Chattonella marina bloom in the coastal sea off Mahe, Southwest India

Harmful algal blooms (HABs) are becoming an increasing problem to human health and environment, including harmful effects on natural and cultured resources, tourism and ecosystems all over the world[1]. Marine raphidophycean algae, *Chattonella* spp., have been implicated in major fish deaths in various parts of the world and are regarded as the most noxious HAB species in Japan and China[2]. Periodic blooms of *Noctiluca scintillans*, *Trichodesmium erythraeum* and *Rhizosolenia* sp. are quite common in Indian waters[3–9]. Blooms of *Coscinodiscus asteromphalus* var. *centralis*, *Prototoperidinium* sp. and *Chattonella marina* have also been reported[9–12] intermittently from the Exclusive Economic Zone (EEZ) of India.

Calicut coast in the southwest of India is vulnerable to algal blooms. The first report of algal bloom from this area was that of *Hornelia marina* (*C. marina*) in 1954 causing severe fish mortalities[13]. After four decades, the blooming of the same species has been reported from the Calicut coast and off Kochi[11,12].

As part of HAB monitoring programme of the Ministry of Earth Sciences, Government of India, seasonal samplings have been carried out from selected coastal and estuarine areas along the Kerala coast since 2006. During 27 October to 1 November 2011, a massive bloom of marine raphidophyte, *C. marina* (Subrahmanyan) Hara et Chihara was observed in the coastal sea off Mahe (11°42′18″N, 75°32′36″E).

Surface water samples were collected on 27 and 29 October and 1 November 2011 (10–11 a.m.). Ten litres of surface water was kept in shade/darkness for 20–30 min to settle down the motile raphidophyte cells and finally the supernatant was poured off and the settled 'thick' mat of *Chattonella* cells was transferred into 250 ml clean polyethylene bottles. A few drops (2 ml) of Lugol's iodine were added as preservative for quantitative analysis. As *Chattonella* spp. is susceptible to preservatives[14], live samples were also collected for qualitative studies. The identification was made on the basis of a standard taxonomic key[15].

Hydrographic variables such as temperature, salinity, pH and dissolved oxygen (DO) were measured *in situ* using standard instruments. Chlorophyll *a* (Chl *a*) nutrients (nitrite, nitrate, phosphate and silicate) were analysed[16,17] with the help of a Hitachi U-3900 UV-visible spectrophotometer. Primary production was measured using Winkler method. Photomicrographs were taken with Leica DM 2000 phase contrast/light microscope with DFC 295-attached digital camera.

The surface water colour was brownish on 27 October and it continued for a week, covering the entire mouth of the estuary. The spreading visible layer (about 1 km in width) extended from the barmouth to both the northern and southern sides due to the blooming of *C. marina* (Figure 1 *a*). The bloom appeared in the coastal areas and extended up to 2 km inside the Mahe (Mayyazhi) Estuary during high tide. A brownish-red coloration was observed in a similar bloom of *C. marina* off Kochi[12]. However, the bloom of the same species had been reported to have appeared in a different colour, green from the same areas earlier[11,13]. The brownish-red water discoloration was due to the dominance of unhealthy cells[12]. Usually, *C. marina* has numerous bright green, disc-shaped chromatophores, uniformly distributed all over the body, around its peripheral region[13]. Here, the chromatophores appeared in golden brown colour instead of the normal green and exhibited almost the same bloom pattern as observed off Kochi, in 2009.

C. marina bloom appeared with a cell abundance of 4.5×10^6 cells l^{-1} and Chl *a* concentration of 10.89 μg l^{-1} on the first day of the study (27 October). There was a gradual decrease in the cell numbers with a decreasing Chl *a* values

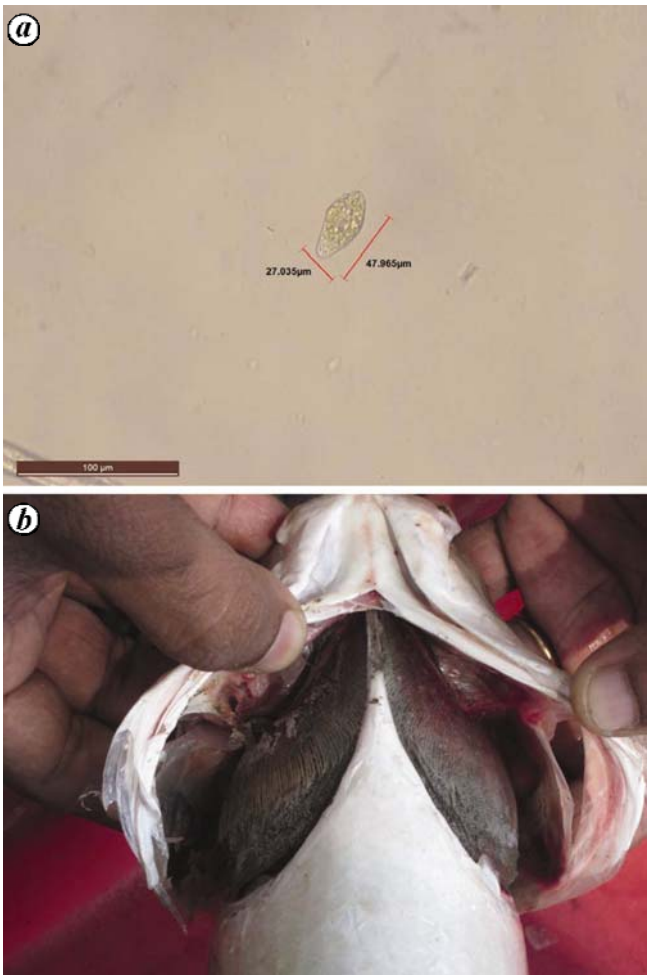

Figure 1. ***a***, Photomicrograph of *C. marina*. ***b***, Gills of Mullet fish being choked by *C. marina* cells.

Table 1. Surface water characteristics in the coastal waters off Mahe during the bloom along with five-year mean values

Parameter	27.10.2011	29.10.2011	01.11.2011	Mean (27 October to 1 November 2011)	Mean (2006–2010 October sampling)
Sea water temperature (°C)	27	26	25	26	26
Salinity (psu)	30	30	28	29.33	31
pH	7.9	8	7.7	7.86	8
DO (ml l^{-1})	4.5	4.8	5.2	4.83	6.6
Phosphate (μM l^{-1})	0.51	0.56	0.49	0.52	1.92
Nitrite (μM l^{-1})	0.13	0.09	0.14	0.12	0.48
Nitrate (μM l^{-1})	12.54	7.54	6.99	9.02	6.59
Silicate (μM l^{-1})	2.38	2.58	3.47	2.81	12.48
Chlorophyll *a* (μg l^{-1})	10.89	9.83	6.69	9.13	3.48
Primary productivity (gC/m^3/day)	1.94	1.2	4.24	2.46	4.27

by 1 November. The five-year mean (result of October 2006–2010 sampling) value of cell abundance and the corresponding Chl *a* was on the lower side (Table 1). A bloom of the same species occurred along the Calicut coast with a very high-standing crop resulting in large-scale fish mortality during September 2002 and 2003 (ref. 11).

Surface water temperature showed a gradual increase, whereas salinity and pH showed a gradual decrease from the first observation (Table 1). The optimum growth of *C. marina* in laboratory conditions was shown to be at 25°C and at a salinity of 30 psu (ref. 18) whereas the SST range was 15–35°C in the Salton Sea during the bloom of the same species[19]. Though *C. marina* preferred an optimal temperature of 25°C and 18°C was the minimum temperature required for its survival[2]; there are other reports which show variations in the optimal conditions for the blooming of *C. marina*[2,20,21]. Intensity of bloom was high on 27 October when temperature was 25°C and the bloom receded with increasing surface water temperature during the following days.

C. marina prefer nitrogen-rich environment for their better growth and survival. Increase in nitrogen, particularly that of nitrate, was thought to be the important cause of the bloom of *C. marina* in the Dapeng Bay[2,21], which is comparable to our findings (Table 1). Phosphate levels were comparatively lower than that of the five-year mean concentration (Table 1). The phosphate concentration was 1.13 μmol l^{-1} when it bloomed off Kochi[12]. Normally, the levels of silicate have shown an increase during early post-monsoon months in this area (mean (2006–2010) silicate concentration is 12.48). During the bloom, silicate was in the range of 2.38–3.27 μmol l^{-1} and hence, it has limited the survival of diatoms.

On 27 October, the bloom was monospecific with only *C. marina*. However, in the following days, i.e. 29 and 1 November, a number of diatom species along with some dinoflagellates also appeared. On 1 November, the bloom intensity had marginally decreased and species such as *Coscinodiscus asteromphalus* (48 cells l^{-1}), *Coscinodiscus radiatus* (18) *Cylindrotheca gracillis* (26), *Pleurosigma estuarii* (18), *Nitzschia closterium* (10), *Hantzschia marian* (8), *Biddulphia aurita* (12), *Chaetoceros decipiens* (12), *Campaylodiscus eccelsianus* (8 cells l^{-1}) and a very less number of dinoflagellates such as *Prorocentrum gracile*, *Ceratium furca*, contributed to the total standing crop. Introduction of the above species along with decrease in *C. marina* cell abundance signifies that the bloom is in the decline phase.

Chattonella spp. have been implicated in major fish deaths in various parts of the world[13,19,22,23]. There are reports that *C. marina* produces toxins[11,12,24]. The gill epithelium of fishes becomes swollen with massive mucous production by *C. marina* and fish appear to suffocate even in well-oxygenated water[25]. In the present study, mortality of a few fishes was observed on 27 October, in the region of 1 km inside the estuary. Pearl spot (*Etroplus suratensis*) and mullet (*Mugil cephalus*) were the main victims. It was found that the gills were fully choked (Figure 1 *b*) with algal cells and the consequent death might have occurred due to suffocation. No other faunal mortalities were observed. Fishes avoiding the bloom areas along this coast were very much evident. Commercially, important shoaling fishes shift from bloom waters to other favourable grounds during *C. marina* blooms, which has taken place in the same coast, earlier[11,13].

Formation of ichthyotoxins during the bloom of *C. marina* has been reported previously[11,12,26]. Conversely, the reactive oxygen species may play a significant role in the fish-killing activity of *C. marina*, rather than certain toxic substances or polysaccharides[27]. However, the impact of HABs depends on the concentration of the harmful species; even the most toxic species must have a minimum cell concentration to exert a harmful effect[1]. The present HAB incident was not at all a prolonged one and other faunal mortalities might be of lower order. It is imperative to comment that even though mortality of a few fishes was observed during the bloom, it may not have been associated with the effect of toxins formed during this bloom event.

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Endophytic bacteria – do they colonize within the plant tissues if applied externally?

The intimate association of endophytic bacteria (EB) with plants makes them potential candidates for biological control. Many bacterial species were reported to form endophytic associations with plants[1]. The beneficial effects that these bacteria confer on plants have made the study of plant–endophyte associations an important research topic for studying biological control of plant diseases[2]. Conventional biocontrol agents are not effective in the management of aerial plant pathogens mainly because of the lack of medium for survival and multiplication on the plant surface. Hence they may get washed away before they can act on the plant. This difficulty is solved to a considerable extent by the use of endophytic microbes which enter through natural openings and reside within the plants. They use plant sap as a medium for growth and systemic movement. Many workers have reported the bio-control efficiency of EB in different patho-systems[3,4]. Endophytes have been isolated from practically all studied plants following standard protocols. But their re-entry has not been proved in most cases.

Endophytes originate from the rhizosphere[5] and some may be transmitted through seeds and natural openings[6]. But if they are applied externally as other biocontrol agents, do they re-enter the plants through natural openings? The best way to find out this is by the radiolabelling technique which gives visual proof for the entry and colonization of labelled endophytes inside the plants. An experiment was conducted using four-month-old cacao seedlings and half matured cacao pods collected from 10-year-old cacao trees. Four bacterial endophytes isolated from different parts of cacao trees by surface sterilization followed by trituration as described by Mc Inroy and Kloepper[7] were used for the experiment.

For isolation of endophytes from feeder roots, tender shoots or leaves, the 1 g samples was exposed to the sterilant followed by washing with three changes of sterile water. The root/shoot/leaf bits were then put in a sterilized mortar containing 10 ml sterile potassium phosphate buffer (PB; 0.1 M, pH 7). The bits were finally washed in the buffer. From the final buffer wash, 1 ml was pipetted out and poured into sterile petri plate. To this molten and cooled medium was added and it served as the sterility check. If microbial growth was observed in the sterility check within four days, the isolates obtained from that particular sample were discarded.

For isolation from cacao pods, half mature pods collected were washed and disinfested with 70% ethanol. They were then cut open aseptically using sterile scalpel. A small piece (1 cm^3 approximately) of placenta of the pod was taken and placed in a pre-weighed, sterile petri dish. The petri dish was weighed along with the piece of tissue. The weight of the tissue was thus calculated and used to find the number of colonies per gram.

The surface-sterilized bits of root/shoot/leaf or the placenta of pod were triturated using sterile mortar and pestle with 9 ml of sterile buffer[7]. The triturate was serially diluted in sterile PB up to 10^{-7}. Then 1 ml of the diluted triturate was pipetted into sterile petri plate and molten and cooled King's B agar medium was added. The plates were