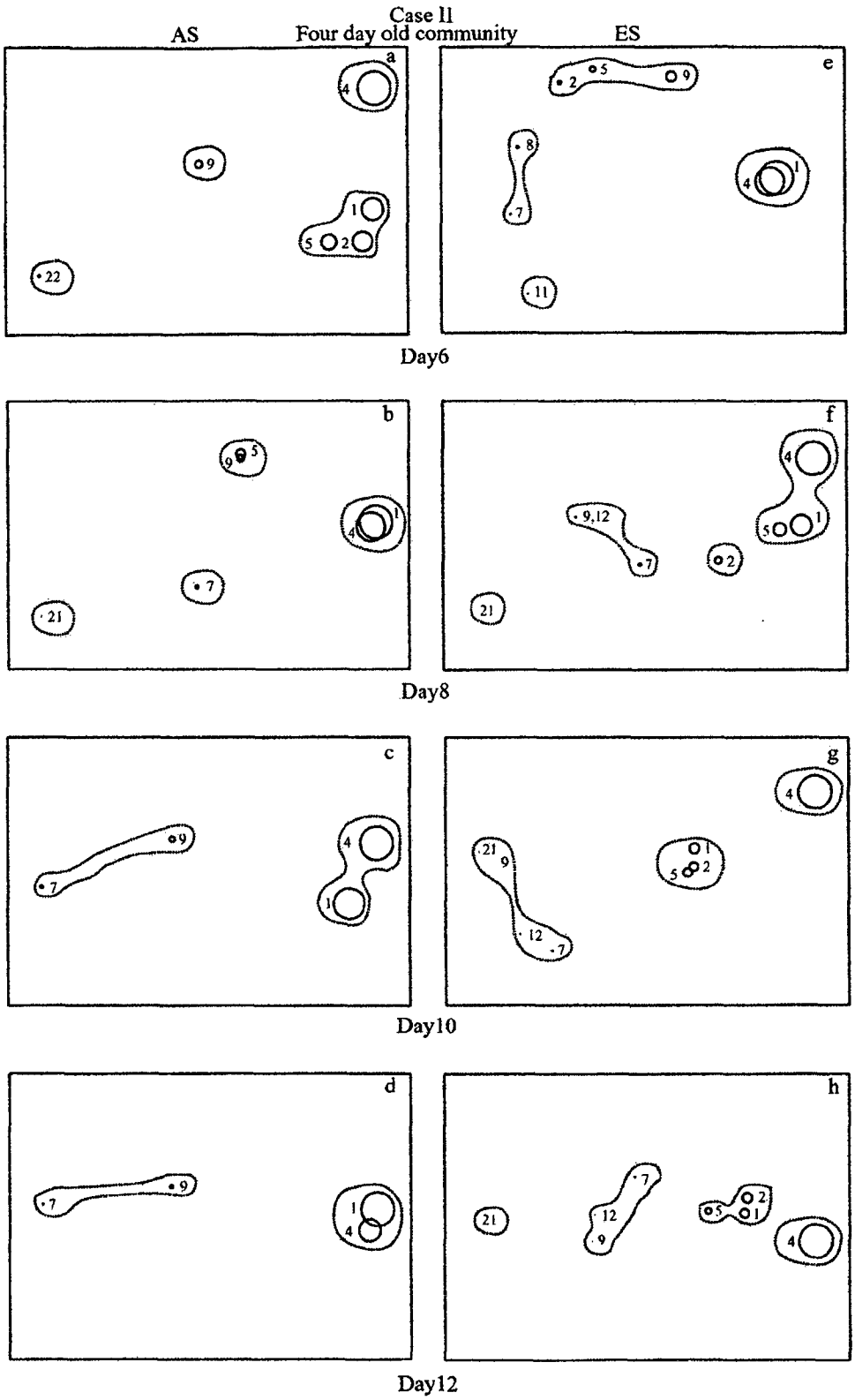
Appendix 11. Multidimensional scaling (MDS) ordinations for the one day old, case II periphytic diatom community incubated in AS (a-d) and ES (e-h) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



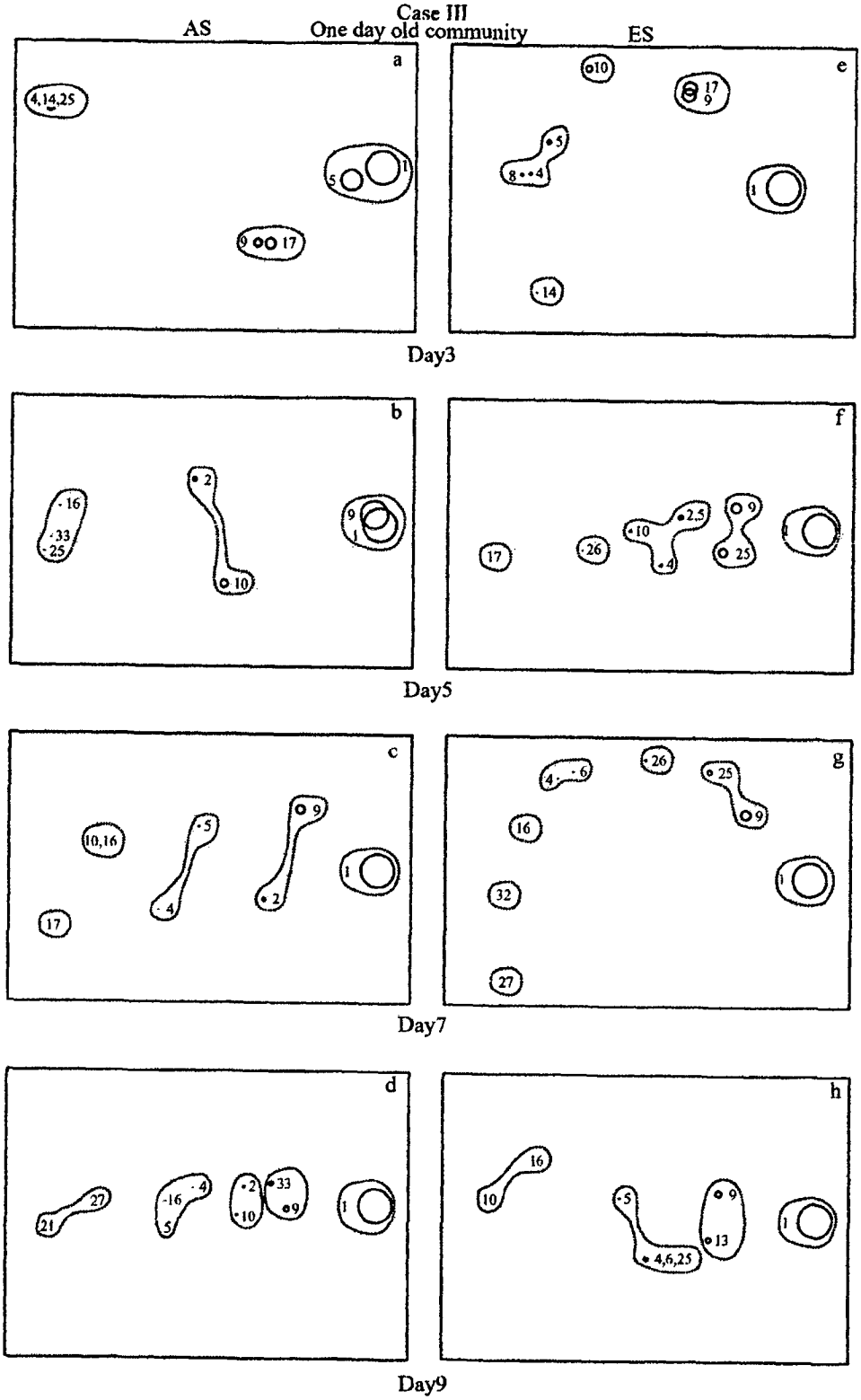
Appendix 12. Multidimensional scaling (MDS) ordinations for the two day old, case II periphytic diatom community incubated in AS (a-f) and ES (g–l) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



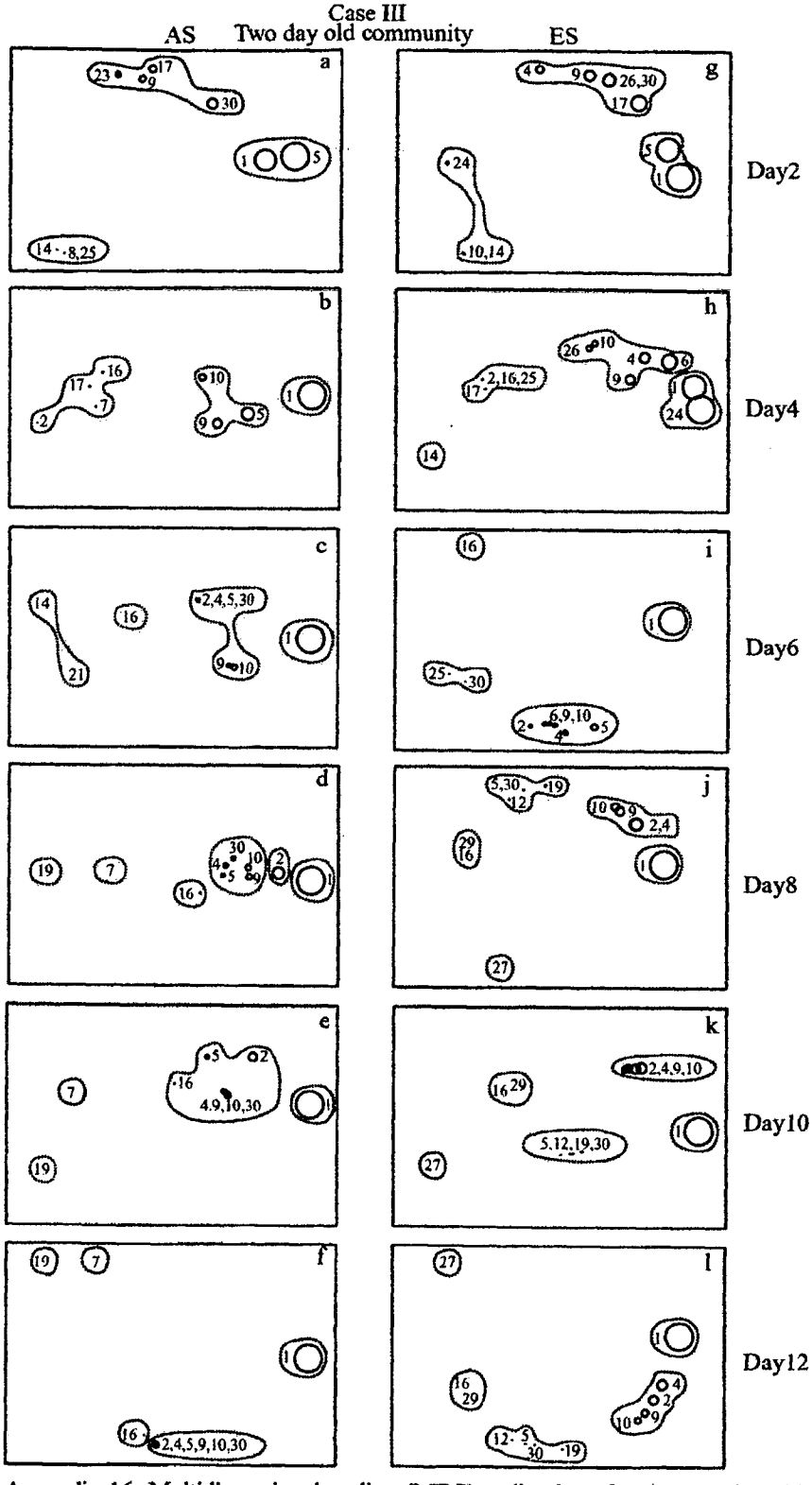
Appendix 13. Multidimensional scaling (MDS) ordinations for the three day old, case II periphytic diatom community incubated in AS (a-f) and ES (g–l) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



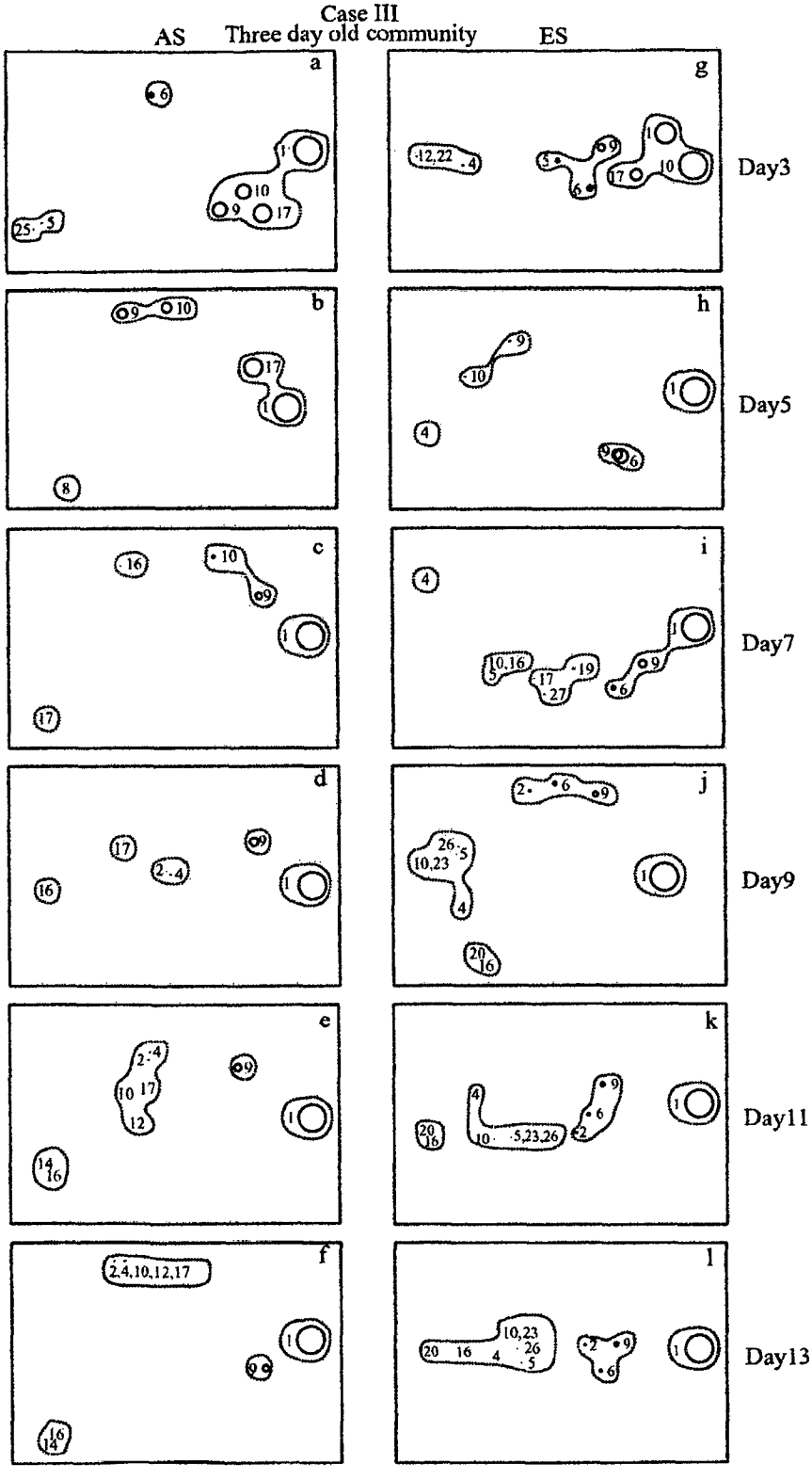
Appendix 14. Multidimensional scaling (MDS) ordinations for the four day old, case II periphytic diatom community incubated in AS (a-d) and ES (e–h) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



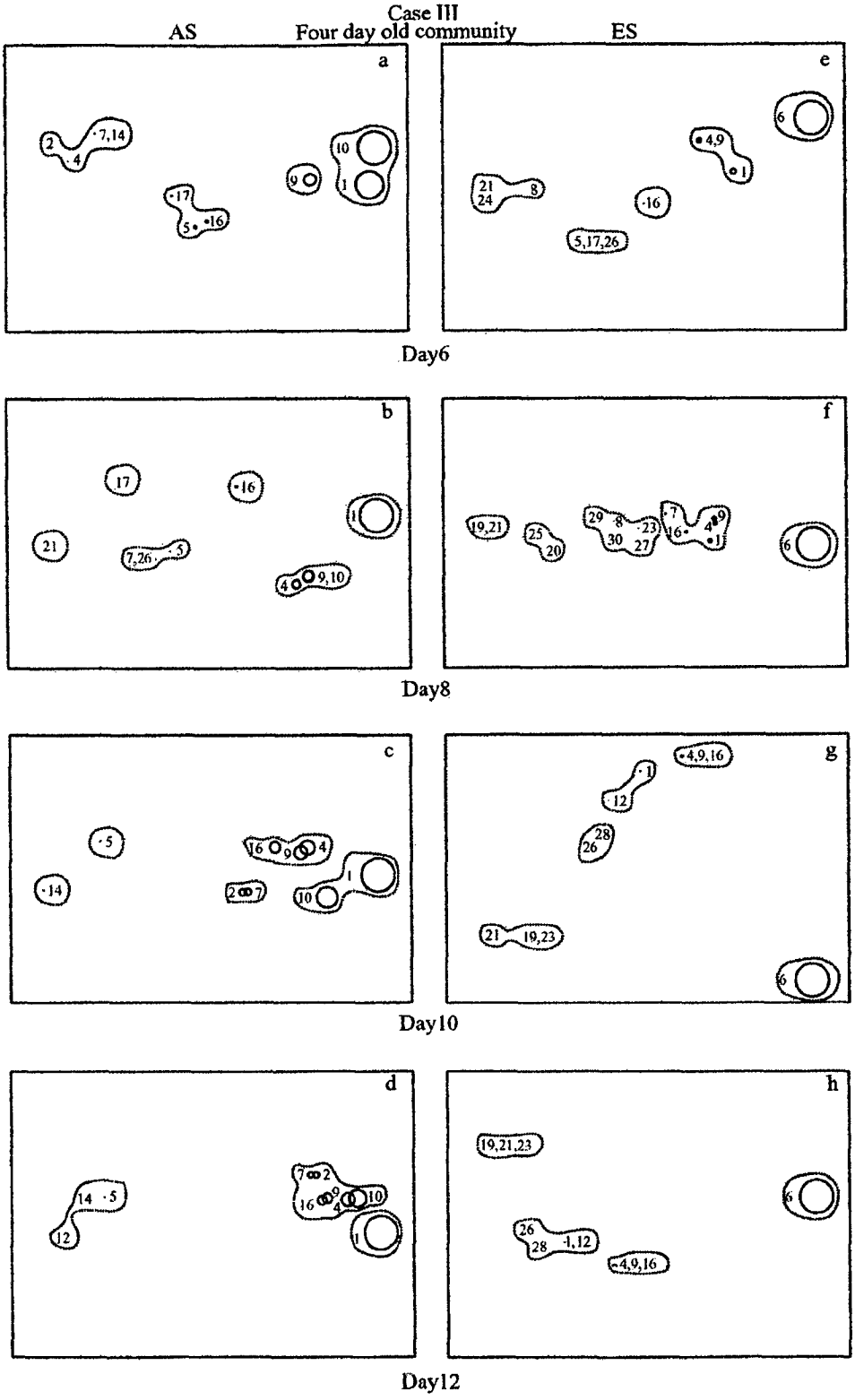
Appendix 15. Multidimensional scaling (MDS) ordinations for the one day old, case III periphytic diatom community incubated in AS (a-d) and ES (e–h) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



Appendix 16. Multidimensional scaling (MDS) ordinations for the two day old, case III periphytic diatom community incubated in AS (a-f) and ES (g–l) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



Appendix 17. Multidimensional scaling (MDS) ordinations for the three day old, case III periphytic diatom community incubated in AS (a-f) and ES (g–l) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.



Appendix 18. Multidimensional scaling (MDS) ordinations for the four day old, case III periphytic diatom community incubated in AS (a-d) and ES (e–h) based on Bray-Curtis similarities. Increasing size of circles indicate increasing abundance of a species (Stress = 0). Hatched lines indicate groups of species.

Publications

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Diatoms of the microphytobenthic community: population structure in a tropical intertidal sand flat

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Abstract Temporal and spatial variations were investigated in the viable diatom population of the microphytobenthic community from an intertidal sand flat of a tropical environment. The presence of diatoms to a sediment depth of 15 cm and their rejuvenation in culture revealed that their viability was not affected by the conditions prevailing at this depth. This depth harbored not only pennate (epipsammic and epipelic) diatoms, some of which are permanent residents of this area, but also centric diatoms of planktonic origin. The occurrence of diatoms such as *Amphora* and *Navicula* throughout the year in the sediments indicated that the two pennate forms were natives of this area. The centric diatom *Thalassiosira*, the vegetative cells of which were not observed in the sediments, must have appeared in culture through the germination of resting stages, which are regularly carried to the study area with coastal sediments and redeposited in intertidal sediments. Nutrients did not play an important role in the case of the pennates, which mainly reside in the sediments; whereas, in centric diatoms, nitrate and phosphate positively influenced their abundance. Multiple regression analysis revealed that some of the grain size fractions served as predictors of diatoms such as *Amphora*, *Grammatophora*, *Pleurosigma* and *Thalasssiothrix*. Wind stimulated the resuspension of the sediment, along with pennate diatoms, down to 5 cm depth. Correlation of chlorophyll *a* with diatom cell numbers, which has been generally used as an indicator of diatom abundance, revealed that chlorophyll *a* concentrations were good predictors of both pennate and centric diatom abundance, to 10 cm depth. However, a negative correlation between chlorophyll *a* and diatoms at the 10–15 cm depth, even when viable diatoms were found in appreciable numbers, suggests survival of these diatoms below the physical disturbance level through the adoption of survival strategies such as resting stage formation.

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Supplementary materials: Additional documentary material has been deposited in electronic form at and can be obtained from http://dx.doi.org/10.1007/s002270100686.

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Introduction

Diatoms constitute an important part of the microphytobenthic community at intertidal sand flats. The diatom population here is usually composed of pennate diatoms, which are either epipsammic (attached to sand grains) or epipelic (motile forms within sediments). Intertidal sand flats are dynamic environments, where the tidally generated water movement and the associated processes of deposition and resuspension of sediment affect the composition and distribution of diatoms. In addition, hydrodynamic processes carry planktonic diatoms present in the ambient water to the intertidal sediment. These planktonic forms can be in either their vegetative or their resting stage, and can contribute to population dynamics. So far, studies on diatom populations have been restricted to the epipelic pennate diatoms, and those forms which reside, although temporarily, on or within the sediment grains have not received due attention. Diatom populations could, however, be better understood by taking into consideration both permanent residents and temporary visitors.

In many studies diatoms were only investigated in the top few centimeters of the sediment (Riznyk et al. 1978; Colijn and Dijkema 1981; Varela and Penas 1985; Lukatelich and McComb 1986). However, the presence of diatoms at a depth of 20 cm has also been reported (Steele and Baird 1968; Colijn and Dijkema 1981; de Jonge and Colijn 1994), based on chlorophyll *a* estimations. In intertidal sand flats a number of factors may be responsible for displacing the diatoms from the surface

sediment layers to the deeper layers. Diatoms can retain photosynthetic capacity in the dark, deeper sediments and thus form an important pool of potential primary producers, which can resume photosynthesis if resurfaced (Fielding et al. 1988).

In the present study, the sediment at the lowest low tide mark was investigated for the temporal variation in diatom abundance to a depth of 15 cm. Chlorophyll *a* content and physico-chemical parameters such as wind speed, sediment characteristics, nutrients and suspended load were correlated with diatom abundance. Such a study will help gain insight into the dynamics of diatom populations, which form an important component of the microphytobenthic community, responsible for the littoral production and thus forming a basic link in intertidal food webs.

Materials and methods

Study area and sampling strategy

Sediment sampling was carried out on a sand flat at Dias Beach (15°27'N; 73°48'E), located near Dona Paula Bay and surrounded by the Mandovi and Zuari estuaries (Fig. 1). This beach is about 200 m in length. The tides are semi-diurnal, with an average spring tide range of ~2 m and neap tide range of ~0.7 m. In this area, the wave heights are > 1.5 m during June–August and low (< 0.7 m) during October–April (Chandramohan et al. 1997). Dias Beach is sheltered, protected on both sides by rocky cliffs. This locality experiences three seasons: the pre-monsoon (February–May), the southwest monsoon (June–September) and the post-monsoon (October–January). The sediment sampling was carried out on a monthly basis for 17 months (May 1998–September 1999) so that a full cycle of the three seasons and a repetition of one season was considered. Sediment samples were collected in triplicate at the low tide mark during the lowest low tide, using a hand-corer with an inner diameter of 4.5 cm. The core length collected was 15 cm. Each core was divided into three sections of 5 cm intervals (0–5, 5–10 and 10–15 cm). Simultaneously, a separate sediment core of the same dimensions was collected for chlorophyll *a* and grain size analysis.

Interstitial water samples were collected by digging the sediment (~15 cm) with a shovel and allowing the water to collect. The water samples were allowed to stand for a few seconds while sand particles settled. Temperature of the surface sediment was recorded at the study site, and analysis of salinity (Mohr–Knudsen titration method) (Strickland and Parsons 1965) and nutrients such as nitrate (NO_3-N), nitrite (NO_2-N), phosphate (PO_4-P) and silicate (Si) along with chlorophyll *a* (corrected for phaeopigments) were carried out by standard procedures (Parsons et al. 1984). Salinity and nutrient values were obtained for the entire 15 cm core, whereas chlorophyll *a* was estimated for each of the three core sections (0–5, 5–10 and 10–15 cm). Simultaneously, surf water samples were also collected from the surface at about 3 m from the low tide mark for the analysis of the water parameters as mentioned above. A known volume of the surf water (500 ml) was transferred to PVC bottles (triplicates) and preserved with Lugol's iodine solution to estimate the diatom population following the method described by Hasle (1978).

Enumeration of diatoms in the sediment

Since this study was carried out on a sandy beach and included the observation of attached forms, direct examination and quantification of samples posed a problem. Even if diatoms in their attached state could be observed directly, there was always a chance of missing cells attached to the other side of the grain. The ultrasonification technique was not of much help either, since some forms did not detach from the substrata and fragile forms were at risk of being ruptured, thus leading to false quantification results. Therefore, the extinction dilution method (most-probable-number method, MPN) (Imai et al. 1984, 1990; Yamochi 1989; Ishikawa and Tamaguchi 1994; Itakura et al. 1997) was employed for quantification of the diatom flora in each section of the sediment core. Subsequent to incubation of the sediment sample, the appearance of centric and pennate diatoms was observed. The appearance of both these forms may have been from two sources: either they were the products of multiplication of vegetative cells or germination of resting stages.

An appropriate amount (1–2 g wet wt) of sediment sample was suspended in *f*/2 medium (Guillard and Ryther 1962) at a concentration of 0.1 g wet wt ml^{-1}. This stock was then subjected to a tenfold serial dilution (10^{-1}–10^{-5}) with the culture medium, and then 1 ml aliquots of diluted suspensions were inoculated into five replicate culture wells. Incubation was carried out at 27 ± 1°C with a 12 h light:12 h dark photocycle. The growth of diatoms in each culture well was examined microscopically after an incubation period of 6–8 days. The wells in which growth was observed were scored as positive. The MPN (for a series of 3 tenfold dilutions) of diatoms in the sediment sample (MPN g^{-1} wet sediment) was then calculated according to a statistical table (Throndsen 1978). This table covers a range of five dilution steps; a set of three dilutions have to be chosen out of the five cultured to get the MPN number. The diatom density per cubic centimeter wet sediment was obtained by multiplying the MPN value with the apparent specific gravity of the wet sediment (Imai and Itakura 1999). The diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyan (1946), Desikachary (1987) and Tomas (1997).

Grain size analysis

Sediment grain size was analyzed by dry sieving (Folk 1968). Categories of grain size included: > 1,000 μm (very coarse sand):

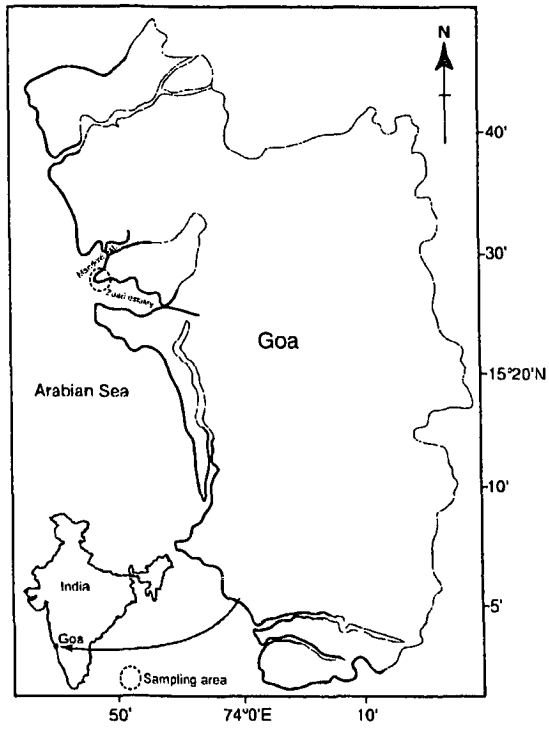


Fig. 1 Location of the study area

500–999 μm (coarse sand), 250–499 μm (medium sand), 125–249 μm (fine sand), 63–124 μm (very fine sand) and < 63 μm (silt and clay). The sediment was moderate to well sorted, with a sorting coefficient ranging from 0.32 to 0.58.

Data analysis

Univariate statistical methods

Univariate techniques included the calculation of the Shannon-Wiener diversity index (H'), richness ($H'_{max.} = \log_2 S$, where S is the number of taxa) and evenness ($J' = H'/H'_{max.}$) of the total diatom population (both benthic and planktonic) (Pielou 1969).

Multivariate statistical methods

The $\log(x+1)$-transformed data on the relative abundance of diatoms in the intertidal sediment was used for the construction of a lower triangular dissimilarity matrix using Bray-Curtis coefficients (Bray and Curtis 1957). This dissimilarity matrix was then subjected to clustering and ordination analysis. Clustering was performed using the weighted pair group average method (Pielou 1984). Ordination was by non-metric multidimensional scaling (NMDS) (Kruskal and Wish 1978). There are advantages in applying more than one method, since each is based on different assumptions and may give different insights (Gray et al. 1988). In the case of the cluster analysis: (1) the individual, once placed in a group, loses its identity; (2) the sequence of individuals is arbitrary; and (3) only the inter-group relationships are shown. In view of these disadvantages, it is advisable to employ an additional method of presentation to show individual relationships such as NMDS (ordination).

The $\log(x+1)$-transformed data on diatom abundance in the water column was subjected to clustering by squared Euclidean distance and the group average method (Pielou 1984).

A two-way analysis of variance (ANOVA) (Sokal and Rohlf 1981) with a 17×3 factorial design (17 months×3 depths) was conducted on the $\log(x+1)$-transformed relative abundance data of each diatom, in order to test the significance of month and depth. The diversity, richness and evenness values of the diatom assemblages were also subjected to two-way ANOVA to evaluate the temporal (month) and spatial (depth) variations.

Multivariate ANOVA (MANOVA) without replication (Sokal and Rohlf 1981) with a 17×3×6 factorial design (17 months, 3 depths, 6 grain size fractions) was performed on the arcsine-transformed grain size values (sediment composition) in order to assess whether any significant variance existed with respect to different sampling periods (months) and depths.

Diatom relative abundance and the arcsine-transformed grain size data for the different depths as well as the $\log(x+1)$-transformed data on water parameters (temperature, salinity and nutrients) were subjected to multiple regression.

Regression was performed on the $\log(x+1)$-transformed data on sediment chlorophyll *a* (linear function), wind speed and suspended load (power function) versus diatom abundance.

Results

Environment

Surf water temperature ranged between 20°C and 31°C, whereas that of the sediment surface ranged between 21°C and 30°C. Salinity was as low as 10 psu during the monsoon period, while during the other seasons it ranged between 28 and 35 psu. The nutrient concentrations in the surface and interstitial waters showed similar trends. Nitrate concentration ranged between 0.08 and 12 μM and was maximum during the monsoon season, with a peak in July, which was followed by a bloom of *Skeletonema costatum* in the ambient waters. Nitrite ranged between 0.013 and 0.8 μM, being highest pre-monsoon (March). During pre-monsoon and post-monsoon seasons a rise was detected in the phosphate concentration of surf and interstitial waters. The surf water silicate concentrations were generally highest during monsoons, whereas, in interstitial waters, they were found to be above 17 μM throughout the investigation period. The observed low salinity and high nitrate and silicate concentrations were due to considerable land runoff during the monsoon season.

Sediment characteristics

Very fine sand (63–124 μm – silt/clay) was found to be the most abundant grain size fraction of the sediment throughout the study period across the 15 cm core (Fig. 2). The contribution of sediment particles > 250 μm was negligible. MANOVA of different months versus grain size and depth versus grain size revealed significant variation (Table 1). Across the three sections, the percentage of the < 63 μm grain size fraction peaked on two occasions. One peak was observed towards the end of the monsoon season (September–October), while the other was evident during February–April (pre-monsoon). During these periods, a simultaneous decrease in the percentage of the 63–124 μm grain size fraction was observed.

Diatoms in surf water

In the water column, 34 species of diatoms belonging to 27 genera were encountered (Table 2). Of these, 19 were centric and 15 pennate. *Navicula delicatula* was found to be the most abundant diatom species.

Clustering of this diatom community at 50% dissimilarity level revealed *N. delicatula* to be the most dissimilar diatom in terms of distribution. Clustering revealed three main groups (Fig. 3). The first group comprised *Navicula*. The third group was represented by *Skeletonema costatum* and *Fragilariopsis cylindrus*, which dominated blooms in the ambient waters during August and September, when a peak was observed in diatom abundance (Fig. 4). The rest of the forms formed the members of the second group.

Diatoms in intertidal sediments

In the sediment, 24 species of diatoms (12 centric, 12 pennate) belonging to 16 (8 each of pennates and centrics) genera were recorded (Table 2). Pennate diatoms were dominant in terms of abundance (Fig. 5). The most abundant pennate diatoms were *Amphora* and *Navicula*; *Thalassiosira* was the most abundant centric diatom.

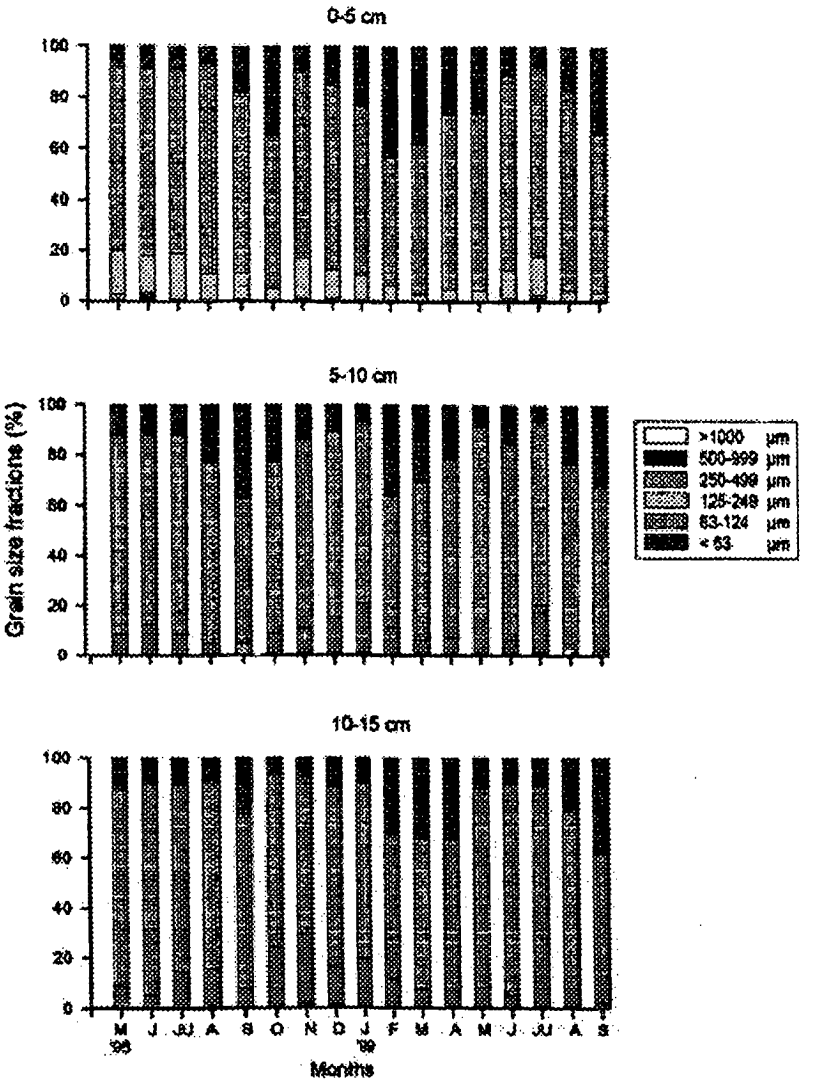


Fig. 2 Temporal variations in grain size fractions (> 1,000, 500–999, 250–499, 125–249, 63–124 and < 63 µm) across the vertical gradient of the core

Diatoms encountered in this area can be divided into three groups based on their abundance; *Amphora*, *Navicula* and *Thalassiosira*, which were present throughout the period of investigation, formed the first group (Fig. 6a). The second group comprised forms that were frequently encountered in the sediment and included eight genera, of which four were centric diatoms (*Biddulphia*, *Melosira*, *Streptotheca* and *Coscinodiscus*) and four were pennates (*Grammatophora*, *Cocconeis*, *Nitzschia* and *Thalassiothrix*) (Fig. 6b, c). The diatoms appearing rarely formed the third group and included *Pleurosigma*, *Fragilariopsis*, *Skeletonema*, *Chaetoceros* and *Bellerochea*.

Table 1 MANOVA (without replication) for the temporal variation in the sediment characteristics along the vertical gradient (0–5, 5–10 and 10–15 cm sediment core sections) (*$P \leq 0.025$; *NS* not significant)

Source of variation	*df*	SS	MS	*F*
Months	16	27.76	1.73	–
Core sections	2	106.72	53.35	–
% Grain size fractions	5	70,930.88	14,186.18	–
Months×Core sections	32	914.22	28.57	0.787^{NS}
Months×% Grain size fractions	80	5,361.15	67.01	1.845*
Core Sections×% Grain size fractions	10	749.10	74.91	2.063*
Months×Core sections×% Grain size fractions	160	5810.9	36.32	–

Table 2 Diatoms recorded in surf water and intertidal sediments (0–15 cm) during the study period

	Centric diatoms	Pennate diatoms
Surf water	*Skeletonema costatum* Greville	*Navicula delicatula* Heimdal
	Coscinodiscus marginatus Ehrenberg	*Navicula subinflata*
	Chaetoceros curvisetus Ehrenberg	*Navicula* sp.
	Chaetoceros diversus Ehrenberg	*Amphora coffeaeformis* (Agardh) Kutzing
	Melosira nummuloides C.A. Agardh	*Amphora rostrata* Wm. Smith
	Streptotheca thamensis Shrubs	*Amphora* sp. Ehrenberg
	Biddulphia mobiliensis (Bailey) Grunow	*Grammatophora marina* Kutzing
	Biddulphia sinensis (Greville) Grunow	*Pleurosigma angulatum* sensu W. Smith
	Cyclotella sp.	*Nitzschia seriata* Cleve
	Planktoniella sol (Wallich) Schutt	*Thalassiothrix nitzschioides* Grun
	Guinardia flaccida (Castracane) H. Peragallo	*Cocconeis scutellum* Ehrenberg
	Rhizosolenia alata Brightwell	*Fragilariopsis cylindrus* (Grunow) Cleve
	Rhizosolenia sp.	*Synedropsis gallioni* Grunow
	Lauderia borealis Cleve	*Surirella* sp.
	Ditylum brightwellii (West) Grunow	*Achnanthes longipes* Agardh
	Leptocylindrus danicus Cleve	
	Eucampia zodiacus Ehrenberg	
	Cerataulina pelagica H. Peragallo	
	Thalassiosira eccentrica Ehrenberg	
Intertidal sediments	*Skeletonema costatum* Greville	*Navicula delicatula* Heimdal
	Coscinodiscus marginatus Ehrenberg	*Navicula subinflata*
	Chaetoceros curvisetus Ehrenberg	*Navicula* sp.
	Chaetoceros diversus Ehrenberg	*Amphora coffeaeformis* (Agardh) Kutzing
	Melosira nummuloides C.A. Agardh	*Amphora rostrata* Wm. Smith
	Streptotheca thamensis Shrubs	*Amphora* sp. Ehrenberg
	Biddulphia mobiliensis (Bailey) Grunow	*Grammatophora marina* Kutzing
	Biddulphia sinensis (Greville) Grunow	*Pleurosigma angulatum* W. Smith
	Thalassiosira sp.	*Nitzschia seriata* Cleve
	Thalassiosira sp.	*Thalassiothrix nitzschioides* Grun
	Thalassiosira eccentrica Ehrenberg	*Cocconeis scutellum* Ehrenberg
	Bellerochea malleus (Brightwell) Van Heurck	*Fragilariopsis cylindrus* (Grunow) Cleve

Temporal and vertical distribution of diatoms in the sediment

The total diatom abundance varied from one sampling period (month) to another, but was found to peak during the pre-monsoon season (Fig. 7). Diatoms belonging to group I (*Amphora*, *Navicula* and *Thalassiosira*) and group II (*Biddulphia*, *Melosira*, *Coscinodiscus*, *Streptotheca*, *Cocconeis*, *Thalassiothrix*, *Nitzschia* and *Grammatophora*) were abundant in the pre-monsoon period, whereas diatoms of group III (*Pleurosigma*, *Fragilariopsis*, *Skeletonema*, *Chaetoceros* and *Bellerochea*) were abundant during the monsoon and post-monsoon seasons.

The temporal variation in abundance of the diatoms across the three individual core sections did not reveal

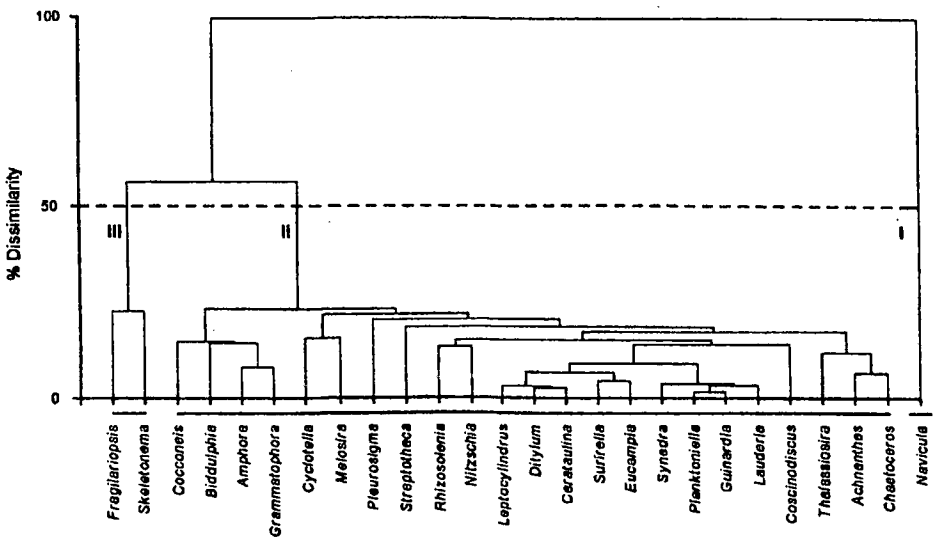


Fig. 3 Cluster dendrogram of the surf water diatom community, constructed by squared Euclidean distance and the group average method

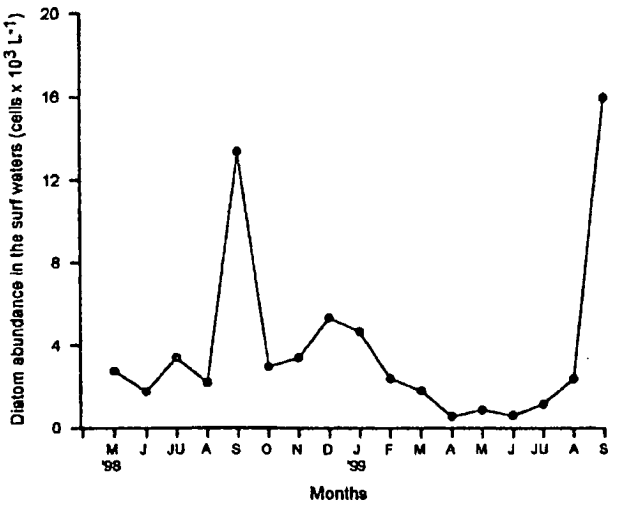


Fig. 4 Temporal variations in total diatom abundance of the surf water

any particular trend. The group I diatoms (*Amphora*, *Navicula* and *Thalassiosira*) were found to peak in February across the three core sections. The distribution of *Amphora* kept a lower profile during the monsoon period, while *Thalassiosira* formed a trough from August to October. *Biddulphia* (group II) peaked during February–March and was not encountered in September down to 15 cm depth. Amongst the group III diatoms, *Bellerochea* was present only in the 0–5 cm stratum (May and June), while *Chaetoceros* was found at 0–5 cm (May and August) and at 10–15 cm (August) depths. *Pleurosigma* was encountered on only one occasion (January) in the 0–5 cm stratum, while at 5–10 cm it was observed during January and August (Fig. 6a–c).

Thus, the diatom data when subjected to two-way ANOVA revealed significant variation with respect to months ($P \leq 0.001$), depths (except for *Skeletonema* and *Streptotheca*) and also the monthxdepth interaction (except for *Skeletonema*) (Table 3).

Diatom diversity was found to decrease drastically with a low evenness value in July at the 0–5 cm depth, as the population was dominated by *Thalassiosira*. During the other months the diversity in the 0–5 cm stratum ranged between 1.5 and 2.3, while at 5–10 and 10–15 cm depths it fluctuated between 0.2 and 2.5. Thus, diatom diversity (H'), richness ($S = H'_{max.}$) and evenness ($J = H'/H'_{max.}$) revealed significant variation with respect to month ($P \leq 0.001$), depth, as well as monthxdepth (Table 4; Fig. 8).

The dendrogram at 50% dissimilarity level revealed five groups of diatoms in the three sediment core sections (Fig. 9a). Group I comprised two sub-groups, IA and IB. Sub-group IA was represented by the most abundant diatoms, i.e. *Thalassiosira*, *Amphora* and *Navicula* for the entire core. *Biddulphia* from the 0–5 and 10–15 cm core sections also formed a member of this sub-group. The abundance of these diatoms ranged up

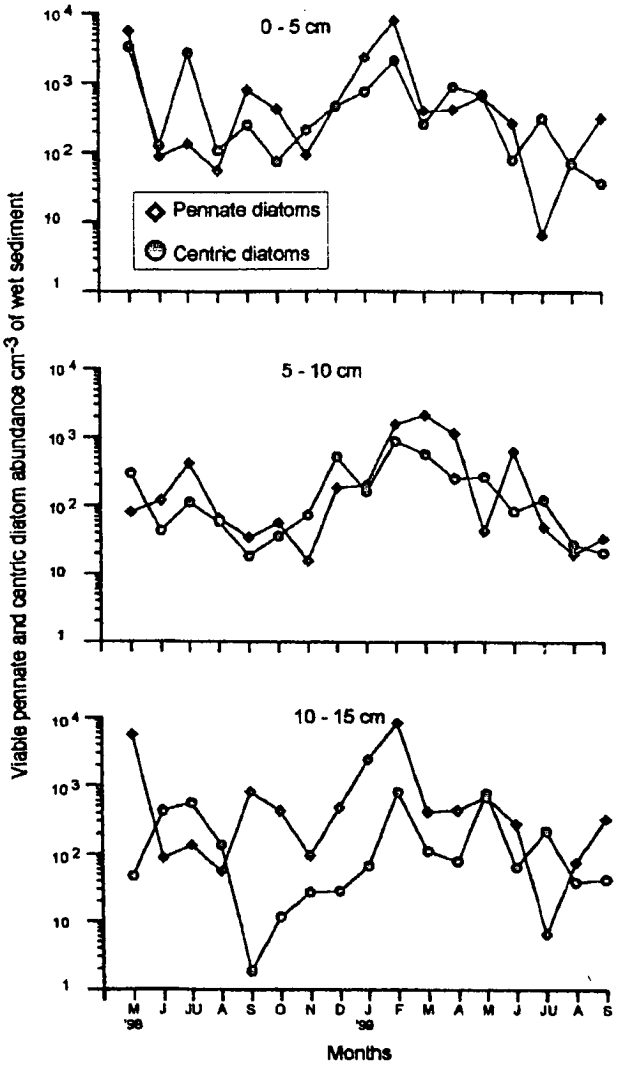
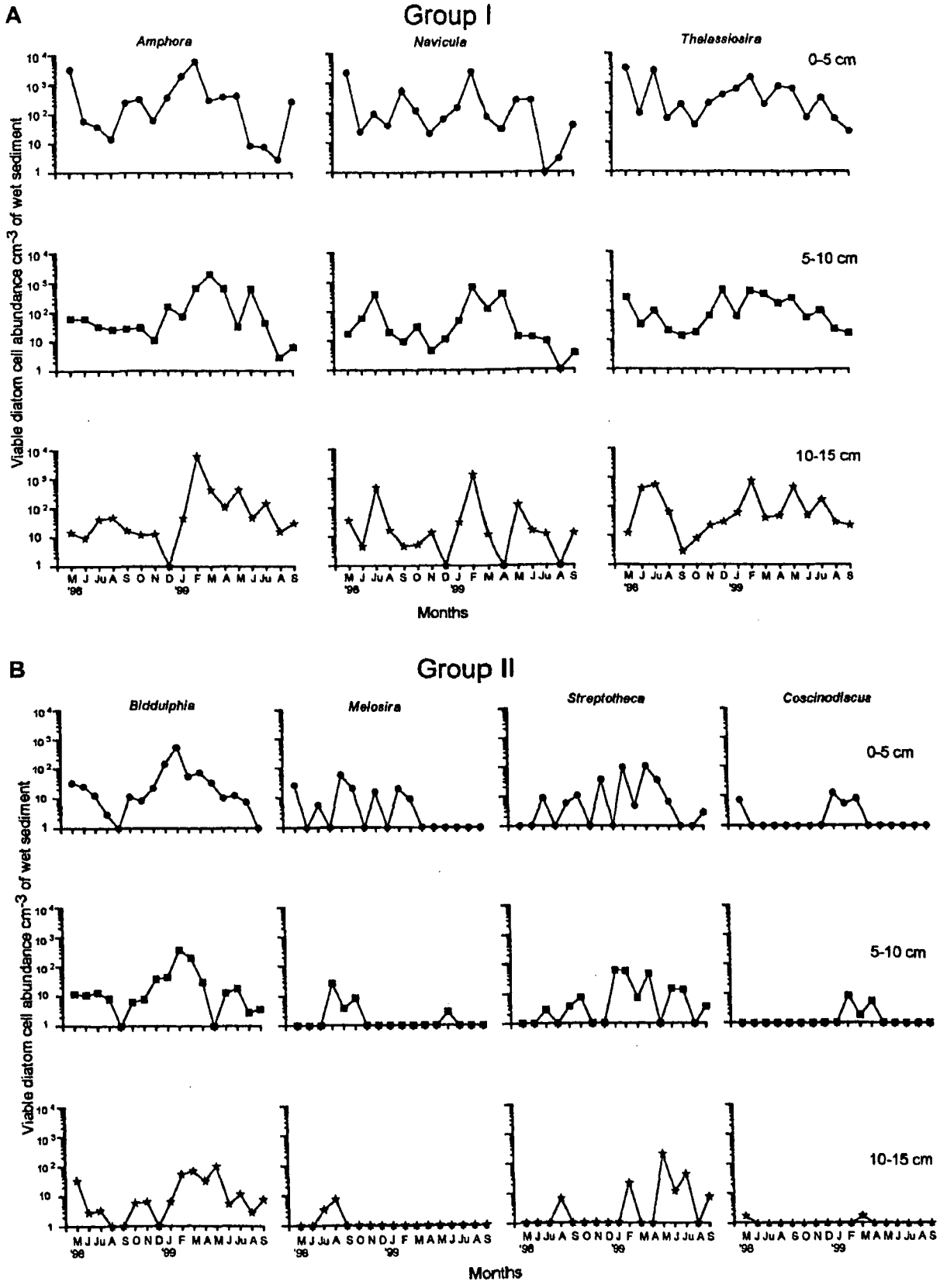


Fig. 5 Temporal variations in pennate and centric diatom abundance across the vertical gradient of the core

to 10^4 cells cm^{-3} wet sediment. The second sub-group (IB) included those forms present at particular depths up to an abundance of 10^2 cells cm^{-3} wet sediment. Group IIA comprised forms occurring rarely and with abundance below 10^2 cells cm^{-3} wet sediment at particular depths during the study period, and group IIB included forms such as *Cocconeis* (10–15 cm) and *Pleurosigma* (0–5 cm) with abundance ranging between 10^2 and 10^3 cells cm^{-3} wet sediment. The abundance of the rest of the forms belonging to this group was below 10^2 cells cm^{-3} wet sediment. Group III included the depths at which the respective forms occurred only once

►

Fig. 6 Temporal variations in diatom abundance across the vertical gradient of the core for group I (a) and group II (b, centric; c, pennate)


A
Group I
Amphora
Navicula
Thelassiosira
0-5 cm
5-10 cm
10-15 cm
Viable diatom cell abundance cm-3 of wet sediment
M J Ju A S O N D J F M A M J Ju A S
'98
'99
Months
B
Group II
Biddulphia
Melosira
Streptotheca
Coscinodiscus
0-5 cm
5-10 cm
10-15 cm
Viable diatom cell abundance cm-3 of wet sediment
M J Ju A S O N D J F M A M J Ju A S
'98
'99
Months

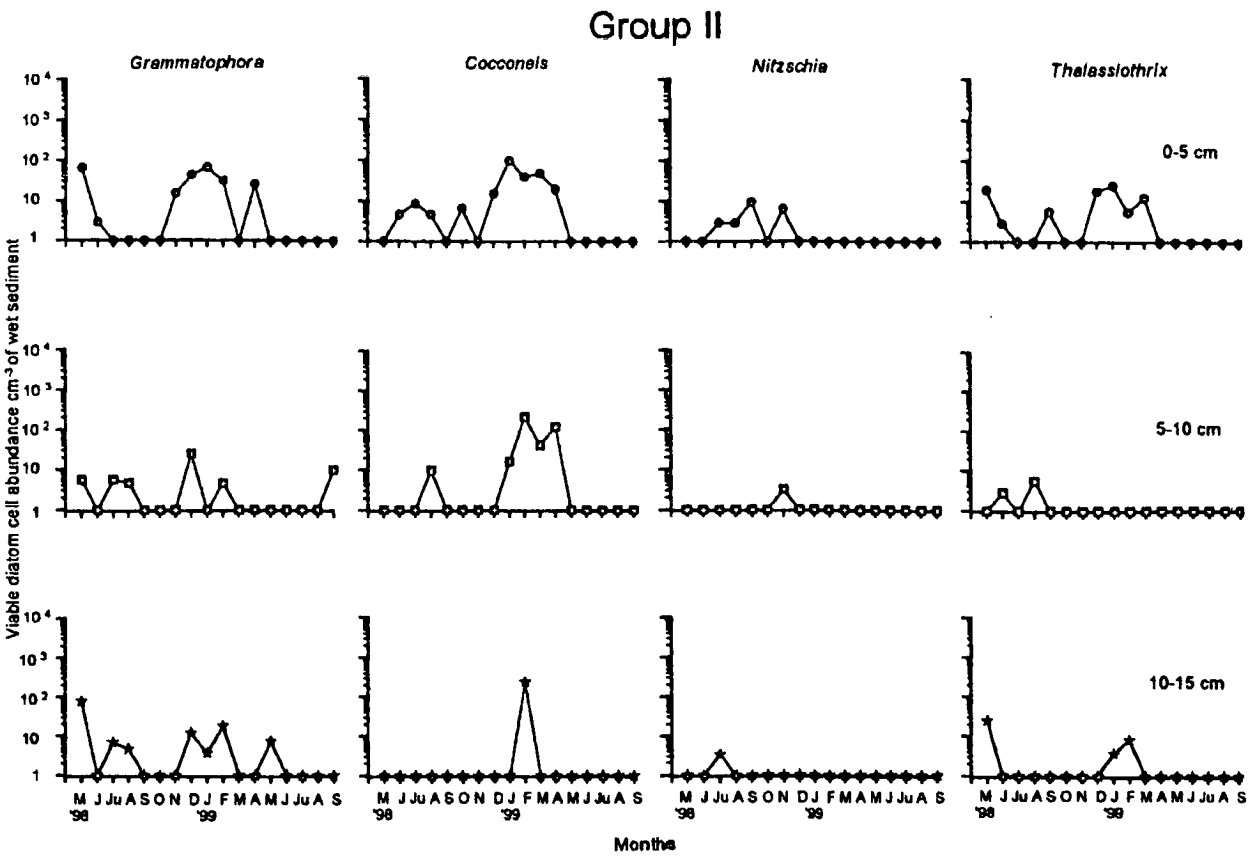


Fig. 6 (Continued)

or twice during the study period. Forms with an abundance ranging between 1 and 10 cells cm^{-3} wet sediment clustered to form group IV. Group V represented the forms, at particular depths, that were absent throughout the study period. The diatom distribution portrayed a similar pattern as that of the cluster analysis when subjected to two-dimensional NMDS ordinations (Fig. 9b).

Relationship with observed interstitial water parameters

Multiple regression was performed on the nutrient (nitrogen, phosphorus, silicate), temperature and salinity values (obtained for the entire 15 cm core) versus the total count of pennate and centric diatoms (0–15 cm). It was observed that, for pennates, which mainly reside in the sediments, nutrients did not play an important role, whereas, for centric diatoms, nitrate and phosphate positively influenced their abundance (Table 5).

Relationship with grain size

Distribution of different grain size fractions was found to differ with the different depths, as well as the different months (Table 1). However, subjection of this data to multiple regression analysis indicated that the grain size could serve as a predictor of only some of the pennate diatoms, e.g. *Amphora*, *Grammatophora*, *Pleurosigma* and *Thalassiothrix* (Table 6). Amongst these, the 250 μm grain size fraction served as a predictor of the abundance of *Amphora* and *Grammatophora* at 0–5 cm depth. *Pleurosigma* was influenced by 125 and <63 μm grain size at 5–10 cm, whereas, for *Thalassiothrix*, the choice of substratum was the 250 μm grain size at the same depth. However, a similar substratum preference of these diatoms at the other depths was not evident. *Navicula*, which was abundant throughout the year was negatively influenced by the 1,000 μm grain size fraction at 0–5 cm. However, the percentage composition of this grain size was negligible. *Fragilariopsis* was negatively influenced by 63 μm grain size at the lowermost depth (10–15 cm).

Wind speed and suspended load

Wind speed in the study area ranged from 2.0 to 4.7 $m\,s^{-1}$. Regression analysis between suspended matter and wind speed was statistically significant. Chlorophyll *a* concentrations and pennate diatom abundance in the 0–5 cm stratum revealed a significant negative correlation between wind speed and suspended load (Fig. 10).

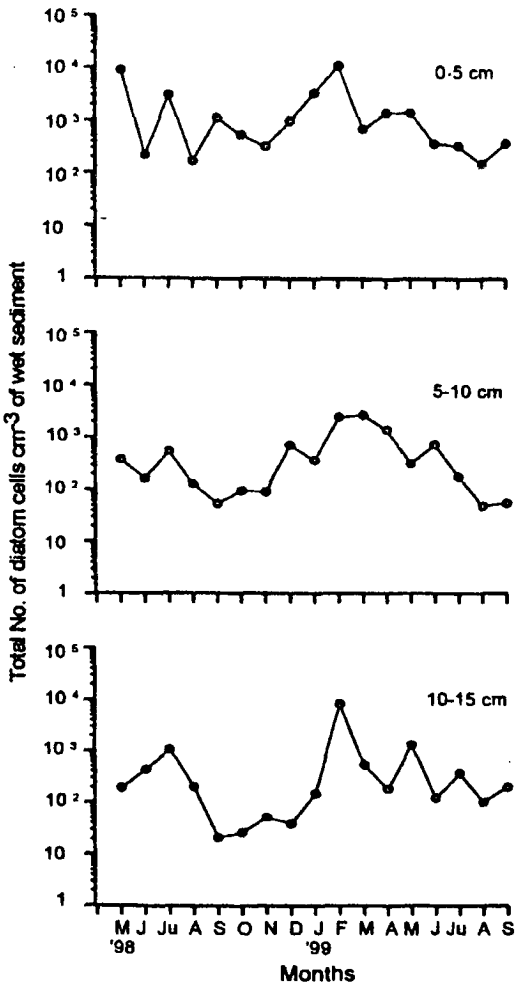


Fig. 7 Temporal variations in total diatom abundance across the vertical gradient of the core

Chlorophyll *a* and diatoms

During monsoons, chlorophyll *a* concentrations were lower irrespective of depth; the highest concentration in this season was observed in the 10–15 cm stratum. During late post-monsoon and pre-monsoon seasons, the 0–5 cm stratum had higher concentrations of chlorophyll *a*; these peaked during December and March (Fig. 11). Regression analysis revealed that the chlorophyll *a* concentrations at 0–5 and 5–10 cm depths were good predictors of pennate as well as centric diatoms, whereas, for the third depth (10–15 cm), this was not the case (Fig. 12). Although the regression analysis showed a negative correlation of diatom abundance with chlorophyll *a* at 10–15 cm depth, the appearance of diatoms in the culture wells provided with growth media and light (prerequisite factors for photosynthesis) confirms the presence of functional chlorophyll *a* at this depth.

Discussion

The sediment-dwelling diatoms form a major component of the microphytobenthic community in the intertidal region (Meadows and Anderson 1968; Round 1979b). This diatom community is generally classified as either epipsammon (particle-attached diatoms) or epipelon (free-living diatoms). However, a third group of diatoms encountered in surf water, i.e. phytoplankton, may also be incorporated into this diatom community (MacIntyre et al. 1996), some of whose members form a permanent component of this community throughout the year. So far investigations on diatoms of the microphytobenthic community have been focussed on epipsammic and epipelic pennate diatoms (Fenchel and Staarup 1971; Admiraal et al. 1982; de Jonge 1985). Steele and Baird (1968) have reported that the distribution of these epipsammic and epipelic autotrophic flora may extend to a depth of 20 cm. Observation under microscope usually reveals

Table 3 Significance values of the two-way ANOVA evaluating the variations in diatom abundance with respect to months and core sections (0–15 cm) (*NS* not significant). For individual comparisons by genus see electronic supplementary materials at http://dx.doi.org/10.1007/s002270100686

Diatoms in the intertidal sediment	Month $P \leq$	Core section $P \leq$	Month×Core section $P \leq$
Navicula	0.001	0.005	0.05
Amphora	0.001	0.005	0.001
Grammatophora	0.001	0.005	0.001
Pleurosigma	0.001	0.025	0.001
Nitzschia	0.001	0.01	0.001
Thalassiothrix	0.001	0.001	0.001
Cocconeis	0.001	0.001	0.001
Fragilariopsis	0.001	0.005	0.001
Bellerochea	0.001	0.001	0.001
Thalassiosira	0.001	0.001	0.005
Biddulphia	0.001	0.001	0.001
Coscinodiscus	0.001	0.005	0.001
Melosira	0.001	0.001	0.001
Skeletonema	0.001	NS	NS
Streptotheca	0.001	NS	0.001
Chaetoceros	0.001	0.005	0.001

Table 4 Two-way ANOVA for diatom diversity, generic richness and evenness of the sediment, with respect to months and core section (*$P \leq 0.001$; **$P \leq 0.05$; ***$P \leq 0.001$)

	df	SS	MS	*F*
Diversity				
Months	16	23.58	1.47	5.28***
Core section	2	3.29	1.64	5.88**
MonthsxCore section	32	24.15	0.75	2.7***
Within subgroup error	102	28.5	0.28	
Total	152	79.52		
Generic richness				
Months	16	21.04	1.31	11.41***
Core section	2	6.15	3.07	26.7***
MonthsxCore section	32	24.6	0.77	6.67***
Within subgroup error	102	11.76	0.11	
Total	152	63.54		
Evenness				
Months	16	1.39	0.087	2.81***
Core section	2	0.3	0.15	4.81*
MonthsxCore section	32	2.07	0.065	2.10**
Within subgroup error	102	3.15	0.03	
Total	152	6.9		

either quite clean-looking grains or grains with a film of mucilage in which bacteria and organic particles are prolific (Round 1981). Due to the difficulties of microscopic observation, the labor involved and the time required, findings have been based on either chlorophyll estimation (Steele and Baird 1968) or epifluorescence microscopic techniques (Grontved 1960; Admiraal et al. 1982; Baillie 1986; Delgado 1989; Cheng et al. 1993). Another widely used method of harvesting diatom cells by using lens tissue is not purposeful here, since it harvests only the motile pennate diatoms without providing any information on the

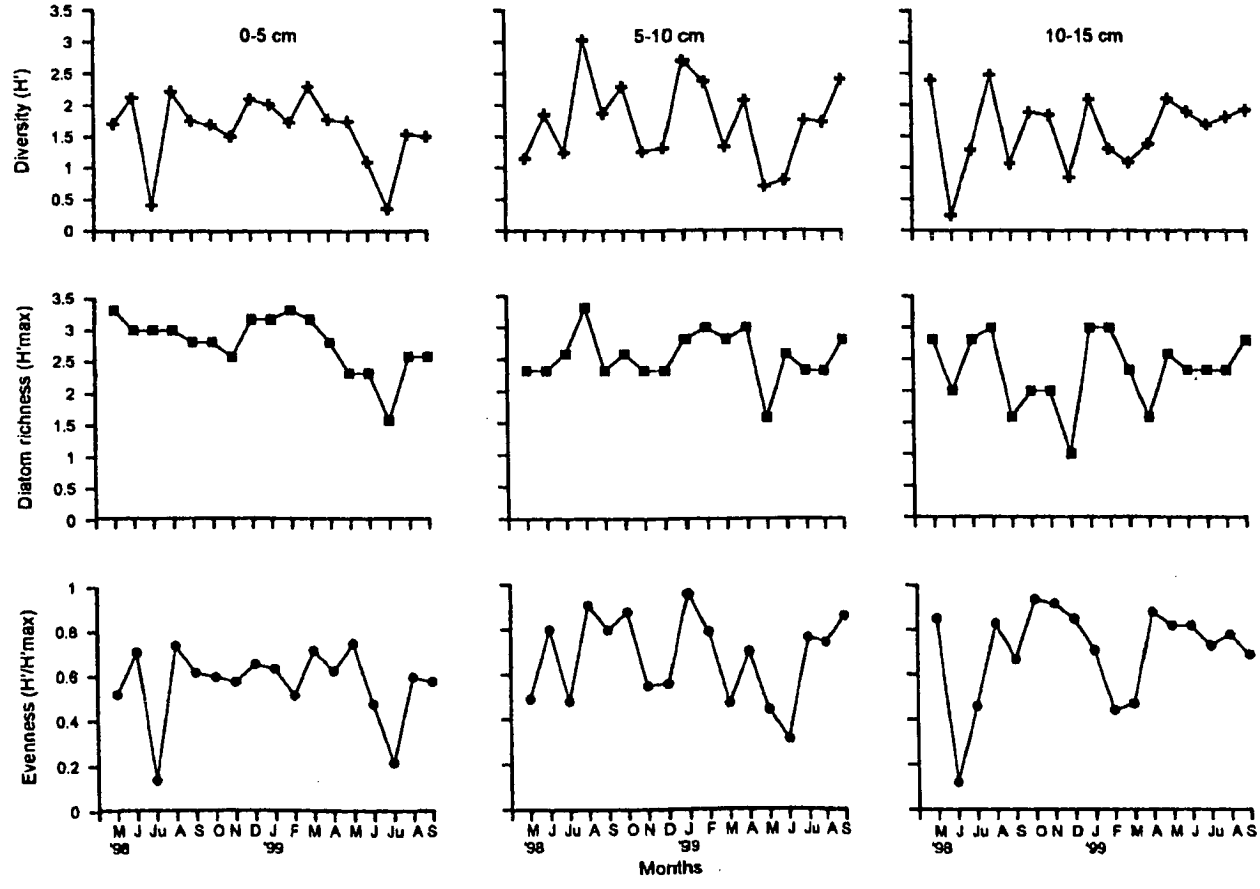


Fig. 8 Diversity (H'), diatom richness (H'_{max}) and evenness (H'/H'_{max}) across the vertical gradient of the core

sub-surface diatom population (Round and Palmer 1966). The ludox-TM density separation method described by de Jonge (1979) gives quantitative results but is laborious. The MPN method employed in this investigation includes incubation of the sample, which allows the diatoms to grow and multiply and thus enables easy observation (Imai et al. 1984, 1990; Yamochi 1989; Ishikawa and Tamaguchi 1994; Itakura et al. 1997). Although this method does not give the exact number of diatom cells, since it is based on the presence or absence of the diatoms (both vegetative and resting), it qualitates the relative abundance of different taxa. In the present study, *f*/2 medium was employed for the growth of diatoms, as it has been extensively used in the culture of phytoplankton (French and Hargraves 1980; Eilertsen et al 1995). However, differences in experimental conditions and the composition of the medium could result in variations that need to be evaluated in detail.

The appearance of diatoms down to a depth of 15 cm revealed that their viability was not affected by the conditions prevailing at this depth. It was observed that this depth harbored, not only pennate diatoms, some of which are permanent residents of this area, but also centric forms, which are usually found in the plankton. The occurrence of group I diatoms in the sediment throughout the year indicated that the two pennate forms were natives of this area. The centric diatom *Thalassiosira*, which was present in surf water but whose vegetative cells were not observed in the sediment, appeared after incubation; this must have occurred through germination of resting stages, which are regularly brought in with coastal sediment and redeposited in the intertidal sediment. Most of the pennate diatoms such as *Navicula* and *Amphora*, which are primarily encountered in bottom sediments, are also often found in the water column (de Jonge and van Beusekom 1992; Tomas 1997) through resuspension, and it seems reasonable to assume that their primary production in the water column is as effective as it is on the tidal flats (de Jonge and van Beusekom 1995). Their presence in the water column is also reflected in the microfouling population of different types of substrata immersed in the sub-surface estuarine waters of the present study area (Smita et al. 1997).

The abundance, diversity, richness and evenness of diatom cells showed spatial and temporal variation (Tables 3, 4; Figs. 6a–c, 8). It was also observed from the dendrogram and ordination plot that, depending on their occurrence and abundance, the diatom genera in the three core sections clustered into five groups. A number of physical, biological and chemical factors may be responsible for the temporal and spatial variation in diatom abundance, diversity, richness and evenness.

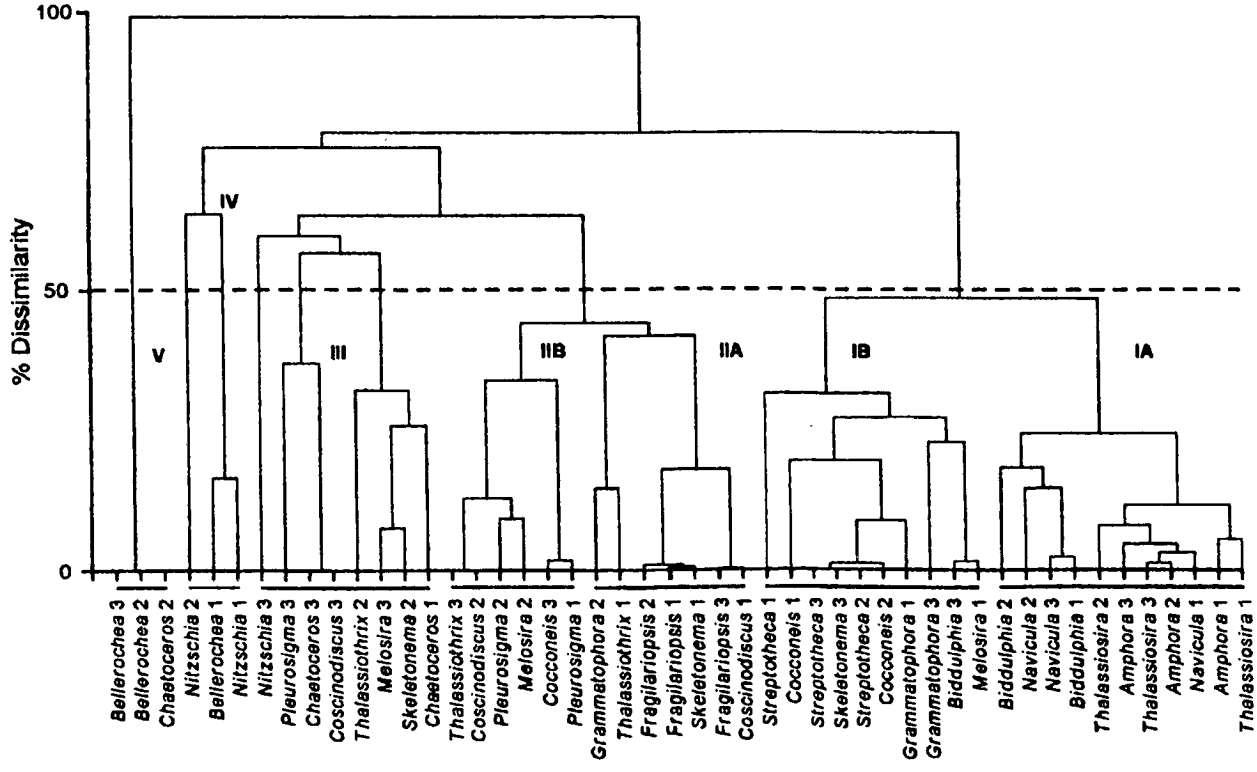


Fig. 9 **a** Cluster dendrogram of diatoms across the vertical gradient of the core using the Bray–Curtis coefficient and group average method. **b** Multidimensional scaling ordination based on the Bray–Curtis dissimilarity coefficient of the diatom community across the three vertical sections

Physical factors

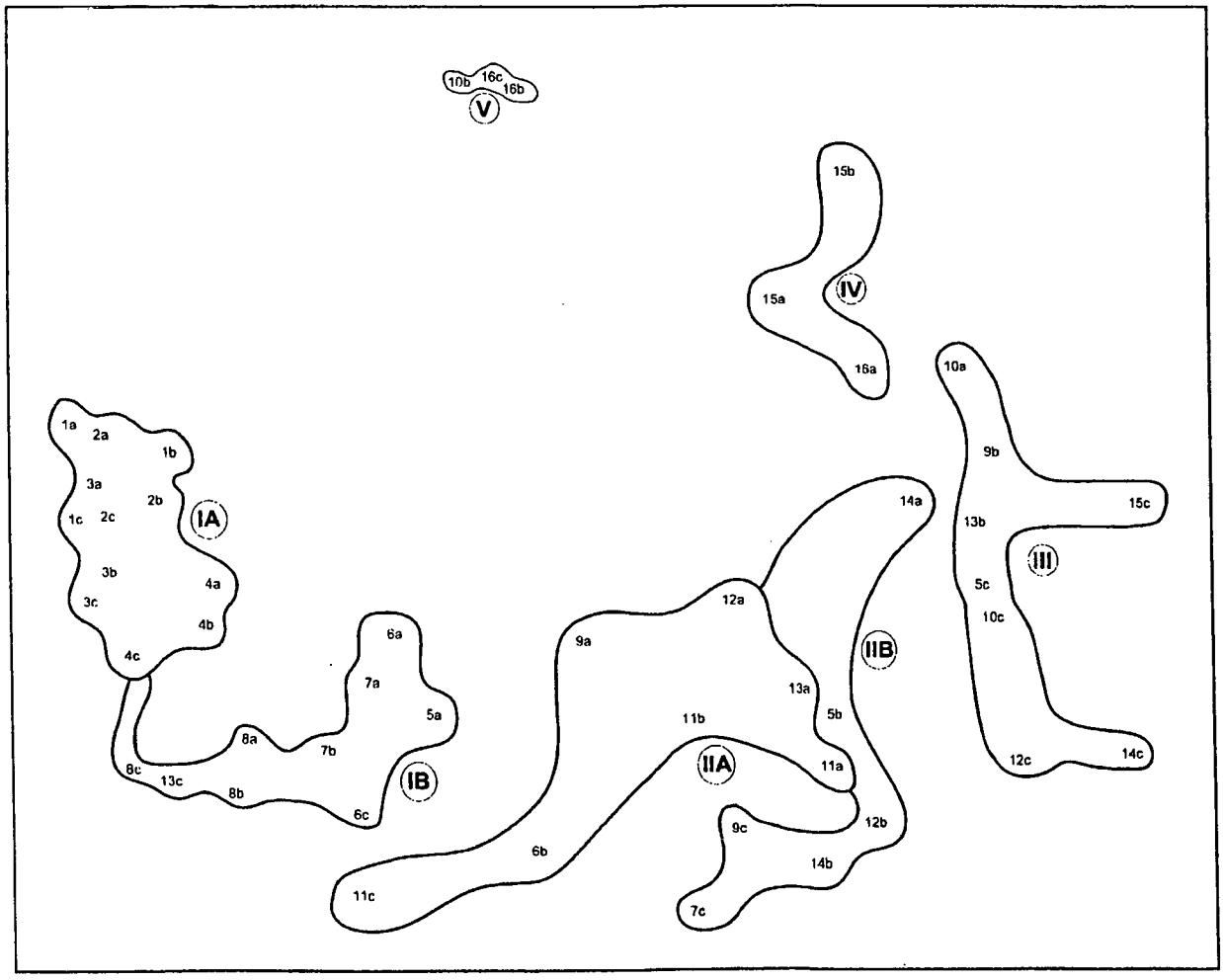
Wind speed and suspended load

Gabrielson and Lukatelich (1985) and de Jonge (1995) showed that chlorophyll *a* increase in the water column was related to resuspension. Resuspension is dependent on the sediment composition of the tidal flats and wind speed (de Jonge 1995). In a study on the resuspension of microphytobenthos and mud, a statistically significant linear relationship was found between wind speed and the fraction of suspended matter < 55 μm in the water above tidal flats in the Ems estuary (de Jonge and van Beusekom 1992). The wind speed ranged between 2 and 14 m s^{-1} in the Ems estuary (de Jonge 1995). This is much higher than the speeds encountered in the present study area, which range from 2.0 to 4.7 m s^{-1}. However, even at such a comparatively low wind speed, a significant relationship was observed between wind speed and suspended load in the 0–5 cm sediment stratum, suggesting that wind speed contributes to resuspension of the sediment. Also, the relation of both wind speed and suspended load with chlorophyll *a* and relative abundance of pennate diatoms in the 0–5 cm stratum was observed to be negative (Fig. 10). This shows that wind stimulates resuspension of the sediment along with pennate diatoms and, thus, chlorophyll *a* in the 0–5 cm stratum.

Grain size

The dynamic habitat of the sandy shore encompasses grains of different size groups, and their proportions were found to alter with month and depth (Fig. 2; Table 1). Across the three core sections, the percentage of the < 63 μm grain size fraction peaked towards the end of the monsoon season (September–October) and during February–April (pre-monsoon). During

Fig. 9 (Continued)



1. *Thalassiosira*; 2. *Amphora*; 3. *Navicula*; 4. *Biddulphia*; 5. *Melosira*; 6. *Grammatophora*; 7. *Cocconeis*; 8. *Streptotheca*; 9. *Thalassiothrix*; 10. *Chaetoceros*; 11. *Fragilaria*; 12. *Coscinodiscus*; 13. *Skeletonema*; 14. *Pleurosigma*; 15. *Nitzschia*; 16. *Bellerochea*

Table 5 Partial regression coefficients (Beta values) for the dependent variable (viable diatoms cm^{-3} wet sediment) and independent variable (water parameters)

Diatoms	Intercept	$R >$	Temperature (°C)	Salinity (psu)	Nitrate (NO_3^-)	Nitrite (NO_2^-)	Phosphate (PO_4^-)	Silicate (SiO_3^-)
Pennate	1.12	0.8	0.186	0.135	0.086	0.057	0.41	-0.84*
Centric	-2.55	0.87	0.307	0.212	0.69*	-0.18	0.83*	-0.8*

Table 6 Partial regression coefficients (Beta values) for the dependent variable (viable diatoms cm^{-3} wet sediment) and independent variable (grain size) (*I* 0-5 cm; *II* 5-10 cm; *III* 10-15 cm) (*$P \leq 0.05$; **$P \leq 0.005$; ***$P \leq 0.001$)

Depth	Diatoms	Intercept	*R*	1,000	500	250	125	63	< 63
I	*Navicula*	-13.7	0.84	-1.02*	0.45	0.38	1.71	1.34	2.97
	Amphora	9.74	0.88	-0.29	-0.28	0.68*	-0.29	-0.99	-0.43
	Grammatophora	-4.48	0.71	-0.36	-0.21	0.96*	0.42	0.49	1.11
II	*Pleurosigma*	-0.025	0.74	0.222	-0.42	-0.154	0.87*	-0.456	0.681*
	Thalassiothrix	-0.004	0.87	-0.028	-0.23	0.8***	-0.39	0.28	-0.19
III	*Fragilariopsis*	2.07	0.85	-0.11	0.074	-0.253	-0.235	-0.668**	-0.082

these periods, a simultaneous decrease in the percentage of the 63–124 μm grain size fraction was observed, compared to the other months. It was observed that although a particular grain size at one depth served as a predictor of some diatoms such as *Amphora*, *Grammatophora*, *Thalassiothrix* and *Pleurosigma*, a similar observation was not evident at other depths (Table 6). This indicates that factors other than grain size play a role in the temporal and vertical distribution of diatoms.

Suspension and deposition

Diatoms such as *Amphora*, *Navicula* and *Thalassiosira*, which were dominant in the surface sediment layers, were also the dominant diatoms throughout the 15 cm core; this included both pennate and centric diatoms (Table 2). In sandy sediments, light irradiance can decrease to 1% at a depth of 2–3 mm (Rasmussen et al. 1983). Therefore, the dominance of the above-men-

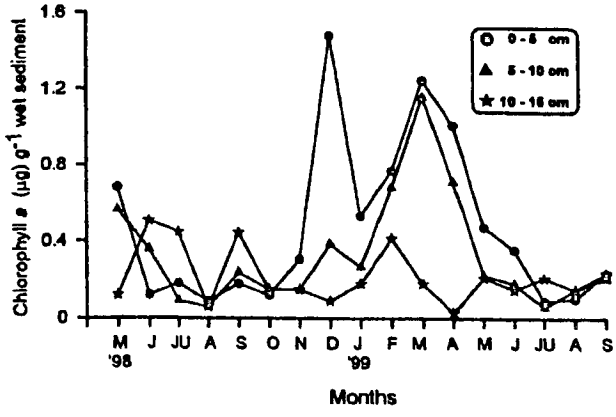
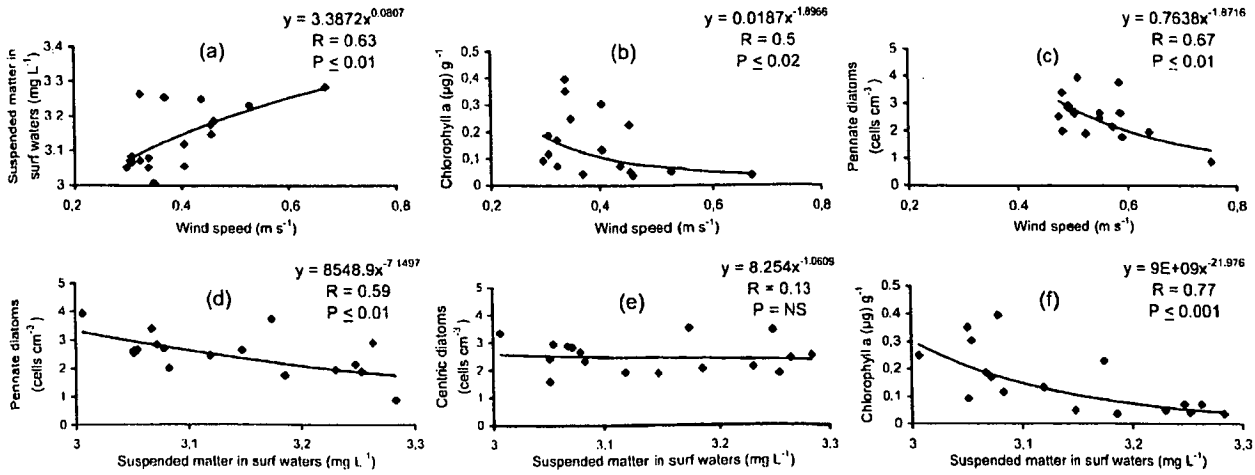


Fig. 11 Concentrations of chlorophyll *a* across the three vertical sections of the sediment core

Fig 10 Linear regression between $\log(x+1)$-transformed values of wind speed and suspended matter (**a**), chlorophyll *a* (μg g^{-1} wet sediment) (**b**) and pennate diatom abundance (**c**); and between suspended matter and pennate diatom abundance (cells cm^{-3} wet sediment) (**d**), centric diatom abundance (cells cm^{-3} wet sediment) (**e**) and chlorophyll *a* (μg g^{-1} wet sediment) (**f**) (*NS* not significant)



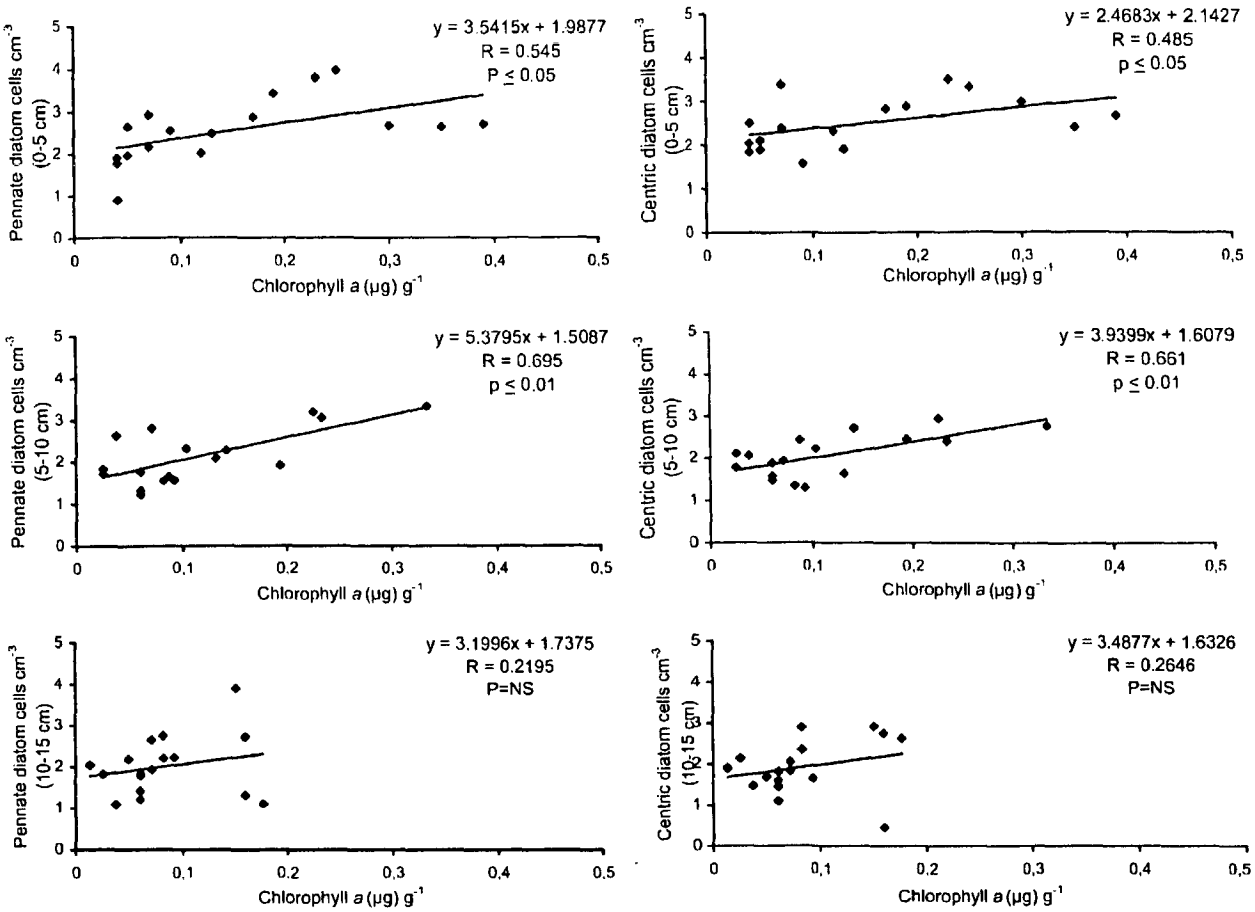


Fig. 12 Linear regression between $\log(x+1)$-transformed values of chlorophyll *a* (µg g^{-1} wet sediment) and pennate and centric diatom abundance (cells cm^{-3} wet sediment) across the three vertical sections of the core (*NS* not significant)

tioned species beyond the photic zone cannot be attributed to cell division. Moreover, since centric diatoms do not possess the ability to move on their own, a migratory mode of transport can also be ruled out for these forms. Hydrodynamic processes such as the waves are responsible for deposition and resuspension of the sediment (Baillie and Welsh 1980; Delgado et al. 1991; de Jonge and van Beusekom 1992, 1995; de Jonge and van den Bergs 1987; de Jonge 1992). The churning of water caused by these processes leads to the suspension of both the diatom-laden sediment particles and the free-living diatom cells in the surface waters. After redistribution, these suspended particles later are again deposited on or within the sediment. During this process, an exchange of diatom cells can take place between the surface and deeper layers of the sediment.

Advection

Advective transport of the diatom cells can occur through the sediment column via percolation of surface water, when the sand is loosened during flood tide. The degree of permeability of the sediment depends on the grain size, being higher for sand than for silty sand (Rusch and Huettel 2000). This vector will be more effective in the case of planktonic diatoms, present in the overlying waters or left behind on the sediment surface by the receding tides (Huettel and Rusch 2000). In the case of pennate diatoms, only those detached will be affected. Although all the forms dominant in surface layers were found throughout the 15 cm core in this investigation, the centric diatom *Thalassiosira* dominated the entire community down to 15 cm (Fig. 9a, b). It can be assumed that in sandy sediments, percolation due to oscillatory wave motion (Reid and Kajiura 1957) will cause water exchange to a sediment depth of at least 20 cm (Steele and Munro 1970). Thus, this vector may act as a selective carrier for loosely attached or the free-living diatom cells, especially the centric forms.

Biological factors

Grazing

Since diatoms are the major autotrophic organisms of this region, they also form the major food source for

consumers, e.g. for the meio- and macrobenthos. An earlier study revealed that macrobenthic organisms are higher in the food chain than meiobenthic and that they are represented by more detritus feeders than suspension feeders [amphipods (40.73%) and polychaetes (32.52%) (Rodrigues 1984)]. It was also observed that the energy increase caused by the wave action and currents at Dona Paula beach is initiated during the pre-monsoon and peaks during the monsoon period, resulting in erosion of the beach (Rodrigues 1984). This results in an increase in the population of these grazers during the post-monsoon and a decrease during the monsoon and pre-monsoon seasons, which may be one of the factors responsible for the higher abundance of group I and group II diatoms during the pre-monsoon period.

Vertical migration

In a dynamic environment, such as the intertidal region, permanent residents will be adapted to the changing environmental conditions and possess mechanisms to overcome difficult situations. The free-living epipelic pennate diatoms are known to exhibit migratory behavior, whereby they move in the sediment, away from or towards light, the former when the tide rises and the latter when the tide recedes. Migration is said to occur in order: (1) to escape bright light conditions and reach a place where the conditions are feasible for primary production, (2) to reduce predation by surface feeders and (3) to avoid transport due to resuspension by the advancing tide (Faure-Fremiet 1951; Ganapati et al. 1959; Palmer and Round 1965; Round 1979a; Heckman 1985). In this investigation, two major pennate diatoms (*Navicula* and *Amphora*) were observed to 15 cm sediment depth (Fig. 6a). Vertical migratory behavior in different sediment types has been related to physical factors such as tidal rhythms and light intensity (Hopkins 1966; Round and Palmer 1966). But many of these field studies were based on a millimeter scale (Hopkins 1963), except one, which reported four diatom species migrating down to 1 cm (Joint et al. 1982). However, work conducted in shallow, outdoor tidal tanks equipped with wave generators showed that vertical migration of the diatom *Hantzschia virgata* can extend beyond 8 cm during high tide (Kingston 1999). Hence, such migratory behavior of these diatoms can result in the transportation of surface diatoms to deeper layers, much beyond the photic zone. However, this self-initiated mode of transportation is only possible for epipelic pennate diatoms, and hence cannot be the only factor responsible for diatom transport.

Bioturbation

During the processes of feeding, defecation and locomotion, deposit feeders such as polychaetes, bivalves, amphipods, gastropods and crustaceans continuously rework the sediment. Burrowing organisms such as polychaetes (e.g. *Scoloplus kerguelensis*) and amphipods (e.g. *Talorchestia*) dominate the macrofauna of this area (Rodrigues 1984). Many field and laboratory studies have demonstrated that continuous sediment reworking by burrowing animals generally stimulates sediment transportation, carrying along with it the attached and adhered microflora and thus acting as another biological factor in the regulation of species diversity and benthic community structure (Pamatmat 1968; Lukatelich and McComb 1986; de Jonge and van den Bergs 1987). The fact that diatoms were observed to 15 cm depth indicates that burrowing organisms play an active role even at this depth. However, in the present investigation, even though diatom numbers did not reveal any particular trend in depth-wise distribution and were encountered down to 15 cm depth in appreciable numbers, the correlation between chlorophyll *a* and diatom numbers was found to be highest in the surface (0–5 cm) stratum, followed by the 5–10 cm core section and negligible at 10–15 cm depth. Hence, this indicates that even if bioturbation activity reached beyond the 10 cm zone, its effects must have been overridden by dormancy.

Chemical factors

Nutrients can play an important role in the distribution of some of the diatoms, especially during the late monsoon period, which is characterized by the renewal of nutrients to surface waters by upwelling and land runoff. *Fragilariopsis* and *Skeletonema* (group III) appeared in the intertidal sediment during August–September (monsoon). The centric diatom *Skeletonema* blooms during August–September in these waters (Smita et al. 1997) and is carried to the intertidal sediment by advective transport. *Fragilariopsis*, a pennate diatom occurring in chains, was found in high numbers during the same period, both in the water column and in the intertidal sediment. Since the subsiding bloom of *Skeletonema* undergoes resting stage formation and *Fragilariopsis* is a resident of the coastal sediment, carried by water masses to the intertidal sediment during bloom formation, these forms are not observed during rest of the year in either the intertidal sediment or the water column (Figs. 4, 7). The abundance of centric diatoms was found to be influenced by nitrate and phosphate, whereas in the case of pennates, which mainly reside in the sediments, nutrients did not play an important role (Table 5).

Survival strategies

Once the diatoms have reached a depth of 15 cm, either voluntarily or forcefully, they have to adapt to some life-saving strategies, at least until they are brought back to the surface by some mechanism. One of the possibilities for survival is that they adapt to spending long periods in the dark, at low metabolic rates and with negligible degradation of pigments (Steele and Baird 1968; French

and Hargraves 1980) and uptake of dissolved organic compounds (Admiraal and Peletier 1979).

Laboratory observations have shown that cells of *Hantzschia virgata* exhibit migration of > 8 cm during high tide (Kingston 1999). Taking this fact into consideration, it is obvious that in an area at the lowest low tide mark, the cells cannot migrate up and down during the short period of exposure at every tidal interval. If these vegetative cells are subjected to high sedimentation rates, their presence at such depths for a period beyond their tolerance limit will expose them to unfavorable conditions, such as darkness, under which photosynthesis will be hindered. Thus, the cell will either undergo transformation from the vegetative to a resting stage, triggered by the surrounding conditions in order to survive the unfavorable environmental condition(s), or face death and degradation. Experimental work has shown that permanent darkness and low nutrients may result in resting stage formation (Anderson 1975; Hargraves and French 1983).

Although chlorophyll *a* in itself is insufficient to describe fluctuations in benthic diatom standing stock (de Jonge 1980), this pigment provides a useful index for the photosynthesis potential of a population (McIntyre et al. 1996). A significant relation between diatom cell numbers and chlorophyll *a* to a depth of 10 cm revealed that both pennate and centric diatoms contributed to the total pool of chlorophyll *a* down to this depth (Fig. 12). In the 0–5 cm stratum, the ability of pennate diatoms to remain attached to the substrate may play an important role in this contribution, whereas centric diatoms may be brought to the sediments by incoming tides. In the 5–10 cm stratum, the positive correlation with chlorophyll *a* revealed that resuspension was not effective down to this depth and that the stock was securely placed. Although viable cells did occur at 15 cm, the reason for the lack of a relationship between chlorophyll *a* and diatom cell number at 10–15 cm depth could be either that there was no sufficient renewal of cells even when advection occurred or that the cells were in a dormant stage. However, in the latter case, they would remain viable as primary producers, and when they resurfaced could play an important role in the benthic community.

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