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Spatio-temporal variability of dinoflagellate assemblages in different salinity regimes in the west coast of India

A A S Alkawri and N Ramaiah*
Biological Oceanography Division
National Institute of Oceanography
(Council of Scientific and Industrial Research)
Dona Paula, Goa 403004
*Corresponding author; email: ramaiah@nio.org

Abstract:

General abundance and species composition of dinoflagellate community in different salinity gradients along the coast of Goa (west coast of India) were studied with a view to understand the annual variations in particular the toxic species. Regular monthly samples were collected from the inter-tidal regions for elucidating the effect of physical and chemical parameters on dinoflagellate assemblage at four disparate locations in terms of their salinity. From this 13-month study, a total number of 25 dinoflagellate species were identified. Of these, ten are known toxic species. *Ceratium furca* was the most dominant dinoflagellate species at all four sampling locations throughout the year. Its highest number, 5763 cells L⁻¹, was observed during September 2008 at the estuarine sampling location off Siridao. The toxic species, *Alexandrium minutum* (1171 cells L⁻¹) followed by *Dinophysis acuminata* (838 cells L⁻¹) were found to attain maximum cell numbers among the 10 different toxic species detected from the study area. It is apparent from our results that the distribution of both general and toxic dinoflagellate species in the study area is affected due to variations in physico-chemical parameters, in particular temperature, salinity and nitrate that in turn are governed by the monsoonal dynamics.

Keywords: Estuaries; Dinoflagellate assemblages; Toxic dinoflagellates; *Dinophysis*, *Alexandrium minutum*, *Prorocentrum micans*

1. Introduction

Dinoflagellates are important components of the phytoplankton in the near-shore and continental shelf environments. Along with diatoms, over half of the present-day dinoflagellates are photosynthetic and are at the base of marine food web (Loeblich, 1984). Some species however, are primary causal agents of paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) and related toxic phenomena (red tides). Such toxic species are of serious economic considerations. Often, the red tide phenomenon has been ascribed to harmful algal blooms (HAB). Over the years, there has been a marked rises in the occurrence of HAB along with their spread to new geographical regions (Anderson et al., 2002a). The apparent global expansion of HAB is linked to intensified anthropogenic eutrophication of coastal waters and to increased load of nutrients (Hallegraeff, 1993; Anderson et al., 2002a and 2002b). Many dinoflagellate species have been implicated in fish kills and consequent economic losses in many regions of the world oceans (Adams et al., 1968; Hoagland et al., 2002). Human fatalities due to PSP, DSP and/or other dinoflagellate related toxicity have been serious public health concerns. In view of the intra- and inter- annual variability in the species composition of dinoflagellates, they are regularly monitored in most European countries, the Americas and Japan. The most countries, however, have not yet set effective monitoring programs, though there are reports of human health concerns as a consequence of HAB (Ramaiah et al., 2005).

In the monsoon affected west coast of India, many studies have documented the occurrence of different genera of dinoflagellates (Devassy and Bhattathiri, 1981; Lingadhal et al., 1998; Tiwari and Nair, 1998; Mendon et al., 2002; Alkershi, 2004). Most of these studies recorded 6 to 8 genera of dinoflagellates to be prevalent with *Protoperidinium* and *Ceratium* as common genera along the central west coast of India. Cases of *Noctiluca* blooms (Sahayak et al., 2007) and fish kills due to dinoflagellate blooms (Naqvi et al., 1998) are also reported in particular from the southern regions. Godhe et al. (2008) have also documented the prevalence of toxic dinoflagellates in Indian coastal waters. Cysts/resting stages of over 30 dinoflagellate species in 10 genera from the coast of Mumbai are reported from an exhaustive recent study by D'Costa et al. (2008). Karunasagar et al. (1990) reported a case of PSP related death.

Most non-parasitic and/or symbiotic dinoflagellate species are marine, numerous in temperate waters and flourish during summer months (Taylor, 1987). Nitrogen and phosphorus concentrations bear a strong influence on stimulation of phytoplankton blooms, including HAB forming species in coastal

areas (Harrison, 1976; Paerl, 1988, 1997; Baek et al., 2008). Some dinoflagellate species may respond well to increased nutrient additions by proliferating in numbers. However, it would be relevant to elucidate their variability in their natural ecosystems subjected to varying physico-chemical characteristics, for instance, during monsoon and non-monsoon periods. In addition, from the perspectives of public health-related issues, it would be necessary to elucidate the differences in the species composition of toxic dinoflagellates from the coastal regions. In view of this, we planned a year-round systematic survey from four locations off Goa where salinity differences/variations are large. We also aimed to document the variations in toxic dinoflagellates in relation to environmental variability from this sparsely investigated region.

2. Material and Methods

2.1 Study Area and Sampling

The west coast of India experiences intense rain fall during the monsoon months of June-September. During the summer season (March-May), both air (28-36 °C) and water temperature (24-32 °C) are higher. The temperature (air: 17-26 °C; water: 21-25°C) is somewhat low during the 'mild' winter period, November-February. The study was carried out in the estuarine and coastal locations of Goa. Based on salinity gradient, four different locations were decided for sampling. Sampling locations (Fig.1) in Chapora estuary (15°36'30.8"N, 73°44'18.6"E) and in Dona Paula Bay (15°27'4.9" N, 73°48'11.8"E) were chosen to represent true estuarine conditions. The station off Anjuna (15°35'5.3"N, 73°44'12.9"E) is truly marine with no influence of river discharges within its 7-8 Km radius. The location off Siridao (15°25'53.1"N, 73°51'43.8"E), usually with lower salinities, is ~5 Km east of the station in Dona Paula Bay. The tidal amplitude off Goa is ~ 1.5 m. Surface samples were collected every month from these locations during the low tide-times from September 2007 to September 2008. The site depths during the low tide-times were 3-5 m at different sampling locations. But for the location off Siridao where the sediment is sandy-silt, the other locations were mostly sandy and at the location off Anjuna, the substrate is rocky.

2.2 Environmental Parameters

Temperature was measured immediately at site using a portable thermometer. The salinity was analyzed by measuring the electrical conductivity ratio, using a salinometer (Autosal, model 8400A, Guildline, Canada; measurement range: 0.005-42 psu). The conductivity ratio readings were converted to salinity

and values expressed in psu. Dissolved oxygen was determined by standard Winkler's method described in Parsons et al. (1984).

2.3 Nutrients

Concentrations of dissolved inorganic nutrients such as nitrate (NO₃-N), nitrite (NO₂-N), phosphate (PO₄-P) were determined spectrophotometrically (Systronics 2202, USA) by following standard methods described in Parsons et al. (1984).

2.4 Chlorophyll *a*

The chlorophyll *a* (chl *a*) concentrations were measured fluorometrically (Turner Designs, USA, 10-AU-005-CE) by filtering two replicates of 1 liter water samples from each sampling location onto 47 mm glass fiber filters (Whatman GF/F) and extracting overnight in 10 ml of 90% acetone under cold and dark conditions.

2.5 Dinoflagellate Assemblage

For quantitative and qualitative analyses of dinoflagellates, cell counts and composition, water sample from each of the above stations were fixed with Lugol's solution (1% wt/vol) and 3% formaldehyde and stored in the dark until analyzed. A settling and siphoning procedure was followed to concentrate samples from 4 L to 100 ml for counting dinoflagellate cells and identification of genera and species, where possible. Three 1-ml replicates of sample concentrates were transferred to Sedgwick-Rafter plankton counting chamber and examined microscopically using 10X eye piece and 20 and/or 40X objective on a Nikon E400 (Nikon, Japan) microscope. All 1,000 squares on the chamber were screened. Cells were enumerated and expressed as cells L⁻¹. They were classified as photosynthetic or heterotrophic forms based on the presence or absence of chloroplasts. Further, the dinoflagellates were identified to their generic/specific level based on the available literature (Taylor 1976; Dodge, 1982; Steidinger and Tangen, 1997; Faust and Gualledge, 2002; Horner, 2002).

2.6 Diversity Indices and Statistical Analyses

The diversity indices of dinoflagellates in the study area were calculated by using the Shannon-Weaver formula:

$$H' = - \sum_{i=1}^S P_i \log_2 P_i$$

where S is total number of species and P_i is the proportion of the numbers of individuals of species i to the total number of individuals $P_i = n_i/N$ (Omori and Ikeda 1984).

Species richness (SR), the number of species recorded from a region, was calculated by Margalef's formula (Margalef, 1951):

$$SR = (S - 1) / \log_n N,$$

where S = total number of species and, N = total individuals present in the sample.

Species evenness (J') was calculated according to Pielou (1966):

$$J' = H' / \log_2 S,$$

where S is total number of species and H' is the Shannon diversity index.

Spearman's rank correlation test was performed to evaluate the relationships between the dinoflagellate species with various observed environmental parameters that may be regulating their population. This test was performed using the software Statistica, version 6.0 (Statsoft, Oklahoma, USA).

Principal component analysis (PCA) was used to reduce the dimensionality of the complex data set using Statistica, version 6.0. This analysis, an orthogonal linear transformation method, converts a number of potentially correlated variables into a smaller number of independent variables, i.e., the principal components, while maintaining most of the original variability in the data. The first principal component accounts for the greatest variability in the data and each succeeding component explains as much of the remaining variability as possible. The Eigen-values and percent variation were calculated using PRIMER.

3. Results

3.1. Physico-chemical parameters

Water temperatures at all sampling stations exhibited a predictable seasonal fluctuation with the minima during the 'winter' month of February and monsoonal month of August (25°C) and the maximum of 33°C was recorded during the summer month of May (Fig 2). The salinity was influenced by the annual cycle of precipitation: spatially, the maximum salinity of 35.92 psu was found off Anjuna, the truly marine location in June. Also on the annual scale, the salinities at this location did not fluctuate widely unlike either off Dona Paula, Siridao or Chapora. The minimum salinity of 0.44 psu was recorded during the rainy month, August 2008 at Chapora, the location most impacted by freshwater discharges

throughout the year. As a consequence of reduced discharges, the salinity increased from October to reach the highest value in June. In general, the salinity was lower during the monsoon months (Fig. 2). Dissolved oxygen concentrations ranged from 2.05 to 7.11 ml L⁻¹ (\equiv 91.5-317.6 μ mol) with the maximum off Anjuna and the minimum off Siridao. The concentration was low only during March 2008 off Dona Paula and Siridao. The pH ranged between 7.52 and 8.48 during the study period.

3.2. Nutrient concentrations

The concentrations of dissolved inorganic nutrients, nitrate, nitrite and phosphate, in the study area varied at all sampled locations (Fig 2). The nitrate concentrations during the sampling period ranged between 0.004 μ mol and 16.35 μ mol. The highest values were observed during September 2007. Nitrite concentrations were usually <1.0 μ mol throughout the sampling period except off Siridao (0.15 to 2.35 μ mol). The silicate concentrations were the highest at Siridao (1.55-128.2 μ mol) followed by Chapora (3.57-105.9 μ mol) when compared with the locations off Dona Paula (1.07-122.67 μ mol) and Anjuna (0.12-74.66 μ mol). Ranging between 0.07 μ mol and 1.34 μ mol, the phosphate concentrations were generally low.

3.3. Chlorophyll *a* concentrations

The chl *a* concentrations (Fig.3) varied from 0.42 to 4.19 μ g L⁻¹ in Chapora and the maximum at this station was in May 2008. At the location off Anjuna, it varied between 0.29 and 2.1 μ g L⁻¹. Off Dona Paula it varied from 0.44 to 2.54 μ g L⁻¹. The highest concentration of 7.50 μ g L⁻¹ was observed off Siridao during September 2008. Its annual range at this location was 1.19 - 7.50 μ g L⁻¹.

3.4. Dinoflagellate Assemblage

The abundance of dinoflagellates was lower than that of diatoms throughout the year at all sampled locations (unpublished data). Distinct temporal and spatial variations in dinoflagellate cell densities were clear (Fig. 3). Some or the other species occurred throughout the period of study in the near shore waters of Goa. Except during September 2008 (8604, 1924 and 1666 cells L⁻¹ off Siridao, Dona Paula and Anjuna, respectively), the abundance of dinoflagellates was mostly <10³ cells L⁻¹. Twenty-five species of planktonic dinoflagellates belonging to seven genera were recorded during this study (Table 1). The number of heterotrophic dinoflagellate species is quite less compared to that of autotrophic ones.

The cell abundances of *Dinophysis* and *Protoperidinium* with seven species each and *Ceratium* with six species were greater than the other genera/species recorded during the present study. The most dominant species in the study area were *Ceratium furca* and the red tide-forming species, *Prorocentrum micans*. The cell counts of *C. furca* were the highest during September 2008 off Siridao and those of *P. micans* during September 2008 off Anjuna. The lowest numbers of species were observed off Anjuna from where the dinoflagellates were not detected during many sampling months during the study period (Table 1).

Of the known toxic species, *Alexandrium minutum*, *Dinophysis acuminata*, *D. caudata* and *Prorocentrum micans* were more abundant in the study area. Cells of *D. hastata*, *D. brevisulcus*, *D. fortii* and *D. miles* were rare though detected at all locations. With its highest numbers in October 2007 (875 cells L⁻¹), *Alexandrium minutum* was generally quite preponderant off Siridao, the low salinity sampling location of this study (Fig 4). In general, before and during this species attained its peak abundance, an increase in nutrient concentrations was observed. Cell counts of *Dinophysis acuminata* and *D. caudata* showed wide fluctuations except during September 2008 when they occurred in high numbers at all locations. Abundance of *D. acuminata* was maximum off Anjuna (392 cells L⁻¹) and Siridao (238 cells L⁻¹) when the salinity was 35 and 31.8 psu respectively at these locations. An increase in nitrate and phosphate were detected before the peak of this species. On the other hand, *Prorocentrum micans* were recorded at all the locations and almost throughout the year.

3.5. Statistical Analyses

Spearman's rank correlation analysis (Table 2) indicated that nutrients, in particular nitrate and phosphate, affect the abundance of dinoflagellates at most locations. The PCA was useful to discern the variance of different parameters lying in different coordinates. At locations off Chapora, Dona Paula and Siridao, the maximal variance of dinoflagellate abundance was loaded in the PC1 or the first coordinate (Fig 5a-c). The percentage variance explainable by PC1 was 43.5 off Dona Paula, 41.4 off Chapora and, 32 off Siridao. Off Anjuna, as much as 35.6% variance of dinoflagellate abundance was explainable by PC3 (Fig 5d) suggesting a different distribution pattern of dinoflagellates at this location. To delineate the percentage variance of different parameters monitored during this study, the coefficients of their Eigen-values were calculated (Table 3). As also discernible from Fig 5, the maximal variation of dinoflagellate abundance at most sampling locations is lying in PC1.

The dinoflagellate species diversity indices were usually <2.00 (Table 4). The highest H' was recorded from off Anjuna followed by Dona Paula during the post-monsoon period. Commensurating with cell abundances and the number of species enumerated, the evenness varied from 0.0 to 1.99 and the species richness from 0.0 to 3.58.

4. Discussion

The seasonal variability in the study area is largely due to the intense rainfall during the monsoon months of June to September, which leads to increased nutrient inputs through upwelling and terrigenous sources. As proposed by Bakun and Broad (2003), the alterations in physical and/or chemical factors disrupt biological settings in which some species can proliferate. Since the early days of phytoplankton ecology, nutrients have been known for controlling the phytoplankton community structure and biomass (Raymont, 1980; Tilman, 1982; Gouda and Panigrahy, 1996, Sawant et al., 2007).

In terms of generic composition, the observations from this study suggest some similarities with those from a few other studies along the west coast of India (Tiwari and Nair, 1998; Alkershi, 2004; D'Costa et al., 2008). Mendon et al. (2002) recorded six genera of dinoflagellates off Mangalore, where *Ceratium furca* was generally more abundant as was the case during this study. Further, several investigators have noted the regular occurrence of *Ceratium* spp (Devassy and Bhattathiri, 1981; Lingadhal, et al., 1998). In the present study, the highest abundance of dinoflagellates at the all locations was during September 2008 followed by October 2007. High temperature and salinity are favorable for the growth of dinoflagellates (Qasim et al., 1972; Taylor, 1973; Joseph and Pillai, 1975). Yoo (1991) reported that changes in salinity, pH, nitrogen and phosphate cause variations in the abundance and composition of dinoflagellates. The low-euryhaline species (occurring exclusively when salinity was <15) was *Alexandrium minutum*, the highly-euryhaline (15-30 psu) species were: *Ceratium furca*, *Prorocentrum micans*, *Pyrophacus horologicum* and stenohaline species (occurring exclusively when salinity was >30) was an unidentified *Dinophysis* sp. It was mostly in the higher salinity periods in particular off Siridao and Dona Paula.

The most dominant species was *Ceratium furca*. Its highest abundance was recorded from 'estuarine' locations off Siridao, Dona Paula and Chapora that are enriched by land-based nutrient inputs. Apparently, the allochthonous nutrient addition seems to contribute to the increased cell densities and

types of dinoflagellates. Our observation thus corroborates with the proposition by Licea et al. (2004) who concluded that nutrients contribute to the bloom formation in the Gulf of Mexico.

While cell densities of *C. furca* were more abundant when the nutrient concentrations were high during the present study, it was found rather frequently, albeit in low abundances, when concentrations of nutrients were lower. As Baek et al. (2008) suggest this species seems to thrive in the low nutrient conditions through physiological acclimation of its specific characteristics for nutrient uptake.

That the nutrients have the great influence on dinoflagellates abundance in our study area is clear from the strong negative correlation between their abundance and nitrite, nitrate as well as phosphate. It is thus inferable that the wide variation in the abundance of dinoflagellates on an annual scale is largely due to variations in nutrient concentrations. It is possible that the temporal difference in their abundance explains the observed variance fitting into the first coordinate. The diversity indices from this study are useful to suggest the existence of a thriving assemblage of dinoflagellates in the locations subjected to salinity variations. Indeed, the H' are generally higher from the estuarine regions when compared with the truly marine, higher salinity location off Anjuna.

Although cysts were not looked for in this study, it is clear from the extensive observations of D'Costa et al. (2008) that there exists a seed population of many of the dinoflagellate species in the shallow coastal sediments, including the known, potentially toxic forms. Similar to the earlier report of Ciminiello et al. (2000) such studies demonstrate the value of cyst sampling to confirm that some species can be missed because they either occur at very low concentration or bloom for very short periods. We believe however that our concentrating as much as 4 liters of seawater and examining three 1 ml replicates of 40X concentrates, helped count even the rarely occurring planktonic dinoflagellates in the water samples.

A literature account of different toxins produced by known toxic dinoflagellates observed during the present study is furnished in Table 5. Of these, *Alexandrium minutum*, known to be potentially toxic (Moestrup et al., 2004), is being observed/reported for the first time in the near shore waters of Goa. Known for PSP production, *A. minutum* was most prevalent off Siridao. It was recorded in the planktonic form off Chapora and Dona Paula as well. In addition to PSP-toxins, Cembella et al. (2000)

and Maclean et al. (2003) suggest that some *Alexandrium* spp can also produce the spirolides, another class of toxins whose effects on humans have not been clarified yet.

The DSP caused mainly due to *Dinophysis* spp has been reported to be the main toxin-related problem in several countries adjoining the Mediterranean Sea, from where mussel contamination due to okadaic acid and dinophysistoxins has been reported (Boni et al., 1993; Belin, 1993; Belin et al., 1995; Delgado et al., 1996; Marasovic' et al., 1998; Koukaras and Nikolaidis, 2004). Further, bioassays with mussels collected from regions where *Dinophysis* are numerous resulted in the death of the mice (Guarino et al., 2002 and Siano et al., 2002). All seven species of *Dinophysis* recorded during this study are reported to be potential causative agents of DSP (Plate I). The most abundant species in the waters off Goa is *D. acuminata* (838 cells L⁻¹) followed by *Dinophysis* sp (508 cells L⁻¹). While recognition of the actual strain(s) producing the toxins is essential, the cell counts of *Dinophysis* spp recorded during this study ought to be considered to denote the presence of potential DSP producing strains.

Ceratium fusus, found in our study at all the locations, can tolerate a great range of salinities (5-70 psu; Taylor et al., 2004). This species can cause harm to invertebrate larvae by an unknown mechanism (Taylor et al., 2004). Rensel and Prentice (1980) and Cardwell et al. (1979) have reported on the adverse effects of *C. fusus* on oyster larvae and shrimps.

The genera *Prorocentrum* and *Protoperidinium*, known to contain toxic species, were found in our samples. In particular, cells of *Prorocentrum micans* were observed at all the stations over wide ranges of water temperatures, salinities and nutrient concentrations. This could be attributed to the fact that their active swimming-cell stages can adapt to the ecological variations as suggested by Dodge (1982); Steidinger and Tangen (1997). Although *P. micans* is capable of forming extensive blooms, it is usually considered harmless (Taylor and Seliger, 1979; Anderson et al., 1985; Graneli et al., 1990). However, there are reports of *P. micans* having caused problems of shellfish kills in Portugal (Pinto and Silva, 1956) and South Africa (Horstman, 1981).

All the potential PSP-toxin producers were rather low in numbers in our study area. Although PSP contamination of shellfish has never been reported along the Goa coast so far, attainment of cell densities >1000 cells L⁻¹ of some known toxic dinoflagellates at least during some period in an annual

cycle is to be taken as an indication of the possibility of PSP in this tourism driven economy. Besides, there are reports that a cell abundance of ~ 400 cells L^{-1} is sufficient to cause DSP (Dr Luis Proenca, personal communication). Our results suggest that salinity effects are very strong on the cell densities of both general and potentially toxic species of dinoflagellates.

The ecophysiology and toxin production of certain dinoflagellate species have been investigated for decades. Unfortunately, these works have been mainly on the species occurring in temperate waters. The effect of environmental changes on toxin production in tropical dinoflagellates species is becoming increasingly evident (Hansen, 1989; Tomas and Baden, 1993; Godhe et al., 2008). Our results are useful to ascertain the effects of salinity on the dinoflagellates. Study on other environmental factors is essential to give a holistic interpretation on growth and toxin production of these tropical species. A better view of salinity–toxicity relationship can only be realized with further examination of natural population of these species.

Outbreaks of shellfish poisoning cases have caused serious economic losses in the fishery industries of the affected countries. Public health concerns arise with increasing poisoning cases due to the consumption of contaminated bivalves. This has been particularly pronounced in countries where seafood is the main source of protein. The perception of dinoflagellate caused-poisoning would change when there are accurate reporting of PSP and DSP as well as there are documentations of toxic species in the near shore waters.

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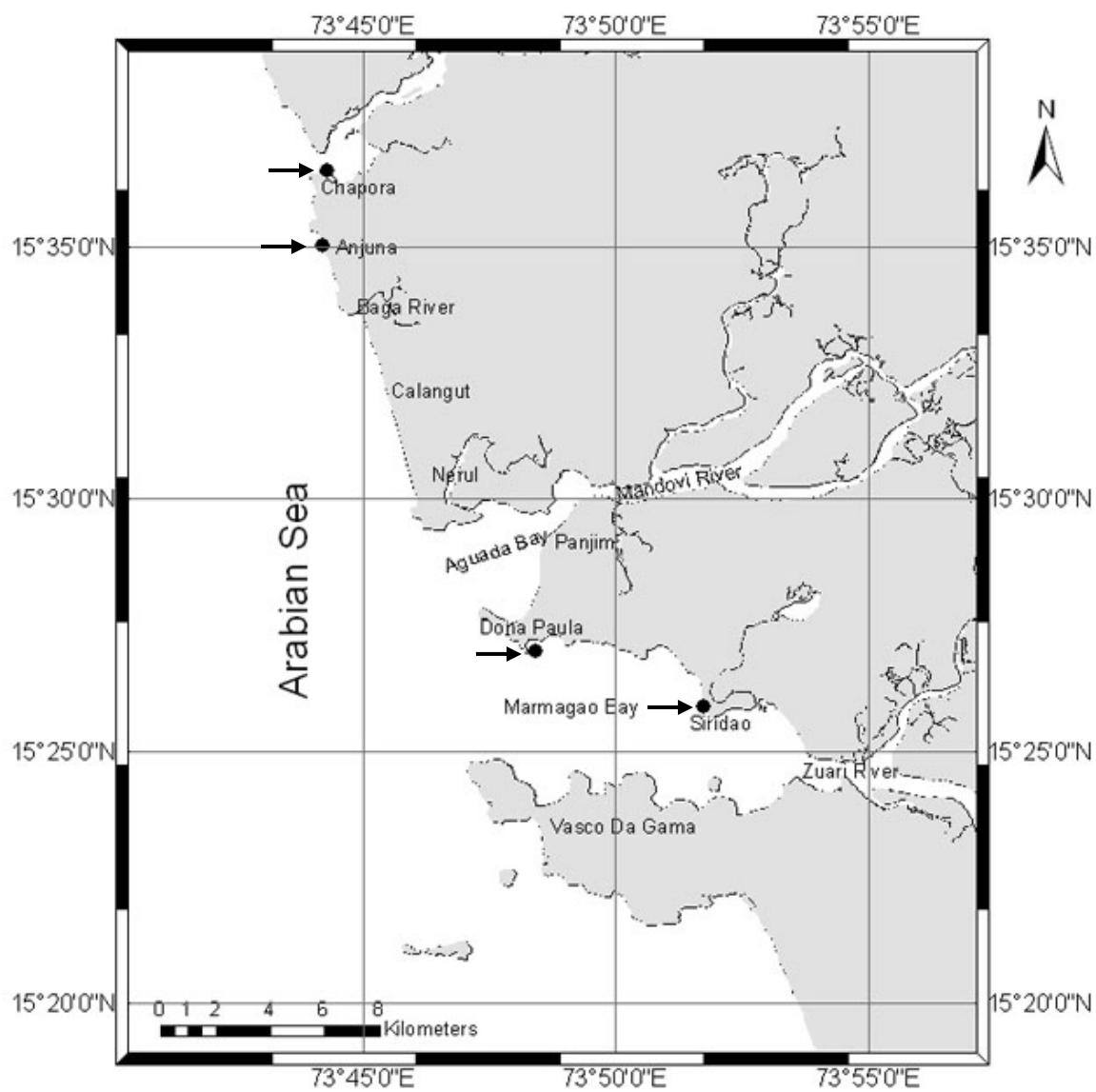


Fig.1. Map showing the sampling locations along the coast of Goa, west coast of India.

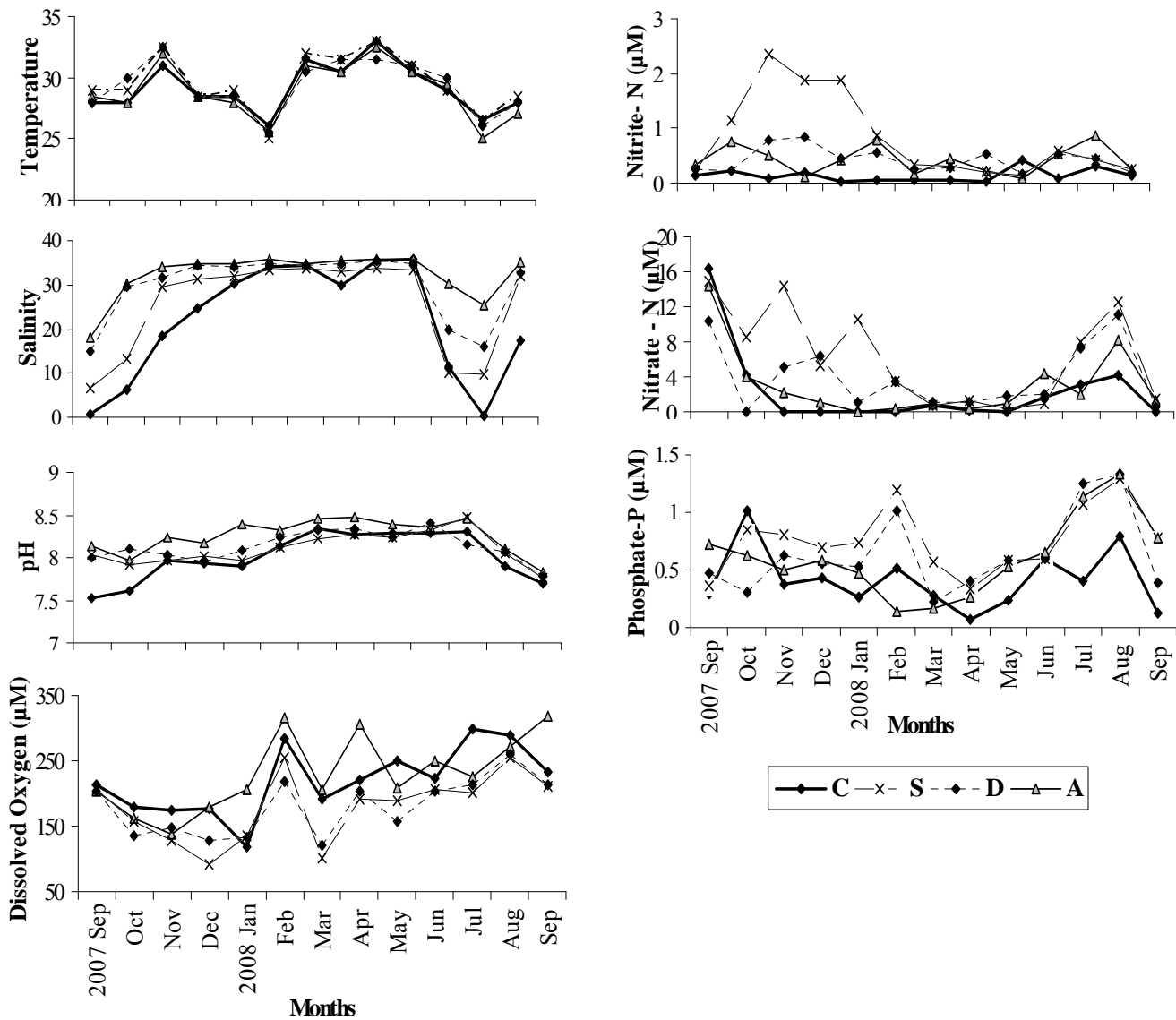


Fig. 2. Variations in temperature, salinity, pH, dissolved oxygen, nitrite, nitrate and phosphate in the surface waters from September 2007 to September 2008 at different sampling locations off Goa, west coast of India (C, Chapora; S, Siridao; D, Dona Paula and A, Anjuna).

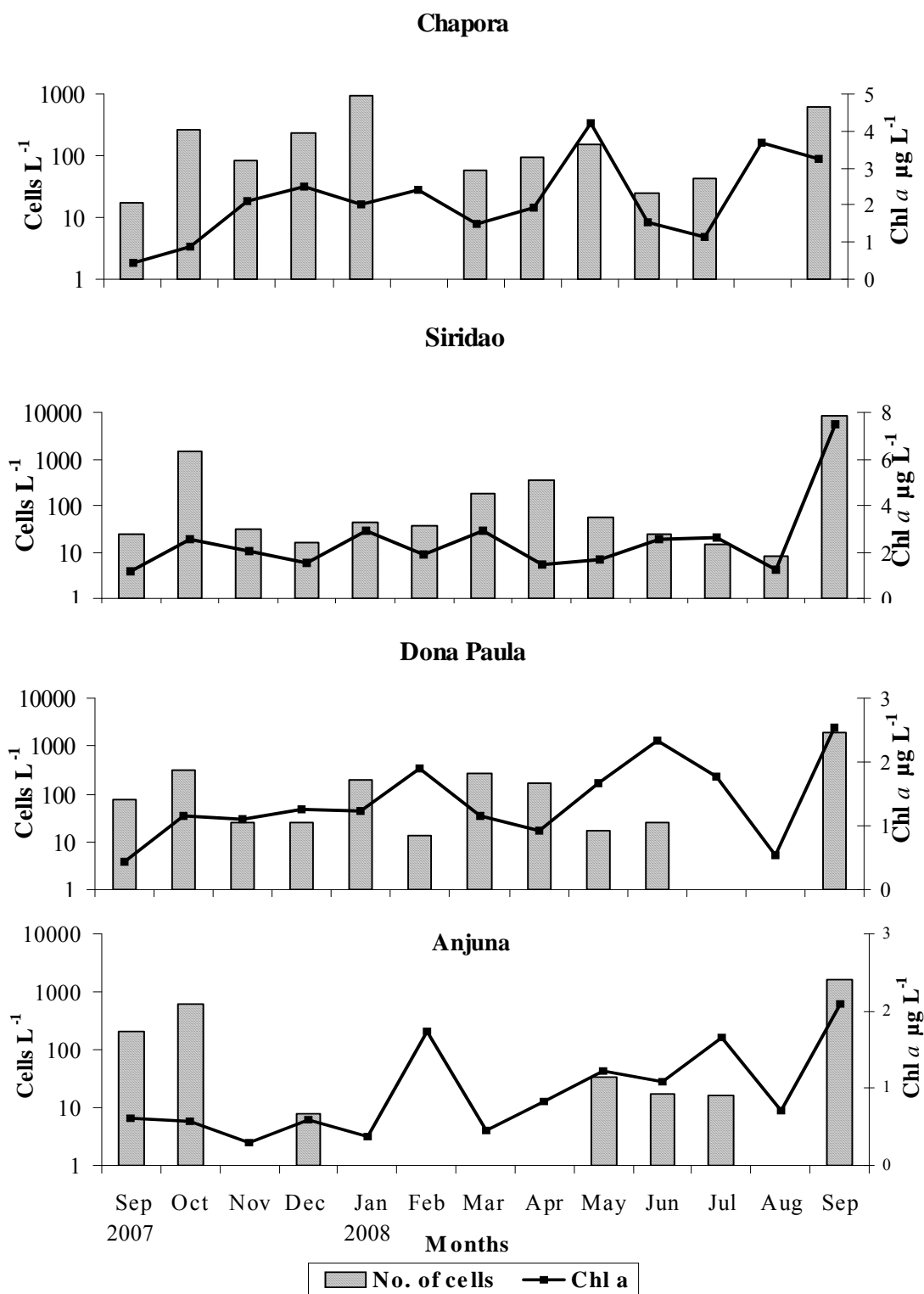


Fig.3. Variations in dinoflagellate cell counts and chlorophyll *a* concentration in the surface waters from September 2007 to September 2008 at different sampling locations off Goa, west coast of India

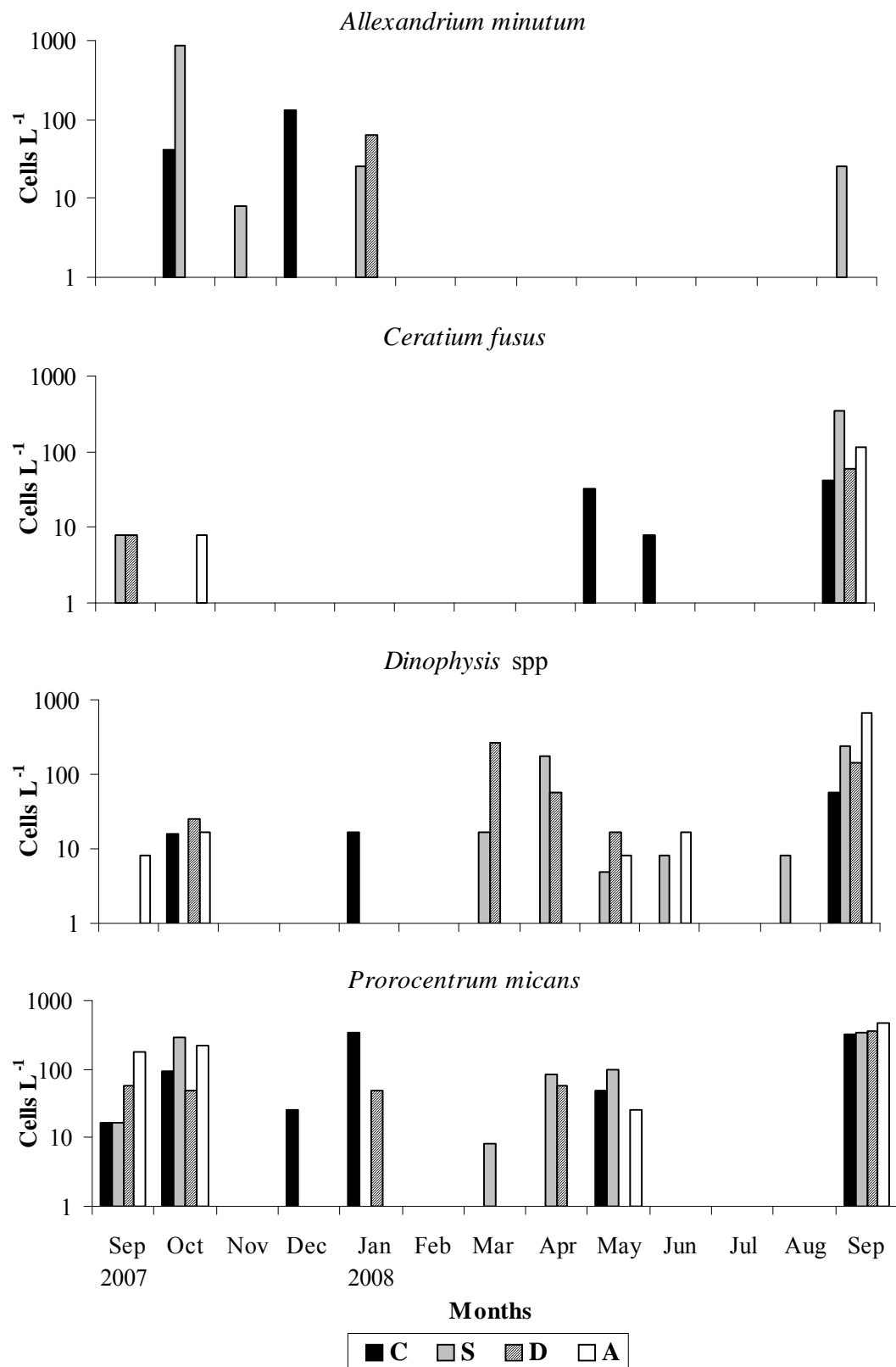
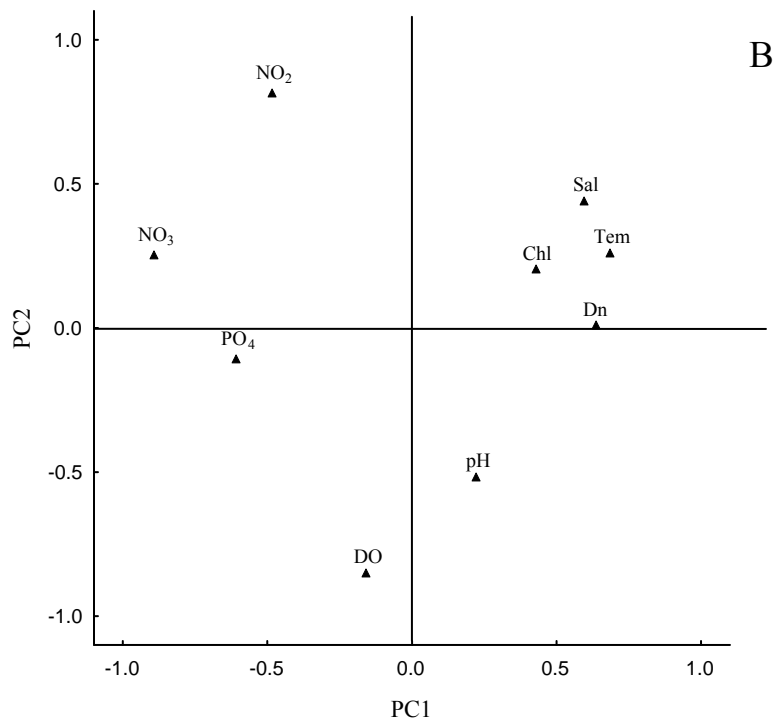
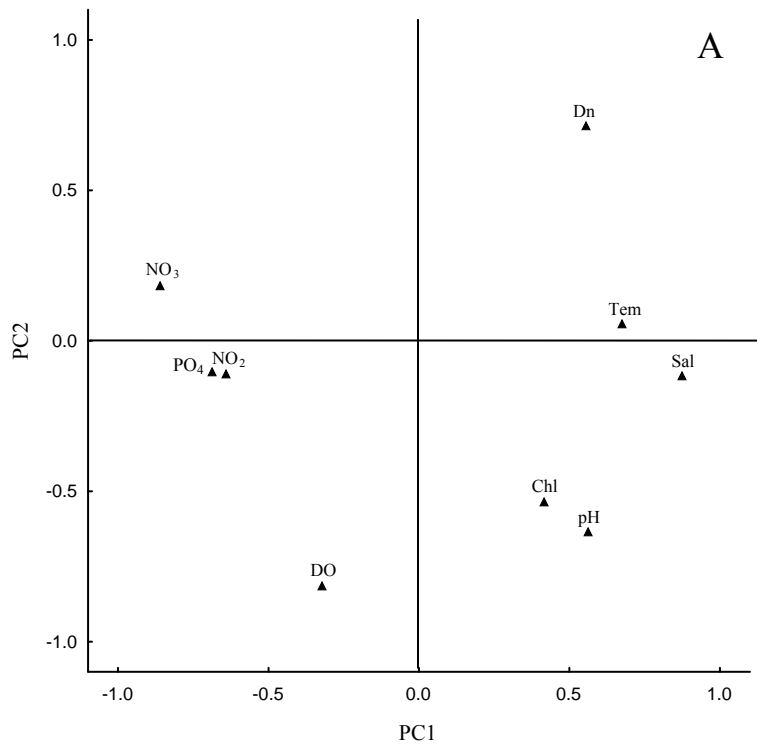


Fig.4. Distribution patterns of a few toxic dinoflagellate species off Goa, west coast of India (C= Chapora, S= Siridao, D= Dona Paula and A= Anjuna) from September 2007 to September 2008



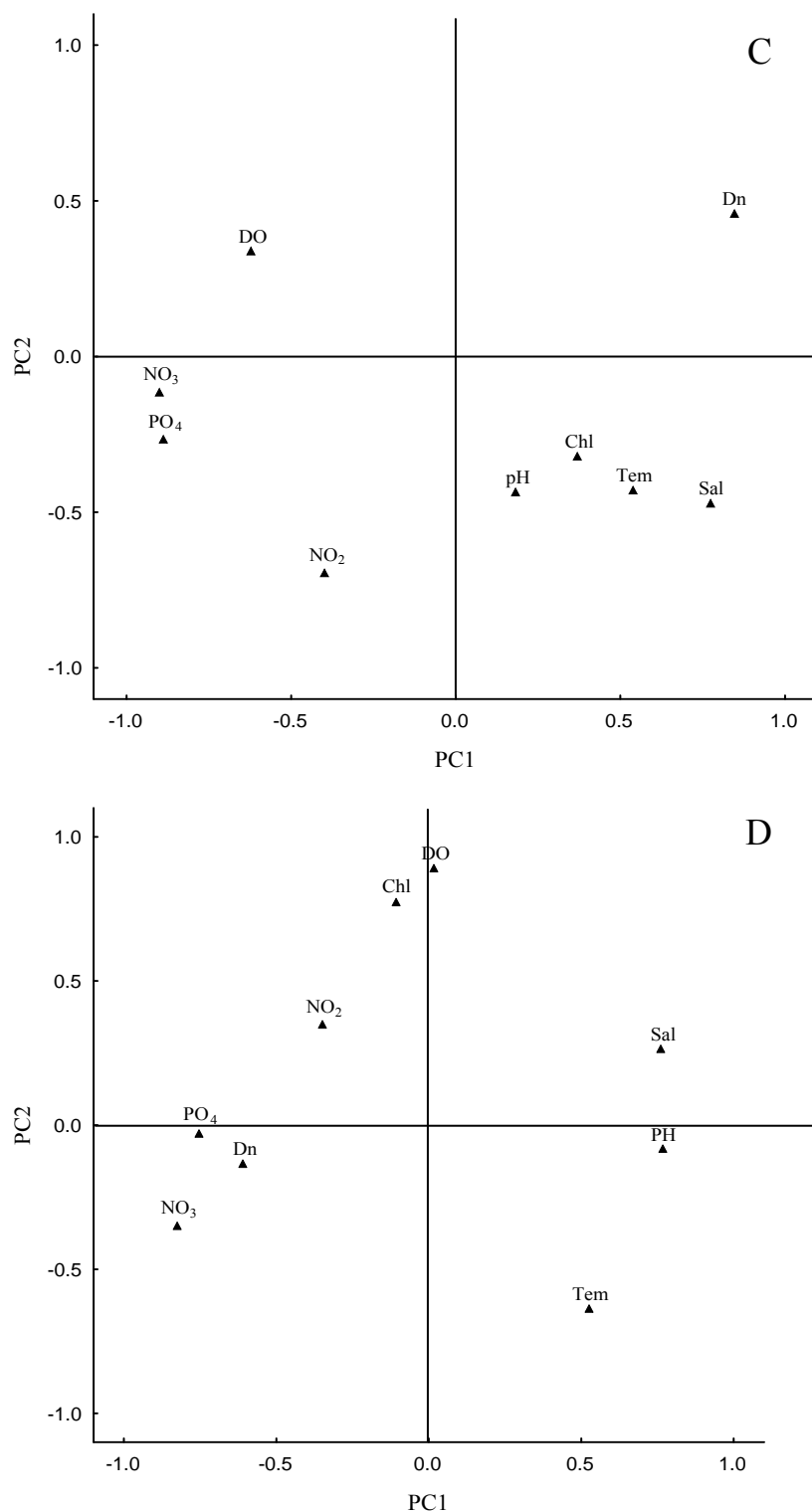


Fig. 5: PCA factor loadings of >0.1 of various parameters measured from four sampling locations (A: Chapora; B: Siridao; C: Dona Paula and, D: Anjuna) off Goa, west coast of India during September 2007- September 2008. Parameters are: Dn- total cell counts of dinoflagellates; Tem- temperature; Sal- salinity; DO- dissolved oxygen; pH; Chl- chlorophyll *a*; NO₂ - nitrite; NO₃- nitrate and, PO₄ - phosphate

Table 1. List of dinoflagellates species, ranges of their cell counts l^{-1} (mean \pm SD) recorded from the surface waters at different sampling locations off Goa, west coast of India

Taxon	Sampling location			
	Chapora	Siridao	Dona Paula	Anjuna
Autotrophic				
<i>Alexandrium minutum</i> *	0-133 (13 \pm 38)	0-875(72 \pm 242)	0-63 (5 \pm 18)	ND [@]
<i>Ceratium breve</i>	0-17(1 \pm 5)	ND	ND	0-8 (1 \pm 2)
<i>Ceratium furca</i>	0-367(54 \pm 103)	0-5763 (492 \pm 1587)	0-1208 (113 \pm 331)	0-175 (26 \pm 57)
<i>Ceratium fusus</i> *	0-42(6 \pm 14)	0-350 (28 \pm 97)	0-58 (5 \pm 16)	0-117 (10 \pm 32)
<i>Ceratium lineatum</i>	ND	0-1713 (136 \pm 474)	0-42 (3 \pm 12)	ND
<i>Ceratium tripos</i>	0-8 (1 \pm 2)	0-50 (4 \pm 14)	0-17(1 \pm 5)	0-25 (2 \pm 7)
<i>Ceratium vultur</i>	0-8 (1 \pm 2)	ND	ND	ND
<i>Dinophysis acuminata</i> *	0-25 (4 \pm 8)	0-238 (19 \pm 66)	0-125 (12 \pm 35)	0-392 (30 \pm 109)
<i>Dinophysis brevisulcus</i> *	ND	ND	0-83 (6 \pm 23)	ND
<i>Dinophysis caudata</i> *	0-33 (3 \pm 9)	ND	ND	0-258 (22 \pm 71)
<i>Dinophysis fortii</i> *	ND	ND	0-8 (1 \pm 2)	ND
<i>Dinophysis hastata</i> *	ND	ND	0-8 (1 \pm 2)	ND
<i>Dinophysis miles</i> *	ND	ND	ND	0-17 (1 \pm 2)
<i>Dinophysis</i> sp*	ND	0-175 (19 \pm 49)	0-183 (20 \pm 52)	ND
<i>Gonyaulax</i> sp	0-8(1 \pm 2)	ND	0-8 (1 \pm 2)	0-25 (3 \pm 2)
<i>Prorocentrum gracile</i>	0-150 (14 \pm 41)	0-17 (3 \pm 6)	0-17 (3 \pm 6)	0-83 (6 \pm 23)
<i>Prorocentrum micans</i> *	0-342 (7 \pm 122)	0-338 (64 \pm 115)	0-358 (44 \pm 98)	0-458 (68 \pm 139)
<i>Pyrophacus horologicum</i>	0-58 (6 \pm 16)	0-142 (17 \pm 39)	0-50 (6 \pm 15)	0- 50 (4 \pm 14)
Heterotrophic				
<i>Protooperidinium conicum</i>	0-17(3 \pm 7)	ND	ND	0-50 (5 \pm 14)
<i>Protooperidinium depressum</i>	0-25(5 \pm 8)	0-38 (3 \pm 6)	0-50 (5 \pm 14)	0-8 (1 \pm 3)
<i>Protooperidinium oceanicum</i>	0-25 (2 \pm 7)	0-63 (5 \pm 17)	0-25 (2.5 \pm 7)	0-25 (2 \pm 7)
<i>Protooperidinium steinii</i>	0-67 (15 \pm 24)	0-117 (13 \pm 32)	0-33 (6 \pm 11)	0-183 (17 \pm 51)
<i>Protooperidinium subinermis</i>	ND	ND	0-8 (1 \pm 2)	ND
<i>Protooperidinium tenuissimum</i>	ND	0-17 (1 \pm 5)	ND	ND
<i>Protooperidinium</i> sp	ND	0-25 (3 \pm 7)	ND	ND

[@] not detected; *known toxic species

Table 2. Spearman's rank correlation coefficients between different environmental variables and total dinoflagellate cell counts from different sampling locations off Goa, west coast of India. Statistically significant values are asterisked.

Parameters	Chapora		Siridao		Dona Paula		Anjuna	
	r	p	r	p	r	p	r	p
Temperature	0.227	0.455	0.064	0.835	0.101	0.741	0.308	0.304
Salinity	0.096	0.754	-0.062	0.839	-0.008	0.978	0.374	0.207
pH	0.283	0.348	-0.544	0.05*	-0.146	0.632	-0.132	0.667
Dissolved oxygen	-0.564	0.044*	-0.153	0.617	-0.534	0.059	-0.093	0.760
Nitrate	-0.566	0.043*	0.489	0.089	-0.796	0.001*	-0.495	0.085
Nitrite	-0.305	0.310	-0.251	0.406	-0.558	0.047*	-0.159	0.602
Phosphate	-0.434	0.137	0.543	0.05*	-0.932	0.000*	-0.156	0.609

Table 3 Variability of different parameters fitting into different coordinates. Variability was derived using principal component analysis

Variable	PC1	PC2	PC3		PC1	PC2	PC3
Chapora				Siridao			
Dn	0.286	0.514	0.079		0.373	0.007	-0.438
Tem	0.348	0.041	-0.465		0.405	0.184	0.351
Sal	0.451	-0.084	0.033		0.351	0.309	0.037
DO	-0.167	-0.584	-0.198		-0.094	-0.595	-0.239
pH	0.291	-0.457	0.370		0.131	-0.362	0.425
Chl	0.214	-0.384	0.598		0.252	0.143	-0.567
NO ₂	0.331	-0.079	0.158		-0.285	0.572	0.016
NO ₃	-0.445	0.123	-0.421		-0.525	0.179	0.041
PO ₄	-0.357	0.079	0.195		-0.358	-0.074	-0.350
Dona Paula				Anjuna			
Dn	0.427	0.362	-0.127		-0.340	0.091	-0.577
Tem	0.272	-0.341	0.044		0.294	-0.436	-0.300
Sal	0.390	-0.374	0.087		0.427	0.180	-0.243
DO	-0.315	0.267	0.525		0.008	0.609	-0.044
pH	0.093	-0.345	0.461		0.427	-0.058	0.240
Chl	0.188	-0.254	0.468		-0.061	0.530	-0.397
NO ₂	-0.200	-0.551	-0.422		-0.195	0.237	0.543
NO ₃	-0.455	0.092	-0.196		-0.462	-0.240	0.075
PO ₄	-0.449	-0.212	0.231		-0.422	-0.018	-0.023

Dn- total cell counts of dinoflagellates; Tem- temperature; Sal- salinity; DO- dissolved oxygen; pH; Chl- chlorophyll *a*; NO₂ - nitrite; NO₃ - nitrate and PO₄ - phosphate

Table 4 Dinoflagellate cell counts (cells L⁻¹), diversity indices (H'), species richness (SR) and evenness (J') at different sampling locations off Goa, west coast of India during September 2007-September 2008

Sampling month	cell counts	no. of species	H'	SR	J'	cell counts	no. of species	H'	SR	J'
Chapora						Siridao				
Sep 2007	17	1	0	0	0	25	2	0.9	0.71	0.9
Oct	266	6	1.93	2.06	0.75	1400	4	1.45	0.95	0.7
Nov	83	2	0.88	0.52	0.88	32	4	2	1.99	1.99
Dec	232	5	1.77	1.69	0.76	16	2	1	0.83	0.83
Jan 2008	934	9	1.97	2.69	0.62	42	2	0.97	0.62	0.97
Feb	ND	ND	ND	ND	ND	38	2	0.92	0.63	0.93
Mar	58	2	0.58	0.57	0.58	184	6	1.76	2.2	0.68
Apr	92	3	1.09	1.02	0.69	342	7	2.04	2.37	0.73
May	149	6	2.32	2.3	1.4	550	6	1.6	1.8	0.63
Jun	25	2	0.9	0.71	0.9	25	2	0.9	0.71	0.9
Jul	42	2	0.97	0.62	0.97	150	2	0.3	0.46	0.3
Aug	ND	ND	ND	ND	ND	8	1	0	0	0
Sep	634	8	2.2	2.5	0.73	8604	11	1.5	2.54	0.44
Dona Paula						Anjuna				
Sep 2007	74	3	0.97	1.07	0.61	208	3	0.76	0.86	1.5
Oct	316	8	2.56	2.8	0.85	616	11	2.7	3.58	0.78
Nov	25	2	0.9	0.71	0.9	8	1	0	0	0
Dec	25	2	0.9	0.71	0.9	8	1	0	0	0
Jan 2008	189	5	2.07	1.76	0.89	ND	ND	ND	ND	ND
Feb	13	1	0	0	0	ND	ND	ND	ND	ND
Mar	274	3	1.06	0.82	0.67	ND	ND	ND	ND	ND
Apr	166	4	1.86	1.35	0.93	ND	ND	ND	ND	ND
May	17	1	0	0	0	33	2	0.8	0.66	0.8
Jun	25	2	0.9	0.71	0.9	17	1	0	0	0
Jul	ND	ND	ND	ND	ND	16	2	1	0.83	1
Aug	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sep	1924	12	1.89	3.35	0.51	1666	9	2.66	2.48	0.84

ND- not detected

Table 5 Occurrence of harmful dinoflagellate species in the coastal waters off Goa, most probable period of their high abundance and their possible harmful effects (taken from literature)

Toxic dinoflagellate	Seasonality	Sampling location	Toxins reported	Impact
<i>Alexandrium minutum</i>	Oct, Dec, Jan, Sep	CPA, SDO, DN-PL	GTXs, NSTX	PSP
<i>Ceratium fusus</i>	Sep, Oct, May, Sep	CPA, SDO, DN-PL, AJN	??	Toxic to marine invertebrate larvae
<i>Dinophysis acuminata</i>	Oct, Jan, Aug- Sep	CPA,SDO, DN-PL, AJN	OA, DTX-1, PTX-2	DSP
<i>Dinophysis brevisulcus</i>	Mar	DN-PL	??	DSP
<i>Dinophysis caudata</i>	Sep-Oct, May, Sep	CPA, AJN	OA, PTX-2	DSP
<i>Dinophysis fortii</i>	Sep	DN-PL	OA, DTX-1, PTX-2	DSP
<i>Dinophysis hastata</i>	Sep	DN-PL	??	DSP
<i>Dinophysis miles</i>	Jun	AJN	OA, DTX-1	DSP
<i>Dinophysis sp</i>	Mar- Jun	SDO, DN-PL	??	??
<i>Prorocentrum micans</i>	Sep-Oct, Dec-Jan, May-Mar, Sep	CPA, SDO, DN-PL, AJN	??	Toxic to shellfish

Abbreviations are: AJN: Anjuna; CPA: Chapora; DO-PL: Dona Paula; SDO: Siridao. DTX-1: dinophysistoxin-1; GTX: gonyautoxin; NSTX: neosaxitoxin; PSP: paralytic shellfish poisoning; DSP: diarrhetic shellfish poisoning; OA: okadaic acid and PTX-2: pectenotoxin-2

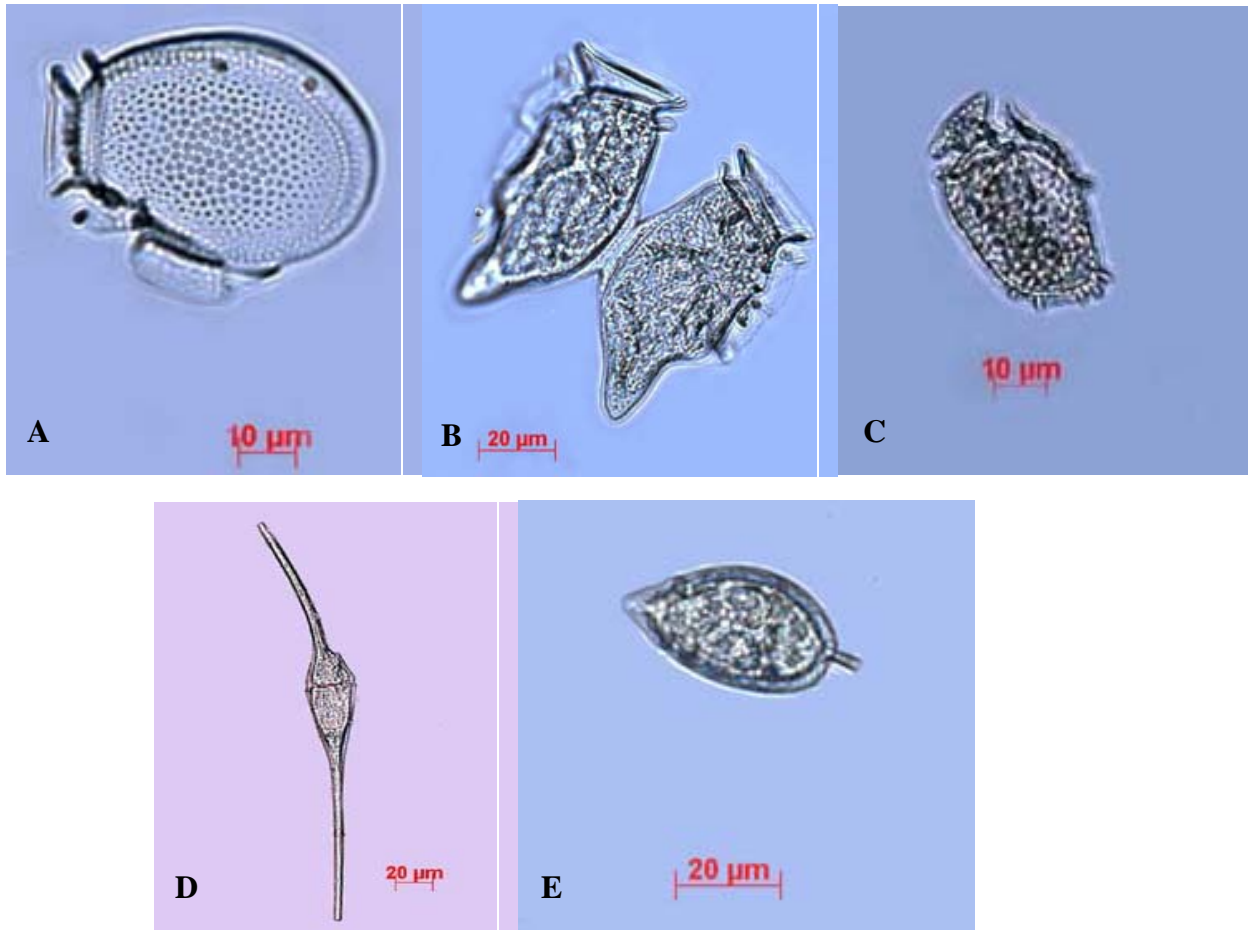


Plate I. Photomicrographs of some harmful dinoflagellates: (A) *Dinophysis acuminata*; (B), *D. caudata*; (C), *Dinophysis* sp(unidentifiable); (D), *Ceratium fusus* and (E), *Prorocentrum micans* from the coastal waters off Goa, West Coast of India.