Environmen tally-related seasonal variation in symbiotic associations of heterotrophic dinoflagellates with cyanobacteria in the western Bay of Bengal

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Abstract

In the western Bay of Bengal, some species of heterotrophic dinoflagellates recurrently show symbiotic associations with cyanobacteria (Synechococcus/Synechocystis). The occurrence of these associations is markedly higher during the spring intermonsoon period compared with the summer and winter monsoon. During the spring intermonsoon, strong stratification causes depleted nitrate concentration in the upper water column (upper 60 m water column had <0.01 µM), possibly favouring the proliferation of cyanobacteria cells. During this period, dissolved oxygen concentration in the surface waters (upper 50 m) of the Bay of Bengal were higher than other seasons. The higher oxygen concentrations could retard the process of nitrogen fixation by inactivating the enzyme involved in nitrogen fixation (nitrogenase). The more frequent occurrence of cyanobacterial cells inside the body of heterotrophic dinoflagellates during the spring intermonsoon may, therefore, be an advantage through exposure to reduced oxygen concentrations. Cyanobacterial cells inside the body of dinoflagellates may be more efficient in nitrogen fixation than their relatives in the surrounding water.

Keywords: Indian Ocean, Bay of Bengal, heterotrophic dinoflagellates, cyanobacteria, symbiosis, nitrogen limitation

1. Introduction

In marine environment, some heterotrophic dinoflagellates contain cyanobacterial symbionts (Norris, 1967; Taylor, 1982), but the ecological significance of this association has remained mysterious for many years. Recent literature suggests that the association has significance in regions where nitrogen limitation is prevalent. In such environments, heterotrophic dinoflagellates provide favourable microenvironments to cyanobacteria for the efficient fixing of molecular nitrogen (Gordon et al., 1994). In the northern Indian Ocean, cyanobacteria play a major role in the pelagic food web especially when the upper euphotic zone is stratified and provided with very low concentrations of nitrate (<0.01 µM, Burkill et al., 1992).

The Bay of Bengal, in the northeastern region of the Indian Ocean, is known for nitrogen limitation in the surface layers due to strong thermohaline stratification. Stratification prevents the advection of nitrate from the subsurface layer resulting in low biological production in the surface waters (Gomes et al., 2000; Madhupratap et al., 2001; Prasanna Kumar et al., 2002). Although upwelling and subsurface eddies were reported in some regions of the BOB during some seasons, its manifestations on primary production is much less compared to the Arabian Sea (Prasanna Kumar et al., 2002, 2004). Even during the summer monsoon period, except along the very coastal and upwelling regions of the Bay of Bengal, nitrate remains depleted in the surface layers. Nitrate input from the rivers is restricted to the coastal regions of the Bay of Bengal and most of the shelf and offshore regions are devoid of any
Symbiotic associations between some species of dinoflagellates and cyanobacteria were identified in the Bay of Bengal during the International Indian Ocean Expedition (IIOE). A few of the cyanobacterial symbionts were isolated and cultured during the expedition (Norris, 1967). However, there is a lack of information on the seasonal occurrence of these associations and its ecological significance in the western Bay of Bengal. The present paper attempts to identify (a) the species of heterotrophic dinoflagellates that show cyanobacterial symbioses, (b) to examine seasonal variations in the occurrence of symbiotic associations, and (c) to provide possible reasons for the symbiotic associations in the western Bay of Bengal.

2. Materials and Methods

Observations were carried out in the western Bay of Bengal on three cruises of FORV Sagar Sampada during the spring intermonsoon (March–April 2001), winter monsoon (December 2001) and summer monsoon (July 2002). During the study, 12 stations (1–12) were sampled along latitudes 11, 13, 15, 17, 19 and 20.5°N except during the summer monsoon where only 11 stations were sampled (Fig. 1). Profiles of temperature and salinity in each station were obtained from respective sensors fitted on a Conductivity Temperature Depth Profiler (Sea Bird Electronics – SBE 911 Plus, USA). Mixed Layer Depth (MLD) was computed as the depth at which density rises by 0.2 units from the surface (Shetye et al., 1996).

Water samples from discrete depths (0, 10, 20, 50, 75, 100 and 120 m) were collected using thoroughly cleaned Go Flo bottles (5 litre capacity) attached to a Conductivity Temperature Depth profiler rosette for collecting biological parameters (heterotrophic dinoflagellates and chlorophyll a). Water samples for nitrate and dissolved oxygen were also collected from different discrete depths (0, 10, 20, 30, 50, 75, 100, 120, 150 and 200 m) using Go Flow bottles. Samples for nitrate were collected into clean glass bottles and analyzed by autoanalyser (SKALAR – Model 51001-1). Dissolved oxygen samples were collected in 125 ml glass bottles and analyzed following the Winkler titration method (Strickland and Parsons, 1972).

During the study, heterotrophic dinoflagellates in the size range 20–200 μm were only considered. Water samples (5–7 litres) from each discrete depth were collected and transferred into black carboys. Initially, water samples from different depths were prefiltered gently through a 200 μm sieve to remove larger zooplankton and the filtered samples were collected into black polythene bottles. Subsequently, these samples filtrate were concentrated by siphoning through PVC tubing, which was tied with a 20 μm Nitex screen at one end. The end of the tubing with Nitex screen was immersed in the samples during the filtration to facilitate the retention of all the MZP of ≥20 μm size at the bottom of the carboy. Thus the samples were concentrated to 100 ml volume and preserved in 1–3% acid Lugol’s solution. The concentrated samples were then used for identification and enumeration of symbiotic species of heterotrophic dinoflagellates by inverted microscopy. Subsamples of microplankton were also collected randomly and preserved in 2% formalin for observation by epifluorescence microscopy for confirming the identity.

Before the analysis of microplankton samples by inverted microscopy, initial sample concentrates (ca. 100 ml) were allowed to settle for 48 hours in a settling chamber. The settled samples were transferred to a Sedgewick Rafter counting chamber and observed under an inverted microscope at 100–400x magnifications. The symbiotic species of heterotrophic dinoflagellates were identified following available publications (Karen and Williams, 1970; Taylor, 1976a,b, 1987). Photomicrographs of the identified species were taken using a Nikon camera and presented. Similarly, samples preserved in 2% formalin were also allowed to settle and transferred to Sedgewick Rafter chamber and observed under inverted microscope. After locating cyanobacterial cells inside the body of dinoflagellates, representative specimens were transferred to a glass slide. After placing a cover slip, specimens were observed under epifluorescence microscope with a green filter set to identify the yellow/orange autofluorescence of the cyanobacterial cells.

Figure 1. Locations of stations.
Two liters of water from each standard depth was filtered through GF/F filters (nominal pore size 0.7 μm) to estimate chlorophyll \( a \). The chlorophyll \( a \) was extracted in 10 ml of 90% acetone (Qualigens AR, Mumbai) in a refrigerator overnight. Samples were brought to room temperature, centrifuged and the absorbance of the supernatant was measured using spectrophotometer (Strickland and Parsons, 1972).

3. Results and Discussion

In the western Bay of Bengal, low surface salinity along with high solar radiation play an important role in controlling water column stratification that is more significant in the inshore regions due to high freshwater influx. High sea surface temperature was observed (28–30.5°C) during the spring intermonsoon and summer monsoon seasons (Fig. 2), due to high solar radiation compared with the winter monsoon. During the winter monsoon, sea surface temperature considerably decreased towards northern latitudes due to the decreasing atmospheric temperature. In all seasons, sea surface salinity was considerably lower in the northern region due to large river influx from the Ganges/Brahmaputra river systems. Sea surface salinity was relatively higher during the spring intermonsoon, compared with other seasons, due to minimum river discharge and precipitation (Ramage, 1984).

Along the inshore region, marked variations of mixed layer depth were found between stations due to varying quantities of fresh water influx. During the winter monsoon, mixed layer depth was low (<30 m) in all inshore stations due to the input of freshwater all along the coast. Also, during spring intermonsoon, a low mixed layer depth was found in the inshore stations of the southern latitudes as a result of high temperatures. A relatively lower mixed layer depth in the northern region during the spring intermonsoon than other seasons was due to high sea surface temperature. Similarly during the summer monsoon, stations in the northern region showed a low mixed layer depth (MLD) (<20 m) due to a high freshwater influx. Along the offshore stations, MLD was minimal (<25 m) during the spring intermonsoon due to thermal stratification. During the winter and summer monsoon, a higher mixed layer depth was found in most of the offshore region due to a relatively lower temperature than in the spring intermonsoon.

Vertical gradation in dissolved oxygen showed marked seasonal variations in the surface layer. Dissolved oxygen concentrations were higher during the spring intermonsoon compared with other monsoon seasons. For any given depth in the surface layer, dissolved oxygen concentrations were 10–20 μM higher during the spring intermonsoon compared to other seasons (Fig. 2). The lowest concentration of
dissolved oxygen concentration was found during the summer monsoon period. Seasonal variations in oxygen concentration in the surface layers may not be related with the seasonal pattern of primary production. In the Bay of Bengal, there are only small variations in primary productivity (Gomes, 2000). Seasonal averages of phytoplankton standing stock (chlorophyll $a$) are also low varying only between 14–18 mg m$^{-3}$ (present study). Hence, a possible reason for the low oxygen concentrations during monsoon periods may be the river influx. Six major rivers – Kavery, Krishna, Godavari, Mahanadi, Ganges and Brahmaputra – empty into the northwestern Bay of Bengal. These rivers bring in large quantities of suspended substances, which includes biogenic and terrigenous materials (Ittekot et al., 1991). During monsoon periods, due to heavy rain over Indian subcontinent, these rivers carry enormous quantity of freshwater and suspended materials. Sediment trap measurements clearly shows a high vertical particle flux in the Bay of Bengal during monsoon periods (Ittekot et al., 1991) and the organic matter of the settling particles is likely to consume dissolved oxygen while undergoing decomposition (Pearl et al., 1987). During monsoon periods, the high-suspended matter present in the Bay of Bengal could be the reason for
the low concentration of dissolved oxygen. The vertical distribution of nitrate (NO$_3$) also showed clear variations with respect to the seasons. During the winter and summer monsoon, the nitracline (1 µM of nitrate) was found at ~40 m depth. However, the nitracline was at a depth of ~60 m during the spring intermonsoon (Fig. 3). Similar seasonal trends in the vertical distribution of nitrate are typical of the northwestern Bay of Bengal (Rao et al., 1994).

Average surface and column chlorophyll $a$ was higher during summer ($0.25$ mg m$^{-3}$ and $18.14$ mg m$^{-2}$) followed by the winter monsoon ($0.16$ mg m$^{-3}$ and $15$ mg m$^{-2}$) (Table 1). The minimum surface and column chlorophyll $a$ was during the spring intermonsoon due to severe depletion of nitrate in the surface layers. Seasonal variations of column chlorophyll $a$ were between $14$ and $18$ mg m$^{-3}$. The low phytoplankton standing stock present in the Bay of Bengal is notably due to the combined effect of nitrate limitation and low solar radiation. The nitracline exists at a relatively shallow depth during the summer and winter monsoon but the solar radiation input during these periods are low (Gomes, 2000). Reduction in the available solar radiation is mainly due to heavy cloud cover, which is more pronounced during the summer monsoon (Gomes et al., 2000). Transparency of the water column is also important during the summer and winter monsoon periods due to increased river discharge that transports large quantities of suspended sediments. In contrast to this, the depletion of nitrogen in the surface layers limits phytoplankton growth during the spring intermonsoon (Gomes et al., 2000). The vertical distribution of chlorophyll $a$ clearly showed subsurface maxima during the spring intermonsoon period (Fig. 5). The increased solar radiation, coupled with a sufficient nitrate concentration in the subsurface layers could be the reason for the relatively deeper subsurface chlorophyll maxima during the spring intermonsoon period (Gomes et al., 2000).

During the spring intermonsoon, three extensive blooms of *Trichodesmium erythraeum* (a colonial cyanobacterium) were found at different locations in the study area. Two blooms were observed along $11^\circ$N and one along $17^\circ$N latitudes (Fig. 6). One of the blooms observed along $11^\circ$N was in the inshore region and the other blooms were in the offshore region. *Trichodesmium* blooms are considered as a biological indication for extended stratification and nitrogen limitation in aquatic systems. Low nutrient concentrations, very clear waters and deep light penetration are ideal for the formation of a *Trichodesmium* bloom (Capone et al., 1998).

Two genera of heterotrophic dinoflagellates (*Ornithocercus* and *Histioneis*) were found, at various times, to contain cyanobacterial cells (*Synechococcus*/ *Synechocystis*). These genera of dinoflagellates are distributed in the deeper waters of tropical and subtropical seas (Gaines et al., 1987; Taylor, 1987) and are devoid of any photosynthetic pigments, feeding by osmotrophy (Droop, 1974). However, in many instances, these dinoflagellates host clusters of cyanobacterial cells in their body. These cyanobacterial symbionts of heterotrophic dinoflagellates are known as "phaeosomes" (Norris, 1967). The cyanobacteria are located externally between the upper and lower cingular list in *Ornithocercus*, whereas in *Histioneis*, the cells are within a chamber on the girdle floor. Under epifluorescence microscope, orange or yellow fluorescence of "phaeosomes" contrasts with the green fluorescence of heterotrophic dinoflagellates (Gordon et al., 1994). During the present study, *Ornithocercus magnificus*, *O. heteroporus*, *O. quadratus*, *O. steinii*, *O. thumi* and *Histioneis hyaline* often showed the presence of "phaeosomes" (Plate 2). Occurrences of these dinoflagellates were confined to a depth of 75 m and *Ornithocercus thumii* and *O. magnificus* were the most common species found in the region.

During the study, occurrence of symbiotic associations varied markedly during different seasons. The total number of individuals of the genera *Ornithocercus* and *Histioneis* observed in the microplankton samples are given in Table 2. The total number of these individuals was highest during spring intermonsoon (1747 individuals) followed by winter monsoon (964 individuals). Similarly, the occurrence of cyanobacterial symbionts was also highest during spring intermonsoon (96% of the total individuals) followed by winter monsoon (15% of the total individuals). In general, during the spring intermonsoon, the occurrence of cyanobacterial symbionts was markedly higher (av. 5,000 individuals m$^{-3}$) than either the summer or winter monsoon (av. 400 and 500 individuals m$^{-3}$, respectively).

The maximum abundance of heterotrophic dinoflagellates and phaeosomes found during the spring intermonsoon was correlated with the concentration of nitrate and dissolved oxygen in the environment. It is known that cyanobacterial cells proliferate in nitrate depleted waters of the global ocean (Johnsen and Sieburth, 1979) and one of these is *Trichodesmium* sp.
Dinoflagellates are also associated with nutrient depleted environments. During the spring intermonsoon, intense nitrogen limitation supports a higher abundance of cyanobacteria and slow growing dinoflagellates. A number of cyanobacteria are known to fix atmospheric nitrogen and possess the enzyme ‘nitrogenase’. However, the enzyme activity is more efficient in less oxygenated conditions, which is very scarce under oligotrophic conditions (Paerl et al., 1987). During the present study, the maximum dissolved oxygen concentration was found during spring intermonsoon (most oligotrophic period).

Due to higher oxygen concentration, the efficiency of nitrogenase activity in the open water could be lower during the spring intermonsoon. In such environments, some cyanobacteria possess ‘heterocysts’ that enable them to create low oxygen microenvironments (Wolk, 1982). Colonial non-heterocystous cyanobacteria form low oxygen tension at the centre of their colonies to enable nitrogenase activity (Kallas et al., 1983; Pausen et al., 1991). Although the efficiency of fixation may be less, non-colonial, non-heterocystous cyanobacteria are also able to fix nitrogen in highly oxygenated, oligotrophic waters (Kallas et al., 1983.)
Cyanobacteria (phaeosomes) are shown to exude a large proportion of their fixed carbon to the host there by accelerate oxygen consumption in their microenvironment. Similarly, the respiration of the cyanobacterial cells itself may reduce the oxygen concentration in their immediate surrounding. These processes help the cyanobacterial cells to retain the nitrogenase for a longer time than their free-living relatives. Thus ‘phaeosomes’ gets advantage in nitrogen fixation by remaining inside the body of heterotrophic dinoflagellates and has special significance in oligotrophic environments where high oxygen tension is normally antagonistic to nitrogenase activity.

During the summer and winter monsoon periods, the low occurrence of symbiotic associations may be due to two reasons: (a) the presence of relatively higher concentration of nitrate in the surface layers result in low abundance of cyanobacteria. This was supported by a study in the Arabian Sea, where the abundance of *Synechococcus* was found to be inversely related with the concentration of nitrate in the surface waters (Burkill et al., 1992) and (b) a relatively low oxygen concentration with higher suspended organic matter concentration that provide a more suitable environments for free-living cyanobacteria to carry out nitrogen fixation.

Symbiotic associations between dinoflagellates and cyanobacteria may be ecologically significant in the Bay of Bengal especially during the spring intermonsoon. The ecological significance of these associations in Bay of Bengal is however questionable due to their low abundance (<5,000 individuals m⁻³). However, microplankton in low abundance may also substantially contribute to the nutrient and plankton dynamics in oligotrophic environments (Goldman, 1993). Our studies support the view that higher occurrence of symbiotic associations between heterotrophic dinoflagellates and cyanobacteria could be considered as bioindicators for extreme and extended stratification and nitrate limitation (Gordon, 1994).

### 4. Conclusion

Six species of heterotrophic dinoflagellates showed symbiotic associations with cyanobacteria in the western Bay of Bengal. The incidence of symbiotic associations was higher during the spring intermonsoon period compared with other seasons. During the spring intermonsoon, nitrate limitation was pronounced in the surface layers (nitracline at 60 m depth) of the western Bay of Bengal. The greater occurrence of cyanobacterial symbiosis with dinoflagellates during the spring intermonsoon, compared with other seasons, could be due to two reasons: (a) extreme and extended nitrogen limitation in the surface waters and (b) higher oxygen concentration in the upper layers, which could inhibit nitrogen fixation. In order to avoid a more oxygenated environment, cyanobacterial cells may enter in to associations with heterotrophic dinoflagellates. The heterotrophic dinoflagellates may provide a microenvironment with relatively low oxygen concentrations that support increased nitrogen fixation.

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### REFERENCES


Kallas, T., Rippka, R., Coursin, T., Rebiere, M.C., Tandeau...


