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Distribution of diatom *Pseudo-nitzschia* and dinoflagellates of *Dinophysis* spp along coast off Goa

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Abstract

As a part of an annual analysis on the phytoplankton distribution and composition, regular monthly sampling carried out during October 2007- September 2008 from salinity gradient zones in the intertidal waters along the coast of Goa. Among the 179 species of phytoplankton observed during this study, as many as 11 of them are recognized, potentially toxic ones. The toxic diatom species, *Pseudo-nitzschia pungens* was quite preponderant, in particular during the pre-monsoon month of May 2008 off Chapora, a perennially low salinity location. Among the 10 toxic dinoflagellate species detected, the known toxic species, Alexandrium minutum followed by *Dinophysis acuminata* were found to attain maximum cell numbers in the study area. It is apparent from our results that the toxic species do occur in all salinity zones sampled and during many months of the year in coastal waters off Goa. Though directly governed by the variations in nutrient concentrations, some of these toxic phytoplankton species attain high cell numbers. It is reasonable for us to therefore caution that the toxic species do prevail in these waters.

Key words

Toxic phytoplankton, Diatom, Dinoflagellates, West coast of India

Introduction

Domoic acid (DA), produced by species of the diatom genus *Pseudo-nitzschia*, was first recognized as a biotoxin to humans when over 100 people became severely ill after consuming mussels harvested near Prince Edward Island, Canada, in 1987 (Wright *et al*., 1989). It causes amnesic shellfish poisoning (ASP) in humans as a consequence of DA poisoning (DAP) following the consumption of contaminated shellfish. It also causes DAP in a variety of marine animals after consumption of contaminated fish (Bates *et al*., 1998). To date, observations on *Pseudo-nitzschia pungens* have received little attention in the Indian waters, despite of it being one of the most commonly reported, potentially toxic representatives of the genus worldwide (Hasle, 2002). Clones of *P. pungens* isolated from various geographic areas exhibited different abilities to produce DA. Its toxic clones have been reported from New Zealand (Rhodes *et al*., 1996), Washington State and Monterey Bay, California (Trainer *et al*., 1998).

Although there is no report on ASP, many studies have reported the occurrence of harmful algal blooms in the Indian waters (Naqvi *et al*., 1998; Sahayak *et al*., 2005; Alkawri and Ramaiah, 2010). Since 1981, cases of paralytic shellfish poisoning (PSP) from coastal Tamil Nadu, Karnataka and Maharashtra are reported with adverse effects (Devassy and Bhat, 1991). In 1981, a PSP outbreak resulted in the hospitalization of 85 people and death of three persons due to the consumption of bloom-affected mussel, *Meretrix casta* in Tamil Nadu. A similar incidence reported from Mangalore in 1983, but in both the causative species were not identified (Bhat and Prabhu Matondkar, 2004). Godhe *et al.* (1996) reported *Gymnodinium catenatum*, a potent PSP species in both planktonic cells and cysts in the sediment, in the coastal waters of Karnataka (off Mangalore). They also noted that the low number of cells would not bring about toxic effects. In September 1997, an outbreak of PSP was reported in three villages of Kerala, resulting in the death of seven persons and hospitalization of over 500, following consumption of mussel, *Pema indica* (Karunasagar *et al*., 1997).

Reports on the occurrence of toxic phytoplankton from this region are rather scanty (Devassy and Goes, 1988; D'Costa *et al*., 2008). In order to highlight the prevalence of quite a number of toxic species, we report here the occurrence, abundance and annual variability of diatoms and dinoflagellates known form other regions of the world oceans as potentially toxic to human health.

Materials and Methods

Various locations sampled for this study are shown in Fig. 1. The sampling location off Anjuna (15°35'5.3"N, 73°44'12.9"E) is truly marine: stable salinity zone of 30-36 psu with no influence of river discharges within its 7-8 Km radius. Location in Chapora estuary (15°36'30.8"N, 73°44'18.6"E;16-35 psu) and in Dona Paula Bay (15°27'4.9" N, 73°48'11.8"E; 0.5-35 psu) and off Siridao (15° 25'53.1"N, 73°51'43.8"E;9-33 psu) were chosen to represent true estuarine, low salinity zones. Surface water samples were collected monthly from all these four locations during the low tide from October 2007 September 2008. They were analyzed for cell counts, identification of phytoplankton (Utermohl, 1958). As many as three replicates of 1 ml 40X concentrates of Lugol's iodine fixed phytoplankton samples were examined microscopically (400X, Nikon E400, Nikon, Japan) for their enumeration and taxonomic identification.

Various chemical parameters (salinity and dissolved oxygen, nitrate-N, nitrite-N, phosphate-P and silicate-S) were measured following standard methods (Parsons, 1984).

Results and Discussion

At each sampling location, the hydrographic and chemical parameters indicated both spatial and temporal differences (Table 1). In brief, nitrate was usually low off Chapora reaching the lowest value of 0.004 μmol during January 2008. Its highest concentrations were off Siridao followed by Dona Paula. Nitrite was invariably below 1μmol but for occasional spikes above this value in particular off Siridao. Phosphate increased from its low concentrations during pre-monsoon to its highs during the monsoon months. The concentrations of silicates were usually more than 1 μmol and generally higher during monsoon at all locations.

From this comprehensive study, it is evident that the toxic phytoplankton species are rather common in the Indian waters. That as many as 11 species known to be potentially toxic were found in the near-shore waters is an indication that any or all of them can be of serious human-health concern when the environmental factors become favorable for their proliferation (Naqvi *et al*., 1998; Ramaiah *et al.,* 2007). The most dominant species among these was the diatom, *Pseudo-nitzschia pungens* (Fig. 2). It was in high abundance off Chapora almost throughout the year. Its bloom proportions, 1.6×10^4 cells l^{-1}, occurred at this location during May 2008. At other locations, it was either in non-detectable levels or, in quite low numbers though its distinct secondary peak cell

Table - 1: Variations in different parameters along with salinity in the surface water samples collected monthly during October 2007 to September 2008 from different sampling locations off Goa

Sampling location	Oct 2007	Nov	Dec	Jan 2008	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Chapora												
Salinity (psu)	6.335	18.57	24.71	30.095	34.10	34.41	30.03	35.45	35.69	11.63	0.44	17.35
Dissolved oxygen(μmol)	180.42	175.5	177.9	119.2	284.5	190.7	221	249	223.6	299.7	289.7	233.8
Nitrate (μmol)	4.18	0.06	0.035	0.004	0.059	0.64	0.094	0.02	1.62	3.15	4.1	0.03
Phosphate (μmol)	1.02	0.38	0.43	0.26	0.51	0.28	0.066	0.24	0.6	0.4	0.8	0.13
Silicate (μmol)	58.92	18.79	14.57	9.8	2.35	4.4	6.33	3.58	5.77	31.16	76.02	52.59
Anjuna												
Salinity (psu)	30.29	34.04	34.876	34.87	35.67	34.95	35.59	35.86	35.92	30.17	25.49	35.02
Dissolved oxygen(μmol)	162.6	137.1	180.4	205.9	315.1	205.9	304.9	208.4	249	226.1	271.9	317.6
Nitrate (μmol)	3.94	2.2	1.14	0.06	0.3	0.98	0.31	0.867	4.29	2.02	8.24	1.32
Phosphate (μmol)	0.62	0.5	0.59	0.474	0.14	0.17	0.26	0.53	0.65	1.13	1.34	0.77
Silicate (μmol)	12.43	3.19	3.86	2.33	0.1	1.47	0.12	1.47	3.34	18.59	58.72	4.01
Dona Paula												
Salinity (psu)	29.47	31.5	34.33	34.17	34.62	34.39	34.62	35.306	34.697	19.677	15.857	32.62
Dissolved oxygen(μmol)	134.8	147.4	127	132.2	218.4	121.9	203.3	157.5	203.3	213.5	259	213.4
Nitrate (μmol)	0.02	5.03	6.33	1.13	3.53	1.03	1.08	1.87	1.98	7.34	11.05	0.46
Phosphate (μmol)	0.31	0.63	0.56	0.526	1.02	0.23	0.4	0.59	0.6	1.25	1.34	0.39
Silicate (μmol)	11.89	15.29	6.29	5.35	5.99	1.37	1.07	2.52	5.47	38.29	122.67	3.48
Siridao												
Salinity (psu)	13.08	29.63	31.22	31.85	33.36	33.87	32.93	33.85	33.53	9.97	9.89	31.88
Dissolved oxygen(μmol)	157.6	127.3	91.5	134.9	254.1	101.6	190.7	188	205.9	200.7	254.1	210.9
Nitrate (μmol)	8.59	14.41	5.26	10.51	3.456	0.76	1.34	0.3	0.85	7.976	12.6	1.37
Phosphate (μmol)	0.85	0.81	0.696	0.74	1.2	0.56	0.33	0.59	0.59	1.07	1.29	0.77
Silicate (μmol)	39.46	15.92	13.86	12.15	8.45	2.25	1.55	1.79	7.9	38.89	128.22	6.23

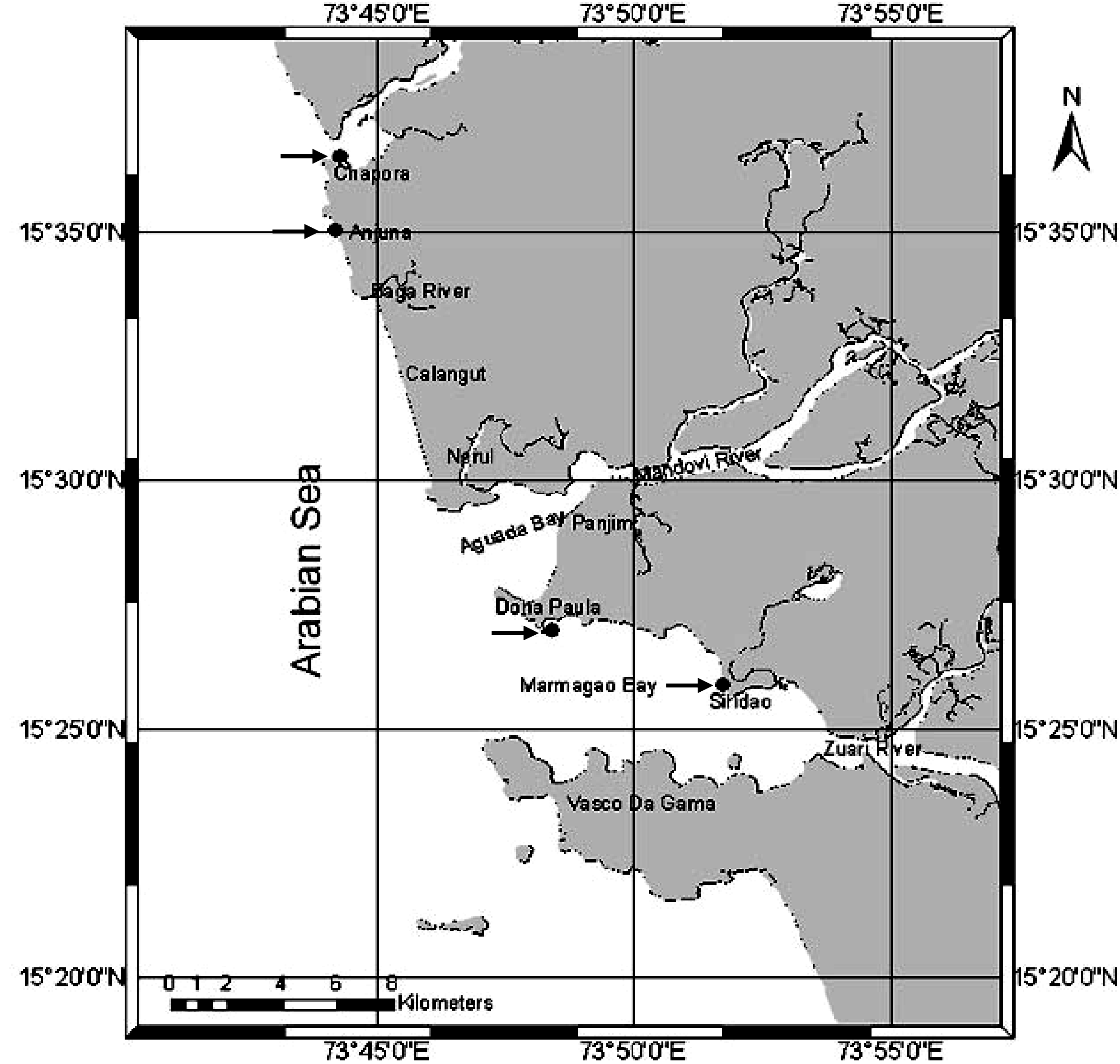

Fig. 1: Map showing the sampling locations along the coast of Goa, West Coast of India

counts of $9.00 \times 10^3 l^{-1}$ were observed during September 2008 off Siridao. Interestingly, its higher abundance and even the bloom densities, in particular off Chapora, coincided with low nitrate, phosphate and silicate concentrations in the ambient waters. Thus, low inorganic nutrients might be favorable for its growth and proliferation. This is in contrast to the abundances of many species of diatoms during the same observational period. Apparently, *P. pungens* is a Table 1. Variations in different parameters along with salinity in the surface water samples collected monthly during October 2007 to September 2008 from different sampling locations off Goa cosmopolitan species (Hasle, 2002), widely distributed along Atlantic coasts and Pacific rims. Further, several strains isolated from New Zealand, Washington and Monterey Bay were observed to produce the DA (Rhodes *et al.*, 1996; Bates *et al.*, 1998; Trainer *et al.*, 1998), which causes amnesic shellfish poisoning (ASP). We have not attempted to examine whether this species is capable of DA production. However, of the 13 known toxic diatom species, as many as nine *Pseudo-nitzschia* spp are reported to produce DA (Moestrup *et al.*, 2004). This is to be taken as a forewarning of a very high probability of *Pseudo-nitzschia* spp (and their strains), with capability to produce DA, occurring in our waters. This is important in particular when nutrient concentrations are low but their abundances are near, and/or at, bloom proportions and, when their cell numbers correlate negatively with ambient nutrient concentrations (for instance, for data from off Chapora, $r=-0.54$, $p=0.07$ between counts of *Pseudo-nitzschia pungens* (*P-n p*) and nitrate; $r=-0.58$, $p= 0.047$ between *P-n p* and phosphate; $r= -0.32$, $p=0.31$ between *P-n p* and silicate). Earlier studies also reported significant negative correlations between abundances of *Pseudo-nitzschia* and the ambient concentrations of silicate, nitrate and nitrite

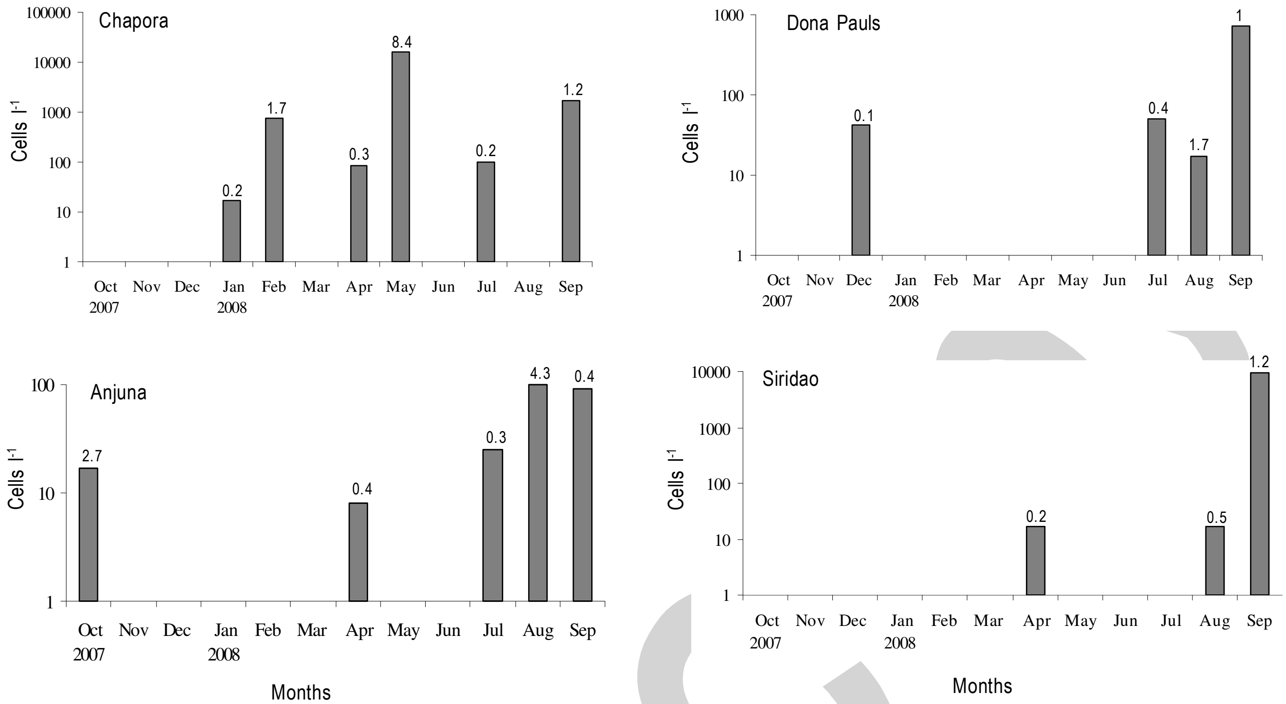

Fig. 2: Abundance (in log no. of cells) and pattern of monthly variations of *Pseudo-nitzschia pungens* off Goa during October 2007 to September 2008. Figures on each histogram denote its percent contribution to total diatom counts

(Anderson *et al.*, 2006; Schnetzer *et al.*, 2007) or phosphate (Schnetzer *et al.*, 2007).

Among the known toxic dinoflagellates, the abundances of *Alexandrium minutum, Dinophysis acuminata, D. caudata* and the red tide-forming *Prorocentrum micans* were remarkable during this study period. Further, cells of *D. hastata, D. brevisulcus, D. fortii* and *D. miles* were rare though detected at all sampling locations in one or the other sampling months (Fig. 3). With its highest numbers in October 2007 (875 cells l^{-1}), *Alexandrium minutum* was generally preponderant off Siridao. In general, an increase in nutrient concentrations was observed before and during this species attained its peak abundance. It is known to be potentially toxic and, observed for the first time in the coast of Goa. It is known to produce toxins which cause paralytic shellfish poisoning (PSP) and 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 clearly elucidated as yet.

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}). An increase in nitrate and phosphate concentration was observed before the peak of these species. The diarrhetic shellfish poisoning (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 okadaic acid and dinophysis-toxins contaminated mussels are reported (Koukaras and Nikolaidis, 2004). All seven species of *Dinophysis* recorded during this study are reported to be potential causative agents of DSP. The most abundant species in the waters off Goa are *D. acuminata* (838 cells l^{-1}) followed by an unidentified *Dinophysis* sp (508 cells l^{-1}; its species name yet to be confirmed). 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, which was found at all the locations we sampled, can cause harm to invertebrate larvae by an unknown mechanism (Taylor, 2004). Its adverse effects on oyster larvae and shrimps are reported (Cardwell *et al*., 1979; Rensel and Prentice, 1980). On the other hand, *Prorocentrum micans* were recorded at all the locations and almost throughout the year over wide ranges of salinity, temperature 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). Although *P. micans* is capable of forming extensive blooms, it is considered harmless (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).

For a harmful bloom to develop at a given site, three conditions must coincide along with the harmful species at that site. Also, such species must reach a threshold concentration, which

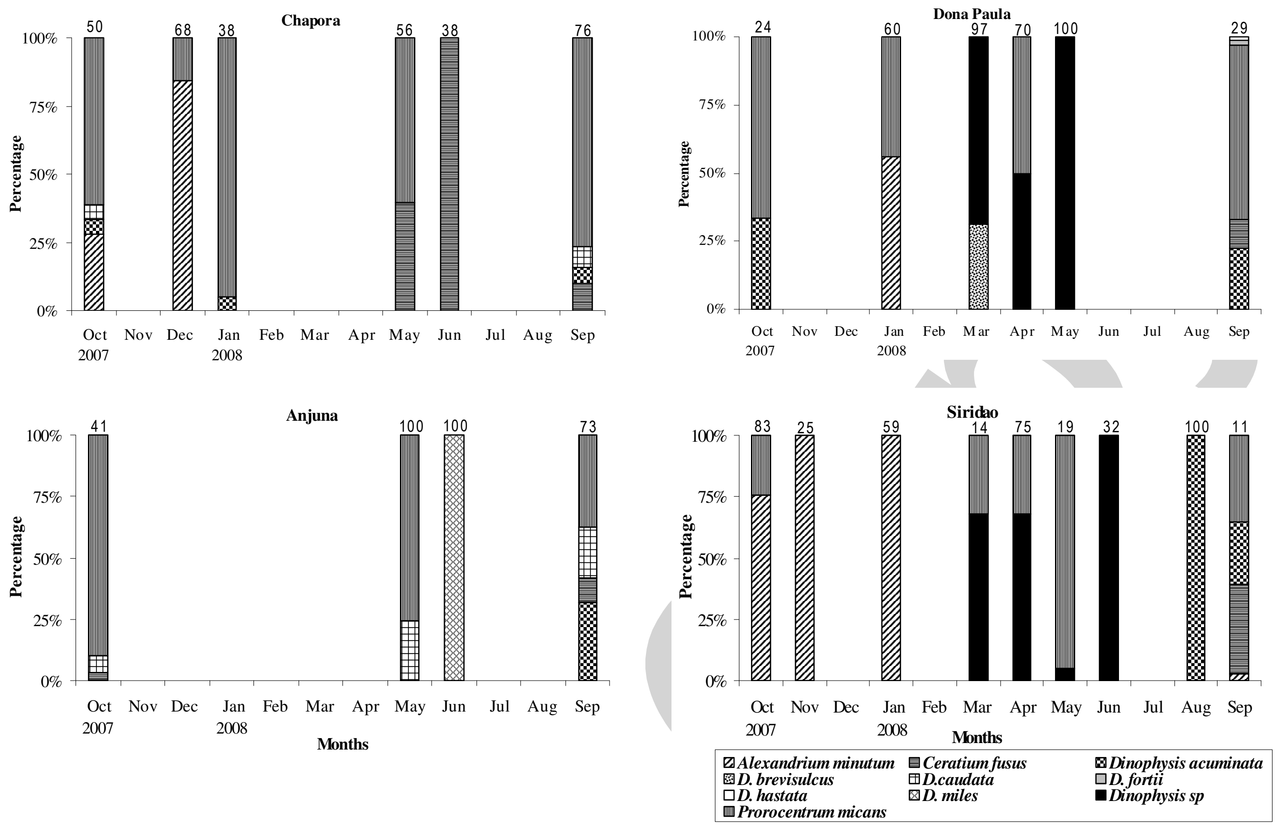

Fig. 3: Abundance and patterns of monthly variations of toxic dinoflagellates species off Goa during October 2007 to September 2008. Figures on each histogram indicate the percent of toxic dinoflagellate species in the total (autotrophic, heterotrophic) dinoflagellate counts

varies from species to species and within the same species in relation to its toxicity and, the bloom must hit the target organism(s) either directly or through vectors (Zingone and Wyatt, 2005). From our analyses, the first condition - the presence of toxic species - is fulfilled at several places and times of the year in coastal waters off Goa. Therefore, it is reasonable for us to caution that the toxic species do prevail in these waters and their contact with target organisms fortunately, so far, may be missing or, hitherto unreported.

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