

Water column characteristics following the September 2004 stench event off southern Malabar coast

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Received 24 May 2006, revised 27 April 2007

An unusual stench emanated from the coastal waters of southwest coast India during September 2004 associated with an algal bloom. Water samples collected from 14 stations from the affected area after three weeks of the event, revealed that phytoplankton were dominated by holococcolithophorids (max 27×10^6 cells Γ^{-1}) in the coastal stations in comparison with the offshore stations (max 8000 cells Γ^{-1}), which contained mainly diatoms. Slightly low oxygen concentration (81-191 μM) associated with relatively lower temperature and high nutrients indicated the prevalence of weak upwelling in the region. Chlorophyll *a* to phaeopigments ratio at the coastal stations indicated that the bloom was in the degrading phase. Resultant microbial activities perhaps led to higher ammonia concentration in the study region. The holococcolithophorids seem to be uncommon to this region, but upon availability of right conditions, presumably temperature and nutrients formed massive bloom and consequently a stench affecting coastal population.

[Key words: Holococcolithophore, phytoplankton bloom, Arabian Sea, nutrients, chlorophyll *a*, phaeopigments, stench event, Malabar coast]

Introduction

Phytoplankton blooms are caused by rapid increase of phytoplankton biomass over a relatively short period of time. Algal blooms that adversely affect other organisms, ecosystems, human health, recreation and/or fisheries are considered harmful¹. About 40 to 50 of phytoplankton species produce toxins that can affect marine populations, as well as human beings^{2,3}. Harmful algal blooms (HAB) have become the subject of intensive research over the past few decades since these blooms are becoming more frequent globally⁴.

Blooms occur frequently in the coastal waters of India and the most reported blooms are *Trichodesmium* and *Noctiluca*. Extensive bloom patches of *Trichodesmium* have been regularly observed in the coastal waters during February to April along the west coast of India^{5,6}. Blooms of dinoflagellates *Noctiluca miliaris* (red tide) have also been reported along the south west coast of India during the monsoon and postmonsoon periods,

leading to fish mortality, which may be due to the oxygen deficiency or the presence of other toxins released by the bloom cells^{7,8}. An unusual stench was reported during 16-17, September 2004, at Kollam and Vizhinjam coast in Kerala, which resulted in breathlessness, nausea and chest pain among the coastal population. Ramaiah *et al.*⁹ collected water samples from the affected coasts and reported that the stench was probably caused by a holococcolithophore bloom. In order to study the changes in water column properties following the bloom, we carried out observation on the physical, chemical and biological characteristics of these coastal areas.

Materials and Methods

Sampling was made from 4-7 October, 2004 at 14 stations located between Kollam and Vizhinjam off the south west coast of Kerala (Fig. 1). The first five stations (stns. 1-5) were close to the coast (where the depth ranged between 18 and 30 m), whereas stations 6-14 were located offshore (35 to 45 m depth). Previously on 23 and 26 September 2004, this affected near shore area was sampled by Ramaiah *et al.*⁹. Therefore, present findings are compared with earlier observations⁹ in the following section.

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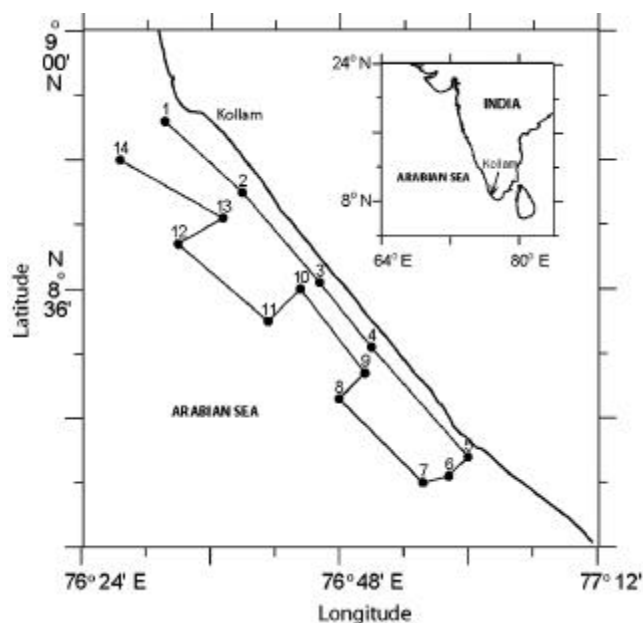


Fig. 1—Station locations off Kerala, South west coast of India.

Temperature and salinity measurements were made by using a portable CTD (Sea Logger, Sea Bird CTD system). Water samples were collected from surface to bottom (at discrete depths) with Niskin bottles (5 litre). Sub-sampling was carried out for dissolved oxygen (DO), nutrients, chlorophyll *a* and phytoplankton speciation. DO was measured by iodometric Winkler titration. Nutrients (nitrate, nitrite, phosphate, silicate and ammonia) were analyzed using a SKALAR autoanalyzer. Chlorophyll *a* and phaeopigments were analyzed by the acetone extraction method¹⁰. Phytoplankton samples (250 ml) fixed with Lugol's iodine (0.5% final concentration)¹¹ were analyzed for abundance after concentrating the phyto-cells through a sedimentation technique followed by microscopic observation¹². For the pigment analysis, samples were immediately filtered on a GF/F filter (pore size 0.7 μm) and stored in liquid nitrogen until the analysis. The frozen filters were extracted in 3 ml of 95% acetone (v/v in deionized water) using a sonicator probe (5 sec, 25 kHz) under low light and temperature (4°C) followed by storage at -20°C for 4 hours. The extract was passed through a Teflon syringe cartridge (Millipore) having a glass fiber pre acrodisc filter to remove the cellular debris. The clarified extract was analyzed by a HPLC (Agilent Technologies) system equipped with an Agilent 1100 pump together with online degasser and Agilent diode array detector following the method

of Van Heukelem¹³. The column used for the analysis was an Eclipse XDB-C8 HPLC column (4.6 \times 150 mm, Agilent Technologies) connected via guard column.

Results and Discussion

Hydrographic parameters

The sampling period was at the end of the southwest monsoon and heavy rainfall was observed in the study area during the first two days. Temperature and salinity profiles for the coastal and offshore stations are given in Fig. 2 (A-D). Towards the northern end (stations 1 and 2), the waters were well mixed with near uniform temperature throughout the water (27.4°C; 0-20 m) but decreased to 26.4°C at 24 m depth. The southern stations (3-5) showed gradual decrease in temperature from 27.4°C to 25.4°C from surface to bottom (Fig. 2A). Surface salinity (34.76 PSU) was patchy and less than that in the deeper waters (Fig. 2C). Column salinities increased from surface to bottom and ranged between 34.63 and 35.48 PSU. The waters along the coastal transect were variously oxygenated with concentrations decreasing from 186 μM at the surface to 94 μM close to the bottom (Table 1, Fig. 3A). Nitrate was found to be increasing with depth, reaching maximal concentrations in bottom waters of stations 4 and 5 (up to 9 μM) [Fig. 3C]. Nitrite was observed throughout the water column, with concentrations varying between 0.19 and 2.06 μM (Table 1). On the other hand, phosphate concentrations did not follow any particular trend spatially, and ranged between 0.18 and 0.44 μM (Table 1). Silicate exhibited the highest concentration at the surface at station 5 (14.26 μM). Higher concentrations of silicate were observed at all the coastal stations (average 10 μM) [Table 1]. A very high ammonia concentration was observed at station 5 at 20 m depth (12 μM) and the levels decreased from surface to bottom at all the stations.

The profiles of temperature, salinity, DO and nitrate along the farther transect comprising stations 7, 8, 11, 12 and 14 are shown in Figs. 2 and 3 (B and D). In comparison to the coastal transect, temperatures at offshore stations decreased gradually from surface (27.8°C) to bottom (23.8°C; ~45 m) [Fig. 2B]. Salinity was found to vary from 34.5 to 35.49 with lower surface salinities in the north (Fig. 2D). Oxygen was found to decrease from 191 μM at surface to 81 μM in bottom waters (Fig. 3B,

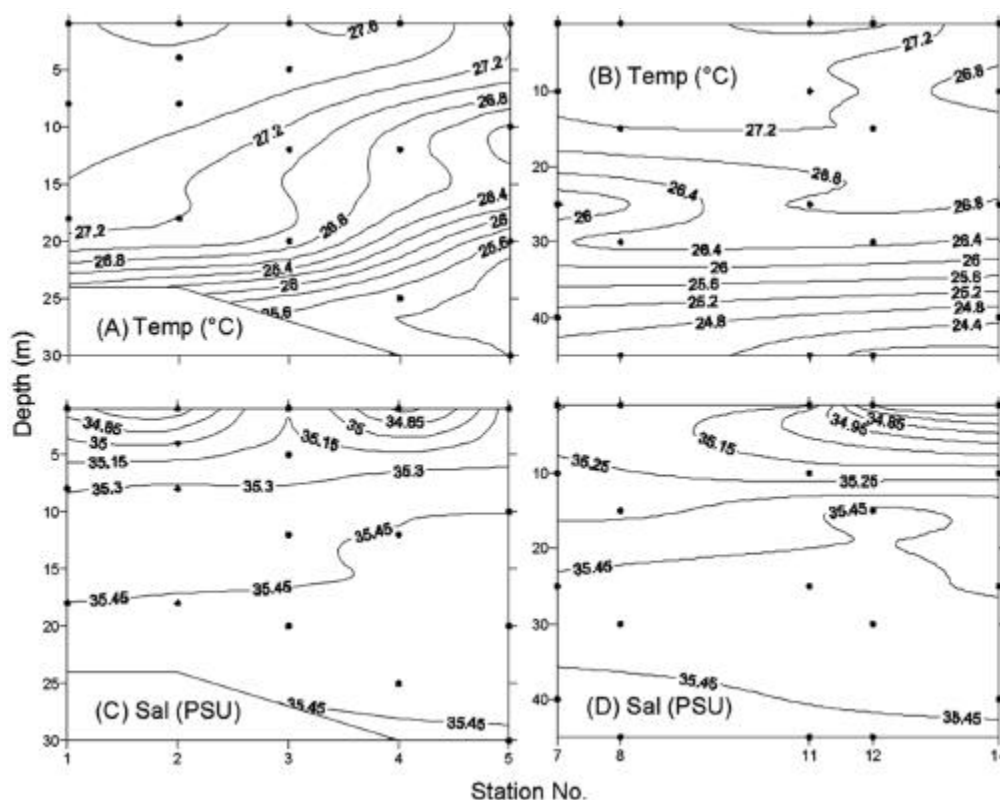


Fig. 2—Variation in temperature for the coastal stations (stns. 1-5) [A], offshore stations (stns. 7, 8, 11, 12 & 14) [B] and salinity for the coastal stations (C) and offshore stations (D).

Table 1—Concentration of various chemical and biological parameters for the coastal (stns. 1-5) and offshore stations (stns. 6-14)

Parameters	Coastal stations		Offshore stations	
	Range	Average	Range	Average
Temp. (°C)	25.4-27.58	(26.96)	23.82-27.83	(26.47)
Sal. (PSU)	34.63-35.48	(35.24)	34.57-35.49	(35.31)
DO (μM)	93.5-185.5	(151.5)	81.2-191.4	(144.7)
Nitrate (μM)	0.69-8.88	(2.52)	0.77-9.97	(3.62)
Nitrite (μM)	0.19-2.06	(0.99)	0.18-2.71	(1.04)
Phosphate (μM)	0.18-0.44	(0.30)	0.06-0.86	(0.32)
Silicate (μM)	5.57-14.26	(10.03)	3.26-13.14	(7.63)
Ammonia (μM)	0.14-11.99	(3.4)	1.12-8.18	(4.06)
Chlorophyll (mg/m^3)	0.05-2.45	(0.5)	0.08-8.97	(1.61)
Phaeopigments (mg/m^3)	0.06-1.11	(0.38)	0.13-3.35	(0.61)

Table 1). The southernmost station showed a significant and sharp drop in oxygen concentrations below 15 m depth. Substantial amounts of nitrate were observed in the surface waters (0-10 m; $\sim 2 \mu\text{M}$) and the concentrations increased up to $9.9 \mu\text{M}$ below 10 m depth (Fig. 3D). Nitrite was also present throughout the water column (0.18 to $2.7 \mu\text{M}$) with maximal levels between 10 and 20 m (Table 1). Similarly, phosphate and silicate were also found in measurable concentrations throughout the water

column (PO_4 , 0.06-0.86 μM ; SiO_4 , 3.26-13 μM) [Table 1]. In the case of phosphate and silicate high concentrations were observed from intermediate (20 m) to bottom depths.

Chlorophyll a, phaeopigments and phytoplankton speciation

Along the coastal transect, chlorophyll *a* concentration was found to vary between 0.05 and 2.45 mg m^{-3} with the maximal levels in the surface waters at station 1 (Fig. 4A). Similar variations were

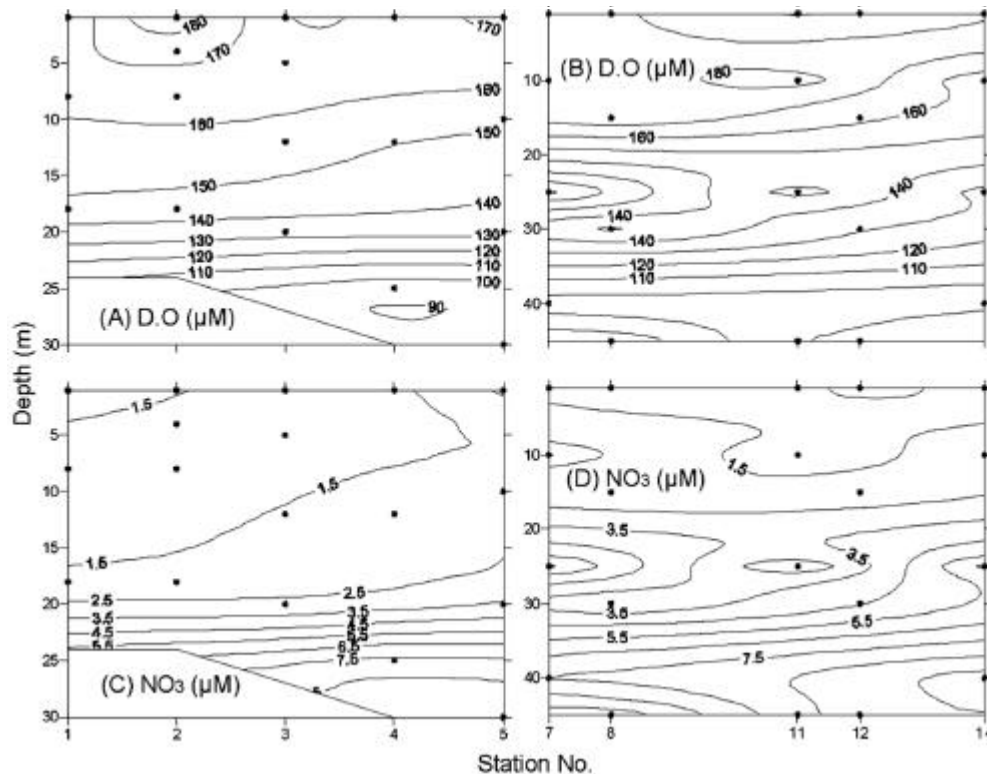


Fig. 3—Variation in dissolved oxygen for the coastal stations (A), offshore stations (B) and nitrate for the coastal stations (C) and offshore stations (D).

observed for phaeopigments, which varied between 0.06 and 1.1 mg m⁻³ (Fig. 4C). The average chlorophyll *a* and phaeopigments for the coastal transect were 0.5 mg m⁻³ and 0.4 mg m⁻³ respectively (Table 1). In comparison, the offshore transect had higher concentrations of chlorophyll *a* (Fig. 4B) and phaeopigments (Fig. 4D). Here, chlorophyll *a* was found to vary between 0.08 to 9 mg m⁻³ with an average concentration of 1.6 mg m⁻³, while phaeopigments varied between 0.13 mg m⁻³ to 3.35 mg m⁻³ with a mean of 0.6 mg m⁻³. In contrast to the coastal transect, the higher chlorophyll *a* in the offshore stations was mostly in subsurface samples. In particular, pigment levels were higher at station 11.

The coastal stations were dominated by the holococcolithophore cells with abundances as high as 27 × 10⁶ cells l⁻¹ at certain stations. The offshore stations were totally dominated by diatoms with few dinoflagellates, and did not contain any bloom cells. Total diatoms and dinoflagellates for coastal stations were lower (35 × 10⁶ cells m⁻²; integrated to 30 m water depth) than for offshore stations (555 × 10⁶ cells m⁻²; integrated to 45 m; Table 2). The predominant diatoms in the coastal stations were

Coscinodiscus radiatus, *Coscinodiscus* sp. (>25%), *Navicula distans* (26%), *Navicula* sp. (19%), *Pleurosigma* sp. (23%) and *Thalassionema nitzschoides* (20%). At the offshore stations (6-14), the above species dominated along with *Chaetoceros curvisetus* (43%), *Guinardia striata* (41%), *Guinardia flaccida* (13.5%), and *Rhizosolenia striata* (23%). Among the dinoflagellates, the major taxa were *Dinophysis* sp. (16%), *Noctiluca* sp. (11%) and *Keratella* sp. (10.4%).

Pigments

HPLC pigment analysis revealed the dominance of specific marker pigments¹⁴ representative of prymnesiophytes at the coastal stations, while offshore stations showed relatively low concentrations of these pigments. The surface concentration of pigments at the coastal and offshore stations is given in the Fig. 5 A and B. Among the marker pigments, fucoxanthin was the most dominant. The average value of fucoxanthin was 156 ng l⁻¹ and its range was 1-1037 ng l⁻¹, with the highest concentration observed in the surface water of station 2. Diadinoxanthin was also found at stations 3 and 5 with average

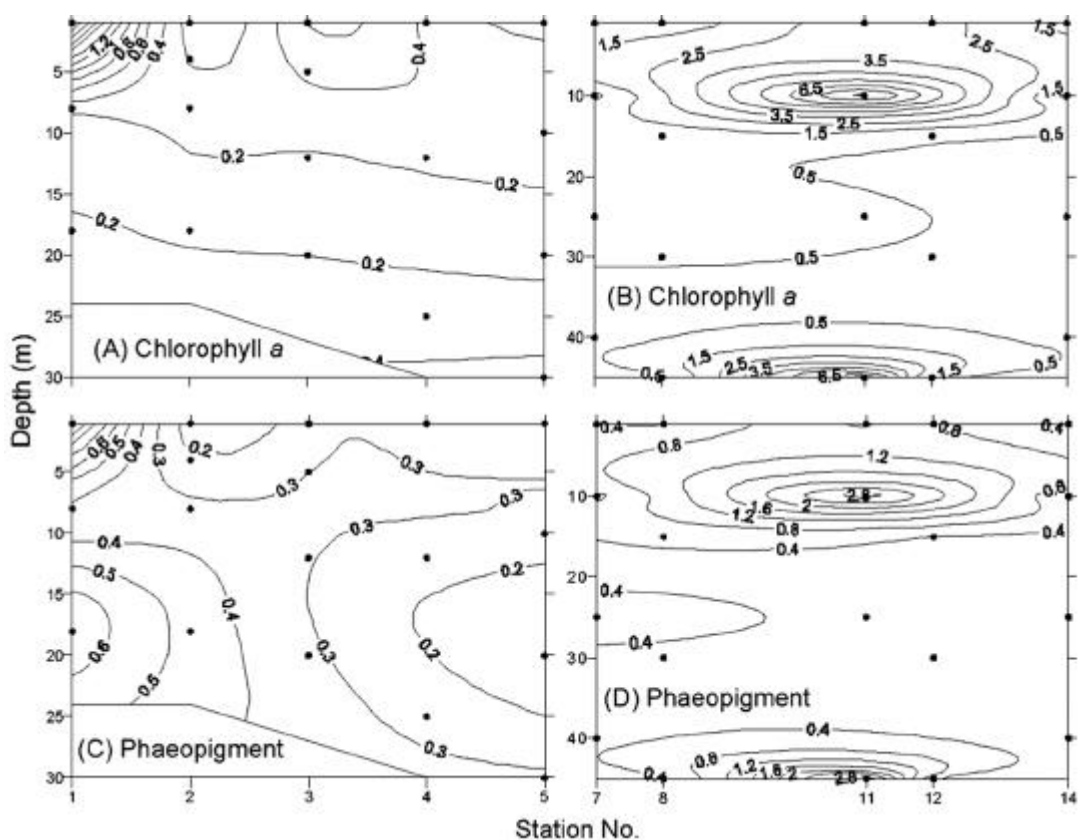


Fig. 4—Variation in chlorophyll *a* (mg m^{-3}) for the coastal stations (A), offshore stations (B) and phaeopigments (mg m^{-3}) for coastal stations (C) and offshore stations (D).

Table 2—Phytoplankton counts for the coastal (stns. 1-5) and offshore stations (stns. 6-14): Range and (Average)

	Coastal stations	Offshore stations
Depth range (m) of integration	0-30 m	0-45 m
Total diatoms and dinoflagellates (Nos. $\times 10^6 \text{ m}^{-2}$)	6.91-115.5 (34.97)	17.76-4480 (555.4)
Total phytoplankton (bloom cells, diatoms and dinoflagellates) (Nos. $\times 10^6 \text{ m}^{-2}$)	811-273955 (105790)	17.76-4480 (555.4)
Diatoms and dinoflagellates (%)	0.01-1.32 (0.29)	100
Bloom cells (%)	98.68-99.99 (99.71)	-

concentration 65 ng l^{-1} and its range was $1\text{-}324 \text{ ng l}^{-1}$. Concurrently, 19'-hexanoyloxyfucoxanthin (19'HF), 19'-butanoyloxyfucoxanthin (19'BF), Chlorophyll c_1c_2 and Chlorophyll c_3 were also present at few stations (Fig. 5A). The marker pigments showed low

concentration in the offshore samples (Fig. 5B), where fucoxanthin showed 16 ng l^{-1} at station 7.

Lower salinities (34.6 PSU) and higher silicate in the surface waters of the coastal transect indicate the effect of runoff following the heavy rainfall. On the other hand, lower dissolved oxygen and temperature in subsurface waters indicate continuing upwelling during this time which is supported by the presence of higher nitrate in subsurface waters. This is in agreement with previous observations along the west coast of India^{15,16}.

Ramaiah *et al.*⁹ reported that the stench along the southern Kerala coast during September, 2004 was perhaps the consequence of a holococcolithophore bloom. Scanning electron photomicrographs of bloom samples indicated that the holococcolithophore belonged to the genus *Helladosphaera*⁹. In the present study also, we observed large number of holococcolithophore cells at the coastal stations (1-5). The counts of bloom forming holococcolithophore at these stations were very high (up to $27 \times 10^6 \text{ cells l}^{-1}$) while there was a marked absence of these cells at the

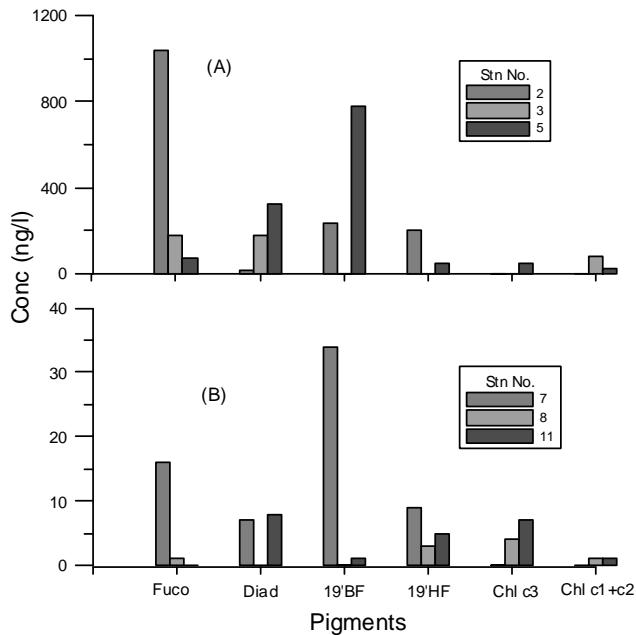


Fig. 5—Pigment concentrations (HPLC) for the coastal stations (2, 3 and 5) [A] and offshore stations (7, 8 and 11) [B].

offshore stations (6-14). This abundance is lesser than that observed by Ramaiah *et al.*⁹ (57.7×10^6 holococcolithophore cells l^{-1}) along the Kollam coast (south west coast of Kerala). Nevertheless, our study showed higher cell numbers compared to Sukhanova *et al.*¹⁷ of 2.8 to 4.5×10^6 cells l^{-1} during a bloom event in the Bering Sea. As observed by Sukhanova *et al.*¹⁷ holococcolithophore cells constituted about 99% of the total phytoplankton count in the present study (Table 2). It is however, surprising that bloom cells were absent in the offshore stations that were only about 5-10 km away from the coastline. Our pigment analysis also revealed high amounts of prymnesiophyte marker pigments like, fucoxanthin, diadinoxanthin and 19'-hexanoylfucoxanthin in the coastal waters, which is supported by the microscopic observation of higher abundance of holococcolithophore cells. Hence this study confirms the finding of Ramaiah *et al.*⁹ that the bloom cells belonged to the prymnesiophyte group.

Phaeopigment concentration in the coastal waters formed about 70% of the chlorophyll *a* content. The high concentration of phaeopigments indicates that the bloom was in the phase of degradation. The enhanced microbial activities resulted in the observed high concentration of ammonia (ca. $12 \mu M$) as also reported by Alldredge *et al.*¹⁸ based on a mesocosm study. Further evidence for this comes from low

dissolved oxygen concentrations (up to $81 \mu M$) in a 30 m water column in association with high nutrient concentrations. Nevertheless, nutrient levels might have also been enhanced by the degrading bloom. In contrast, phaeopigments in the offshore stations (dominated by diatoms) comprised of about 40% of the total chlorophyll *a* indicating the active growth in these phytoplankton cells.

The seeding of bloom, alien to the Indian Ocean region may be due to human interference through exchange of ballast waters. Holococcolithophore or other HABs proliferate when right conditions with respect to nutrients, light and temperature become prevalent⁹, which were available in the study area during this period. Immediately following the bloom (stench event), a bloom of dinoflagellates (*Noctiluca miliaris*) was also observed on 29th September 2004 along the southern Kerala coast where the water showed low temperature and high salinity⁸. The difference was that this bloom had formed around 20 miles off the coast while our study and that of Ramaiah *et al.*⁹ concentrated closer to the coast. Bloom formation is a natural process which enhances biological productivity, but toxic blooms can lead to fish mortality (either due to anoxia or due to the damage of fish gills) and hazards to human health¹⁹. The other adverse effect of blooms is the toxins produced enter the food chain of clams, mussels etc. lead to paralytic shell fish poisoning²⁰. In the present study the stench event reported along the south west Kerala coast affected the children and created panic among the coastal population. Sukhanova *et al.*¹⁷ reported the re-occurrence of holococcolithophore bloom in the subsequent years, hence systematic samplings and studies will help to create awareness among the people, residing coastal areas.

Conclusion

The present study confirmed the presence of prymnesiophyte bloom along the southern Kerala coast that probably resulted in stench during September 2004. The abundance of bloom cells during the present investigation was ca. 60% less than that observed just two weeks previously by Ramaiah *et al.*⁹. The holococcolithophore cells were confined to near shore stations whereas the deeper samples were dominated by active diatoms. The harmfulness of algal blooms including coccolithophores were reported earlier but the occurrence of these blooms in the coastal waters of Arabian Sea were observed for the first time⁹.

Acknowledgement

Authors wish to thank the Director, NIO for the infrastructural facilities and support. The authors acknowledge Drs. S. W. A. Naqvi, M. Dileep Kumar, P.V. Narvekar and P. V. Bhaskar for their valuable suggestions to improve the manuscript and thank the crew of CRV Sagar Sukti and Mr. Sudesh Gaonkar for their onboard help. A.K. Pratihary and Jane T. Paul thank CSIR for support through fellowship. The data presented here are a part of the ongoing CSIR-Network project CMM-0009. This is NIO's contribution No. 4249.

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