Chapter 6

Summary
In the context of the seas around India, several studies have explained the dynamics of phytoplankton communities (Subrahmanyan 1946; 1968; Devassy and Bhattathiri 1974; Taylor 1976; Desikachary and Prema 1987; Devassy and Goes 1988; Mitbavkar and Anil 2000; Saravanane et al. 2000; Habeebrehman et al. 2008; Patil and Anil 2008). Most of these studies were restricted to coastal dynamics of phytoplankton. However, very little is known about spatio-temporal variations in phytoplankton in the open ocean. In fact, most studies have focused on microphytoplankton (diatoms and dinoflagellates). The contribution of smaller phytoplankton groups (pico- and nano-plankton) has been underestimated due to the limitations of routine microscopy.

In view of the above, the present study was carried out in coastal and oceanic waters of India using the ballast water management programme and the Indian XBT observation programme respectively. Several aspects of phytoplankton community structure were addressed. These aspects were (1) spatio-temporal variation in dinoflagellates with reference to Harmful Algal Bloom (HAB) species, (2) regional and seasonal variations in phytoplankton community structure and the contribution of each group to the total community based on pigment studies, and (3) community structure of phytoplankton in two different coastal environments in relation to environmental settings. In the process of such evaluation, *Karlodinium veneficum* (a dinoflagellate) and 2 *Skeletonema* spp. (diatoms) were reported for the first time in Indian waters. Subsequently, the allelopathic effect of *K. veneficum* on *Skeletonema costatum* was evaluated.
To address the spatio-temporal variation in dinoflagellates with reference to HAB species, in the Bay of Bengal (BOB), surface waters were sampled using the ‘ships-of-opportunity’ programme. Sampling was carried out onboard passenger vessels plying between two transects, Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) during November 2003-September 2006. The samples were collected from 12 different locations at CP and 10 different locations at PK transects at one degree intervals. A total of 134 dinoflagellate species were recorded, with abundance ranging from 0-94 cells L\(^{-1}\). Mixotrophs dominated in both transects on most occasions. *Ceratium* was the most dominant mixotrophic genus whereas *Protoperidinium* was the most dominant heterotrophic form. The highest dinoflagellate abundance was observed in September 2006, the period of withdrawal of the South West Monsoon (SWM) during which cloud cover reduces. The variations in species abundance could be attributed to the seasonal variations in the stratification observed in the BOB, which restricts the transport of nutrients from deeper layers to the surface (Prasanna Kumar et al. 2002).

The present study is the first of its kind detailing the HAB species from the stratified surface waters of the BOB and their seasonal occurrence. Nineteen potentially harmful species which accounted for approximately 14% of the total identified dinoflagellates species were encountered in this study. Among these, the frequently occurring HAB species (FOS) were low in abundance (≤40 cell L\(^{-1}\)) and included *Ceratium furca, C. fusus, Dinophysis* sp., *Noctiluca scintillans* and *S. trochoidea*. The low abundance of FOS in the region may not be a growth issue but they may serve as inocula for future blooms if coupled with population-triggering
physical processes like eddies and cyclones in the region. In this scenario, the predominance of FOS like *C. furca* and *N. scintillans* in the BOB during cyclone-prone months, make their candidature stronger for future blooms in the region.

The regional and seasonal variations in phytoplankton community structure and the contribution of each group to the total community were analyzed based on pigment studies. Surface water samples were collected from CP and PK transects for phytoplankton and pigment analysis through High Performance Liquid Chromatography (HPLC). Using the diagnostic indices, spatial and temporal variations in surface water phytoplankton pigment distribution in the BOB were studied during the spring intermonsoon (SpIM, February-April 2007) and the commencement of the summer monsoon (CSM, May-June 2007). The Diagnostic Pigment (DP) index was defined as the sum of seven different pigments (DP= ZEA + Chl b + ALLO + 19'- HF + 19'- BF + FUC + PER); each of these pigments have their taxonomic significance. The use of the DP index was extended by Vidussi et al. (2001) and more recently by Barlow et al. (2007) to derive size-equivalent pigment indices which roughly correspond to the biomass proportions of pico-, nano- and microplankton. Thus, the DP index can be used to understand the biomass structure of an area purely based on pigment sums and ratios. Prokaryotic diagnostic pigment (ProkDP) and Flagellate diagnostic pigment (FlagDP) represents the biomass from pico- and nano- phytoplankton respectively. Diatom diagnostic pigments (DiatDP) together with dinoflagellate diagnostic pigments (DinoDP) represent the biomass of micro-phytoplankton.
The BOB is generally dominated by ProkDP followed by FlagDP. In fact, ProkDP dominated at all the oceanic stations whereas FlagDP was dominant at the near coastal stations. Changes in the pigment pattern were observed at the onset of the monsoon, indicating the influence of rainfall especially in near coastal waters. During the commencement of summer monsoon, an oscillation in the dominance of ProkDP and FlagDP was observed in the Central Oceanic Bay (COB), whereas flagellates and diatoms were dominant at the near coastal stations. Comparative studies between microscopic counts and diagnostic pigment indices suggest coupling pigment composition analysis with microscopic analysis of natural assemblages to establish valid biogeochemical and ecosystem models. Notably, the components of dinoflagellate communities could be missed by pigment analysis alone.

The community structure of phytoplankton in 2 different coastal environments was evaluated in relation to environmental settings. Seasonal studies were carried out at 2 distinct ports environments namely Mormugao Port (MPT) and Visakhapatnam Port (VPT) located along the west and east coast of India respectively. Both the study locations differed in their environmental settings. MPT, situated on the mouth of the Zuari, was influenced by tidal flushing and the SWM whereas VPT, situated on the east coast, was influenced by the North East Monsoon (NEM) in addition to the SWM. VPT was also subjected to anthropogenic pressure along with several aspects of pollution (Subba Rao and Venkateswara Rao 1980, Sarma et al. 1996). Samples were collected from surface and near bottom waters for phytoplankton analysis and for estimation of physico-chemical variables. Sampling at both the study areas were carried out on different occasions [premonsoon (May 05), postmonsoon (December
05, and withdrawal of SWM (September 06) at MPT and NEM (November-December 07, premonsoon (April 08) and SWM (August 08) at VPT]. Diatoms and dinoflagellates were analyzed separately.

The coastal micro-phytoplankton community structure from the 2 contrasting study areas (MPT and VPT) differed. The environment under the monsoonal flushing (MPT) exhibited variation in the phytoplankton community structure, resembling natural seasonal cycling. In fact, distinct seasonal trends in the diatom communities in surface and near bottom waters were observed. Seasonal changes in the dinoflagellate community were clearer in surface waters compared to near bottom waters. Mixotrophic dinoflagellates dominated in both surface and near bottom waters. However, dinoflagellates were not observed in any of the 16 stations sampled during PrM, possibly due to their tendency to migrate towards favourable surface waters. In contrast to these conditions, the influence of eutrophication on the phytoplankton community was observed in VPT, the semi-enclosed water body. This was reflected in the prevalence of a single dominant genus (Skeletonema) irrespective of seasons. Autotrophic dinoflagellates consistently dominated in surface waters; near bottom waters showed an oscillation between auto- and heterotrophic dominance.

Detailed taxonomic studies on preserved/cultured micro-phytoplankton from these 2 coastal locations revealed 2 new reporting of diatoms and 1 potentially toxic, non-thecate dinoflagellate. Though the planktonic diatom genus Skeletonema is common in Indian coastal waters, the high diversity in this genus has only recently been revealed. Therefore, it is expected that several species occur in the highly diverse
marine habitats along the Indian coastline. However, most Indian studies on phytoplankton diversity consider only *Skeletonema costatum*, the type species. In the present study, a *S. costatum* culture, raised from water samples taken from Goa was analyzed in addition to 2 preserved samples, 1 from the BOB and 1 from the Arabian Sea (AS). Samples were examined using Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The Goan culture strain was also examined using Transmission Electron Microscopy (TEM) and characterized using its LSU rDNA sequence. The Goan culture strain belonged to *S. costatum sensu stricto*. The sample from the BOB was identified as *S. grevillei* whereas the sample from the AS was identified as *S. tropicum*. The reporting of these species in Indian waters has wide-ranging implications. Firstly, it is an important blooming diatom in Indian waters. Secondly, it influences trophic dynamics by providing cues for intermittently breeding invertebrates (Barnes 1962, Desai and Anil 2005). Even though the present study involved the analysis of a limited number of samples, it highlights the need for ultrastructural and molecular taxonomic studies to unravel the diversity of *Skeletonema* in Indian waters. Regular monitoring is needed to uncover not only the diversity of this genus, but also habitat preferences and seasonal variation of the detected species.

*Karlodinium veneficum*, a non-thecate, potentially toxic dinoflagellate was isolated from Goa for the first time. This species has both toxic and non-toxic strains and due to its bloom forming capability, is known to influence co-existing phytoplankton. Its detection in the coastal waters of India was possible because of live sample analysis. This species often gets overlooked due to its smaller size (de Salas et al. 2005) (< 20
Artifacts due to fixation could be another reason; this has already been reported as a significant factor of bias in phytoplankton analysis (Throndsen 1978, Booth 1987, Stoecker et al. 1994, Mender-Deuer et al. 2001, Zarauz and Irigoien 2008).

Two experiments on *K. veneficum* isolated from Indian waters were conducted. The first experiment analyzed the effect of 4 different preservatives on characteristic morphological features of *K. veneficum*. The second experiment analyzed the allelopathic effect of *K. veneficum* on *S. costatum*, a co-existing diatom. Experiments with preservatives/fixatives suggest that it is possible to recognize morphological features of *K. veneficum* for as long as 30 days, when Lugol's iodine is used. The allelopathic effect of *K. veneficum* on the growth of the diatom *S. costatum* suggests its potential ecological implications. However, several factors influencing this allelopathic effect, for e.g., cell concentration (dose-dependence), its relation with nutrient concentrations, and the role of predation-induced defensive metabolites need to be elucidated in future experiments.

Overall, this study investigates the role of different environmental factors on phytoplankton community structure in both oceanic and coastal waters. The results of this study suggest that climatic factors (for e.g., rainfall) play an important role in the dynamics of these communities. However, anthropogenic pressures influence the seasonality in these communities in coastal waters. New reports of diatom and dinoflagellate species from the region during the course of this study and observations on their biological aspects highlight the need for further studies in this direction.