

Monospecific bloom of noxious raphidophyte *Chattonella marina* in the coastal water of South West coast of India

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Abstract

A massive monospecific bloom of toxic marine raphidophyte *Chattonella marina* was observed off Kochi along the southwest coast of India during late September 2009 with very high cell density (1.59×10^7 cells L⁻¹) and wide spatial distribution. The tear drop shaped cells were 38-65 μ m long, 25-30 μ m wide and having large number of chloroplasts. Almost 90% of the phytoplankton population was composed of *C. marina* in the bloom area. Other phytoplankters were few in number represented by *Skeletonema costatum*, *Rhizosolenia* spp., *Chaetoceros* spp., *Pseudo-nitzschia* spp. etc. and among these *Skeletonema costatum* was dominant with cell density 2.3×10^4 cells L⁻¹. The concentration of photosynthetic pigment, chlorophyll *a* was 8.3 μ g L⁻¹ in the bloom area. Toxicity test using the alcohol extracts of the *Chattonella* bloom samples showed characteristic neurotoxic symptoms in fishes leading to mortality.

Key words: Raphidophyte, noxious bloom, *Chattonella marina*, HABs, southwest coast of India.

Introduction

The raphidophyte flagellate, *Chattonella marina* (Subrahmanyam) Hara et Chihara is a well known causative organism of red tides and associated mass mortality of marine fauna throughout the world oceans. *C. marina* is able to produce haemolytic, haemagglutinating compounds and reactive oxygen species (ROS) including superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) (Onoue and Nozawa, 1989; Oda et al., 1994), the maximum production of which occurs during the exponential growth phase. They are also known to produce neurotoxins, which were originally characterised from the dinoflagellate *Gymnodinium breve*. These polyether compounds are ichthyotoxic at nanomolecular concentrations with the gills acting as the main absorptive area for brevetoxins from the water column. *C. marina* has caused mass mortality of fishes, leading to great economic losses in many countries, and ROS production has been implicated as one of the major factors leading to fish mortality (Marshall et al., 2002; Kawano et al., 1996).

The occurrence of red tides along the coasts of India has been fairly wide spread however, only a few reports are available on toxic red tides and associated mortality of marine fauna and shellfish poisoning (Hornell, 1917; Subrahmanyam, 1954; Karunasagar, 1992; Naqvi et al., 1998; Jugnu and Kripa, 2009). *Chattonella marina* (= *Hornellia marina*) was first described by Subrahmanyam (1954) from the coastal waters of Southwest coast of India with green discolouration of surface water and accompanied mortality among fishes and crustaceans. From the published reports it was clear that the *Chattonella* bloom was earlier recorded only twice from the Indian EEZ (Subrahmanyam, 1954; Jugnu and Kripa, 2009). Blooms of marine raphidophyte *Chattonella marina* is known to have deleterious effects on the marine fauna. A massive bloom of this raphidophyte observed along the south west coast of India during late summer monsoon period has been studied in detail. Characterization of the bloom forming species as well as the physico chemical parameters and phytoplankton- zooplankton assemblages of the bloom area was studied.

Investigation on the ichthyotoxicity of the bloom in the juveniles of *Oreochromis mossambicus* by means of alcohol extraction was also done. Since south west coast of India is one among the important fishery potential zone, such ichthyotoxic bloom studies are of considerable importance.

Materials and methods

For the qualitative and quantitative analysis of phytoplankton, 10 litres of discoloured surface water samples were taken using clean plastic bucket and filtered through 20 μ bolting silk and the filtrates were collected. Since the fixed *Chattonella* cells were deformed and shrank making it difficult to identify, unpreserved samples were first examined for identification and counted with Sedgwick-Rafter counting chamber using a *Nikon Eclipse* microscope following planktonic marine flagellate identification key by Throndsen (1997), and then preserved in 3% buffered neutral formalin/ Lugol's iodine solution for further analysis. The phytoplankton species were identified using standard keys (Tomas, 1997). Size analysis of cells was done using an ocular-micrometer. Temperature and salinity at the sampling stations were recorded using *Seabird 911 plus CTD*. The Sea Surface Temperature was measured using a bucket thermometer. Salinity values from the CTD were calibrated against the values obtained using the *Guildline Autosol* onboard. Dissolved oxygen was estimated following Winkler method. pH was measured using an electronic *Ino Lab* (WTW series) pH meter. Chlorophyll *a* was determined by filtering one litre of water sample through GF/F Whatman filter paper. Filter papers with filtrates were placed in extraction vials containing 10 ml of 90% acetone and the extraction were performed under cold temperature in the dark over a 24 hours period. Chlorophyll *a* was measured spectrophotometrically (*Thermo UV1*) according to the methods described by Parsons et al (1984). Nutrients (nitrite, nitrate, phosphate and silicate) were analysed using segmented flow Auto Analyzer (*SKALAR*) onboard the vessel by following standard procedures (Grasshoff et al., 1983). Meteorological parameters were obtained by continuous Automated Weather Station (*AWS*). Degree of stratification in terms of static stability parameter (*E*) was computed following Pond and Pickard (1983), $E = -1/\rho \partial\rho/\partial z - g/C^2$, where 'g' is the acceleration due to gravity, 'ρ' is the density which is a function of salinity, temperature and pressure, 'C' is the speed of sound. Procedure by Onoue and Nozawa (1989) was used for the

separation of neurotoxin from the *C. marina* bloom samples and ichthyotoxicity test were carried out using juveniles of *Oreochromis mossambicus*. Mesozooplankton samples were collected from mixed layer by Multiple Plankton Net (*Hydrobios*) and from surface by Bongo nets. The samples were preserved in 4% formalin prepared in sea water after determining the biomass by displacement volume method.

Results and discussion

During the routine monitoring of harmful algal blooms along the west coast of India onboard FORV Sagar Sampada, a rusty brownish red discolouration of surface water was observed off Kochi (Fig. 1, Lat.10°00.13 N, Long.75°58.86 E) on 26th September 2009. The bloom area was located approximately 3 nautical miles from the Cochin bar mouth, which discharges large quantities of estuarine waters to Arabian Sea during the South West monsoon. From the microscopic analysis of fresh bloom samples, it was observed that the discolouration was by the blooming of an ichthyotoxic raphidophycean member *Chattonella marina* (Subrahmanyam) Hara et Chihara (Fig 2).



Fig. 1. *Chattonella marina* bloom site off Kochi (Southwest coast of India).

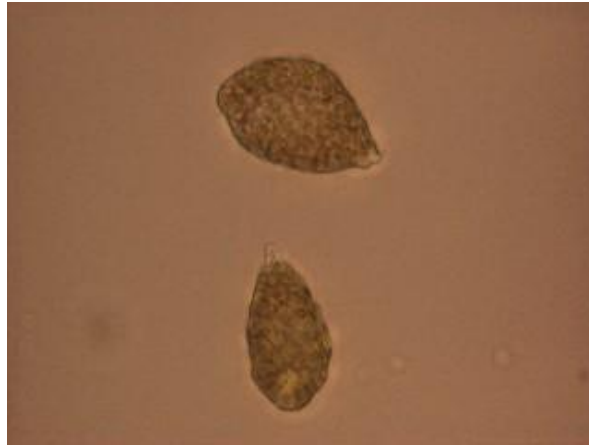


Fig. 2. Microphotographs of live cells of *C. marina* x 400.

The present *Chattonella marina* bloom was characterized by rusty brownish red discolouration of surface waters. Healthy individual of *Chattonella* normally contains numerous bright green, disc shaped chloroplasts distributed all over the body at the peripheral region. In previous reports of *Chattonella* bloom from Indian EEZ (Hornell, 1917; Subrahmanyam, 1954; Jugnu and Kripa, 2009) the water discolouration was greenish, showing the healthy and growing phase of the bloom. But here in our observation the brownish red discolouration was due to the dominance of unhealthy cells with yellow chloroplasts, showing the declining phase of the bloom. Phototaxis of *Chattonella* was observed during the present study. Brownish patches which aggregated along the ships shade during the course of sample collection indicated that *Chattonella* preferred lower light regime.

The physico-chemical parameters of the *Chattonella marina* bloom area was shown in figure 3A and 3B. Temperature has been recognized as a major factor that controls *Chattonella* abundance (Nakamura and Watanabe, 1983a). The observed sea surface temperature (SST) and salinity of the *Chattonella* bloom area was 25.56°C and 34.87 psu respectively, and is in the suitable range for the *Chattonella marina* bloom as reported by Yamaguchi et al (1991). Thermohaline stratification of the water column and the weak wind, which strengthens the stratification, is an important triggering factor to the development of harmful algal blooms (HABs) and was well documented (Amano et al., 1998; Smayda, 1990). The vertical distribution of temperature and salinity (Fig. 4A and 4B) in the *Chattonella* bloom area indicated thermohaline stratification in the water column with difference in temperature 2.14°C and salinity 0.21 psu. The degree of stratification is examined in terms of the static

stability parameter (E) computed and presented in (Fig. 5). The bloom station which is located in the shelf waters off Kochi (depth < 30 m), the stability maximum occurs at 8 m depth. These stratifications favours the *Chattonella* cells in their vertical movements (Handy et al., 2005) thereby minimizing zooplankton grazing pressure and allowing the cells to obtain nutrients at depths and light at the surface.

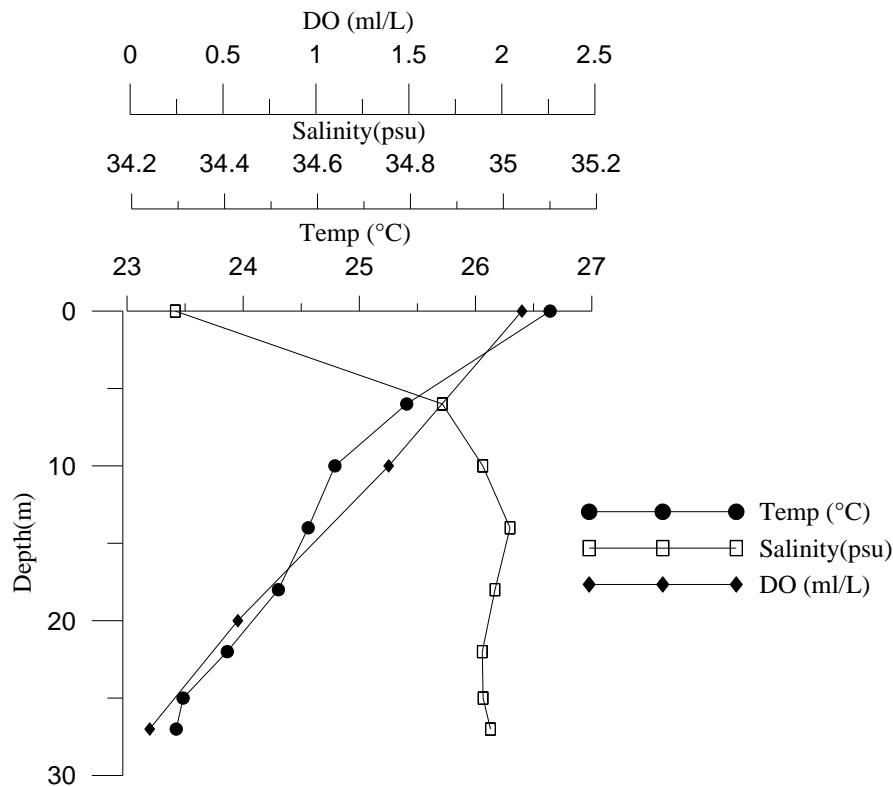


Fig. 3A. Vertical profiles of Temperature (°C), Salinity (psu) and DO (ml L⁻¹) of *C. marina* bloom area

Microscopic observations of unfixed bloom samples revealed the presence of *Chattonella marina* in the bloom area with cell density 1.59×10^7 cells L⁻¹. Other phytoplankters were few in number represented by *Coscinodiscus asteromphalus* var. *centralis*, *Thalassiosira* sp., *Nitzschia longissima*, *Skeletonema costatum*, *Guinardia delicatula*, *Rhizosolenia* spp., *Pseudo-nitzschia* spp., *Ceratium* spp., *Dinophysis* spp., and *Prorocentrum* sp. Among these *Skeletonema costatum* was dominant with cell density 2.3×10^4 cells L⁻¹. The present bloom was preceded by a multispecies diatom bloom dominated by *Skeletonema* and *Thalassiosira* spp. in the previous month (August 2009, from FORV data centre). A unique or unusual feature of the *Chattonella* bloom is that it occurred in cold water right after the diatom bloom, which developed during the early upwelling periods. The relation between bloom of

Skeletonema costatum prior or in combination with other (Raphidophycean) red tide species were previously reported (Graneli et al., 1995). *S. costatum* probably produces stimulants for growth of red tide species (Iwasaki, 1979).

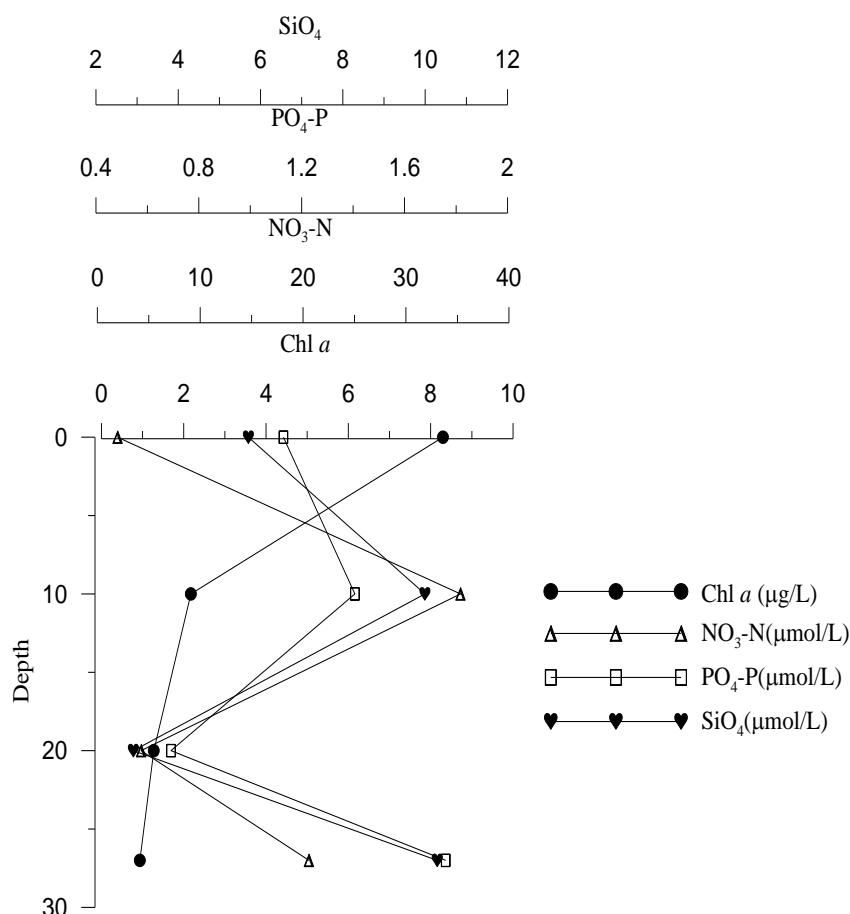


Fig. 3B. Vertical profiles of Chlorophyll a ($\mu\text{g L}^{-1}$) and nutrient concentrations ($\mu\text{mol L}^{-1}$) of *C. marina* bloom area.

Dissolved oxygen (DO) values in the *Chattonella* bloom area (2.1 ml L^{-1}) was very low compared to the normal values reported from the southwest coast during the monsoon period (4.5 to 6.5 ml L^{-1} , Balachandran et al., 1989). The low DO value in the present observation shows that the bloom was in declining phase.

Zooplankton bio-volume was low in *Chattonella* bloom area (1.37 ml m^{-3}) compared to that of non bloom area (3.14 ml m^{-3}). Copepods represented the major group with 1.36×10^5 individuals m^{-3} . Zooplankton grazing did not appear to have a major impact on the bloom of *Chattonella* sp., thus reflecting zooplankton avoidance of the *Chattonella* bloom or high death rates of zooplankton exposed to the bloom (Hansen et al., 2001). Reduced zooplankton grazing pressure during massive red

tide could be attributed to the dense population HAB species, and also due to the production of grazing inhibiting compounds or toxins that deter grazers or lethal to grazers.

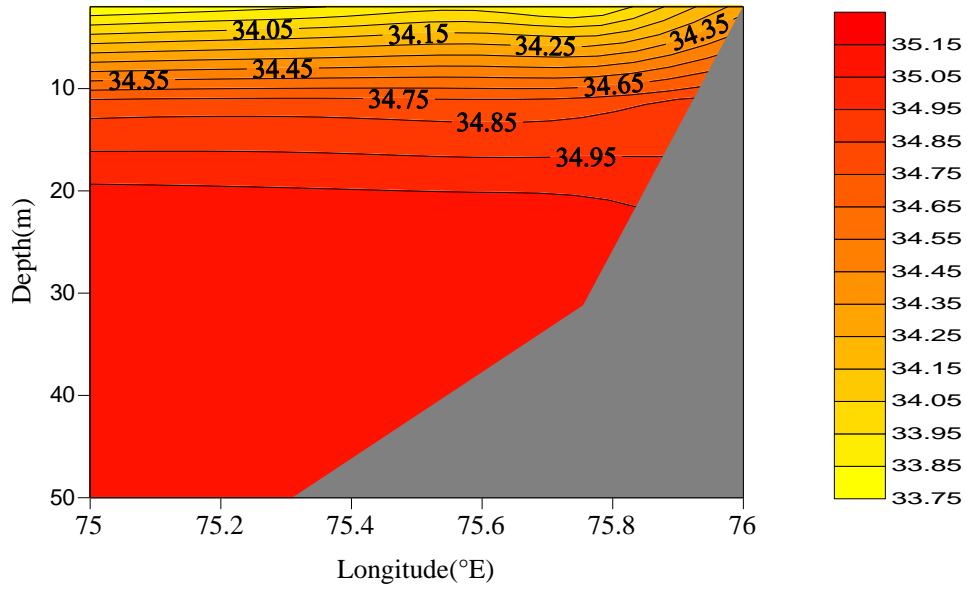


Fig. 4A. Vertical section of Salinity along 10°N.

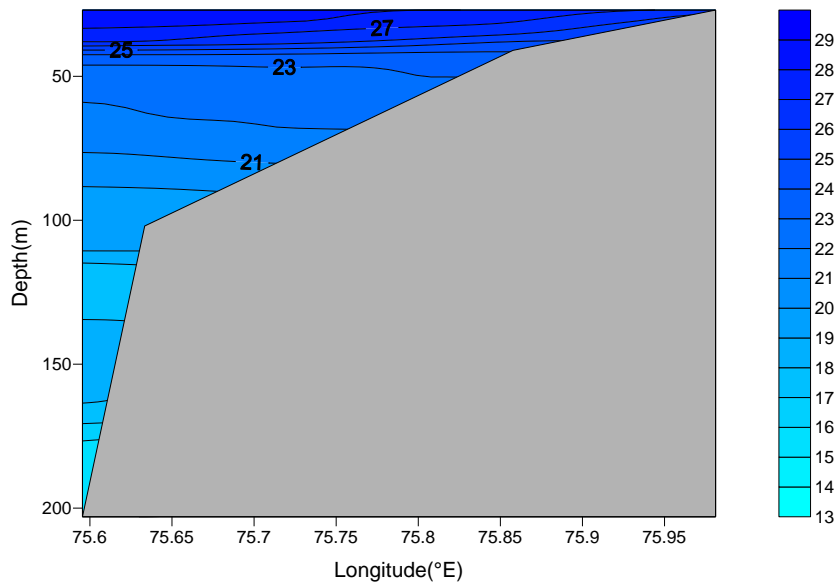


Fig. 4B. Vertical section of temperature along 10°N.

Mikhail (2007) reported chlorophyll *a* value of $90 \mu\text{g L}^{-1}$ from Alexandria waters during a *Chattonella sp.* bloom. In this observation, concentration of chlorophyll *a* was $8.3 \mu\text{g L}^{-1}$ showing the declining phase of the bloom. Nutrient levels were on a higher side in the *Chattonella* bloom area with $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and SiO_4 concentration of $1.96 \mu\text{mol L}^{-1}$, $1.13 \mu\text{mol L}^{-1}$ and $5.70 \mu\text{mol L}^{-1}$ respectively. Nutrient measurements made during the bloom imply that high nutrients, particularly inorganic nitrogen, may have played a role in initiation of this bloom. Eutrophication caused by the upwelling and heavy estuarine discharge during the SW monsoon is the reason for increased nutrient concentrations. Since *Chattonella* blooms are considered to start after the germination of cysts, the presence of nutrient rich surface water seems to play an important role in supporting a relatively hasty growth of cells during the initial phase of the bloom.

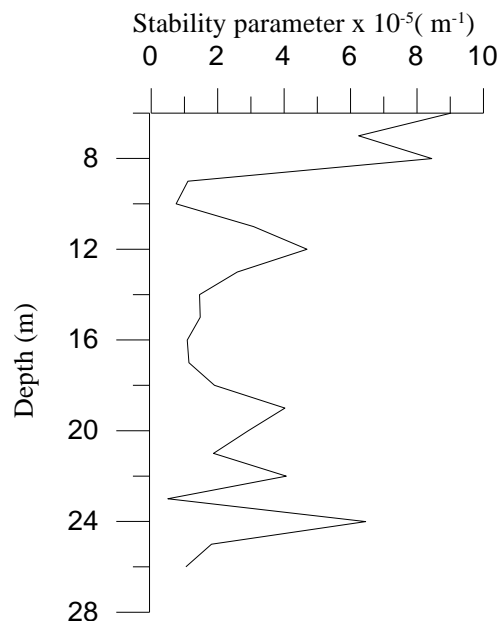


Fig. 5. Graph showing the degree of stratification in terms of static stability parameter 'E'.

According to Hara *et al* (1994) the genus *Chattonella* comprises seven species, among these two species, viz., *C. marina* and *C. antique* are capable of producing extremely potent toxins fatal to diverse animal groups as well as to human beings. Hence, it was decided to test the toxicity, if any, in the case of current *C. marina* bloom. 'Alcohol extracts' of the *Chattonella* cells concentrated from the fresh bloom samples were used for bioassay on *Oreochromis mossambicus*. A series of toxicity tests were conducted and in each case the characteristic neurotoxic symptoms leading to death of the fish were observed. A few dead fishes and crabs were

observed along with the present *C. marina* bloom and might be due to its detrimental effects to fish associated with reduction in DO, obstruction of oxygen exchange in gills by ROS production and also due to the excretion of neurotoxins which result in haemoagglutination and haemolysis in fish blood.

Novel blooms of raphidophycean members and associated fish mortality is increasing globally in the recent years. The same holds true along the West coast of India where mortality of fish and other marine organisms caused by ichthyotoxic phytoplankton, bacterial/viral pathogens and anoxia is on the rise, while little is known about the raphidophycean *Chattonella* blooms. The present observation gives an insight to the *Chattonella* bloom and associated hydrographical features in the coastal waters of the west coast of India. Detailed study is required to elucidate the role of cyst germination in the bloom onset, development, and toxin production and associated environmental parameters since it is essential for effective management and mitigation of *Chattonella* bloom outbreaks.

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