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Dedicated to my late father

Shri. Krishna P. Naik and sister Deepa

Contents

Statement

As required under the University ordinance 0.19.8 (vi), I state that the present thesis titled *"Studies on phytoplankton with reference to dinoflagellates"* 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.

(Ravidas K. Naik)

Certificate

This is to certify that the thesis entitled *"Studies on phytoplankton with reference to dinoflagellates"* submitted by **Mr. Ravidas K. Naik** for the award of the degree of Doctor of Philosophy in Marine Sciences is based on his original studies carried out by him 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 Referees have been incorporated.

10/6/2011

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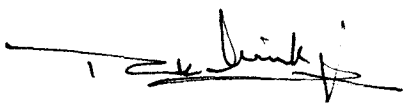


(Ravidas K. Naik)

Chapter 1

General introduction

Phytoplankton, the microscopic, floating, plant life of all fresh and marine water bodies, has a truly global distribution. In fact, they contribute over 25% of the total vegetation of the planet (Jeffrey and Hallegraeff 1990). The word 'phytoplankton' is derived from the Greek word phyton = plant, and planktons = wanderer. On July 27th 1676, in Netherlands, van Leeuwenhoeck was probably the first person to observe marine phytoplankton under the microscope. Some 200 years after this observation, Victor Hensen, a scientist from Germany, was convinced that these microscopic organisms were the base for the marine food chain (de Baar 1994). They include diatoms, dinoflagellates, coccolithophores, red algae, green algae and blue green algae (cyanobacteria), with sizes ranging from 0.2 μm to several millimeters. Based on size, phytoplankton can be classified into three classes: the microplankton (20-200 μm), nanoplankton (2-20 μm) and picoplankton (0.2-2 μm) (Table 1.1). Diatoms (Class Bacillariophyta) occur in all 3 size classes whereas dinoflagellates (Class Dinophyta) are represented in the micro- and nanoplankton groups (Jeffrey and Hallegraeff 1990).

Phytoplankton exhibit seasonal variation in abundance and type; they also vary from one water body to another, and even within a small region in the same water body. Classically, phytoplankton are recognized as the basis of all animal production in the open ocean, which fuels the food web upon which world fisheries are based. It is important to monitor these populations, since failure in abundance and timing of phytoplankton blooms can lead to collapse of fisheries (Lasker 1975). In the recent past, the importance of phytoplankton studies has moved beyond the context of

fisheries, to aspects like global warming and climate change. These studies have also extended to include the effects on human health due to Harmful Algal Blooms (HABs) (Fig. 1.1).

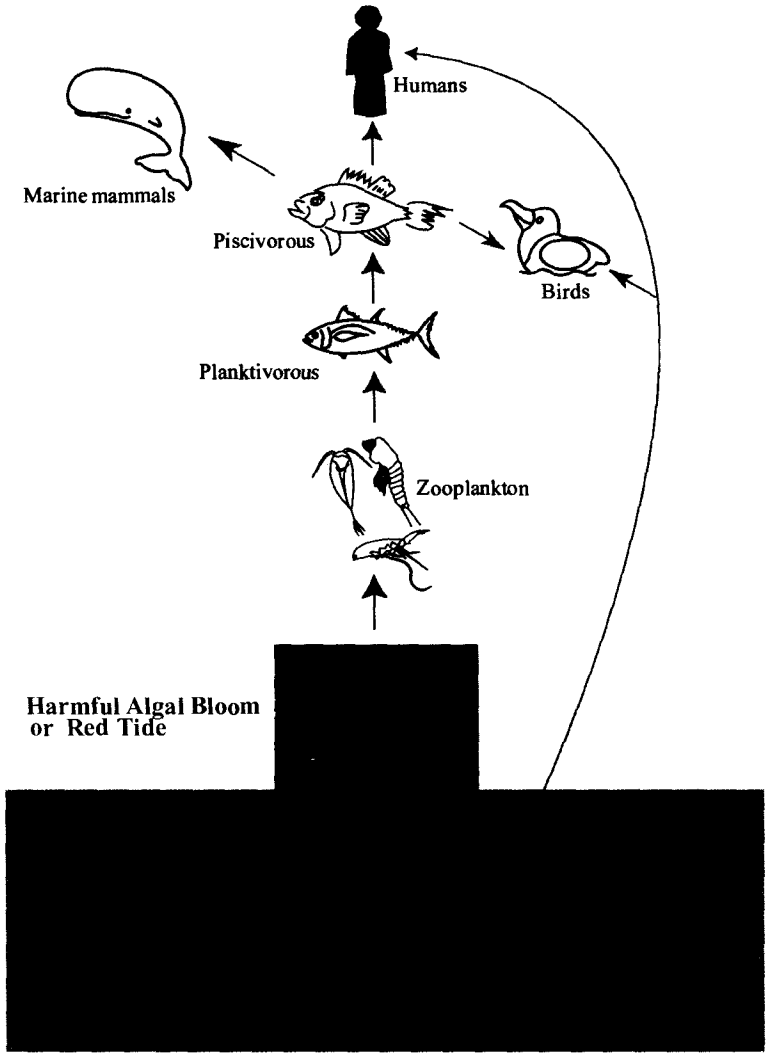


Fig. 1.1. The consequences of Harmful Algal Blooms (HABs) for the ecosystem and human health.

Among the total marine phytoplankton species, approximately 7% are capable of forming algal blooms (red tides) (Sournia 1995). Dinoflagellates are the most important contributors to HABs, accounting for 75% of the total HAB species (Smayda 1997). They form an important component of marine and freshwater phytoplankton. They consist of around 1,800 species, of which approximately 50 produce some sort of toxic compounds (Godhe 2002).

The biology of dinoflagellates is distinct from that of other phytoplankton groups. Dinoflagellates range in size from about 2-200 μm, although *Noctulica* can be up to 2 mm. The cell wall is complex and variable, consisting of layer of vesicles with or without cellulosic plates. If the plates are present, the cells are called thecate or armored (Fig. 1.2).

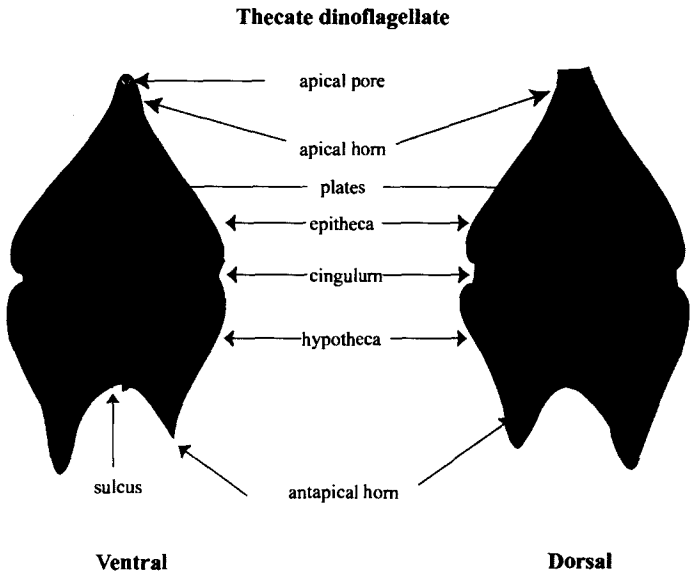


Fig. 1.2. Morphological details of thecate dinoflagellates.

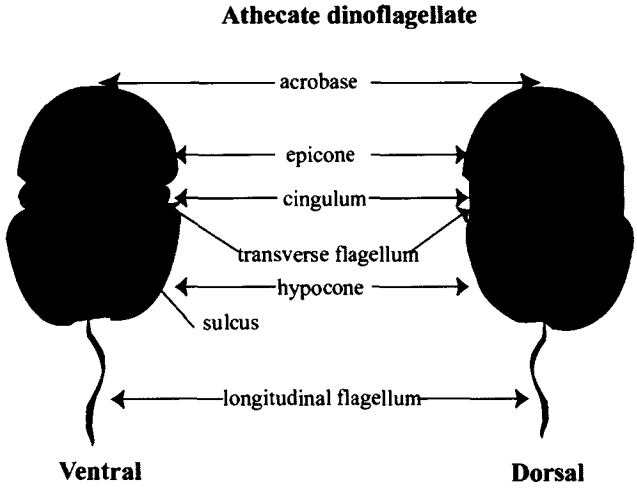


Fig. 1.3. Morphological details of athecate dinoflagellates.

Dinoflagellates without cellulosic plates are termed athecate, unarmored or naked dinoflagellates (Fig. 1.3). The shape and arrangement of plates varies depending on the genus. The theca may have horns, spines or lists and the plates may be ornamented with pores, depressions, spines, ridges and reticulations. Most dinoflagellate cells are divided into two parts by a horizontal groove or cingulum. The anterior part is the epitheca (epicone) and the posterior part is the hypotheca (hypocone). The two parts may be equal or unequal size and the cingulum placement helps define genera, especially in athecate forms. A vertical groove or sulcus splits the hypotheca and may extend onto the epitheca.

Dinoflagellates are biflagellate, motile cells with two dissimilar flagella. Depending on where the flagella arise from, they can be classified into 2 groups: desmokonts and dinokonts (Fig. 1.4).

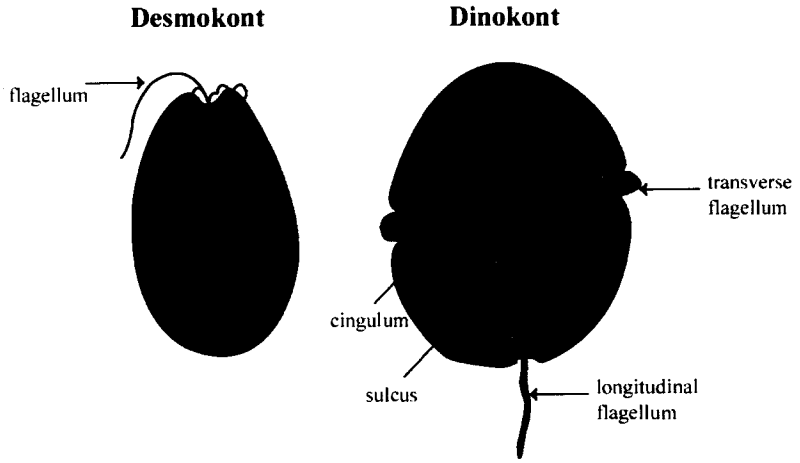


Fig. 1.4. Arrangement of flagella in desmokont and dinokont dinoflagellates.

In desmokonts, the flagella arise from the anterior part of the cell, as in *Prorocentrum.* In dinokonts, the flagella are inserted ventrally; the transverse flagellum is located in the cingulum whereas the longitudinal flagellum is situated in the sulcus, for e.g., *Protoperidinium.* The transverse flagellum allows the cell to move forward or backward by spinning in circles in order to propel it in either direction. The longitudinal flagellum acts mainly as a means of steering. It also provides extra pressure to help force the cell in the direction it wants to head.

Nutritional modes of dinoflagellates vary from autotrophy (in chloroplast-containing cells) to heterotrophy (cells lacking chloroplasts) with some autotrophic dinoflagellates now known to be mixotrophs. Heterotrophic forms can obtain nutrients through various ways, ranging from direct resorption (osmotrophy), ingestion of particulate food (phagotrophy) to pallium feeding (Schnepf and Elbrachter 1992). They may also have specialized structures, such as peduncles, used

for phagocytising other organisms (Schnepf and Elbrachter 1992). Food reserves in dinoflagellates are typically unsaturated fatty acids and starch (Dodge 1973).

Reproduction is either asexual or sexual. In asexual reproduction, haploid (1N) cells divide by binary division. In sexual reproduction, haploid gametes are produced which fuse to form a diploid (2N) zygote. The zygote is a non-motile, resting stage (hypnozygote), which settles to the bottom and remains dormant for some periods of time. The zygote undergoes meiosis to produce haploid vegetative cells. The hynozygote or resting cyst morphology is very different from that of vegetative cells.

Some dinoflagellates have been reported to form temporary cysts when cultured with certain species of diatoms (Uchida et al. 1996). This may be due to chemical stimulation or cell contact between dinoflagellates and diatoms (Uchida 2001). Chan et al. (1980) reported that filtrate extracts of the dinoflagellate, *Scrippsiella sweeneyae* was inhibitory to the diatoms – *Cylindrotheca fusiformis*, *Navicula pelliculosa* and *Nitzschia angularis*, indicating the importance of the interactions between dinoflagellates and diatoms. In addition, dinoflagellates are also affected by other factors, for e.g., allelopathy, predation and symbiosis.

Knowledge of the combined effects of different factors is essential to understand not only the spatio-temporal variations in dinoflagellate communities, but also phytoplankton communities in general. Such data represents decisive data in environmental monitoring, food-web studies and ecosystem modeling.

Phytoplankton diversity dynamics varies from coastal to oceanic environments based on the governing factors in the respective environments (Margalef 1978).

Generally, large phytoplankton tend to be abundant in turbulent, high nutrient, coastal waters, while smaller cells are prominent in stratified and/or oceanic waters (Chisholm 1992, Cullen et al. 2002). In this manner, coastal and oceanic environments represent varied environmental settings and thus support different communities. One of the processes which interlink these communities is offshore-seeding HAB-forming organisms. For example, transport of offshore-seeded *Prorocentrum* and *Ceratium* blooms to inshore waters (Pitcher and Boyd 1996). Thus, understanding phytoplankton community structure and its spatio-temporal variations in both - coastal and oceanic environments will provide the basis for interlinking studies.

In the context of the seas around India, several studies have illustrated the diversity and dynamics of phytoplankton communities (Subrahmanyan 1946, 1968; Devassy and Bhattathiri 1974, Taylor 1976, Desikachary and Prema 1987, Devassy and Goes 1988, Mitbavkar and Anil 2000, Saravanane et al. 2000, Habeebrehman et al. 2008, Patil and Anil 2008). Most of these studies were restricted to coastal dynamics of phytoplankton. However, very little is known about spatio-temporal variations in phytoplankton in the open ocean. In fact, most studies have focused on the micro-phytoplankton group (diatoms and dinoflagellates) in near-shore coastal waters. The contribution of smaller phytoplankton groups (pico- and nano-plankton) has been underestimated due to the limitations of routine microscopy.

In view of the above, the present study was carried out to analyze the spatio-temporal variations in phytoplankton communities in the open ocean using the 'ships-

of-opportunity' programme. Considering the limitations of microscopic methods, pigment analysis was included along with routine microscopy to quantify the contribution of smaller phytoplankton groups in oceanic waters. Efforts were also made to elucidate the characteristics of the phytoplankton communities in coastal habitats by evaluating two diverse habitats of Mormugao and Vishakhapatnam ports.

In the process of such evaluation, the effect of preservation on morphology of dinoflagellates and on their quantification was carried out with *Karlodinium veneficum,* a newly reported, non-thecate dinoflagellate from Indian waters. Subsequently, the influence of its culture filtrate and cell extracts on the growth of *Skeletonema costatum*, a key stone species in Indian waters (Patil and Anil 2008, D'Costa and Anil 2010) was evaluated.

The studies carried out are presented in the following chapters:

- *Spatio-temporal variation in surface water dinoflagellates in the Bay of Bengal*
- *Primary description of surface water phytoplankton pigment patterns in the Bay of Bengal*
- *Micro-phytoplankton community structure at Mormugao and Visakhapatnam ports*
- *Effect of preservation on the morphology of* Karlodinium veneficum, *a non-thecate, potentially harmful dinoflagellate and allelopathy in relation to* Skeletonema costatum

Chapter 2

Spatio-temporal variation in surface water dinoflagellates in the Bay of Bengal

2.1. Introduction

In the contemporary ocean, large scale estimates of phytoplankton biomass and its production is possible with remote sensing of ocean color monitoring. These studies indicate climatic-driven changes in phytoplankton biomass and production (Behrenfeld et al. 2006, Doney 2006). It is also important to understand the taxonomic and physiological components of these changes (Doney 2006). This points to the need for studies on spatial and temporal variation in phytoplankton community structure. The taxonomic database of phytoplankton will not only help to understand the above query, but also to understand Harmful Algal Bloom (HAB) related problems (Hallegraeff 2010).

HABs are natural phenomena; historical records indicate their occurrence long before the advent of human activities in coastal ecosystems. Recent surveys have demonstrated a dramatic increase and geographic spread in HAB events in the last few decades (Anderson 1989, Smayda 1990, Hallegraeff 1993). Thus, knowledge of the present geographic distribution and seasonal fluctuations in HAB species is important to understand globally spreading HAB events (GEOHAB 2001). In fact, Hallegraeff (2010) has reported that unpreparedness for such significant range expansions or spreading of HAB problems in poorly monitored areas will be one of the greatest problems for human society in the future.

Among the total marine phytoplankton species, approximately 7% are capable of forming algal blooms (red tides) (Sournia 1995). Dinoflagellates are the most important group producing toxic and HABs (Steidinger 1983 and 1993, Anderson

1989, Hallegraeff 1993) accounting for 75% of the total HAB species (Smayda 1997).

The causative factors for such blooms, results from a coupling mechanism involving physical, chemical and biological factors, whereas the role or mechanism of chemical and biological factors are now reasonably understood (Fistarol et al. 2004, Solé et al. 2006, GEOHAB 2006, Adolf et al. 2007, Waggett et al. 2008). However comparable understanding about physical factors is lacking except for few examples (Maclean 1989, Karl et al. 1997, Belgrano 1999, Yin et al. 1999). Impacts of these factors in combination with local inter-annual meteorological conditions will vary from one geographical location to the other and will thus influence bloom dynamics differentially.

HAB studies in Indian waters indicate reasonable reports on HABs and their impacts along the west coast of India. Direct impacts of HABs on human health have also been reported from Mangalore (Karunasagar et al. 1984). Dinoflagellate toxins have also been recorded in shellfish from surrounding estuaries near Mangalore in 1985 and 1986 (Segar et al. 1989). Planktonic and cyst forms of *Gymnodinium catenatum*, a PSP-producing dinoflagellate from this region were detected later on (Godhe et al. 1996) and the importance of close monitoring of coastal waters, sediment and shellfish was highlighted.

Compared to the above regions, the Bay of Bengal (BOB), the eastern arm of the Indian Ocean, remains relatively unexplored in the context of HAB studies. The BOB is known for its unique characteristic features: large volume of freshwater input from

river discharge and rainfall, warmer sea surface temperatures, monsoonal clouds and reversal of currents. The riverine input into this area injects loads of nutrients and suspended sediment in the BOB (Gordon et al. 2002, Mukhopadhyay et al. 2006). These features point to the suitability of the BOB as a zone prone for algal blooms including HAB events. However, the strongly stratified surface layer of the BOB restricts the transport of nutrients from deeper layers to the surface (Prasanna Kumar et al. 2002). Thus, it is very interesting to understand the seasonal variations in dinoflagellate community structure in this inimitable geographic region.

Therefore, the present study, the first of its kind from the region, was undertaken to elucidate the spatial and temporal distribution of dinoflagellates in the BOB, with emphasis on HAB species. To do so, taking oceanic cruises on regular intervals is not cost-effective. Thus, the 'ships-of-opportunity' programme, supported by the Indian Expendable Bathythermograph (XBT) project, was used in the present study. The following objectives were addressed. (1) Dinoflagellate distribution in the surface waters of the BOB and (2) Detailing of the HAB species present and their seasonal occurrence.

2.2. Materials and Methods

2.2.1. Study area and sampling

This study was conducted with the support of the XBT programme. Surface water samples were collected using steel buckets from the moving ship. This method was selected in order to minimize the physical damage to cells compared to the method of

collecting samples using a pump. A similar approach was used by Smetacek et al. (2002). The samples were collected on 2 transects [Chennai to Port Blair (CP, 12

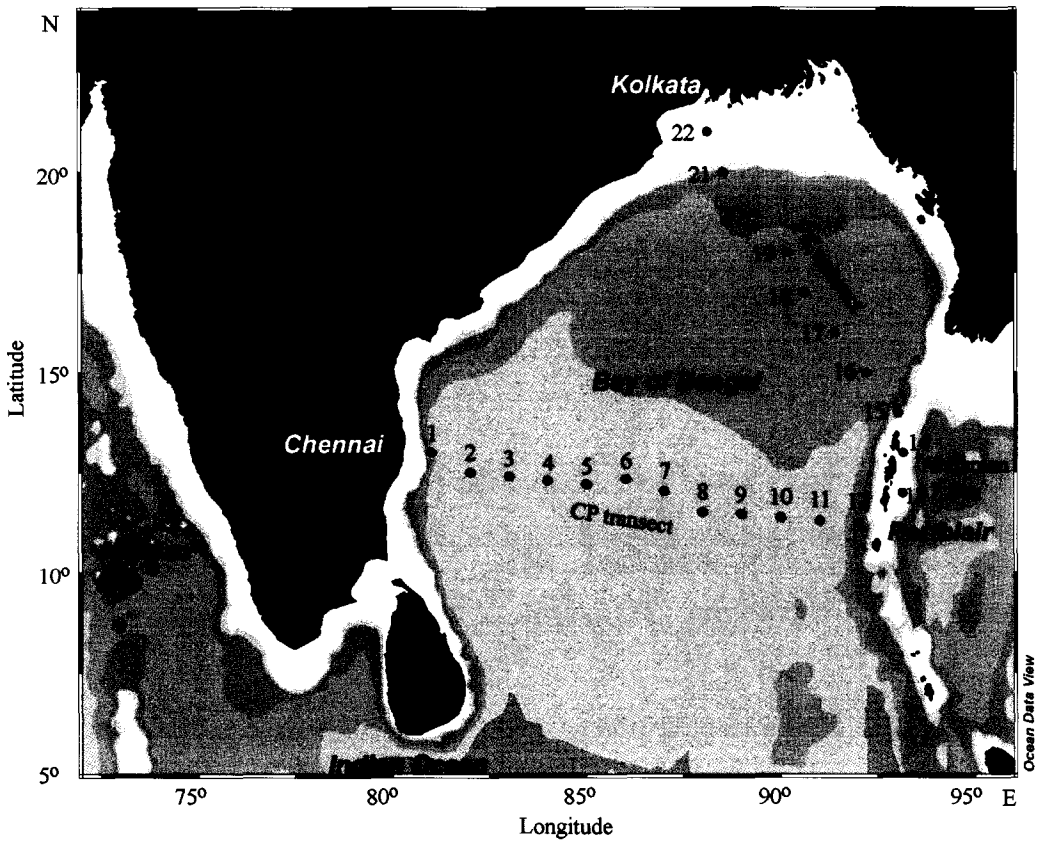


Fig. 2.1. Study area map showing station locations along the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects in the Bay of Bengal.

stations) and Port Blair to Kolkata (PK, 10 stations)] (Fig. 2.1), from passenger ships plying along these transects. Samples were collected at one degree intervals along both transects, from November 2003 to September 2006 covering different seasons. 1L of sample was fixed with Lugol's iodine solution for the microscopic enumeration and identification of dinoflagellates to the lowest possible taxonomic level.

2.2.2. Microscopic analysis

Samples (1L) were brought down to 10 mL final concentration by the settling and siphoning technique. Three mL of the concentrated sample was taken in a petri dish and examined under an Olympus inverted microscope at 100X to 400X magnification. Identification of the dinoflagellate taxa was carried out using the keys provided by Subrahmanyan (1968), Taylor (1976), Tomas (1997), Horner (2002) and Hallegraeff (2003).

2.2.3. Data analyses

To evaluate seasonal differences, the observational period was classified into three seasons: Pre-Monsoon (PrM – February-March 04), South West Monsoon (SWM – June 04, July 04, August 04, September 06) and Post-Monsoon (PoM – November 03, October 04).

Univariate measures [Shannon-Wiener diversity index (H'), Margalef's species richness (d) and Pielou's evenness (J')] were analyzed using PRIMER (version 5) and the variations in these were determined by two-way ANOVA. Two-way ANOVA was also performed on the log (x+1)-transformed dinoflagellate abundance data to evaluate spatial and temporal variation (Sokal and Rohlf 1981). Spatial variation in the dinoflagellate community and the abundance profiles of the five most dominant taxa during each sampling period in both transects are presented as SURFER plots using the SURFER 8 program. The percentage contribution of autotrophic,

mixotrophic and heterotrophic forms of dinoflagellates in each sampling period was also calculated and is presented as pie charts.

2.3. Results

2.3.1. Dinoflagellate assemblages

A total of 134 species of dinoflagellates were recorded in surface waters of the BOB during the observation period (Table 2.1). Dinoflagellate abundance ranged from 0 – 94 cells L^{-1} throughout the observation period at both CP and PK transects (Fig. 2.2). Further grouping of these identified species based on their nutritional mode, revealed 40 autotrophic, 50 mixotrophic and 44 heterotrophic species, indicated the dominance of mixotrophic forms (Table 2.1). Autotrophic dinoflagellates ranged from 0 – 58 cells L^{-1} (Fig. 2.3), mixotrophic dinoflagellates from 0 – 48 cells L^{-1} (Fig. 2.4) and heterotrophic dinoflagellates from 0 – 60 cells L^{-1} (Fig. 2.5), in both transects.

2.3.2. Spatial and temporal variation in dinoflagellate assemblages

Overall, the dinoflagellate assemblages did not show any significant spatial variation in abundance, species evenness, richness and diversity measures. The only exceptions were species richness and diversity in the PK transect (Table 2.2). Significant seasonal variation in dinoflagellate abundance was observed in the CP transect. Species richness and diversity varied significantly in the PK transect;

Table 2.1. Dinoflagellates observed during the study period along the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects (* potential HAB species, † new reporting). The values outside the bracket indicate the abundance range and values inside the bracket indicate the frequency of occurrence at CP (12 stations) and PK (10 stations) transects.

Dinoflagellates	CP transect					PK transect				
	Feb 04	Jun 04	Jul 04	Oct 04	Sep 06	Nov 03	Mar 04	Aug 04	Oct 04	Sep 06
Autotrophic										
Alexandrium concavum		0-2 (1)								
Alexandrium spp.			0-2 (2)		0-9 (1)			0-2 (1)	0-20 (1)	0-6 (1)
Amphidinium sp.*	0-7 (5)	0-2 (1)	0-2 (1)	0-5 (3)	0-12 (10)			0-8 (2)	0-5 (1)	0-9 (8)
Amphisolenia bidentata		0-2 (2)	0-8 (7)	0-3 (1)	0-3 (3)			0-2 (1)		0-9 (3)
Amphisolenia globifera										0-3 (2)
Blepharocysta sp.		0-4 (2)	0-4 (3)	0-8 (2)				0-4 (5)	0-8 (6)	0-3 (1)
Ceratocorys armata	0-2 (1)						0-2 (1)			
Ceratocorys horrida		0-4 (2)		0-3 (1)		0-2 (2)				
Corythodinium constrictum		0-2 (1)					0-4 (2)			
Corythodinium elegans			0-4 (1)	0-3 (1)	0-3 (1)					0-3 (1)
Corythodinium michaelsarsi				0-8 (2)						
Corythodinium reticulatum	0-2 (2)						0-2 (2)			
Corythodinium tesselatum		0-2 (2)	0-2 (1)	0-3 (2)	0-3 (3)	0-2 (2)				0-3 (2)
Corythodinium sp.			0-2 (1)		0-3 (1)					
Ensiculifera sp.									0-5 (1)	
Gambierdiscus sp.*†								0-4 (1)		
Goniodoma polyedricum		0-2 (2)				0-6 (2)	0-2 (1)		0-5 (1)	
Goniodoma sphaericum	0-6 (3)				0-3 (1)		0-6 (1)	0-2 (2)	0-5 (1)	0-3 (2)

Continued...

Table 2.1. ...Contd.

Dinoflagellates	CP transect					PK transect				
	Feb 04	Jun 04	Jul 04	Oct 04	Sep 06	Nov 03	Mar 04	Aug 04	Oct 04	Sep 06
Autotrophic										
Gonyaulax brevisulcata										0-3 (1)
Gonyaulax digitalis				0-5 (1)						
Gonyaulax grindleyi	0-2 (1)									
Gonyaulax monospina†					0-3 (1)					0-3 (1)
*Gonyaulax polyedra**	0-4 (4)			0-3 (1)			0-2 (1)			
*Gonyaulax polygramma**	0-8 (1)	0-2 (1)	0-2 (1)	0-8 (4)	0-6 (1)	0-4 (2)	0-4 (1)	0-4 (2)	0-8 (3)	0-6 (2)
Gonyaulax scrippsae	0-4 (1)		0-2 (2)		0-3 (1)	0-6 (3)				
*Gonyaulax spinifera**	0-4 (4)			0-3 (1)		0-4 (1)	0-2 (2)			
Gonyaulax sp.	0-2 (1)	0-2 (2)	0-2 (2)	0-3 (1)	0-6 (6)	0-2 (1)	0-4 (2)	0-6 (1)	0-3 (2)	0-3 (5)
Gymnodinium spp.*			0-2 (1)	0-5 (1)	0-6 (3)			0-6 (1)		0-6 (3)
Oxytoxum globosum	0-26 (1)									
Oxytoxum laticeps				0-3 (1)	0-6 (3)	0-2 (1)				0-6 (4)
Oxytoxum scolopax	0-3 (5)	0-2 (2)	0-4 (6)	0-8 (4)	0-3 (5)		0-6 (2)	0-2 (2)	0-5 (1)	0-3 (4)
Oxytoxum sceptrum		0-2 (1)	0-4 (5)	0-3 (4)				0-2 (3)		0-3 (1)
Oxytoxum sp.	0-6 (3)	0-2 (1)					0-2 (2)			0-3 (1)
Podolampas bipes		0-2 (3)		0-3 (1)	0-3 (2)	0-2 (1)			0-5 (1)	0-6 (2)
Podolampas elegans	0-4 (3)						0-2 (1)			
Podolampas palmipes	0-4 (4)	0-2 (2)	0-2 (3)	0-3 (3)	0-6 (1)		0-2 (1)	0-2 (1)	0-5 (1)	0-3 (1)
Podolampas spinifera		0-2 (1)	0-2 (2)	0-3 (1)					0-5 (1)	
Pyrophacus steinii	0-2 (1)			0-3 (1)		0-2 (3)	0-2 (1)			0-18 (1)
Pyrophacus spp.				0-5 (2)						
Torodinium teredo†					0-3 (2)					

Continued...

Table 2.1. ...Contd.

Dinoflagellates	CP transect					PK transect				
	Feb 04	**Jun 04**	**Jul 04**	**Oct 04**	**Sep 06**	**Nov 03**	**Mar 04**	**Aug 04**	**Oct 04**	**Sep 06**
Mixotrophic										
*Alexandrium minutum**	0-10 (1)									
Ceratium arietinum									0-3 (2)	
Ceratium azoricum					0-3 (1)	0-2 (1)				
Ceratium candelabrum f. *depressum*			0-2 (1)	0-3 (1)						0-3 (1)
Ceratium contortum	0-5 (3)						0-4 (1)			
Ceratium declinatum		0-2 (1)	0-2 (1)	0-5 (4)	0-9 (4)				0-5 (1)	0-9 (3)
Ceratium deflexum				0-3 (1)					0-8 (1)	
Ceratium dens					0-3 (1)					
Ceratium extensum							0-4 (4)	0-6 (1)		
*Ceratium furca**	0-2 (1)		0-2 (2)	0-5 (4)	0-9 (5)	0-20 (5)		0-6 (4)	0-13 (4)	0-18 (4)
*Ceratium fusus**	0-6 (4)	0-2 (4)	0-2 (3)	0-13 (9)	0-6 (4)	0-20 (1)	0-4 (1)	0-6 (5)	0-8 (7)	0-12 (3)
Ceratium gibberum	0-2 (1)									
Ceratium horridum			0-2 (2)		0-6 (2)			0-2 (1)		0-3 (1)
Ceratium karstenii	0-2 (4)						0-2 (1)			
Ceratium kofoidii		0-2 (1)	0-2 (1)			0-2 (1)				
Ceratium lineatum			0-2 (2)	0-3 (2)	0-3 (1)				0-5 (1)	0-3 (1)
Ceratium lunula							0-2 (1)			
Ceratium macroceros		0-2 (3)	0-2 (1)			0-2 (3)		0-2 (1)		
Ceratium pentagonum					0-9 (7)			0-2 (1)		0-3 (1)
Ceratium schmidtii			0-2 (2)	0-5 (3)	0-3 (1)				0-8 (2)	
Ceratium symmetricum	0-10 (4)			0-3 (1)			0-2 (2)			

Continued...

Table 2.1. ...Contd.

Dinoflagellates	CP transect					PK transect				
	Feb 04	Jun 04	Jul 04	Oct 04	Sep 06	Nov 03	Mar 04	Aug 04	Oct 04	Sep 06
Mixotrophic										
Ceratium teres		0-8 (6)	0-8 (7)	0-8 (7)	0-9 (7)	0-4 (1)		0-2 (1)	0-3 (2)	0-12 (3)
Ceratium trichoceros	0-2 (2)						0-8 (1)			
Ceratium tripos		0-2 (1)	0-2 (1)			0-2 (1)				
Ceratium vultur	0-4 (1)									0-3 (1)
Ceratium spp.			0-2 (1)	0-3 (2)	0-3 (3)				0-5 (1)	
*Dinophysis caudata**								0-2 (1)	0-5 (1)	
*Dinophysis miles**								0-12 (1)		
Dinophysis schuetii				0-3 (1)						
Dinophysis spp.	0-4 (5)		0-2 (2)	0-5 (2)			0-6 (6)		0-5 (1)	0-3 (1)
Dissodium asymmetricum						0-2 (1)				
Prorocentrum arcuatum†	0-2 (1)						0-4 (1)			
*Prorocentrum belizeanum**†		0-24 (1)								
Prorocentrum compressum		0-2 (1)		0-5 (1)				0-2 (1)		
Prorocentrum gracile				0-8 (4)						
Prorocentrum lenticulatum	0-4 (1)									
*Prorocentrum lima**							0-2 (1)			
*Prorocentrum micans**	0-20 (1)		0-2 (2)	0-3 (1)	0-3 (2)			0-10 (1)	0-10 (2)	0-9 (1)
*Prorocentrum minimus**	0-2 (1)		0-2 (1)	0-3 (1)				0-2 (1)		
*Prorocentrum mexicanum**	0-5 (6)									
Prorocentrum obtusum				0-8 (3)					0-3 (2)	0-3 (2)
Prorocentrum scutellum†								0-2 (1)		
*Prorocentrum sigmoides**			0-2 (1)		0-3 (1)					0-9 (4)
Prorocentrum sp.	0-2 (2)	0-14 (2)	0-4 (3)	0-8 (5)	0-9 (7)	0-4 (4)	0-4 (1)	0-6 (2)	0-8 (3)	0-6 (5)

Continued...

Table 2.1. ...Contd.

Dinoflagellates	CP transect					PK transect				
	Feb 04	Jun 04	Jul 04	Oct 04	Sep 06	Nov 03	Mar 04	Aug 04	Oct 04	Sep 06
Mixotrophic										
Pyrocystis fusiformis			0-2 (1)	0-3 (1)						
Pyrocystis hamulus	0-2 (1)		0-4 (2)			0-2 (1)				
Pyrocystis lunula	0-4 (1)									
Pyrocystis spp.		0-2 (1)	0-2 (1)	0-3 (1)	0-3 (2)	0-14 (5)	0-6 (1)		0-5 (2)	0-3 (1)
*Scrippsiella trochoidea**	0-14 (9)		0-2 (1)	0-3 (1)	0-9 (7)		0-26 (6)	0-4 (1)	0-5 (2)	0-15 (7)
Scrippsiella spp.		0-2 (1)	0-6 (5)	0-10 (3)			0-2 (1)	0-6 (3)	0-8 (1)	
Heterotrophic										
Gotoius abei†		0-6 (4)		0-3 (1)		0-10 (5)			0-8 (4)	0-3 (1)
Histioneis carinata†	0-2 (1)				0-3 (1)		0-4 (2)			
Histioneis costata†	0-2 (1)	0-2 (1)						0-2 (1)		
Histioneis depressa		0-2 (2)	0-2 (2)							
Histioneis spp.					0-3 (1)					
Katodinium sp.		0-2 (3)	0-4 (5)	0-10 (2)	0-3 (2)			0-2 (2)	0-5 (1)	
Noctiluca scintillans		0-2 (1)				0-40 (1)	0-2 (1)			
Noctiluca spp.	0-2 (1)									
Ornithocercus heteroporus	0-6 (2)						0-2 (1)			
Ornithocercus magnificus		0-2 (2)	0-4 (2)	0-18 (2)	0-3 (1)				0-5 (1)	0-6 (2)
Ornithocercus quadratus		0-2 (1)	0-2 (1)	0-3 (1)					0-5 (1)	
Ornithocercus steinii			0-2 (1)			0-4 (2)	0-2 (1)	0-2 (1)	0-5 (1)	
Ornithocercus thumii		0-2 (1)				0-4 (2)		0-2 (1)	0-5 (1)	
Ornithocercus spp.								0-2 (1)		0-3 (1)
Oxyphysis spp.					0-3 (1)					
Pentapharsodinium sp.†		0-2 (1)				0-4 (1)				

Continued...

Table 2.1. ...Contd.

Dinoflagellates	CP transect					PK transect				
	Feb 04	Jun 04	Jul 04	Oct 04	Sep 06	Nov 03	Mar 04	Aug 04	Oct 04	Sep 06
Heterotrophic										
Phalacroma argus	0-2 (1)									
Phalacroma cuneus								0-2 (1)	0-5 (1)	
Phalacroma rapa	0-2 (2)	0-2 (1)		0-3 (1)						
Phalacroma rotundatum					0-3 (1)			0-2 (1)	0-8 (2)	0-3 (2)
Phalacroma spp.		0-2 (2)	0-2 (2)			0-2 (1)				
Pronoctiluca pelagica							0-2 (1)			
Pronoctiluca spinifera		0-2 (2)	0-2 (1)	0-3 (1)	0-3 (1)					
Protoperidinium asymmetricum	0-4 (3)						0-2 (1)			
Protoperidinium brevipes		0-2 (1)								
*Protoperidinium crassipes**	0-2 (1)						0-2 (1)			
Protoperidinium depressum	0-2 (4)	0-2 (1)					0-4 (2)	0-2 (1)	0-5 (1)	
Protoperidinium divergens		0-2 (1)	0-4 (3)	0-5 (6)	0-3 (1)	0-2 (1)		0-2 (2)	0-3 (5)	
Protoperidinium elegans				0-3 (2)						
Protoperidinium grande	0-4 (1)									
Protoperidinium leonis		0-2 (1)								
Protoperidinium minutum										0-3 (1)
Protoperidinium oblongum						0-2 (1)				
Protoperidinium ovatum	0-4 (8)						0-26 (2)			
Protoperidinium pacificum		0-2 (1)		0-10 (2)		0-6 (2)	0-4 (2)		0-8 (3)	

Continued...

Table 2.1. ...Contd.

Dinoflagellates	CP transect					PK transect				
	Feb 04	Jun 04	Jul 04	Oct 04	Sep 06	Nov 03	Mar 04	Aug 04	Oct 04	Sep 06
Heterotrophic										
Protoperidinium pallidum			0-2 (1)							
Protoperidinium pedunculatum	0-4 (3)	0-2 (2)		0-8 (4)		0-4 (1)	0-2 (3)	0-4 (1)	0-5 (1)	
Protoperidinium pellucidum	0-4 (2)				0-3 (1)	0-4 (1	0-2 (3)	0-4 (1)		
Protoperidinium pentagonum		0-2 (1)				0-10 (1)		0-2 (1)		
Protoperidinium pyriforme							0-2 (1)			
Protoperidinium steinii			0-2 (1)	0-3 (2)	0-6 (1)	0-4 (1)		0-8 (2)	0-3 (4)	
Protoperidinium subinerme				0-3 (1)				0-2 (2)		
Protoperidinium sp.		0-4 (4)	0-4 (6)	0-3 (4)	0-9 (8)	0-2 (1)	0-4 (2)	0-8 (3)	0-5 (1)	0-9 (9)
Zygabikodinium sp.		0-2 (1)								

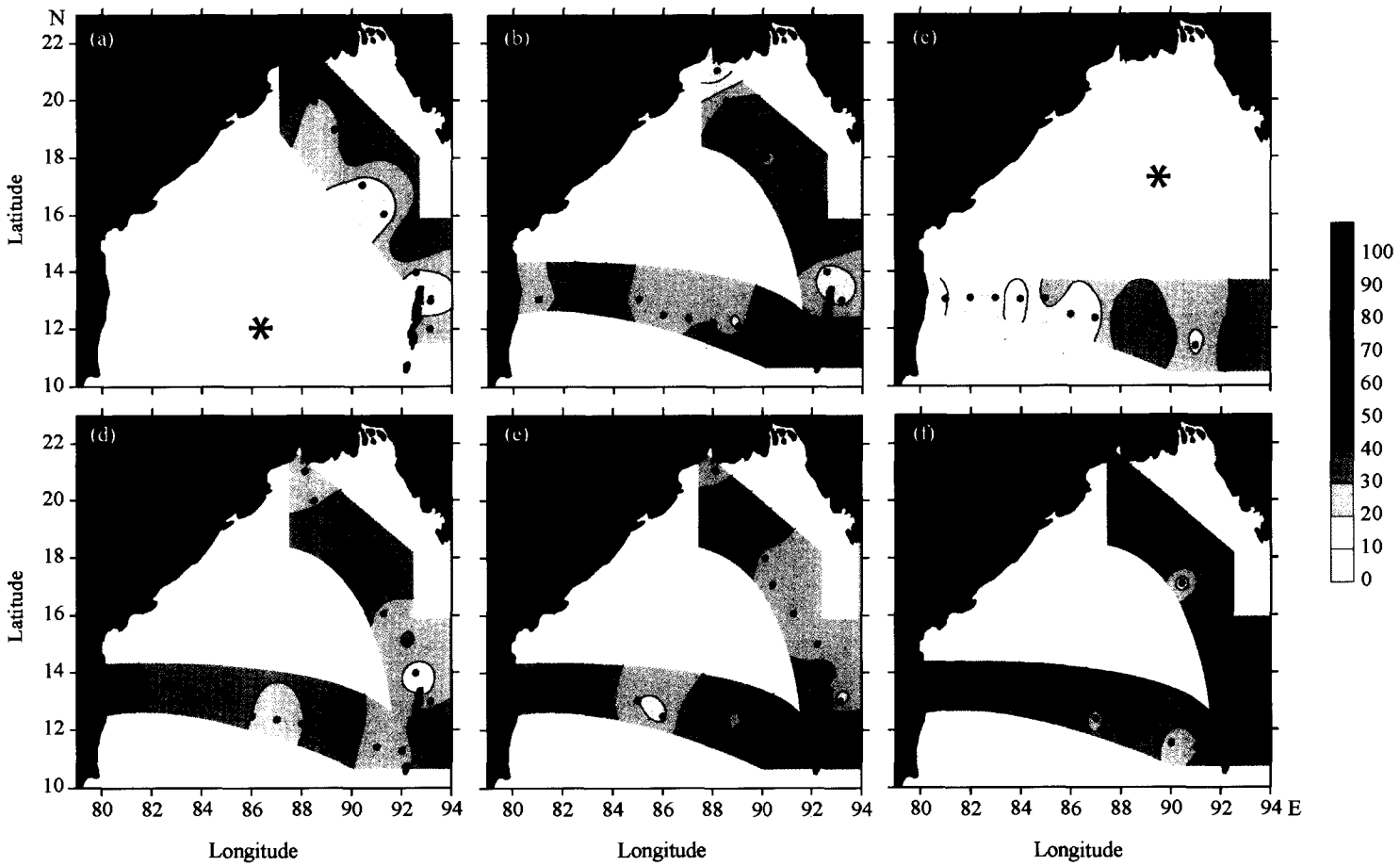


Fig. 2.2. Spatio-temporal variation in total dinoflagellate abundance (cells L^{-1}) along the Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects in (a) Nov 03, (b) Feb-Mar 04, (c) Jun 04, (d) Jul-Aug 04, (e) Oct 04 and (f) Sep 06. * transect not sampled.

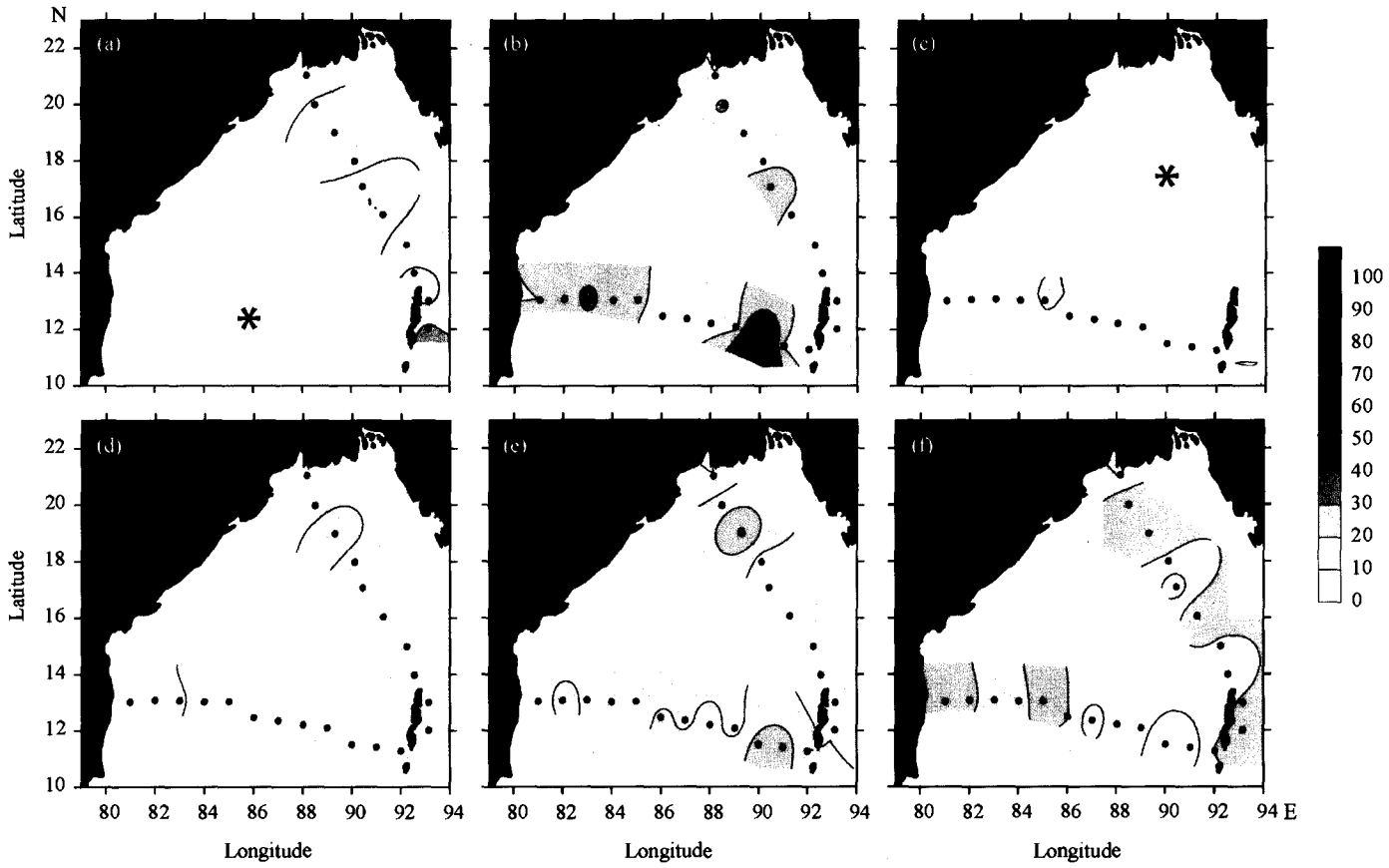


Fig. 2.3. Spatio-temporal variation in autotrophic dinoflagellate abundance (cells L^{-1}) along the Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects in (a) Nov 03, (b) Feb-Mar 04, (c) Jun 04, (d) Jul-Aug 04, (e) Oct 04 and (f) Sep 06. * transect not sampled.

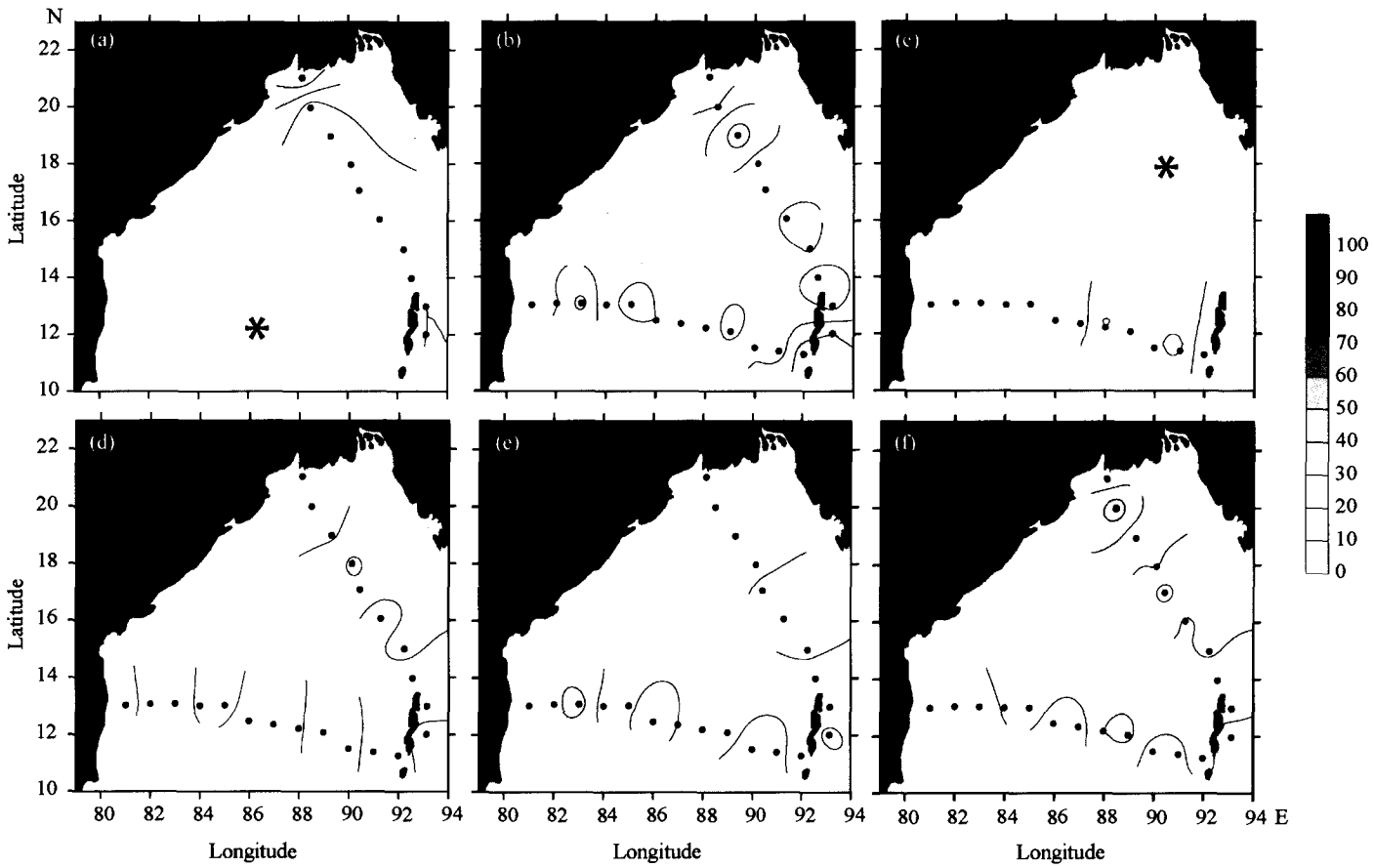


Fig. 2.4. Spatio-temporal variation in mixotrophic dinoflagellate abundance (cells L^{-1}) along the Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects in (a) Nov 03, (b) Feb-Mar 04, (c) Jun 04, (d) Jul-Aug 04, (e) Oct 04 and (f) Sep 06. * transect not sampled.

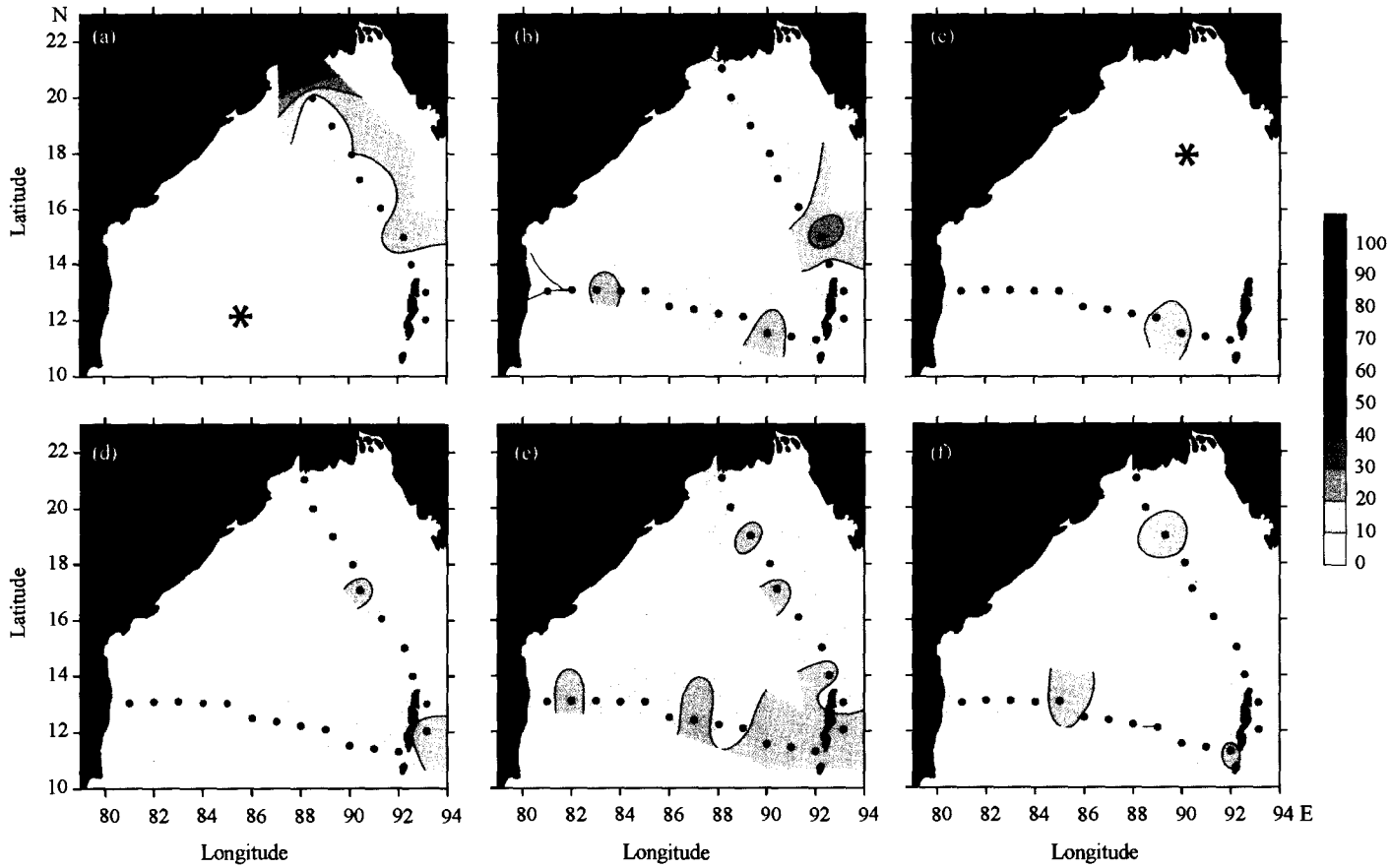


Fig. 2.5. Spatio-temporal variation in heterotrophic dinoflagellate abundance (cells L^{-1}) along the Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects in (a) Nov 03, (b) Feb-Mar 04, (c) Jun 04, (d) Jul-Aug 04, (e) Oct 04 and (f) Sep 06. * transect not sampled.

Table 2.2. Two-way ANOVA to evaluate the variation in dinoflagellate abundance, species richness, evenness and diversity along the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. Values in bold indicate significant variation.

Factor	CP transect				PK transect			
	df	SS	MS	*p-value*	*df*	SS	MS	*p-value*
Abundance								
Stations	11	0.5781	0.0526	0.3281	9	1.1035	0.1226	0.3102
Seasons	4	1.2082	0.3021	**0.0002**	4	0.7474	0.1869	0.1373
Within sub-group error	44	1.9596	0.0445		36	3.6006	0.1000	
Total	59	3.7459			49	5.4516		
Species richness								
Stations	10	10.1732	1.0173	0.1661	9	9.5642	1.0627	**0.0180**
Seasons	4	0.4200	0.1050	0.9585	4	2.7963	0.6991	0.1614
Within sub-group error	40	26.6678	0.6667		36	14.4171	0.4005	
Total	54	37.2610			49	26.7776		
Species evenness								
Stations	10	0.0504	0.0050	0.1922	9	0.2111	0.0235	0.2578
Seasons	4	0.0170	0.0043	0.3145	4	0.1674	0.0419	0.0710
Within sub-group error	40	0.1385	0.0035		36	0.6365	0.0177	
Total	54	0.2059			49	1.0151		
Species diversity								
Stations	10	2.6394	0.2639	0.1033	9	3.7540	0.4171	**0.0338**
Seasons	4	0.6172	0.1543	0.4078	4	1.8929	0.4732	**0.0483**
Within sub-group error	40	6.0408	0.1510		36	6.4055	0.1779	
Total	54	9.2974			49	12		

species richness only across stations and diversity across both stations and seasons (Table 2.2). The highest abundance of dinoflagellates was observed during September 06 followed by October 04 whereas low abundance was recorded during June, July and August 04 (Fig. 2.2). Mixotrophic dinoflagellates dominated across both transects, with the exception of October 04 at CP and September 06 at PK transects,

that were dominated by heterotrophic forms (Fig. 2.6). Autotrophic dinoflagellates contributed 18-41% whereas heterotrophic forms contributed in the range of 13-49% (Fig. 2.6).

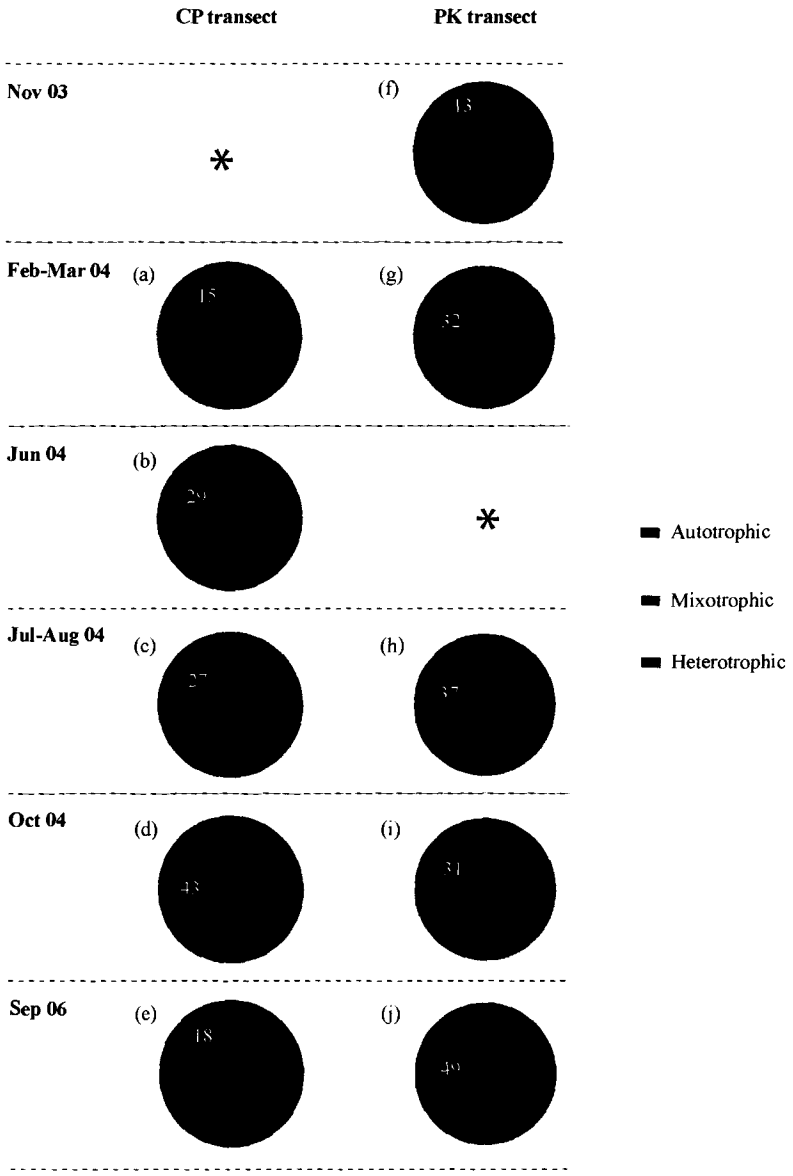


Fig. 2.6. The percentage contribution of autotrophic, mixotrophic and heterotrophic dinoflagellates along the (a-e) Chennai–Port Blair (CP) and (f-j) Port Blair–Kolkata (PK) transects. (a) Feb 04, (b) Jun 04, (c) Jul 04, (d) Oct 04, (e) Sep 06, (f) Nov 03, (g) Mar 04, (h) Aug 04, (i) Oct 04 and (j) Sep 06. * transect not sampled.

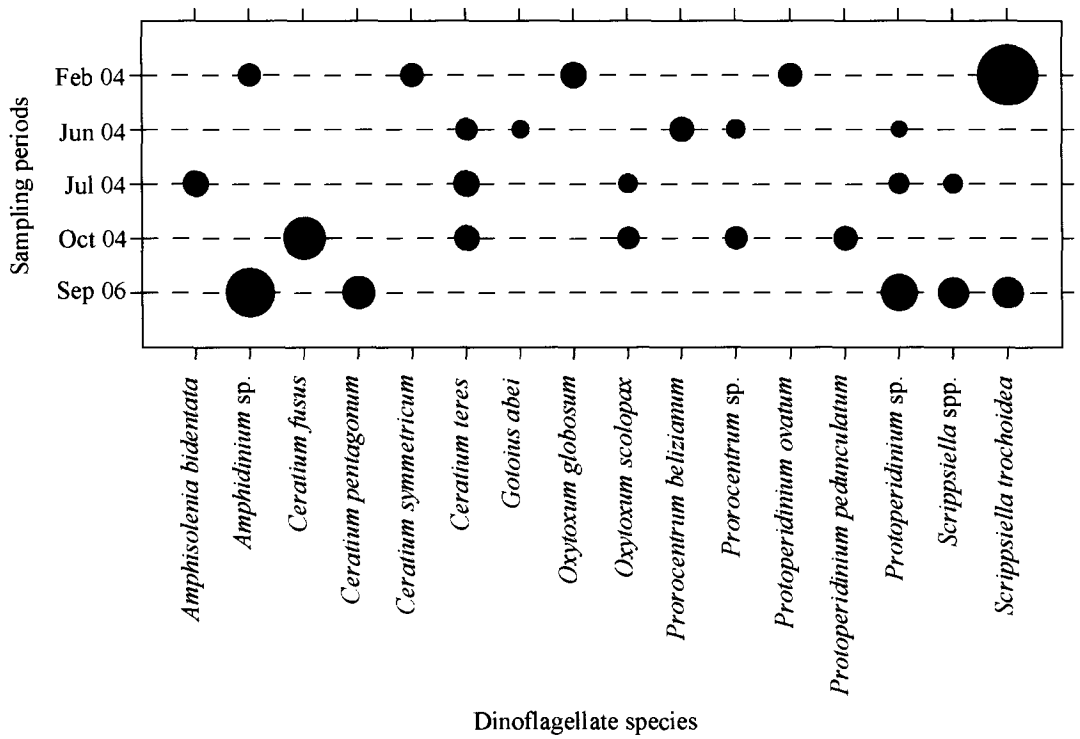


Fig. 2.7. The five most abundant dinoflagellate species during each sampling period along the Chennai–Port Blair (CP) transect. The maximum diameter of the circles corresponds to an average abundance of 8 cells L^{-1}.

Ceratium was the dominant genus among the mixotrophic forms whereas *Protoperidinium* was the dominant heterotrophic form. *Ceratium fusus*, *C. teres*, *Gotoius abei*, *Oxytoxum scolopax*, *Protoperidinium ovatum* and *Scrippsiella trochoidea* were among the abundant forms in both transects. *Amphisolenia bidentata*, *C. pentagonum*, *C. symmetricum*, *O. globosum* and *Prorocentrum belizianum* were abundant only in CP transect (Fig. 2.7).

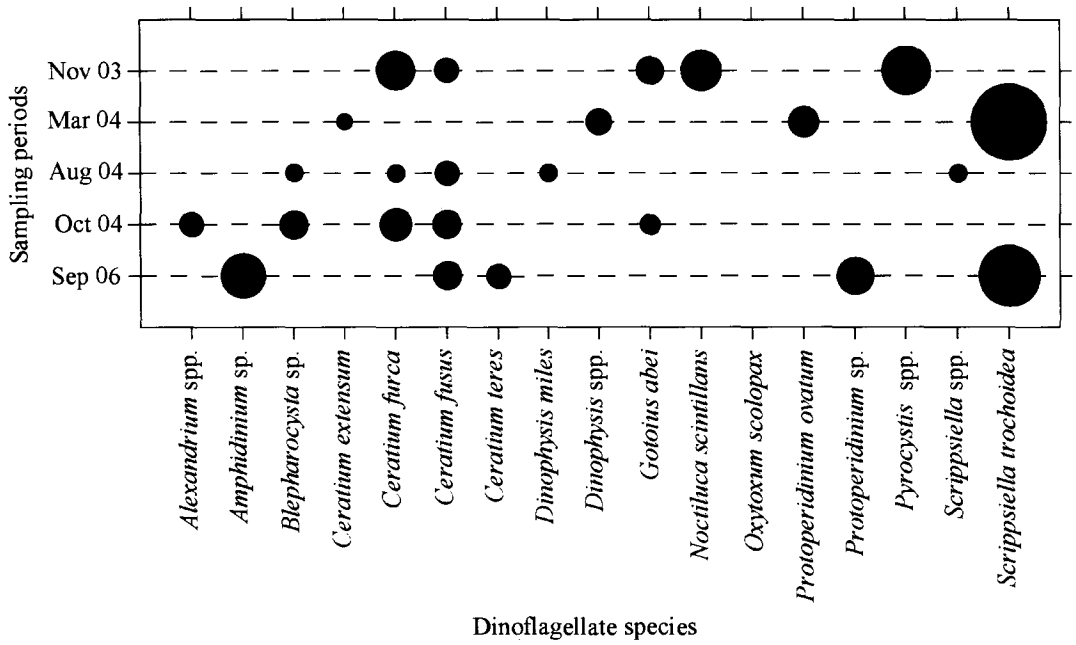


Fig. 2.8. The five most abundant dinoflagellate species during each sampling period along the Port Blair-Kolkata (PK) transect. The maximum diameter of the circles corresponds to an average abundance of 8 cells L^{-1}.

C. extensum, *C. furca*, *Dinophysis miles* and *Noctiluca scintillans* were abundant only in the PK transect (Fig. 2.8).

2.3.3. Seasonal variation in HAB species

Overall, 19 potentially HAB species were encountered in this area (Table 2.1; some species in Fig.2.9). Some HAB species were present during all the seasons throughout the study period. These frequently occurring HAB species were *C. furca, C. fusus, Dinophysis* sp., *Gonyaulax polygramma*, *Gonyaulax* sp., *N. scintillans*, *Prorocentrum* sp., *S. trochoidea*, *Scrippsiella* sp. (Table 2.1). Season-specific trends were also observed. *Alexandrium minutum*, *Prorocentrum lima* and *Protoperidinium*

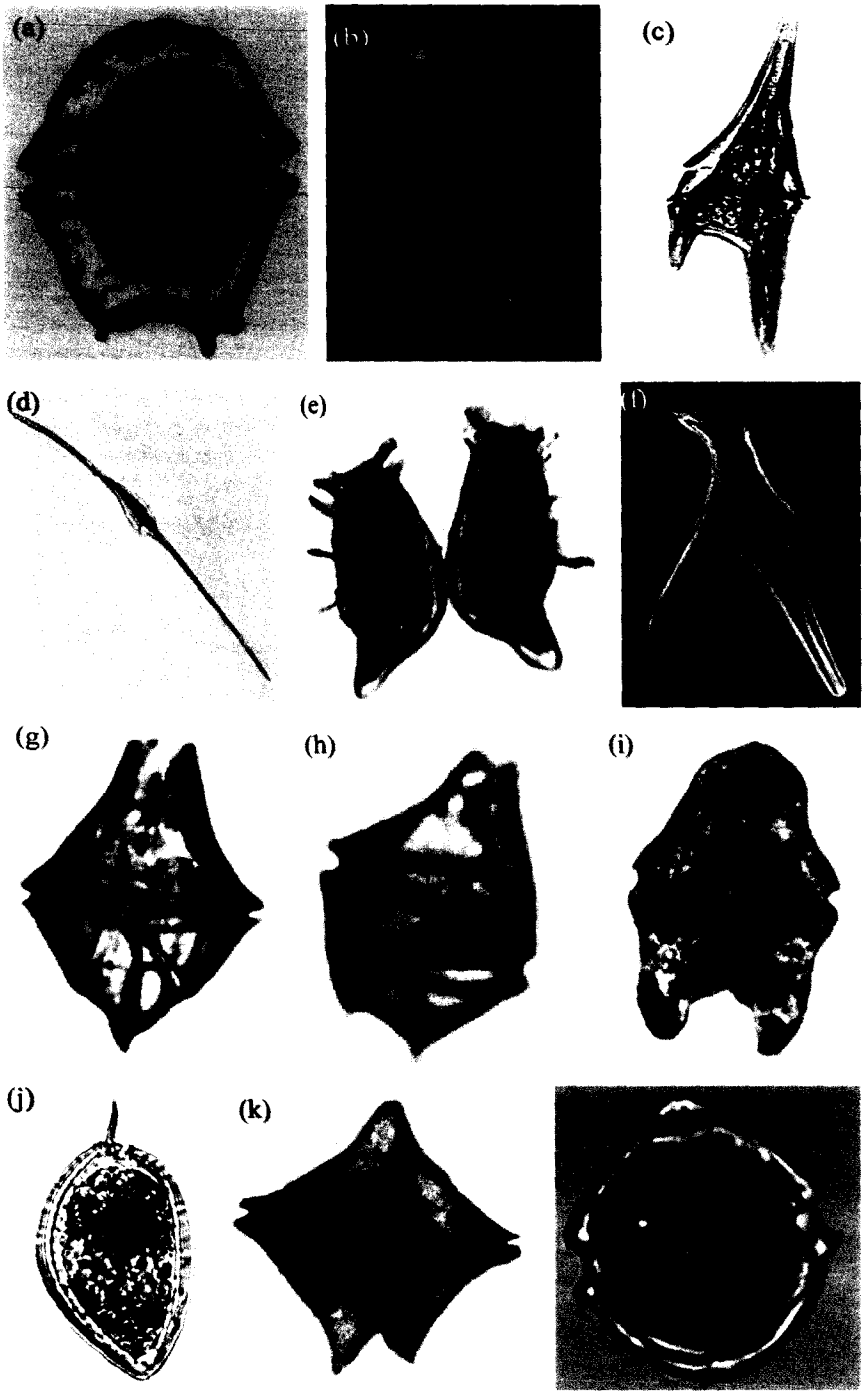


Fig. 2.9. Potential HAB species. (a) *Alexandrium* sp., (b) *Amphidinium* sp., (c) *Ceratium furca*, (d) *Ceratium fusus*, (e) *Dinophysis caudata*, (f) *Dinophysis miles*, (g) *Gonyaulax polygramma*, (h) *Gonyaulax spinifera*, (i) *Gymnodinium* sp., (j) *Prorocentrum micans*, (k) *Protoperidinium crassipes* and (l) *Scrippsiella trochoidea.*

crassipes occurred only during PrM whereas *Dinophysis caudata*, *D. miles*, *Prorocentrum micans*, *P. mexicanum*, *P. minimus*, *Gymnodinium* sp. and *Gambierdiscus* sp. were found during SWM and PoM periods. *Gonyaulax polyedra* and *G. spinifera* occurred in PrM and PoM but not during SWM. In contrast to this, *P. belizeanum* and *P. sigmoides* occurred only during the SWM (Table 2.1).

2.4. Discussion

The BOB is a unique geographic region; it is characterized by a stratified water column that restricts the vertical transport of nutrients from the bottom layers to the surface, and therefore phytoplankton productivity as well (Prasanna Kumar et al. 2002). The stratification is especially intense during the SWM period due to the enormous influx of freshwater through precipitation and riverine discharges that leads to the formation of a low salinity cap at the surface. This study revealed that dinoflagellate abundance in surface waters was generally in the range of 0-94 cells L^{-1} and did not vary significantly across stations (Table 2.2). This could be related to the environmental conditions of the study area. Stratified water columns, with their characteristic oligotrophic conditions, tend to promote stasis of the resident population, rather than promoting growth or blooms (Smayda 2002). In fact, many studies (McGill 1973, Paul et al. 2008) have reported that the surface waters of the BOB are nitrate-deficient. Yet, even though the stratified BOB environment supports a rather homogeneous dinoflagellate community in surface waters, significant seasonal variations in abundance and species diversity of the dinoflagellate

community were observed (Table 2.2). Minimum values were observed during SWM, correlating to the intensification of the stratified layer, and maximum during the withdrawal of the SWM and PoM (Fig. 2.2).

The dominance of mixotrophic dinoflagellates in the study area (Fig. 2.6) could be a consequence of the prevalent low light and/or nutrient scarcity, conditions that are known to promote mixotrophy (Legrand et al. 1998, Stoecker et al. 2006). *Ceratium* and *Protoperidinium* were the most abundant representatives of the mixotrophic and heterotrophic forms in the BOB respectively (Figs. 2.7&2.8); their unique characteristics of vertical migration, and heterotrophy are reasonably well understood (Baek et al. 2006 and 2007, Baek et al. 2008 a, b and c, Latz and Jeong 1996), and probably confer on them a competitive advantage in both coastal and oceanic environments.

Comparison of the present study with earlier reports from the same area (Taylor 1976, Jyothibabu et al. 2003, Paul et al. 2007) indicated 10 new dinoflagellate species (Table 2.1 species marked with †; some species in Fig. 2.10). Approximately 14% of the identified species were potential HAB species (Table 2.1 species marked with *). Among the frequently occurring HAB species (hereafter FOS) recorded in the region; *C. furca* and *C. fusus* showed a characteristic transect-specific distribution. *C. furca* was abundant only in the PK transect. *C. fusus* which was predominant in the PK transect (Fig. 2.8) was abundant only during October 04 in the CP transect (Fig. 2.7).

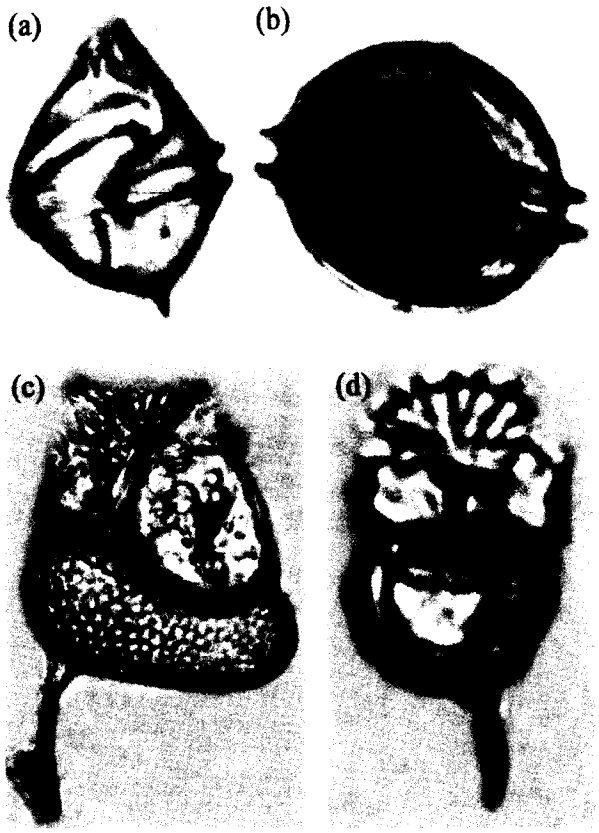


Fig. 2.10. Newly reported species from the region. (a) *Gonyaulax monospina*, (b) *Gotoius abei*, (c) *Histioneis carinata* and (d) *Histioneis costata*.

C. furca and *C. fusus* are characteristically found in stable stratified water columns (Baek et al. 2006 and 2007). However, both species differ in several characteristics. In recent observations in Sagami Bay, Japan, Baek et al. (2009) reported that *C. furca* had a competitive edge over *C. fusus*, because of its efficient diel vertical migration capability (a 'biological' factor). *C. fusus* was stimulated by low salinity and showed dependence on external environmental conditions such as enhanced nutrient concentrations following fresh water discharge by heavy rainfall (combination of 'physical' and 'chemical' factors). In light of this, the observations in this study point

out that the water mass of the PK transect is influenced by riverine discharge to a much greater extent compared to the CP transect. Additionally, the differential capabilities of *Ceratium* species to acclimatize to such niches can be an important factor in determining their diversity and spatio-temporal distribution.

S. trochoidea, another FOS in the region, was abundant during September 06/ February-March 04 (Figs. 2.7, 2.8). September is known as the period of withdrawal of SWM, during which cloud cover reduces, whereas March is known for clear sky and with no rainfall. Studies on the factors triggering the growth/bloom formation of *S. trochoidea*, point to different regulating factors. For e.g., in Hong Kong waters, the initiation, maintenance and disappearance of a *S. trochoidea* red tide was not directly driven by changes in nutrients (Yin et al. 2008). Subsequently, Zhuo-Ping et al. (2009) observed that the cell density of *S. trochoidea* was positively influenced by high irradiance and further enhanced by iron concentration. These conditions of high irradiance could possibly be responsible for the predominance of *S. trochoidea* in March.

Blooms of *N. scintillans*, yet another FOS in the region, have been reported from Indian waters (Raghu Prasad 1953 and 1956, Santha Joseph 1975, Naqvi et al. 1998, Eashwar et al. 2001, Mohanty et al. 2007, Gomes et al. 2008). Most of these blooms occurred during the SWM. However, as discussed above for *S. trochoidea*, additional studies in other parts of the world reveal different triggering factors. For e.g., a *N. scintillans* bloom observed in the Gulf of Thailand was mainly influenced by the SWM (Sriwoon et al. 2008). Since the SWM is a major meteorological event

influencing the BOB, it is absolutely necessary to investigate in detail the factors sustaining the population of *N. scintillans* in the BOB and their bloom dynamics.

Dinophysis sp., another FOS in the region, was abundant during March at PK transect (Fig. 2.8). *Dinophysis* are known to increase in cell density immediately after a storm-induced mixing event (Nishitani et al. 2005). They can also migrate through strong gradients and survive under unfavorable conditions (Setala et al. 2005). In a recent observation in Portuguese waters, Escalera et al. (2010) found that the increased numbers of *Dinophysis* was a result of physically-driven accumulation due to long-shore transport. They also found the bloom to be associated with much warmer temperatures. In these observation, its predominance during March indicates its preference for high temperature since this period is considered a warmer season in the BOB (Narvekar and Prasanna Kumar 2006).

Given that the FOS observed in this study ranged from 0-40 cells L^{-1}, their presence may not be a population growth issue, as suggested by Smayda (2002). However, they may play a significant role in the development of pelagic seed banks, which can serve as inocula for bloom events elsewhere, on the onset of favorable conditions. An earlier study (Avaria 1979) suggested that the Chilean frontal zone located 100 km offshore supported a *P. micans* bloom. Transport of offshore-seeded *Prorocentrum* and *Ceratium* blooms to inshore waters (Pitcher and Boyd 1996) also supports the above assumption.

Another intriguing aspect to be considered is the enhancement of phytoplankton biomass to bloom levels by physical processes occurring in the BOB like eddies

(Gomes et al. 2000, Prasanna Kumar et al. 2004) and cyclones (Madhu et al. 2002, Vinayachandran and Mathew 2003, Rao et al. 2006). In both eddies and cyclones, bloom formation takes place due to transport of nutrients from bottom layers to the surface. During cyclones, due to strong wind speed, the stratified layer breaks and deepens the mixed layer, leading to introduction of nutrients into the surface layers whereas during eddies, Ekman pumping plays an important role in transporting nutrients to surface waters. Eddies are most likely to occur during the SWM (Prasanna Kumar et al. 2004) whereas cyclones are common during November (Madhu et al. 2002, Vinayachandran and Mathew 2003, Rao et al. 2006). The combination of such physical effects including turbulence and advection, with the diverse behavioral characteristics of dinoflagellates (e.g., migration, physiological adaptation) holds the key to understanding HAB dynamics in stratified oceanic areas. Even though some of these physical processes may play a crucial role in the formation of HABs, these processes are not well defined and thus knowledge in this context remains weak (GEOHAB 2003). For e.g., the above studies were based on remote sensing (chlorophyll *a*) and primary productivity values and none of the reported blooms/enhancement of phytoplankton biomass was taxonomically characterized. In this context, the present investigation pointing out the presence of *N. scintillans* and *C. furca* during November (Table 2.1) further strengthens their probable candidature for bloom formation in the region.

Summarizing the results, the present study is the first of its kind detailing the HAB species from the stratified surface waters of the BOB and their seasonal occurrence.

The frequently occurring HAB species indicate their ability to survive even under such conditions. Their low abundance in the region may not be a growth issue but they may serve as inocula for blooms if coupled with population-triggering physical processes like eddies and cyclones in the region. In this scenario, the characteristic ability of FOS like *C. furca* and *N. scintillans* and their predominance during cyclone-prone months, make their candidature stronger for future bloom occurrences in the region.

Chapter 3

Primary description of surface water phytoplankton pigment patterns in the Bay of Bengal

3.1. Introduction

Phytoplankton, the microscopic floating plant life of all water bodies, include among their components: diatoms, dinoflagellates, coccolithophores, green algae, blue green algae and other groups. All together these marine phytoplankton are important contributors to global carbon fluxes. Qualitative and quantitative analysis of such groups is important to understand the community structure and dynamics of any ecosystem. To accomplish this, one approach is the identification and enumeration of phytoplankton through microscopic analysis. This approach is time-consuming and requires a high level of taxonomic expertise. There is also the risk of missing out smaller groups of phytoplankton (picophytoplankton, < 2μm) in routine microscopic analysis. Pigment analysis using chromatography, is another approach considered as a powerful tool for characterization and monitoring of phytoplankton abundance and composition of field populations (Wright and Jeffrey 2006). The northern Indian Ocean has been studied using this approach on several occasions (Latasa and Bidigare 1998, Barlow et al. 1999, Goericke et al. 2000, Roy et al. 2006, Barlow et al. 2008). The seasonal pigment pattern of surface phytoplankton from the southern hemisphere was also studied in the recent past (Barlow et al. 2007). Most of these studies concentrated on the Arabian Sea as the study area. The characteristic features of the Arabian Sea, such as monsoon, upwelling and current patterns made it an area of interest, while its counterpart, the Bay of Bengal (BOB), has remained relatively unexplored.

The BOB, the eastern arm of the northern Indian Ocean is characterized by seasonal reversing of winds, currents influenced by monsoon and riverine flux causing the near-surface circulation to be thermohaline in nature. These features make the BOB a unique oceanic area and thus, understanding the spatio-temporal variations in the distribution of phytoplankton pigments in this area will provide novel information on contribution of specific groups to the total pigment pool. This information will be important in developing applications of remote sensing in biogeochemistry and ecosystem models.

3.2. Materials and Methods

3.2.1. Sampling strategy

Under the Indian Expendable Bathythermograph (XBT) Programme, surface water was collected with bucket on two transects [Chennai-Port Blair (CP) and Port Blair-Kolkata (PK)] (Fig. 3.1), from passenger ships plying along these transects. Samples were collected at one degree intervals from 22 stations on 4 occasions, from February 2007 to June 2007. Surface water was collected for the enumeration and identification of micro-phytoplankton to the lowest possible taxonomic level using light microscopy, and for pigment analysis using High Performance Liquid Chromatography (HPLC).

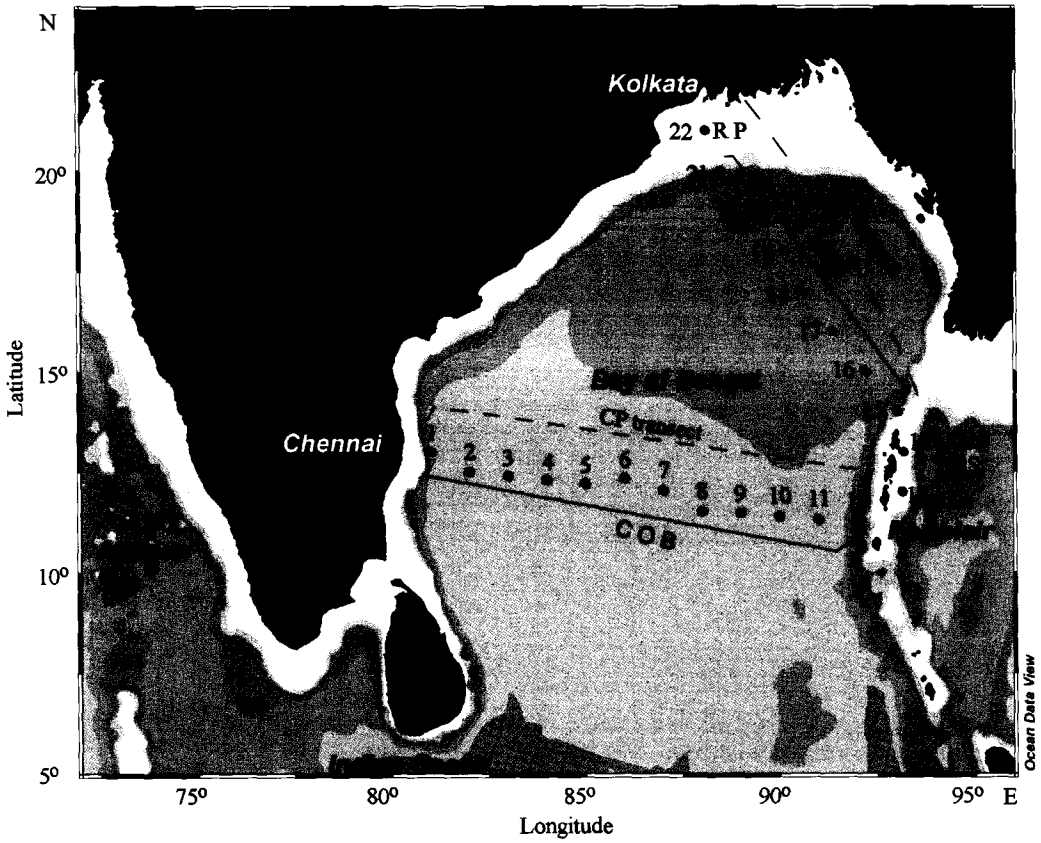


Fig. 3.1. Study area showing station locations and regions (COB-Central Oceanic Bay, AS-Andaman Sea, NOB-Northern Oceanic Bay and RP- River plume) along Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects.

3.2.2. Pigment analysis

Seawater (2-5 L) was filtered through 47 mm GF/F filters. The filters were stored in liquid nitrogen until further analysis in the shore laboratory. Phytoplankton pigments were extracted using 3 mL 95% acetone for 5 min in an ultrasonic bath filled with ice-water. The extracts were stored overnight at -20°C for HPLC analysis. The entire extraction procedure was carried out in dim light conditions and at low temperature to minimize degradation of pigments. The HPLC analysis was carried out following the method of van Heukelem (2002).

3.2.3. Pigment calibrations and estimation of the analytical detection limit

Pigment calibration standards were purchased from Sigma-Aldrich Company and DHI Water and Environment (Hørsholm, Denmark). At least 6-7 replicate injections were run for each standard. Single point calibrations were made except for chlorophyll *a* for which multipoint calibrations were used and corresponding response factors were determined. The analytical detection limit (ADL) which is typically given by a signal to noise ratio (S/N) = 3-5 was experimentally determined by the technique of serial dilutions, which were in the order of 0.0002-0.0005 mg m^{-3}. Injections of a mix standard were routinely done along with a set of samples to check the accuracy of the response factors and changes in the retention time. Pigment identification in samples was done manually by comparing HPLC in-line diode array detector spectra with those of standards and published spectra. The method followed gave a good response for tropical and subtropical waters with analytical separations of monovynyl chlorophyll *a* from divinyl chlorophyll *a* forms.

3.2.4. Data analyses

Grouping of pigments permits the formulation of variables which is often useful in photo-physiological studies (Bidigare et al. 1987) and have been presented in detail in Table 3.1. For example, the pool of accessory non-chlorophyll *a* pigments are useful in remote-sensing investigations (Trees et al. 2000). Carotenoids are functionally classified as photosynthetic (PSC) and non-photosynthetic (mainly photoprotective,

PPC) (Bidigare et al. 1990, Babin et al. 1996). The PSC pigments include fucoxanthin and the 19′-hexanoyloxyfucoxanthin pigments, and are prominent in regions of high productivity (Barlow et al. 2002). The PPC group includes zeaxanthin, diadinoxanthin and diatoxanthin. This group is dominant in surface waters with low chlorophyll and can account for up to 70% of the total pigment pool (Gibb et al. 2001).

The ratios that can be derived from these pooled variables, for e.g., PSC/TChla, are dimensionless and have the advantage of automatically scaling the comparison of results from different areas and pigment concentrations. The diagnostic pigment (DP) criteria was introduced by Claustre (1994) to estimate a pigment derive analog to the f- ratio (the ratio of new production to total production). The use of DP was extended by Vidussi et al. (2001) and more recently by Barlow et al. (2007) to derive size-equivalent pigment indices which roughly correspond to the biomass proportions of pico, nano and microplankton. Thus, DP can be used to understand the biomass structure of an area purely based on pigment sums and ratios.

Table 3.1. The pigment symbol, names, formulae and taxonomic groups designation (Jeffrey and Vesk 1997) for diagnostic pigment sums and pigment indices (Barlow et al. 2007) including some from Vidussi et al. (2001)*.

Symbol	Pigment	Designation of phytoplankton groups
Chla	Chlorophyll *a* (plus allomers and epimers)	
Chlb	Chlorophyll *b*	Chlorophytes
Chlc1	Chlorophyll c_1	
Chlc2	Chlorophyll c_2	
Chlc3	Chlorophyll c_3	
Chlidea	Chlorophyllide *a*	
DVChla	Divinyl chlorophyll *a*	Prochlorophytes
DVChlb	Divinyl chlorophyll *b*	Prochlorophytes
All	Alloxanthin	Cryptophytes
But	19' -Butanoyloxyfucoxanthin	Crysophytes
Caro	ββ-Carotene + βε-carotene	
Diad	Diadinoxanthin	
Diato	Diatoxanthin	
Fuc	Fucoxanthin	Diatoms
Lut	Lutein	
Hex	19'-Hexanoyloxyfucoxanthin	Prymnesiophytes
Per	Peridinin	Dinoflagellates
Viol	Violaxanthin	
Zea	Zeaxanthin	Cyanobacteria
	Pigment sum	**Formula**
TChla	Total chlorophyll *a*	Chla + DVChlab + Chlidea
Chlbc	Sum of chlorophyll *b* and *c*	Chlb + $Chlc_1$ + $Chlc_2$ +$Chlc_3$
PPC	Photoprotective carotenoids	All + Caro + Diad + Diato + Lut + Viol + Zea
PSC	Photosynthetic carotenoids	But + Fuc + Hex + Per
TPig	Total pigments	TChla + Chlbc + PPC + PSC
DP	Diagnostic pigments	All + But + Chlb + Fuc + Hex + Per + Zea
	Pigment index	**Formula**
DVChla/Tchla	Divinyl chlorophyll *a* to total chlorophyll *a*	DV Chla/TChla
$TChla_{TP}$	Total chlorophyll *a* to total pigments	TChla/Tpig
PPC_{TP}	Photoprotective carotenoids to total pigment	PPC/Tpig
PSC_{TP}	Photosynthetic carotenoids to total pigment	PSC/Tpig
$Diat_{DP}$	Diatom proportion of DP	Fuc/DP
*$Dino_{DP}$	Dinoflagellate proportion of DP	Per/DP
$Flag_{DP}$	Flagellate proportion of DP	(All + But + Chlb + Hex)/DP
$Prok_{DP}$	Prokaryote proportion of DP	Zea/DP

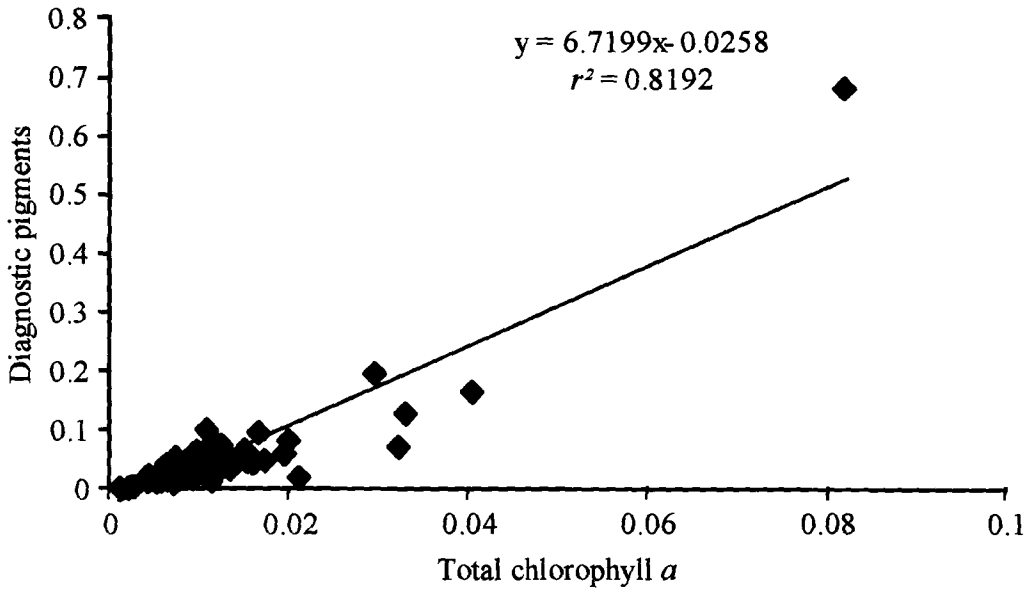


Fig. 3.2. Linear regression between total chlorophyll *a* and diagnostic pigments.

A linear regression between total chlorophyll *a* and diagnostic pigments (DP) showed a significant relationship ($r^2 = 0.8192$, $n = 85$, $p < 0.001$) (Fig. 3.2). Thus, DP was used as an index to understand the pattern of community dominance in the sampling area.

In order to see the regional variation in pigment patterns with respect to the sampled seasons, the two transects in the study area, CP and PK, were partitioned into different regions: Central Oceanic Bay (COB), Andaman Sea (AS), Northern Oceanic Bay (NOB) and the region influenced by river Hooghly, referred to here as River Plume (RP). These regions were partitioned according to oceanic and coastal nature based on the bathymetry of study area (Fig. 3.1). TRMM_3B42 data was downloaded for the grid area, Latitude 5°N-25°N, Longitude 78°E-95°E from Mirador data access (http://mirador.gsfc.nasa.gov/collections/TRMM_3B42_006.shtml) to

obtain rainfall data and to follow changes in the monsoon during the observed period (Fig. 3.3).

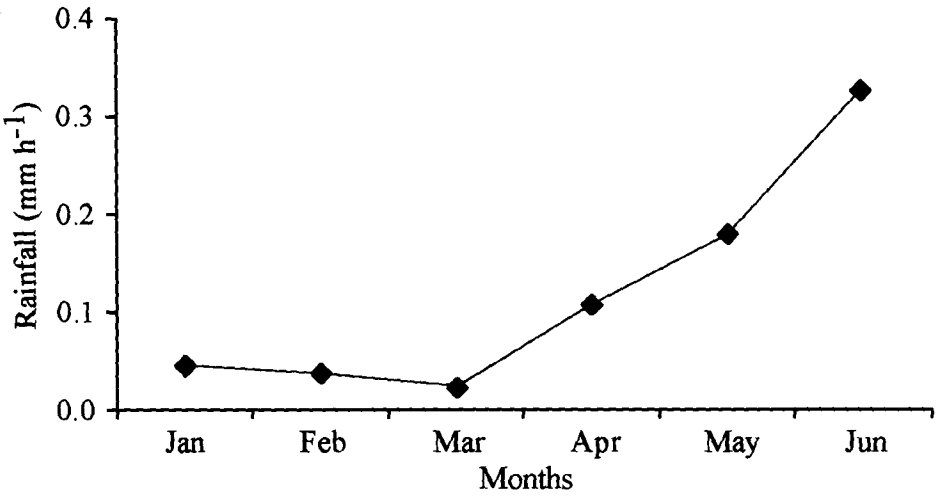


Fig. 3.3. Rainfall (mm h^{-1}) data for the BOB during the period January-June 2007.

The observational period was categorized into two seasons, spring intermonsoon (SpIM, Feb-April 2007) and commencement of the summer monsoon (CSM, May-June 2007).

3.3. Results

3.3.1. The Analytical Detection Limit and total chlorophyll a concentrations

The Analytical Detection Limit (ADL) in this study (the S/N ratio) = 10. Total chlorophyll *a* (TChla) ranged from 0.002-0.067 mg m^{-3} at the CP transect and 0.001-0.681 mg m^{-3} at the PK transect (Figs. 3.4-3.7a,b, Table 3.2). The lowest concentrations of pigments, close to the ADL, were generally associated with the CSM (Fig. 3.7a,b).

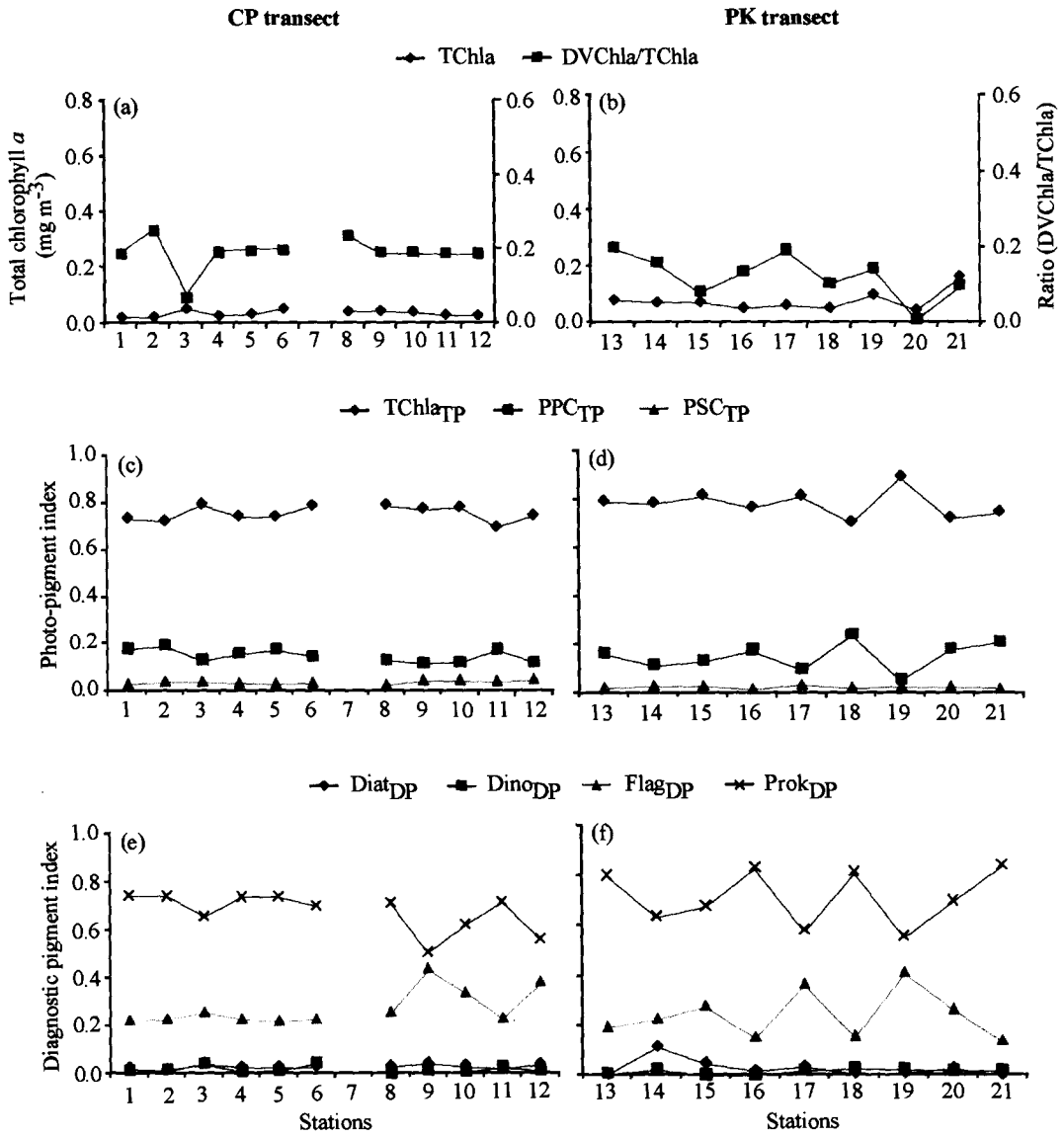


Fig. 3.4. Pigment indices for the period February-March 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll *a* (mg m^{-3}) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.

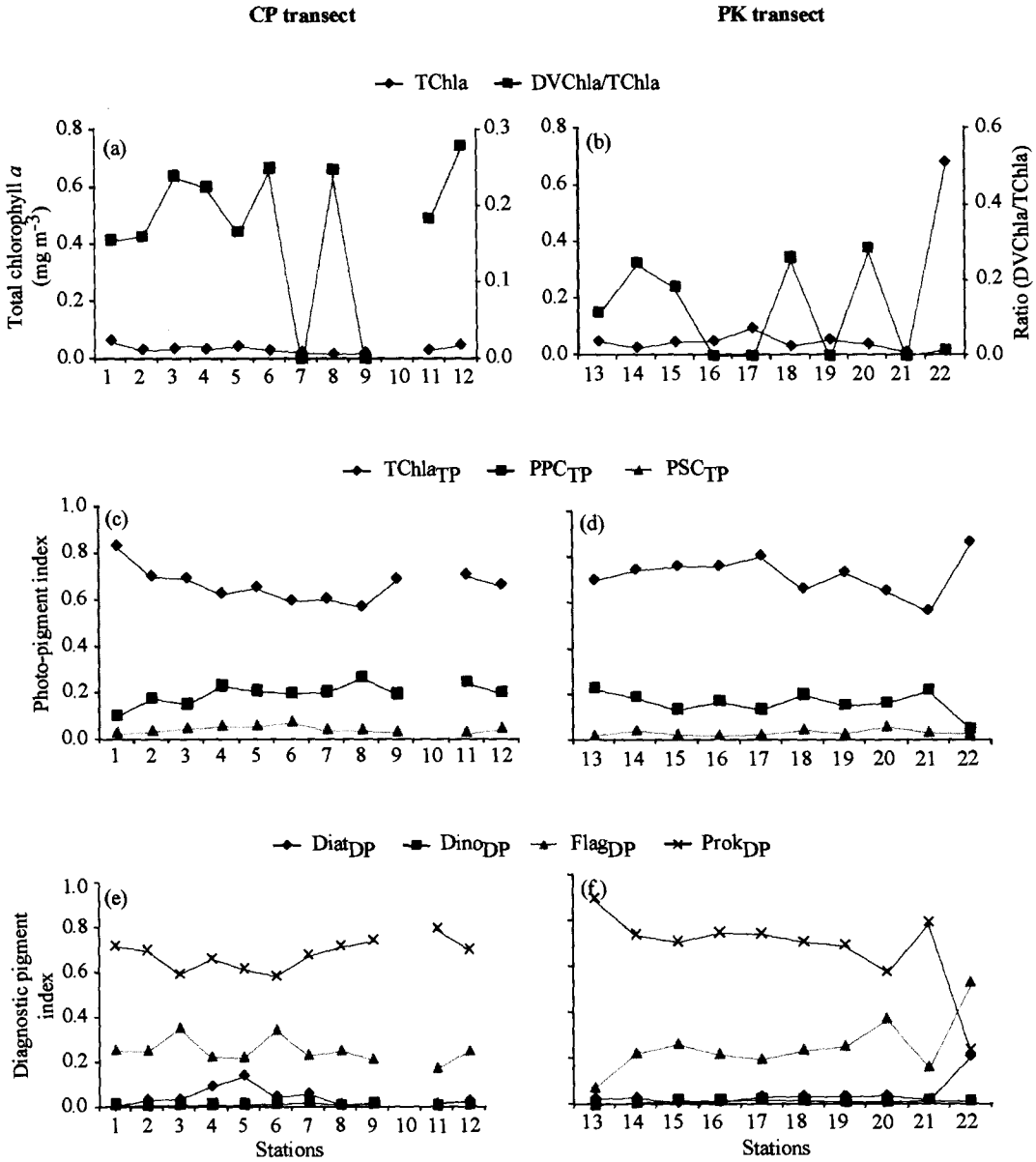


Fig. 3.5. Pigment indices for the period April 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll *a* (mg m^{-3}) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.

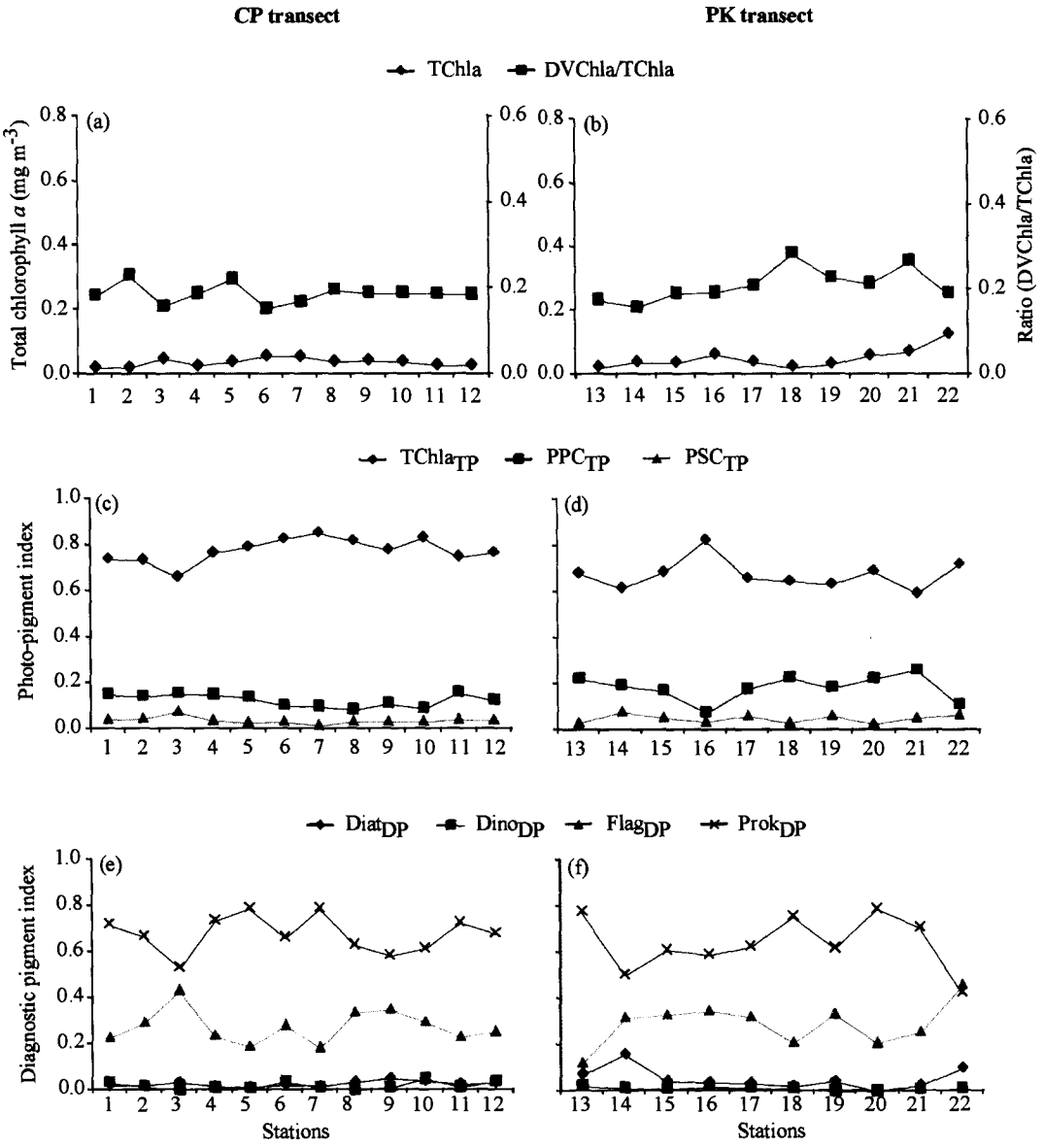


Fig. 3.6. Pigment indices for the period April-May 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll *a* (mg m^{-3}) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.

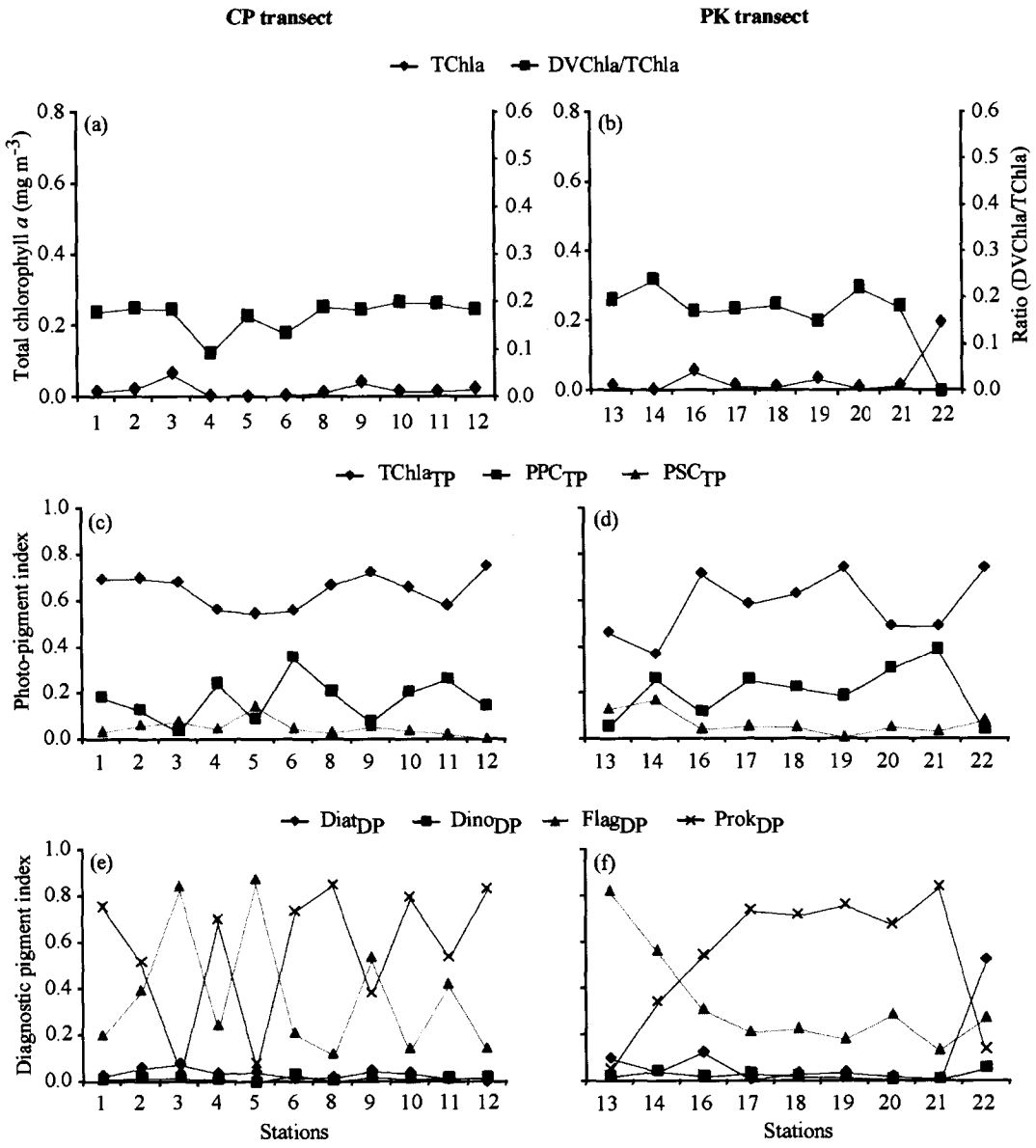


Fig. 3.7. Pigment indices for the period May-June 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll *a* (mg m^{-3}) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.

Table 3.2. Minimum and maximum total chlorophyll *a* (mg m^{-3}) in Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects during the period February-June 2007.

Sampling period	CP transect	PK transect
February-March 2007	0.020-0.051	0.047-0.165
April-2007	0.018-0.064	0.013-0.681
April-May 2007	0.020-0.055	0.023-0.128
May-June 2007	0.002-0.067	0.001-0.196

3.3.2. Photo-pigment indices

The photo-pigment indices revealed the dominance of $TCha_{TP}$ (Total chlorophyll *a*/total pigments) over PPC_{TP} (Photoprotective carotenoids/total pigments) and PSC_{TP} (Photosynthetic carotenoids/total pigments) whereas PPC_{TP} dominance was observed over PSC_{TP} in all the observations at CP and PK transects (Fig. 3.4-3.7c,d).

3.3.3. Diagnostic pigment indices

An evaluation of the diagnostic pigment indices point out the dominance of $Prok_{DP}$ followed by $Flag_{DP}$ throughout the observed region during SpIM (Feb-May 2007), except at the last station of PK (station 22) (Fig. 3.4-3.6e,f). $Diat_{DP}$ followed by $Dino_{DP}$ were present in low proportions. However, during the period CSM (May-June 2007), we could see oscillations in the dominance between $Prok_{DP}$ and $Flag_{DP}$ in the CP transect (Fig. 3.7e), whereas, in the PK transect, $Flag_{DP}$ dominated in the AS and $Prok_{DP}$ was dominant in the NOB(Fig. 3.7f).

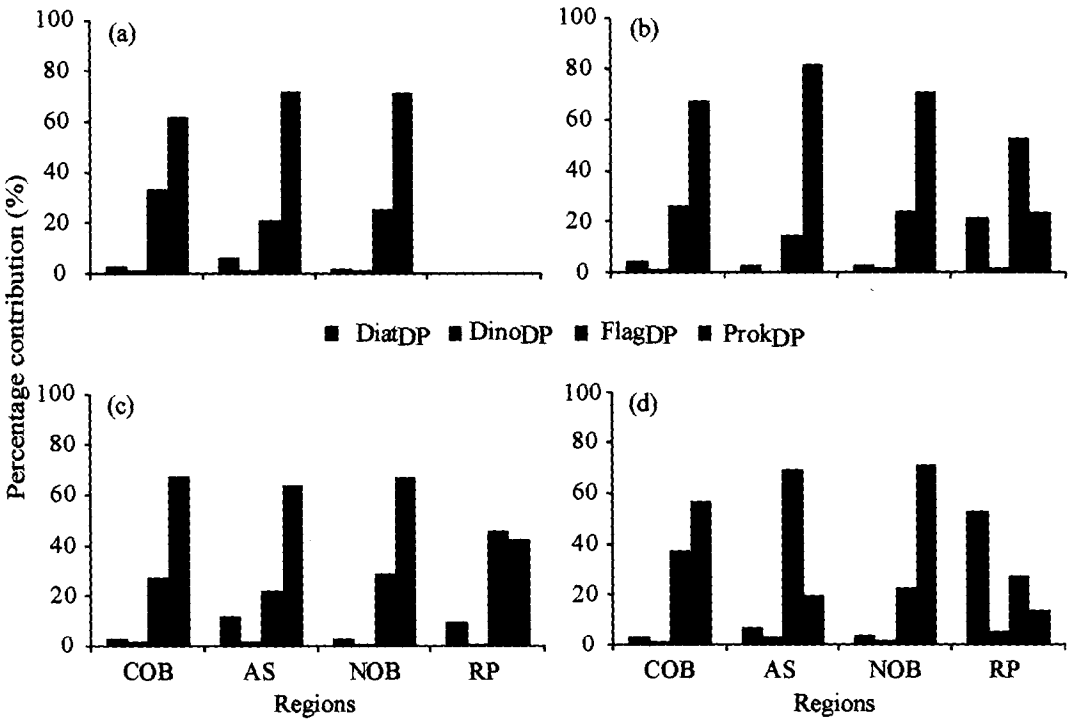


Fig. 3.8. Percentage contribution of diagnostic pigment indices in each region (COB, AS, NOB and RP) in (a) February-March, (b) April, (c) April-May and (d) May June. Description of diagnostic pigment indices in Table 3.1.

3.3.4. Changes in pigment patterns in different regions

Throughout the observation period, the COB and NOB regions were dominated by $Prok_{DP}$ followed by $Flag_{DP}$, whereas a decrease in the percentage contribution of $Prok_{DP}$ and an increase in $Flag_{DP}$ was observed during CSM, as compared to the SpIM (Fig. 3.8a-d). The AS showed a pattern similar to COB and NOB except for the fact that $Flag_{DP}$ dominated over $Prok_{DP}$ during CSM. In the RP region, diagnostic pigment indices for all groups ($Prok_{DP}$, $Flag_{DP}$, $Diat_{DP}$ and $Dino_{DP}$) exhibited a completely different pattern compared to the other three regions (COB, AS and NOB)

(Fig. 3.8a-d). The $Flag_{DP}$ diagnostic pigment index was dominant during the SpIM observations (Fig. 3.8a-c), whereas the $Diat_{DP}$ index was dominant during the CSM (Fig. 3.8d). A considerable increase in $Dino_{DP}$ was also observed during SpIM at the RP region (Fig. 3.8d).

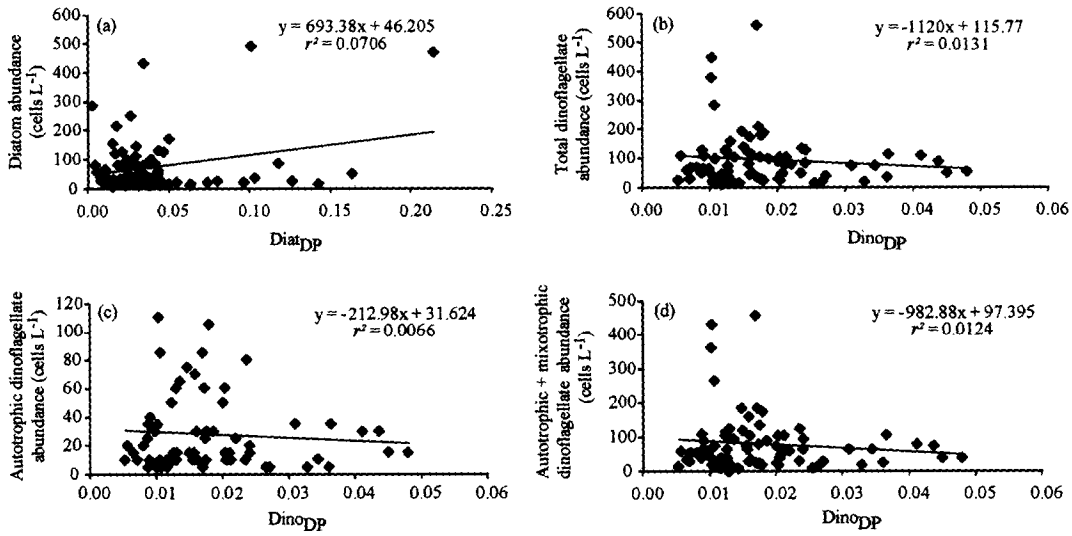


Fig. 3.9. Linear regression between (a) diatom diagnostic pigment and diatom abundance, (b) dinoflagellate diagnostic pigment and total dinoflagellate abundance, (c) dinoflagellate diagnostic pigment and autotrophic dinoflagellate abundance, and (d) dinoflagellate diagnostic pigment and autotrophic + mixotrophic dinoflagellate abundance.

A linear regression between $Diat_{DP}$ and microscopic cell counts of diatoms showed a significant relationship ($r^2 = 0.0706$, $n = 80$, $p < 0.02$) (Fig. 3.9a), whereas $Dino_{DP}$ against dinoflagellate cell counts showed a non-significant relationship ($r^2 = 0.0131$, $n = 74$, p = ns) (Fig. 3.9b). The correlation between $Dino_{DP}$ and dinoflagellate cell counts did not improve even when the mixotrophic and heterotrophic forms were omitted from the total dinoflagellate counts (Fig. 3.9c-d).

3.4. Discussion

The phytoplankton biomass evaluated so far in the region (Radhakrishna et al. 1978, Bhattathiri et al. 1980, Radhakrishna et al. 1982, Devassy et al. 1983, Sarma and Aswanikumar 1991, Gomes et al. 2000, Prasanna Kumar et al. 2002, Madhupratap et al. 2003) is based on fluorometer and spectrophotometer estimations and remote-sensing values. The present study, based on HPLC pigment characterization, is the first report from this area. In this study, the pigment composition was characterized and evaluated in relation to the microscopic cell counts of diatoms and dinoflagellates. The BOB is considered less productive as compared to the Arabian Sea, due to strongly stratified surface waters (Prasanna Kumar et al. 2002). It has been observed that such stratified conditions support the dominance of prokaryotic groups (Chisholm 1992, Cullen et al. 2002). The observations also indicated the dominance of $Prok_{DP}$ in the oceanic waters of the BOB (Fig. 3.4-3.7e,f). In a recent study from the BOB, Hegde et al. (2008) observed that stratified conditions support the prevalence of *Trichodesmium*, which is a prokaryote with the ability to fix nitrogen. Thus, this prokaryote will have a substantial input in new production in stratified waters. In the Baltic Sea, it has been observed that nitrogen fixation by diazotrophs leads to the transfer of newly fixed nitrogen to picoplanktonic organisms and supports the microbial foodweb (Ohlendieck et al. 2000). In the present work, the DVChla/TChla ratio indicated the contribution of *Prochlorococcus* sp. among the picoplankton group (Fig. 3.4-3.7a,b). The metabolic properties of *Prochlorococcus* (Vaulot and Partensky 1992, Casey et

al. 2007, Martiny et al. 2009a) give them a flexible metabolism and the ability to assimilate nitrate and nitrite (Martiny et al. 2009b). Hence, *Prochlorococcus* can assimilate newly fixed nitrogen by micro-prokaryotes like *Trichodesmium* and maintain its dominance in oceanic waters.

Earlier studies on accessory pigments from tropical latitudes demonstrated the greater presence of PPC_{TP} in surface, low chlorophyll waters (Stuart et al. 1998, Gibb et al. 2000, Barlow et al. 2004). The observations from this study also indicate that PPC_{TP} tend to be high in surface waters during the SpIM period (Fig. 3.4-3.7c,d). However, notable changes in the accessory pigments were observed with increase of PSCs during CSM period at few stations of CP transect (Fig. 3.7c). Similar changes were observed in the Atlantic, Pacific, Gulf of Oman and Arabian Sea (Trees et al. 2000, Veldhuis and Kraay 2004). They observed that the change in community structure is a physiological response to the changing environment thereby resulting in changes in accessory pigments. In the present work, the change in accessory pigments during CSM might be due to the responses of the community to the changing environment influenced by rainfall. This indicates that environmental and meteorological conditions may alter phytoplankton dynamics through a chain of linked processes. These variations in accessory pigments in turn are likely to affect the optical properties of phytoplankton which has implications for ocean colour remote-sensing (Sathyendranath et al. 2005).

The second dominant group was the flagellates and their dominance in the AS and RP near coastal regions (Fig. 3.7f) indicate their preference for nutrient-rich coastal waters. Similarly, $Diat_{DP}$ also showed a preference for nutrient-rich turbulent waters, being the dominant group at RP during the CSM (Fig. 3.7f). This change in community structure could be linked to the increased rainfall during this season (Fig. 3.3). Thus a more significant change in community structure can be expected as rainfall reaches its peak. Comparison of diagnostic pigments and microscopic cell counts indicates that though a significant relationship between $Diat_{DP}$ and diatom abundance was observed (Fig. 3.9a), it is pertinent to note that this significance was arrived at even without considering the contribution of haptophytes, which also contain fucoxanthin. In the case of $Dino_{DP}$ versus dinoflagellate abundance (Fig. 3.9b-d) the relationship was not significant. This suggests that peridinin as a marker pigment did not work well for the dinoflagellate population in the region. In view of this, further research comparing the HPLC pigment composition of dinoflagellates with live cell abundances (to eliminate artifacts due to preservatives) should be considered.

Summarizing the results, phytoplankton community structure in the BOB was dominated by $Prok_{DP}$ followed by $Flag_{DP}$ with a low biomass of TChla, during the study period. Changes in the pigment pattern were observed at the onset of the monsoon, indicating the influence of rainfall especially in near coastal regions like AS and RP. Comparative studies between microscopic counts and diagnostic pigment

indices suggest coupling pigment composition analysis with microscopic analysis of natural assemblages to establish valid biogeochemical and ecosystem models. Notably, the components of dinoflagellate communities could be missed by pigment analysis alone.

Chapter 4

Micro-phytoplankton community structure at Mormugao and Visakhapatnam ports

4A.1. Introduction

Phytoplankton dynamics is influenced by the interactions between biotic and abiotic factors. Abiotic factors include physico-chemical conditions such as currents, light, nutrients, salinity and temperature. Biotic factors include interactions with other organisms, including pathogens, parasitoids and grazers (Smetacek et al. 2004).

Seasonal changes in these environmental variables lead to shifts in phytoplankton community structure; these variations are finally linked to climatic factors (Rantajarvi et al 1998, Schiever 1998). Given the wide range of factors governing phytoplankton community structure, it should be noted that the effect of such factors may differ from one geographical area to another depending on the strength and duration of the physical forcing (Longhurst 1998) and also the anthropogenic pressure prevalent in the region. Therefore, qualitative and quantitative analyses of phytoplankton groups in the context of the regulating environmental variables are important to understand the community structure and dynamics of any coastal ecosystem.

The relative significance of biotic and abiotic factors varies over geographical regions and also across seasonal cycles. Such variations are especially prominent in tropical environments such as along the coasts of India, that are influenced by the monsoons. Monsoon events result in alterations in the level of available nutrients to phytoplankton, and also affect mortality through effects of turbulence on physiological processes like cell division (White 1976).

Two distinct monsoon systems influence the coasts of India – the South West Monsoon (SWM) and the North East Monsoon (NEM). This is due to the Indian subcontinent being embraced by different water bodies, the Arabian Sea (AS) in the west and the Bay of Bengal (BOB) in the east. Due to the proximity of the Eurasian land mass and the heating and cooling of air masses over it, the AS and the BOB experience strong monsoonal wind forcing that reverses seasonally. The SWM blows from the southwest toward the northeast in the summer months (June to September), and the northeast monsoon (NEM) blows in the opposite direction during the winter months (December through March) (Schott and McCreary 2001). During the monsoons, precipitation and run-off originating from terrigeneous sources result in increased nutrient discharge into coastal water bodies. Additionally, stormy weather and high wind speed during the monsoons leads to increased suspended load in the coastal water column.

Diatoms followed by dinoflagellates are important groups contributing to coastal water phytoplankton dynamics. Diatoms and dinoflagellates exhibit distinct differences in morphology, mode of nutrition and physiological traits. Diatoms are sessile organisms, largely autotrophic and can assimilate organic matter heterotrophically (Tuchman 1996). Dinoflagellates vary in their mode of nutrition, from auto-, mixo- to heterotrophy. They are motile and can contribute to Harmful Algal Blooms (HABs) (Anderson et al. 2002).

In the present study, two distinct port environments, influenced by region-specific monsoon systems, were sampled - Mormugao Port (MPT) located along the west coast of India and Visakhapatnam Port (VPT) located along the east coast. MPT is mainly influenced by the South West Monsoon (SWM) whereas VPT is influenced by the North East Monsoon (NEM) as well as the SWM.

The aims of the present study were to elucidate (1) the seasonal variations in diatom and dinoflagellate communities in both contrasting environments, and (2) the physico-chemical variables governing these changes.

4A.2. Materials and methods

4A.2.1. Study areas

Mormugao Port (MPT), located at 15°25'N and 73°47'E, is situated at the mouth of the Zuari estuary along the west coast of India (Fig. 4A.1a). Due to its geographical location, it opens to the AS and thus has a high influence of tidal flushing. It is mainly influenced by the SWM.

Visakhapatnam Port (VPT) is located at 17°41'N and 83°16'E, along the east coast of India (Fig. 4A.1b). It is a semi-enclosed water body, with inadequate flushing and stagnant water conditions (Sarma et al 1982, Kadam and Bhangle 1993). This area is also subjected to anthropogenic pressures and various aspects of pollution (Subba Rao and Venkateswara Rao 1980, Sarma et al 1996). VPT is influenced by the NEM as well as the SWM.

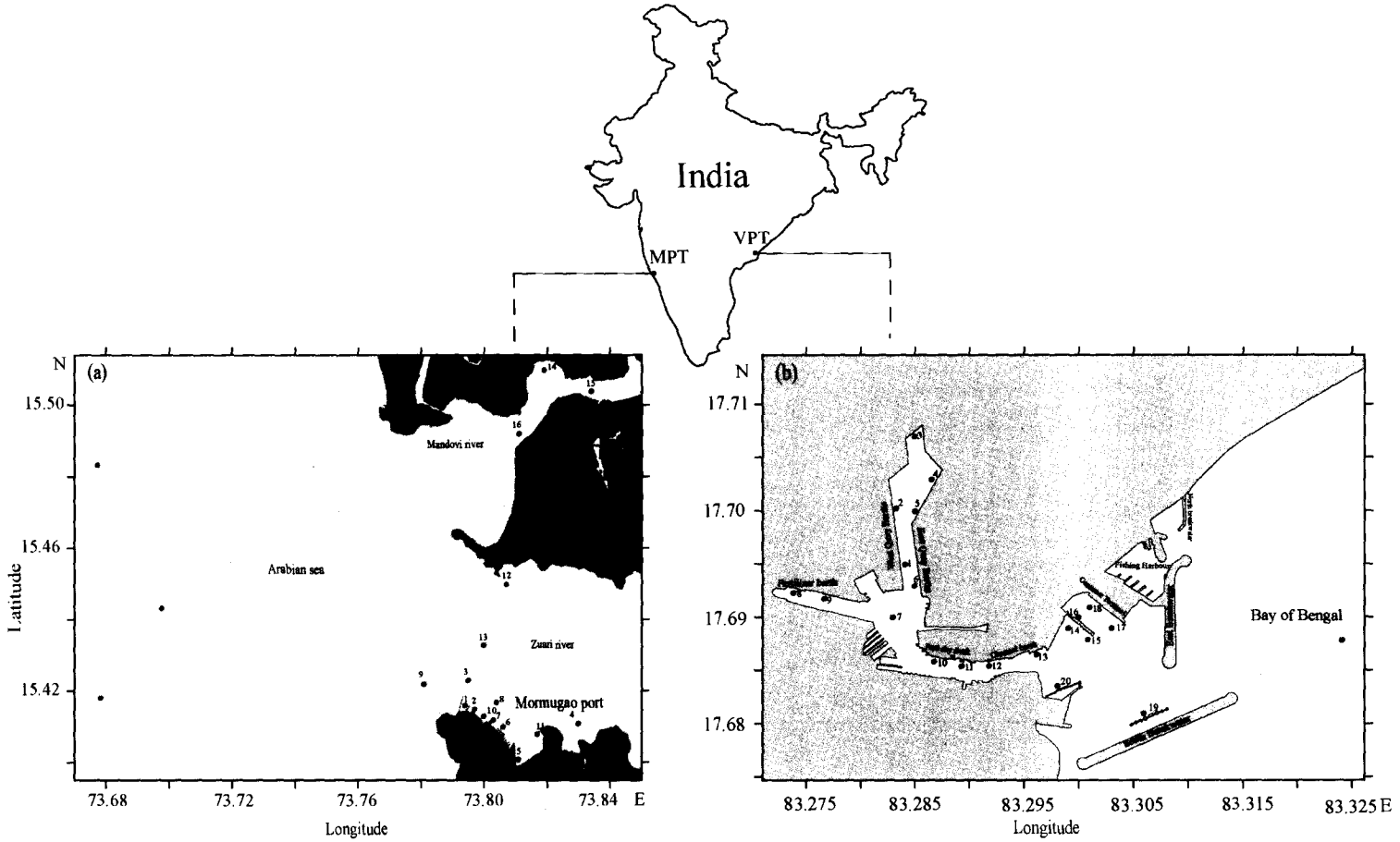


Fig. 4A.1. Location of sampling stations. (a) Mormugao Port (MPT) along the west coast and (b) Visakhapatnam Port (VPT) along the east coast of India.

4A.2.2. Sampling strategy

Surface and near bottom waters at MPT (and adjoining areas) and VPT were sampled for the analysis of phytoplankton and environmental variables. Only those stations that were sampled during all the 3 sampling periods have been considered for further analysis (16 and 20 stations at MPT and VPT respectively) (Tables 4A.1, 4A.2).

Table 4A.1. Details of stations in Mormugao Port (MPT), India.

Station no.	Station name	Latitude	Longitude
1	MPT, Berth-1	15.416	73.794
2	MPT, Berth-3	15.415	73.797
3	MPT, Channel Marker Buoy-6	15.423	73.795
4	Goa Shipyard	15.411	73.830
5	MPT, Berth-11	15.401	73.811
6	MPT, Berth-9	15.410	73.806
7	MPT, Berth-8	15.412	73.803
8	MPT, Mooring Dolphin-2	15.417	73.804
9	MPT, Channel Marker Buoy-7	15.422	73.781
10	MPT, Berth-5	15.413	73.800
11	MPT, Vasco Bay	15.408	73.817
12	Dona Paula Jetty	15.450	73.807
13	MPT – Dona Paula, Mid Point	15.433	73.800
14	Mandovi, Verem Bay	15.510	73.819
15	Mandovi, RND Jetty	15.504	73.834
16	Mandovi, (Youth Hostel)	15.492	73.811

Sampling was conducted on different occasions - May 2005/pre-monsoon (PrM), December 2005/post-monsoon (PoM) and September 2006/withdrawal of the SWM (WSWM) at MPT and November-December 2007 (NEM), April 2008 (PrM) and August 2008 (SWM) at VPT. Surface and near bottom seawater samples (1L) were

collected using a bucket and Niskin sampler, respectively. Sampling details are provided in Appendices 1 (MPT) and 2 (VPT).

4A.2.3. Analysis of environmental variables

Temperature, pH and nutrients such as nitrate (NO_3-N), nitrite (NO_2-N), phosphate (PO_4-P), silicate (Si) and ammonia (NH_4) (SKALAR autoanalyzer), were analyzed using standard procedures (Parsons et al. 1984). Ammonia concentrations were analyzed only in Visakhapatnam.

Table 4A.2. Details of stations in Visakhapatnam Port (VPT), India.

Station no.	Station name	Latitude	Longitude
1	West Quay Berth-1	17.695	83.284
2	West Quay Berth-4	17.700	83.283
3	East Quay Berth-9	17.707	83.285
4	East Quay Berth-7	17.703	83.286
5	East Quay Berth-5	17.700	83.285
6	East Quay Berth-1	17.693	83.285
7	Turning Basin	17.690	83.283
8	Fertilizer Wharf	17.692	83.274
9	Oil Refinery Berth-2	17.692	83.277
10	Port Dry Dock	17.686	83.286
11	DC Jetty	17.686	83.292
12	Boat Basin Berth-3	17.686	83.289
13	Dredger Berth	17.686	83.296
14	General Cargo Berth N	17.689	83.299
15	Ore Berth–1	17.688	83.301
16	Ore Berth–2	17.690	83.300
17	Container Berth-1	17.689	83.303
18	Container Berth-2	17.691	83.301
19	Oil Berth	17.681	83.306
20	LPG Berth	17.684	83.298

4A.2.4. Analysis of phytoplankton

For analysis of phytoplankton, seawater samples (1L) were preserved in Lugol's iodine immediately after collection. The samples were allowed to settle for 48 hours, decanted to a volume of 100 mL, and subsequently observed using light microscopy at 100X and 200X magnification. Enumeration and identification was carried out to the lowest possible taxonomic level using the keys provided by Subrahmanyan (1946), Desikachary (1987) and Tomas (1997) and references therein. Phytoplankton abundance was expressed as cells L^{-1}.

4A.2.5. Data analyses

Canonical Correspondence Analysis (CCA) (ter Braak 1995) was performed to evaluate the temporal variations in the effect of the environmental characteristics of the water column on the diatom and dinoflagellate communities at MPT and VPT, using the Multi-Variate Statistical Package program version 3.1 (Kovach 1998).

The CCA results are presented as 2 graphs (station and species biplots), that indicate the relationship between the species, stations and environmental variables. The physico-chemical variables are plotted as arrows, which point in the direction of increasing values of the environmental variable. The lengths of the arrows are directly proportional to their importance. The angle between the arrow and an ordination axis signifies the extent of correlation between them; the smaller the angle, the stronger the correlation. A perpendicular line drawn from an arrow through a site

or species points to the relative location of that site or species along an environmental gradient (Palmer 1993).

The univariate measures of the diatom community (species richness, evenness and diversity) were analyzed using PRIMER (version 5) and the variations in these were determined by two-way ANOVA. Two-way ANOVA was also performed on the diatom and dinoflagellate abundance data to evaluate spatial and temporal variation (Sokal and Rohlf 1981). The seasonal variations in phytoplankton, diatoms and dinoflagellates and the abundance profiles of the five most dominant taxa during each sampling period in both MPT and VPT are presented as SURFER plots using the SURFER 8 program. The pennate:centric (P:C) ratio of diatoms and the percentage contribution of autotrophic, mixotrophic and heterotrophic forms of dinoflagellates was also calculated.

4A.3. Results

4A.3.1. Mormugao Port (MPT)

4A.3.1a. Environmental variables

The highest temperature was observed during PrM (31-32°C) (Fig. 4A.2a). During PoM and WSWM it was lower by 3°C (Fig. 4A.2a). pH was maximum (8.34-8.35) during PoM, and minimum (7.86-7.91) during WSWM (Fig. 4A.2b). Nutrient (NO_2, NO_3, SiO_4 and PO_4) concentrations were highest during the WSWM and minimum during PrM (Fig. 4A.2c-f).

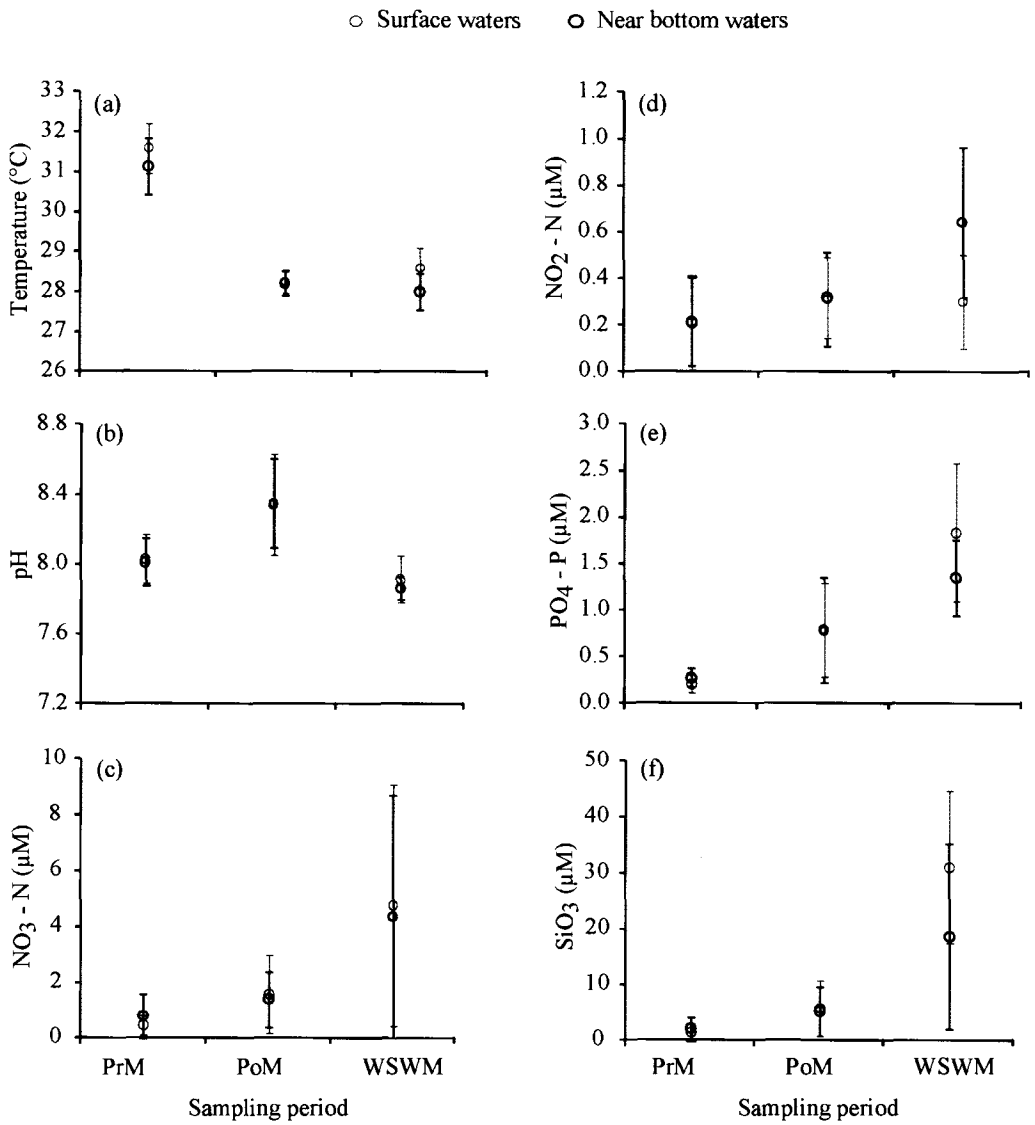


Fig. 4A.2. Seasonal variation in physico-chemical variables in Mormugao Port (MPT), India. (a) Temperature (°C), (b) pH, (c) Nitrate (µM), (d) Nitrite (µM), (e) Phosphate (µM) and (f) Silicate (µM). PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon.

4A.3.1b. Phytoplankton community

Overall, 207 species belonging to 77 genera were recorded in the water column (Table 4A.3). The abundance range and frequency of occurrence are given in Appendix 3).

Table 4A.3. Phytoplankton species recorded in surface (S) and near bottom (NB) waters in Mormugao Port (MPT), India during pre-monsoon (PrM), post-monsoon (PoM) and withdrawal of the South West Monsoon (WSWM).

Taxon	Species code	CCA code	PrM		PoM		WSWM	
			S	NB	S	NB	S	NB
Diatoms								
Centric								
Asterolampra marylandica	AslM	1				+		
Asterolampra sp.	Aslsp	2			+	+	+	+
Asteromphalus cleveanus	AspC	3		+				
Bacteriastrum sp.	Baps	9	+			+		
Bellerochea sp.	Belrsp	10		+				+
Biddulphia biddulphiana	BidB	11	+					+
Cerataulina bergonii	CetBr	12						+
Cerataulina bicornis	CetB	13	+	+	+			+
Cerataulina pelagica	CetP	14			+	+		
Cerataulina sp.	Cets	15					+	
Chaetoceros affinis	ChA	16	+	+	+	+	+	+
Chaetoceros compressus	ChCmp	17	+	+		+	+	
Chaetoceros curvisetus	ChCr	18	+	+	+	+	+	
Chaetoceros danicus	ChDa	19	+	+	+		+	
Chaetoceros decipiens	ChDe	20	+	+	+	+	+	+
Chaetoceros didymus	ChDid	21			+	+		
Chaetoceros diversus	ChDiv	22	+					
Chaetoceros laciniosus	ChLac	23	+					
Chaetoceros lorenzianus	ChLo	24	+				+	+
Chaetoceros messanensis	ChMe	25			+	+		
Chaetoceros peruvianus	ChPe	26	+	+				
Chaetoceros tortissimus	ChTo	28			+			
Chaetoceros sp.	Chs	29	+	+	+	+	+	+
Corethron criophilum	CorC	30	+	+				
Corethron hystrix	CorH	31	+	+				
Corethron sp.	Cors	32	+					
Coscinodiscus curvatulus	CosCur	33		+				
Coscinodiscus eccentricus	CosE	34		+				
Coscinodiscus gigas var. *punctiformis*	CosGi	35				+	+	+

Table 4A.3. Contd....

...Table 4A.3. Contd.

Taxon	Species code	CCA code	PrM		PoM		WSWM	
			S	NB	S	NB	S	NB
Coscinodiscus granii	CosG	36	+	+	+			
Coscinodiscus lineatus	CosL	37	+	+	+			+
Coscinodiscus marginatus	CosM	38	+	+	+	+	+	+
Coscinodiscus nodulifer	CosN	39			+	+		
Coscinodiscus radiatus	CosR	40	+	+	+	+	+	+
Coscinodiscus sp.	Coss	41	+	+	+	+	+	+
Cyclotella caspia	CyC	42	+	+	+	+	+	
Cyclotella littoralis	CyL	43		+		+	+	+
Cyclotella sp.	Cys	44	+	+	+	+	+	+
Dactyliosolen fragilissimus	DaF	45		+				
Ditylum brightwellii	DitB	46	+	+	+	+	+	+
Ditylum sol	Dits	47	+	+	+	+	+	+
Guinardia delicatula	GuD	49	+					
Guinardia flaccida	GuF	51	+	+			+	+
Guinardia striata	GuS	52	+	+	+	+	+	+
Guinardia sp.	Gus	53	+	+	+	+		
Helicotheca tamesis	HeT	54	+	+	+	+	+	+
Hemiaulus hauckii	HmH	55	+	+	+			
Hemiaulus membranaceus	HmM	57			+			
Hemiaulus sinensis	HmS	58	+		+			
Hemidiscus hardmanianus	HemH	59	+					
Lauderia annulata	LaA	60	+	+			+	+
Lauderia sp.	Las	61					+	+
Leptocylindrus danicus	LeD	62	+		+		+	+
Leptocylindrus minimus	LeMi	63			+			
Lithodesmium sp.	Lis	64			+			
Melosira nummuloides	MeN	65	+	+				
Odontella aurita	OdA	66	+	+		+	+	+
Odontella mobiliensis	OdM	67	+	+		+	+	+
Odontella sinensis	OdS	68	+	+			+	+
Odontella sp.	Ods	69	+		+			+
Paralia sulcata	ParS	70	+	+		+		+
Planktoniella sol	PlaS	71	+	+	+	+		+

Table 4A.3. Contd....

...Table 4A.3. Contd.

Taxon	Species code	CCA code	PrM		PoM		WSWM	
			S	NB	S	NB	S	NB
Proboscia alata	PrA	72				+	+	+
Rizosolenia curvata	RhC	75						+
Rhizosolenia formosa	RhF	76	+					+
Rhizosolenia hebetata	RhH	77				+		+
Rhizosolenia setigera	RhSe	79			+		+	+
Rhizosolenia styliformis	RhSt	80	+		+		+	+
Rhizosolenia sp.1	Rhs	81					+	+
Skeletonema costatum	SkC	82	+		+	+	+	+
Skeletonema sp.	Sks	84					+	+
Stephanopyxis sp.	Sts	85						+
Thalassiosira punctigera	ThsP	86					+	
Thalassiosira subtilis	ThsS	87	+					
Thalassiosira sp.	Thss	88	+		+	+	+	+
Triceratium caudatum	TcerC	89			+			
Triceratium favus	TcerF	90			+			+
Triceratium sp.	Tcers	91	+				+	
Pennate								
Achnanthes sp.	Achs	93	+		+	+		
Amphiprora gigantea	AmpGi	94	+	+	+	+		+
Amphiprora sp.	Amps	95	+	+	+	+		+
Amphora sp.	Ams	96	+	+		+	+	
Asterionellopsis glacialis	AsnG	97	+	+				
Asterolampra marylandica	AstlM	98			+			
Bacillaria paxillifera	BacP	99	+	+				
Caloneis sp.	Cals	100			+			
Climacosphaenia sp.	Clims	101			+			
Cylindrotheca closterium	CylC	102	+	+	+	+	+	+
Diploneis crabro	DipC	103	+	+	+	+		+
Diploneis sp.	Dips	104	+	+	+	+		+
Ephemera planamembranacea	EphP	105		+		+		
Fragilaria striatula	FraS	107						+
Fragilariopsis cylindrus	FrsC	108		+		+		
Fragilariopsis sp.	Frss	109			+			+
Gyrosigma sp.	Gyrs	110	+		+	+		
Haslea sp.	Hss	113	+	+		+	+	+

Table 4A.3. Contd....

...Table 4A.3. Contd.

Taxon	Species code	CCA code	PrM		PoM		WSWM	
			S	NB	S	NB	S	NB
Licmophora undulatum	LiU	114				+		
Licmophora sp.	Lis	115	+		+			
Meuniera membranacea	MuM	116	+	+				+
Navicula sp. 1	Nvs1	117	+	+		+		
Navicula transitans var. *derasa* f. *delicatula*	NvTDe	118	+	+	+	+	+	+
Navicula transitans var. *derasa*	NvTDs	119	+	+	+	+	+	+
Navicula granii	NvG	121			+	+		
Navicula sp.	Nvs	122	+	+	+	+	+	+
Nitzschia bilobata	NiB	124		+				
Nitzschia delicatissima	NiDe	125	+	+		+		
Nitzschia longissima	NiL	126				+		
Nitzschia panduriformis	NiP	127	+	+	+	+		
Nitzschia seriata	NiSe	128	+	+	+			
Nitzschia sigma	NiSi	129	+	+	+	+	+	+
Nitzschia sp.	Nis	130	+	+	+	+	+	+
Pinnularia rectangulata	PiR	131	+		+	+		+
Pinnularia spp.	Pis	132						+
Phaeodactylum tricornutum	PhTr	133		+				
Pleurosigma angulatum	PleA	136	+	+	+	+		+
Pleurosigma directum	PleD	137	+	+	+	+		+
Pleurosigma elongatum	PleE	138	+	+	+	+		
Pleurosigma fasciola	PleFa	139	+	+				
Pleurosigma formosa	PleFo	140	+					
Pleurosigma naviculaceum	PleNa	141	+		+	+		
Pleurosigma normanii	PleNo	142	+		+	+	+	+
Pleurosigma sp.	Ples	143	+		+	+	+	+
Pseudo-nitzschia australis	PsnA	144			+	+		
Pseudo-nitzschia delicatissima	PsnD	145	+					
Pseudo-nitzschia pungens	PsnPu	147	+		+	+		
Pseudo-nitzschia sp.1	Psns1	148			+			
Pseudo-nitzschia sp.	Psns	149	+		+	+	+	+
Surirella fastuosa	SuF	150	+		+	+		+
Surirella ovata	SuOt	151			+	+		
Surirella sp.	Sus	152					+	+

Table 4A.3. Contd....

...Table 4A.3. Contd.

Taxon	Species code	CCA code	PrM		PoM		WSWM	
			S	NB	S	NB	S	NB
Synedra pulchella	SyP	153	+		+	+		
Synedra sp.	Sys	154			+	+	+	+
Thalassionema bacillare	ThnB	155	+		+	+	+	+
Thalassionema frauenfeldii	ThnF	156						+
Thalassionema nitzschioides	ThnN	157	+		+	+	+	
Thalassionema sp.	Thns	158				+		
Tropidoneis sp.	Trops	159	+		+			
Dinoflagellates								
Autotrophic								
Alexandrium catenatum	AxCt	160	+		+	+		
Alexandrium compressum	AxCm	161			+			
Alexandrium sp.	Axs	163	+		+	+	+	+
Amphidinium sp.	Ampds	164	+		+	+		+
Blepharocysta sp.	Bleps	165	+					
Ensiculifera sp.	Ensis	166			+			
Gambierdiscus sp.	Gambs	167	+		+	+		+
Gonyaulax digitale	GnxDle	168	+		+			
Gonyaulax monospina	GnxMo	169			+	+		
Gonyaulax polygramma	GnxP	170	+		+			
Gonyaulax scrippsae	GnxS	171						
Gonyaulax spinifera	GnxSp	172	+		+			
Gonyaulax verior	GnxVer	173			+			
Gonyaulax sp.	Gnxs	174	+		+	+	+	
Gymnodinium sp.	Gyms	175	+		+	+	+	+
Oxytoxum scolopax	OxSc	177				+		
Oxytoxum sp.	Oxs	178					+	
Peridinium quinquecorne	Prqn	179			+		+	
Podolampas bipes	PoB	180			+			
Pyrophacus spp.	PyhH	182					+	
Torodinium teredo	TorT	183				+		
Torodinium sp.	Tors	184						+

Table 4A.3. Contd....

...Table 4A.3. Contd.

Taxon	Species code	CCA code	PrM		PoM		WSWM	
			S	NB	S	NB	S	NB
Mixotrophic								
Ceratium dens	CerDn	185						
Ceratium extensum	CerEx	186			+			
Ceratium furca	CerFr	187	+		+	+	+	+
Ceratium fusus	CerFs	188			+	+	+	
Ceratium lineatum	CerL	191			+	+		
Ceratium macroceros	CerM	192	+					
Ceratium pentagonum	CerP	193			+	+		
Ceratium tripos	CerTp	196				+		
Dinophysis acuminata	DyA	197	+		+	+		
Dinophysis acuta	DyAc	198	+				+	
Dinophysis caudata	DyC	199	+				+	+
Dinophysis norvegica	DyN	201	+		+			
Dinophysis sp.	Dys	202			+	+		
Prorocentrum gracile	ProG	203	+		+	+	+	+
Prorocentrum micans	ProM	204	+		+	+	+	+
Prorocentrum minimus	ProMi	205					+	
Prorocentrum scutellum	ProS	207				+		
Prorocentrum sigmoides	ProSg	208	+		+	+	+	+
Prorocentrum sp.	Pros	209	+		+	+	+	+
Pyrocystis hamulus	PysH	210			+	+		
Scrippsiella trochoidea	ScT	211	+		+	+	+	+
Scrippsiella sp.	Scs	212	+		+			
Heterotrophic								
Diplopsalis lenticulata	DipL	213					+	
Diplopsalis sp.	Dips	214						+
Gotoius abei	GotA	215	+		+	+		+
Gyrodinium sp.	Gyds	216	+		+	+	+	+
Katodinium sp.	Kats	217			+			
Phalacroma rotundata	PhR	218	+		+	+		+

Table 4A.3. Contd....

...Table 4A.3. Contd.

Taxon	Species code	CCA code	PrM		PoM		WSWM	
			S	NB	S	NB	S	NB
Protoperidinium brevipes	PpBr	220	+		+	+		
Protoperidinium conicum	PpC	221	+			+		+
Protoperidinium depressum	PpDe	222	+		+			
Protoperidinium divergens	PpDi	223	+			+	+	+
Protoperidinium excentricum	PpEx	224	+		+	+		
Protoperidinium leonis	PpLe	225	+					
Protoperidinium minutum	PpMi	226	+		+	+	+	+
Protoperidinium oblongum	PpOb	227	+		+			
Protoperidinium pacificum	PpPc	228			+	+		
Protoperidinium pallidum	PpPal	229	+		+		+	+
Protoperidinium pedunculatum	PpPd	230	+		+			
Protoperidinium pellucidum	PpPl	231	+		+		+	+
Protoperidinium pentagonum	PpPn	232			+		+	+
Protoperidinium pyriforme	PpPy	233						+
Protoperidinium steinii	PpSt	234	+				+	
Protoperidinium subinerme	PpSu	235	+		+	+		
Protoperidinium stellatum	PpSt	236			+			
Protoperidinium sp.	Pps	237	+		+	+	+	+
Warnowia sp.	Wars	238			+	+		
Zygabikodinium sp.	Zygs	239	+					

Phytoplankton abundance varied from 1.3 x 10^4 to 2.8 x 10^5 cells L^{-1} in surface waters and from 1.2 x 10^4 to 5.8 x 10^4 cells L^{-1} in near bottom waters (average and standard deviation values in Fig. 4A.3a). Phytoplankton abundance (N), Margalef's species richness (*d*), Pielou's evenness (*J'*) and Shannon-Wiener diversity (*H'*) in surface and near bottom waters did not show significant variation across stations (spatial abundance details in Fig. 4A.3'). However, significant temporal variation was observed in all the measures except species richness in surface waters (two-way ANOVA, Tables 4A.4. 4A.5).

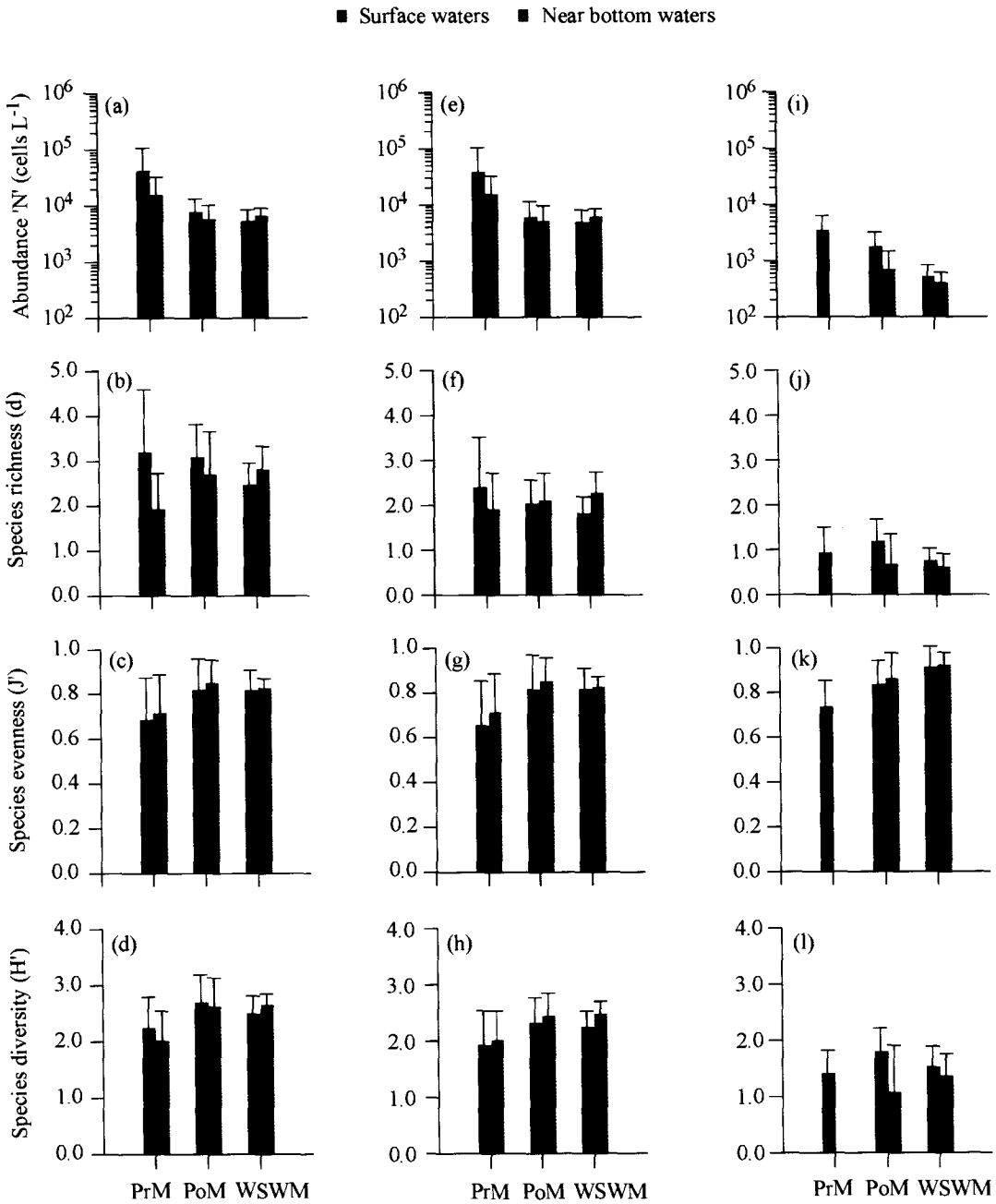


Fig. 4A.3. Seasonal variation in abundance (cells L^{-1}), species richness, evenness and diversity of the (a-d) phytoplankton, (e-h) diatom and (i-l) dinoflagellate communities in Mormugao Port (MPT), India. PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon.

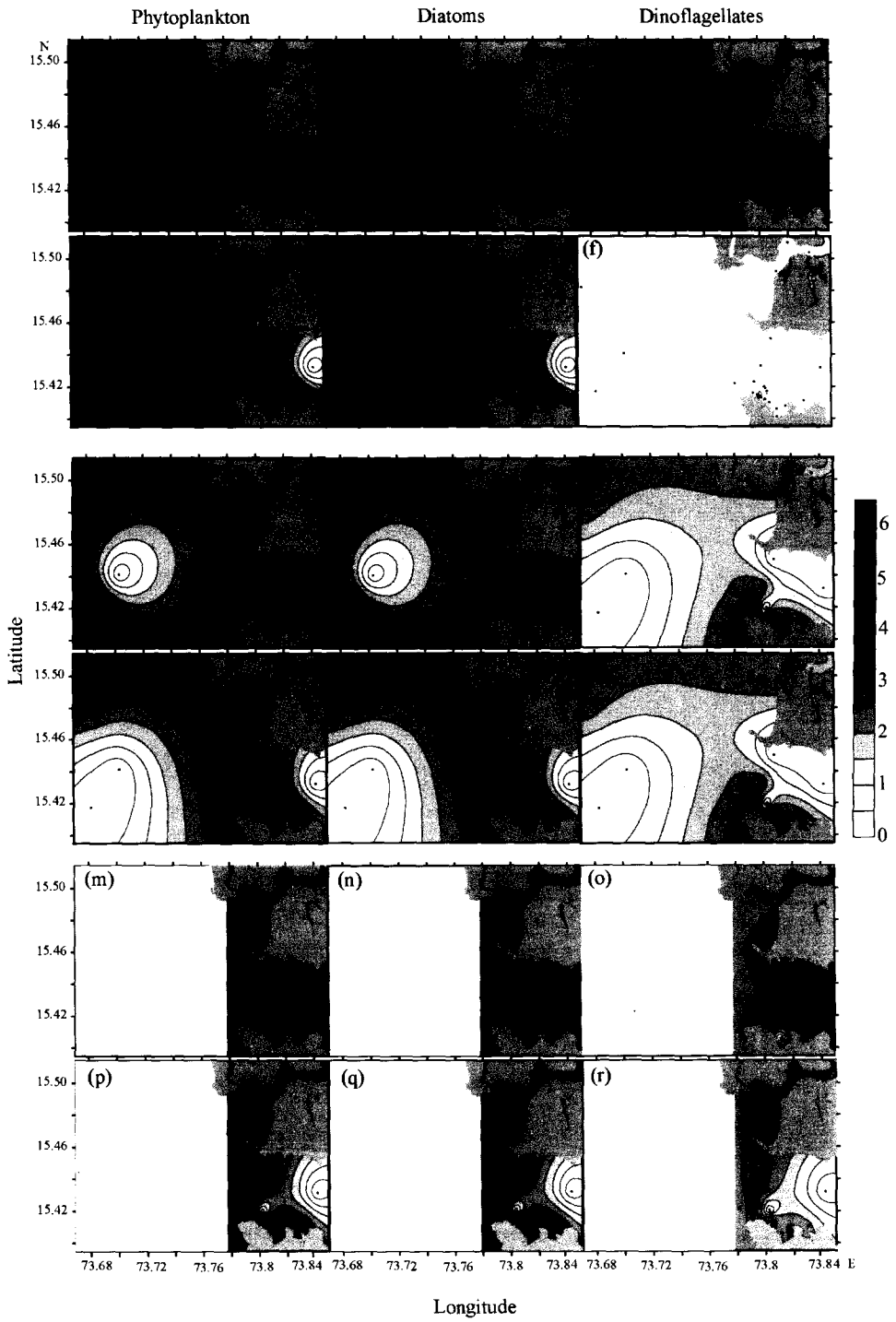


Fig. 4A.3'. Spatial variation in abundance (cells L^{-1}) of phytoplankton, diatoms and dinoflagellates during (a-f) PrM, (g-l) PoM (m-r) WSWM in Mormugao Port (MPT), India. a-c, g-i, m-o: surface waters; d-f, j-l, p-r: near bottom waters. PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon. Scale: log(x+1).

Table 4A.4. Two-way ANOVA to evaluate the variation in abundance, species richness, evenness and diversity of phytoplankton, diatom and dinoflagellates in surface waters of Mormugao port (MPT), India. Values in bold indicate significant variation.

Factor	Phytoplankton				Diatoms				Dinoflagellates			
	df	SS	MS	*p-value*	*df*	SS	MS	*p-value*	*df*	SS	MS	*p-value*
Abundance (N)												
Station	15	111.09	7.41	0.1942	15	111.65	7.44	0.3091	15	28.94	1.93	0.1523
Season	2	172.40	86.20	**0.0000**	2	169.92	84.96	**0.0001**	2	53.43	26.71	**0.0000**
Within sub-group error	30	154.84	5.16		30	182.68	6.09		30	37.54	1.25	
Total	47	438.33			47	464.25			47	119.90		
Species richness (*d*)												
Station	15	12.58	0.84	0.5719	15	7.40	0.49	0.6227	15	3.76	0.25	0.2175
Season	2	4.92	2.46	0.0878	2	2.76	1.38	0.1108	2	1.50	0.75	**0.0260**
Within sub-group error	30	27.95	0.93		30	17.47	0.58		30	5.43	0.18	
Total	47	45.44			47	27.63			47	10.69		
Species evenness (*J'*)												
Station	15	0.31	0.02	0.5216	15	0.24	0.02	0.8721	15	0.16	0.01	0.4989
Season	2	0.19	0.09	**0.0226**	2	0.28	0.14	**0.0147**	2	0.26	0.13	**0.0002**
Within sub-group error	30	0.65	0.02		30	0.85	0.03		30	0.34	0.01	
Total	47	1.14			47	1.36			47	0.76		
Species diversity (*H'*)												
Station	15	3.59	0.24	0.3339	15	0.24	0.02	0.8721	15	2.39	0.16	0.4156
Season	2	1.73	0.86	**0.0233**	2	0.28	0.14	**0.0147**	2	1.24	0.62	**0.0248**
Within sub-group error	30	6.06	0.20		30	0.85	0.03		30	4.44	0.15	
Total	47	11.39			47	1.36			47	8.07		

Table 4A.5. Two-way ANOVA to evaluate the variation in abundance, species richness, evenness and diversity of phytoplankton, diatom and dinoflagellates in near bottom waters of Mormugao Port (MPT), India. Values in bold indicate significant variation.

Factor	Phytoplankton				Diatoms				Dinoflagellates			
	df	SS	MS	*p-value*	*df*	SS	MS	*p-value*	*df*	SS	MS	*p-value*
Abundance (N)												
Station	15	61.16	4.08	0.2290	15	60.99	4.07	0.2563	15	18.96	1.26	0.3356
Season	2	31.11	15.56	**0.0116**	2	42.15	21.08	**0.0037**	2	207.04	103.52	**0.0000**
Within sub-group error	30	89.85	3.00		30	93.13	3.10		30	32.06	1.07	
Total	47	182.12			47	196.28			47	258.06		
Species richness (*d*)												
Station	15	5.69	0.38	0.8894	15	3.54	0.24	0.9195	15	2.76	0.18	0.3669
Season	2	7.45	3.72	**0.0099**	2	1.01	0.50	0.3547	2	4.27	2.14	**0.0001**
Within sub-group error	30	20.70	0.69		30	14.07	0.47		30	4.85	0.16	
Total	47	33.84			47	18.62			47	11.88		
Species evenness (*J'*)												
Station	15	0.22	0.01	0.4262	15	0.23	0.02	0.3912	15	0.78	0.05	0.5215
Season	2	0.17	0.08	**0.0063**	2	0.17	0.08	**0.0057**	2	7.20	3.60	**0.0000**
Within sub-group error	30	0.41	0.01		30	0.41	0.01		30	1.64	0.05	
Total	47	0.80			47	0.81			47	9.62		
Species diversity (*H'*)												
Station	15	2.66	0.18	0.5225	15	2.50	0.17	0.4157	15	4.93	0.33	0.2360
Season	2	4.20	2.10	**0.0002**	2	2.15	1.07	**0.0033**	2	16.83	8.41	**0.0000**
Within sub-group error	30	5.58	0.19		30	4.65	0.15		30	7.32	0.24	
Total	47	12.44			47	9.30			47	29.08		

In surface waters, phytoplankton abundance and species richness was highest in PrM; Pielou's evenness (J') and Shannon-Wiener diversity (H') were maximum in PoM (Fig. 4A.3). In near bottom waters, abundance was highest in PrM. Species evenness was highest in PoM whereas species richness and diversity were maximum in WSWM (Fig. 4A.3).

4A.3.1c. Diatom community

Diatoms constituted 77-100% of the phytoplankton community (Fig. 4A.4). A total of 137 diatom species (78 centric, 59 pennate) belonging to 52 genera (26 centric, 26 pennate) were observed (Table 4A.3).

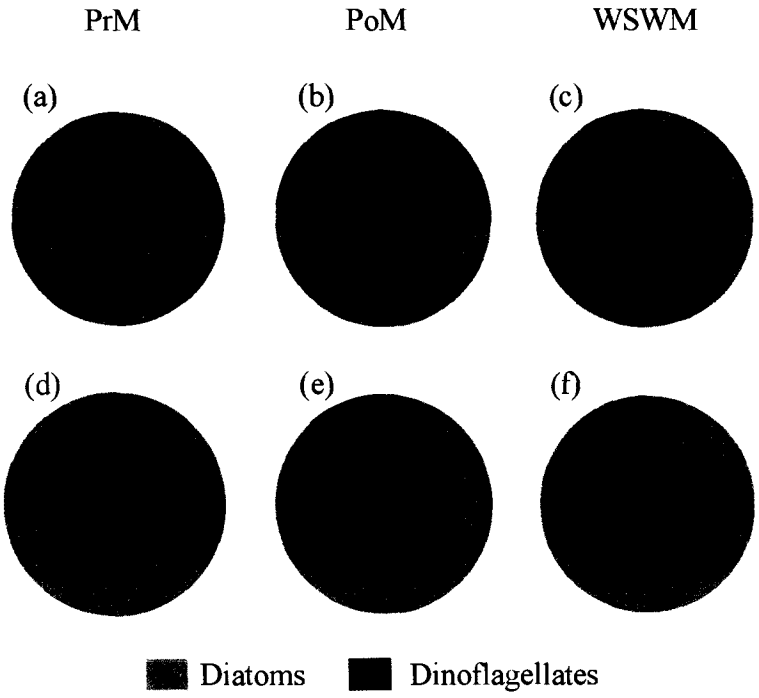


Fig. 4A.4. Phytoplankton community composition (%) in (a-c) surface and (d-f) near bottom waters in Mormugao Port (MPT), India. PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon.

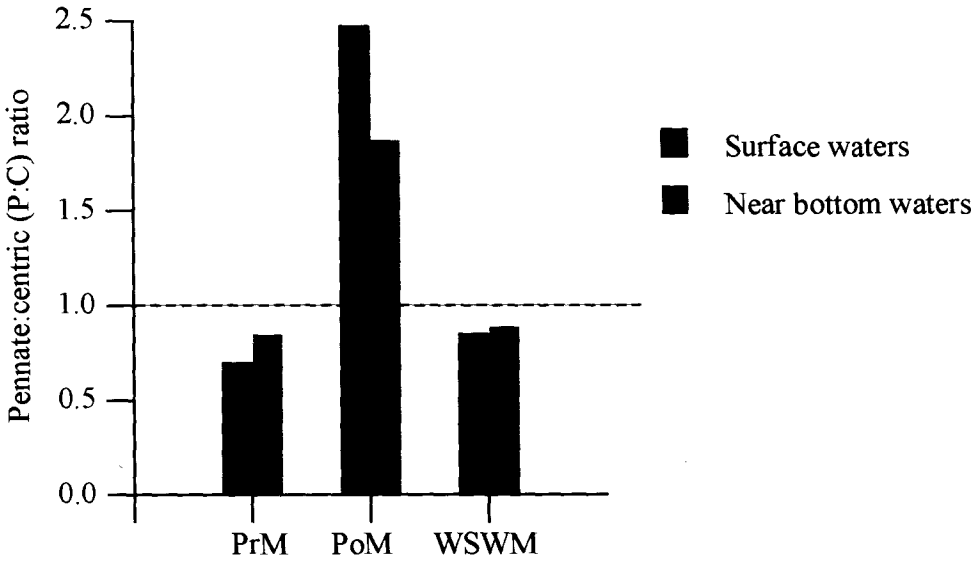


Fig. 4A.5. Pennate:centric ratio of the diatom community in surface and near bottom waters in Mormugao Port (MPT), India. PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon.

Diatom abundance varied from 1.2 x 10^4 to 2.6 x 10^5 cells L^{-1} in surface waters and from 1.1 x 10^4 to 5.8 x 10^4 cells L^{-1} in near bottom waters (average and standard deviation values in Fig. 4A.3e). The diatom community in surface and near bottom waters was centric-dominated during PrM and WSWM, and pennate-dominated during PoM observation (Fig.4A.5). Diatom abundance, species richness, evenness and diversity did not show significant variation across stations. However, significant temporal variation in all the above measures, except species richness, in surface and near bottom waters was observed (Tables 4A.4, 4A.5). The temporal changes in the diatom community in surface and near bottom waters (Fig. 4A.3e-h) mirrored the trends in the phytoplankton community.

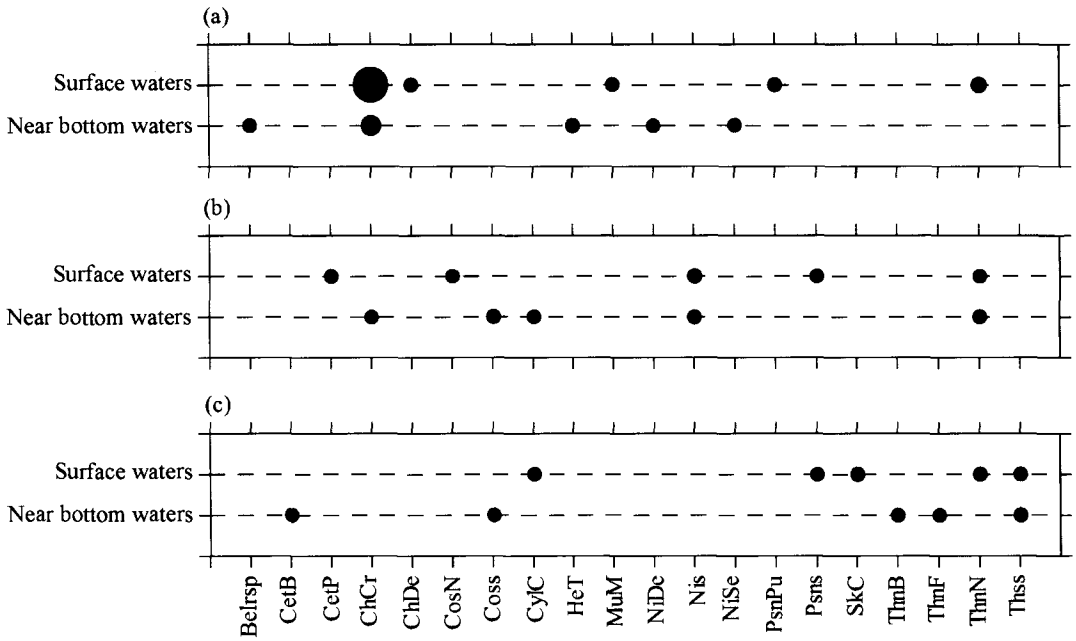


Fig. 4A.6. The five most abundant diatom species in surface and near bottom waters in Mormugao Port (MPT), India during (a) Pre-monsoon, (b) Post-monsoon and (c) withdrawal of the South West Monsoon. Species codes are given in Table 4A.3. The maximum diameter of the circles corresponds to an average abundance of 2.6×10^4 cells L^{-1}.

Characteristic shifts in dominance patterns of diatoms in surface and near bottom waters were observed (Fig. 4A.6). *Chaetoceros curvisetus, Nitzschia* sp., *Thalassionema nitzschioides* and *Thalassiosira* sp. dominated in both, surface and near bottom waters, but only during specific sampling periods. Dominance of certain diatoms was restricted to specific sampling periods. For e.g., *Bellerochea* sp., *Chaetoceros decipiens*, *Helicotheca tamesis*, *Meuniera membranacea*, *Nitzschia delicatissima, Nitzschia seriata* and *Pseudo-nitzschia pungens* were among the most dominant diatoms only during PrM. *Cerataulina pelagica, Coscinodiscus nodulifer, Nitzschia* sp. were the most dominant only during PoM, and *Cerataulina bicornis,*

Skeletonema costatum, Thalassionema bacillare, Thalassionema frauenfeldii and *Thalassiosira* sp. were the most dominant only during WSWM (Fig. 4A.6).

4A.3.1d. Effect of environmental variables on the diatom community in surface and near bottom waters

In the CCA biplots (Fig. 4A.7a-d), 4 axes explaining 91% and 93% of the relationship between diatoms and environmental variables were extracted in surface and near bottom waters respectively (details in Appendix 4). Temperature and phosphate were the most important environmental variables influencing diatom community structure in surface and near bottom waters. In addition to these variables, silicate and pH were also relevant in surface and near bottom waters respectively (Fig. 4A.7a-d). The diatom communities in surface and near bottom waters showed distinct seasonal variation with respect to the environmental variables, as evident in the clustering patterns of stations in the station biplots (Fig. 4A.7a,c). The accompanying species biplots revealed the preference of species for particular environmental variables. For e.g., *Corethron criophilum* and *Navicula* sp.1 (CCA codes 30 and 117 respectively) showed a consistent preference for elevated temperature in both, surface and near bottom waters (Fig. 4A.7b,d). These species appeared to be restricted to/were most dominant in the PrM period (Fig. 4A.7b,d).

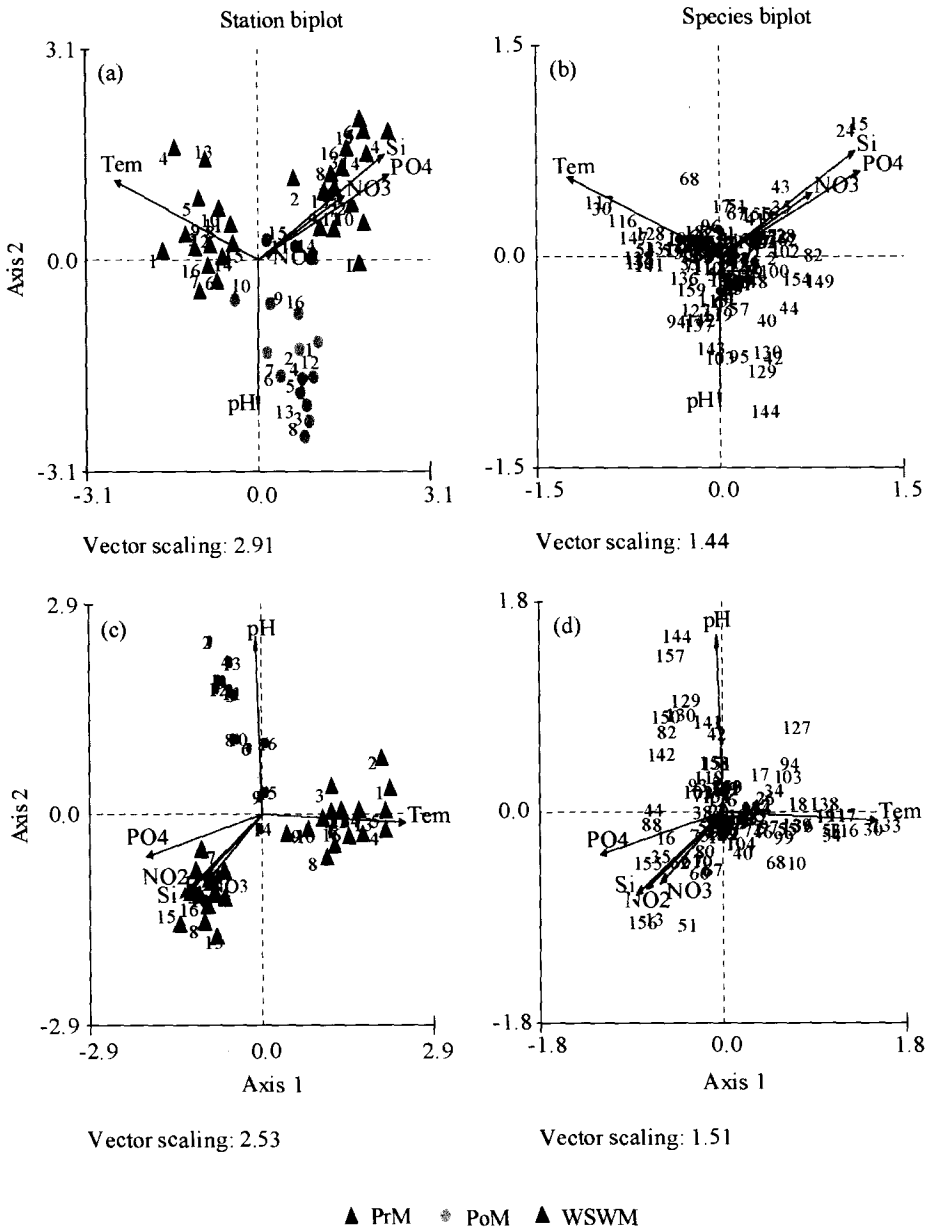


Fig. 4A.7. Ordination diagrams for species and stations based on Canonical Correspondence Analysis (CCA) of the diatom communities in (a,b) surface and (c,d) near bottom waters in Mormugao Port (MPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate and silicate) are indicated by arrows labeled Tem, NO3, NO2, PO4 and Si respectively. Station codes are given in Table 4A.1, species codes are given in Table 4A.3. PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon. Expanded species biplots in Appendix 5.

4A.3.1e. Dinoflagellate community

Dinoflagellates constituted 0-23% of the phytoplankton community (Fig. 4A.4). Seventy dinoflagellate species (22 autotrophic, 22 mixotrophic, 26 heterotrophic) belonging to 25 genera (12 autotrophic, 5 mixotrophic, 8 heterotrophic) were recorded (Table 4A.3). Mixotrophic dinoflagellates dominated in all the observation periods (Fig. 4A.8). Dinoflagellate abundance ranged from 1.3 x 10^3 cells L^{-1} to 1.1 x 10^4 cells L^{-1} in surface waters and from 0 to 3 x 10^3 cells L^{-1} in near bottom waters (average and standard deviation values in Fig.4A.3i).

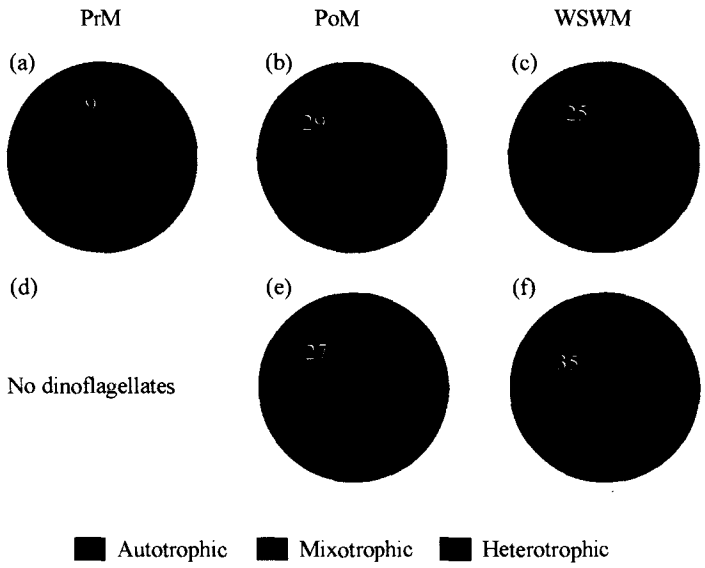


Fig. 4A.8. Dinoflagellate community composition (%) based on mode of nutrition in (a-c) surface and (d-f) near bottom waters in Mormugao Port (MPT), India. PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon.

Similar to the diatom community, the dinoflagellate community did not show any significant variation in abundance, species richness, evenness and diversity across stations. However, significant seasonal variation in these measures was observed (Tables 4A.4, 4A.5). In surface waters, dinoflagellate abundance was highest in PrM (Fig. 4A.3i). Species evenness was maximum in WSWM whereas species richness

and species diversity were highest in PoM (Fig. 4A.3j-l). In near bottom waters, dinoflagellates were not recorded in PrM in all of the 16 stations sampled. Species evenness and diversity were maximum in WSWM (Fig. 4A.3k,l).

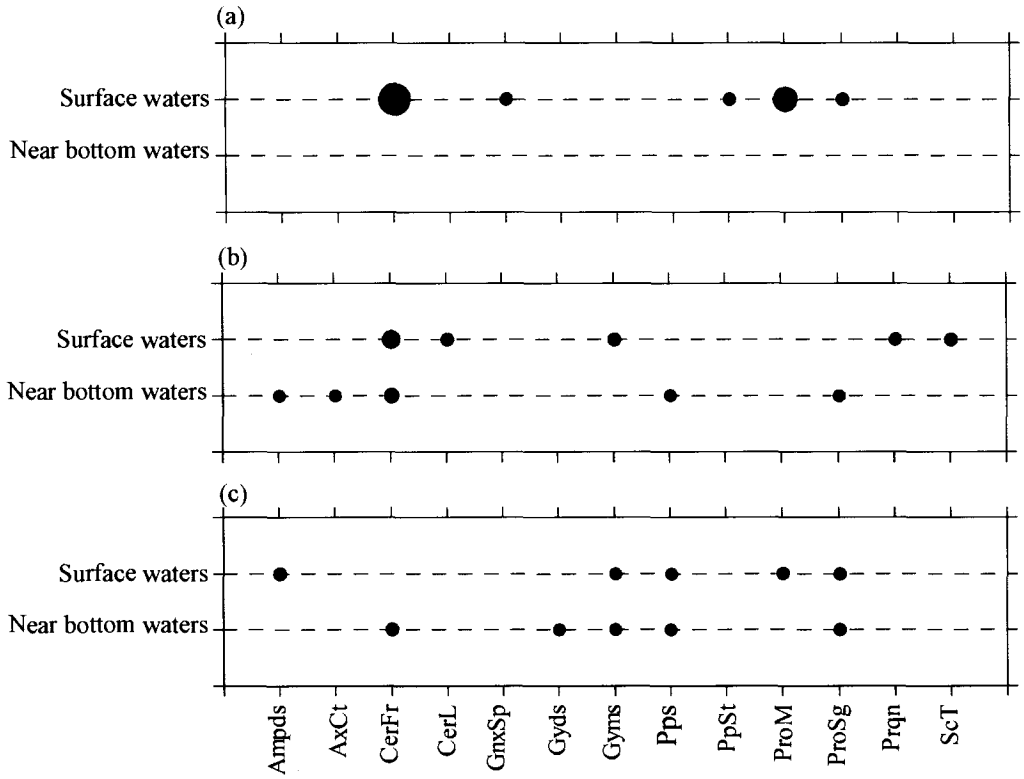


Fig. 4A.9. The five most abundant dinoflagellate species in surface and near bottom waters in Mormugao Port (MPT), India during (a) Pre-monsoon, (b) Post-monsoon and (c) withdrawal of the South West Monsoon. Species codes are given in Table 4A.3. The maximum diameter of the circles corresponds to an average abundance of 1.5×10^3 cells L^{-1}.

Characteristic shifts in dominance patterns of dinoflagellates in surface and near bottom waters were observed (Fig. 4A.9). *Ceratium furca*, *Gymnodinium* sp., *Protoperidinium* sp. and *Prorocentrum sigmoides* dominated in both, surface and near bottom waters, but only during specific sampling periods. Dominance of certain dinoflagellates was restricted to specific sampling periods. For e.g., *Gonyaulax spinifera* and *Protoperidinium steinii* were among the most dominant dinoflagellates

only during PrM. *Alexandrium catenatum, Ceratium lineatum, Peridinium quinquecorne* and *Scrippsiella trochoidea* were the most dominant only during PoM, and *Gyrodinium* sp. was among the most dominant only during WSWM (Fig. 4A.9).

4A.3.1f. Effect of environmental variables on the dinoflagellate community in surface and near bottom waters

In the CCA biplots (Fig. 4A.10a-d), 4 axes explaining 86% and 83% of the relationship between dinoflagellates and environmental variables were extracted in surface and near bottom waters respectively (details in Appendix 4). Temperature, pH and phosphate were the most important environmental variables influencing dinoflagellate community structure in surface waters, whereas temperature, nitrate and silicate were the most important in near bottom waters (Fig. 4A.10a-d). Seasonal variation in the dinoflagellate communities with respect to the environmental variables was noticed in only surface waters (Fig. 4A.10a). Interestingly, dinoflagellates were not recorded in near bottom waters during PrM (Fig.4A.8). The accompanying species biplots revealed the preference of species for particular environmental variables (Fig. 4A.10b,d).

Interestingly, dinoflagellates were not recorded in near bottom waters during pre-monsoon (Fig.4A.8). In the CCA biplots for near bottom waters (Fig. 4A.10c,d), 4 axes explaining 83% of the relationship between dinoflagellates and environmental variables were extracted (details in Appendix 4). Temperature and nitrate were the most important environmental variables influencing dinoflagellate community structure in bottom waters (Fig. 4A.10c,d).

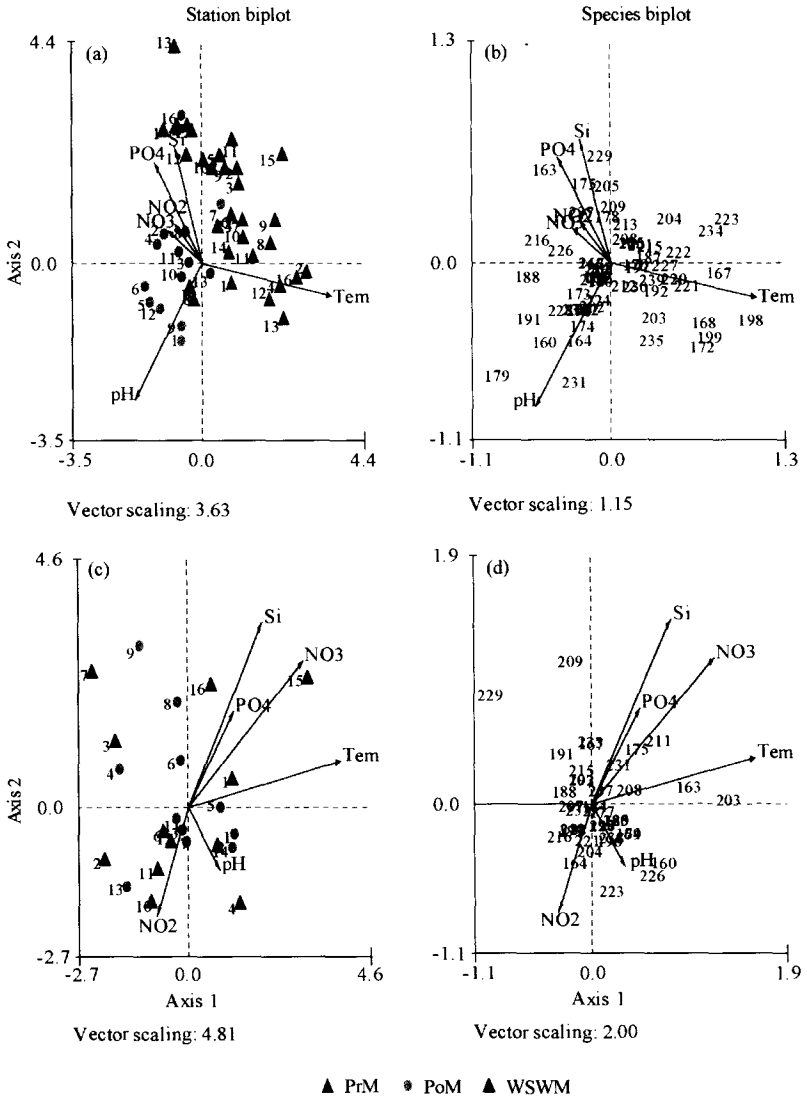


Fig. 4A.10. Ordination diagrams for species and stations based on Canonical Correspondence Analysis (CCA) of the dinoflagellate communities in (a,b) surface and (c,d) near bottom waters in Mormugao Port (MPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate and silicate) are indicated by arrows labeled Tem, NO3, NO2, PO4 and Si respectively. Station codes are given in Table 4A.1, species codes are given in Table 4A.3. PrM: Pre-monsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon. Expanded species biplots in Appendix 6.

4A.3.2. Visakhapatnam Port (VPT)

4A.3.2a. Environmental variables

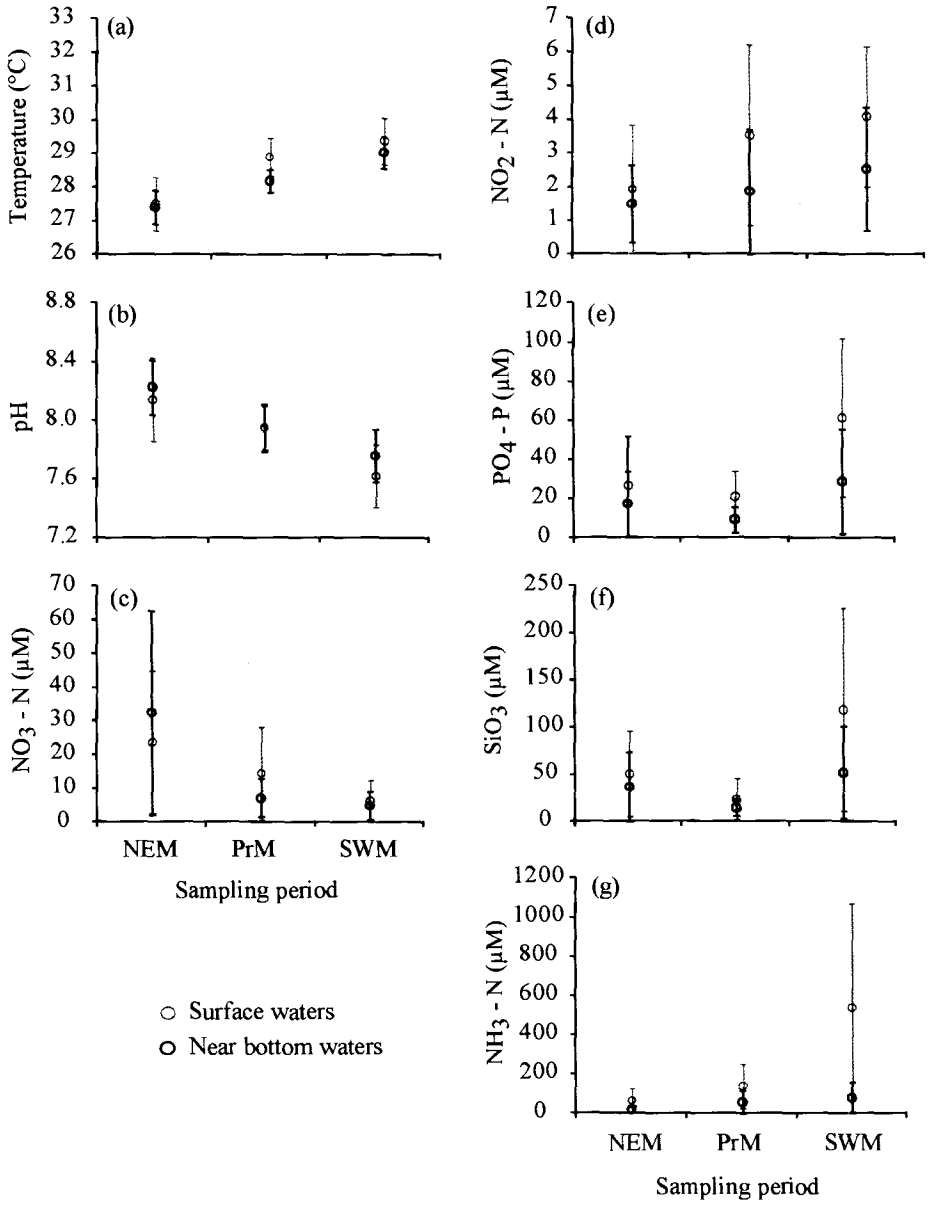


Fig. 4A.11. Seasonal variation in physico-chemical variables in Visakhapatnam Port (VPT), India. (a) Temperature (°C), (b) pH, (c) Nitrate (µM), (d) Nitrite (µM), (e) Phosphate (µM), (f) Silicate (µM) and (g) Ammonia (µM). NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon.

The highest temperature during the observation period (29°C) was recorded during SWM whereas the minimum (27-27.5°C) was recorded during NEM (Fig. 4A.11a). pH appeared to have an inverse relation to temperature, with highest values during NEM and lowest during SWM (Fig. 4A.11b). Concentrations of NO_2, PO_4, SiO_4 and NH_3 were highest during SWM and showed considerable variation (Fig. 4A.11d-g). Nitrate (NO_3) was maximum during NEM (Fig. 4A.11c).

4A.3.2b. Phytoplankton community

Overall, 76 species belonging to 45 genera were recorded in the water column (Table 4A.6). The abundance range and frequency of occurrence are given in Appendix 7).

Table 4A.6. Phytoplankton species recorded in surface (S) and near bottom (NB) waters in Visakhapatnam Port (VPT), India during north east monsoon (NEM), pre-monsoon (PrM) and South West Monsoon (SWM).

Taxon	Species code	CCA code	NEM		PrM		SWM	
			S	NB	S	NB	S	NB
Diatoms								
Centric								
Asteromphalus sp.	Asps	2	+					
Bacteriastrum furcatum	BaF	3	+	+			+	+
Bacteriastrum hyalinum	BaH	4	+	+			+	+
Bacteriastrum sp.	Bas	5	+					
Chaetoceros affinis	ChA	6		+		+		
Chaetoceros curvisetus	ChCr	9	+	+				+
Chaetoceros danicus	ChDa	10						
Chaetoceros decipiens	ChDe	11	+	+	+		+	+
Chaetoceros diversus	ChDiv	12		+				
Chaetoceros peruvianus	ChPe	15	+					
Chaetoceros spp.	Chs	18	+	+	+	+	+	

...Table 4A.6. Contd.

Table 4A.6. Contd....

Taxon	Species code	CCA code	NEM		PrM		SWM	
			S	NB	S	NB	S	NB
Coscinodiscus marginatus	CosM	20	+	+	+	+		+
Coscinodiscus sp.	Coss	21	+	+				+
Cyclotella sp.	Cys	22	+	+				
Ditylum brightwellii	DiB	24		+				
Guinardia flaccida	GuF	28				+		
Guinardia striata	GuS	29	+		+	+	+	+
Guinardia sp.	Gus	30	+	+		+		
Helicotheca tamesis	HeT	31		+				
Hemiaulus hauckii	HeH	32	+				+	+
Hemiaulus sinensis	HmS	34	+					
Hemidiscus sp.	Hems	35		+				
Leptocylindrus danicus	LeD	37	+			+	+	
Leptocylindrus minimus	LeM	38		+			+	+
Odontella sinensis	OdS	40						+
Paralia sulcata	ParS	41	+					
Planktoniella sol	PlaS	42	+					
Rhizosolenia setigera	RhSe	48						+
Rhizosolenia styliformis	RhSt	49				+	+	
Rhizosolenia sp.	Rhs	50						+
Skeletonema costatum	SkC	51	+	+	+	+	+	+
Skeletonema tropicum	SkT	52	+	+	+	+	+	+
Skeletonema sp.	Sks	53	+	+	+	+	+	+
Thalassiosira sp1	Thss1	54	+	+	+	+	+	+
Pennate								
Amphora sp.	Ams	57				+		+
Cylindrotheca closterium	CylC	62		+	+	+		
Diploneis crabro	DipC	63				+		+
Meuniera membranacea	MuM	68	+	+		+		
Navicula delicatula	NvTDe	69	+					
Navicula derasa	NvTDs	70						+
Navicula sp.	Nvs	73	+	+	+	+	+	+
Nitzschia sp.	Nis	76				+		

...Table 4A.6. Contd.

...Table 4A.6. Contd.

Taxon	Species code	CCA code	NEM		PrM		SWM	
			S	NB	S	NB	S	NB
Pleurosigma sp.	Ples	83				+		
Pseudo-nitzschia sp.	Psns	87	+	+	+	+	+	+
Thalassionema bacillare	ThnB	90				+	+	
Thalassionema nitzschioides	ThnN	92	+	+	+	+	+	+
Tropidoneis sp.	Trops	95		+				
Dinoflagellates								
Autotrophic								
Alexandrium sp.	Axs	96	+	+	+			
Amphidinium sp.	Ampds	97	+	+	+	+		+
Amylax triacantha	AmxT	98			+			
Gambierdiscus sp.	Gambs	101			+			
Goniodoma sphaericum	GoniS	102		+				
Gonyaulax polyedra	GnxP	104			+			
Gonyaulax scrippsae	GnxSc	105			+			
Gonyaulax verior	GnxVer	107	+	+	+			
Gonyaulax sp.	Gnxs	108	+	+	+	+	+	+
Gymnodinium sanguineum	GymS	109			+			
Gymnodinium sp.	Gyms	110			+			
Heterocapsa sp.	Htrs	111	+					
Oxytoxum sp.	Oxs	112	+					
Peridinium quinquecorne	Prqn	113	+	+	+	+		+
Mixotrophic								
Ceratium furca	CerFr	114	+		+	+		
Ceratium fusus	CerFs	115	+		+			
Ceratium sp.	Cers	116		+	+			

Table 4A.6. Contd....

...Table 4A.6. Contd.

Taxon	Species code	CCA code	NEM		PrM		SWM	
			S	NB	S	NB	S	NB
Dinophysis caudata	DyC	117	+					
Prorocentrum gracile	ProG	118			+	+		
Prorocentrum micans	ProM	119	+		+	+	+	
Prorocentrum sigmoides	ProSg	121			+			
Prorocentrum sp.	Pros	122	+	+	+	+		+
Scrippsiella trochoidea	ScT	123	+	+	+	+		
Heterotrophic								
Gotoius abei	GotA	126		+				
Phalacroma sp.	Phs	127	+					
Protoperidinium divergens	PpDi	130		+	+	+		
Protoperidinium pallidum	PpPal	133			+	+		
Protoperidinium pellucidum	PpPl	134	+	+		+		
Protoperidinium sp.	Pps	138	+	+	+	+	+	+

Phytoplankton abundance varied from 1.3 x 10^5 to 9.5 x 10^5 cells L^{-1} in surface waters and from 5.3 x 10^5 to 1.3 x 10^6 cells L^{-1} in near bottom waters (average and standard deviation values in Fig.4A.12a). No significant spatial variation in the abundance, species richness, evenness and diversity of the phytoplankton community in surface and near bottom waters was observed (Tables 4A.7, 4A.8) (abundance details in Fig. 4A.12'). However, significant seasonal variation in these measures was observed (Tables 4A.7, 4A.8). In both surface water and near bottom waters, phytoplankton abundance and diversity were highest in PrM (Fig.4A.12a,d). Species richness was maximum in NEM whereas species evenness was highest in SWM (Fig. 4A.12b,c).

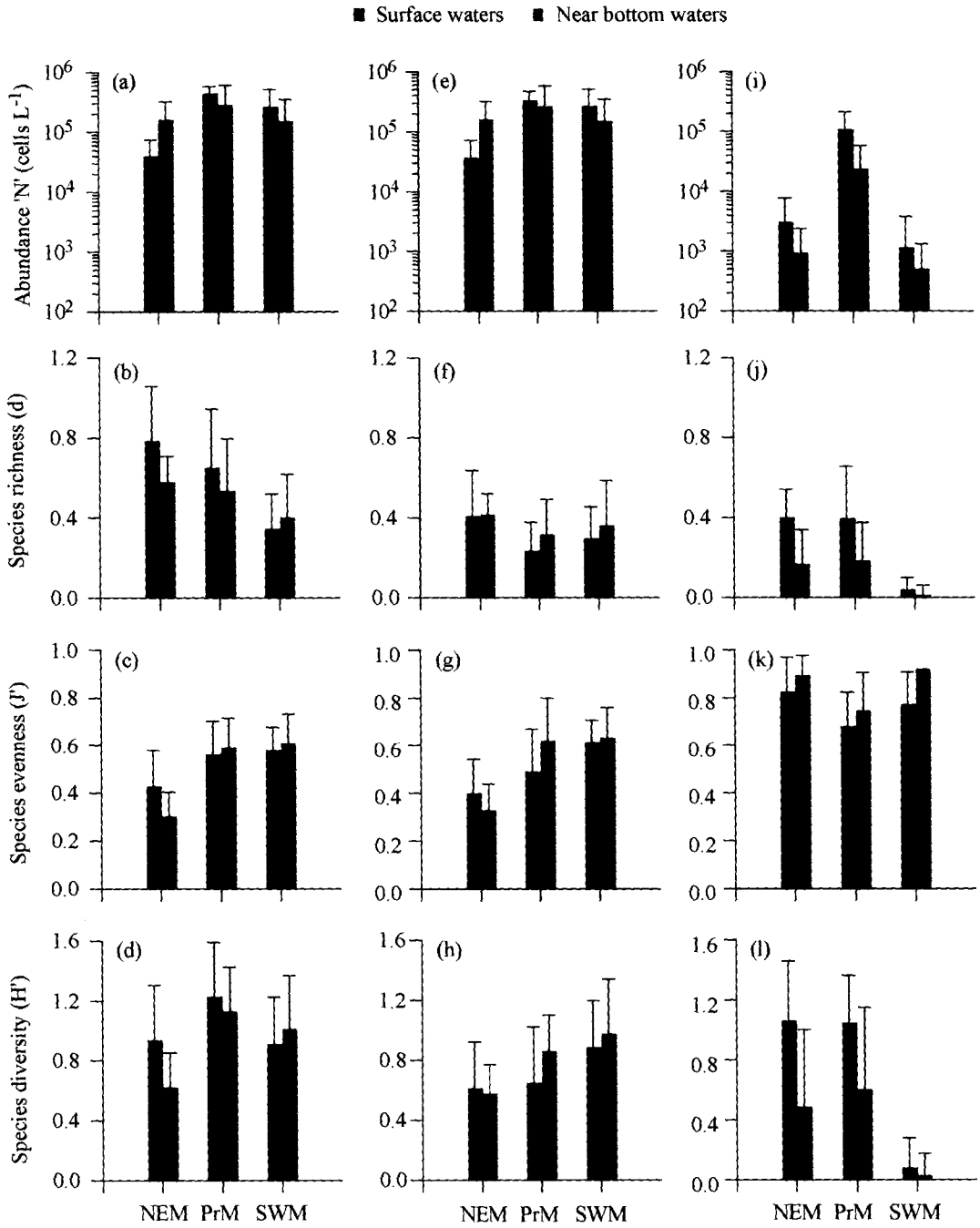


Fig. 4A.12. Seasonal variation in abundance (cells L^{-1}), species richness, evenness and diversity of the (a-d) phytoplankton, (e-h) diatom and (i-l) dinoflagellate communities in Visakhapatnam Port (VPT), India. NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon.

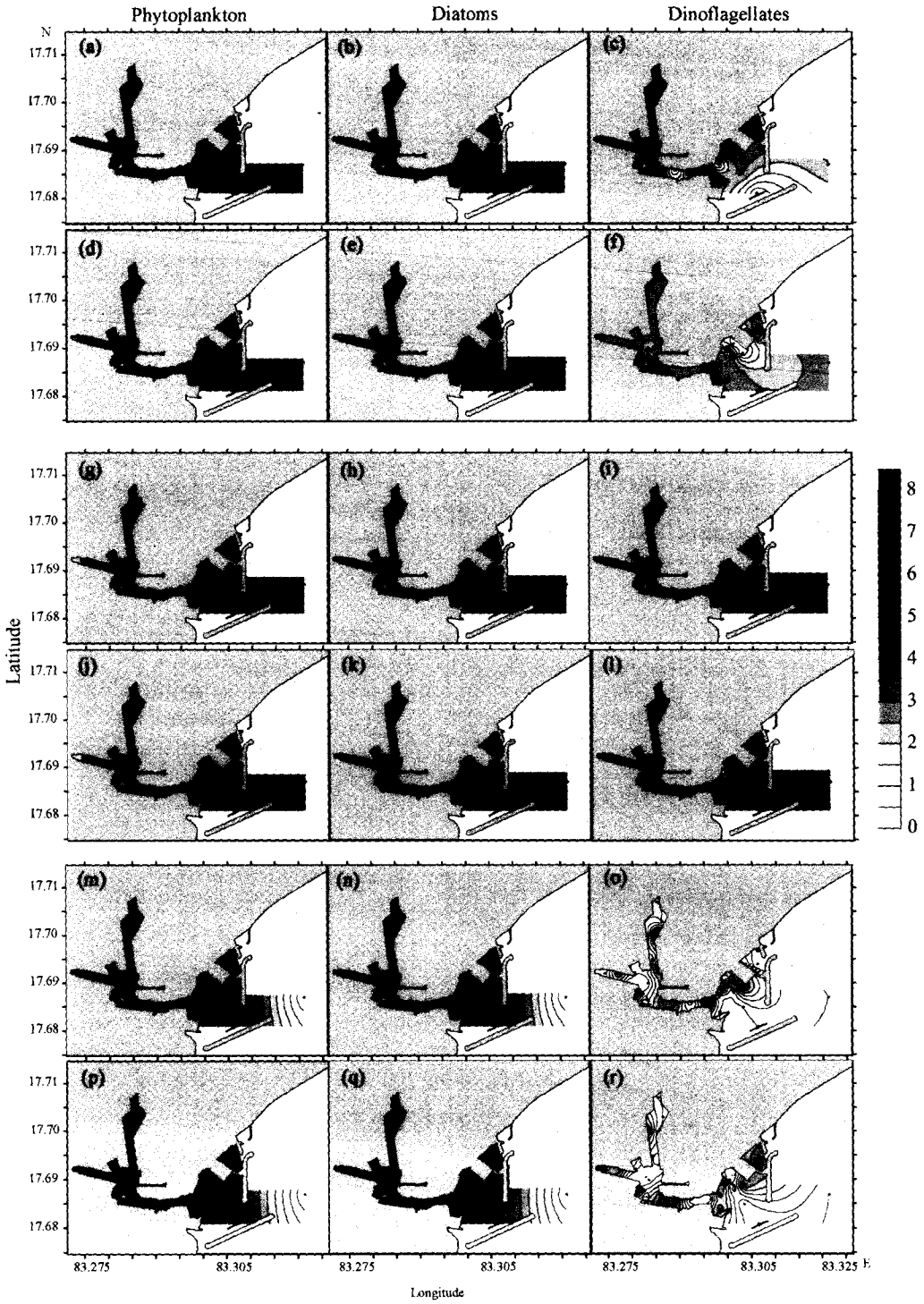


Fig. 4A.12'. Spatial variation in abundance (cells L^{-1}) of phytoplankton, diatoms and dinoflagellates during (a-f) PrM, (g-l) PoM (m-r) WSWM in Visakhapatnam Port (VPT), India. a-c, g-i, m-o: surface waters; d-f, j-l, p-r: near bottom waters. NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon. Scale: log(x+1).

Table 4A.7. Two-way ANOVA to evaluate the variation in abundance, species richness, evenness and diversity of phytoplankton, diatom and dinoflagellates in surface waters of Visakhapatnam port (VPT), India. Values in bold indicate significant variation.

Factor	Phytoplankton				Diatoms				Dinoflagellates			
	df	SS	MS	*p-value*	*df*	SS	MS	*p-value*	*df*	SS	MS	*p-value*
Abundance (N)												
Station	19	286.65	15.09	0.0713	19	347.18	18.27	**0.0110**	19	175.45	9.23	0.8721
Season	2	1515.50	757.75	**0.0000**	2	1234.15	617.07	**0.0000**	2	2011.36	1005.68	**0.0000**
Within sub-group error	38	328.76	8.65		38	290.53	7.65		38	571.80	15.05	
Total	59	2130.91			59	1871.86			59	2758.61		
Species richness (*d*)												
Station	19	1.42	0.07	0.2596	19	0.65	0.03	0.3888	19	0.65	0.03	0.3568
Season	2	2.02	1.01	**0.0000**	2	0.31	0.16	**0.0121**	2	1.85	0.92	**0.0000**
Within sub-group error	38	2.23	0.06		38	1.19	0.03		38	1.15	0.03	
Total	59	5.67			59	2.15			59	3.65		
Species evenness (*J'*)												
Station	19	0.31	0.02	0.5257	19	0.42	0.02	0.3512	19	1.32	0.07	0.1038
Season	2	0.27	0.14	**0.0013**	2	0.46	0.23	**0.0001**	2	5.16	2.58	**0.0000**
Within sub-group error	38	0.66	0.02		38	0.73	0.02		38	1.64	0.04	
Total	59	1.24			59	1.61			59	8.13		
Species diversity (*H'*)												
Station	19	2.37	0.12	0.4441	19	2.02	0.11	0.5293	19	2.24	0.12	0.2288
Season	2	1.24	0.62	**0.0102**	2	0.90	0.45	**0.0260**	2	12.67	6.34	**0.0000**
Within sub-group error	38	4.55	0.12		38	4.23	0.11		38	3.40	0.09	
Total	59	8.16			59	7.15			59	18.32		

Table 4A.8. Two-way ANOVA to evaluate the variation in abundance species richness, evenness and diversity of phytoplankton, diatom and dinoflagellates in near bottom waters of Visakhapatnam port (VPT), India. Values in bold indicate significant variation.

Factor	Phytoplankton				Diatoms				Dinoflagellates			
	df	SS	MS	*p-value*	*df*	SS	MS	*p-value*	*df*	SS	MS	*p-value*
Abundance (N)												
Station	19	434.96	22.89	0.2779	19	444.32	23.39	0.2971	19	205.27	10.80	0.4680
Season	2	152.41	76.21	**0.0238**	2	103.09	51.54	**0.0818**	2	719.15	359.57	**0.0000**
Within sub-group error	38	700.90	18.44		38	732.04	19.26		38	404.67	10.65	
Total	59	1288.27			59	1279.44			59	1329.09		
Species richness (*d*)												
Station	19	0.97	0.05	0.2287	19	0.67	0.04	0.2305	19	0.48	0.03	0.2451
Season	2	0.33	0.17	**0.0205**	2	0.10	0.05	0.1633	2	0.33	0.16	**0.0009**
Within sub-group error	38	1.47	0.04		38	1.02	0.03		38	0.74	0.02	
Total	59	2.77			59	1.79			59	1.54		
Species evenness (*J'*)												
Station	19	0.97	0.05	0.2287	19	0.23	0.01	0.9268	19	3.19	0.17	0.1753
Season	2	0.33	0.17	**0.0205**	2	1.19	0.59	**0.0000**	2	2.63	1.31	**0.0002**
Within sub-group error	38	1.47	0.04		38	0.88	0.02		38	4.49	0.12	
Total	59	2.77			59	2.30			59	10.31		
Species diversity (*H'*)												
Station	19	0.97	0.05	0.2287	19	1.36	0.07	0.5252	19	4.82	0.25	0.1173
Season	2	0.33	0.17	**0.0205**	2	1.71	0.85	**0.0001**	2	3.68	1.84	**0.0001**
Within sub-group error	38	1.47	0.04		38	2.85	0.07		38	6.15	0.16	
Total	59	2.77			59	5.92			59	14.65		

4A.3.2c. Diatom community

Diatoms constituted 77-100% of the phytoplankton community in the water column (Fig. 4A.13).

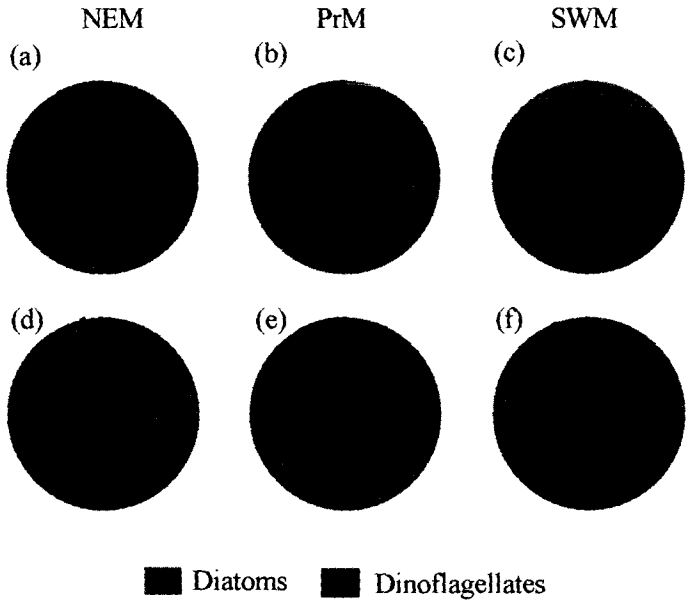


Fig. 4A.13. Phytoplankton community composition (%) in (a-c) surface and (d-f) near bottom waters in Visakhapatnam port (VPT), India. NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon.

A total of 47 diatom species (34 centric, 13 pennate) belonging to 27 genera (17 centric, 10 pennate) were recorded (Table 4A.6). Diatom abundance varied from 7.1 x 10^5 to 9.1 x 10^5 cells L^{-1} in surface waters and from 5.3 x 10^5 to 1.3 x 10^6 cells L^{-1} in near bottom waters (average and standard deviation values in Fig. 4A.12e).

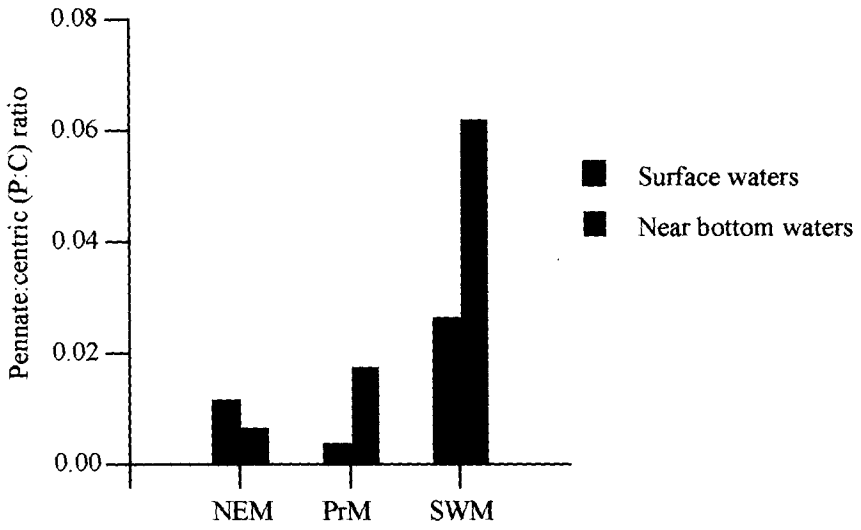


Fig. 4A.14. Pennate:centric ratio of the diatom community in surface and near bottom waters in Visakhapatnam port (VPT), India. NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon.

The diatom community showed consistent domination of centric diatoms (Fig. 4A.14). Significant spatial variation across stations was observed only in abundance in surface waters (Table 4A.7). Significant temporal variation was observed in all the measures except species richness in near bottom waters (Table 4A.8).

Chaetoceros spp., *Guinardia* sp., *Pseudo-nitzschia* sp., *Skeletonema costatum*, *Skeletonema tropicum*, *Skeletonema* sp. and *Thalassiosira* sp.1 were the most dominant diatoms during the observation period. *Chaetoceros* spp., *Guinardia* sp. and *Pseudo-nitzschia* sp. showed season-specific dominance (Fig. 4A.15).

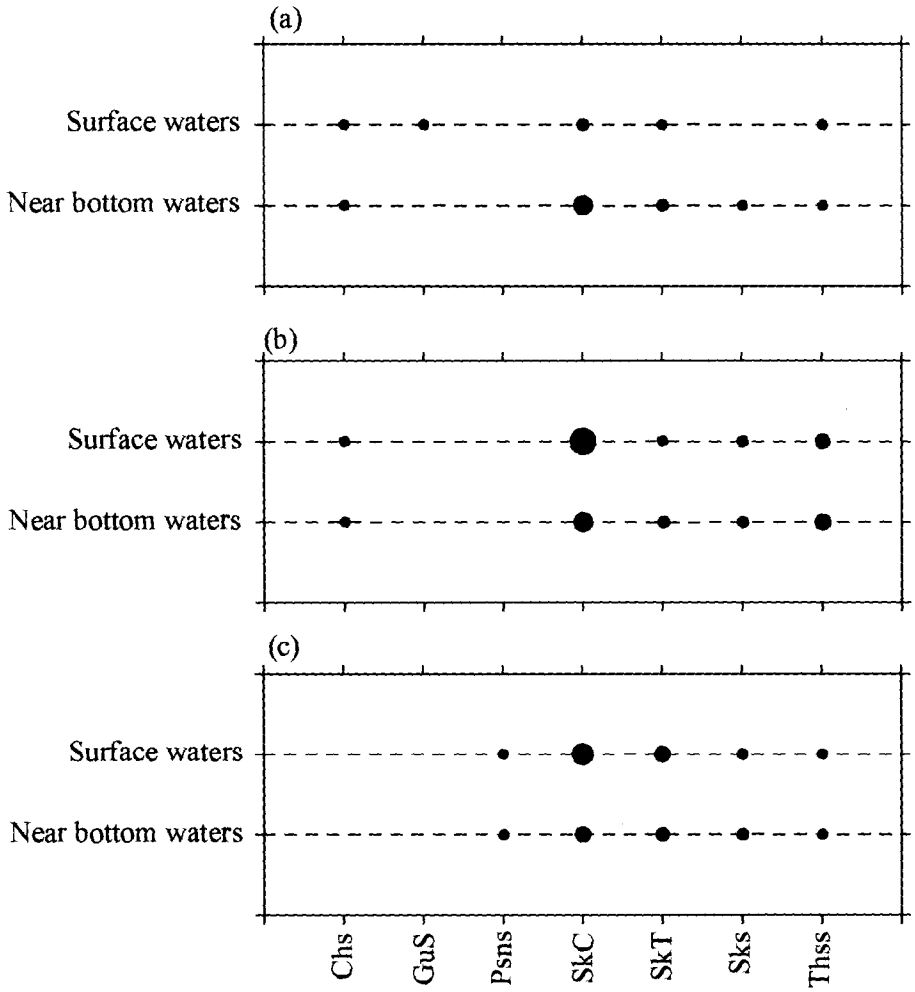


Fig. 4A.15. The five most abundant diatom species in surface and near bottom waters in Visakhapatnam port (VPT), India during (a) North East Monsoon, (b) Pre-monsoon and (c) South West Monsoon. Species codes are given in Table 4A.6. The maximum diameter of the circles corresponds to an average abundance of 2.4 x 10^5 cells L^{-1}.

4A.3.2d. Effect of environmental variables on the diatom community in surface and near bottom waters

In the CCA biplots (Fig. 4A.16a-d), 4 axes explaining 92% and 86% of the relationship between diatoms and environmental variables were extracted in surface and near bottom waters respectively (details in Appendix 4).

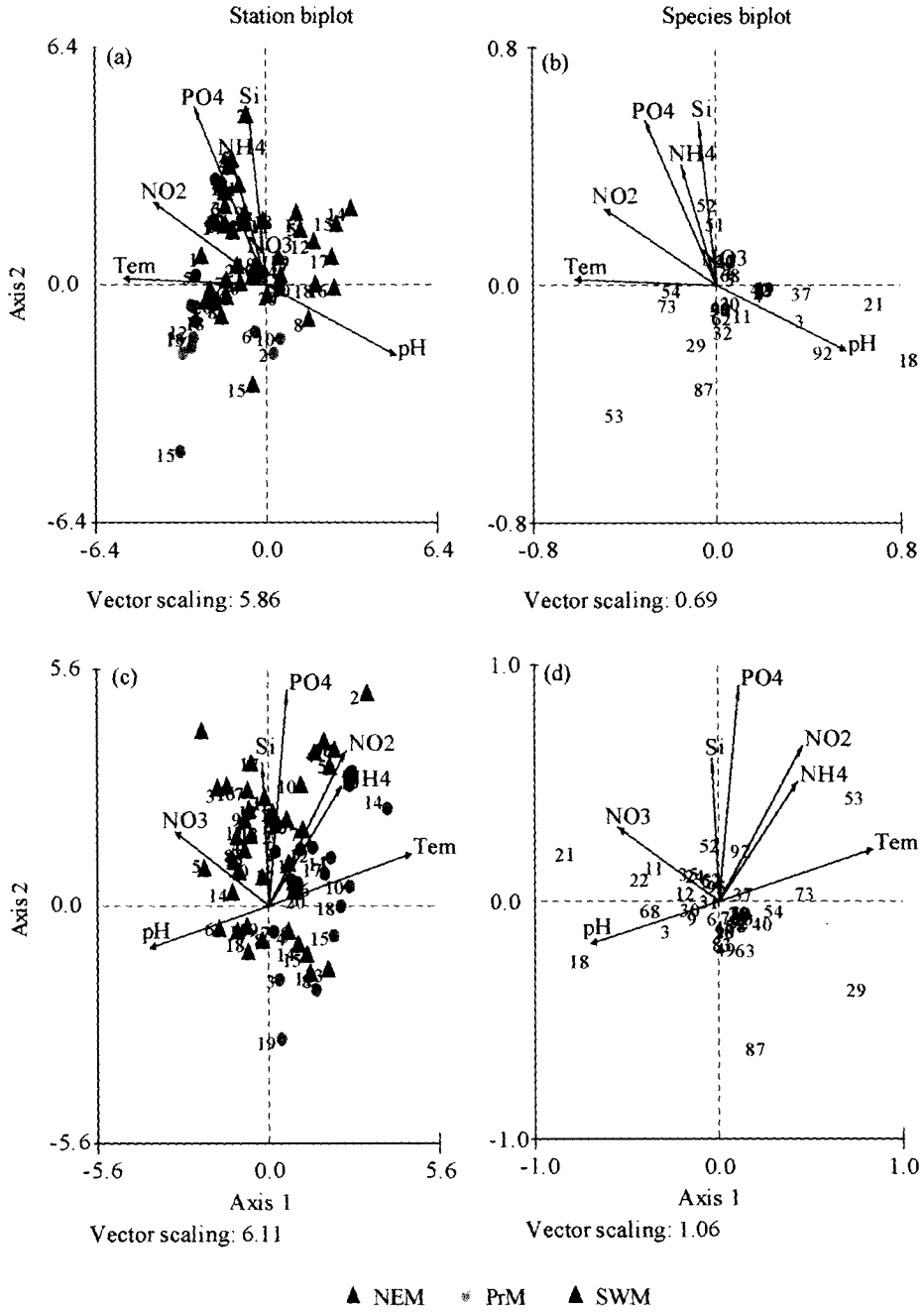


Fig. 4A.16. Ordination diagrams for species and stations based on Canonical Correspondence Analysis (CCA) of the diatom communities in (a,b) surface and (c,d) near bottom waters in Visakhapatnam Port (VPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate, silicate and ammonia) are indicated by arrows labeled Tem, NO3, NO2, PO4, Si and NH3 respectively. Station codes are given in Table 4A.2, species codes are given in Table 4A.6. NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon. Expanded species biplots in Appendix 8.

Temperature and pH were the most important environmental variables influencing diatom community structure in surface and near bottom waters. In addition to these variables, nitrite and phosphate were also relevant in surface and near bottom waters respectively (Fig. 4A.16a-d). The station biplots clearly show that the NEM sampling is distinct from the other two samplings in both surface and near bottom waters (Fig. 4A.16b,d). The accompanying species biplots revealed the preference of species for particular environmental variables (Fig. 4A.16b,d). For e.g., *Bacteriastrum furcatum* (CCA code 3), *Skeletonema tropicum* (CCA code 52) and *Navicula* sp. (CCA code 73) showed a preference for elevated pH, silicate and temperature respectively. This was observed in both, surface and near bottom waters (Fig. 4A.7b).

4A.3.2e. Dinoflagellate community

Dinoflagellates constituted 0-9% of the phytoplankton community (Fig. 4A.13). Overall, 29 dinoflagellate species (14 autotrophic, 9 mixotrophic, 6 heterotrophic) belonging to 18 genera (11 autotrophic, 4 mixotrophic, 3 heterotrophic) were recorded in the water column (Table 4A.6). Heterotrophic dinoflagellates dominated in all the observation periods (Fig. 4A.17).

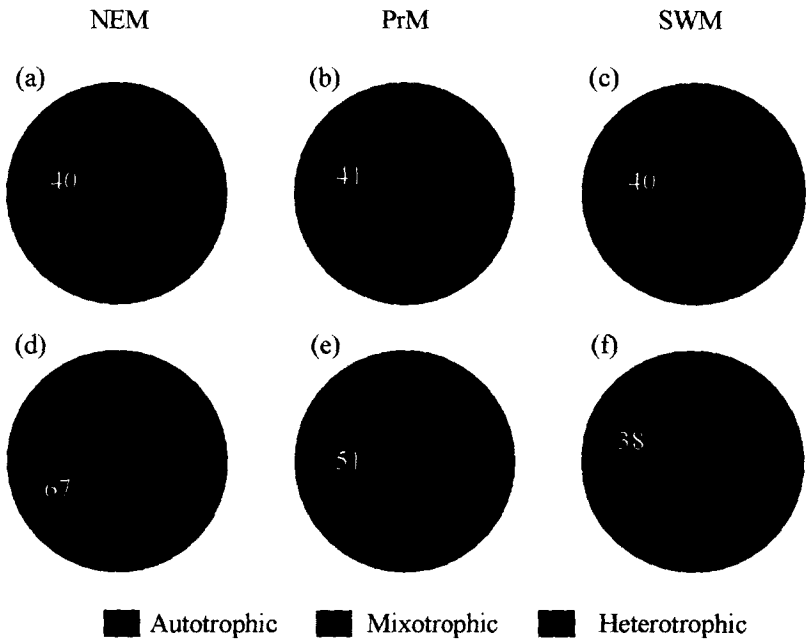


Fig. 4A.17. Dinoflagellate community composition (%) based on mode of nutrition in (a-c) surface and (d-f) near bottom waters in Visakhapatnam Port (VPT), India. NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon.

Dinoflagellate abundance varied from 1.1 x 10^4 cells L^{-1} to 3.3 x 10^5 cells L^{-1} in surface waters and from 2.4 x 10^3 to 1.2 x 10^5 cells L^{-1} in near bottom waters (average and standard deviation values in Fig. 4A.12i). The dinoflagellate community did not show any significant variation in abundance, species richness, evenness and diversity across stations. However, a significant temporal variation in these measures was observed (Tables 4A.7, 4A.8). In surface waters, the dinoflagellate community showed maximum abundance in PrM. Species richness, evenness and diversity was highest in NEM (Fig. 4A.12j-l). In near bottom waters, dinoflagellate abundance, species richness and diversity were highest in PrM; species evenness was maximum in SWM (Fig. 4A.12i-l).

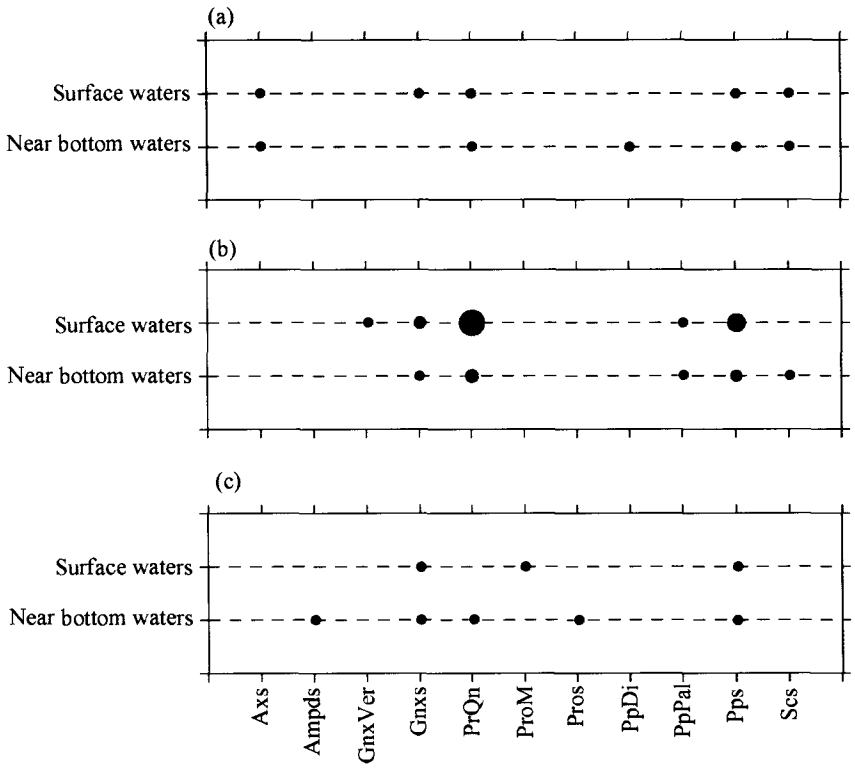


Fig. 4A.18. The five most abundant dinoflagellate species in surface and near bottom waters in Visakhapatnam port (VPT), India during (a) North East Monsoon, (b) Pre-monsoon and (c) South West Monsoon. Species codes are given in Table 4A.6. The maximum diameter of the circles corresponds to an average abundance of 6.1 x 10^4 cells L^{-1}.

Some dinoflagellate species were predominant during the observation period. These were *Alexandrium* sp., *Amphidinium* sp., *Gonyaulax verior*, *Gonyaulax* sp., *Peridinium quinquecorne*, *Prorocentrum micans*, *Prorocentrum* sp., *Protoperidinium divergens*, *Protoperidinium pallidum*, *Protoperidinium* sp. and *Scrippsiella trochoidea*. Shift in dominance with respect to seasons was observed (Fig. 4A.18).

4A.3.2f. Effect of environmental variables on the dinoflagellate community in surface and near bottom waters

In the CCA biplots (Fig. 4A.19a-d), 4 axes explaining 86% and 91% of the relationship between dinoflagellates and environmental variables were extracted in surface and near bottom waters respectively (details in Appendix 4). Temperature, nitrite and pH were the most important environmental variables influencing dinoflagellate community structure in surface waters whereas pH, silicate and phosphate were the most relevant in near bottom waters (Fig. 4A.19a-d). Seasonal variation in the dinoflagellate communities with respect to the environmental variables was noticed in only surface waters (Fig. 4A.19b). The accompanying species biplots showed a rather diverse spread of species with regard to the environmental variables (Fig. 4A.19b,d).

4A.4. Discussion

The present study, emphasizing on the seasonal variations in diatom and dinoflagellate communities in two contrasting port environments along the coasts of India, unravel the environmental variables that shape these communities.

At MPT, the characteristic temporal variations observed in the diatom and dinoflagellate communities along with the shift in predominant taxa/forms, resembles the natural cycling of phytoplankton, and also the concept put forward by Fogg and Thake (1987) of phytoplankton varying in abundance and kind with season, from one water body to another, and even within a small space in the same water body.

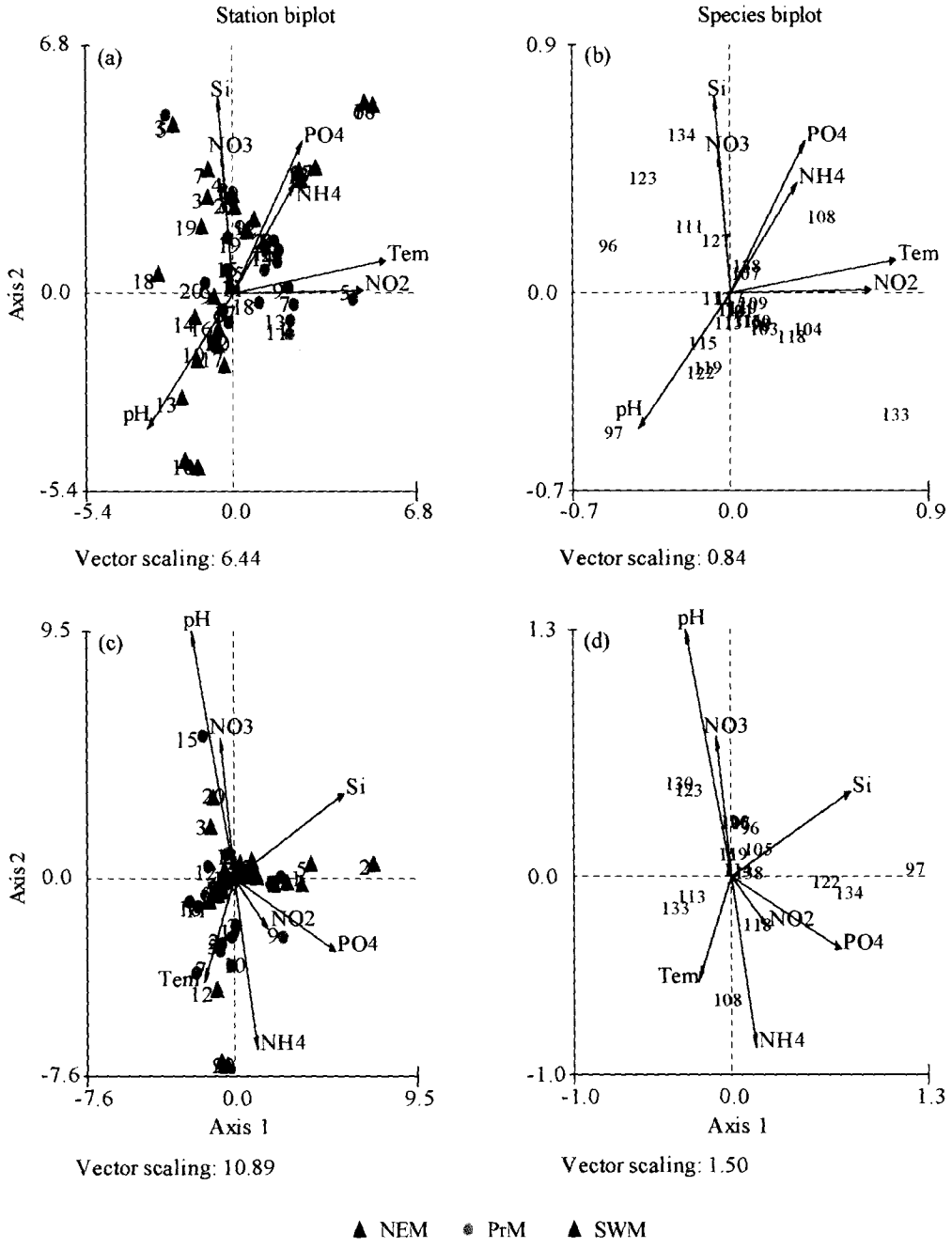


Fig. 4A.19. Ordination diagrams for species and stations based on Canonical Correspondence Analysis (CCA) of the dinoflagellate communities in (a,b) surface and (c,d) near bottom waters in Visakhapatnam Port (VPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate, silicate and ammonia) are indicated by arrows labeled Tem, NO3, NO2, PO4, Si and NH3 respectively. Station codes are given in Table 4A.2, species codes are given in Table 4A.6. NEM: North East Monsoon; PrM: Pre-monsoon; SWM: South West Monsoon. Expanded species biplots in Appendix 9.

The general understanding about coastal water phytoplankton community dynamics is that several environmental variables have a role in determining the productivity of phytoplankton in coastal ecosystems (Downing 1997, Lara Lara et al. 1990, Mallin et al. 1999). The environmental variables in the present study showed seasonal variation. PrM, PoM and WSWM were characterized by highest values of temperature, pH and nutrient concentrations respectively (Fig. 4A.2). The effect of such variations in environmental variables on diatom and dinoflagellate communities was obvious in the CCA analysis (Figs. 4A.7,10,16,19).

The seasonal changes in diatoms, the major contributors to the phytoplankton community, mirrored the variations in the phytoplankton community (Fig. 4A.3). Among all the sampling periods, WSWM was characterized by the highest nutrient concentrations along with low temperature values (Fig. 4A.2), and had minimum diatom abundance (Fig. 4A.3e). However, species evenness and diversity during WSWM were lower compared to the other seasons (Fig. 4A.12g,h). On the contrary, PrM was characterized by highest temperature values but the lowest nutrient concentrations (Fig. 4A.2), and supported the highest diatom abundance (Fig. 4A.3e).

The low diatom abundance during WSWM may be due to the effect of rainfall that brings nutrient loads and land runoff through rivers. This results in elevation of nutrient concentration and probably, high turbidity during WSWM in the study area. Besides nutrient enrichment and turbidity, the study area also faces monsoonal cloud cover which restricts solar radiation during same period (Patil and Anil 2008). Light is considered a first order factor in controlling phytoplankton biomass (Kewlayanga et al. 2007). Nutrient uptake is an energy-demanding process and is, therefore, partl

light-dependent (Kooistra et al. 2007). Because of this, less light can restrict the build-up of phytoplankton biomass, and could be one of the reasons for the low diatom abundance during the WSWM. The influence of these environmental variables on the diatom community is evident in the distinct clustering pattern of stations in the CCA biplots (Figs. 4A.7,10,16,19).

Even during the WSWM, some of the dominant diatoms in surface and near bottom waters differed. *Cyclotella caspia*, *Pseudo-nitzschia* sp., *Skeletonema costatum*, *Thalassionema nitzschioides* and *Thalassiosira* sp. dominated in surface waters, whereas *Cerataulina bicornis*, *Coscinodiscus* sp., *Thalassionema bacillare*, *Thalassionema frauenfeldii* and *Thalassiosira* sp. dominated in near bottom waters (Fig. 4A.6c). These shifts in dominance trends could arise due to differences in the environmental characteristics governing diatom communities in surface and near bottom waters. For e.g., nutrient concentrations which differed markedly in surface and near bottom waters (Fig. 4A.2c-f).

The highest abundance of diatoms during PrM characterized by low nutrient concentrations can be understood in the context of the changes in environmental variables. The PrM was a comparatively warmer season, with high levels of solar irradiance (Patil and Anil 2008). Therefore, under such conditions, the availability of light no longer seems to be the limiting factor. Though differences in the dominant diatoms were observed (Fig. 4A.6a), the diatom community in both surface and near bottom waters shifted to centric dominance during the PrM. Many of the centric diatoms that occurred in the PrM period were large diatoms that, due to certain physiological features, tend to be 'storage-adapted' according to Sommer (1984).

Generally, large diatoms have lower growth rates but high maximum nutrient uptake rates. They have disproportionately large vacuoles compared to smaller, pennate diatoms (Sicko-Goad et al. 1984), where nitrate is stored (Raven 1987). All these characteristics, leading to the 'storage-adapted' strategy, may be responsible for the dominance of centric diatoms during the PrM 'comparatively low nutrient' period.

PoM was high in pH and low nutrient concentrations as compared to the WSWM (Fig. 4A.2b-f). In fact, pH was an important environmental variable influencing diatom and dinoflagellate community structure in near bottom (Figs. 4A.7,10) and surface waters (Figs. 4A.7,10) respectively. pH is considered to be a very important environmental variable in coastal ecosystems. A change in pH can affect the growth of phytoplankton and thus the rate of primary production (Goldman et al. 1982a, b, Schmidt and Hansen 2001, Hansen 2002). It may also play a role in the seasonal succession of phytoplankton species (Hinga 2002) since many species are sensitive to high pH (Hansen 2002). The effect of pH may vary from species to species; some species may have a higher tolerance towards pH variations (Hinga 2002).

Abundance, species richness and diversity of the dinoflagellate communities were low compared to those of diatoms (Fig. 4A.3i,j,l). Shifts in predominant species from season to season and surface waters to near bottom waters indicated the influence of environmental settings, as observed in the diatom community. However, the seasonal trends in the dinoflagellate community with regard to the environmental variables were apparent only in surface waters (Fig. 4A.10). Interestingly, dinoflagellates were not recorded in near bottom waters in PrM in all of the 16 stations sampled. This correlates well with a time-series study (24 hrs observations at 1 hour intervals)

carried out in the vicinity of Mormugao Port during May 2007 (personal observations). During this study, it was observed that during the day hours, dinoflagellates were more abundant in surface waters compared to near bottom waters. In some instances, dinoflagellates were below detectable numbers in near bottom waters. This could be due to the ability of dinoflagellates to migrate towards favourable conditions, in this case, higher irradiance in surface waters.

Mixotrophic forms (*Ceratium furca*, *Prorocentrum micans* and *Prorocentrum sigmoides)* dominated in surface waters during the PrM, and together with other mixotrophs contributed upto 80% to the dinoflagellate community in surface waters (Fig. 4A.8a). In the context of dinoflagellates occurring below detectable levels in near bottom waters in PrM, it is logical to infer that the mixotrophic forms dominant at MPT would migrate towards surface waters to take advantage of the higher irradiance conditions in surface waters.

At VPT, the average phytoplankton abundance was higher compared to MPT throughout the observation period, consistently $\geq 10^5$ cells L^{-1} (Fig. 4A.12a). Phytoplankton abundance was highest during PoM and minimum during NEM (Fig. 4A.12a). Though diatoms were the major contributors to the phytoplankton community, the seasonal variations in species richness and diversity of the phytoplankton community were not reflected in the diatom component (Fig. 4A.12f,h). These differences could be due to the consistent dominance of two centric genera (*Skeletonema* and *Thalassiosira*) throughout the observation period. Shifts in dominance trends were noticed only for *Chaetoceros* spp., *Guinardia* sp. and *Pseudo-nitzschia* sp. (Fig. 4A.15).

The consistent dominance of *Skeletonema* and *Thalassiosira* could be linked to the comparatively high nutrient concentrations in VPT. *Skeletonema* has a high growth rate and nitrate uptake rate, and can adapt to warmer temperatures and lower salinity compared to other diatoms (Liu et al. 2005, Mitbavkar and Anil 2000 and references therein). Due to these reasons, it can proliferate rapidly under high nutrient conditions. They also secrete aldehyde compounds as part of their defence mechanisms, which allows them to escape grazing pressure (d'Ippolito et al. 2002) and maintain a high abundance. In fact, it is a common blooming diatom in many tropical coastal areas (Mitbavkar and Anil 2000, Patil and Anil 2008, D'Costa and Anil 2010 and references therein). In the present study, *Skeletonema* contributed substantially to the diatom community throughout the observation period and may play the role of a keystone species, as reported earlier by D'Costa and Anil (2010). However, considering the high diversity of this genus, this strategy of defense may vary from species to species and also from one bioregion to another. A detailed study on the diversity of this genus from Indian waters (including the present study areas) is explained in chapter 4B.

Thalassiosira is the other genus which was dominant in the water column throughout the study period. Patil and Anil (2008) reported *Thalassiosira* blooms during non-monsoon periods, especially following inputs of nitrate. Similar to *Skeletonema*, this genus also has the ability to escape grazing pressure by aldehyde production (Pohnert 2005); this could be one of the reasons for its dominance in VPT irrespective of the season.

The dinoflagellate community in VPT, with marked variations in abundance, species richness, evenness and diversity, was very distinct from the diatom community. A two-fold increase in dinoflagellate abundance was observed in PrM. Compared to diatoms, species evenness was high during all the seasons (Fig. 4A.12k). The high species evenness was maintained even under the SWM conditions wherein species richness and diversity were markedly reduced (Fig. 4A.12j,l). Shifts in predominant species with respect to season also indicate the influence of environmental variables on the dinoflagellate community as compared to diatoms.

As opposed to dominance of mixotrophic dinoflagellates at MPT, oscillations in dominance trends were observed at VPT. Though surface waters were consistently dominated by autotrophic forms, near bottom waters varied from heterotrophic dominance in NEM and PrM to autotrophic dominance in SWM (Fig. 4A.17). An armoured dinoflagellate - *Peridinium quinquecorne,* contributed substantially to autotrophic dominance. *P. quinquecorne* photosynthesizes using chloroplasts originating from their symbionts. Studies by Horiguchi (2006) and Takano et al. (2008) have indicated that the *P. quinquecorne* chloroplast, originated from centric diatoms: *Thalassiosira/Skeletonema* (Takano et al. 2008) or *Chaetoceros* (Horiguchi 2006). This could be an example of utilization of symbiont chloroplasts to enhance nutrient assimilation through photosynthesis, and may prove to be an efficient adaptation strategy in dinoflagellates in the study area.

Overall, differences in the micro-phytoplankton community structure at MPT and VPT were observed. The environment under the monsoonal flushing (MPT) exhibited variation in the phytoplankton community structure, resembling natural

seasonal cycling. In contrast to these conditions, the influence of eutrophication on the phytoplankton community was observed in VPT, the semi-enclosed water body. This was reflected in the prevalence of a single dominant genus (*Skeletonema*) irrespective of seasons.

Marked changes in the dinoflagellate assemblages, influenced by prevalent environmental conditions, were also noticed. Mixotrophic dinoflagellates dominated at MPT whereas autotrophic and heterotrophic forms showing an oscillatory trend in dominance at VPT, especially in near bottom waters. Consistent with the observations of this study, theoretical studies demonstrate that mixotrophy is advantageous in oligotrophic environments whereas auto- and heterotrophy is predominant in eutrophic environments (Troost et al 2005 a, b). Mixotrophy may also be advantageous in fluctuating, monsoon-influenced environments (D'Costa and Anil 2010).

Heterotrophic dinoflagellates contributed 40-41% in surface and 38-67% in near bottom waters (Fig. 4A.17). Heterotrophic dinoflagellates usually dominate when prey organisms are available in plenty (Harland et al. 2006). This is reflected in the present observations in VPT, where the high silicate concentrations correlated with high abundance of *Skeletonema* species (Fig.4A.16b,d), as well as the occurrence of *Protoperidinium pellucidum* (a heterotrophic dinoflagellate) (Fig.4A.19b,d). This highlights the possible links between environmental variables, diatom and dinoflagellate communities.

Detailed taxonomic studies on preserved/cultured microphytoplankton from these 2 coastal locations revealed 2 new reportings of diatoms. Details regarding these new reportings are given in Part B of this chapter.

4B.1. Introduction

The diatom genus *Skeletonema* Greville emend. Sarno et Zingone is common in the plankton of coastal marine habitats throughout the tropical and temperate regions (Hasle 1973, Castillo et al. 1995, Chen et al. 2007, Huang et al. 2007). The first species to be described in this genus is *Skeletonema costatum* (Greville) Cleve (Greville 1866) and in most studies, the genus is considered synonymous with this species. However, such a treatment constitutes a serious underestimation of the generic diversity because several species have been recognized in *Skeletonema*. These are: *S. ardens* Sarno et Zingone (Sarno et al. 2007), *S. costatum* (Greville) Cleve emend. Zingone *et* Sarno (*sensu stricto; s.s.*) (Zingone et al. 2005), *S. dohrnii* Sarno et Kooistra (Sarno et al. 2005), *S. grethae* Zingone et Sarno (Sarno et al. 2005), *S. grevillei* Sarno et Zingone (Zingone et al. 2005, Sarno et al. 2007), *S. japonicum* Zingone et Sarno (Sarno et al. 2005), *S. marinoi* Sarno et Zingone (Sarno et al. 2005), *S. menzelii* Guillard, Carpenter and Reimann (Guillard et al. 1974), *S. potamos* (Weber) Hasle (Weber 1970, Hasle and Evensen 1976), *S. pseudocostatum* Medlin emend. Zingone et Sarno (Medlin et al. 1991, Sarno et al. 2005), *S. tropicum* Cleve (Cleve 1900) and *S. subsalsum* (A. Cleve) Bethge (Bethge 1928, Hasle and Evensen 1975). The taxonomic treatment of *Skeletonema* is a work in progress because some of the proposed species are not well separated genetically and/or morphologically (Alverson and Kolnick 2005, Godhe et al. 2006, Alverson 2008, Ellegaard et al. 2008, Kooistra et al. 2008). Moreover, cryptic diversity abounds in *S. menzelii* and *S. tropicum* (Kooistra et al. 2008).

In a recent biogeographical study, molecular and morphological approaches were integrated to identify numerous strains of *Skeletonema* collected from all over the world (Kooistra et al. 2008). Results indicated that although most *Skeletonema* species appear to be widely distributed, none seems to be truly cosmopolitan. However, tropical regions were severely under represented and Indian waters were missing in their survey.

In Indian waters, Subrahmanyan reported *S. costatum* as early as 1946 (Subrahmanyan 1946). The genus has since then been reported throughout the year, both in the Arabian Sea (AS) and the Bay of Bengal (BOB) (Devassy and Bhattathiri 1974, Desikachary and Prema 1987, Desikachary et al. 1987, Devassy and Goes 1988, Mitbavkar and Anil 2000, Madhu et al. 2006, Paul et al. 2007, Habeebrehman et al. 2008, D'Costa and Anil 2010). Along the west coast of India, *Skeletonema* blooms during the onset of the South West Monsoon (SWM) after an intermittent break, when salinity is low and nutrient concentrations are higher compared to other months of the year (Mitbavkar and Anil 2000, Patil and Anil 2008). Along the east coast, *Skeletonema* dominates the phytoplankton community in the northern part of the BOB during the SWM and it does likewise at the onset of the north-east monsoon at Kalpakkam, in the southern part of BOB, when salinity and nutrient concentrations are low (Saravanane et al. 2000, Paul et al. 2007). These lower nutrient concentrations associated with dominance of *Skeletonema* in the BOB are in contrast to the elevated nutrient concentrations associated with *Skeletonema* blooms along the west coast of India. In spite of these apparent differences, all these blooms are believed to consist of *S. costatum*. However, this treatment does not take into account

the recent taxonomic appraisal of the genus. The goal of the present study is to give a first account of *Skeletonema* species diversity in Indian waters according to the updated taxonomy.

4B.2. Materials and methods

4B.2.1. Culture strain

One culture strain was examined. The culture strain was isolated from the waters of Dona Paula Bay, Goa, along the west coast of India in May 2007. It was maintained in f/2 medium (Guillard and Ryther 1962) at 25°C with a 12 h:12h light:dark cycle and transferred to fresh f/2 medium every 10 days. It is presently in the culture collection of the Marine Corrosion and Materials Research Division (MCMRD), National Institute of Oceanography, Goa, India.

4B.2.2. Preserved samples

Two preserved phytoplankton samples were selected for analysis. These samples were collected from the east coast (Bay of Bengal; 21°01'N, 88°13'E; October 2006) and west coast (Arabian Sea; 15°27'N, 73°48'E; August 2007) of India.

4B.2.3. Light microscopy (LM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

The culture and preserved samples were prepared for Light Microscopy (LM), and Scanning Electron Microscopy (SEM) following Sarno et al. (2005). Samples were treated with acids (1:1:4, sample:HNO_3:H_2SO_4) to remove organic matter, followed

by boiling for a few seconds and washing with distilled water. Samples were observed using an Axiophot light microscope (Carl Zeiss, Oberkochen, Germany), equipped with a camera. For SEM, acid-cleaned material was mounted on stubs, sputter-coated with gold-palladium, and observed using a JEOL JSM-6700F field emission scanning electron microscope. Fixed samples that were not subjected to cleaning were dehydrated in an ethanol series, critical point-dried (substituting 100% ethanol with CO_2), sputter-coated with gold-palladium prior to SEM analysis. For transmission electron microscopy (TEM), the acid-cleaned material was mounted onto Formvar-coated grids and observed using a Philips 400 transmission electron microscope.

4B.2.4. Molecular analysis of the culture strain

The culture strain was also examined utilizing a molecular approach. DNA extraction, PCR amplification and sequencing of the first ca. 700 base pairs of the large subunit nuclear ribosomal RNA coding region (LSU rDNA), and alignment of this marker with other sequences was carried out as described in Sarno et al. (2005).

4B.3. Results

4B.3.1. Microscopic identification of the culture strain and preserved samples

The culture strain was identified as *S. costatum sensu stricto* (Fig. 4B.1a) whereas *S. grevillei* (Fig. 4B.1b) and *S. tropicum* (Fig. 4B.1c) were identified in preserved samples from the east and west coasts of India respectively.

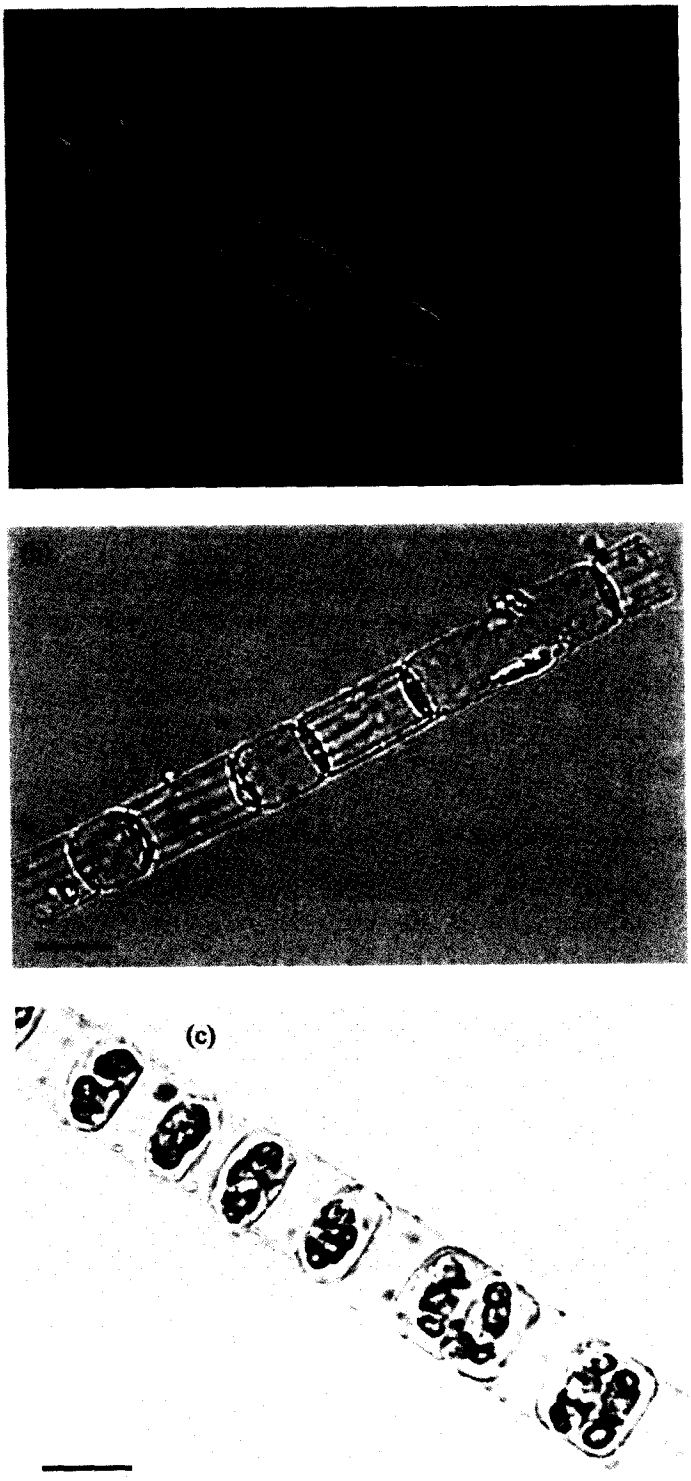
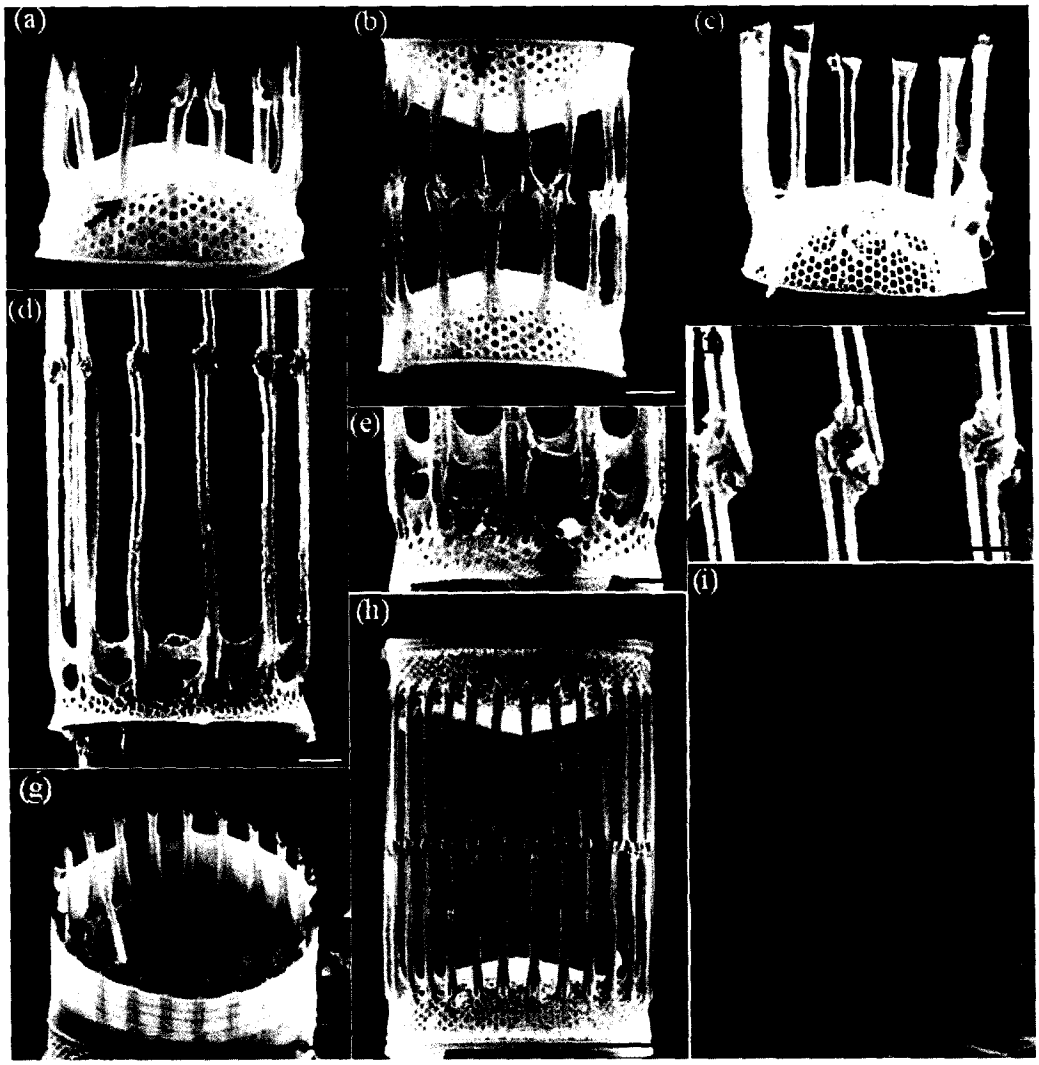


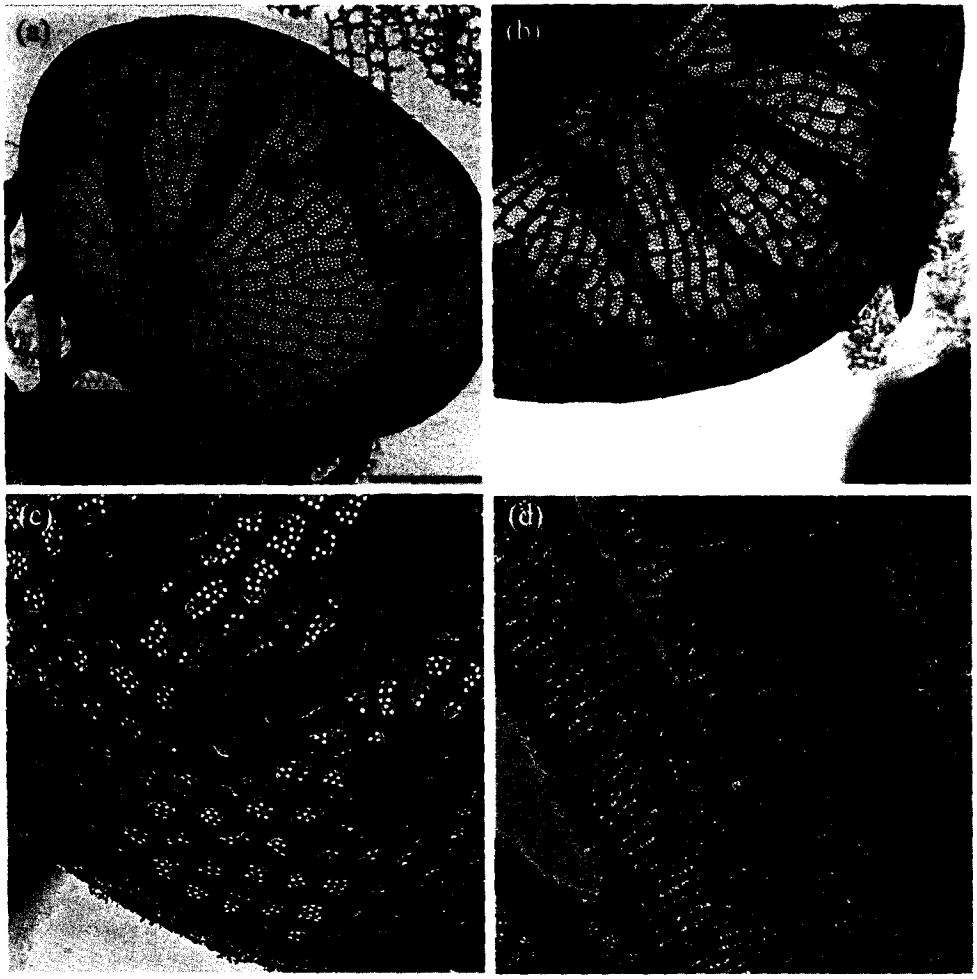
Fig. 4B.1. Light photomicrographs of (a) *S. costatum sensu stricto,* (b) *S. grevillei* and (c) *S. tropicum*. Scale bar = 10 μm.

Skeletonema costatum (Fig. 4B.2a-c): Cells are 4-12 μm in diameter and contain 1-2 chloroplasts. In terminal valves, fultoportulae have open processes that end with a claw-like protrusion (Fig. 4B.2a). In intercalary valves, processes of fultoportulae are closed, with a small hole often visible at their base and a longitudinal suture running from the hole towards the process end (Fig. 4B.2b). Each process generally connects to two processes of the sibling valve (1:2 junction). In both intercalary and terminal valves, the rimoportula is located near the marginal ring of fultoportulae and has a long external process (Fig. 4B.2b). Details of the marginal fultoportulae processes and cingular ultrastructure are shown in the accompanying TEM pictures (Fig. 4B.3a-d).

Skeletonema grevillei (Figs. 4B.2d-f): Cells are 5-8 μm in diameter and contain 1-2 chloroplasts. The external parts of fultoportulae are open along their entire length in both terminal and intercalary valves. In terminal valves, processes of fultoportulae are irregularly truncated at their tips and pointed at their lateral ends (Fig. 4B.2d). In intercalary valves, the processes of the fultoportulae are long (7-8 μm), each joining one process of the adjacent cells (1:1 junction) with a knuckle-like connection (Fig. 4B.2e-f). Several series of siliceous ridges link the bases of the processes to each other and to the valve mantle, delimiting a series of bigger, oval holes between the fultoportulae and a series of smaller, circular holes at the external base of the fultoportulae (Fig. 4B.2d-f). In terminal valves of the colony the rimoportula is located close to the valve face margin, near the marginal ring of fultoportulae, and it bears a long process.



Figs. 4B.2. General morphology of *Skeletonema* (SEM pictures). (a) *S. costatum,* terminal valve with the marginal terminal rimoportula process (arrow). Scale bar = 1 μm. (b) *S. costatum,* intercalary valve with the long rimoportula process (arrow). Scale bar = 1 μm. (c) *S. grevillei,* terminal valve. Scale bar = 1 μm. (d) *S. grevillei,* intercalary valve. Scale bar = 1 μm. (e) *S. grevillei,* detail of an intercalary valve showing the well expanded silica ridges joining the bases of the intercalary fultoportula processes. Scale bar = 1 μm. (f) *S. grevillei,* detail of knuckle-like junction. Scale bar = 1 μm. (j) *S. tropicum,* terminal valve with fultoportula processes and subcentral rimoportula process. Scale bar = 5 μm. (k, l) *S. tropicum,* intercalary valve. Scale bar = 5 μm.



Figs. 4B.3. General morphology of *S. costatum* (TEM pictures). (a) Valve view showing the marginal fultoportulae. Scale bar = 5 μm. (b) Valve view showing positions of the fultoportulae processes and junctions. Scale bar = 5 μm. (c) Details of the fultoportulae process base. Scale bar = 2.5 μm. (d) Cingular band ultrastructure. Scale bar = 2.5 μm.

Skeletonema tropicum (Figs. 4B.2g-i): Cells are 9-15 μm in diameter and contain several chloroplasts (1-7). The tips of the fultoportulae of terminal processes are irregularly truncated or, more often, show a claw-like projection (Fig. 4B.2g). The processes of the intercalary fultoportulae connect tightly to those of the next cell (Fig. 4B.2h). The junction is generally of the 1:1 type, but occasionally 1:2 junctions are

visible (Fig. 4B.2i). The processes of the fultoportulae are straight (Fig. 4B.2h) or curved (Fig. 4B.2i). Both types of processes have been observed in a single colony. The rimoportula of the terminal valves has a long external process, located midway between the annulus and the valve margin (Fig. 4B.2g).

4B.3.2. Molecular analysis

The partial LSU rDNA sequence of the culture strain was identical to those of *S. costatum* strains B and C (Lagoa dos Patos, Brazil, Genbank numbers EF423397 and EF423398) and strain SZN-B212 (Montevideo, Uruguay, Genbank number EF423396).

4B.4. Discussion

S. costatum s. s., S. grevillei and *S. tropicum* were identified for the first time in Indian waters. The morphological features of the *S. costatum* strain in this study corresponded to those reported in Zingone et al. (2005), which re-examined the type material of the species, and in Sarno et al. (2007) which observed the species as live material. Some specimens of *S. grevillei* from the east coast showed a larger development of the silica ridges (Fig. 4.5) compared to the original description of the species (Zingone et al. 2005).

The microscopic identification of the culture specimen as *S. costatum s. s.* was further corroborated by the results of the molecular analysis. Considering the other 2 *Skeletonema* species, Kooistra et al. (2008) uncovered cryptic diversity in *S. tropicum*

However, in the present study, genetic information for this material and the *S. grevillei* material could not be accessed.

S. costatum s. s. and *S. grevillei* have a wide geographical range, occurring in both temperate and tropical waters (Sarno et al. 2007, Kooistra et al. 2008). The third species, *S. tropicum*, has been reported by Kooistra et al. (2008) in tropical locations as well as in the Mediterranean Sea, the East China Sea and in southern Brazil during summer and autumn. The reporting of these species in this study points to the hidden diversity of *Skeletonema* in Indian waters. *Skeletonema* plays an important role in influencing trophic dynamics in Indian waters. Firstly, it is an important blooming diatom in Indian waters. Secondly, it influences trophic dynamics by providing cues for intermittently breeding invertebrates, e.g., barnacles, to release their larvae (Barnes 1962, Desai and Anil 2005). Therefore, the potential high diversity of *Skeletonema* in Indian waters has wide-ranging implications.

Summarizing the results, *S. costatum s. s.,* in addition to 2 other *Skeletonema* species (*S. grevellei* and *S. tropicum*), were identified for the first time in Indian waters, in this study involving the analysis of a limited number of samples. This highlights the need for ultrastructural and molecular taxonomic studies to unravel the diversity of *Skeletonema* in Indian waters. Regular monitoring is needed to uncover not only the diversity of this genus, but also habitat preferences and seasonal variation of the detected species.

Chapter 5

Effect of preservation on the morphology of Karlodinium veneficum, a non-thecate, potentially harmful dinoflagellate and allelopathy in relation to Skeletonema costatum

5.1. Introduction

In 1950, near Plymouth Sound UK, a non-thecate dinoflagellate was isolated by Parke and later described and named as *Gymnodinium veneficum* by Ballantine (1956). Further studies on the same strain, using light and electron microscopy and partial LSU rDNA, found matching features with *Karlodinium micrum* and thus Bergholtz et al. (2005) suggested a change of name for both *G. veneficum* and *K. micrum* to *Karlodinium veneficum.*

K. veneficum (as *K. micrum*) is a widespread species, reported from various bioregions, Australia (Hallegraeff 2002), North America (Deeds et al. 2002), Southern Africa (Tengs et al. 2001) and Europe (Bjornland and Tangen 1979). Bergholtz et al. (2005) also described its wide distribution around different water bodies. This species often gets overlooked due to its small size (de Salas et al. 2005) (< 20 μm). Artifacts due to fixation could be another reason, since previous studies showed artifacts induced by fixation are a potentially significant factor of bias to phytoplankton sample analysis (Throndsen 1978, Booth 1987, Stoecker et al. 1994, Mender-Deuer et al. 2001, Zarauz and Irigoien 2008). The majority of earlier studies conducted in Indian waters are preservative-based. Identification of species belonging to *Karlodinium* often requires live samples (Bergholtz et al. 2005). In this study, in the course of routine monitoring programme involving collection of phytoplankton samples with the intention of raising cultures, we have isolated and cultured *K. veneficum.* This is the first report of this species from waters around the subcontinent of India.

K. veneficum is considered as a common member of the coastal phytoplankton, generally found at relatively low cell abundance, but capable of forming blooms under appropriate conditions. Such blooms have resulted in fish mortality (Abbott and Ballantine 1957, Deeds et al. 2002, Fensin 2004, de Salas et al. 2005, Place 2005, Adolf et al. 2008). Karlotoxins produced by certain strains of *K. veneficum* and its mixotrophic capabilities are likely to be important factors contributing to the formation and continuation of blooms (Adolf et al. 2007, Waggett et al. 2008). But, in some of the cases, by releasing secondary metabolites such as allelopathic chemicals, some toxic algae can also negatively influence co-occurring phytoplankton (Legrand et al. 2003). Such production of toxins or release of organic compounds is considered as part of the defense mechanisms or competition strategies among phytoplankton (Smetacek 2001, Tillmann and John 2002, Tillmann 2003, Fistarol et al. 2004, Tillmann 2004).

In light of the above, two experiments on *K. veneficum* isolated from Indian waters were conducted. The first experiment analyzed the effect of four different fixatives on characteristic morphological features of *K. veneficum.* The second experiment analyzed the allelopathic effect of *K. veneficum* on *Skeletonema costatum,* a common diatom in coastal, marine habitats throughout the tropical and temperate regions (Hasle 1973, Castillo et al. 1995, Chen et al. 2007, Haung et al. 2007).

5.2. Materials and methods

5.2.1. Dinoflagellate culture

Dinoflagellates were isolated from seawater samples collected from the vicinity of Dona Paula Bay along the central west coast of India (15°27′N, 73°48′E). The isolated dinoflagellates were maintained in f/2 medium (Guillard and Ryther 1962) without silicate at 22±2°C using a 12h:12h light:dark cycle.

5.2.2. Morphological characterization

The culture was examined using light and Scanning Electron Microscopy (SEM). For SEM, samples were fixed in 1% osmium tetraoxide, placed on a Nucleopore polycarbonate filter, and dehydrated in ethanol series consisting of 25%, 50%, 75%, 95% and 100% ethanol. Samples were kept for 15 min in each ethanol concentration except for 100% where it was kept for 30 min. The samples were then critical point dried. The filters were mounted on a stub, sputter-coated with gold and examined with a JEOL JSM-6700F field emission scanning microscope (at 5.0kV). Samples were also stained with 4'-6-diamidino-2-phenylindole (DAPI), kept under dark for 30 min and then observed under epifluorescence microscopy to determine the arrangement of the nucleus and chloroplasts. Light and epifluorescence photomicrographs were taken using Olympus BX51 microscope equipped with Olympus DP71 camera (12.5 megapixels) and ImagePro-plus software.

5.2.3. Effect of preservatives/fixatives

An experiment was designed to study the effect of fixatives on morphology of *K. veneficum*. The preservatives/fixatives used were buffered formalin, glutaraldehyde, Lugol's iodine and osmium tetraoxide. After fixation, samples were kept in a cool, dark place for a maximum period of 30 days. Samples were observed at 4 different time intervals (days 1, 7, 15 and 30), under a light microscope using 100X, 200X, 400X and 1000X magnification. Observations at each magnification were captured using a DP71 Olympus digital camera. All the images were tiled together using ImagePro-plus software for easy comparison.

5.2.4. Allelopathy experimental set-up

Culture filtrate (CF) and cell extracts (CE) were obtained during the exponential phase of *K. veneficum* culture (cell density 2.7 x 10^4 cells mL^{-1}). The CF was obtained by gravity filtration of *K. veneficum* culture (250 mL) through GF/F (glass fiber) filters. For getting the CE, 250 mL of culture was centrifuged for 15 min at 5000 rpm followed by sonication with a 40T Titanium needle probe (max. power 50 W) for 2 min with 30 sec intervals (3 cycles). After sonication, the contents were filtered through GF/F filter to obtain the CE.

S. costatum culture was inoculated in f/2 medium at a concentration of 100 cells mL^{-1}. Three sets of culture flasks (150 mL) in triplicate, containing 100 mL of *S. costatum* culture were used. One replicate was treated with CF (7 mL), the second

with CE (7 mL) and the control was leveled by adding same amount of plain f/2-silicate medium (7 mL), which was used for *K.veneficum* culture. Samples (2 mL) for cell count were taken from control as well as from CF and CE-treated flasks every alternate day, starting from the 2nd day of the experiment. Cell counting was carried out using a haemocytometer. Counting was possible only up to 12th day and thereafter the counting was stopped in order to avoid error due to clumping of *Skeletonema* cells.

5.2.5. Data analyses

The allelopathic effect was calculated using the equation, AE= [(Ctn-Ftn)/Ctn] x 100 (Fistarol et al. 2004) wherein, the difference between the cell numbers in the controls (Ctn) and in the filtrate treatments (Ftn) for the same sampling occasion, was normalized by the cell numbers in the control, and expressed as percentage. It represents the percentage of decrease or increase of cells in the filtrate relative to the control.

The log-transformed *S. costatum* abundance data [in controls and treatments (CF/CE)] was subjected to two-way ANOVA to assess the variation across treatments and days.

5.3. Results

5.3.1. Morphological characterization

The isolated dinoflagellate was identified as *K. veneficum* (Ballantine) J. Larsen. The identification of the species was carried out based on the characteristic features described in Bergholtz et al. (2005), using light and scanning electron microscopy.

5.3.2. Light microscopy (LM)

The cell outline is oval, epicone and hypocone are of about equal size (Fig. 5.1a). The cell dimensions ranged between 9-12 µm length and 6-9 µm width. This range is smaller compared to the range given by Bergholtz et al. (2005). Each cell has two to four large chloroplasts, 1-2 in each epicone and hypocone (Fig. 5.1a,b). The nucleus is large, round and situated centrally in the cell (Fig. 5.1c).

5.3.3. Scanning electron microscopy (SEM)

The apical groove and ventral pore are clearly noticeable just above the sulcal extension (Fig. 5.1d). The apical groove is deep and approximately 0.4 µm wide (Fig. 5.1e,f). The ventral pore is elongated, approximately 1.7 µm in length and 0.2 µm in width and situated on the left side of the apical groove (Fig. 5.1f). The outline of the epicone as well as hypocone is round (Fig. 5.1d-f). The transverse (Fig. 5.1d-f) and longitudinal flagella can be seen very clearly (Fig. 5.1d).

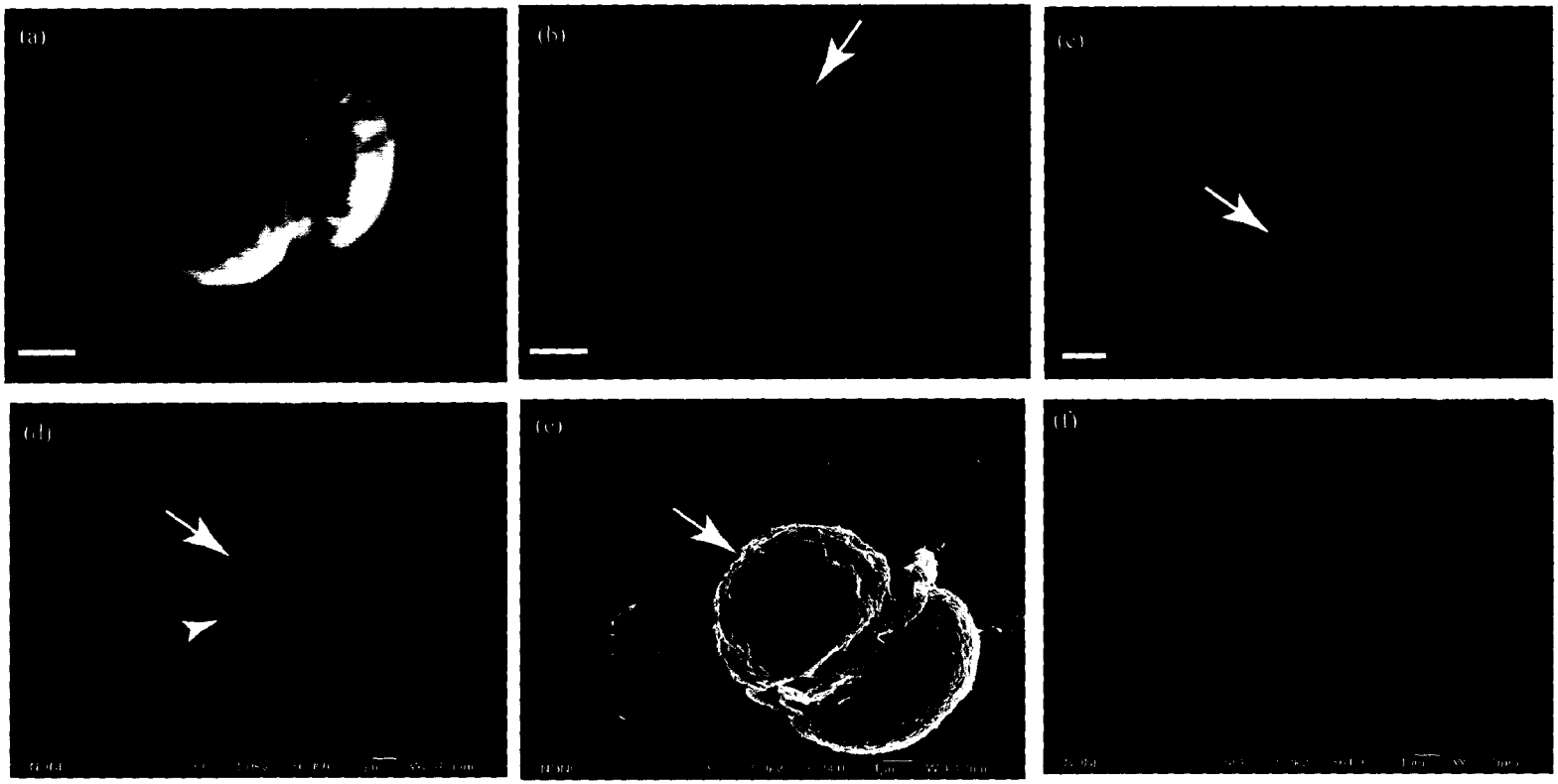


Fig. 5.1. Light, epifluorescence and scanning electron photomicrographs of *Karlodinium veneficum.* (a) Cell showing four large chloroplasts, two each in epicone and hypocone. Scale bar = 2 μM. (b) Depression of apical groove as observed in epicone (arrow). Scale bar = 2 μM. (c) Cell stained in DAPI showing the position of nucleus (arrow). Scale bar = 2.5 μM. (d) Ventral pore (arrow) and sulcal intrusion on ventral side of the epicone (arrowhead). Scale bar = 1 μM. (e,f) Dorsal view of cell showing apical groove (arrow) and transverse flagella. Scale bar = 1 μM.

5.3.4. Effect of preservatives/fixatives

These observations suggest the probable identification problems during light microscopy and also about the suitability of preservatives/fixatives. However, it is difficult to identify *K. veneficum* up to species level using a light microscope alone. Some minute characteristic features like apical groove, apical pore and sulcal intrusion are important for its identification (Bergholtz et al. 2005) but cannot be seen clearly under a light microscope.

On observing under 100X and 200 X magnification, *K. veneficum* appears like a pinhead structure (Fig. 5.2) and it is quite possible that it will be overlooked during routine microscopic analysis. The observation under 400X magnification can provide identifiable features at genus level (*Karlodinium/Gymnodinium*) due to a slight, dumbbell-shaped appearance (Fig. 5.3). Under 1000X magnification (Fig. 5.3), it was possible to identify the genus and by carefully observing the chloroplast structure and arrangement, species level features could also be visualized. But this observation is possible only if analysis is done within one week of fixation. With increasing number of days in preservatives/fixatives, the morphology changes, leading to identification errors. The results obtained indicate that till 7 days, all the preservatives/fixatives provided similar results. However, observations on days 15 and 30 indicated that the species in buffered formalin and glutaraldehyde had artifacts, such as no clear demarcation between epicone and hypocone, and chloroplast disruption. However, in Lugol's iodine and osmium tetraoxide, the demarcation between epicone and hypocone were still visible and artifacts were less obvious (Fig. 5.3).

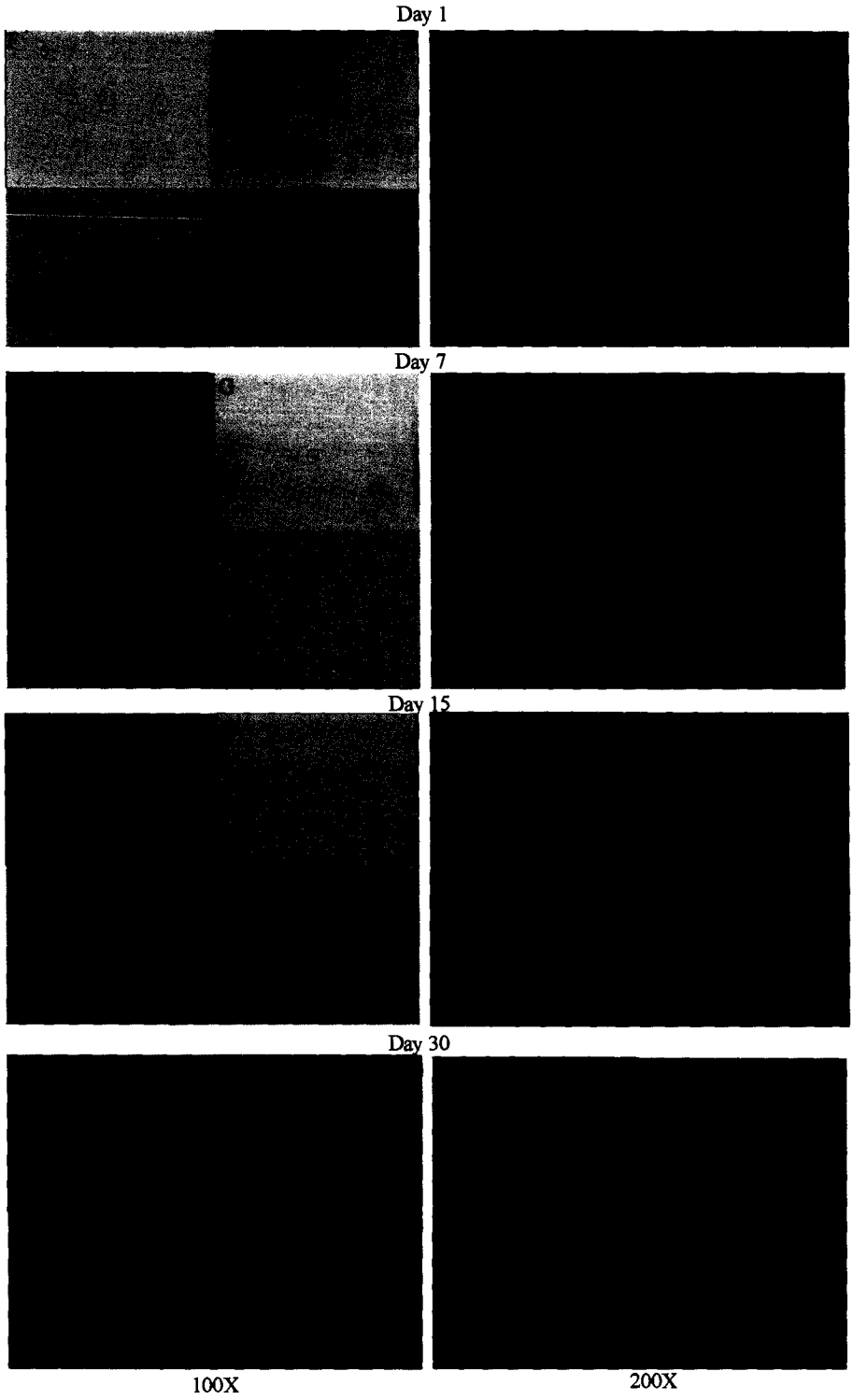


Fig. 5.2. Light photomicrographs of preserved *K. veneficum* (100X and 200X magnification) taken at different time intervals (days 1,7,15 and 30). F-Formalin, G-Glutaraldehyde, L-Lugol's iodine and O-Osmium tetraoxide.

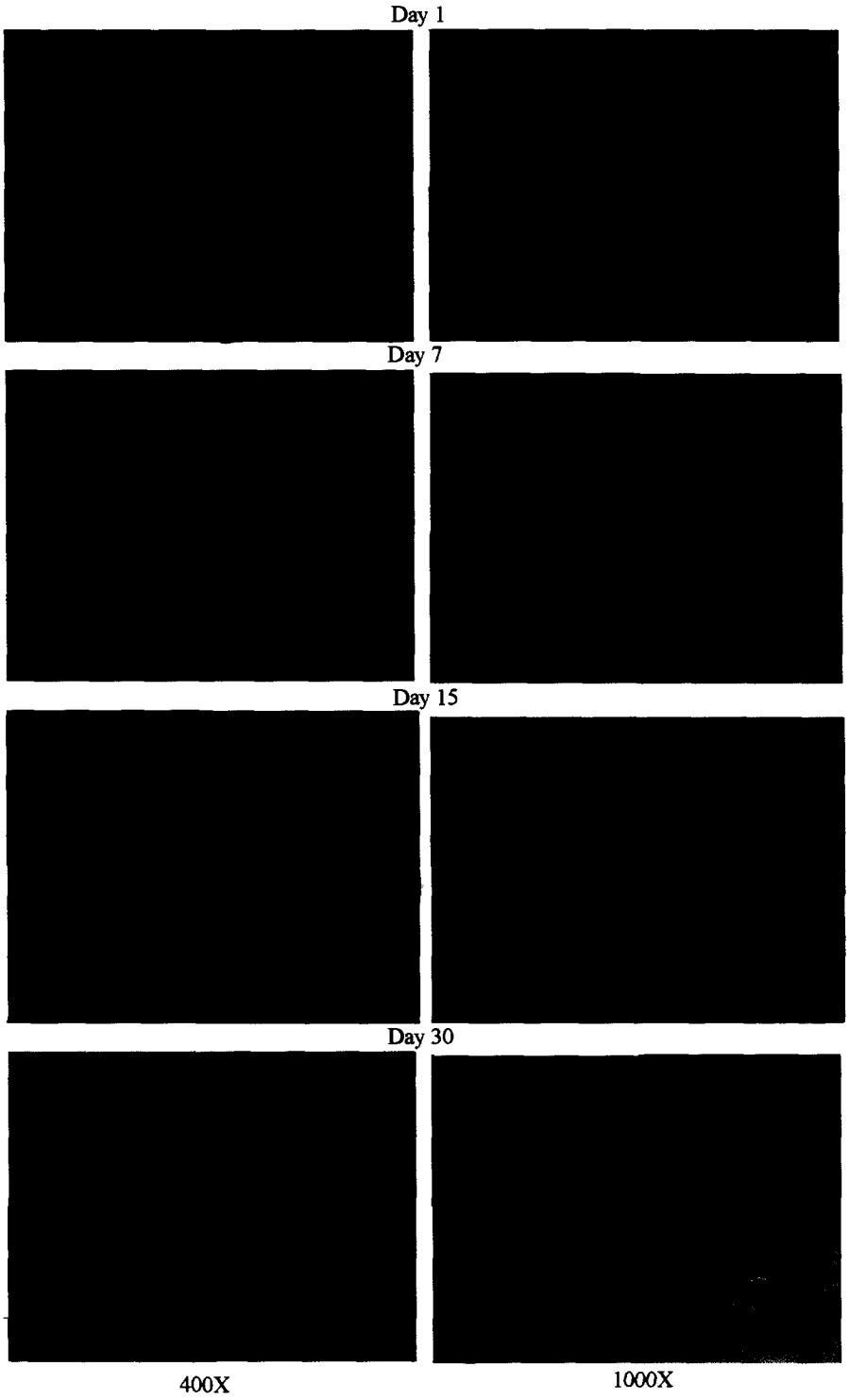


Fig. 5.3. Light photomicrographs of preserved *K. veneficum* (400X and 1000X magnification) taken at different time interval (days 1,7,15 and 30). F-Formalin, G-Glutaraldehyde, L-Lugol's iodine and O-Osmium tetraoxide.

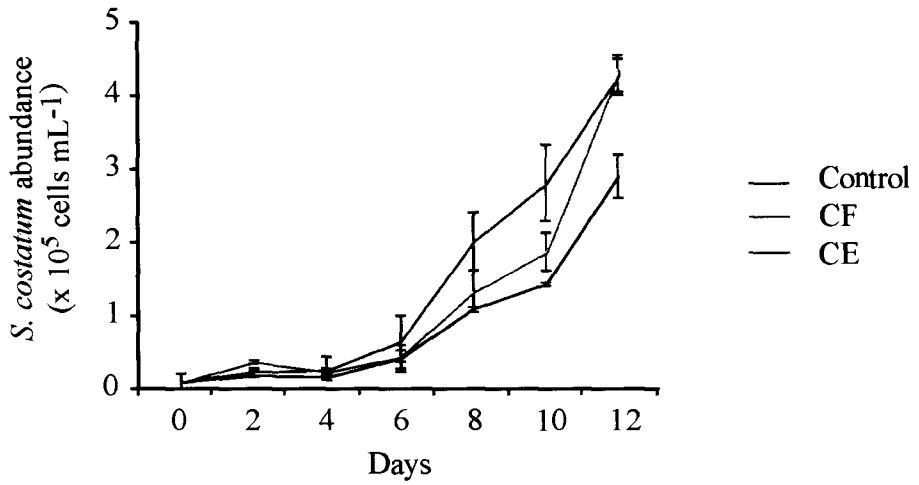


Fig. 5.4. Growth curve of *S. costatum* in control, with Culture Filtrate (CF) and Cell Extract (CE).

5.3.5. *Allelopathic effect of Culture Filtrate (CF)*

Initially till day 4, the CF was not inhibitory to *S. costatum.* In fact, the cell count of *S. costatum* in CF treated flasks (in triplicates) was higher till day 4 as compared to the control count. However, from day 6 onwards, an inhibitory effect was observed, which lasted till day 10 (Fig. 5.4). The highest AE/percentage of inhibition (33.9%) was observed on day 8 (Fig. 5.5).

Statistical analysis indicated that *S. costatum* abundance in the CF treatment did not vary significantly from that in the control. Significant variation was observed only across days (two-way ANOVA, Table 5.1).

Table 5.1. Two-way ANOVA to evaluate the variation in abundance of *S. costatum* in the Culture Filtrate (CF) and Cell Extract (CE) treatments.

Factor	*df*	SS	MS	*p-value*
Culture Filtrate				
Treatments	1	0.0108	0.0108	0.3000
Days	6	4.0933	0.6822	**1.75E-05**
Within sub-group error	6	0.0502	0.0084	
Total	13	4.1543		
Cell Extract				
Treatments	1	0.1409	0.1049	**0.0033**
Days	6	3.9916	0.6653	**3.59E-06**
Within sub-group error	6	0.0287	0.0048	
Total	13	4.1251		

5.3.6. Allelopathic effect of Cell Extract (CE)

Effect of CE inhibited the growth of *S.costatum* throughout the observation period (Fig. 5.4). Maximum AE (48.9%) was observed on day 10 (Fig. 5.5). However, the inhibitory effect lowered from day 10 to day 12 (Fig. 5.5).

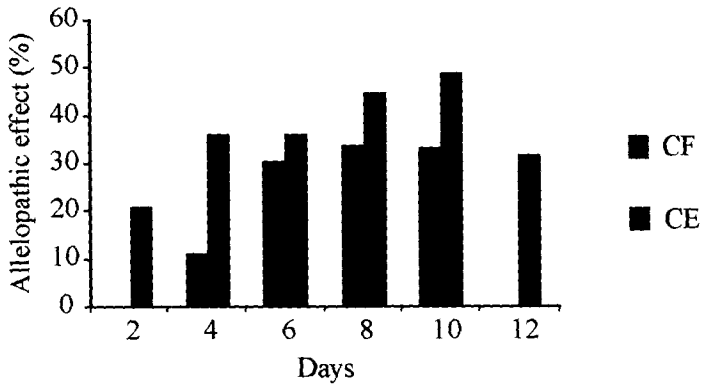


Fig. 5.5. Allelopathic effect of *K. veneficum* in Culture Filtrate (CF) and Cell Extract (CE)-treated *S. costatum*.

Statistical analysis indicated that *S. costatum* abundance in the CE treatment varied significantly from that in the control and also across days (two-way ANOVA, Table 5.1).

5.4. Discussion

Identification of *K. veneficum* from coastal waters of India adds one more geographic location in the information about its global distribution. Non-reporting of this species from the region can possibly be related to preservative-induced artifacts. Each method of preservation has its own merits and demerits in preservation of taxa (Throndsen 1978). For example, the most important advantage of the Lugol's iodine method is that flagellates do not lose their flagella. Ingredients for preparation of Lugol's iodine are relatively easy to obtain and this stock solution keeps well for many years. The demerits of this method are that samples fixed in Lugol's iodine need monitoring during storage as iodine oxidizes with time. The formaldehyde method maintains coccolithophorids, diatoms and thecate dinoflagellates in identifiable condition whereas the demerit of this method is that formaldehyde fixation bleaches the cell content and thus, it becomes difficult to distinguish between pigmented and non-pigmented cells (Throndsen 1978). In view of this, the method of preservation depends on the objectives of the work and the targeted taxa (Gifford and Caron 2000). The goal of the first experiment was to verify the temporal changes in morphological characteristic features of *K. veneficum* in different preservatives/fixatives and to determine the suitable choice for taxonomic identification of this species. The results obtained indicated that Lugol's iodine and

osmium tetraoxide provide better results. Nevertheless, the danger of using osmium tetraoxide outside a fume-hood, its expense, and the possibility of sample staining black limits its value (Taylor 1978). Lugol's iodine, considered to be a widely used fixative (Menden-Deuer et al. 2001, Zarauz and Irigoien 2008), was recommended earlier for preserving ciliates and flagellates (Throndsen 1978, Leakey et al. 1994, Karayanni et al. 2004). *K. veneficum* fixed in Lugol's iodine, provide better images depicting the characterstic outline of the epicone and hypocone. However, excess of Lugol's iodine can stain the chloroplast and render one of the identifying features of *K. veneficum* (chloroplast structure, 2 each in epicone and hypocone) non-recognizable. However, this problem can be solved by using sodium thiosulphate for cleaning the iodine stain (Throndsen 1978).

Apart from the cell outline, structure of the epicone and hypocone and chloroplasts, which are visible using light microscopy, there are several additional features which are necessary to identify *K. veneficum* up to species level. These features are the presence and position of the ventral pore and structure of the apical groove and sulcal intrusion, and cannot be discerned in preserved samples using light microscopy. In such cases, the use of electron microscopy facilitates identification upto species-level (Hasle 1978), and therefore must be used in conjunction with light microscopy whenever possible.

The detection of *K. veneficum* from the study area may not be surprising, since the species is known as a commonly found dinoflagellate in coastal waters around the world. But what make its detection interesting are the characteristic features of the west coast of India where the study area is located. The west coast of India is

influenced by the South West Monsoon (SWM) coupled with upwelling that results in high nutrient conditions (de Souza et al. 1996). This, in turn, triggers high primary production (Raghukumar and Anil 2003). The SWM also has a role in changing the dinoflagellate community structure (D'Costa et al. 2008). The appropriate biological and ecological mechanisms of this species to attain a large biomass in nature are not clear (Adolf et al. 2007). But the general understanding or the occurrence concepts of dinoflagellate blooms are linked with eutrophication or altered nutrient ratios (GEOHAB 2006 and references therein). Therefore, the presence of *K. veneficum* in the coastal waters of India (west coast) is of ecological interest. The Indian SWM conditions may provide appropriate conditions for *K. veneficum* to bloom. The bloom-forming capability of *K. veneficum*, its toxin-producing potential and the consequences for the ecosystem have been reported for other water bodies in earlier studies (Abbott and Ballantine 1957, Deeds et al. 2002, Fensin 2004, Place 2005, de Salas et al. 2005, Adolf et al. 2008); these studies indicate its adverse effect on fish and copepods.

It has been reported that karlotoxin produced by *K. veneficum* confers not only self-protection from grazers but also to co-occurring species as well (Adolf et al. 2007). Sheng et al. (2010) showed that karlotoxins are a vital instrument in the predation process. In the present study, the possibility of extracellular compounds released by *K. veneficum* as CF and intracellular compounds as CE, was considered. The results point out that the intracellular compounds can be more inhibitory to the co-existing diatom *S. costatum* compared to the extracellular compounds. The extracellular compounds appear to be relatively short-lived. This could be one of the

reasons for the substantial increase in *S. costatum* growth (compared to the control) on the 12th day in presence of CF. This aspect needs to be explored further.

The relatively high allelopathic effect of CE indicates the important role of contents of the organism. Adolf et al. (2007) and Waggett et al. (2008) have pointed out that, under grazing pressure, *K. veneficum* secretes anti-grazing metabolites. In view of this, it will be important to understand the differences between grazing-induced defensive mechanisms and population sustenance interactions with co-existing phytoplankton. To elucidate this aspect, further studies on prey-predator interactions and competition with other phytoplankton through micro- or mesocosm experiments will be a step forward.

Summarizing the results, the experiments carried out on the effect of fixatives, point out that it is possible to recognize morphological features of *K. veneficum* as long as 30 days, when Lugols iodine is used as a fixative. The allelopathic effect of *K. veneficum* on the growth of the diatom *S. costatum* observed in the study, suggests its potential ecological implications. However, several factors influencing the allelopathic effect of *K. veneficum*, for e.g., cell concentration (dose-dependence), its relation with nutrient concentrations, and the role of predation-induced defensive metabolites need to be elucidated in future experiments.

Chapter 6

Summary

In the context of the seas around India, several studies have explained the dynamics of phytoplankton communities (Subrahmanyan 1946; 1968; Devassy and Bhattathiri 1974; Taylor 1976; Desikachary and Prema 1987; Devassy and Goes 1988; Mitbavkar and Anil 2000; Saravanane et al. 2000; Habeebrehman et al. 2008; Patil and Anil 2008). Most of these studies were restricted to coastal dynamics of phytoplankton. However, very little is known about spatio-temporal variations in phytoplankton in the open ocean. In fact, most studies have focused on micro-phytoplankton (diatoms and dinoflagellates). The contribution of smaller phytoplankton groups (pico- and nano-plankton) has been underestimated due to the limitations of routine microscopy.

In view of the above, the present study was carried out in coastal and oceanic waters of India using the ballast water management programme and the Indian XBT observation programme respectively. Several aspects of phytoplankton community structure were addressed. These aspects were (1) spatio-temporal variation in dinoflagellates with reference to Harmful Algal Bloom (HAB) species, (2) regional and seasonal variations in phytoplankton community structure and the contribution of each group to the total community based on pigment studies, and (3) community structure of phytoplankton in two different coastal environments in relation to environmental settings. In the process of such evaluation, *Karlodinium veneficum* (a dinoflagellate) and 2 *Skeletonema* spp. (diatoms) were reported for the first time in Indian waters. Subsequently, the allelopathic effect of *K. veneficum* on *Skeletonema costatum* was evaluated.

To address the spatio-temporal variation in dinoflagellates with reference to HAB species, in the Bay of Bengal (BOB), surface waters were sampled using the 'ships-of-opportunity' programme. Sampling was carried out onboard passenger vessels plying between two transects, Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) during November 2003-September 2006. The samples were collected from 12 different locations at CP and 10 different locations at PK transects at one degree intervals. A total of 134 dinoflagellate species were recorded, with abundance ranging from 0-94 cells L^{-1}. Mixotrophs dominated in both transects on most occasions. *Ceratium* was the most dominant mixotrophic genus whereas *Protoperidinium* was the most dominant heterotrophic form. The highest dinoflagellate abundance was observed in September 2006, the period of withdrawal of the South West Monsoon (SWM) during which cloud cover reduces. The variations in species abundance could be attributed to the seasonal variations in the stratification observed in the BOB, which restricts the transport of nutrients from deeper layers to the surface (Prasanna Kumar et al. 2002).

The present study is the first of its kind detailing the HAB species from the stratified surface waters of the BOB and their seasonal occurrence. Nineteen potentially harmful species which accounted for approximately 14% of the total identified dinoflagellates species were encountered in this study. Among these, the frequently occurring HAB species (FOS) were low in abundance ($\leq$40 cell L^{-1}) and included *Ceratium furca, C. fusus, Dinophysis* sp., *Noctiluca scintillans* and *S. trochoidea*. The low abundance of FOS in the region may not be a growth issue but they may serve as inocula for future blooms if coupled with population-triggering

physical processes like eddies and cyclones in the region. In this scenario, the predominance of FOS like *C. furca* and *N. scintillans* in the BOB during cyclone-prone months, make their candidature stronger for future blooms in the region.

The regional and seasonal variations in phytoplankton community structure and the contribution of each group to the total community were analyzed based on pigment studies. Surface water samples were collected from CP and PK transects for phytoplankton and pigment analysis through High Performance Liquid Chromatography (HPLC). Using the diagnostic indices, spatial and temporal variations in surface water phytoplankton pigment distribution in the BOB were studied during the spring intermonsoon (SpIM, February-April 2007) and the commencement of the summer monsoon (CSM, May-June 2007). The Diagnostic Pigment (DP) index was defined as the sum of seven different pigments (DP= ZEA + Chl *b* + ALLO + 19'- HF + 19'- BF + FUC + PER); each of these pigments have their taxonomic significance. The use of the DP index was extended by Vidussi et al. (2001) and more recently by Barlow et al. (2007) to derive size-equivalent pigment indices which roughly correspond to the biomass proportions of pico-, nano- and microplankton. Thus, the DP index can be used to understand the biomass structure of an area purely based on pigment sums and ratios. Prokaryotic diagnostic pigment ($Prok_{DP}$) and Flagellate diagnostic pigment ($Flag_{DP}$) represents the biomass from pico- and nano- phytoplankton respectively. Diatom diagnostic pigments ($Diat_{DP}$) together with dinoflagellate diagnostic pigments ($Dino_{DP}$) represent the biomass of micro-phytoplankton.

The BOB is generally dominated by $Prok_{DP}$ followed by $Flag_{DP}$. In fact, $Prok_{DP}$ dominated at all the oceanic stations whereas $Flag_{DP}$ was dominant at the near coastal stations. Changes in the pigment pattern were observed at the onset of the monsoon, indicating the influence of rainfall especially in near coastal waters. During the commencement of summer monsoon, an oscillation in the dominance of $Prok_{DP}$ and $Flag_{DP}$ was observed in the Central Oceanic Bay (COB), whereas flagellates and diatoms were dominant at the near coastal stations. Comparative studies between microscopic counts and diagnostic pigment indices suggest coupling pigment composition analysis with microscopic analysis of natural assemblages to establish valid biogeochemical and ecosystem models. Notably, the components of dinoflagellate communities could be missed by pigment analysis alone.

The community structure of phytoplankton in 2 different coastal environments was evaluated in relation to environmental settings. Seasonal studies were carried out at 2 distinct ports environments namely Mormugao Port (MPT) and Visakhapatnam Port (VPT) located along the west and east coast of India respectively. Both the study locations differed in their environmental settings. MPT, situated on the mouth of the Zuari, was influenced by tidal flushing and the SWM whereas VPT, situated on the east coast, was influenced by the North East Monsoon (NEM) in addition to the SWM. VPT was also subjected to anthropogenic pressure along with several aspects of pollution (Subba Rao and Venkateswara Rao 1980, Sarma et al. 1996). Samples were collected from surface and near bottom waters for phytoplankton analysis and for estimation of physico-chemical variables. Sampling at both the study areas were carried out on different occasions [premonsoon (May 05), postmonsoon (December

05, and withdrawal of SWM (September 06) at MPT and NEM (November-December 07, premonsoon (April 08) and SWM (August 08) at VPT]. Diatoms and dinoflagellates were analyzed separately.

The coastal micro-phytoplankton community structure from the 2 contrasting study areas (MPT and VPT) differed. The environment under the monsoonal flushing (MPT) exhibited variation in the phytoplankton community structure, resembling natural seasonal cycling. In fact, distinct seasonal trends in the diatom communities in surface and near bottom waters were observed. Seasonal changes in the dinoflagellate community were clearer in surface waters compared to near bottom waters. Mixotrophic dinoflagellates dominated in both surface and near bottom waters. However, dinoflagellates were not observed in any of the 16 stations sampled during PrM, possibly due to their tendency to migrate towards favourable surface waters. In contrast to these conditions, the influence of eutrophication on the phytoplankton community was observed in VPT, the semi-enclosed water body. This was reflected in the prevalence of a single dominant genus (*Skeletonema*) irrespective of seasons. Autotrophic dinoflagellates consistently dominated in surface waters; near bottom waters showed an oscillation between auto- and heterotrophic dominance.

Detailed taxonomic studies on preserved/cultured micro-phytoplankton from these 2 coastal locations revealed 2 new reportings of diatoms and 1 potentially toxic, non-thecate dinoflagellate. Though the planktonic diatom genus *Skeletonema* is common in Indian coastal waters, the high diversity in this genus has only recently been revealed. Therefore, it is expected that several species occur in the highly diverse

marine habitats along the Indian coastline. However, most Indian studies on phytoplankton diversity consider only *Skeletonema costatum,* the type species. In the present study, a *S. costatum* culture, raised from water samples taken from Goa was analyzed in addition to 2 preserved samples, 1 from the BOB and 1 from the Arabian Sea (AS). Samples were examined using Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The Goan culture strain was also examined using Transmission Electron Microscopy (TEM) and characterized using its LSU rDNA sequence. The Goan culture strain belonged to *S. costatum sensu stricto.* The sample from the BOB was identified as *S. grevillei* whereas the sample from the AS was identified as *S. tropicum.* The reporting of these species in Indian waters has wide-ranging implications. Firstly, it is an important blooming diatom in Indian waters. Secondly, it influences trophic dynamics by providing cues for intermittently breeding invertebrates (Barnes 1962, Desai and Anil 2005). Even though the present study involved the analysis of a limited number of samples, it highlights the need for ultrastructural and molecular taxonomic studies to unravel the diversity of *Skeletonema* in Indian waters. Regular monitoring is needed to uncover not only the diversity of this genus, but also habitat preferences and seasonal variation of the detected species.

Karlodinium veneficum, a non-thecate, potentially toxic dinoflagellate was isolated from Goa for the first time. This species has both toxic and non-toxic strains and due to its bloom forming capability, is known to influence co-existing phytoplankton. Its detection in the coastal waters of India was possible because of live sample analysis. This species often gets overlooked due to its smaller size (de Salas et al. 2005) (< 20

μm). Artifacts due to fixation could be another reason; this has already been reported as a significant factor of bias in phytoplankton analysis (Throndsen 1978, Booth 1987, Stoecker et al. 1994, Mender-Deuer et al. 2001, Zarauz and Irigoien 2008).

Two experiments on *K. veneficum* isolated from Indian waters were conducted. The first experiment analyzed the effect of 4 different preservatives on characteristic morphological features of *K. veneficum.* The second experiment analyzed the allelopathic effect of *K. veneficum* on *S. costatum,* a co-existing diatom. Experiments with preservatives/fixatives suggest that it is possible to recognize morphological features of *K. veneficum* for as long as 30 days, when Lugol's iodine is used. The allelopathic effect of *K. veneficum* on the growth of the diatom *S. costatum* suggests its potential ecological implications. However, several factors influencing this allelopathic effect, for e.g., cell concentration (dose-dependence), its relation with nutrient concentrations, and the role of predation-induced defensive metabolites need to be elucidated in future experiments.

Overall, this study investigates the role of different environmental factors on phytoplankton community structure in both oceanic and coastal waters. The results of this study suggest that climatic factors (for e.g., rainfall) play an important role in the dynamics of these communities. However, anthropogenic pressures influence the seasonality in these communities in coastal waters. New reports of diatom and dinoflagellate species from the region during the course of this study and observations on their biological aspects highlight the need for further studies in this direction.

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Short Communications

Skeletonema (Bacillariophyceae) in Indian waters: A reappraisal

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The planktonic diatom genus *Skeletonema* is common in Indian coastal waters. Recent taxonomic studies have uncovered high diversity in this genus, and it is expected that several species occur also in the highly diverse marine habitats along the Indian coastline. In the present study, material of a culture raised from a specimen isolated from a water sample taken from Goa and material from two Lugol fixed samples, one from the Bay of Bengal and one from the Arabian Sea, were examined. Samples were examined in LM and SEM. The culture strain from Goa was also characterized using its LSU rDNA sequence. Results revealed that the above strain belongs to *S. costatum sensu stricto.* The sample from the Bay of Bengal contained *S. grevillei* and the sample from the Arabian Sea, *S. tropicum.*

[**Keywords:** *Skeletonema costatum sensu stricto, S. grevillei, S. tropicum,* Indian waters]

The diatom genus *Skeletonema* Greville emend. Sarno et Zingone is common in the plankton of coastal marine habitats throughout the tropical and temperate regions[1-4]. The first species to be described in this genus is *S. costatum* (Greville) Cleve[5] and in most studies, the genus is considered synonymous with this species. However, such a treatment constitutes a serious underestimation of the generic diversity because several species have been recognized in *Skeletonema*. These are: *S. ardens* Sarno et Zingone[6], *S. costatum* (Greville) Cleve emend. Zingone et Sarno (*sensu stricto; s.s.*)[7], *S. dohrnii* Sarno et Kooistra[8], *S. grethae* Zingone et Sarno[8], *S. grevillei* Sarno et Zingone[6,7], *S. japonicum* Zingone et Sarno[8], *S. marinoi* Sarno et Zingone[8], *S. menzelii* Guillard, Carpenter and Reimann[9], *S. potamos* (Weber) Hasle[10,11], *S. pseudocostatum* Medlin emend. Zingone et Sarno[8,12], *S. tropicum* Cleve[13] and *S. subsalsum* (A. Cleve) Bethge[14,15]. The taxonomic treatment of *Skeletonema* is a work in progress because some of the proposed species are not well separated genetically and/or morphologically[16-20]. Moreover, cryptic diversity abounds in *S. menzelii* and *S. tropicum*[20].

In a recent biogeographical study, molecular and morphological approaches were integrated to identify numerous strains of *Skeletonema* collected from all over the world[20]. Results indicated that although most *Skeletonema* species appear to be widely distributed, none seems to be truly cosmopolitan. However, tropical regions were severely underrepresented and Indian waters were missing in their survey.

In Indian waters, Subrahmanyan[21] reported *S. costatum.* The genus has since then been reported throughout the year, both in the Arabian Sea and the Bay of Bengal[22-29]. Along the west coast of India, *Skeletonema* blooms during the onset of the south-west monsoon after an intermittent break, when salinity is low and nutrient concentrations are higher compared to other months of the year[26,30]. Along the east coast, *Skeletonema* dominates the phytoplankton community in the northern part of the Bay of Bengal during the south-west monsoon and it does likewise at the onset of the north-east monsoon at Kalpakkam, in the southern part of Bay of Bengal, when salinity and nutrient concentrations are low[28,31]. These lower nutrient concentrations associated with dominance of *Skeletonema* in the Bay of Bengal are in contrast to the elevated nutrient concentrations associated with *Skeletonema* blooms along the west coast of India. In spite of these apparent differences, all these blooms are believed to consist of *S. costatum.* However, this treatment does not take into account the recent taxonomic appraisal of the genus. The goal of the present study is to give a first account

of *Skeletonema* species diversity in Indian waters according to the updated taxonomy.

To accomplish this goal, we examined one culture strain and two preserved phytoplankton samples. The culture strain was isolated from the waters of Dona Paula Bay, Goa, along the west coast of India in May 2007. It was maintained in f/2 medium[32] at 25°C with 12 h : 12h L :D cycle and transferred to fresh f/2 medium every 10 days. It is presently in the culture collection of Marine Corrosion and Materials Research Division (MCMRD), National Institute of Oceanography, Goa, India. The preserved phytoplankton samples were collected from the east coast (Bay of Bengal; 21°01'N, 88°13'E; October 2006) and west coast (Arabian Sea; 15°27'N, 73°48'E; August 2007) of India. Culture and the preserved samples were prepared for LM and SEM following Sarno *et al.*[8]. Samples were observed using an Axiophot (Carl Zeiss, Oberkochen, Germany) and a JEOL JSM-6700F field emission scanning microscope.

The culture strain was also examined utilizing a molecular approach. DNA extraction, PCR amplification and sequencing of the first ca. 700 base pairs of the large subunit nuclear ribosomal RNA coding region (LSU rDNA), and alignment of this marker with other sequences was carried out as described in Sarno *et al.*[8]

Culture strain was identified as *S. costatum s. s.* based on molecular and morphological results, whereas *S. grevillei* and *S. tropicum* were identified based on morphological characters in samples from the east and west coast of India respectively.

Skeletonema costatum (Figures 1 and 2): Cells are 4-12 µm in diameter and contain 1-2 chloroplasts. In terminal valves, fultoportulae have open processes that end with a claw-like protrusion (Figure 1). In intercalary valves, processes of fultoportulae are closed, with a small hole often visible at their base and a longitudinal suture running from the hole towards the process end (Figure 2). Each process generally connects to two processes of the sibling valve (1:2 junction). In both intercalary and terminal valves, the rimoportula is located near the marginal ring of fultoportulae and has a long external process (Figure 2). The morphological features of this strain corresponded to those reported in Zingone *et al.*[7], which re-examined the type material of the species, and in Sarno *et al.*[6] which observed the species as live material. Results of the molecular identification procedure showed that the partial LSU rDNA sequence of the culture strain was identical to those of *S. costatum* strains B and C (Lagoa dos Patos, Brazil, Genbank numbers EF423397 and EF423398) and strain SZN-B212 (Montevideo, Uruguay, Genbank number EF423396).

Skeletonema grevillei (Figures 3-6): Cells are 5-8 µm in diameter and contain 1-2 chloroplasts. The external parts of fultoportulae are open along their entire length in both terminal and intercalary valves. In terminal valves, processes of fultoportulae are irregularly truncated at their tips and pointed at their lateral ends (Figure 3). In intercalary valves, the processes of the fultoportulae are long (7-8 µm), each joining one process of the adjacent cells (1:1 junction) with a knuckle-like connection (Figures 4, 6). Several series of siliceous ridges link the bases of the processes to each other and to the valve mantle, delimiting a series of bigger, oval holes between the fultoportulae and a series of smaller, circular holes at the external base of the fultoportulae (Figures 3-5). In terminal valves of the colony the rimoportula is located close to the valve face margin, near the marginal ring of fultoportulae, and it bears a long process. The morphological features of some specimens found in the East coast (Figure 5) show a larger development of the silica ridges compared to the original description of the species[7].

Skeletonema tropicum (Figures 7-9): Cells are 9-15 µm in diameter and contain several chloroplasts (1-7). Tips of the fultoportulae of terminal processes are irregularly truncated or, more often, show a claw-like projection (Figure 7). The processes of the intercalary fultoportulae connect tightly to those of the next cell (Figure 8). The junction is generally of the 1:1 type, but occasionally 1:2 junctions are visible (Figure 9). Processes of the fultoportulae are straight (Figure 8) or curved (Figure 9). Both types of processes have been observed in a single colony. Rimoportula of the terminal valves has a long external process, located midway between the annulus and the valve margin (Figure 7). Hasle[1] showed spiraling processes connecting sibling cells in specimens from Peru and from Atlantida (Uruguay) identified as *S. costatum.* Kooistra *et al.*[20] uncovered cryptic diversity in *S. tropicum.* However, in the present study, we did not have access to genetic information for this material.

Skeletonema costatum s. s. and *S. grevillei* have a wide geographical range, occurring in both temperate and tropical waters[6,20]. The third species, *S. tropicum*, has been reported by Kooistra *et al.*[20] in tropical locations as well as in the Mediterranean

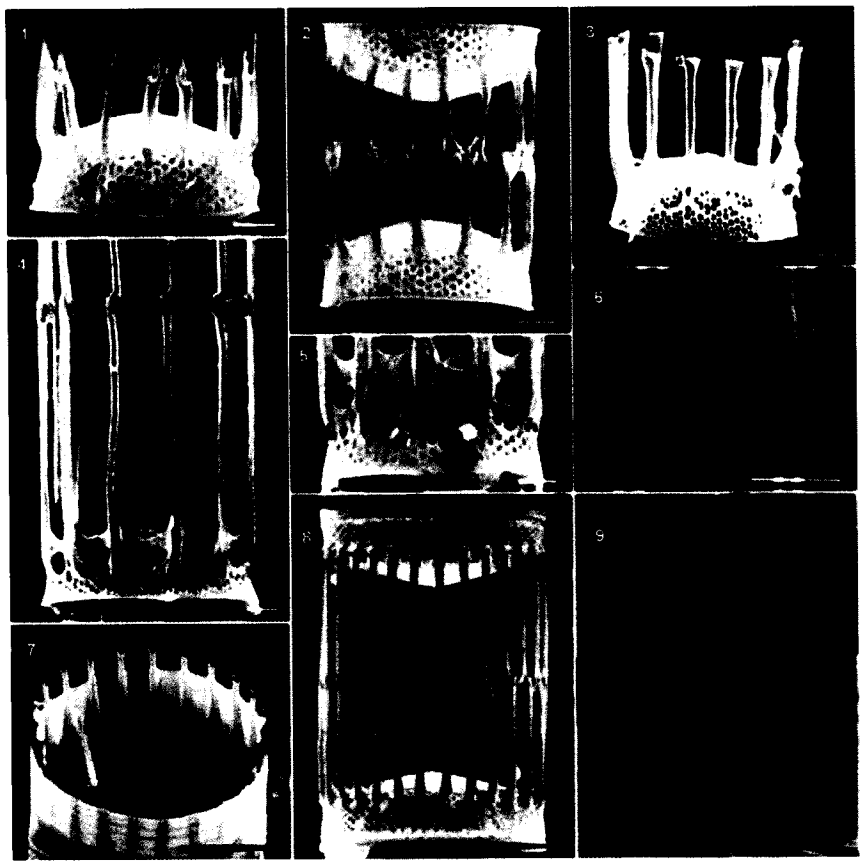

Plate 1

Plate 1— General morphology of *Skeletonema* (SEM pictures). (1) *S. costatum,* terminal valve with the marginal terminal rimoportula process (arrow). Scale bar = 1 μm. (2) *S. costatum,* intercalary valve with the long rimoportula process (arrow). Scale bar = 1 μm. (3) *S. grevillei,* terminal valve. Scale bar = 1 μm. (4) *S. grevillei,* intercalary valve. (5) *S. grevillei,* detail of an intercalary valve showing the well expanded silica ridges joining the bases of the intercalary fultoportula processes. Scale bar = 1 μm. (6) *S. grevillei,* detail of knuckle-like junction. Scale bar = 1 μm. (7) *S. tropicum,* terminal valve with fultoportula processes and subcentral rimoportula process. Scale bar = 5 μm. (8, 9) *S. tropicum,* intercalary valve. Scale bar = 5 μm

Sea, the East China Sea and in southern Brazil during summer and autumn.

The observation of two other *Skeletonema* species (*S. grevellei* and *S. tropicum*) in addition to *S. costatum s. s.* in this study highlights the need for ultrastructural and molecular taxonomic studies to unravel the diversity of *Skeletonema* in Indian waters. Regular monitoring is needed to uncover not only the diversity of this genus, but also habitat preferences and seasonal variation of the detected species.

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Karlodinium veneficum in India: effect of fixatives on morphology and allelopathy in relation to *Skeletonema costatum*

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Dinoflagellates form an important component of marine and freshwater phytoplankton. They are a remarkably diverse and complex group with various modes of nutrition and ability to produce toxins, and are major contributors to harmful algal blooms. In the present work, we identify the presence of *Karlodinium veneficum* in India. Its detection in the coastal waters of India was possible because of live sample analysis. This species has both toxic and nontoxic strains and due to its bloom-forming capability, it is known to influence the coexisting phytoplankton community structure. In this communication we provide a description of this species, possible methods for its identification in preserved samples and effect of its extracellular and intracellular extract on the growth of *Skeletonema costatum*, an important and abundant diatom in the phytoplankton community in the Indian waters.

Keywords: Allelopathy, cell extract, harmful algal blooms, *Karlodinium veneficum*, phytoplankton, *Skeletonema costatum*.

IN 1950, near Plymouth Sound, UK, a non-thecate dinoflagellate was isolated by Parke and later described and named as *Gymnodinium veneficum* by Ballantine[1]. Further studies on the same strain using light and electron microscopy and partial LSU rDNA, found matching features with *Karlodinium micrum*. Thus Bergholtz *et al.*[2] suggested a change of name for both *G. veneficum* and *K. micrum* to *Karlodinium veneficum*.

K. veneficum (as *K. micrum*) is a widespread species, reported from various bioregions, Australia[3], North America[4], southern Africa[5] and Europe[6]. Bergholtz *et al.*[2] also described its wide distribution around different water bodies. This species often gets overlooked due to its small size (< 20 μm)[7]. Artifacts due to fixation could be another reason, since previous studies showed that artifacts induced by fixation are a potentially significant factor of bias to phytoplankton sample analysis[8,9]. The majority of earlier studies conducted in the Indian waters are preservative-based. Identification of species belonging to *Karlodinium* often requires live samples[2]. In this study, in the course of routine monitoring programme involving collection of phytoplankton samples with the intention of raising cultures, we have isolated and cultured *K. veneficum*. This is the first report of this species from waters around the subcontinent of India.

K. veneficum is considered as a common member of the coastal phytoplankton, generally found at relatively low cell abundance, but capable of forming blooms under appropriate conditions. Such blooms have resulted in fish mortality[4,7,10,11]. Karlotoxins produced by certain strains of *K. veneficum* and its mixotrophic capabilities are likely to be important factors contributing to the formation and continuation of blooms[12,13]. But, in some of the cases, by releasing secondary metabolites such as allelopathic chemicals, some toxic algae can also negatively influence co-occurring phytoplankton[14]. Such production of toxins or release of organic compounds is considered a part of the defence mechanisms or competition strategies among phytoplankton[15,16].

In light of the above, we conducted two experiments on *K. veneficum* isolated from the Indian waters. The first experiment analysed the effect of four different fixatives on characteristic morphological features of *K. veneficum*. The second experiment analysed the allelopathic effect of *K. veneficum* on *Skeletonema costatum*, a common diatom in coastal, marine habitats throughout the tropical and temperate regions[17,18].

Dinoflagellates were isolated from sea-water samples collected from the vicinity of Dona Paula Bay along the central west coast of India (15°27′N, 73°48′E). The isolated dinoflagellates were maintained in f/2 medium[19] without silicate at 22 ± 2°C using a 12 h : 12 h light : dark cycle.

The culture was examined using light microscopy (LM) and scanning electron microscopy (SEM). For SEM, the samples were fixed in 1% osmium tetraoxide, placed on a nucleopore polycarbonate filter, and dehydrated in ethanol series consisting of 25%, 50%, 75%, 95% and 100% ethanol. Samples were kept for 15 min in each ethanol concentration except for 100%, where it was kept for 30 min. The samples were then critical-point dried. The filters were mounted on a stub, sputter-coated with gold and examined with a JEOL JSM-6700F field emission scanning microscope (at 5.0 kV). Samples were also stained with 4′-6-diamidino-2-phenylindole (DAPI), kept in dark for 30 min and then observed under epifluorescence microscopy to determine the arrangement of the nucleus and chloroplasts. Light and epifluorescence photomicrographs were taken using Olympus BX51 microscope equipped with Olympus DP71 camera (12.5 megapixels) and ImagePro-plus software.

An experiment was designed to study the effect of fixatives on the morphology of *K. veneficum*. The fixatives used were buffered formalin, glutaraldehyde, Lugol's iodine and osmium tetraoxide. After fixation, the samples were kept in a cool, dark place for a maximum period of 30 days. Samples from all fixatives were observed at four different time intervals (days 1, 7, 15 and 30), under a

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light microscope using 100×, 200×, 400× and 1000× magnification. Observations at each magnification were captured using an Olympus DP71 digital camera. All the images were tiled together using ImagePro-plus software for easy comparison.

Culture filtrate (CF) and cell extracts (CE) were obtained during the exponential phase of *K. veneficum* culture (cell density 2.7×10^4 cells ml^{-1}). The CF was obtained by gravity filtration of *K. veneficum* culture (250 ml) through GF/F (glass fibre) filters. To obtain the CE, 250 ml of the culture was centrifuged for 15 min at 5000 rpm followed by sonication with a 40T Titanium needle probe (max power 50 W) for 2 min with 30 s interval (three cycles). After sonication, the contents were filtered through GF/F filter to obtain the CE.

S. costatum culture was inoculated in f/2 medium at a concentration of 100 cells ml^{-1}. Three sets of culture flasks (150 ml) in triplicate, containing 100 ml *S. costatum* culture were used. One replicate was treated with CF (7 ml), the second with CE (7 ml) and the control was levelled by adding same amount of plain f/2-silicate medium (7 ml), which was used for *K. veneficum* culture. Samples (2 ml) for cell count were taken from the control as well as from CF and CE-treated flasks every alternate day, starting from the second day of the experiment. Cell counting was carried out using a haemocytometer. Counting was possible only up to 12th day and thereafter the counting was stopped in order to avoid error due to clumping of *Skeletonema* cells.

The allelopathic effect (AE) was calculated using the equation[16]: AE = [(Ctn-Ftn)/Ctn] × 100, wherein the difference between the cell numbers in the controls (Ctn) and in the filtrate treatments (Ftn) for the same sampling, was normalized by the cell numbers in the control, and expressed as percentage. It represents the percentage of decrease or increase of cells in the filtrate relative to the control.

The log-transformed *S. costatum* abundance data [in controls and treatments (CF/CE)] was subjected to two-way ANOVA to assess the variation across treatments and days.

The isolated dinoflagellate was identified as *K. veneficum* (Ballantine) J. Larsen. Identification of the species was carried out based on the characteristic features described in Bergholtz *et al.*[2] using LM and SEM.

In the case of LM, the cell outline is oval; the epicone and hypocone are of about equal size (Figure 1 *a*). The cell dimensions ranged from 9 to 12 μm length, and 6 to 9 μm width. This range is smaller compared to that given by Bergholtz *et al.*[2]. Each cell has four large chloroplasts, two each in the epicone and hypocone (Figure 1 *a* and *b*). The nucleus is large, round and situated centrally in the cell (Figure 1 *c*).

In the case of SEM the apical groove and ventral pore are clearly noticeable just above the sulcal extension (Figure 1 *d*). The apical groove is deep and approximately 0.4 μm wide (Figure 1 *e* and *f*). The ventral pore is elongated, approximately 1.7 μm in length and 0.2 μm in

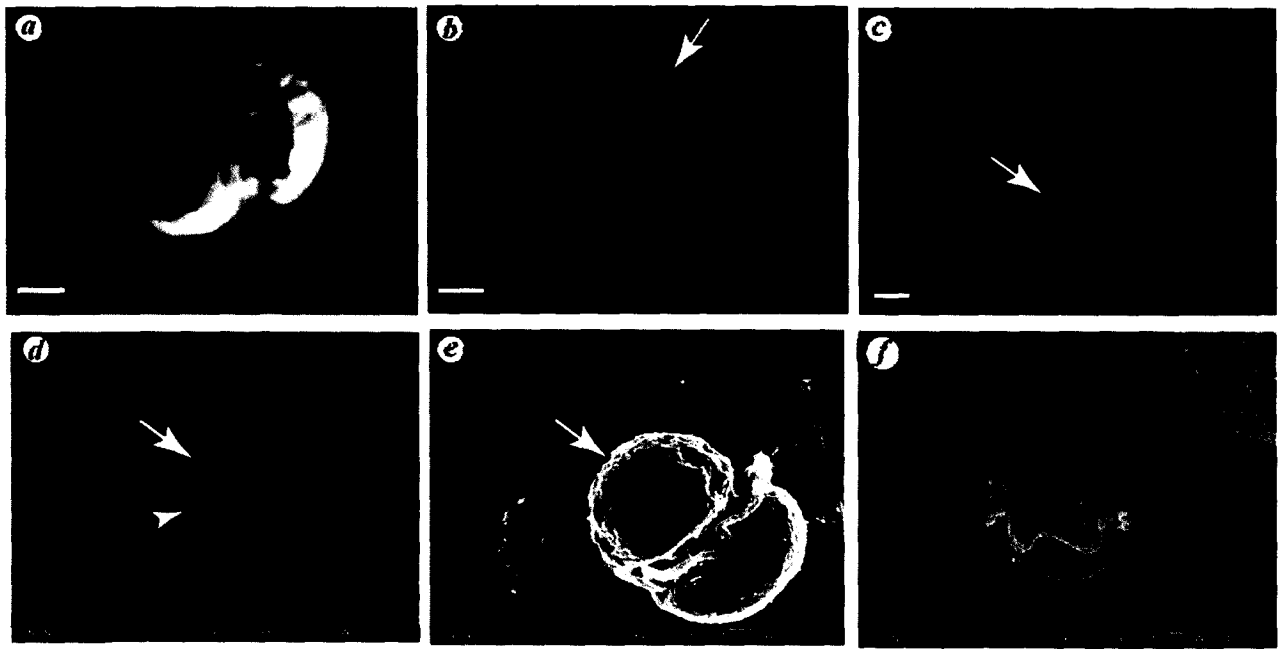

Figure 1. Light, epifluorescence and scanning electron photomicrographs of *Karlodinium veneficum*. ***a***, Cell showing four large chloroplasts, two each in the epicone and hypocone. Scale bar = 2 μM. ***b***, Depression of apical groove as observed in epicone (arrow). Scale bar = 2 μM. ***c***, Cell stained with DAPI showing the position of the nucleus (arrow). Scale bar = 2.5 μM. ***d***, Ventral pore (arrow) and sulcal intrusion on the ventral side of the epicone (arrowhead). Scale bar = 1 μM. ***e***, ***f***, Dorsal view of the cell showing apical groove (arrow) and transverse flagella. Scale bar = 1 μM.

width and situated on the left side of the apical groove (Figure 1 *f*). The outline of the epicone and the hypocone is round (Figure 1 *d–f*). The transverse and longitudinal flagella can be seen clearly (Figure 1 *d*).

We carried out an evaluation of the effect of fixatives on the morphology of *K. veneficum*. It is generally difficult to identify *K. veneficum* up to species level using light microscope alone. Some minute characteristic features like apical groove, apical pore and sulcal intrusion are important for its identification[2], but cannot be seen clearly under a LM.

Observation of *K. veneficum* under 100X and 200X magnification provides a pinhead appearance, and it is quite possible that it will be overlooked during routine microscopic analysis. Observation under 400X magnification can provide clues to identifiable features at the genus level (*Karlodinium/Gymnodinium*) due to a slight, dumbbell-shaped appearance. Under 1000X magnification, our observation indicated that it is possible to identify the genus and by carefully observing the chloroplast structure and arrangement, species-level features could also be visualized. But this observation is possible only if the analysis is done within one week of fixation. With increasing number of days in fixatives, the morphology changes, leading to identification errors. The results obtained indicate that till seven days, all the fixatives provided similar results. However, observations on days 15 and 30 indicated that the species in buffered formalin and glutaraldehyde had artifacts, such as no clear demarcation between the epicone and hypocone, and chloroplast disruption. However, in Lugol's iodine and osmium tetraoxide, the demarcation between epicone and hypocone was still visible and artifacts were less obvious (Figure 2).

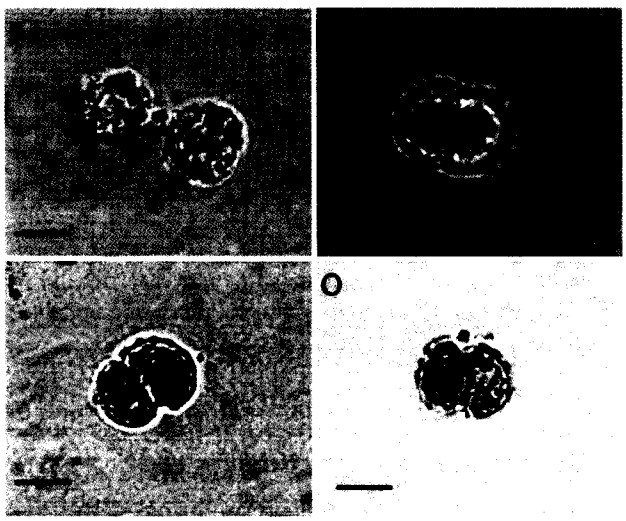

Figure 2. Light photomicrograph of *K. veneficum* taken at 1000X magnification on day-30 showing the effect of fixatives. F, Formalin; G, Glutaraldehyde; L, Lugol's iodine and O, Osmium tetraoxide. Scale bar = 5 μM.

In the case of allelopathic evaluation, initially till day-4, the CF was not inhibitory to *S. costatum*. In fact, the cell count of *S. costatum* in CF-treated flasks (in triplicates) was higher till day-4 compared to the control count. However, from day-6 onwards, an inhibitory effect was observed, which lasted till day-10 (Figure 3 *a*). The highest AE/percentage of inhibition (33.9) was observed on day-8 (Figure 3 *b*).

Statistical analysis indicated that *S. costatum* abundance in the CF treatment did not vary significantly from that in the control (two-way ANOVA, P = not significant); it varied significantly only across days (two-way ANOVA, $P = 0.00002$).

CE inhibited the growth of *S. costatum* throughout the observation period (Figure 3 *a*). Maximum AE (48.9%) was observed on day-10 (Figure 3 *b*). However, the inhibitory effect lowered from day-10 to day-12 (Figure 3 *b*).

Statistical analysis indicated that *S. costatum* abundance in the CE treatment varied significantly from that in the control (two-way ANOVA, $P = 0.00338$) and also across days (two-way ANOVA, $P = 0.00004$).

Identification of *K. veneficum* from coastal waters of India adds one more geographic location in the information about its global distribution. Non-reporting of this species from the region can possibly be related to preservative-induced artifacts. Each method of preservation

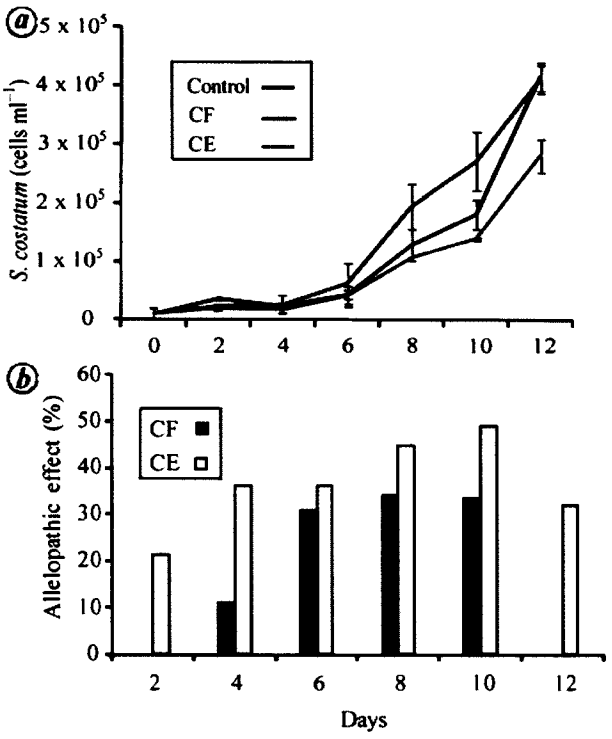

Figure 3. Allelopathic effect of *K. veneficum* on the growth of the diatom *Skeletonema costatum*. ***a***, Growth curve of *S. costatum* in control, with culture filtrate (CF) and culture extract (CE). ***b***, Allelopathic effect (%) in CF and CE-treated *S. costatum*.

has its own merits and demerits in preservation of taxa[8]. For example, the most important advantage of the Lugol's iodine method is that flagellates do not lose their flagella. Ingredients for the preparation of Lugol's iodine are relatively easy to obtain and this stock solution keeps well for many years. The demerits of this method are that samples fixed in Lugol's iodine need monitoring during storage as iodine oxidizes with time. The formaldehyde method maintains coccolithophorids, diatoms and thecate dinoflagellates in identifiable condition, whereas the demerit of this method is that formaldehyde fixation bleaches the cell content and thus, it becomes difficult to distinguish between pigmented and non-pigmented cells[8]. In view of this, the method of preservation depends on the objectives of the work and the targeted taxa[20]. The goal of our first experiment was to verify the temporal changes in morphological characteristic features of *K. veneficum* in different fixatives and to determine the suitable fixative for taxonomic identification of this species. The results obtained indicate that Lugol's iodine and osmium tetraoxide provide better results. Nevertheless, the danger of using osmium tetraoxide outside a fumehood, its expense and the possibility of the sample staining black limits its value[21]. Lugol's iodine, considered to be a widely used fixative[9], was recommended earlier for preserving ciliates and flagellates[8]. *K. veneficum* fixed in Lugol's iodine provides better images depicting the characteristic outline of the epicone and hypocone. However, excess of Lugol's iodine can stain the chloroplast and render one of the identifying features of *K. veneficum* (chloroplast structure, two each in epicone and hypocone) non-recognizable. However, this problem can be solved by using sodium thiosulphate for cleaning the iodine stain[8].

Apart from the cell outline, structure of the epicone and hypocone and chloroplasts, which are visible using LM, there are several additional features which are necessary to identify *K. veneficum* up to species level. These features are the presence and position of the ventral pore and structure of the apical groove and sulcal intrusion, and cannot be discerned in preserved samples using LM. In such cases, the use of electron microscopy facilitates identification up to species level, and therefore must be used in conjunction with LM whenever possible.

The detection of *K. veneficum* from the study area may not be surprising, since the species is known as a commonly found dinoflagellate in coastal waters around the world. But what makes its detection interesting are the characteristic features of the west coast of India, where the study area is located. This area is influenced by the southwest monsoon and enhanced nutrient loading from land[22] that is known to trigger primary productivity[23]. The southwest monsoon also has a role in changing the dinoflagellate community structure[24]. The appropriate biological and ecological mechanisms of this species to attain a large biomass in nature are not clear[12]. But the general understanding or the occurrence concepts of dinoflagellate blooms are linked with eutrophication or altered nutrient ratios[25]. Therefore, the presence of *K. veneficum* in the coastal waters of India (west coast) is of ecological interest. The Indian southwest monsoon may provide appropriate conditions for *K. veneficum* to bloom. The bloom-forming capability of *K. veneficum*, its toxin-producing potential and the consequences for the ecosystem have been reported for other water bodies in earlier studies[4,7,10]. These studies indicate its adverse effect on fish and copepods.

It has been reported that karlotoxin produced by *K. veneficum* confers not only self-protection from grazers, but also to co-occurring species as well[12]. Sheng *et al.*[26] showed that karlotoxins are a vital instrument in the predation process. In the present study, we examined the possibility of extracellular compounds released by *K. veneficum* as CF and intracellular compounds as CE. The results point out that the intracellular compounds can be more inhibitory to the co-existing diatom, *S. costatum* compared to the extracellular compounds. The extracellular compounds appear to be relatively short-lived. This could be one of the reasons for the substantial increase in *S. costatum* growth (compared to the control) on the 12th day in the presence of CF. This aspect needs to be explored further.

The relatively high allelopathic effect of CE indicates the important role of contents of the organism. Adolf *et al.*[12] and Waggett *et al.*[13] have pointed out that, under grazing pressure, *K. veneficum* secretes anti-grazing metabolites. In view of this, it will be important to understand the differences between grazing-induced defensive mechanisms and population sustenance interactions with the co-existing phytoplankton. To elucidate this aspect, further studies on prey–predator interactions and competition with other phytoplankton through micro- or mesocosm experiments will be a step forward.

Experiments carried out on the effect of fixatives point out that it is possible to recognize morphological features of *K. veneficum* for as long as 30 days, when Lugol's iodine is used as a fixative. The allelopathic effect of *K. veneficum* on the growth of the diatom *S. costatum* observed in the study, suggests its potential ecological implications. However, several factors influencing the allelopathic effect of *K. veneficum*, e.g. cell concentration (dose-dependence), its relation with nutrient concentration, and the role of predation-induced defensive metabolites need to be elucidated in future experiments.

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European *Cryptothallus mirabilis* v. Malmb. matured in India – its morphological and LM & SEM palynological studies, with comments on utility for modern and fossil studies

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The present communication provides an account of the Scottish *Cryptothallus mirabilis* – a unique, non-chlorophyllous thalloid bryophyte which grows hidden underneath the ground layer. The studied plants were immature during collection, but have successfully matured in India under the artificial conditions. Plants show unbranched–branched fleshy thalli and enlarged calyptra which protects the sporophyte. Palynological studies has shown that spores remain adherent in their tetrad after maturity, crytopolar type, showing irregularly reticulate sculpturing at exposed surface (i.e. distal), which under SEM exhibits double ornamentation. This taxon is widely distributed but the extent of distribution is not certain due to subterranean habit. The present study will be useful in both modern and fossil studies.

Keywords: *Cryptothallus mirabilis*, fossil studies, morphological–palynological studies, Scotland India.

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Dinoflagellate community structure from the stratified environment of the Bay of Bengal, with special emphasis on harmful algal bloom species

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Abstract Harmful algal blooms (HABs) have been documented along the coasts of India and the ill effects felt by society at large. Most of these reports are from the Arabian Sea, west coast of India, whereas its counterpart, the Bay of Bengal (BOB), has remained unexplored in this context. The unique characteristic features of the BOB, such as large amount of riverine fresh water discharges, monsoonal clouds, rainfall, and weak surface winds make the area strongly stratified. In this study, 19 potentially harmful species which accounted for approximately 14% of the total identified species (134) of dinoflagellates were encountered in surface waters of the BOB during November 2003 to September 2006. The variations in species abundance could be attributed to the seasonal variations in the stratification observed in the BOB. The presence of frequently occurring HAB species in low abundance ($\leq$40 cell L^{-1}) in stratified waters of the BOB may not be a growth issue. However, they may play a significant role in the development of pelagic seed banks, which can serve as inocula for blooms if coupled with local physical processes like eddies and cyclones. The predominance of *Ceratium furca* and *Noctiluca scintillans*, frequently occurring HAB species during cyclone-prone seasons, point out their candidature for HABs.

Keywords *Ceratium furca* · *Noctiluca scintillans* · Bay of Bengal · Stratification · Cyclones · Eddies

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Introduction

Harmful algal blooms (HABs) are natural phenomena; historical records indicate their occurrence long before the advent of human activities in coastal ecosystems. Recent surveys have demonstrated a dramatic increase and geographic spread in HAB events in the last few decades (Anderson 1989; Smayda 1990; Hallegraeff 1993). Thus, knowledge of the present geographic distribution and seasonal fluctuations in HAB species is important to understand globally spreading HAB events (GEOHAB 2001). In fact, Hallegraeff (2010) has reported that unpreparedness for such significant range expansions or spreading of HAB problems in poorly monitored areas will be one of the greatest problems for human society in the future.

Among the total marine phytoplankton species, approximately 7% are capable of forming algal blooms (red tides; Sournia 1995); dinoflagellates

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are the most significant group producing toxic and harmful algal blooms (Steidinger 1983, 1993; Anderson 1989; Hallegraeff 1993) accounting for 75% of the total HAB species (Smayda 1997).

Blooms result from a coupling mechanism involving physical, chemical, and biological factors. Though dynamics of blooms is complex, the role or mechanism of chemical and biological factors are now reasonably understood (Fistarol et al. 2004; Solé et al. 2006; GEOHAB 2006; Adolf et al. 2007; Waggett et al. 2008). However, comparable understanding about physical factors is lacking except for few examples (Maclean 1989; Karl et al. 1997; Belgrano et al. 1999; Yin et al. 1999). Impacts of these factors in combination with local inter-annual meteorological conditions will vary from one geographical location to the other and will thus influence bloom dynamics differentially.

HAB studies in Indian waters indicate reasonable reports on HABs and their impacts along the west coast of India. Direct impacts of HABs on human health have also been reported from Mangalore (Karunasagar et al. 1984); they related the death of a boy to an outbreak of paralytic shellfish poisoning (PSP) following consumption of clams. Dinoflagellate toxins have also been recorded in shellfish from surrounding estuaries near Mangalore in 1985 and 1986 (Segar et al. 1989). Planktonic and cyst forms of *Gymnodinium catenatum*, a PSP-producing dinoflagellate from this region were detected later on (Godhe et al. 1996) and the importance of close monitoring of coastal waters, sediment, and shellfish was highlighted.

Compared to the above regions, the Bay of Bengal (BOB), the eastern arm of the Indian

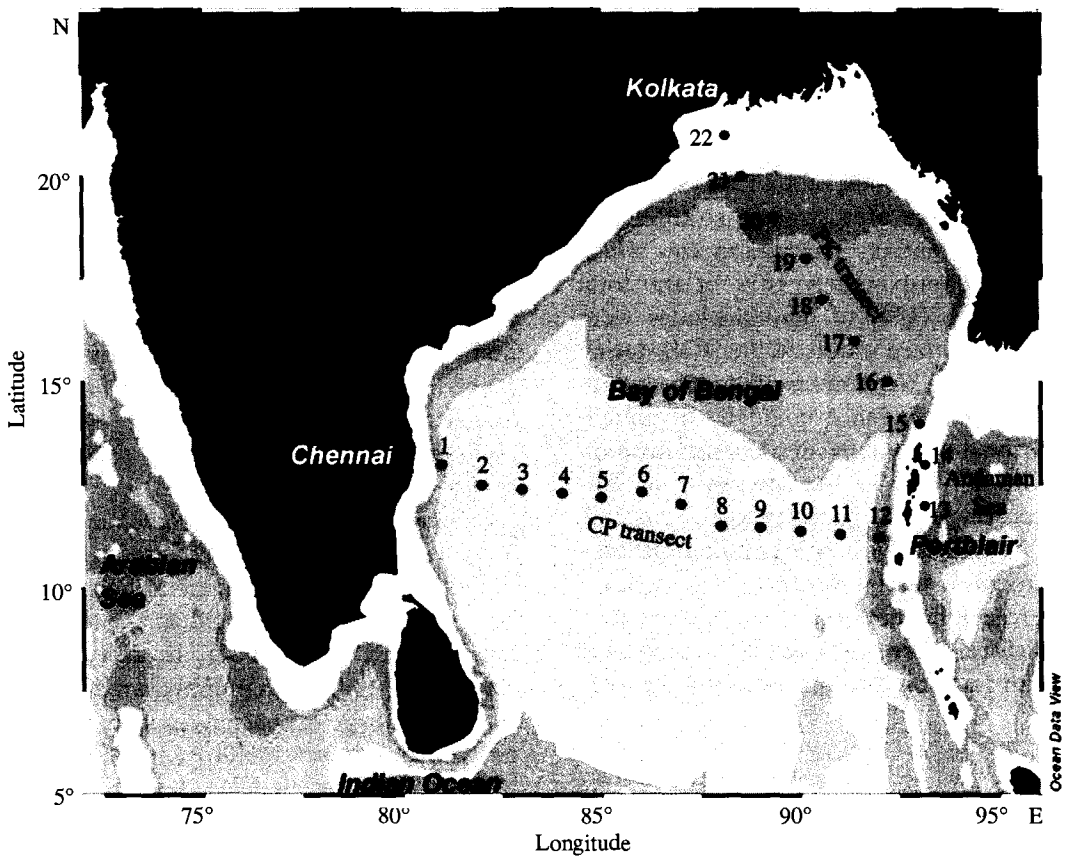

Fig. 1 Study area map showing station locations along the Chennai–Port Blair (*CP*) and Port Blair–Kolkata (*PK*) transects in the Bay of Bengal

Ocean, remains relatively unexplored in the context of HAB studies. The BOB is known for its unique characteristic features: large volume of freshwater input from river discharge and rainfall, warmer sea surface temperatures, monsoonal clouds and reversal of currents. The riverine input into this area injects loads of nutrients and suspended sediment in the BOB (Gordon et al. 2002; Mukhopadhyay et al. 2006). These features point to the suitability of the BOB as a zone prone for algal blooms including HAB events. However, the strongly stratified surface layer of the BOB restricts the transport of nutrients from deeper layers to the surface (Prasanna Kumar et al. 2002). Thus, it is very interesting to understand the seasonal variations in dinoflagellate community structure in this inimitable geographic region.

Since taking oceanic cruises on regular intervals is not cost-effective, the "ships of opportunity" program, supported by the Indian Expendable Bathythermograph (XBT) project, was used. Therefore, the present study on the spatial and temporal distribution of dinoflagellates in the BOB, with emphasis on HAB species, is the first of its kind from the region. The following objectives were addressed: (1) dinoflagellate distribution in the surface waters of the BOB and (2) detailing of the HAB species present and their seasonal occurrence.

Materials and methods

Study area and sampling

This study was conducted with the support of the XBT program. Surface water samples were collected using steel buckets from the moving ship. This method was selected in order to minimize the physical damage to cells compared to the method of collecting samples using a pump. The samples were collected on two transects [Chennai to Port Blair (CP, 12 stations) and Port Blair to Kolkata (PK, 10 stations)] (Fig. 1), from passenger ships plying along these transects. Samples were collected at one-degree intervals along both transect, from November 2003 to September 2006 covering different seasons. Among the sampling stations from CP (central BOB) and PK (northern BOB) transects, the majority were oceanic whereas, few stations are near the coast (Table 1). Samples (1 L) were fixed with Lugol's iodine solution for the laboratory enumeration and identification of dinoflagellates to the lowest possible taxonomic level.

Table 1 Station details of Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects

Stations	Lat (N)	Long (E)
CP transect		
1	13.04	81.07
2	13.09	82.03
3	13.09	83
4	13.04	84.05
5	13.05	85.05
6	12.49	86
7	12.38	87
8	12.23	88.03
9	12.1	89
10	11.52	90.05
11	11.41	91.04
12[a]	11.28	92.07
PK transect		
13[a]	12.03	93.14
14[a]	13.01	93.14
15	14	92.56
16	15.01	92.24
17	16.08	91.29
18	17.09	90.43
19	18	90.12
20	19	89.3
21	20	88.5
22[a]	21.05	88.14

[a]Near coastal stations

Microscopic analysis

The 1-L sample was kept settling for 48 h. After that, the volume was brought down to 100 ml and then to 10 ml final concentration after another 48-h settling period (method modified from Hasle 1978). From this 10-ml final concentration, 3 ml concentrated sample was taken in a petri dish (3.8 cm diameter) and examined under an Olympus inverted microscope at ×100 to ×400 magnification. Identification of the dinoflagellate taxa was carried out using the keys provided by Subrahmanyan (1968), Taylor (1976), Tomas (1997), Horner (2002), and Hallegraeff et al. (2003).

Table 2 Taxonomic list of identified dinoflagellates during the study period along the CP and PK transects (* potentially HAB species, † new reporting)

	CP transect					PK transect				
	Feb04	Jun04	Jul04	Oct04	Sep06	Nov03	Mar04	Aug04	Oct04	Sep06
Autotrophic dinoflagellates										
Alexandrium concavum		0–2 (1)								
Alexandrium spp.			0–2 (2)		0–9 (1)			0–2 (1)	0–20 (1)	0–6 (1)
Amphidinium sp.*	0–7 (5)	0–2 (1)	0–2 (1)	0–5 (3)	0–12 (10)			0–8 (2)	0–5 (1)	0–9 (8)
Amphisolenia bidentata		0–2 (2)	0–8 (7)	0–3 (1)	0–3 (3)			0–2 (1)		0–9 (3)
Amphisolenia globifera										0–3 (2)
Blepharocysta sp.		0–4 (2)	0–4 (3)	0–8 (2)				0–4 (5)	0–8 (6)	0–3 (1)
Ceratocorys armata	0–2 (1)						0–2 (1)			
Ceratocorys horrida		0–4 (2)		0–3 (1)		0–2 (2)				
Corythodinium constrictum		0–2 (1)					0–4 (2)			
Corythodinium elegans			0–4 (1)	0–3 (1)	0–3 (1)					0–3 (1)
Corythodinium michaelsarsi				0–8 (2)						
Corythodinium reticulatum	0–2 (2)						0–2 (2)			
Corythodinium tesselatum		0–2 (2)	0–2 (1)	0–3 (2)	0–3 (3)	0–2 (2)				0–3 (2)
Corythodinium sp.			0–2 (1)		0–3 (1)					
Ensiculifera sp.									0–5 (1)	
Gambierdiscus sp.* †								0–4 (1)		
Goniodoma polyedricum		0–2 (2)				0–6 (2)	0–2 (1)		0–5 (1)	
Goniodoma sphaericum	0–6 (3)				0–3 (1)		0–6 (1)	0–2 (2)	0–5 (1)	0–3 (2)
Gonyaulax brevisulcata										0–3 (1)
Gonyaulax digitalis				0–5 (1)						
Gonyaulax grindleyi	0–2 (1)									
Gonyaulax monospina †					0–3 (1)					0–3 (1)
*Gonyaulax polyedra**	0–4 (4)			0–3 (1)			0–2 (1)			
*Gonyaulax polygramma**	0–8 (1)	0–2 (1)	0–2 (1)	0–8 (4)	0–6 (1)	0–4 (2)	0–4 (1)	0–4 (2)	0–8 (3)	0–6 (2)
Gonyaulax scrippsae	0–4 (1)		0–2 (2)		0–3 (1)	0–6 (3)				
*Gonyaulax spinifera**	0–4 (4)			0–3 (1)		0–4 (1)	0–2 (2)			
Gonyaulax sp.	0–2 (1)	0–2 (2)	0–2 (2)	0–3 (1)	0–6 (6)	0–2 (1)	0–4 (2)	0–6 (1)	0–3 (2)	0–3 (5)
Gymnodinium spp.*			0–2 (1)	0–5 (1)	0–6 (3)			0–6 (1)		0–6 (3)
Oxytoxum globosum	0–26 (1)									
Oxytoxum laticeps				0–3 (1)	0–6 (3)	0–2 (1)				0–6 (4)
Oxytoxum scolopax	0–3 (5)	0–2 (2)	0–4 (6)	0–8 (4)	0–3 (5)		0–6 (2)	0–2 (2)	0–5 (1)	0–3 (4)
Oxytoxum sceptrum		0–2 (1)	0–4 (5)	0–3 (4)				0–2 (3)		0–3 (1)
Oxytoxum sp.	0–6 (3)	0–2 (1)					0–2 (2)			0–3 (1)

Podolampas bipes		0–2 (3)		0–3 (1)	0–3 (2)	0–2 (1)			0–5 (1)	0–6 (2)
Podolampas elegans	0–4 (3)						0–2 (1)			
Podolampas palmipes	0–4 (4)	0–2 (2)	0–2 (3)	0–3 (3)	0–6 (1)		0–2 (1)	0–2 (1)	0–5 (1)	0–3 (1)
Podolampas spinifera		0–2 (1)	0–2 (2)	0–3 (1)					0–5 (1)	
Pyrophacus steinii	0–2 (1)			0–3 (1)		0–2 (3)	0–2 (1)			0–18 (1)
Pyrophacus spp.				0–5 (2)						
Torodinium teredo †					0–3 (2)					
Mixotrophic dinoflagellates										
*Alexandrium minutum**	0–10 (1)								0–3 (2)	
Ceratium arietinum					0–3 (1)	0–2 (1)				
Ceratium azoricum			0–2 (1)	0–3 (1)						0–3 (1)
Ceratium candelabrum f. *depressum*	0–5 (3)						0–4 (1)			
Ceratium contortum		0–2 (1)	0–2 (1)	0–5 (4)	0–9 (4)				0–5 (1)	0–9 (3)
Ceratium declinatum				0–3 (1)					0–8 (1)	
Ceratium deflexum					0–3 (1)					
Ceratium dens							0–4 (4)	0–6 (1)		
Ceratium extensum			0–2 (2)	0–5 (4)	0–9 (5)	0–20 (5)		0–6 (4)	0–13 (4)	0–18 (4)
*Ceratium furca**	0–2 (1)									
*Ceratium fusus**	0–6 (4)	0–2 (4)	0–2 (3)	0–13 (9)	0–6 (4)	0–20 (1)	0–4 (1)	0–6 (5)	0–8 (7)	0–12 (3)
Ceratium gibberum	0–2 (1)									
Ceratium horridum			0–2 (2)		0–6 (2)			0–2 (1)		0–3 (1)
Ceratium karstenii	0–2 (4)						0–2 (1)			
Ceratium kofoidii		0–2 (1)	0–2 (1)			0–2 (1)				
Ceratium lineatum			0–2 (2)	0–3 (2)	0–3 (1)				0–5 (1)	0–3 (1)
Ceratium lunula							0–2 (1)			
Ceratium macroceros		0–2 (3)	0–2 (1)			0–2 (3)		0–2 (1)		
Ceratium pentagonum					0–9 (7)			0–2 (1)		0–3 (1)
Ceratium schmidtii			0–2 (2)	0–5 (3)	0–3 (1)				0–8 (2)	
Ceratium symmetricum	0–10 (4)			0–3 (1)			0–2 (2)			
Ceratium teres		0–8 (6)	0–8 (7)	0–8 (7)	0–9 (7)	0–4 (1)		0–2 (1)	0–3 (2)	0–12 (3)
Ceratium trichoceros	0–2 (2)						0–8 (1)			
Ceratium tripos		0–2 (1)	0–2 (1)			0–2 (1)				
Ceratium vultur	0–4 (1)									0–3 (1)
Ceratium spp.			0–2 (1)	0–3 (2)	0–3 (3)				0–5 (1)	
*Dinophysis caudata**								0–2 (1)	0–5 (1)	
*Dinophysis miles**								0–12 (1)		
Dinophysis schuettii				0–3 (1)						
Dinophysis spp.	0–4 (5)		0–2 (2)	0–5 (2)			0–6 (6)		0–5 (1)	0–3 (1)
Dissodium asymmetricum						0–2 (1)				

Table 2 (continued)

	CP transect					PK transect				
	Feb04	Jun04	Jul04	Oct04	Sep06	Nov03	Mar04	Aug04	Oct04	Sep06
Prorocentrum arcuatum †	0–2 (1)						0–4 (1)			
*Prorocentrum belizeanum** †		0–24 (1)								
Prorocentrum compressum		0–2 (1)		0–5 (1)				0–2 (1)		
Prorocentrum gracile				0–8 (4)						
Prorocentrum lenticulatum	0–4 (1)									
*Prorocentrum lima**							0–2 (1)			
*Prorocentrum micans**	0–20 (1)		0–2 (2)	0–3 (1)	0–3 (2)			0–10 (1)	0–10 (2)	0–9 (1)
*Prorocentrum minimus**	0–2 (1)		0–2 (1)	0–3 (1)				0–2 (1)		
*Prorocentrum mexicanum**	0–5 (6)									
Prorocentrum obtusum				0–8 (3)					0–3 (2)	0–3 (2)
Prorocentrum scutellum †								0–2 (1)		
*Prorocentrum sigmoides**			0–2 (1)		0–3 (1)					0–9 (4)
Prorocentrum sp.	0–2 (2)	0–14 (2)	0–4 (3)	0–8 (5)	0–9 (7)	0–4 (4)	0–4 (1)	0–6 (2)	0–8 (3)	0–6 (5)
Pyrocystis fusiformis			0–2 (1)	0–3 (1)						
Pyrocystis hamulus	0–2 (1)		0–4 (2)			0–2 (1)				
Pyrocystis lunula	0–4 (1)									
Pyrocystis spp.		0–2 (1)	0–2 (1)	0–3 (1)	0–3 (2)	0–14 (5)	0–6 (1)		0–5 (2)	0–3 (1)
*Scrippsiella trochoidea**	0–14 (9)		0–2 (1)	0–3 (1)	0–9 (7)		0–26 (6)	0–4 (1)	0–5 (2)	0–15 (7)
Scrippsiella spp.		0–2 (1)	0–6 (5)	0–10 (3)			0–2 (1)	0–6 (3)	0–8 (1)	
Heterotrophic dinoflagellates										
Gotoius abei†		0–6 (4)		0–3 (1)		0–10 (5)			0–8 (4)	0–3 (1)
Histioneis carinata †	0–2 (1)				0–3 (1)		0–4 (2)			
Histioneis costata †	0–2 (1)	0–2 (1)						0–2 (1)		
Histioneis depressa		0–2 (2)	0–2 (2)							
Histioneis spp.					0–3 (1)					
Katodinium sp.		0–2 (3)	0–4 (5)	0–10 (2)	0–3 (2)			0–2 (2)	0–5 (1)	
Noctiluca scintillans		0–2 (1)				0–40 (1)	0–2 (1)			
Noctiluca spp.	0–2 (1)									
Ornithocercus heteroporus	0–6 (2)						0–2 (1)			
Ornithocercus magnificus		0–2 (2)	0–4 (2)	0–18 (2)	0–3 (1)				0–5 (1)	0–6 (2)
Ornithocercus quadratus		0–2 (1)	0–2 (1)	0–3 (1)					0–5 (1)	
Ornithocercus steinii			0–2 (1)			0–4 (2)	0–2 (1)	0–2 (1)	0–5 (1)	
Ornithocercus thumii		0–2 (1)				0–4 (2)		0–2 (1)	0–5 (1)	

Ornithocercus spp.								0–2 (1)		0–3 (1)
Oxyphysis spp.					0–3 (1)					
Pentapharsodinium sp. †		0–2 (1)				0–4 (1)				
Phalacroma argus	0–2 (1)									
Phalacroma cuneus								0–2 (1)	0–5 (1)	
Phalacroma rapa	0–2 (2)	0–2 (1)		0–3 (1)						
Phalacroma rotundatum					0–3 (1)			0–2 (1)	0–8 (2)	0–3 (2)
Phalacroma spp.		0–2 (2)	0–2 (2)			0–2 (1)				
Pronoctiluca pelagica							0–2 (1)			
Pronoctulica spinifera		0–2 (2)	0–2 (1)	0–3 (1)	0–3 (1)					
Protoperidinium asymmetricum	0–4 (3)						0–2 (1)			
Protoperidinium brevipes		0–2 (1)								
*Protoperidinium crasipes**	0–2 (1)						0–2 (1)			
Protoperidinium depressum	0–2 (4)	0–2 (1)					0–4 (2)	0–2 (1)	0–5 (1)	
Protoperidinium divergens		0–2 (1)	0–4 (3)	0–5 (6)	0–3 (1)	0–2 (1)		0–2 (2)	0–3 (5)	
Protoperidinium elegans				0–3 (2)						
Protoperidinium grande	0–4 (1)									
Protoperidinium leonis		0–2 (1)								
Protoperidinium minutum										0–3 (1)
Protoperidinium oblongum						0–2 (1)				
Protoperidinium ovatum	0–4 (8)						0–26 (2)			
Protoperidinium pacificum		0–2 (1)		0–10 (2)		0–6 (2)	0–4 (2)		0–8 (3)	
Protoperidinium pallidum			0–2 (1)							
Protoperidinium pedunculatum	0–4 (3)	0–2 (2)		0–8 (4)		0–4 (1)	0–2 (3)	0–4 (1)	0–5 (1)	
Protoperidinium pellucidum	0–4 (2)				0–3 (1)	0–4 (1)	0–2 (3)	0–4 (1)		
Protoperidinium pentagonum		0–2 (1)				0–10 (1)		0–2 (1)		
Protoperidinium pyriforme							0–2 (1)			
Protoperidinium steinii			0–2 (1)	0–3 (2)	0–6 (1)	0–4 (1)		0–8 (2)	0–3 (4)	
Protoperidinium subinerme				0–3 (1)				0–2 (2)		
Protoperidinium sp.		0–4 (4)	0–4 (6)	0–3 (4)	0–9 (8)	0–2 (1)	0–4 (2)	0–8 (3)	0–5 (1)	0–9 (9)
Zygabikodinium sp.		0–2 (1)								

The values outside bracket indicate the range of abundance during that month and values inside the bracket indicates the frequency of occurrence at CP (total 12 stations) and PK (total 10 stations) transects during the respective sampling months

Data analyses

To evaluate seasonal differences, the observational period was classified into three seasons: Pre-monsoon (PrM—February to March 04), South west monsoon (SWM—June 04, July 04, August 04, September 06) and Post-monsoon (PoM—November 03, October 04).

Univariate measures [Shannon–Wiener diversity index (H'), Margalef's species richness (d) and Pielou's evenness (J')] were analyzed using PRIMER (version 5, developed by PRIMER-E Limited, UK) and the variations in these were determined by two-way ANOVA. Two-way ANOVA was also performed on the dinoflagellate abundance data to evaluate spatial and temporal variation (Sokal and Rohlf 1981). Spatial variation in the dinoflagellate community and the abundance profiles of the five most dominant taxa during each sampling period in both transects are presented as SURFER plots using the SURFER 8 program (developed by Golden Software Inc., USA). The percentage contribution of autotrophic, mixotrophic, and heterotrophic forms of dinoflagellates in each sampling period was also calculated.

Results

Taxonomic identification revealed 134 species of dinoflagellates in surface waters of the BOB during the observation period (Table 2). Further grouping of these identified species based on their nutritional mode, revealed 40 autotrophic, 50 mixotrophic, and 44 heterotrophic species, indicated the dominance of mixotrophic forms (Table 2). Comparison of this report with earlier reported species from the same area (Taylor 1976; Jyothibabu et al. 2003; Paul et al. 2007) indicated

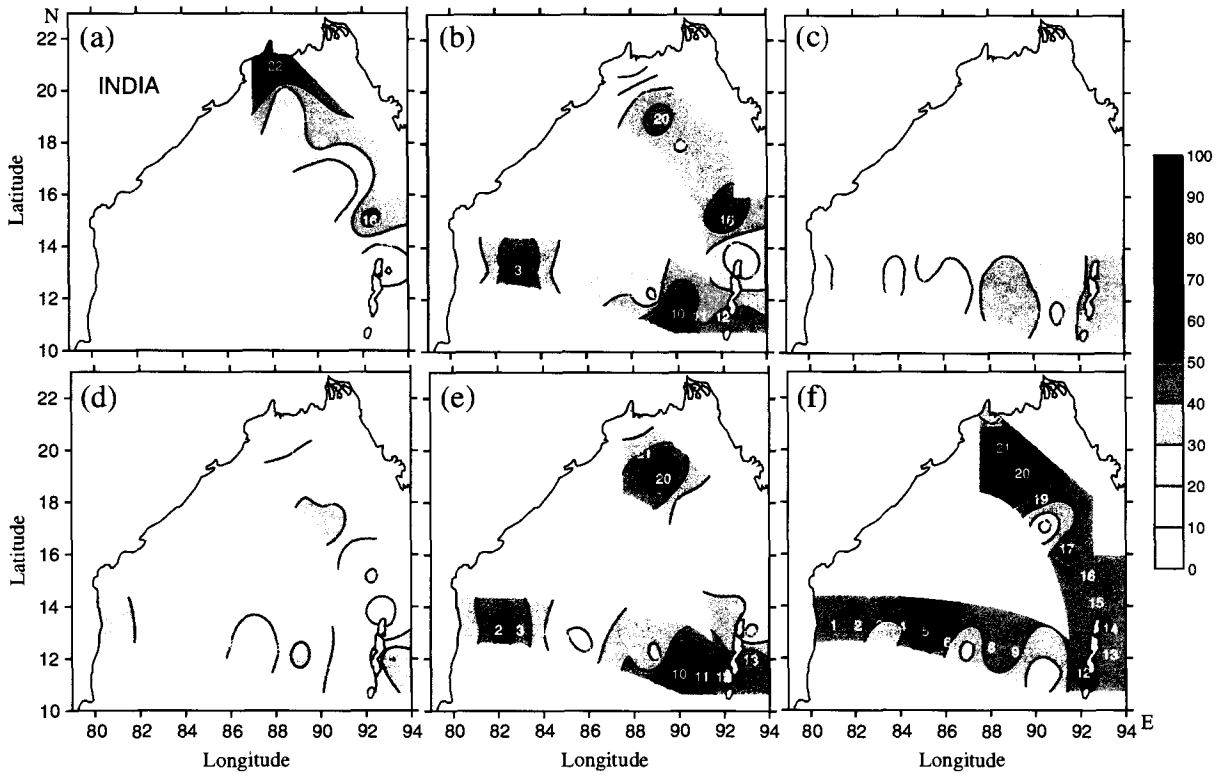

Fig. 2 Spatio-temporal variation in total dinoflagellate abundance (cell L^{-1}) along the Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects in **a** Nov 03, **b** Feb–Mar 04, **c** Jun 04, **d** Jul–Aug 04, **e** Oct 04 and **f** Sep 06

Table 3 Two-way ANOVA to evaluate the variation in total dinoflagellate abundance, species richness, evenness, and diversity along the CP and PK transects

	CP transect					PK transect				
	df	SS	MS	Fs	*P* value	*df*	SS	MS	Fs	*P* value
Abundance										
Stations	11	3319	302	1	0.2817	9	3092	344	1	0.4943
Seasons	4	5025	1256	5	*0.0016*	4	3493	873	2	0.0663
Within sub-group error	44	10576	240			36	12994	361		
Total	59	18920				49	19579			
Species evenness										
Stations	10	0	0	1	0.1922	9	0	0	1	0.2578
Seasons	4	0	0	1	0.3145	4	0	0	2	0.0710
Within sub-group error	40	0	0			36	1	0		
Total	54	0				49	1			
Species richness										
Stations	10	10	1	2	0.1661	9	10	1	3	*0.0180*
Seasons	4	0	0	0	0.9585	4	3	1	2	0.1614
Within sub-group error	40	27	1			36	14	0		
Total	54	37				49	27			
Species diversity										
Stations	10	3	0	2	0.1033	9	4	0	2	*0.0338*
Seasons	4	1	0	1	0.4078	4	2	0	3	*0.0483*
Within sub-group error	40	6	0			36	6	0		
Total	54	9				49	12			

ten new dinoflagellate species (Table 2 species marked with †). Approximately 14% of the identified species were potential HAB species (Table 2 species marked with *).

Spatial and temporal distribution of dinoflagellate assemblages

The abundance of dinoflagellates ranged from 0–94 cell L^{-1} throughout the observation period at both CP and PK transects (Fig. 2). Though seasonal variation in dinoflagellate abundance was observed in both transects, the variation was statistically significant at only the CP transect (Table 3). The highest average abundance of dinoflagellates was observed during September 06 (48 cell L^{-1} at PK) followed by October 04 (40 cell L^{-1} at CP) whereas low abundance was recorded during June (21 cell L^{-1} at CP) and August 04 (22 cell L^{-1} at PK; Fig. 2). The spatial variation in dinoflagellate abundance was not statistically significant in both transects (Table 3). Species richness and diversity varied significantly in the PK transect; species richness only across stations and diversity across both stations and seasons (Table 3). Mixotrophic dinoflagellates dominated across both transects, with the exception of October 04 at CP and September 06 at PK transects, that were dominated by heterotrophic forms (Fig. 3). *Ceratium* was the dominant genus among the mixotrophic forms whereas *Protoperidinium* was the dominant heterotrophic form. *Ceratium fusus*, *Ceratium teres*, *Gotoius abei*, *Oxytoxum scolopax*, *Protoperidinium ovatum*, and *Scrippsiella trochoidea* were among the abundant forms in both the transects. *Amphisolenia bidentata*, *Ceratium pentagonum*, *Ceratium symmetricum*, *Oxytoxum globosum*, and *Prorocentrum belizianum* were abundant only in CP transect (Fig. 4a) whereas *Ceratium extensum*, *Ceratium furca*, *Dinophysis miles* and *Noctiluca scintillans* were abundant only in the PK transect (Fig. 4b).

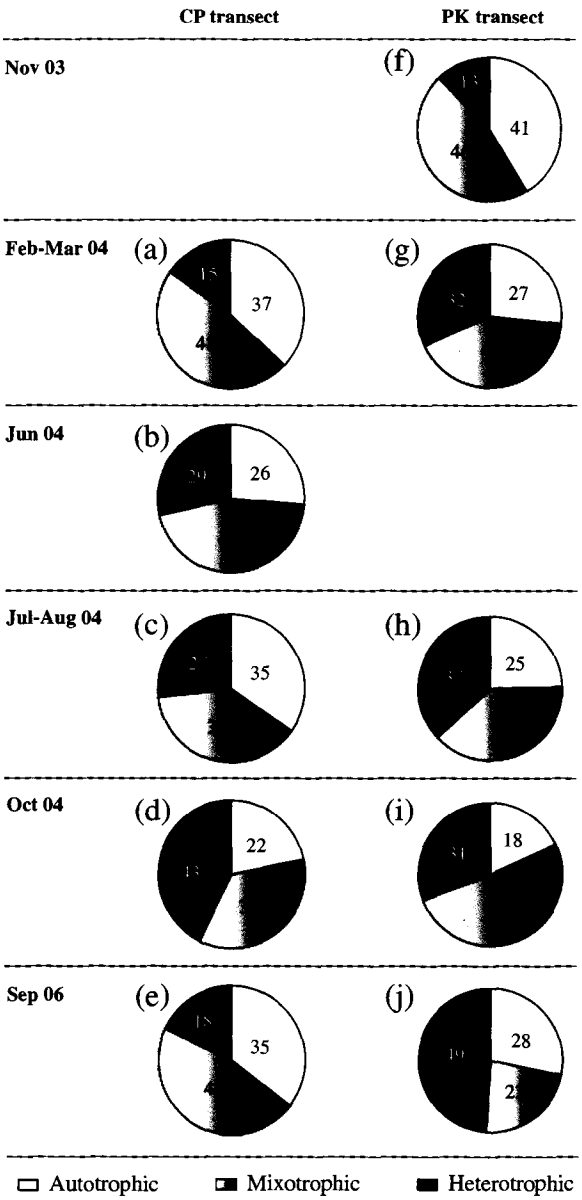

Fig. 3 The percentage contribution of autotrophic, mixotrophic and heterotrophic dinoflagellates along the **a–e** Chennai–Port Blair (CP) and **f–j** Port Blair–Kolkata (PK) transects. **a** Feb 04, **b** Jun 04, **c** Jul 04, **d** Oct 04, **e** Sep 06, **f** Nov 03, **g** Mar 04, **h** Aug 04, **i** Oct 04 and **j** Sep 06

Seasonal variation in HAB species

Overall, 19 potentially HAB species were encountered in this area (Table 2). Some HAB species were present during all the seasons throughout the study period. These frequently occurring species were *C. furca*, *C. fusus*, *Dinophysis* sp., *Gonyaulax polygramma*, *Gonyaulax* sp., *N. scintillans*, *Prorocentrum* sp., *S. trochoidea*, *Scrippsiella* sp. (Table 2). Season-specific trends were also observed. *Alexandrium minutum*, *Prorocentrum*

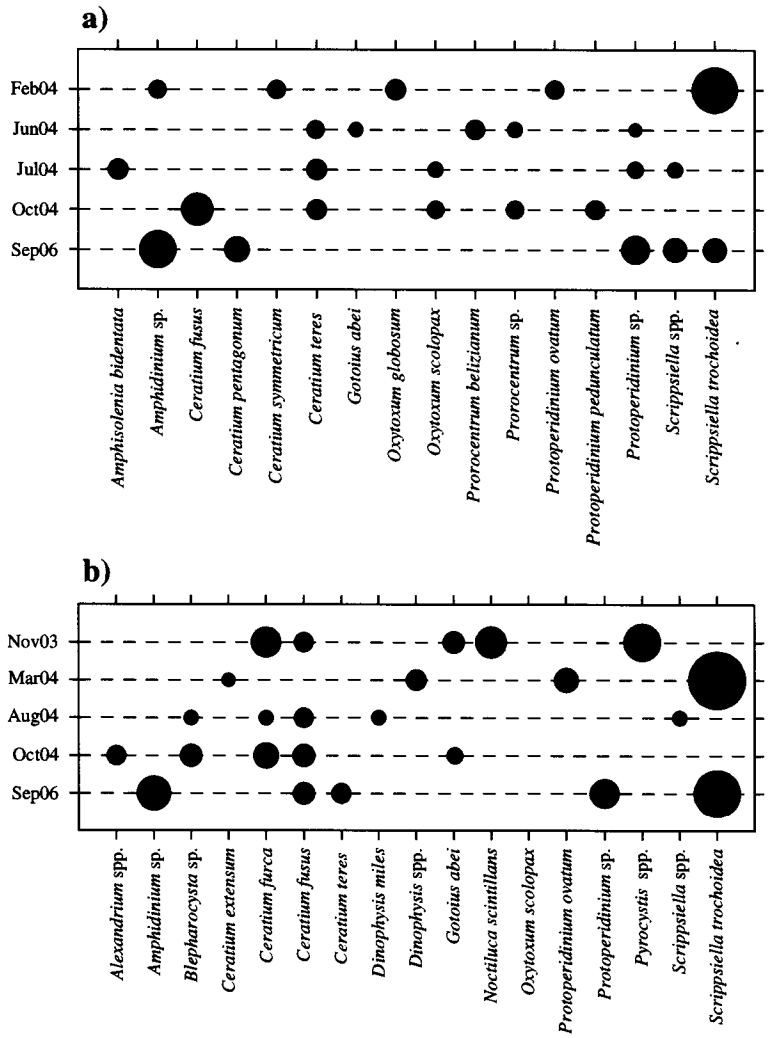

Fig. 4 The five most abundant species during each sampling period at the (**a**) Chennai–Port Blair and (**b**) Port Blair–Kolkata transects. The maximum diameter of circle corresponds to average 9 cell L^{-1}

lima, and *Protoperidinium crassipes* occurred only during PrM whereas *Dinophysis caudata*, *D. miles*, *Prorocentrum micans*, *P. mexicanum*, *P. minimus*, *Gymnodinium* sp. and *Gambierdiscus* sp. were found during SWM and PoM periods. *Gonyaulax polyedra* and *G. spinifera* occurred in PrM and PoM but not during SWM. In contrast to this, *P. belizianum* and *P. sigmoides* occurred only during the SWM.

Discussion

The BOB is a semi-enclosed tropical basin, distinguished by a strongly stratified surface layer and a seasonally reversing circulation (Shetye et al. 1996). It is also influenced by monsoonal winds and enormous freshwater influx. The surface stratified water column of the BOB restricts the vertical transport of nutrients from the bottom layers to the surface, and therefore phytoplankton productivity as well (Prasanna Kumar et al. 2002). The stratification is especially intense during the SWM period (Prasanna Kumar et al. 2004) due to the influx of freshwater through precipitation and riverine discharges. The average annual riverine discharge varies from the northern to southern bay with maximum discharge at north (10^{12} m^3 from Ganges and Brahmaputra), medium at central (8.5×10^{10} m^3 from Krishna

and Godavari) and minimum at southern bay (UNESCO 1988) resulting in variation in surface water salinity. Our study revealed that dinoflagellate abundance in surface waters was generally in the range of 0–94 cell L^{-1} and did not vary significantly across stations (Table 3). This could be related to the environmental conditions of the study area. Stratified water columns, with their characteristic oligotrophic conditions, tend to promote stasis of the resident population, rather than promoting growth or blooms (Smayda 2002). In fact, many studies (McGill 1973; Paul et al. 2008) have reported that the surface waters of the BOB are nitrate-deficient. Low surface PO_4^{3-}-P values of 0.1 μg-at L^{-1} have also been recorded in the Bay of Bengal and Andaman Sea (Kabanova 1964; Rozanov 1964). Though the stratified BOB environment supported a rather homogeneous dinoflagellate community in surface waters, significant seasonal variations in abundance and species diversity of the dinoflagellate community were observed (Table 3). Minimum values were observed during SWM, correlating to the intensification of the stratified layer, and maximum during the withdrawal of the SWM and PoM (Fig. 2).

The dominance of mixotrophic dinoflagellates in the study area could be a consequence of the prevalent low light and/or nutrient scarcity, conditions that are known to promote mixotrophy (Legrand et al. 1998; Stoecker et al. 2006). *Ceratium* and *Protoperidinium* were the most abundant representatives of the mixotrophic and heterotrophic forms in the BOB respectively (Fig. 4); their unique characteristics of vertical migration, and heterotrophy are reasonably well understood (Baek et al. 2006, 2007; Baek et al. 2008a, b, c; Latz and Jeong 1996; and references therein), and probably confer on them a competitive advantage in both coastal and oceanic environments.

In the context of the frequently occurring HAB species (hereafter FOS) recorded in the region; *C. furca* and *C. fusus* showed a characteristic transect-specific distribution. *C. furca* was abundant only in the PK transect. *C. fusus* which was predominant in the PK transect (Fig. 3b) was abundant only during October 04 in the CP transect (Fig. 4a).

C. furca and *C. fusus* are characteristically found in stable stratified water columns (Baek et al. 2006, 2007). However, both species differ in several characteristics. In recent observations in Sagami Bay, Japan, Baek et al. (2009) reported that *C. furca* had a competitive edge over *C. fusus*, because of its efficient diel vertical migration capability (a 'biological' factor). *C. fusus* was stimulated by low salinity and showed dependence on external environmental conditions such as enhanced nutrient concentrations following fresh water discharge by heavy rainfall (combination of 'physical' and 'chemical' factors). In light of this, our observations point out that the water mass of the PK transect (which is in the northern BOB) is influenced by riverine discharge to a much greater extent compared to the CP transect (in the central BOB). Additionally, the differential capabilities of *Ceratium* species to acclimatize to such niches can be an important factor in determining their diversity and spatio-temporal distribution.

S. trochoidea, another FOS in the region, was abundant during September/February–March (Fig. 4). September is known as the period of withdrawal of SWM, during which cloud cover reduces, whereas March is known for clear sky and with no rainfall. Studies on the factors triggering the growth/bloom formation of *S. trochoidea*, point to different regulating factors. For e.g., in Hong Kong waters, the initiation, maintenance and disappearance of a *S. trochoidea* red tide was not directly driven by changes in nutrients (Yin et al. 2008). Subsequently, Zhuo-Ping et al. (2009) observed that the cell density of *S. trochoidea* was positively influenced by high irradiance and further enhanced by iron concentration. These conditions of high irradiance could possibly be responsible for the predominance of *S. trochoidea* in March.

Blooms of *N. scintillans*, yet another FOS in the region, have been reported from Indian waters (Raghu Prasad 1953, 1956; Santha Joseph 1975; Naqvi et al. 1998; Eashwar et al. 2001; Mohanty et al. 2007; Gomes et al. 2008). Most of these blooms occurred during the SWM. Sriwoon et al. (2008) observed that *N. scintillans* blooms in the Gulf of Thailand were mainly influenced by the SWM. Since the SWM is a major meteorological event influencing the BOB, it is absolutely

necessary to investigate in detail the factors sustaining the population of *N. scintillans* in the BOB and their bloom dynamics.

Dinophysis sp., another FOS in the region, was abundant during March at PK transect (Fig. 4b). *Dinophysis* are known to increase in cell density immediately after a storm-induced mixing event (Nishitani et al. 2005). They can also migrate through strong gradients and survive under unfavorable conditions (Setala et al. 2005). In a recent observation in Portuguese waters, Escalera et al. (2010) found that the increased numbers of *Dinophysis* was a result of physically-driven accumulation due to long-shore transport. They also found the bloom to be associated with much warmer temperatures. In our observation, its predominance during March indicates its preference for high temperature since this period is considered a warmer season in the BOB (Narvekar and Prasanna Kumar 2006).

Given that the FOS observed in our study ranged from 0–40 cell L^{-1}, their presence may not be a population growth issue, as suggested by Smayda (2002). However, they may play a significant role in the development of pelagic seed banks of vegetative cells (Smayda 2002), which can serve as inocula for bloom events elsewhere, on the onset of favorable conditions. An earlier study (Avaria 1979) suggested that the Chilean frontal zone located 100 km offshore supported a *P. micans* bloom. Transport of offshore-seeded *Prorocentrum* and *Ceratium* blooms to inshore waters (Pitcher and Boyd 1996) also supports the above assumption.

Another intriguing aspect to be considered is the enhancement of phytoplankton biomass to bloom levels by physical processes occurring in the BOB like eddies (Gomes et al. 2000; Prasanna Kumar et al. 2004) and cyclones (Madhu et al. 2002; Vinayachandran and Mathew 2003; Rao et al. 2006). In both eddies and cyclones, bloom formation takes place due to transport of nutrients from bottom layers to the surface. During cyclones, due to strong wind speed, the stratified layer breaks and deepens the mixed layer, leading to introduction of nutrients into the surface layers whereas during eddies, Ekman pumping plays an important role in transporting nutrients to surface waters. Eddies are most likely to occur during the SWM (Prasanna Kumar et al. 2004) whereas cyclones are common during November (Madhu et al. 2002; Vinayachandran and Mathew 2003; Rao et al. 2006). The combination of such physical effects including turbulence and advection, with the diverse behavioral characteristics of dinoflagellates (e.g., migration, physiological adaptation) holds the key to understanding HAB dynamics in stratified oceanic areas. Even though some of these physical processes may play a crucial role in the formation of HABs, these processes are not well defined and thus knowledge in this context remains weak (GEOHAB 2003). For example, the above studies were based on remote sensing (chlorophyll *a*) and primary productivity values and none of the reported blooms/enhancement of phytoplankton biomass was taxonomically characterized. In this context, the present investigation pointing out the presence of *N. scintillans* and *C. furca* during November (Table 1) further strengthens their probable candidature for bloom formation in the region.

However, it should be noted that the present findings are based on the surface water distribution of dinoflagellates from the BOB, but taking in to account the fact that phytoplankton tend to gather more in sub-surface rather than surface waters, future studies on the depth-wise distribution of dinoflagellates and notably, HAB species, will be a step forward.

Conclusions

The present study is the first of its kind detailing the HAB species from the stratified surface waters of the BOB and their seasonal occurrence. The frequently occurring HAB species indicate their ability to survive even under such conditions; their low abundance in the region may not be a growth issue but they may serve as inocula for blooms if coupled with population triggering physical processes like eddies and cyclones in the region. In this scenario, the characteristic ability of FOS like *C. furca* and *N. scintillans* and their predominance during cyclone-prone months, make their candidature stronger for future blooms in the region.

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Primary description of surface water phytoplankton pigment patterns in the Bay of Bengal

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ABSTRACT

Spatial and temporal variations in surface water phytoplankton pigment distribution in the Bay of Bengal were studied during the spring intermonsoon (SpIM, February–April) and the commencement of the summer monsoon (CSM, May–June), using pigment and diagnostic indices. The Prokaryotic pigment index ($Prok_{DP}$) was dominant at all the oceanic stations whereas the Flagellate pigment index ($Flag_{DP}$) was dominant at the near coastal stations. However, during the commencement of summer monsoon, an oscillation in the dominance of $Prok_{DP}$ and $Flag_{DP}$ was observed in the central oceanic bay, whereas flagellates and diatoms were dominant at the near coastal stations. This change in pigment pattern is possibly related to the influence of rainfall. Comparison of pigment data with microscopic cell counts indicated a significant relationship between the diatom pigment index ($Diat_{DP}$) and diatom abundance. However, the relationship between the dinoflagellate pigment index ($Dino_{DP}$) and dinoflagellate abundance was not significant. Studies coupling pigment composition analysis with microscopic analysis of phytoplankton in natural conditions should thus be a prerequisite in establishing valid biogeochemical and ecosystem models.

1. Introduction

Phytoplankton, the base of food webs in all water bodies, include diatoms, dinoflagellates, coccolithophores, green algae, cyanobacteria and other groups. All together, these marine phytoplankton are important contributors to global carbon fluxes; their contribution through photosynthetic carbon fixation, leads to the formation of ~45 Gt of organic carbon per annum, of which 16 Gt are exported to the ocean interior (Falkowski et al., 1998).

Qualitative and quantitative analyses of phytoplankton composition are important to understand the community structure and dynamics of any ecosystem. To accomplish this, one approach is the identification and enumeration of phytoplankton through microscopic analysis. This approach is time-consuming and requires a high level of taxonomic expertise. There is also the risk of missing out smaller groups of phytoplankton (picophytoplankton, <2 μm in size) in routine microscopic analysis. Pigment analysis using liquid chromatography, is another approach considered as a powerful tool for characterization and monitoring of phytoplankton abundance and composition of field populations (Wright and Jeffrey, 2006). This method allows the quantification of over 50 phytoplankton pigments compared to other methods used to analyze chlorophyll (Jeffrey et al., 1997). Additionally, the composition of phytoplankton communities and their physiological status can also be inferred (Roy et al., 2006). The northern Indian Ocean has been studied using this approach on several occasions (Latasa and Bidigare, 1998; Barlow et al., 1999, 2008; Goericke et al., 2000; Roy et al., 2006). The seasonal pigment pattern of surface phytoplankton from the southern hemisphere was also studied in the recent past (Barlow et al., 2007). Most of these studies focused on the Arabian Sea, whose characteristic features such as monsoon, upwelling and current patterns made it an area of interest, while its counterpart, the Bay of Bengal (BOB), remained unexplored.

The BOB, the eastern arm of the northern Indian Ocean is characterized by special features such as seasonal reversal of winds, surface currents and fresh water influx from the adjacent rivers controlling the stratification in the near-surface layers (Shetye et al., 1991,1993). These features make the BOB a unique oceanic area and thus, understanding the spatio-temporal variations in the distribution of phytoplankton pigments in this area will provide novel information on the contribution of specific phytoplankton groups to the total pigment pool. The objective of this study was to characterize the pigment composition in different regions of the BOB and to evaluate it in relation to the microscopic cell counts of diatoms and dinoflagellates. This information will be important in developing applications of remote sensing in biogeochemical and ecosystem models.

2. Materials and methods

Under the Indian Expendable Bathythermograph (XBT) Programme, surface water was collected with bucket on two transects [Chennai to Port Blair (CP) and Port Blair to Kolkata (PK)] (Fig. 1),

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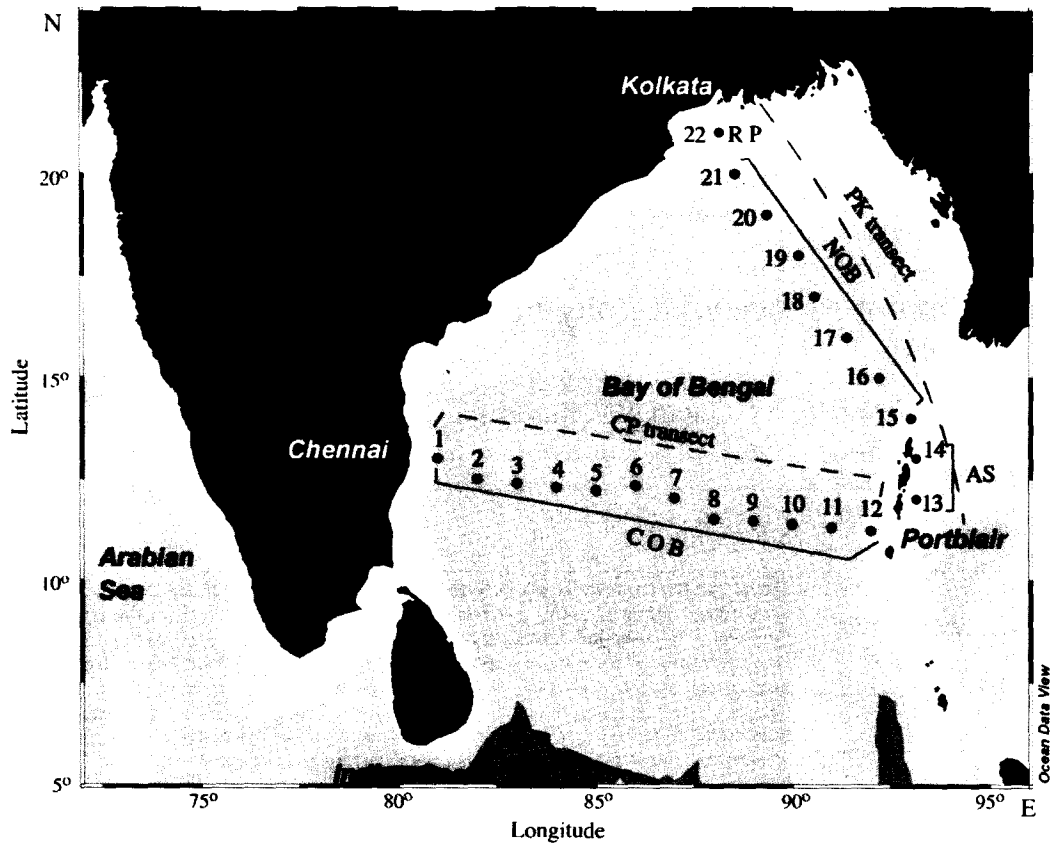

Fig. 1. Study area showing station locations and regions (COB–Central Oceanic Bay, AS–Andaman Sea, NOB– Northern Oceanic Bay and RP– River plume) along Chennai – Port Blair (CP) and Port Blair – Kolkata (PK). Figure also represents the bathymetry of the study area.

from passenger ships plying along these transects. Samples were collected at one degree intervals from 22 stations on four occasions (February–March, April, April–May and May–June 2007). Surface water was collected for the enumeration and identification of microphytoplankton to the lowest possible taxonomic level using light microscopy, and for pigment analysis using high performance liquid chromatography (HPLC). For pigment analysis, seawater samples (5 L except station 22 where 2 L) were filtered through 47-mm GF/F filters and stored in liquid nitrogen until analysis in the shore laboratory. Phytoplankton pigments were extracted using 3 ml 95% acetone for 5 min in an ultrasonic bath filled with ice-water and the extracts were stored overnight at −20 °C. The entire extraction procedure was carried out in dim light conditions and at low temperature to minimize degradation of pigments. The HPLC analysis was carried out following the method of van Heukelem (2002). According to the standard practice, pigment indices were calculated (Table 1) following the method of Barlow et al. (2007). A linear regression between diagnostic pigments (DP) and total chlorophyll *a* showed a significant relationship ($r^2 = 0.91$, $n = 84$, $p < 0.01$). Thus, indices derived from DP were used to understand the pattern of community dominance in the sampling area.

In order to see the regional variation in pigment patterns with respect to the sampled seasons, the two transects in the study area, CP and PK, were partitioned into different regions as Central Oceanic Bay (COB), Andaman Sea (AS), Northern Oceanic Bay (NOB) and the region influenced by river Hooghly, named as River Plume (RP). These regions were partitioned according to oceanic and coastal nature based on the bathymetry of the study area (Fig. 1).

Chlorophyll images were downloaded and processed (Level 3 MODIS, 4 km resolution). For the SpIM period, seasonal composite images were downloaded whereas for CSM, monthly images were downloaded. TRMM_3B42 data was downloaded for the grid area, Latitude 5°N–25°N, Longitude 78°E–95°E from Mirador data access (http://mirador.gsfc.nasa.gov/collections/TRMM_3B42__006.shtml) to obtain rainfall data and to follow changes in the monsoon during the observed period (Fig. 2). The observational period was categorized into two seasons, spring intermonsoon (SpIM, Feb–May 2007) and commencement of the summer monsoon (CSM, May–June 2007).

2.1. Data analyses

Three samplings carried out during SpIM (February–May) were clubbed together and presented as SpIM, with standard deviation error bars whereas a single sampling was carried out during CSM.

The regional variation in community structure was determined by two-way ANOVA (alpha value 0.05). This was done separately for SpIM and CSM periods. Absolute values (Fucoxanthin, Peridinin, Alloxanthin + 19′ -Butanoyloxyfucoxanthin + Chlorophyll *b* + 19′ -Hexanoyloxyfucoxanthin, Zeaxanthin representing diatoms, dinoflagellates, flagellates and prokaryotes respectively) were used to perform two-way ANOVA.

Regression analysis was carried out between respective diagnostic pigment indices and microscopic counts of a) diatoms, b) total dinoflagellates, c) autotrophic dinoflagellates and d) autotrophic + mixotrophic dinoflagellates. In the regression analysis for diatoms, data for station number 22 was omitted as microscopic counts were not available.

3. Results

Total chlorophyll *a* (TChla) ranged from 0.013 to 0.681 mg/m^3 during the SpIM period with a minimum value at NOB and maximum

Table 1
The pigment symbol, names, formulae and taxonomic group designation (Jeffrey et al., 1997) for diagnostic pigment sums and pigment indices (Barlow et al., 2007) including one index from Vidussi et al. (2001)*.

Symbol	Pigment	Designation of phytoplankton groups
Chla	Chlorophyll a (plus allomers and epimers)	
Chlb	Chlorphyll *b*	Chlorophytes
Chlc1	Chlorophyll c_1	
Chlc2	Chlorophyll c_2	
Chlc3	Chlorophyll c_3	
Chlidea	Chlorophyllide *a*	
DVChla	Divinyl chlorophyll *a*	Prochlorophytes
DVChlb	Divinyl chlorophyll *b*	Prochlorophytes
All	Alloxanthin	Cryptophytes
But	19′ -Butanoyloxyfucoxanthin	Crysophytes
Caro	ββ- Carotene + βε-carotene	
Diad	Diadinoxanthin	
Diato	Diatoxanthin	
Fuc	Fucoxanthin	Diatoms
Lut	Lutein	
Hex	19′- Hexanoyloxyfucoxanthin	Prymnesiophytes
Per	Peridinin	Dinoflagellates
Viol	Violaxanthin	
Zea	Zeaxanthin	Cynobacteria
	Pigment sum	Formula
TChla	Total chlorophyll *a*	Chla + DVChlab + Chlidea
Chlbc	Sum of chlorophyll *b* and *c*	Chlb + Chlc1 + Chlc2 + Chlc3
PPC	Photoprotective carotenoids	All + Caro + Diad + Diato + Lut + Viol + Zea
PSC	Photosynthetic carotenoids	But + Fuc + Hex + Per
TPig	Total pigments	Tchla + Chlbc + PPC + PSC
DP	Diagnostic pigments	All + But + Chlb + Fuc + Hex + Per + Zea
	Pigment index	Formula
DVChla/Tchla	Divinyl Chlorophyll *a* to total chlorophyll *a*	DV Chla/Tchla
$TChla_{TP}$	Total chlorophyll a to total pigments	TChla/Tpig
PPC_{TP}	Photoprotective carotenoids to total pigment	PPC/Tpig
PSC_{TP}	Photosynthetic carotenoids to total pigment	PSC/Tpig
$Diat_{DP}$	Diatom proportion of DP	Fuc/DP
$*Dino_{DP}$	Dinoflagellate proportion of DP	Per/DP
$Flag_{DP}$	Flagellate proportion of DP (excluding dinoflagellates)	(All + But + Chlb + Hex)/DP
$Prok_{DP}$	Prokaryote proportion of DP	Zea/DP

at RP (Table 2). During CSM, TChla ranged from 0.001 to 0.196 mg/m^3 with minimum at AS and maximum at RP (Table 2). The lowest concentrations of pigments, close to the limit of quantification (LOQ), were generally associated with the CSM. The LOQ in this study was defined as signal (S) to noise (N) ratio (S/N = 10) which is more

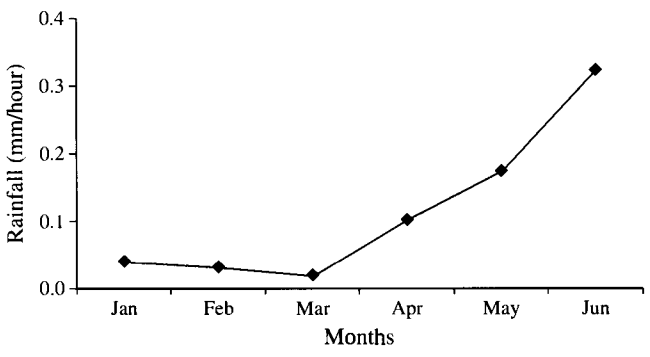

Fig. 2. Rainfall (mm/hour) data for BOB for the period January–June 2007.

Table 2
Range of total chlorophyll a (mg/m^3) in the different regions (COB, AS, NOB and RP) of Bay of Bengal during SpIM and CSM periods.

Sampling seasons	Regions			
	COB	AS	NOB	RP
SpIM	0.018–0.064	0.023–0.082	0.013–0.165	0.681
CSM	0.002–0.067	0.001–0.015	0.009–0.057	0.196

widely used and acceptable in HPLC pigment analysis. These were experimentally determined by the method of serial dilutions for chlorophyll *a* and fucoxanthin which were in the order of 0.0002–0.0005 mg/m^3. These were used as a reference to derive the LOQ for other marker pigments observed during these investigations as suggested by Hooker et al. (2005). However, it should be noted that the limit of detection (LOD) of the instrument is marginally lower than what was used in this analysis and typically represent (S/N = 3–5). In most cases, the observed concentrations in the central BOB suggested extreme oligotrophic conditions. The remote-sensing images also indicated low range of chlorophyll *a* during SpIM and CSM (Fig. 3). The photo-pigment indices revealed the dominance of $TChla_{TP}$ (total chlorophyll *a* to total pigment) over PPC_{TP} (photoprotective carotenoids to total pigments) and PSC_{TP} (photosynthetic carotenoids to total pigments) during SpIM and CSM periods (Figs. 4c and 5c). During SpIM period, dominance of PPC_{TP} over PSC_{TP} was observed at all the stations (Fig. 4c). However this pattern of dominance was altered during CSM at a few stations (Fig. 5c). An evaluation of the diagnostic pigment indices point out the dominance of $Prok_{DP}$ followed by $Flag_{DP}$ throughout the observed region during SpIM (Fig. 4a) except at the last station of PK. $Diat_{DP}$ followed by $Dino_{DP}$ was present in low proportions (Fig. 4a). However, during the CSM period, we could see oscillations in the dominance between $Prok_{DP}$ and $Flag_{DP}$ in the CP transect (Fig. 5a), whereas, in the PK transect, $Flag_{DP}$ dominated in the Andaman Sea and $Prok_{DP}$ was dominant in the northern oceanic Bay (Fig. 5a).

The changes in pigment patterns at the different regions were also studied. It was observed that during the SpIM period, all the regions except RP were dominated by $Prok_{DP}$ followed by $Flag_{DP}$ (Fig. 6a). In the RP region, $Flag_{DP}$ was dominant followed by $Prok_{DP}$ (Fig. 6a). The community structure observed during CSM was different than that observed during SpIM (Fig. 6a and b). In the region AS, the community was dominated by $Flag_{DP}$ followed by $Prok_{DP}$ whereas the region RP was dominated by $Diat_{DP}$ followed by $Flag_{DP}$ (Fig. 6b). During CSM, the contribution of $Dino_{DP}$ was high compared to that in the SpIM period (Fig. 6a and b). Though regional variations in community structure were observed in both seasons (SpIM and CSM), the variation was statistically significant only during SpIM (Table 3).

Microscopic analysis of diatoms and dinoflagellates was used to quntify their cell abundance during SpIM and CSM period (Fig. 7a and b). During SpIM, dinoflagellates dominated and the maximum abundance was observed in the RP region (station 22) (Fig. 7a) whereas, diatoms were abundant during the CSM (Fig. 7b). Among the dinoflagellates, mixotrophic forms were dominant during the SpIM (Fig. 7c) and the CSM (Fig. 7d).

A linear regression between $Diat_{DP}$ and microscopic cell counts of diatoms showed a significant relationship (Fig. 8a; $n = 79$, $p < 0.05$). However, fucoxanthin is also found in significant levels in Prymnesiophyceae, Chrysophyceae and Raphidophyceae (Jeffrey et al., 1997) and due to this reason the samples with $Diat_{DP}$ above 0.05 but with diatom abundance less than 100 cells/L were checked for their level of ChlC3 and 19′ But (two pigments found in high concentration in these 3 nanoflagellate groups but not in diatoms). The concentrations of ChlC3 and 19′ But were negligible. Thus, it had no effect on the above significant relationship between $Diat_{DP}$ and microscopic cell counts of diatoms. Regression between $Dino_{DP}$ and total dinoflagellate cell counts showed a non-significant relationship (Fig. 8b; $n = 73$, p = ns).

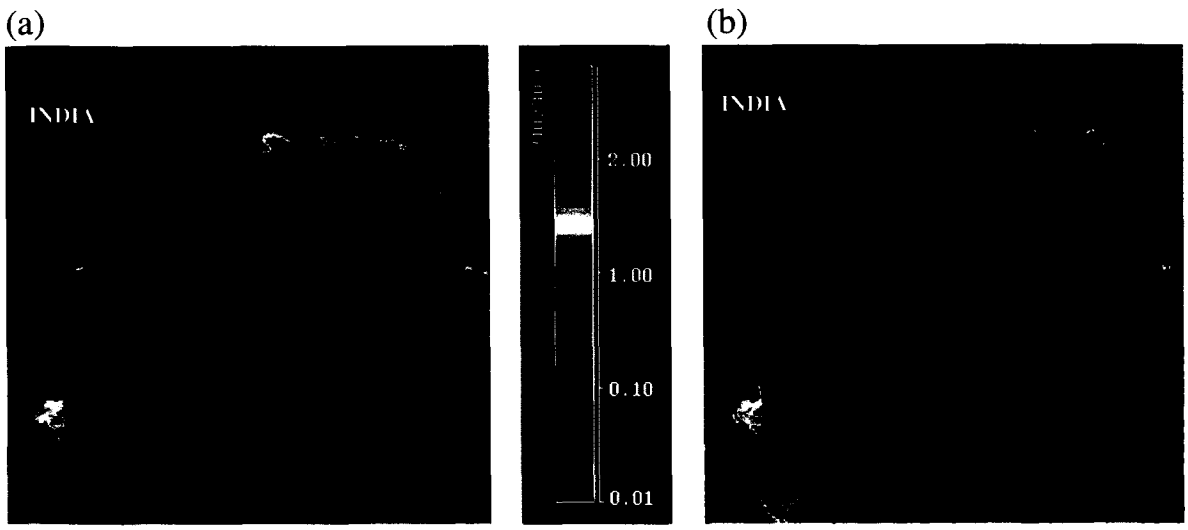

Fig. 3. Satellite-derived chlorophyll *a* concentration (mg /m^3) : (a) SpIM, 2007 (b) monthly image (June 2007) for CSM.

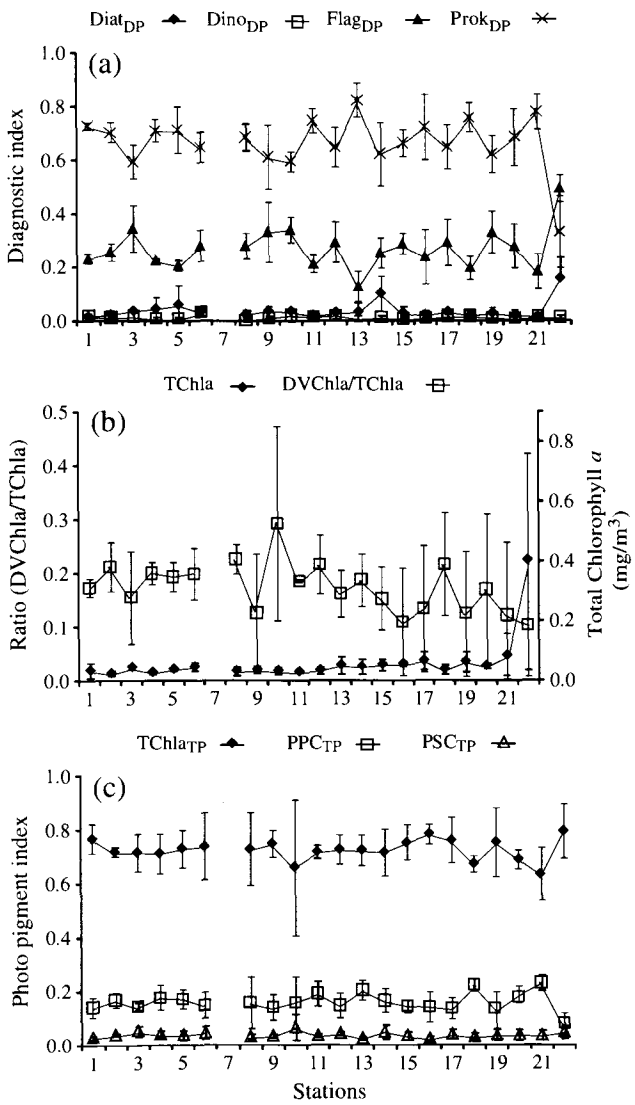

Fig. 4. Pigment indices for the SpIM period: (a) diagnostic indices (b) Tchla and DVChla/Tchla, (c) photo-pigment indices. See Table 1 for symbols and formulae.

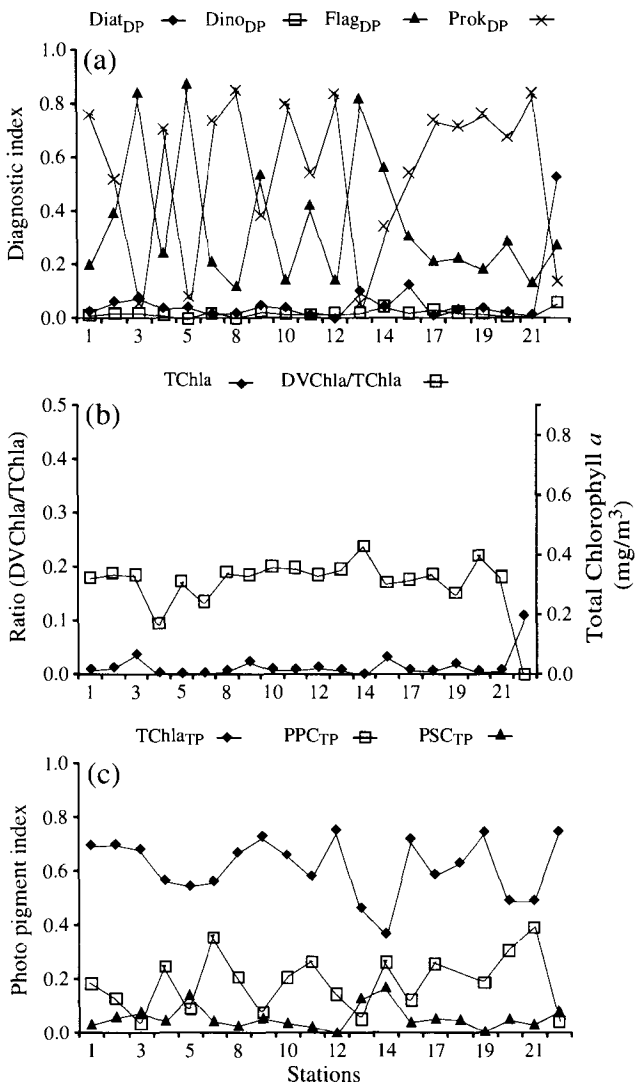

Fig. 5. Pigment indices for the CSM period: (a) diagnostic indices (b) Tchla and DVChla/Tchla, (c) photo-pigment indices. See Table 1 for symbols and formulae.

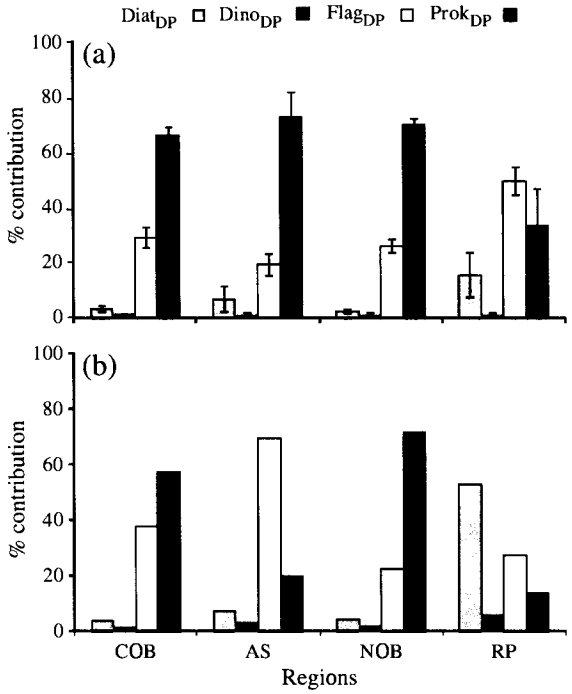

Fig. 6. Percentage contribution of diagnostic indices to each region (COB, AS, NOB and RP) (a) SpIM and (b) CSM. See Table 1 for symbols.

The correlation between Dino$_{DP}$ and dinoflagellate cell counts did not improve even when the mixotrophic and heterotrophic forms were omitted from the total dinoflagellate counts (Fig. 8c and d).

4. Discussion

The phytoplankton biomass evaluated so far in the region (Radhakrishna et al., 1978, 1982; Bhattathiri et al., 1980; Devassy et al., 1983; Sarma and Aswanikumar, 1991; Gomes et al., 2000; Kumar et al., 2002; Madhupratap et al., 2003) is based on fluorometer and spectrophotometer estimations and remote-sensing values. To the best of our knowledge, the present study, based on HPLC pigment characterization, is the first report from this area. The BOB is considered less productive as compared to the Arabian Sea, due to strongly stratified surface waters (Kumar et al., 2002). It has been observed that such stratified conditions support the dominance of prokaryotic groups (Chisholm, 1992; Cullen et al., 2002). Our observations also indicated the dominance of Prok$_{DP}$ in the oceanic waters of the BOB (Figs. 4a and 5a). In a recent study from the Bay of Bengal, Hegde et al. (2008) observed that stratified conditions support the prevalence of *Trichodesmium*, which is a prokaryote with the ability to fix nitrogen. In the Baltic Sea, it has been observed that nitrogen fixation by diazotrophs leads to the transfer of newly fixed nitrogen to picoplanktonic organisms and supports the microbial foodweb (Ohlendieck et al., 2000). In the present work, the DVChla/TChla ratio indicated the contribution of *Prochlorococcus* sp. to the picoplankton group and this contribution was higher during SpIM compared to CSM (Figs. 4b and 5b). The metabolic properties of *Prochlorococcus* (Vaulot and Partensky, 1992; Casey et al., 2007; Martiny et al., 2009a) give them a flexible metabolism and the ability to assimilate nitrate and nitrite (Martiny et al., 2009b). Hence, *Prochlorococcus* can assimilate newly fixed nitrogen by micro-prokaryotes like *Trichodesmium* and maintain its dominance in oceanic waters.

Earlier studies on accessory pigments from tropical latitudes demonstrated the greater presence of PPCs in surface, low chlorophyll waters (Stuart et al., 1998; Gibb et al., 2000; Barlow et al., 2004). Our observations also indicate that PPCs tend to be high in surface waters during the SpIM period (Fig. 4c). However, notable changes in the accessory pigments were observed with increase in the relative contribution of PSCs during the CSM period at a few stations of CP transect (Fig. 5c). Similar changes were observed in the Atlantic, Pacific, Gulf of Oman and Arabian Sea (Trees et al., 2000; Veldhuis and Kraay, 2004). They observed that the change in community structure is a physiological response to the changing environment thereby resulting in changes in accessory pigments. In the present work, the change in accessory pigments during CSM might be due to the responses of the community to the changing environment influenced by rainfall. This indicates that environmental and meteorological conditions may alter phytoplankton dynamics through a chain of linked processes. These variations in accessory pigments, in turn, are likely to affect the optical properties of phytoplankton which has implications for ocean colour remote-sensing (Sathyendranath et al., 2005).

The second dominant group was the flagellates and their dominance in the AS and RP near coastal regions (Fig. 6a and b) indicate their preference for nutrient-rich areas. Similarly, Diat$_{DP}$ also showed a preference for nutrient-rich turbulent waters, being the dominant group at RP during the CSM (Fig. 6b). This change in community structure could be linked to the increased rainfall during this season (Fig. 2). Thus a more significant change in community structure can be expected as rainfall reaches its peak. Comparison of diagnostic pigments and microscopic cell counts indicates that though a significant relationship between Diat$_{DP}$ and diatom abundance was observed (Fig. 8a; $n=79$, $p<0.05$), in the case of Dino$_{DP}$ versus dinoflagellate abundance, the relationship was not significant (Fig. 8b). This suggests that peridinin as a marker pigment did not work well for the dinoflagellate population in the region. In view of this, further research comparing the HPLC pigment composition of dinoflagellates with live cell abundances (to eliminate artifacts due to preservatives) should be considered.

Table 3
Two-way ANOVA to evaluate the variation in community structure in the different regions in Bay of Bengal during SpIM and CSM periods.

	SpIM		CSM	
	df	*p-value*	*df*	*p-value*
Community	3	0.0736	3	0.4756
Regions	3	0.0382	3	0.0929

5. Conclusions

Phytoplankton community structure in the Bay of Bengal is generally dominated by prokaryotes followed by flagellates with a low biomass of total chlorophyll *a*. Changes in the community structure were observed at the onset of the monsoon, indicating the influence of rainfall especially in near coastal regions like Andaman Sea and River plume. Comparative studies between microscopic counts and diagnostic pigment indices suggest coupling pigment composition analysis with microscopic analysis of natural assemblages to establish valid biogeochemical and ecosystem models. Notably, the components of dinoflagellate communities could be missed by pigment analysis alone.

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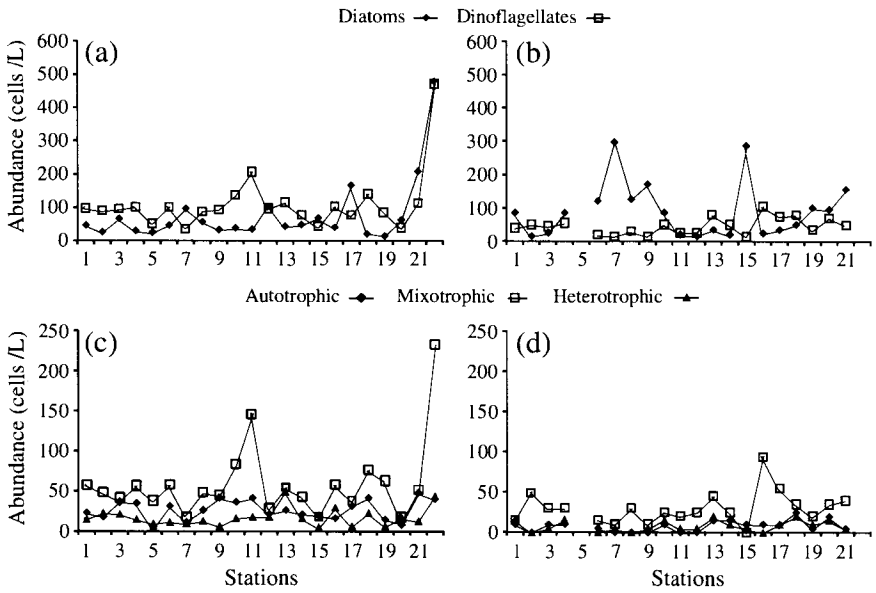

Fig. 7. Microscopic counts of diatoms and dinoflagellates (a) SpIM and (b) CSM and abundance of autotrophic, mixotrophic and heterotrophic dinoflagellate during (c) SpIM and (d) CSM.

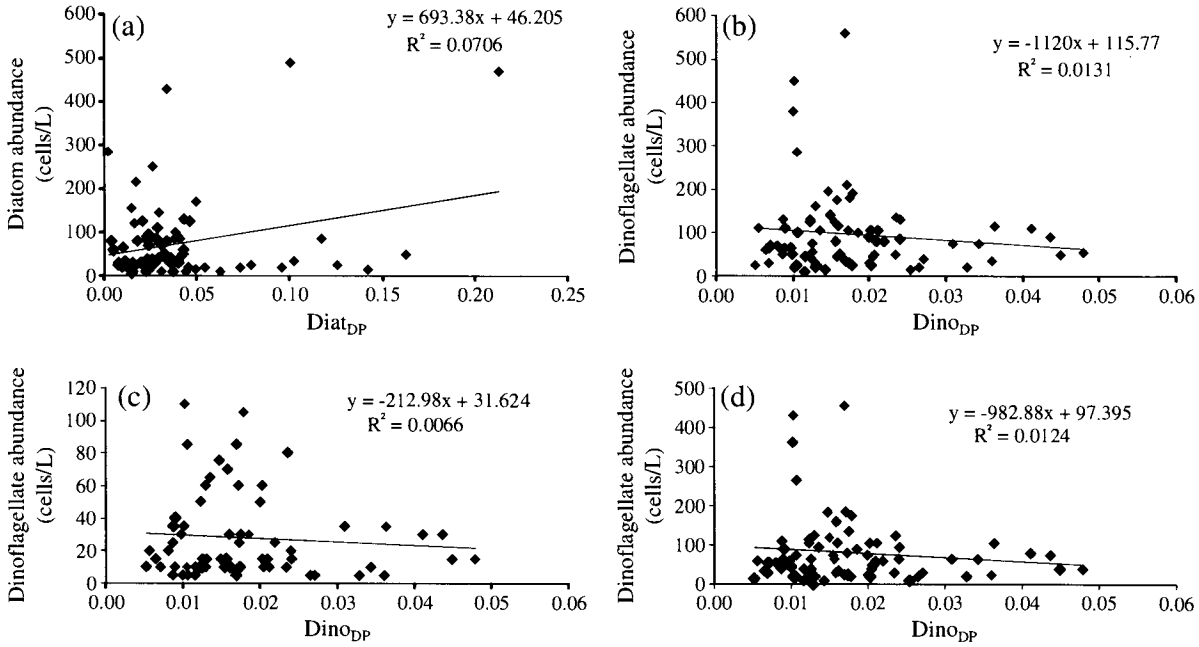

Fig. 8. Linear regression between : (a) diatom pigment index and diatom abundance, (b) dinoflagellate pigment index and dinoflagellate abundance, (c) dinoflagellate pigment index and autotrophic dinoflagellate abundance and (d) dinoflagellate pigment index and autotrophic + mixotrophic dinoflagellate abundance.

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Appendices

Chapter 4A

Appendix 1: Details of stations and sampling dates in Mormugao Port (MPT). PrM: Pre-monsoon, PoM: Post-monsoon, WSWM: Withdrawal of the South West Monsoon.

Station code	Station name	Date of sampling			Time of sampling		
		PrM	PoM	WSWM	PrM	PoM	WSWM
1	MPT, Berth-1	05-04-2005	12-06-2005	27-09-2006	08:45	09:35	08:35
2	MPT, Berth-3	05-04-2005	12-06-2005	27-09-2006	10:00	10:40	09:35
3	MPT, Channel Marker Buoy-6	05-04-2005	12-06-2005	28-09-2006	11:15	12:05	09:00
4	Goa Shipyard	05-04-2005	12-06-2005	29-09-2006	13:45	14:25	10:20
5	MPT, Berth-11	05-06-2005	12-08-2005	27-09-2006	09:35	11:00	14:10
6	MPT, Berth-9	05-06-2005	12-10-2005	27-09-2006	12:40	09:25	12:20
7	MPT, Berth-8	05-08-2005	12-08-2005	27-09-2006	09:15	12:45	11:30
8	MPT, Mooring Dolphin-2	05-10-2005	12-08-2005	28-09-2006	09:45	10:05	11:00
9	MPT, Channel Marker Buoy-7	05-10-2005	12-10-2005	28-09-2006	11:25	11:35	09:45
10	MPT, Berth-5	05-10-2005	12-10-2005	27-09-2006	12:15	12:20	10:35
11	MPT, Vasco Bay	05-10-2005	12-08-2005	29-09-2006	14:00	14:35	09:00
12	Dona Paula Jetty	05-12-2005	12-08-2005	29-09-2006	08:35	15:40	12:00
13	MPT – Dona Paula, Mid Point	05-04-2005	12-06-2005	29-09-2006	14:45	15:10	09:35
14	Mandovi, Verem Bay	05-08-2005	12-12-2005	25-09-2006	09:50	15:00	12:30
15	Mandovi, RND Jetty	05-08-2005	12-12-2005	25-09-2006	10:30	12:20	14:20
16	Mandovi, (Youth Hostel)	05-08-2005	12-12-2005	25-09-2006	09:00	11:30	15:15

Chapter 4A

Appendix 2: Details of stations and sampling dates in Visakhapatnam Port (VPT). NEM: North East Monsoon, PoM: Post-monsoon, SWM: South West Monsoon.

Station code	Station name	Date of sampling			Time of sampling		
		NEM	PoM	SWM	NEM	PoM	SWM
1	West Quay Berth-1	30-11-2007	04-09-2008	24-08-2008	09:40	12:30	09:00
2	West Quay Berth-4	29-11-2007	04-09-2008	24-08-2008	13:00	10:15	09:30
3	East Quay Berth- 9	29-11-2007	04-09-2008	24-08-2008	11:30	08:30	08:20
4	East Quay Berth- 7	29-11-2007	04-09-2008	24-08-2008	12:45	09:15	08:40
5	East Quay Berth- 5	29-11-2007	04-09-2008	24-08-2008	12;15	09:45	09:00
6	East Quay Berth- 1	30-11-2007	04-09-2008	23-08-2008	10:00	11:30	10:30
7	Turning Basin	28-11-2007	04-08-2008	23-08-2008	12:40	12:30	10:00
8	Fertilizer Wharf	28-11-2007	04-08-2008	21-08-2008	10:00	09:45	10:00
9	Oil Refinery Berth - 2	28-11-2007	04-08-2008	21-08-2008	11:00	11:00	11:00
10	Port Dry Dock	28-11-2007	04-08-2008	21-08-2008	13:20	13:00	11:45
11	DC Jetty	29-11-2007	04-10-2008	23-08-2008	10:00	09:15	08:00
12	Boat Basin Berth - 3	30-11-2007	04-10-2008	21-08-2008	10:30	08:45	12:15
13	Dredger Berth	30-11-2007	04-10-2008	23-08-2008	11:15	09:55	10:30
14	General Cargo Berth N	30-11-2007	04-11-2008	23-08-2008	12:15	10:00	11:00
15	Ore Berth – 1	30-11-2007	04-11-2008	23-08-2008	13:15	10:30	11:30
16	Ore Berth – 2	12-01-2007	04-10-2008	22-08-2008	10:30	12:00	10:00
17	Container Berth-1	12-01-2007	04-10-2008	22-08-2008	11:00	11:00	10:30
18	Container Berth-2	12-01-2007	04-10-2008	22-08-2008	12:00	10:30	11:00
19	Oil Berth	12-01-2007	04-11-2008	22-08-2008	09:30	11:00	08:15
20	LPG Berth	12-02-2007	04-11-2008	22-08-2008	11:00	08:45	09:00

Appendix 3: Phytoplankton recorded in surface (S) and near bottom (NB) waters in Mormugao Port (MPT). PrM: Pre-monsoon, PoM: Post-monsoon, WSWM: withdrawal of the South West Monsoon. The values outside the bracket indicate the abundance range (cells L^{-1}) and the values inside the bracket indicate the frequency of occurrence at 16 stations.

Taxon	PrM		PoM		WSWM	
	S	NB	S	NB	S	NB
Diatoms						
Centric						
AslM				0-50(1)		
Aslsp			0-50(1)	0-50(1)	0-50(2)	0-50(1)
AspC		0-167(1)				
Baps	0-100(1)			0-50(1)		
Belrsp		0-4342(5)				0-400(2)
BidB	0-150(2)					0-100(1)
CetBr						0-50(1)
CetB	0-50(1)	0-150(1)	0-63(1)			0-1050(13)
CetP			0-3063(2)	0-2563(2)		
Cets					0-200(11)	
ChA	0-668(2)	0-150(1)	0-500(2)	0-100(2)	0-200(3)	0-200(6)
ChCmp	0-600(3)	0-334(2)		0-100(1)	0-200(3)	
ChCr	0-235600(15)	0-38750(11)	0-875(8)	0-1938(9)	0-650(8)	
ChDa	0-1200(5)	0-167(5)	0-100(1)		0-100(1)	
ChDe	0-3400(13)	0-1500(6)	0-500(9)	0-1050(8)	0-950(12)	0-650(9)
ChDid			0-813(1)	0-125(1)		
ChDiv	0-250(2)					
ChLac	0-500(3)					
ChLo	0-100(1)				0-250(9)	0-200(4)
ChMe			0-188(1)	0-375(1)		
ChPe	0-150(2)	0-200(2)				
ChTo			0-450(1)			
Chs	0-1200(6)	0-500(2)	0-875(4)	0-500(5)	0-500(2)	0-200(1)
CorC	0-1050(14)	0-1000(12)				
CorH	0-100(1)	0-100(2)				
Cors	0-200(200)					

Appendix 3 Contd.....

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Taxon	PrM		PoM		WSWM	
	S	NB	S	NB	S	NB
CosCur		0-50(1)				
CosE		0-167(2)				
CosGi				0-50(1)	0-50(3)	0-200(5)
CosG	0-250(3)	0-150(5)	050(1)			
CosL	0-167(1)	0-2004(1)	0-250(1)			0-50(1)
CosM	0-2505(6)	0-334(8)	0-400(7)	0-250(11)	0-7000(14)	0-650(15)
CosN			0-5500(2)	0-63(1)		
CosR	0-100(2)	0-300(4)	0-100(6)	0-150(1)	0-500(2)	0-100(3)
Coss	0-1169(12)	0-668(11)	0-1125(8)	0-6000(11)	0-600(15)	0-1300(16)
CyC	0-100(1)	0-250(3)	0-188(4)	0-300(5)	0-100(2)	
CyL		0-334(1)		0-63(1)	0-100(3)	0-50(1)
Cys	0-350(2)	0-50(1)	0-300(8)	0-200(9)	0-250(8)	0-550(13)
DaF		0-1000(1)				
DitB	0-1150(14)	0-835(14)	0-188(8)	0-125(8)	0-450(8)	0-250(10)
Dits	0-334(10)	0-450(10)	0-813(8)	0-200(8)	0-100(7)	0-200(6)
GuD	0-8050(2)					
GuF	0-250(3)	0-200(2)			0-100(3)	0-300(6)
GuS	0-3006(7)	0-3006(4)	0-688(8)	0-550(6)	0-350(9)	0-350(10)
Gus	0-1550(7)	0-2350(5)	0-250(3)	0-63(1)		
HeT	0-4350(12)	0-7750(13)	0-100(3)	0-50(2)	0-100(1)	0-150(2)
HmH	0-501(3)	0-550(3)	0-50(1)			
HmM			0-50(2)			
HmS	0-100(1)		0-50(1)			
HemH	0-100(1)					
DitB	0-1150(14)	0-835(14)	0-188(8)	0-125(8)	0-450(8)	0-250(10)
Dits	0-334(10)	0-450(10)	0-813(8)	0-200(8)	0-100(7)	0-200(6)
GuD	0-8050(2)					
GuF	0-250(3)	0-200(2)			0-100(3)	0-300(6)
GuS	0-3006(7)	0-3006(4)	0-688(8)	0-550(6)	0-350(9)	0-350(10)
Gus	0-1550(7)	0-2350(5)	0-250(3)	0-63(1)		
HeT	0-4350(12)	0-7750(13)	0-100(3)	0-50(2)	0-100(1)	0-150(2)
HmH	0-501(3)	0-550(3)	0-50(1)			
HmM			0-50(2)			
HmS	0-100(1)		0-50(1)			
HemH	0-100(1)					

Appendix 3 Contd....

.....Appendix 3 Contd.

Taxon	PrM		PoM		WSWM	
	S	NB	S	NB	S	NB
LaA	0-150(4)	0-200(1)			0-100(1)	0-150(4)
Las					0-150(2)	0-350(4)
LeD	0-334(1)		0-500(3)		0-300(3)	0-700(4)
LeMi			0-63(1)			
Lis			0-50(1)			
MeN	0-550(2)	0-50(1)				
OdA	0-334(2)	0-167(4)		0-188(3)	0-50(1)	0-150(3)
OdM	0-334(3)	0-1000(5)		0-63(2)	0-100(3)	0-450(11)
OdS	0-334(8)	0-500(8)			0-100(5)	0-100(7)
Ods	0-167(1)		0-50(2)			0-50(1)
ParS	0-668(3)	0-334(2)		0-63(1)		0-500(3)
PlaS	0-100(3)	0-250(4)	0-50(1)	0-50(2)		0-50(1)
PrA				0-63(1)	0-50(3)	0-300(9)
RhC						0-150(2)
RhF	0-50(2)					0-50(1)
RhH				0-125(2)		0-50(1)
RhSe			0-50(1)		0-50(1)	0-100(1)
RhSt	0-50(2)		0-50(1)		0-50(1)	0-150(3)
Rhs					0-150(1)	0-50(1)
SkC	0-200(2)		0-2200(3)	0-900(4)	0-4500(7)	0-1400(2)
Sks					0-2600(1)	0-400(1)
Sts						0-100(1)
ThsP					0-150(1)	
ThsS	0-750(1)					
Thss	0-2171(14)		0-438(14)	0-375(14)	0-5800(14)	350-2250(16)
TcerC			0-63(1)			
TcerF			0-63(1)			0-50(1)
Tcers	0-50(1)				0-50(1)	
Pennate						
Achs	0-50(1)		0-63(2)	0-63(4)		
AmpGi	0-350(10)	0-668(10)	0-250(7)	0-300(7)		0-50(1)
Amps	0-100(2)	0-100(4)	0-250(5)	0-500(2)		0-50(1)
Ams	0-50(2)	0-150(1)		0-63(1)	0-50(1)	

Appendix 3 Contd....

.....Appendix 3 Contd.

Taxon	PrM		PoM		WSWM	
	S	NB	S	NB	S	NB
AsnG	0-600(3)	0-550(2)				
AstlM			0-50(1)			
BacP	0-3500(2)	0-4000(3)				
Cals			0-188(4)			
Clims			0-50(1)			
CylC	0-1650(9)	0-650(5)	0-1050(12)	0-1500(7)	0-1400(14)	0-950(11)
DipC	0-16793)	0-334(9)	0-100(5)	0-150(5)		0-50(2)
Dips	0-150(40	0-167(2)	0-63(1)	0-63(1)		0-100(1)
EphP		0-50(1)		0-63(1)		
FraS						0-400(1)
FrsC		0-35(2)		0-188(1)		
Frss			0-200(1)			0-150(1)
Gyrs	0-334(2)		0-63(1)	0-50(1)		
Hss	0-50(1)	0-167(2)		0-50(1)	0-50(1)	0-150(6)
LiU				0-63(1)		
Lis	0-150(2)		0-63(3)			
MuM	0-3950(8)	0-2672(6)				0-200(1)
Nvs1	0-510(8)	0-550(11)		0-188(2)		
NvTDe	0-1450(8)	0-550(9)	0-550(7)	0-150(7)	0-150(6)	0-400(12)
NvTDs	0-350(6)	0-1000(4)	0-375(7)	0-300(9)	0-50(1)	0-350(8)
NvG			0-313(1)	0-63(1)		
Nvs	0-400(8)	0-200(12)	0-188(7)	0-750(8)	0-200(11)	0-250(9)
NiB		0-100(1)				
NiDe	0-501(1)	0-6346(2)		0-63(2)		
NiL				0-188(2)		
NiP	0-334(4)	0-501(6)	0-150(3)	0-150(6)		
NiSe	0-1600(6)	0-3100(5)	0-125(2)			
NiSi	0-200(3)	0-100(1)	0-350(7)	0-600(6)	0-50(2)	0-50(2)
Nis	0-650(3)	0-100(1)	0-7000(8)	0-6600(10)	0-150(4)	0-200(3)
PiR	0-334(5)		0-50(1)	0-50(1)		0-50(1)
Pis						0-100(1)
PhTr		0-668(11)				
PleA	0-900(4)	0-1200(10	0-63(2)	0-125(20		0-50(1)
PleD	0-200(5)	0-250(5)	0-200(5)	0-150(5)		0-100(4)
PleE	0-300(11)	0-1503(10)	0-438(2)	0-125(4)		
PleFa	0-501(2)	0-501(3)				

Appendix 3 Contd....

.....Appendix 3 Contd.

Taxon	PrM		PoM		WSWM	
	S	NB	S	NB	S	NB
PleFo	0-500(1)					
PleNa	0-550(8)		0-100(2)	0-150(3)		
PleNo	0-1002(10)		0-350(8)	0-350(8)	0-50(3)	0-200(7)
Ples	0-501(4)		0-150(5)	0-200(6)	0-100(1)	0-50(3)
PsnA			0-450(6)	0-300(7)		
PsnD	0-300(1)					
PsnPu	0-6500(9)		0-500(1)	0-813(6)		
Psnsl			0-812(1)			
Psns	0-450 (3)		0-1700(13)	0-450(12)	0-1050(13)	0-650(13)
SuF	0-668(11)		0-100(3)	0-100(7)		0-150(3)
SuOt			0-100(1)	0-250(2)		
Sus					0-100(1)	0-50(1)
SyP	0-167(1)		0-100(1)	0-200(1)		
Sys			0-250(2)	0-250(3)	0-550(3)	0-350(6)
ThnB	0-650(9)		0-150(5)	0-100(3)	0-600(12)	50-900(16)
ThnF						350-3450(16)
ThnN	0-7400(14)		0-2750(14)	0-3300(13)	350-2400(16)	
Thns				0-438(3)		
Trops	0-150(4)		0-188(2)			
Dinoflagellates						
Autotrophic						
AxCt	0-100(1)		0-650(5)	0-550(2)		
AxCm			0-400(1)			
Axs	0-167(1)		0-350(5)	0-100(2)	0-100(5)	0-200(3)
Ampds	0-150(2)		0-300(5)	0-200(6)		0-100(5)
Bleps	0-167(1)					
Ensis			0-63(1)			
Gambs	0-600(6)		0-50(1)	0-50(1)		0-100(1)
GnxDle	0-167(4)		0-50(1)			

Appendix 3 Contd....

.....Appendix 3 Contd.

Taxon	PrM		PoM		WSWM	
	S	NB	S	NB	S	NB
GnxMo			0-100(2)	0-200(1)		
GnxP	0-100(2)		0-63(1)			
GnxSp	0-334(5)		0-100(2)			
GnxVer			0-100(2)			
Gnxs	0-50(1)		0-100(2)	0-100(1)	0-100(1)	
Gyms	0-334(5)		0-750(9)	0-125(5)	0-150(8)	0-150(6)
OxSc				0-50(1)		
Oxs					0-50(1)	
Prqn			0-800(7)		0-50(1)	
PoB			0-50(1)			
PyhH					0-250(2)	
TorT				0-50(1)		
Tors						0-50(1)
Mixotrophic						
CerEx			0-50(1)			
CerFr	0-4008(15)		0-1700(11)	0-1000(8)	0-600(10)	0-300(11)
CerFs			0-100(5)	0-100(3)	0-150(2)	
CerL			0-688(4)	0-188(1)		
CerM	0-167(1)					
CerP			0-50(2)	0-50(1)		
CerTp				0-50(1)		
DyA	0-167(1)		0-50(1)	0-100(2)		
DyAc	0-167(5)				0-50(1)	
DyC	0-501(4)				0-100(1)	0-50(1)
DyN	0-167(1)		0-63(1)			
Dys			0-63(2)	0-50(1)		
ProG	0-501(5)		0-150(5)	0-50(2)	0-50(1)	0-50(3)
ProM	0-2338(14)		0-125(7)	0-50(2)	0-150(13)	0-100(3)
ProMi					0-50(1)	
ProS				0-63(1)		
ProSg	0-600(8)		0-300(8)	0-100(6)	0-500(7)	0-250(5)
Pros	0-300(4)		0-100(5)	0-63(3)	0-100(5)	0-100(2)
PysH			0-63(1)	0-63(1)		
ScT	0-200(4)		0-800(6)	0-150(4)	0-100(6)	0-50(2)
Scs	0-50(1)		0-63(1)			

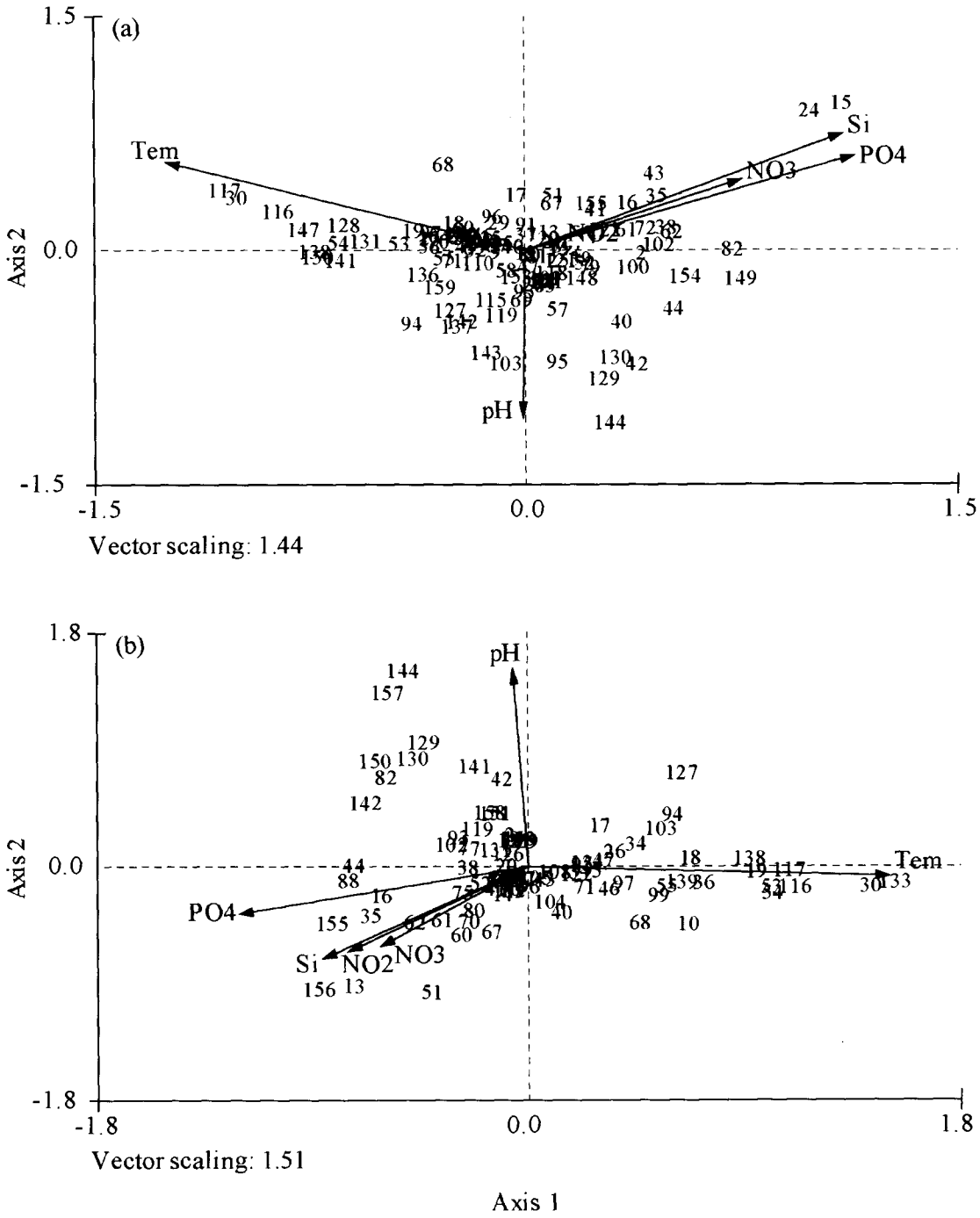
Appendix 3 Contd....

.....Appendix 3 Contd.

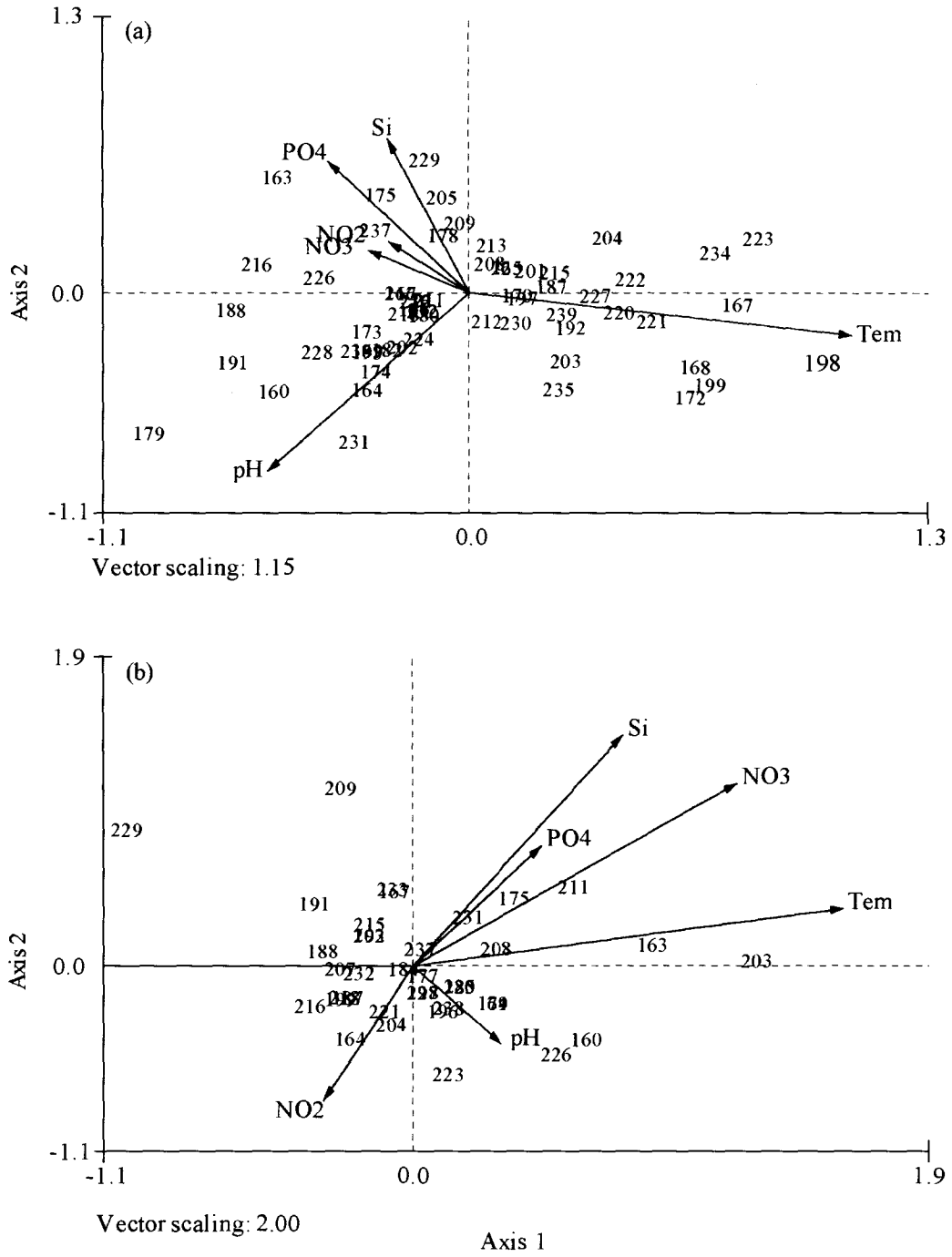
Taxon	PrM		PoM		WSWM	
	S	NB	S	NB	S	NB
Heterotrophic						
DipL					0-50(1)	
Dips						0-50(1)
GotA	0-150(5)		0-150(2)	0-50(3)		0-50(2)
Gyds	0-50(2)		0-63(8)	0-100(4)	0-100(2)	0-150(6)
Kats			0-63(1)			
PhR	0-50(1)		0-63(2)	0-50(3)		0-50(1)
PpBr	0-300(3)		0-50(1)	0-50(1)		
PpC	0-167(3)			0-50(1)		0-50(1)
PpDe	0-50(3)		0-63(1)			
PpDi	0-200(6)			0-100(2)	0-50(3)	0-50(3)
PpEx	0-300(1)		0-250(4)	0-188(3)		
PpLe	0-167(1)					
PpMi	0-167(1)		0-200(5)	0-100(2)	0-150(3)	0-150(2)
PpOb	0-167(3)		0-50(1)			
PpPc			0-150(3)	0-100(2)		
PpPal	0-167(2)		0-63(2)		0-100(5)	0-100(5)
PpPd	0-167(1)		0-63(1)			
PpPl	0-167(2)		0-150(6)		0-100(1)	0-100(2)
PpPn			0-50(1)		0-50(2)	0-50(2)
PpPy						0-50(1)
PpSt	0-900(4)				0-50(1)	
PpSu	0-167(3)		0-200(3)	0-50(1)		
PpSt			0-63(1)			
Pps	0-150(5)		0-600(8)	0-200(6)	0-250(9)	0-200(8)
Wars			0-100(3)	0-50(2)		
Zygs	0-167(1)					

Appendix 4: Cumulative constrained percentages of the 4 axes extracted in the CCA analysis for diatoms and dinoflagellates in surface and near bottom waters in MPT and VPT.

Factors	Axis 1	Axis 2	Axis 3	Axis 4
MPT				
Diatoms - surface waters	47.80	72.05	83.44	90.88
Diatoms - near bottom waters	53.46	81.78	87.93	93.19
Dinoflagellates - surface waters	38.98	61.39	74.89	85.83
Dinoflagellates - near bottom waters	27.49	50.22	70.51	83.09
VPT				
Diatoms - surface waters	39.86	68.75	86.03	92.13
Diatoms - near bottom waters	42.17	63.14	75.13	86.23
Dinoflagellates - surface waters	35.55	61.46	77.82	86.23
Dinoflagellates - near bottom waters	40.37	66.21	79.87	90.81



Appendix 5: Ordination diagrams for species based on Canonical Correspondence Analysis (CCA) of the diatom communities in (a) surface and (b) near bottom waters in Mormugao Port (MPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate and silicate) are indicated by arrows labeled Tem, NO3, NO2, PO4 and Si respectively. Species codes are given in Table 4A.3.



Appendix 6: Ordination diagrams for species based on Canonical Correspondence Analysis (CCA) of the dinoflagellate communities in (a) surface and (b) near bottom waters in Mormugao Port (MPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate and silicate) are indicated by arrows labeled Tem, NO3, NO2, PO4 and Si respectively. Species codes are given in Table 4A.3.

Appendix 7: Phytoplankton recorded in surface (S) and near bottom (NB) waters in Visakhapatnam Port (VPT), India. NEM: North East Monsoon, PrM: Pre-monsoon, SWM: South West Monsoon. The values outside the bracket indicate the abundance range (cells L^{-1}) and the values inside the bracket indicate the frequency of occurrence at 20 stations.

Taxon	NEM		PrM		SWM	
	S	NB	S	NB	S	NB
Diatoms						
Centric						
Asps	0-100(1)					
BaF	0-700(4)	0-600(4)			0-1600(2)	0-1600(1)
BaH	0-400(2)	0-200(1)			0-1600(1)	0-800(1)
Bas	0-200(1)					
ChA		0-800(1)		0-4800(1)		
ChCr	0-5200(2)	0-6800(2)				0-2400(1)
ChDe	0-200(1)	0-800(5)	0-6400(1)		0-3200(1)	0-1600(1)
ChDiv		0-600(2)				
ChPe	0-300(1)					
Chs	0-1200(10)	0-3200(10)	0-14400(5)	0-17600(3)	0-7200(1)	
Cors						0-1600(1)
CosM	0-100(1)	0-400(1)	0-1600(1)	0-1600(2)		0-2400(2)
Coss	0-200(4)	0-400(7)				0-2400(2)
Cys	0-200(1)	0-800(4)				
DiB		0-200(1)				
GuF				0-3200(1)		
GuS	0-400(1)		0-2400(2)	0-3200(6)	0-2400(4)	0-3200(4)
Gus	0-7600(1)	0-1600(3)		0-3200(1)		
HeT		0-400(1)				
HeH	0-200(1)				0-1600(2)	0-1600(1)
HmS	0-100(1)					
Hems		0-200(1)				
LeD	0-400(2)			0-3200(1)	0-10400(1)	
LeM		0-1400(1)			0-6400(2)	0-7200(2)
OdS						0-1600(3)
ParS	0-1000(1)					
PlaS	0-100(1)					

Appendix 7 Contd.....

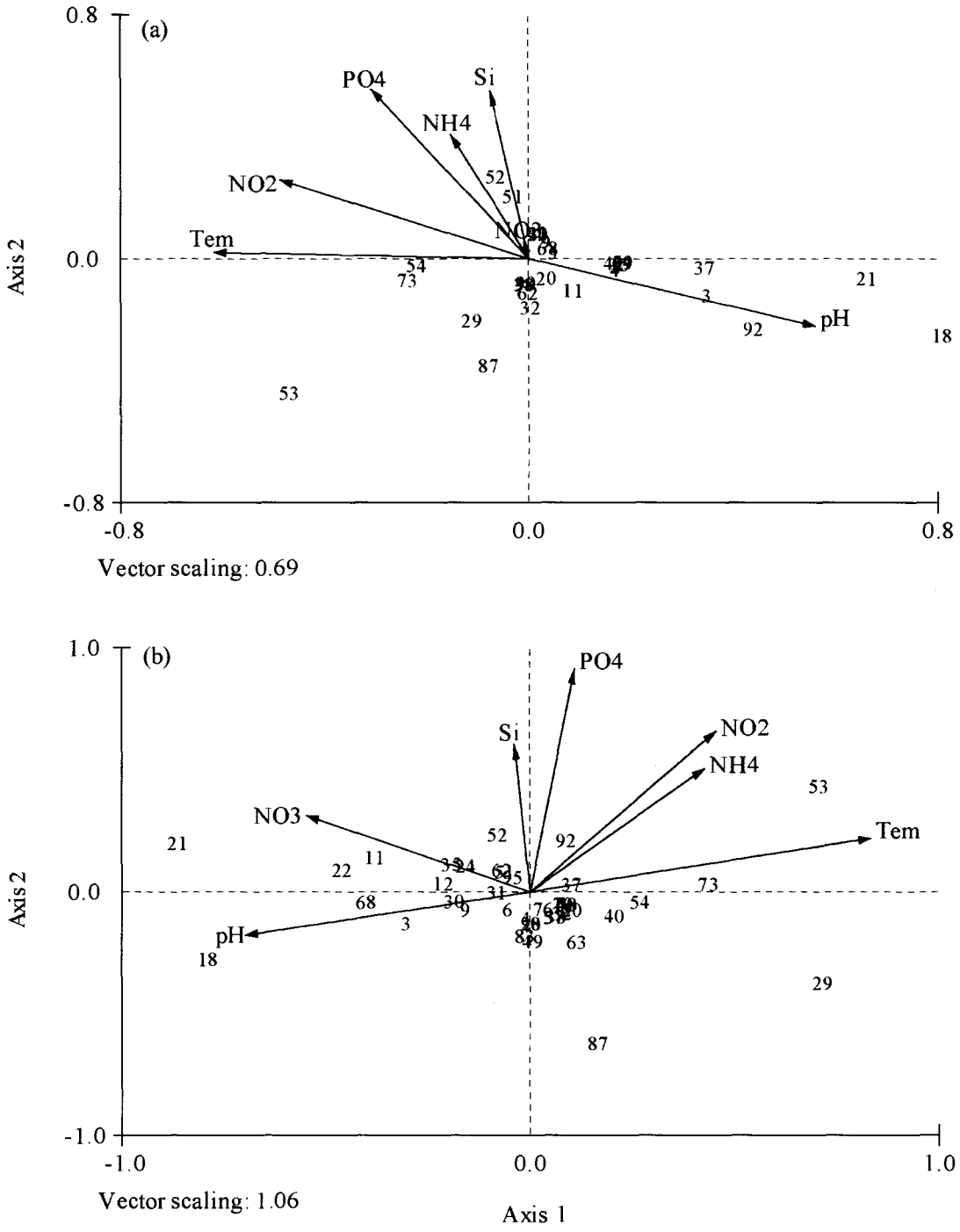
.....Appendix 7 Contd.

Taxon	NEM		PrM		SWM	
	S	NB	S	NB	S	NB
RhSe						0-1600(1)
RhSt				0-1600(2)	0-800(1)	
Rhs						0-800(1)
SkC	11230-122400(20)	67000-377800(20)	155780-366020(20)	30988-915200(20)	32000-559200(20)	0-382400(18)
SkT	0-6500(14)	0-155200(19)	0-256000(19)	0-275200(18)	0-350400(19)	0-292000(19)
Sks	0-1200(1)	0-5300(2)	0-64000(9)	0-150400(9)	0-143200(7)	0-387200(5)
Thss	0-4200(14)	0-4000(10)	0-68800(10)	0-192000(7)	0-36000(15)	0-8800-(15)
Pennate						
Ams				0-3200(1)		0-800(1)
CylC		0-4000(3)	0-800(2)	0-1600(1)		
DipC				0-1600(2)		0-800(1)
MuM	0-400(1)	0-1200(5)		0-3200(2)		
NvTDe	0-100(1)					
NvTDs						0-800(1)
Nvs	0-400(3)	0-200(3)	0-3200(2)	0-8000(5)	0-3200(4)	0-2400(8)
Nis				0-1600(1)		
Ples				0-1600(2)		
Psns	0-300(8)	0-400(4)	0-3200(7)	0-8000(8)	0-9600(8)	0-10400(8)
ThnB				0-1600(1)	0-1600(1)	
ThnN	0-400(5)	0-600(7)	0-3200(2)	0-3200(4)	0-4000(2)	0-3200(4)
Trops		0-800(1)				
Dinoflagellates						
Autotrophic						
Axs	0-3600(4)	0-2200(2)	0-1600(2)			
Ampds	0-460(4)	0-200(2)	0-1600(4)	0-1600(2)		0-800(1)
AmxT			0-9600(2)			
Gambs			0-100(1)			
GnxDig			0-1600(2)			
GnxP			0-3200(1)			
GnxSc		0-400(1)				
GnxVer	0-1200(4)	0-200(1)	0-19200(4)			
Gnxs	0-1600(6)	0-800(1)	0-35200(17)	0-12000(9)	0-2400(5)	0-800(3)
GymS			0-1600(1)			
Gyms			4800(1)			
Htrs	0-200(2)					
Oxs	0-200(1)					
Prqn	0-9200(16)	0-1600(6)	0-224000(18)	0-99200(11)		0-1600(1)

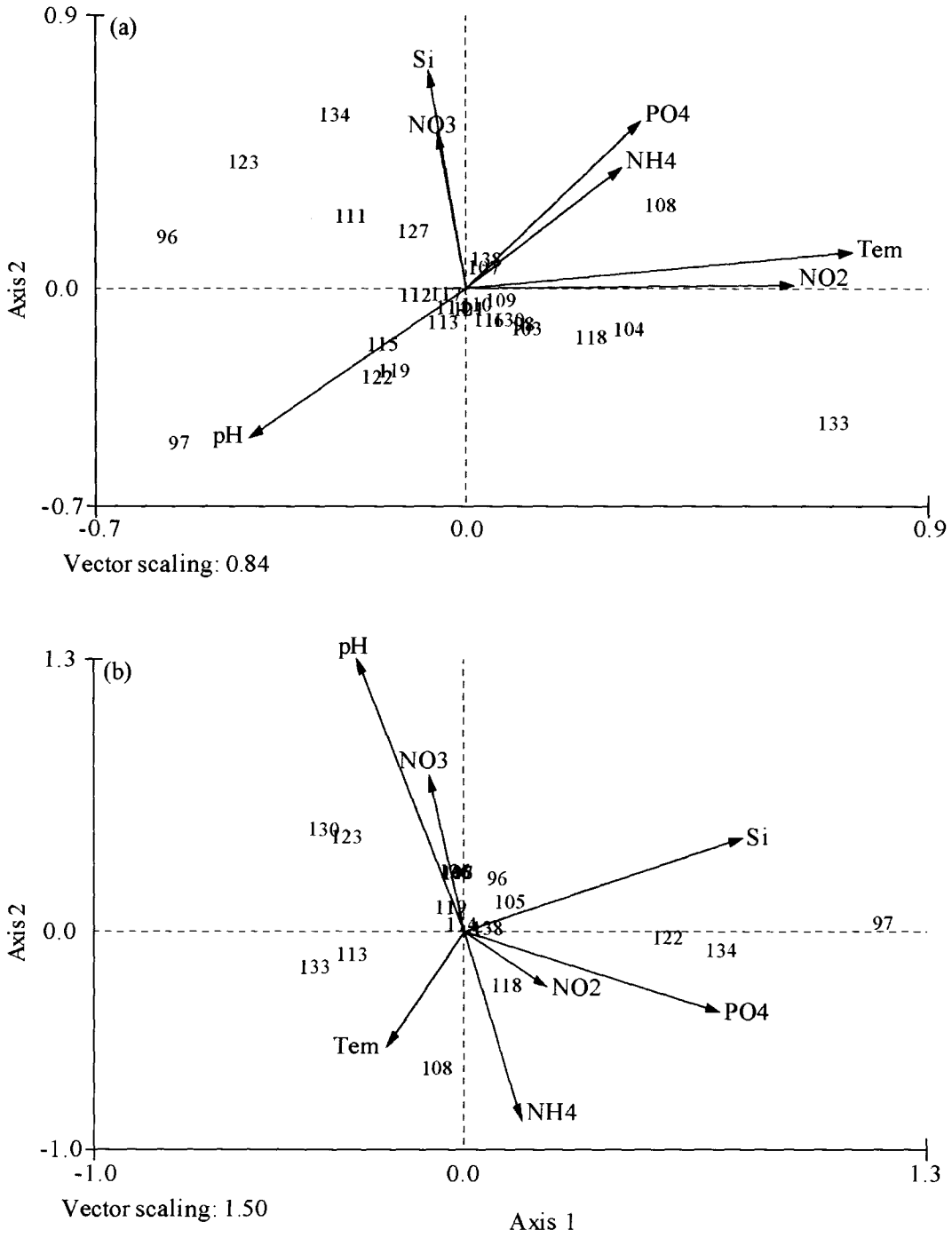
Appendix 7 Contd....

.....Appendix 7 Contd.

Taxon	NEM		PrM		SWM	
	S	NB	S	NB	S	NB
Mixotrophic						
CerFr	0-200(2)		0-4800(2)	0-800(1)		
CerFs	0-200(1)		0-1600(1)			
Cers		0-200(1)	0-1600(2)			
DyC	0-200(1)					
ProG			0-3200(3)	0-1600(1)		
ProM	0-600(4)		0-5600(8)	0-3200(2)	0-800(2)	
ProSg			0-1600(1)			
Pros	0-200(1)	0-100(1)	0-1600(3)	0-1600(2)		0-800(1)
ScT	0-3000(9)	0-800(3)	0-3200(7)	0-3200(4)		
Heterotrophic						
GotA		0-200(1)				
Phs	0-100(1)					
PpDi		0-1600(2)	0-800(2)	0-800(2)		
PpPal			0-8000(7)	0-3200(4)		
PpPl	0-600(3)	0-200(3)		0-1600(2)		
Pps	0-3000(19)	0-1200(14)	1200-112000(20)	0-32000(15)	0-8800(5)	0-2400(3)



Appendix 8: Ordination diagrams for species based on Canonical Correspondence Analysis (CCA) of the diatom communities in (a) surface and (b) near bottom waters in Visakhapatnam Port (VPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate and silicate) are indicated by arrows labeled Tem, NO3, NO2, PO4 and Si respectively. Species codes are given in Table 4A.3.



Appendix 9: Ordination diagrams for species based on Canonical Correspondence Analysis (CCA) of the dinoflagellate communities in (a) surface and (b) near bottom waters in Visakhapatnam Port (VPT), India. The physico-chemical variables (temperature, pH, nitrate, nitrite, phosphate and silicate) are indicated by arrows labeled Tem, NO3, NO2, PO4 and Si respectively. Species codes are given in Table 4A.3.