Indian Journal of Geo Marine Sciences
Vol. 47 (08), August 2018, pp. 1658-1664

Subsurface bloom of dinoflagellate *Gonyaulax polygramma* Stein in the shelf waters off Mangalore- South Eastern Arabian Sea

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Received 13 February 2017; revised 07 April 2017

A monospecific bloom of thecate dinoflagellate *Gonyaulax polygramma* (5x 10^8 cells L^{-1}) was observed from the shelf waters off Mangalore (South Eastern Arabian Sea) during late summer monsoon. The bloom was observed to be subsurface (~5m depth) with chlorophyll *a* maxima of 13.15 µg L^{-1}. The species observed to be photosynthetic and no visible food vacuoles were observed within the cell. Water column stability with sufficient inorganic nutrients during the fag end of summer monsoon might have favoured the bloom species. The region observed complete exclusion of zooplankton and can suggestively due to unpalatability of the bloom species.

[**Keywords***:* *Gonyaulax polygramma*; Harmful Algal Blooms; Ecdysis cysts; Water column stability; South Eastern Arabian Sea]

Introduction

Dinoflagellate blooms resulting in red tides along the coastal waters are of considerable interest owing to its multidimensional relationship with the hydrographical and physicochemical factors. Initiation, proliferation and crashing of bloom events correlate very well with the ambient hydrobiology. Seasonal variation in these factors contributes directly or indirectly to the bloom dynamics. In the marine ecosystems like that of Arabian sea characterized with changing physico-chemical characteristics and productivity patterns governed by biannually reversing monsoon, the events of algal blooms particularly harmful algal blooms gains much scientific attention.

Phytoplankton communities in the Arabian Sea relates very well to the availability of nutrient and changing pattern of temperature and sunlight during the south west and north east monsoons. Besides, large volume of freshwater meets the sea during the monsoon periods, which causes variability in salinity along the horizontal and vertical planes. Annual outbreaks of Harmful Algal Blooms (HABs) occur during the summer monsoon (June-September) along the coastal waters of South Eastern Arabian Sea (SEAS) [1-8]. The negative effects of harmful algae are many and diverse, ranging from economical losses in aquaculture, fisheries and tourism industry to major environmental impacts and human health hazards. Numerous mechanisms in relation to the hydrographic characteristics operating over various spatial scales have been proposed for being responsible for the formation of red tides. The dinoflagellate *Gonyaulax polygramma* Stein is considered a warm- temperate to tropical species with a preference for neritic waters. The species has long been associated with red tides and was first reported from the Bay of Agu, Japan[9]. It is one of the most abundant phytoplankton in the near shore coastal waters during calm, warm weather, in the upwelling seasons[10]. Any harmful or deleterious effects from the bloom of *G. polygramma* are often associated with the oxygen depletion resulting from the decay of massive blooms as well as with the release of ammonia and sulphide with the cell wall decomposition[11].

In Indian EEZ, particularly along the west coast the incidence, intensity and occurrence of the red tides has increased in recent past[12]. The *G. polygramma* bloom was earlier reported twice from the west coast of India[13, 14] and the bloom was non-toxic and no fish mortality was reported. Subsequently, there have been a number of reports on algal blooms and related red tides from the Indian EEZ, but none was attributed to *Gonyaulax* sp. The present study describes on an observation of a subsurface bloom of *G. polygramma* from the coastal waters of eastern Arabian sea along

the region off Mangalore during late summer monsoon period. The paper details on the hydrobiological characteristics of the bloom region, physiology of the bloom and the possible environmental impacts associated with the bloom.

Materials and Methods

During the regular monitoring for Harmful Algal Blooms along Indian EEZ as a part of the Marine Living Resources programme (MLR) onboard *FORV Sagar Sampada*, a thick reddish brown layer, located in the subsurface water were observed at off Mangalore (Lat. 12°51.831'N Long. 74°20.002'E-Figure 1) during late phase of summer monsoon (October 2008). Meteorological parameters such as air temperature, wind speed and wind direction were recorded through the Automated Weather Station (AWS) onboard FORV Sagar Sampada. Vertical profiling of hydrographical parameters such as temperature, salinity and density of the bloom area was done using a Conductivity–Temperature–Depth profiler (*CTD: SBE 9, Sea-Bird Electronics, USA*) attached with sensors for understanding oceanic processes. The water samples were taken using Niskin bottles attached to the rosette sampler of CTD for chemical and biological analysis of various parameters. pH was measured immediately after sampling using *Ino Lab* (*WTW series*) pH meter. Dissolved oxygen was measured by the standard Winkler method[15]. Major nutrients (nitrate, phosphate and silicate) were analysed using a segmented flow Auto Analyzer (SKALAR) onboard by following UNESCO-JGOFS protocol[16]. Chlorophyll *a* measurements was made spectrophotometrically[17] using UV-Visible spectrophotometer (PERKIN ELMER Lambda 25). The stability of the water column was calculated using Brunt-Vaisala frequency[18].

Numerical investigations of phytoplankton was carried out by filtering ~ 20 litres of subsurface water collected by using Niskin bottles attached to CTD unit and filtered through 20µm net. The filtrates were immediately analysed onboard for live materials and then fixed with 1-3% formaldehyde-Lugol's iodine solution for further laboratory analysis. Quantitative estimation and species identification of phytoplankton was done by employing Sedgewick–Rafter counting cell (1 ml in triplicate) under Nikon Eclipse E200 microscope following standard identification keys[19-21]. Zooplankton samples were collected using oblique hauls of Bongo nets (300µm mesh size) and biovolume was estimated through displacement volume method[22].

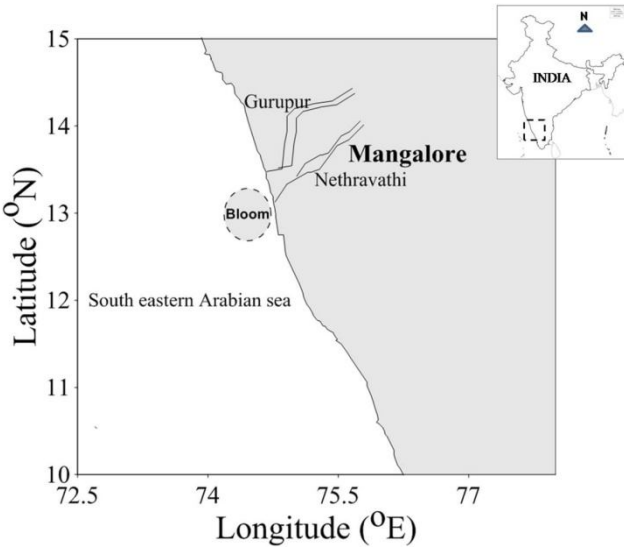

Fig. 1 — Map showing the area of subsurface bloom of *Gonyaulax polygramma* off Mangalore (South Eastern Arabian Sea)

Results

Observation of bloom and identification of bloom species

Subsurface discoloration was observed in the inshore waters off Mangalore, South west coast of India during the fag end of summer monsoon (Figure 2). Being a bright sunny day with an extremely calm sea, it was possible to distinguish the reddish brown discolouration of the subsurface layer of water extending from 3.5 to 10 m below the surface. Microscopic examination of the red water samples collected revealed that the discolouration was

Fig. 2 — Subsurface bloom of *Gonyaulax polygramma* off Mangalore

by an intense bloom of *G. polygramma* Stein with a cell density of $5x10^8$ cells L^{-1}. *G. polygramma* is an elongate, elegant, armored dinoflagellate with a thick outer coat. *G. polygramma* identified with elongated cells, tapered epitheca with a moderate apical horn, rounded hypotheca with two antapical horns (Figure 3a). Theca was reticulate and striated in matured cells. This species was pigmented and photosynthetic. *G. polygramma* is nontoxic but is known to cause fish mortality due to low dissolved oxygen levels. Along with this bloom a number of ecdysed cells (= ecdysis cyst *ie* stressed cells without theca), were also found (Figure 3b).

Physicochemical conditions of the bloom area

The bloom area experienced weak northerly winds (2.8 m/s), and the Ekman depth was only up to 5m. Surface water temperature was 29.55 ºC and did not vary much in the subsurface depth where bloom was observed (Figure 4). Influence of freshwater discharge from Nethravathi River was evident up to 60 km from coast (Figure 5) (SSS, 34-34.2; SST, 29.5°C). Presence of fresh water and weak mixing resulted in strong stratification of the water column and was established in the calculated Brunt Vaisala frequency (0- $6.24x10^{-4}$ s^{-1}). Mixed layer depth was shallow (13 m). Pycnocline was observed at 15 m in the area. Dissolved oxygen in the subsurface bloom waters were ~4.8 ml L^{-1}. pH values varied from 8.46 in the surface to 8.28 in the subsurface layers. The nutrient concentrations did not show much variation in the upper 20 m depth and beyond which rapid increase was observed (Figure 6). Inorganic nitrate values in the bloom region was 1.65 µmol L^{-1} in the bloom depth where the inorganic phosphate values were 0.6 µmol L^{-1}. Dissolved silicate was ~10 µmol L^{-1}. Chlorophyll *a* concentration in the subsurface bloom layer (bloom layer) was higher (13.145 µg L^{-1}) compared to that of surface layer (5.213 µg L^{-1}).

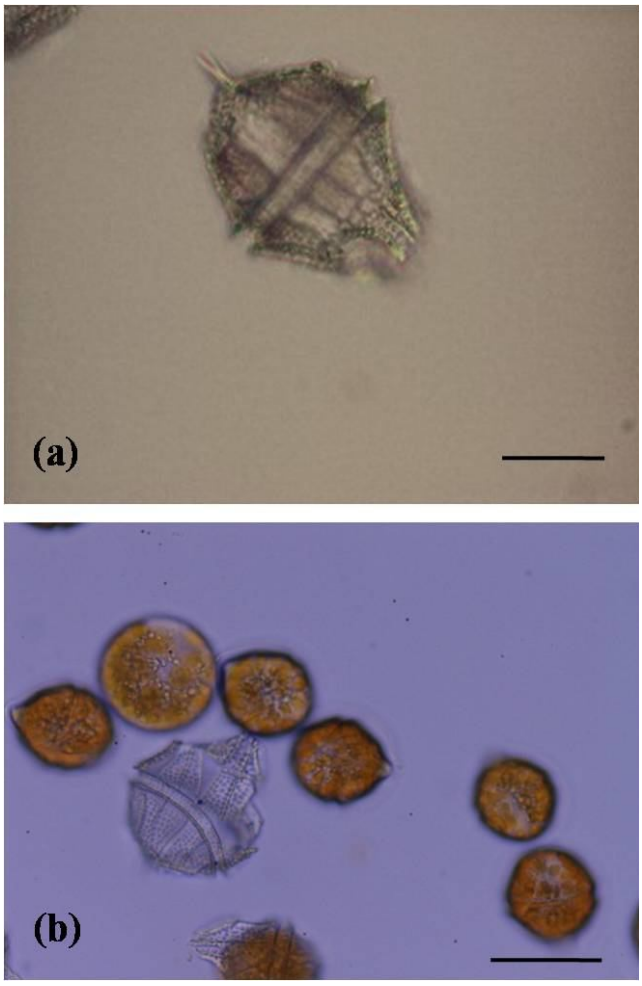

Fig. 3 — Microphotographs of **(a)** *Gonyaulax polygramma* **(b)** *Gonyaulax* ecdysal cyst (Scale bar: 10µm)

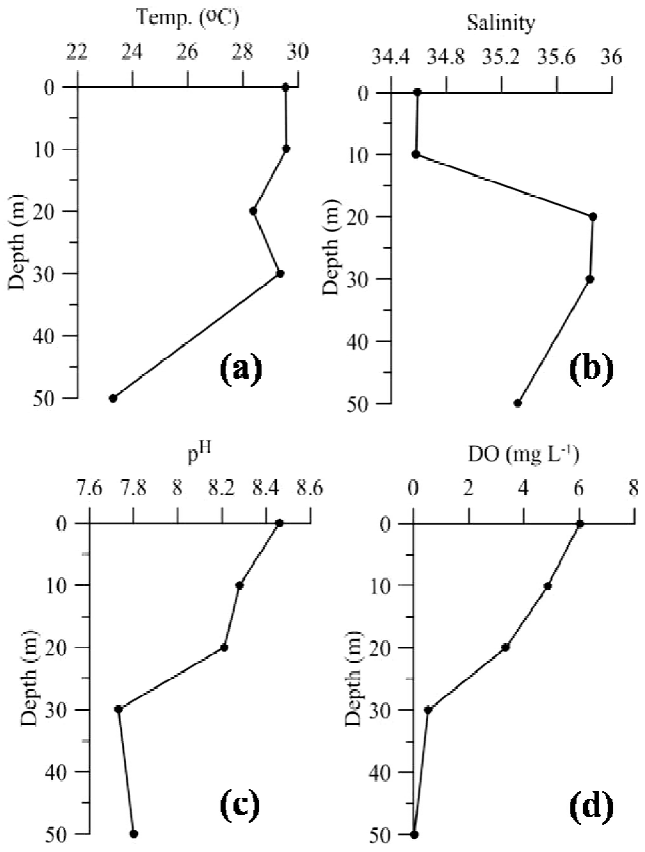

Fig. 4 — Vertical distribution of **(a)** Temperature **(b)** Salinity **(c)** pH and **(d)** Dissolved Oxygen in the *Gonyaulax polygramma* bloom area

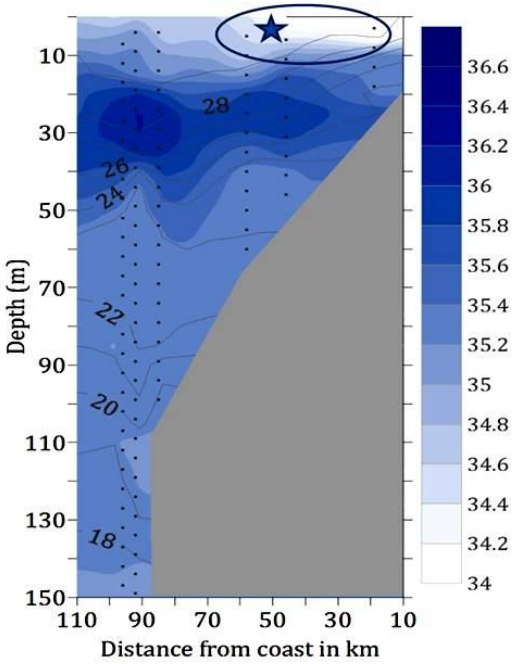

Fig. 5 — Vertical distribution of salinity (colour gradient) and temperature (contour) in the *Gonyaulax polygramma* bloom region (★) off Mangalore.

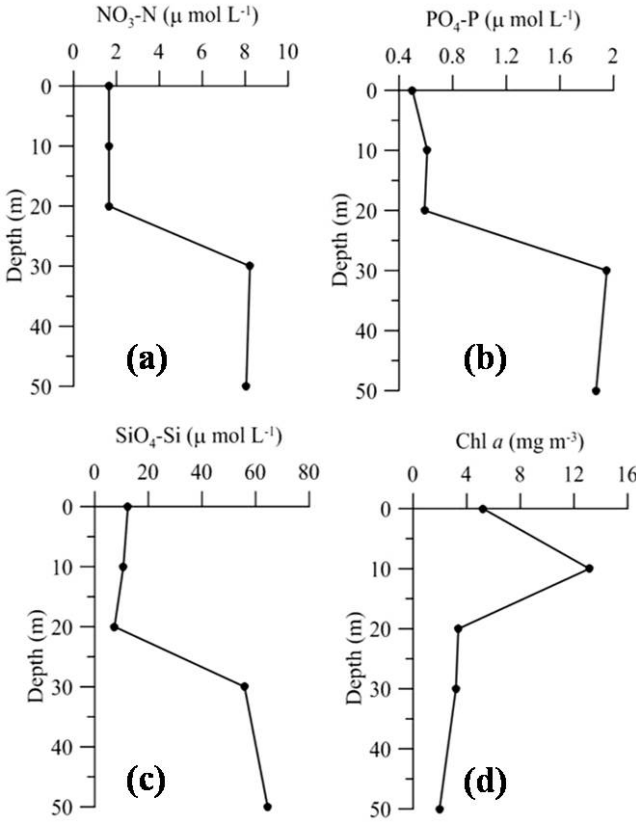

Fig. 6— Vertical distribution of **(a)** NO_3-N **(b)** PO_4-P **(c)** SiO_4 and **(d)** Chlorophyll *a* in *Gonyaulax polygramma* bloom area

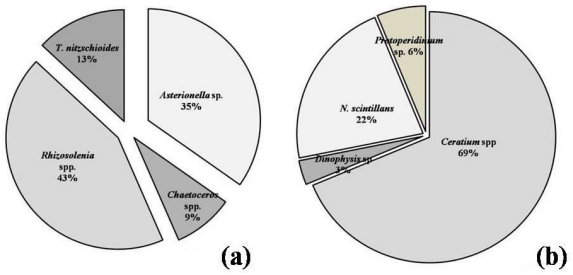

Fig. 7— Composition of other phytoplankton in the *Gonyaulax* bloom area **(a)** diatoms **(b)** dinoflagellates other than bloom species.

Biological conditions of the bloom area

The bloom is almost monospecific in nature comprising 99% of the total number of microalgae present. Qualitative and quantitative analyses of phytoplankton revealed that the *G. polygramma* cell density was 5x 10^7 cells L^{-1} in bloom area and other phytoplankton species were very insignificant in numbers and were represented by diatoms like *Rhizosolenia* spp., *Asterionella* sp., *Chaetoceros* spp., and *Thalassionema nitzschioides* (Figure 7a). Dinoflagellates were represented by *Ceratium* spp., *Protoperidinium* sp., and fewer cells of *Noctiluca scintillans*, *Dinophysis* sp.etc (Figure 7b). Along with the *G. polygramma* cells, the bloom contains their cysts in the germination stage. The bloom region observed almost a complete exclusion of zooplankton, however few copepods were collected along with bloom patch through oblique bongo net hauls.

Discussion

The study describes a subsurface bloom of dinoflagellate *G. polygramma* along the shelf waters of South Eastern Arabian sea, off Mangalore during late summer monsoon period. The coastal waters off Mangalore were reported to be an area of diverse fishery and the region observes high primary production during monsoonal nutrient enrichment. Riverine input through adjacent Nethravathi and Gurupur estuaries substantially influence the physicochemical characteristics of these coastal waters. Physiology of the bloom along with the responses of ecosystem towards the event is discussed briefly. The dinoflagellate species *G. polygramma*, a red tide causing species is cosmopolitan with worldwide distribution. The organism has not yet been identified to produce toxin[23], however the harmful effects are reported due to formation of

anoxic conditions and elevated sulphide and ammonia concentrations following cell decomposition. Previously mass mortality of fishes and shellfishes was reported from Uwajima Bay, Japan following intense bloom of *G. polygramma*[11].

The subsurface bloom of *G. polygramma* off Mangalore during the withdrawal phase of summer monsoon upwelling was characterized by high chlorophyll *a* concentrations (13.15 µg L^{-1}). The dinoflagellate has photosynthetic pigments and was earlier recognized as an autotrophic species. The high chlorophyll *a* concentration observed in the region could be attributed to this photosynthetic species. Later studies revealed the presence of food vacuoles inside the cells revealing the mixotrophic characteristics of *G. polygramma*, by engulfing the prey through the apical horn and sulcus[24]. However such food vacuole were, absent in *G. polygramma* collected during the present study.

Upwelling systems are highly susceptible to the occurrence and deleterious effects of harmful algal blooms[25]. Red tide events initiate and establishes with the slackening of upwelling signatures and instigation of thermal stratification[26]. The physical conditions in the bloom area reveals stable conditions of the water column with shallow Ekmann depth (5m), supported by weak winds (2.8 m/s), and comparable higher sea surface temperatures. Moreover, the influence of fresh water influx reinforced the process. The bloom was observed at a depth of 10 m of the water column and pycnocline of the column was observed at ~15 m depth that underlies the occurrence of *Gonyaulax* sp above the pycnocline. Stratified waters are crucial for the development of red tides[27]. Most of the earlier red tide events reveals that blooms of dinoflagellates both seasonally and regionally are associated with water column stratification[28]. The 'Margalef mandala' suggests *Gonyaulax* sp. as a *K*-strategist that thrives in conditions with sufficient nutrients and stable waters[29], unlike diatoms (*r*-strategist) that can tolerate water column turbulence and shear stress, dinoflagellates prefer stable waters to flourish.

Despite the water column stability another possible factor responsible for the occurrence of bloom in the subsurface waters can be due to their vertical migration pattern. *Gonyaulax* and many other dinoflagellates migrate vertically during daily changes of light intensity. Studies on the bloom dynamics of *Gonyaulax* sp. observed that cells form vertically migrating red patches or aggregations[30]. The *Gonyaulax* sp. swimming speed varies from 2 to 20 m day^{-1} [31]. The subsurface discolouration of this bloom was observed during the afternoon hours (14:00 hrs) this indicates that the bloom species might have moved towards the bottom to avoid the high light intensity.

Inorganic nutrients concentrations were significantly high in the blooms region. Previously higher concentration of nitrate and other nutrients were recorded during the post monsoon season (October- January) from the coastal waters off Mangalore[32]. This is attributed to the enormous run off from Nethravathi- Gurpur estuarine systems to Arabian Sea. Heavy rainfall and wind, is believed to be the meteorological parameters of paramount importance in governing the mechanism of increasing incidences of red tide occurrence[33]. Rainfall through river discharge brings in macro and micronutrients to the coastal waters thereby creating conditions conducive for algal blooms in coastal waters. This coincides with the generally productive fishing season in the area. Even though, *G. polygramma* is reported to be less dependent on the higher nutrient status of water column[34] the excystment or cyst germination is favoured with higher nutrient concentrations in addition to the major controlling factor on cyst germination, temperature[35].

Most of the dinoflagellates species undergo cyst formation during stressed or unfavourable environmental conditions. The germination of cysts is reported before the initiation of bloom in many instances with the onset of favourable environmental conditions[36]. Along with the active cells of *G. polygramma*, ecdysed cells were observed in the subsurface waters in the bloom region. Cysts are generally found in sediments in the vicinity of bloom areas[37]. The most important conditions that favour cyst germination (excystment) include the presence of stable overlying water mass, increase in temperature, influx of organic matter and inorganic nutrients, and accumulation of ammonia[38]. Under stressed condition the cells cast off their outer theca. The ecdysed cells settles as temporary cysts in the sediments can develop into vegetative cells under favourable conditions[36]. The presence of ecdysed cells along with the active cells strongly suggest that the bloom is nearing the end phase and active dividing stage might have ceased. Studies on tracking the dinoflagellate cyst assemblages have identified *Gonyaulax* cyst assemblages from the sediments off Mangalore and

have correlated it with the terrigenous input[39]. The previous reports on *G. polygramma* bloom from the Arabian sea, [13, 14] recorded neither harmful nor non-toxic events. Even though no toxic events were reported in the present bloom conditions, complete exclusion of zooplankton occurred in the area, except for one or two copepods, is suggestive of the unpalatability of the bloom species, which could be attributed to the complete absence of zooplankton in the bloom area[7].

Conclusion

HABs and red tides are observed to be on an increasing trend along the Indian coastal waters for the last few years. Urbanization and anthropogenic influences are considered to be the major factors for the proliferation of red tide species. Although occurrence of potentially harmful *G. polygramma* has been reported from the Indian waters, the bloom event of the species was recorded only once from the region. Lowering of water temperature along with the nutrient enrichment associated with summer monsoon upwelling and rainfall can be considered as the possible reasons for the sub surface bloom of *G. polygramma* in the region. While no harmful effects were recorded in the present event, possible chances of deleterious effects from unfavourable aftermaths such as anoxia can be resulted with the recurrent blooming of such species.

Acknowledgement

The authors are grateful to all the participants of *FORV Sagar Sampada* cruise 258 for the help rendered in sampling. This observation was made under the Marine Living Resources (MLR) Programme of the Ministry of Earth Sciences, Government of India, New Delhi.

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