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.





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).

Station	Station name	Latituda	Longitudo
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

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

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 (NO₃-N), nitrite (NO₂-N), phosphate (PO₄-P), silicate (Si) and ammonia (NH₄) (SKALAR autoanalyzer), were analyzed using standard procedures (Parsons et al. 1984). Ammonia concentrations were analyzed only in Visakhapatnam.

Station	Station name	Latituda	Longitudo
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

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

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 (NO₂, NO₃, SiO₄ and PO₄) 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 ($^{\circ}$ C), (b) pH, (c) Nitrate (μ M), (d) Nitrite (μ M), (e) Phosphate (μ M) and (f) Silicate (μ M). 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).

Tayon	Species	CCA	Pr	M	Po	М	WS	SWM
	code	code	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	+	+				
<i>Corethron</i> sp.	Cors	32	+					
Coscinodiscus curvatulus	CosCur	33		+				
Coscinodiscus eccentricus	CosE	34		+				
Coscinodiscus gigas var.								
punctiformis	CosGi	35				+	+	+

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).

Tayon	Species	CCA	Pr	М	Po	М	WS	SWM
1 4 X 0 II	code	code	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		+		+	+	+
<i>Cyclotella</i> 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	+	+	+	+	+	+
<i>Guinardia</i> 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	+	+			+	+
<i>Lauderia</i> 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	+	+			+	+
<i>Odontella</i> sp.	Ods	69	+		+			+
Paralia sulcata	ParS	70	+	+		+		+
Planktoniella sol	PlaS	71	+	+	+	+		+

Tayon	Species	CCA	Pr	М	Pol	М	WS	SWM
Taxon	code	code	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	+		+		+	+
<i>Rhizosolenia</i> sp.1	Rhs	81					+	+
Skeletonema costatum	SkC	82	+		+	+	+	+
<i>Skeletonema</i> 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 marvlandica	AstlM	98			+			
Bacillaria paxillifera	BacP	99	+	+				
Caloneis sp.	Cals	100			+			
<i>Climacosphaenia</i> sp.	Clims	101			+			
Cvlindrotheca 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	+	+		+	+	+

Tavan	Species	CCA	Pr	Μ	Pol	Μ	WS	SWM
1 22011	code	code	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			+	+		
<i>Navicula</i> 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	+		+	+		
<i>Pseudo-nitzschia</i> sp.1	Psns1	148			+			
Pseudo-nitzschia sp.	Psns	149	+		+	+	+	+
Surirella fastuosa	SuF	150	+		+	+		+
Surirella ovata	SuOt	151			+	+		
Surirella sp	Sust	152					+	+

Tayon	Species	CCA	Pr	M	Pol	М	WS	SWM
Taxon	code	code	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	+		+	+	+	
<i>Thalassionema</i> sp.	Thns	158				+		
<i>Tropidoneis</i> 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						+

Tayon	Species	CCA	Pr	Μ	Pol	Μ	WS	SWM
	code	code	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	+		+	+	+	+
<i>Scrippsiella</i> sp.	Scs	212	+		+			
Heterotrophic								
Diplopsalis lenticulata	DipL	213					+	
Diplopsalis sp.	Dips	214						+
Gotoius abei	GotA	215	+		+	+		+
<i>Gyrodinium</i> sp.	Gyds	216	+		+	+	+	+
Katodinium sp.	Kats	217			+			
Phalacroma rotundata	PhR	218	+		+	+		+

Tayon	Species	CCA	PrM	I	PoN	1	WS	WM
	code	code	S	NB	S	NB	S	NB
Protoperidinium brevipes	PpBr	220	+		+	+		
Protoperidinium conicum	РрС	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	РрРу	233						+
Protoperidinium steinii	PpSt	234	+				+	
Protoperidinium subinerme	PpSu	235	+		+	+		
Protoperidinium stellatum	PpSt	236			+			
Protoperidinium sp.	Pps	237	+		+	+	+	+
<i>Warnowia</i> sp.	Wars	238			+	+		
Zygabikodinium sp.	Zygs	239	+					

Phytoplankton abundance varied from 1.3×10^4 to 2.8×10^5 cells L⁻¹ in surface waters and from 1.2×10^4 to 5.8×10^4 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: Premonsoon; PoM: Post-monsoon; WSWM: withdrawal of the South West Monsoon. Scale: log(x+1).

Dinoflagellates	Diatoms	Phytonlankton
'alues in bold indicate significant variation.	gao port (MPT), India. V	liatom and dinoflagellates in surface waters of Mormug
ies richness, evenness and diversity of phytoplankton,	tion in abundance, speci	Table 4A.4. Two-way ANOVA to evaluate the variation

	Phy	/toplankt	on		Dia	toms			Din	oflagella	tes	
Factor								-d				
	df	SS	MS	p-value	df	SS	MS	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	0	169.92	84.96	0.0001	3	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	0	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	0	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	0	1.73	0.86	0.0233	0	0.28	0.14	0.0147	0	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		

	Phy	vtoplank	ton		Dia	toms			Dir	oflagella	ntes	
Factor								- <i>d</i>				
	df	SS	SM	p-value	df	SS	SM	value	df	SS	SM	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	0	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	0	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	0	0.17	0.08	0.0063	0	0.17	0.08	0.0057	0	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	0	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		

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.

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: Postmonsoon; 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 1.2×10^4 to 2.6×10^5 cells L⁻¹ in surface waters and from 1.1×10^4 to 5.8×10^4 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 x 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: Postmonsoon; 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 1.3×10^3 cells L⁻¹ to 1.1×10^4 cells L⁻¹ in surface waters and from 0 to 3×10^3 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: Premonsoon; 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 premonsoon (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).

85



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: Postmonsoon; WSWM: withdrawal of the South West Monsoon. Expanded species biplots in Appendix 6.

4A.3.2a. Environmental variables



Fig. 4A.11. Seasonal variation in physico-chemical variables in Visakhapatnam Port (VPT), India. (a) Temperature ($^{\circ}$ C), (b) pH, (c) Nitrate (μ M), (d) Nitrite (μ M), (e) Phosphate (μ M), (f) Silicate (μ M) and (g) Ammonia (μ M). 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 ($27-27.5^{\circ}$ 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 NO₂, PO₄, SiO₄ and NH₃ were highest during SWM and showed considerable variation (Fig. 4A.11d-g). Nitrate (NO₃) 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), premonsoon (PrM) and South West Monsoon (SWM).

Tayan	Species	CCA	NE	М	PrN	1	SWI	M
1 8 2011	code	code	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	+	+	+	+	+	
				-		1 4 4	60	

....Table 4A.6. Contd.

Table 4A.6. Contd....

Tavon	Species	CCA	NE	Μ	Pr	N	SW	M
1 8 2011	code	code	S	NB	S	NB	S	NB
Coscinodiscus								
marginatus	CosM	20	+	+	+	+		+
Coscinodiscus sp.	Coss	21	+	+				+
<i>Cyclotella</i> sp.	Cys	22	+	+				
Ditylum brightwellii	DiB	24		+				
Guinardia flaccida	GuF	28				+		
Guinardia striata	GuS	29	+		+	+	+	+
<i>Guinardia</i> 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								
stvliformis	RhSt	49				+	+	
<i>Rhizosolenia</i> sp.	Rhs	50						+
Skeletonema costatum	SkC	51	+	+	+	+	+	+
Skeletonema tropicum	SkT	52	+	+	+	+	+	+
Skeletonema sp.	Sks	53	+	+	+	+	+	+
<i>Thalassiosira</i> sp1	Thss1	54	+	+	+	+	+	+
Pennate								
Amphora sp.	Ams	57				+		+
Cylindrotheca	1 1110							
closterium	CvlC	62		+	+	+		
Dinloneis crahro	DinC	63		-		+		+
Meuniera	Dipe	00						
membranacea	MuM	68	+	+		+		
Navicula delicatula	NvTDe	69	+					
Navicula derasa	NyTDs	70	•					+
Navicula sp	Nvs	73	+	+	+	+	+	+
Nitzschia sp	Nis	76				+		

....Table 4A.6. Contd.

Tawar	Species	CCA	NE	Μ	PrN	M	SW	M
1 8 2011	code	code	S	NB	S	NB	S	NB
Pleurosigma sp.	Ples	83				+		
<i>Pseudo-nitzschia</i> 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	+	+	+			
<i>Gonyaulax</i> sp.	Gnxs	108	+	+	+	+	+	+
Gymnodinium								
sanguineum	GymS	109			+			
<i>Gymnodinium</i> 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		+	+			

Tayon	Species	CCA	NE	Μ	PrN	[SWN	M
	code	code	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 1.3×10^5 to 9.5×10^5 cells L⁻¹ in surface waters and from 5.3×10^5 to 1.3×10^6 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).

	Phy	/toplankte	00		Dia	toms			Din	oflagellat	es	
Factor	df	SS	SM	p- value	df	SS	SM	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	0	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	0	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	0	0.27	0.14	0.0013	0	0.46	0.23	0.0001	0	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	0	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.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.

	Ph	ytoplankte	uc		Dia	toms			Din	oflagellat	es	
Factor	df	SS	WS	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	7	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	0	0.33	0.17	0.0205	0	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	0	0.33	0.17	0.0205	0	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		

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.

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: Premonsoon; 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) Premonsoon 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 1.1×10^4 cells L⁻¹ to 3.3×10^5 cells L⁻¹ in surface waters and from 2.4×10^3 to 1.2×10^5 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) Premonsoon 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 6.1×10^4 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, partly 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 Due to these reasons, it can proliferate rapidly under high nutrient therein). They also secrete aldehyde compounds as part of their defence conditions. 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:HNO₃:H₂SO₄) 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 CO₂), 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 = $10 \mu m$.

Skeletonema costatum (Fig. 4B.2a-c): Cells are 4-12 μ m 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 μ m 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 μ m), 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 μ m. (b) *S. costatum*, intercalary valve with the long rimoportula process (arrow). Scale bar = 1 μ m. (c) *S. grevillei*, terminal valve. Scale bar = 1 μ m. (d) *S. grevillei*, intercalary valve. Scale bar = 1 μ m. (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 μ m. (f) *S. grevillei*, detail of knuckle-like junction. Scale bar = 1 μ m. (j) *S. tropicum*, terminal valve with fultoportula processes and subcentral rimoportula process. Scale bar = 5 μ m. (k, l) *S. tropicum*, intercalary valve. Scale bar = 5 μ m.



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

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.