

Identification of non-indigenous phytoplankton species dominated bloom off Goa using inverted microscopy and pigment (HPLC) analysis

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An unusual phytoplankton bloom dominated by unidentified green coloured spherical algal cells ($\sim 5 \mu\text{m}$ diameter) and dinoflagellates (*Heterocapsa*, *Scripsiella* and *Gymnodinium*) was encountered along the coast of Goa, India during 27 and 29 January, 2005. Pigment analysis was carried out using both fluorometric and HPLC methods. Seawater samples collected from various depths within the intense bloom area showed high concentrations of Chl *a* (up to 106 mg m^{-3}) associated with low bacterial production (0.31 to $0.52 \text{ mg C m}^{-3} \text{ h}^{-1}$) and mesozooplankton biomass (0.03 ml m^{-3}). Pigment analyses of the seawater samples were done using HPLC detected marker pigments corresponding to prasinophytes, dinoflagellates and diatoms. Chlorophyll *b* (36–56%) followed by peridinin (15–30%), prasinoxanthin (11–17%) and fucoxanthin (7–15%) were the major diagnostic pigments while pigments of cryptophytes and cyanobacteria including alloxanthin and zeaxanthin formed $<10\%$. Although microscopic analysis indicated a decline in the bloom, pheophytin concentrations in the water column measured by both techniques were very low, presumably due to fast recycling and/or settling rate. The unique composition of the bloom and its probable causes are discussed in this paper.

1. Introduction

Coastal and near-shore waters are more productive regions in the marine environment nourished by nutrients derived by regeneration, upwelling and land run-off. Phytoplankton blooms in these waters follow a seasonal pattern that shapes the coastal marine ecosystem. Environmental forcing, nutrient availability, predator communities and land-driven inputs are the major factors that control coastal and near-shore phytoplankton community and blooms. Several anthro-

pogenic factors in these environments can result in alteration of coastal water quality (eutrophication), introduction of non-native species (ballast action), alteration of predator community (over-fishing), etc. As a result, non-periodic and exceptional blooms of both noxious and/or toxic phytoplankton species lasting for few weeks to months are frequently reported, influencing the seasonal patterns of dominant phytoplankton community structure, thereby affecting coastal and estuarine biogeochemical processes (Cloern 1996).

Keywords. Dinoflagellates; prasinoxanthin; HPLC; pigment composition; phytoplankton bloom.

The primary production and phytoplankton community in the waters along the coast of India are largely influenced by monsoonal forcings, resulting in changes in the coastal currents, surface temperatures, nutrient availability, etc. For example, Banse *et al* (1996) hypothesized increased nutrient supply as principal factor controlling primary production in the western coastal waters of India. Along with nutrient injection in the coastal waters, seasonal changes in the surface coastal current directions (Shetye *et al* 1994) coupled with changes in salinity and temperature regulate the phytoplankton diversity along west coast of India (Qasim 1977). Phytoplankton diversity, blooms and their life stages have been exhaustively studied in these waters following the classical microscopy methods (Subrahmanyam 1958; Dehadrai and Bhargava 1972; Devassy *et al* 1978; Sawant and Madhupratap 1996), remote sensing (Desa *et al* 2005; Gomes *et al* 2008), molecular techniques (Godhe *et al* 2001) and of late using pigment identification by HPLC technique (Roy *et al* 2006; Parab *et al* 2006; Roy 2010). All these studies show that diatoms are the pre-dominant forms of phytoplankton followed by seasonal blooms of dinoflagellates and *Trichodesmium*.

In the recent past, 'unusual blooms' of phytoplankton species unrelated to the seasonally recurring communities have been reported. Some of

these blooms have been either toxic (Sahayak *et al* 2005), caused nuisance to coastal population (Ramaiah *et al* 2005) or led to discolouration of coastal waters (Nayar *et al* 2001). Moreover, the frequency of these blooms in the near-shore waters of the west coast of India has increased which reportedly coincide with increased commercial shipping in these waters (Anil *et al* 2002). One common trait in all these blooms – harmful or otherwise was their sporadic nature; most of them were never encountered earlier and their occurrences in these waters were largely uncommon. Here we report one such occurrence of phytoplankton bloom in the near-shore waters of Goa, west coast of India dominated by cells hitherto not observed in these waters. Moreover, an attempt is made to explain possible cause of this bloom in these waters.

2. Material and methods

2.1 Study site and sampling

The mixed phytoplankton bloom was observed during one of the monthly sampling at the Candolim time series (CaTS) transect (figure 1) in the near-shore waters off Goa, west coast of India. Seawater was sampled on two days (27 and 29 January,

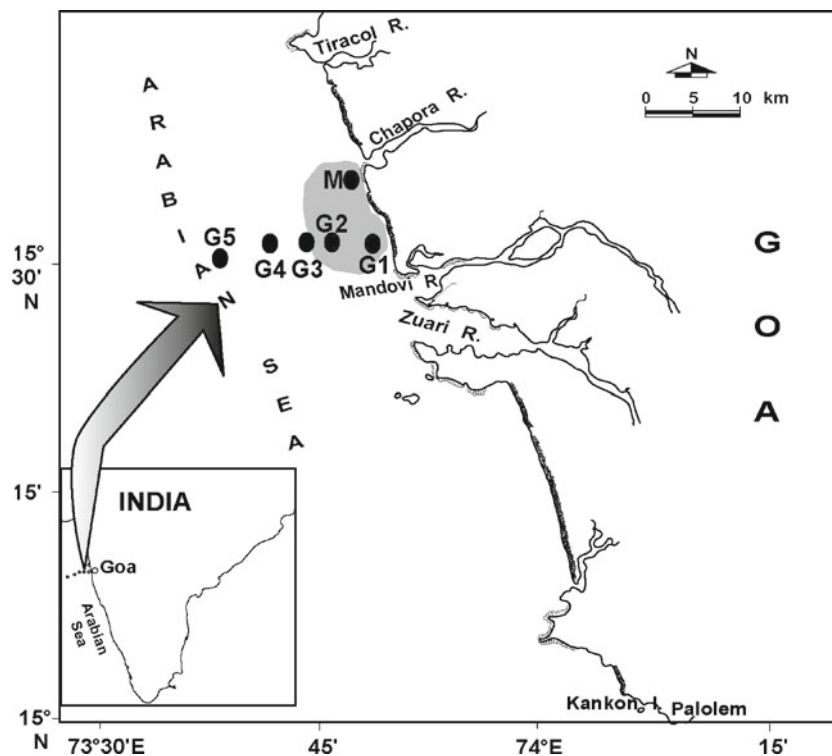


Figure 1. Map showing the CaTS (Candolim time-series) stations G1 to G5 and one station off Morjim north of CaTS. The approximate spread of the bloom is indicated by the shaded portion.

2005). On the first day of sampling, seawater from three stations (G1, G2, G3) along the CaTS transect (table 1) were sampled. On the subsequent day, sampling was restricted to G1, G2 and station off Morjim (M) (figure 1).

Seawater was collected using a 5 litre Niskin sampler and sub-samples were collected for phytoplankton enumeration and its generic composition, chlorophyll *a* (Chl *a*), pigment composition and bacterial production.

2.1.1 Chl *a* and other pigments

Aliquots of seawater were filtered on GF/F (0.7 μm) filters for chlorophyll *a* and pigment composition. The Chl *a* filters were extracted immediately after filtration whereas pigment samples were stored in liquid nitrogen prior to analyses. Both Chl *a* and pigment samples were extracted in 90% acetone under refrigeration.

2.1.2 Phytoplankton abundance

Samples for phytoplankton abundance and composition were fixed with Lugol's iodine and stored in dark prior to microscopic examination.

2.1.3 Bacterial production

Ten millilitre of unfiltered samples were spiked with 20 μl of $^3\text{H}_1$ -thymidine (1 mCi; specific activity: 12,000 mCi mmol^{-1}) in triplicates and incubated for 60 minutes in the dark in ambient conditions. The uptake was stopped by adding 0.2 μm filtered formalin (4% final concentration). Formalin prefixed samples were used as controls and were incubated similarly.

Additional sample for mesozooplankton biomass was collected using towing method (HT net, 200 μm mesh) and the samples were preserved in 4% formalin. All the samples were brought back

to the laboratory within 3 hours of sampling for further processing and were stored in ice during transport.

2.2 Analyses

Chlorophyll *a* (Chl *a*) and phaeophytin concentrations were measured fluorometrically on a Turner fluorometer (UNESCO 1994). For estimation of Chl *a*, the filter paper containing the sample was crushed and the pigments were extracted in 10 ml of 90% acetone in dark for 24 h in the refrigerator. After extraction, the fluorescence was measured before (F_o) and after acidification (F_a) with two drops of 1.2 N HCl. The actual concentrations of Chl *a* and phaeophytin pigments were then calculated following JGOFS protocol (UNESCO 1994).

The total pigment samples were analyzed following a slightly modified method described by van Heukelem (2002) as described by Roy *et al* (2006). The HPLC system was equipped with an Eclipse XDB C-8 reverse phase column (4.6 \times 150 mm) attached to a guard column, an Agilent 1100 pump, online degasser and diode array detector (Agilent Technologies Model 1100). The column was maintained at 60°C and the elution was carried out using a linear gradient program over 36 min at the rate of 1.1 ml min^{-1} . The eluant was a mixture of solvent A (methanol (70:30) + 1 M ammonium acetate (pH 7.2)) and solvent B (methanol). The solvents A and B were mixed from 95/5% to 5/95% for the first 22 min followed by an isocratic separation with 95% solvent B up to 30 min to elute α - and β -carotenes. The column was equilibrated for 6 min between two successive analyses. The pigments were detected using a UV-diode-array detector. Pigment standards (DHI Waters, Denmark and Sigma-Aldrich, USA) for Chl *a* and 15 different marker pigments were used to identify and quantify various phytoplankton groups. The marker pigments were chlorophyll c_2 (Chl c_2),

Table 1. Distribution of chlorophyll *a*, phaeophytins and total bacterial production (BP) during the bloom period in the near shore waters off Goa.

	27/01/2005							29/01/2005				
	G1		G2		G3			G1		G2		Off Morjim
Location	73.45.6°E 15.31.2°N		73.44.4°E 15.31.2°N		73.43.8°E 15.31.2°N			73.45.6°E 15.31.2°N		73.44.4°E 15.31.2°N		73.43.4°E 15.37.4°N
Parameters	1 m	4 m	1 m	9 m	1 m	7 m	14 m	1 m	4 m	1 m	1 m	6 m
Chlorophyll <i>a</i>	106.8	3.2	17.6	1.4	6.5	3.4	0.7	22.9	11.7	19.8	18.7	3.8
Phaeophytins	0.84	0.37	0	0.73	0	0.1	0.73	0	0	0.4	0.3	1.1
BP	nd	nd	nd	nd	nd	nd	nd	0.52	0.33	nd	0.31	0.34

Chlorophyll *a* and phaeophytins concentrations are in mg m^{-3} ; bacterial production (BP) is in $\text{mg-C m}^{-3} \text{h}^{-1}$. nd: not done.

chlorophyll c_3 (Chl c_3), peridinin (Per), fucoxanthin (Fuc), 19' butanoyloxyfucoxanthin (19' BF), neoxanthin (Neo), violaxanthin (Viola), prasinoxanthin (Pra), alloxanthin (Allo), 19' hexanoyloxyfucoxanthin (19' HF), diadinoxanthin (Diad), zeaxanthin (Zea), lutein (Lut), chlorophyll b (Chl b), total carotene ($\alpha + \beta$, Tcar), chlorophyll a (Chl a) and phaeophytin (Phaeo a). Diagnostic pigment (DP) concentrations were calculated as described by Roy *et al* (2006) after including Pra as prasinoxanthin also contributed significantly to the pigment concentration. The standards for carotenes (α - and β -types) were purchased individually from DHI Waters and calibrated as a mixture. Both forms of carotenes had similar elution time and could not be resolved properly. Therefore, the two carotenes were grouped together and expressed as Tcar. All the other chemicals used were procured from Merck (Germany).

The samples collected for phytoplankton composition and enumeration were examined following sedimentation technique using Utermohl's inverted microscope (Hasle 1978).

The samples for bacterial production was filtered onto 0.2 μm cellulose nitrate filters, extracted in cold trichloroacetate (TCA), the paper dissolved in scintillation cocktail (UNESCO 1994) and activity measured using a scintillation counter (Wallac model 2000). Zooplankton biomass was estimated by volume displacement method (Madhupratap and Haridas 1990).

3. Results

On day 1 of sampling, the sampled waters at G1 and G2 were dirty brown in colour compared to surface waters outside the patch (figure 2). The

patch was tracked up to Morjim and appeared to be concentrated in near shore waters. A similar distinct horizontal and vertical spatial variation was also observed in all the major parameters (table 1). The surface Chl a concentration decreased from 106.8 mg m^{-3} at G1 to 6.5 mg m^{-3} at G3. A similar decrease with depth was also observed at all stations (table 1). The depth and spatial profile of Chl a indicated that the bloom cells were concentrated close to the shores. During the subsequent sampling, the surface Chl a value at G1 dropped to 22.9 mg m^{-3} with a substantial increase in near bottom chlorophyll concentrations to 11.7 mg m^{-3} . The spatial spread of the bloom up to Morjim was evident as the surface Chl a concentrations remained nearly same. The phaeophytin concentration after acidification of Chl a samples was always less than 1 mg m^{-3} during the sampling.

HPLC analysis of the pigment samples detected a range of diagnostic pigments (DP) specific for dinoflagellates, prasinophytes, diatoms, cyanobacteria and cryptophytes (figure 3). Unlike the fluorometric method, the HPLC method measured low Chl a concentrations (4.1–6.5 mg m^{-3}) in the surface waters at G1 and Morjim. Based on the DP calculation, the bloom was dominated by Chl b (36–56%), peridinin (15–30%), prasinoxanthin (11–17%) and fucoxanthin (7–15%) (figure 3). The concentration of the prasinoxanthin was higher in surface waters compared to other pigments. Other DP including diadinoxanthin, zeaxanthin, alloxanthin, etc., contributed <5%, respectively.

Microscopic observations showed that the bloom was dominated by unidentified unicellular green coloured algal cells during both days of sampling (figure 4). On the first day of sampling, the bloom cells completely covered the field area and formed aggregates. The cells were covered with mucus

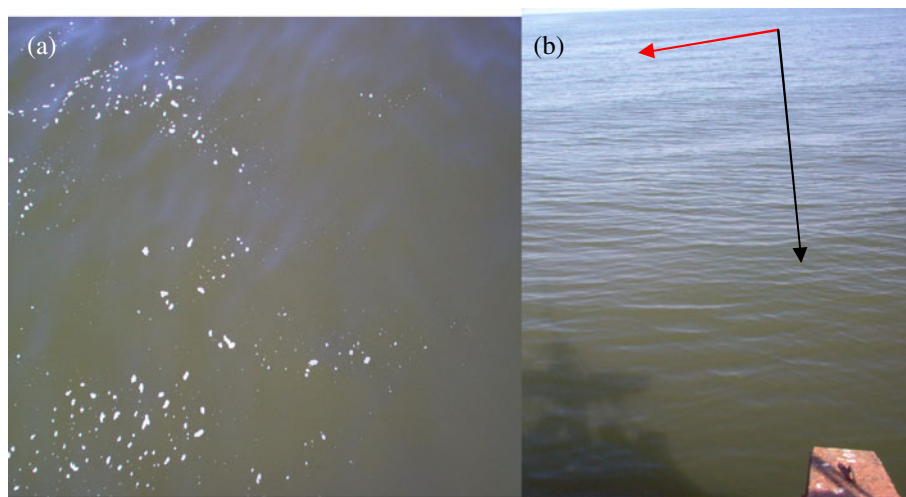


Figure 2. Photographs of (a) surface waters from within the bloom patch showing distinct muddy colour and (b) edge of the bloom patch showing different colours outside (red arrow) and inside (black arrow) the bloom patch.

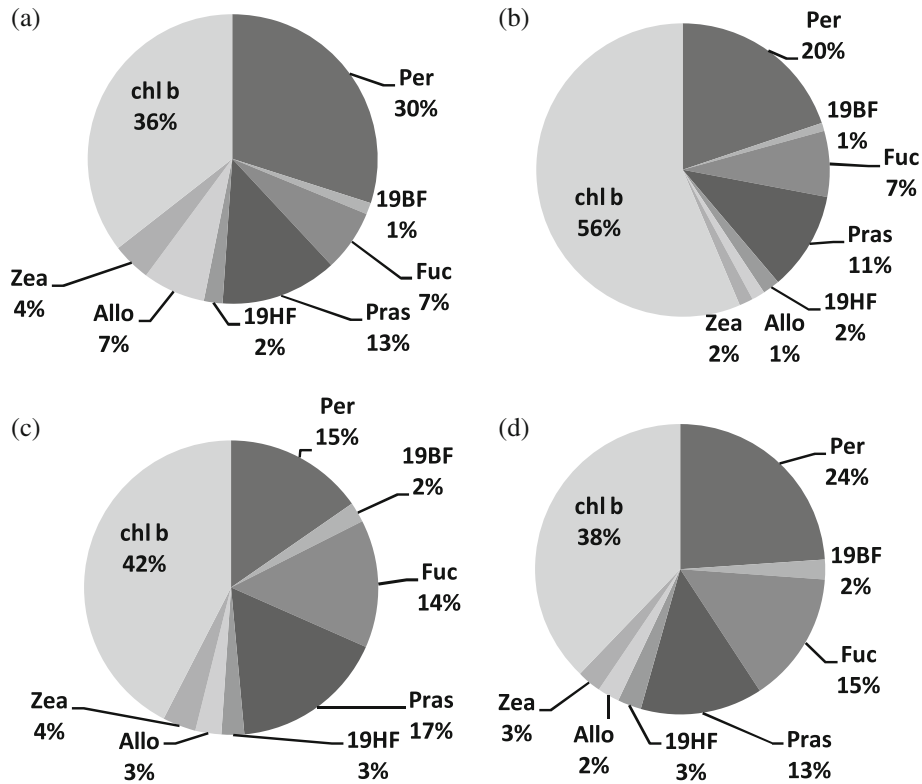


Figure 3. Percent distribution of major diagnostic pigments identified using HPLC method in samples collected from (a) G1 surface, (b) G1 4 m, (c) Morjim surface, and (d) Morjim 6 m.

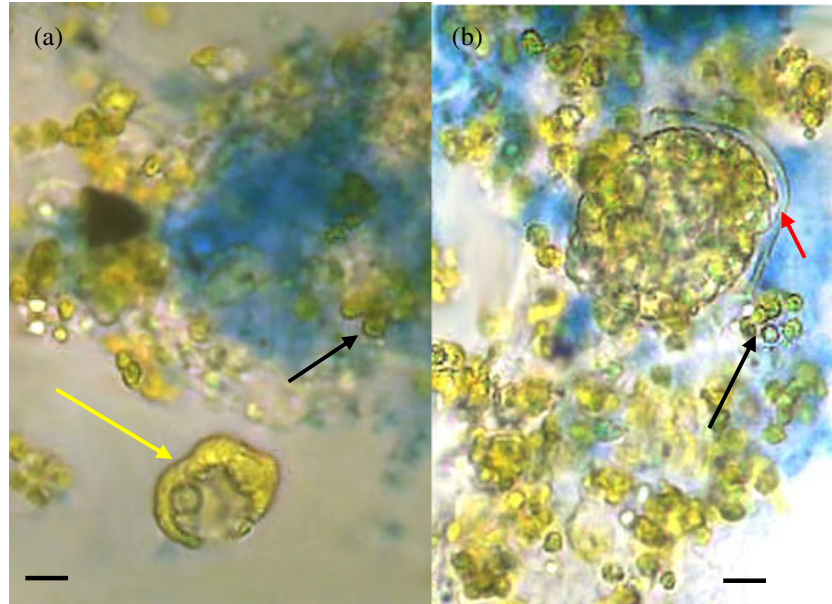


Figure 4. Microphotographs of the bloom sample showing (a) broken dinoflagellates cell (yellow arrow) and (b) an intact dinoflagellate containing green unidentified cells (red arrow). Also note the unidentified green cells and the mucus stained with alcian blue in the background (black arrows). Scale in each photograph equals 10 μ m.

which could be stained with Alcian blue. The cells could not be disaggregated without causing cell breakage and hence were difficult to count. The samples contained broken as well as intact green

coloured unidentified algal cells as well as dinoflagellates (figure 4a and b). The ruptured cell-walls of algae as observed microscopically suggested that many of these cells were in a state of decay and

Table 2. The composition of the major phytoplankton genera identified in the surface waters at G1 station.

Phytoplankton composition	27/01/2005	29/01/2005
Diatoms		
<i>Amphora</i> sp.	—	300
<i>Navicula</i> sp.	8000	200
<i>Nitzschia</i> sp.	1333	200
<i>Cocconeis</i> sp.	2000	—
<i>Coscinodiscus</i> sp.	—	800
<i>Pleurosigma</i> sp.	800	100
Dinoflagellates		
<i>Amphidinium</i> sp.	4000	—
<i>Ceratium</i> sp.	—	600
<i>Dinophysis</i> sp.	—	100
<i>Gymnodinium</i> sp.	8000	200
<i>Gyrodinium</i> sp.	12000	200
<i>Heterocapsa</i> sp.	34667	—
<i>Prorocentrum</i> sp.	38666	400
<i>Protoperidinium</i> sp.	4000	500
<i>Scripsiella</i> sp.	145333	—
Silicoflagellates		
<i>Dictyocha</i> sp.	1333	—
<i>Distephanus</i> sp.	—	200
Unidentified algal cells	NC	1150000
Total cells L ⁻¹	263866	1153800

NC: Not counted.

the bloom was in the declining phase. The surface samples collected subsequently showed that the unidentifiable cells amounted to ~99% of total phytoplankton abundance ($\sim 1.15 \times 10^6$ cells l⁻¹; table 2). Phytoplankton cells other than the bloom cells decreased from 2.6×10^5 cells l⁻¹ on the first day of sampling to 0.04×10^5 cells l⁻¹ during the subsequent sampling. Among these phytoplankton cells, dinoflagellates constituted ~93.5% (2.4×10^5 cells l⁻¹) on the first day of sampling. During the second day of sampling, the contribution of dinoflagellate decreased to ~56%. *Heterocapsa* sp., *Scripsiella* sp. and *Gymnodinium* sp. dominated the dinoflagellates community (table 2). Diatoms formed <4% of the total phytoplankton population.

Bacterial production rates were low and ranged from 0.31 to 0.52 mg C m⁻³ h⁻¹ (table 1). Copepods formed the bulk of the mesozooplankton population (>200 μ m) and the total mesozooplankton biomass was 0.03 ml m⁻³.

4. Discussion

A mixed phytoplankton bloom at the CaTS stations was a chance observation and unique for

these waters. The bloom was largely composed of green coloured spherical cells which were 4–5 μ m in diameter and covered in mucus exudates (figure 4a and b). Moreover, the bloom was mainly confined to the shallow waters (depth 12 m), forming clumps of cells. The west coast of India largely experiences weak sea-breeze (1.5 to 5 m s⁻¹) between November and April and wind direction is almost along the coastline at the CaTS. The cross shore component of the winds changes from land–sea in the mornings to sea–land in the afternoons (Neetu et al 2006). The sampling stations experience the West India Coastal Currents (WICC) that brings in low saline, warm and nutrient depleted waters from the south (Shetye 1999; Naqvi et al 2003). Such hydrographic conditions support diatoms and dinoflagellates (Devassy and Goes 1989) which were also observed in this mixed phytoplankton bloom. Moreover, earlier reports suggest that diatoms (55%) and dinoflagellates (36%) constitute the phytoplankton population in the waters around CaTS (Parab et al 2006). The existing meteorological and hydrodynamic conditions suggest that the bloom might have developed off-shore and migrated to the coast over time, resulting in extremely high concentrations of Chl *a* in the surface waters.

This is the first report of a bloom in these waters wherein prasincoxanthin contribution was up to 17% of the diagnostic pigments. The waters in and around CaTS area is predominantly diatom based ecosystem with seasonal blooms of other phytoplankton species like *Trichodesmium* and a few dinoflagellates (Devassy and Goes 1988; Desa et al 2005) and prasincoxanthin pigment generally constitutes <5% of the pigment suite analyzed in these waters (Parab et al 2006). Our assessment of the mixed bloom is based on the combination of microscopic observations (table 2; figure 4) and measurements of marker pigments using HPLC (figure 3). Prasincoxanthin is a biomarker pigment for cells of class *Prasinophyceae* whereas Chl *b* appears in a range of phytoplankton groups including prasinophytes, euglenophytes and chlorophytes (Gibb et al 2001). All the microscopically identified phytoplankton species in this bloom do not contain prasincoxanthin and also do not contain endosymbionts that may have the marker pigment. Since the unidentified cells were the most abundant cells and the dinoflagellates identified in this bloom were not the source of prasincoxanthin, we believe that the presence of prasincoxanthin in the samples could be attributed to these cells. In the absence of clear taxonomic information using microscopy, we can only speculate that the unidentified algal cell may belong to class *Prasinophyceae*.

Table 3. Blooms of non-indigenous species reported from west coast of India.

No.	Bloom events	Dominant sp.	Bloom effect	Reference
1	Trichodesmium bloom	<i>Trichodesmium</i> sp.	Dark brown discolouration	Devassy <i>et al</i> (1978)
2	Dinoflagellate bloom	<i>Gymnodinium nagasakiense</i>	Red discolouration	Karunasagar and Karunasagar (1992)
3	Dinoflagellate bloom	<i>Noctiluca</i> sp.	Red discolouration & fish kill	Naqvi <i>et al</i> (1998)
4	Dinoflagellate bloom	<i>Noctiluca milaris</i>	Red discolouration	Nayak <i>et al</i> (2000)
5	Dinoflagellate bloom	<i>Noctiluca scintillans</i>	Red discolouration	Nayar <i>et al</i> (2001)
6	Trichodesmium bloom	<i>Trichodesmium</i> sp.	Dark brown discolouration	Sarangi <i>et al</i> (2004)
7	Dinoflagellate bloom	<i>Noctiluca milaris</i>	Red discolouration	Sahayak <i>et al</i> (2005)
8	Holoccolithophorid bloom	Unidentified holococcolithophore	Obnoxious stench	Ramaiah <i>et al</i> (2005)
9	Mixed phytoplankton bloom	Unidentified prasincoxanthin containing algal cells	Dirty brown discolouration	Present study

Pigment to Chl *a* ratios are widely used to estimate the relative contribution of different phytoplankton groups (Mackey *et al* 1996) and is a function of the physiological state of the cells, ambient nutrient levels and photoadaptive stress (Falkowski and LaRoche 1991). The Pras: Chl *a* (0.01–0.03) and Chl *b*: Chl *a* ratios (0.034–0.132) in the bloom samples were much lower than those reported in culture studies of prasinophytes (Pras: Chl *a* – 0.097 to 0.305; Chl *b*: Chl *a* – 0.618 to 1.313) (Latasa *et al* 2004). The lower pigment ratios in this study could be attributed to the age of the bloom, growth conditions and physiological stress. Another factor that may have skewed the pigment ratios in the present study was the contribution of Chl *a* from diverse phytoplankton population. A direct comparison of pigment ratios of a mixed population to monoculture-based studies is therefore highly subjective.

The age of the bloom could not be ascertained and contrary to the low phaeophytin *a* concentrations, the bloom had already entered the decline phase as evident from the decrease in Chl *a* concentrations over the sampling days (table 1) and microscopy (figure 4). The dominance of dinoflagellates and copepods in the bloom samples may have resulted in the low phaeophytin *a* concentrations. Accumulation of phaeophytin *a* differs with different grazers and need not always increase during the decline of a bloom. Engelkes (1985) found that copepod grazing did not form phaeophytin *a* from Chl *a* whereas protozoans could digest chlorophylls to colourless residues (Burkill *et al* 1987). Moreover, microzooplankton ingest fecal pellets

resulting in extensive degradation of pigments and fast recycling of pigments within water column (Strom 1993).

The bloom was unusual for various reasons including exceptionally high concentration of Chl *a* by fluorometry, unique composition, presence of prasincoxanthin pigments and low biological activity. High Chl *a* concentrations by fluorometry was not reflected in HPLC method which may be due to combination of factors. Fluorometric analyses do not differentiate Chl *a* and Chl *b* and presence of phytoplankton groups with high amounts of Chl *b* can produce a significant fluorescence anomaly due to changes in the accessory pigment composition (Lorenzen and Jeffrey 1980; Trees *et al* 1985, 2000). Variations in extraction procedures can result in discrepancy of up to 87% between the two analytical techniques (van Heukelem 2002). Other sources of discrepancies include differences in sample collection, sample storage, nature of standards used for sample analysis, etc. Despite these discrepancies, HPLC pigment measurement is considered to be more reliable as it separate and quantifies various pigments and their derivatives (Jeffrey *et al* 1999).

This bloom was one-time event in these waters and has never been encountered previously (table 3). Subsequent sampling during the same month in following years did not show any exceptional Chl *a* values or phytoplankton composition. The stations along CaTS have been monitored continuously since mid 1990s and have been reported for its biogeochemistry (Naqvi *et al* 2003). Moreover, primary production and phytoplankton

communities in these waters have been monitored regularly since 1970s (Dehadrai and Bhargava 1972; Qasim 1977; Devassy and Goes 1989; Parab *et al* 2006). The occurrences of 'unusual' bloom events have been reported globally with increasing frequency. One of the common features of all the 'unusual' blooms in coastal waters is the dominance of non-indigenous species (NIS) that may reoccur sporadically with or without adverse effect (Cloern 1996). For example, nuisance brown tides in the northeast coast of United States have been attributed to sporadic bloom of *Aureococcus anophagefferens* (Bricelj and Lonsdale 1997). Similarly, intense blooms of toxic dinoflagellate and holococcolithophorids causing large scale fish kill and foul smell production in the southwest coast of India have been recorded periodically (Karunasagar and Karunasagar 1992; Karunasagar *et al* 1989, 1990; Ramaiah *et al* 2005). Unlike toxic blooms, non-toxic bloom of a prymnesiophyte *Phaeocystis globosa* was first reported in the Arabian Sea by Madhupratap *et al* (2000). Nevertheless, non-toxic blooms of NIS generally go unreported due to lack of adequate attention and continuous monitoring of the coastal waters.

The presence of prasinoxanthin in the bloom samples raises questions about its origin. Prasinoxanthin pigments in bloom samples are generally limited to higher latitudes (Reigman and Kraay 2001; Schluter and Møhlenberg 2003; Ansotegui *et al* 2003; Yu *et al* 2007). Nevertheless, cells having prasinoxanthin have been reported from Gulf of Mexico (Guillard *et al* 1991), tropical waters (Hallaegraeff and Jeffrey 1984; McManus and Dawson 1994) and equatorial waters (Mackey *et al* 1998; Gin *et al* 2003). Based on the archival data (table 3) and the nature of the bloom, we believe that the dominant phytoplankton cells were alien to these waters and were accidentally introduced. The sampling area is close to Mormugao Port, which is one of the major shipping ports along the west coast of India (figure 1), and experiences intense shipping activity largely with China, Korea and southeast Asian countries. The introduction of alien microalgal species in Indian waters is well established (Subba Rao 2005); nine dinoflagellate species and one diatom species belonging to the coastal waters of China, Japan, Mediterranean, western Atlantic and Australia have been reportedly introduced in and around Indian waters in the last 50 years. With increased maritime activity in and around the sampling waters, it is likely that this non-native phytoplankton could have been transported via ballast waters of the ships and released into the sampling station.

Blooms of non-indigenous species are sporadic and depend upon the adaptability of these forms to new environment and avoid potential predators/

consumers. Apart from possible toxic/harmful effects, there is little information on the impacts of such blooms on the organic carbon composition and the stress on existing microbial food-web and associated biological communities. During the unusual bloom at CaTS, phytoplankton carbon (Chl *a*-C) estimated using OC:Chl *a* of 80 (Banse 1977) ranged from 8.5 g C m⁻³ at G1 (surface) to 56 mg C m⁻³ at G3 (14 m) on the first day sampling. Some of our values were much higher than those reported which could be attributed to the Chl *a* based carbon calculation adopted and under estimation of total organic carbon produced since dinoflagellates produce significant fraction of organic carbon as mucus exudates (figure 4b). The low microbial activity might be a reflection of the nature of organic matter produced during the bloom. Similar microbial response to organic carbon has been reported for a *Phaeocystis* bloom (Janse *et al* 2001). The low microbial activity and zooplankton biomass suggested that significant fraction of carbon fixed during the bloom escaped the food-web. The eventual sedimentation of such blooms and the shallow depth of the sampling stations would have increased organic carbon supply to the benthic communities that may influence their biomass and fecundity.

The ever increasing maritime activities around Indian waters raises concern about the vulnerability of the region to NIS through ballast water. It is very likely that similar blooms might be occurring along the Indian coastline but go largely unnoticed for want of continuous monitoring, awareness and effective mitigation measures both at policy formulation and implementation levels. Under Global Ballast Water Management Program of International Maritime Organization (IMO), extensive research on various aspects including baseline biota data, risk scenario development and analysis, GIS based database, development of demonstrable technologies for collection, supply and treatment of ballast water, etc. has been initiated at selected ports around the Indian coastline from 2001 (<http://www.bwmindia.com/>). These initiatives need to be further strengthened by proper documentation of such events and identification of NIS and related harmful events combined with effective coastal management tools is the need of the hour.

5. Conclusions

An unusual phytoplankton bloom dominated by unidentified cells and prasinoxanthin as one of the dominant pigments was encountered in a diatom-dominated marine environment in the near-shore waters off Goa. Based on the timing of the bloom,

morphology and pigment composition, we believe that this bloom was caused by a non-indigenous species which might have been introduced via ballast water discharge. The accumulation of bloom cells and dinoflagellates in a small patch in shallow waters combined with low bacterial production and zooplankton biomass resulted in unusually high concentrations of organic carbon. Although no immediate harmful impact of the bloom was evident, further studies are required to ascertain the impact of similar blooms on the microbial population, microbial loop and benthic fauna.

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