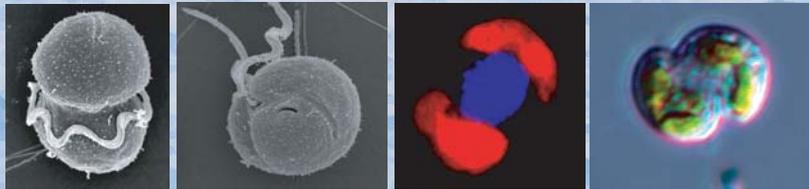


# Studies on Phytoplankton with reference to Dinoflagellates

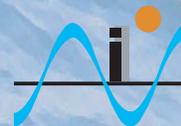


Thesis submitted to the Goa University  
for the degree of  
Doctor of Philosophy  
in  
Marine Sciences

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September, 2010

*Dedicated to my late father  
Shri. Krishna P. Naik and sister Deepa*

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# Statement

As required under the University ordinance 0.19.8 (vi), I state that the present thesis titled "*Studies on phytoplankton with reference to dinoflagellates*" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

**(Ravidas K. Naik)**

# Certificate

This is to certify that the thesis entitled “*Studies on phytoplankton with reference to dinoflagellates*” submitted by **Mr. Ravidas K. Naik** for the award of the degree of Doctor of Philosophy in Marine Sciences is based on his original studies carried out by him under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any Universities or Institutions.

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Lastly, I offer my regards and prayers to all of those who supported me in any respect during the completion of the thesis.

(Ravidas K. Naik)

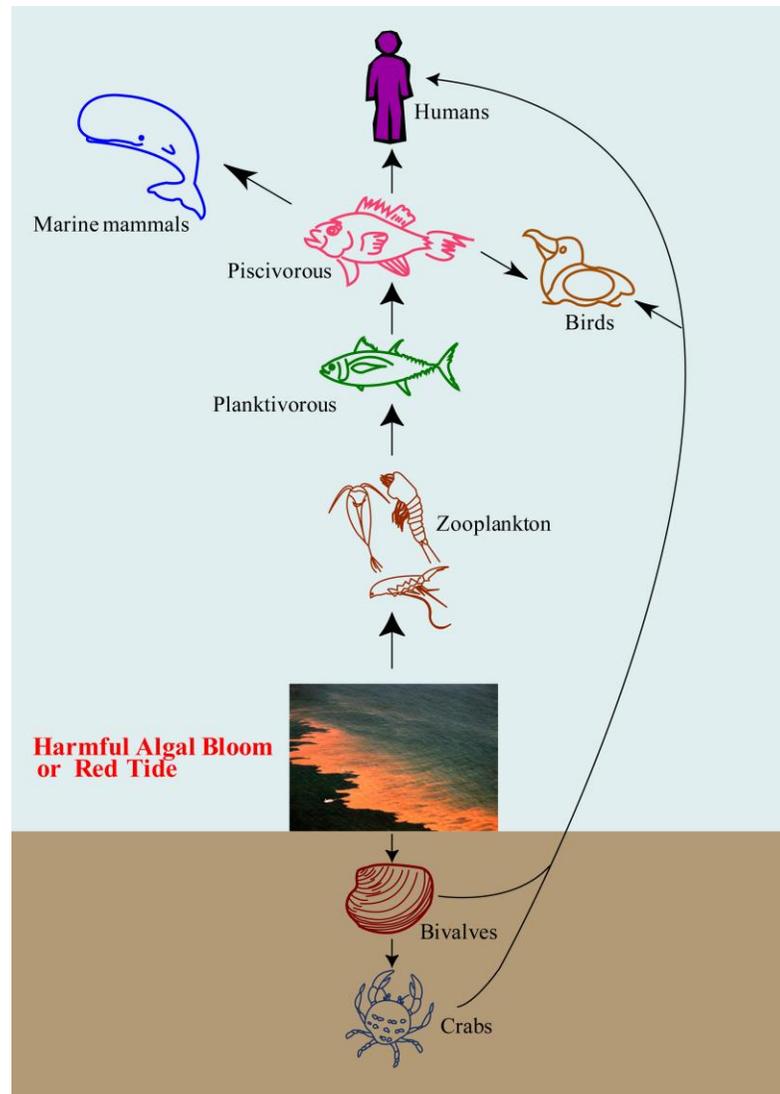
# *Chapter 1*

## *General introduction*

Phytoplankton, the microscopic, floating, plant life of all fresh and marine water bodies, has a truly global distribution. In fact, they contribute over 25% of the total vegetation of the planet (Jeffrey and Hallegraeff 1990). The word 'phytoplankton' is derived from the Greek word phyton = plant, and planktons = wanderer. On July 27<sup>th</sup> 1676, in Netherlands, van Leeuwenhoeck was probably the first person to observe marine phytoplankton under the microscope. Some 200 years after this observation, Victor Hensen, a scientist from Germany, was convinced that these microscopic organisms were the base for the marine food chain (de Baar 1994). They include diatoms, dinoflagellates, coccolithophores, red algae, green algae and blue green algae (cyanobacteria), with sizes ranging from 0.2  $\mu\text{m}$  to several millimeters. Based on size, phytoplankton can be classified into three classes: the microplankton (20-200  $\mu\text{m}$ ), nanoplankton (2-20  $\mu\text{m}$ ) and picoplankton (0.2-2  $\mu\text{m}$ ) (Table 1.1). Diatoms (Class Bacillariophyta) occur in all 3 size classes whereas dinoflagellates (Class Dinophyta) are represented in the micro- and nanoplankton groups (Jeffrey and Hallegraeff 1990).

Phytoplankton exhibit seasonal variation in abundance and type; they also vary from one water body to another, and even within a small region in the same water body. Classically, phytoplankton are recognized as the basis of all animal production in the open ocean, which fuels the food web upon which world fisheries are based. It is important to monitor these populations, since failure in abundance and timing of phytoplankton blooms can lead to collapse of fisheries (Lasker 1975). In the recent past, the importance of phytoplankton studies has moved beyond the context of

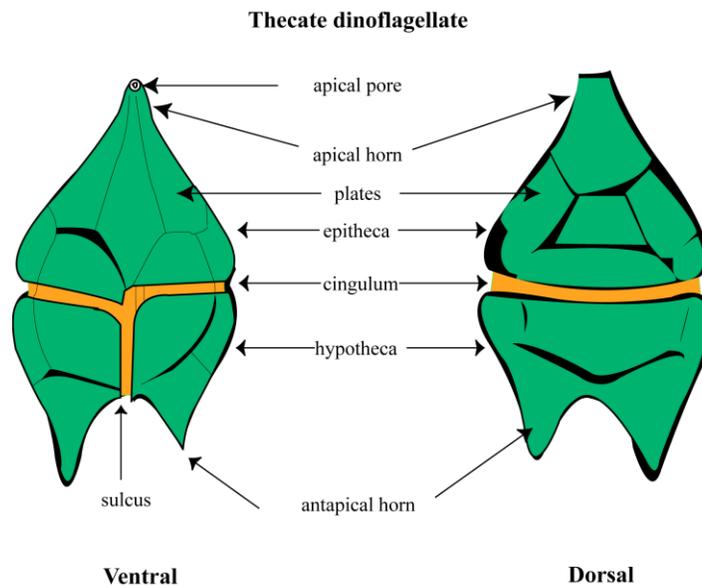
fisheries, to aspects like global warming and climate change. These studies have also extended to include the effects on human health due to Harmful Algal Blooms (HABs) (Fig. 1.1).



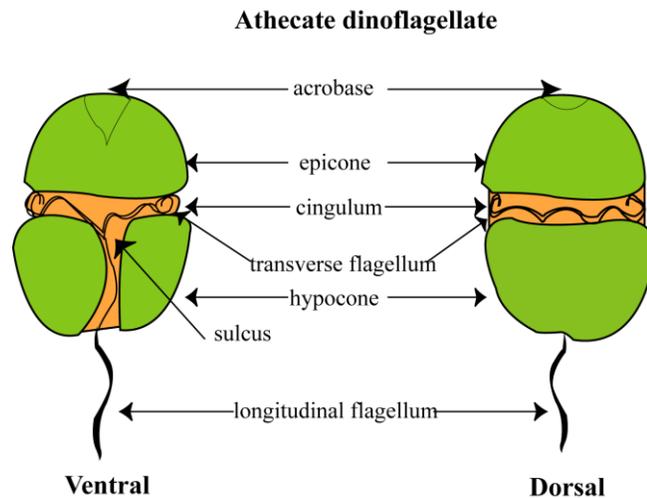
**Fig. 1.1.** The consequences of Harmful Algal Blooms (HABs) for the ecosystem and human health.

Among the total marine phytoplankton species, approximately 7% are capable of forming algal blooms (red tides) (Sournia 1995). Dinoflagellates are the most important contributors to HABs, accounting for 75% of the total HAB species (Smayda 1997). They form an important component of marine and freshwater phytoplankton. They consist of around 1,800 species, of which approximately 50 produce some sort of toxic compounds (Godhe 2002).

The biology of dinoflagellates is distinct from that of other phytoplankton groups. Dinoflagellates range in size from about 2-200  $\mu\text{m}$ , although *Noctulica* can be up to 2 mm. The cell wall is complex and variable, consisting of layer of vesicles with or without cellulosic plates. If the plates are present, the cells are called thecate or armored (Fig. 1.2).



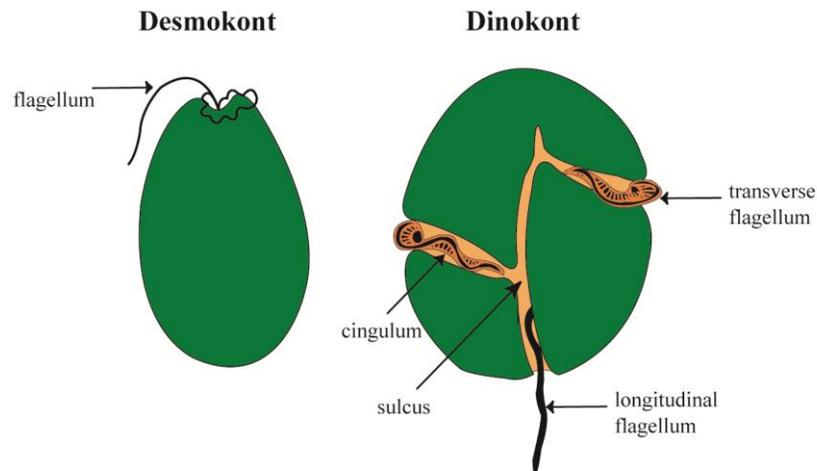
**Fig. 1.2.** Morphological details of thecate dinoflagellates.



**Fig. 1.3.** Morphological details of athecate dinoflagellates.

Dinoflagellates without cellulosic plates are termed athecate, unarmored or naked dinoflagellates (Fig. 1.3). The shape and arrangement of plates varies depending on the genus. The theca may have horns, spines or lists and the plates may be ornamented with pores, depressions, spines, ridges and reticulations. Most dinoflagellate cells are divided into two parts by a horizontal groove or cingulum. The anterior part is the epitheca (epicone) and the posterior part is the hypotheca (hypocone). The two parts may be equal or unequal size and the cingulum placement helps define genera, especially in athecate forms. A vertical groove or sulcus splits the hypotheca and may extend onto the epitheca.

Dinoflagellates are biflagellate, motile cells with two dissimilar flagella. Depending on where the flagella arise from, they can be classified into 2 groups: desmokonts and dinokonts (Fig. 1.4).



**Fig. 1.4.** Arrangement of flagella in desmokont and dinokont dinoflagellates.

In desmokonts, the flagella arise from the anterior part of the cell, as in *Prorocentrum*. In dinokonts, the flagella are inserted ventrally; the transverse flagellum is located in the cingulum whereas the longitudinal flagellum is situated in the sulcus, for e.g., *Proto-peridinium*. The transverse flagellum allows the cell to move forward or backward by spinning in circles in order to propel it in either direction. The longitudinal flagellum acts mainly as a means of steering. It also provides extra pressure to help force the cell in the direction it wants to head.

Nutritional modes of dinoflagellates vary from autotrophy (in chloroplast-containing cells) to heterotrophy (cells lacking chloroplasts) with some autotrophic dinoflagellates now known to be mixotrophs. Heterotrophic forms can obtain nutrients through various ways, ranging from direct resorption (osmotrophy), ingestion of particulate food (phagotrophy) to pallium feeding (Schnepf and Elbrachter 1992). They may also have specialized structures, such as peduncles, used

for phagocytising other organisms (Schnepf and Elbrachter 1992). Food reserves in dinoflagellates are typically unsaturated fatty acids and starch (Dodge 1973).

Reproduction is either asexual or sexual. In asexual reproduction, haploid (1N) cells divide by binary division. In sexual reproduction, haploid gametes are produced which fuse to form a diploid (2N) zygote. The zygote is a non-motile, resting stage (hypnozygote), which settles to the bottom and remains dormant for some periods of time. The zygote undergoes meiosis to produce haploid vegetative cells. The hypnozygote or resting cyst morphology is very different from that of vegetative cells.

Some dinoflagellates have been reported to form temporary cysts when cultured with certain species of diatoms (Uchida et al. 1996). This may be due to chemical stimulation or cell contact between dinoflagellates and diatoms (Uchida 2001). Chan et al. (1980) reported that filtrate extracts of the dinoflagellate, *Scrippsiella sweeneyae* was inhibitory to the diatoms – *Cylindrotheca fusiformis*, *Navicula pelliculosa* and *Nitzschia angularis*, indicating the importance of the interactions between dinoflagellates and diatoms. In addition, dinoflagellates are also affected by other factors, for e.g., allelopathy, predation and symbiosis.

Knowledge of the combined effects of different factors is essential to understand not only the spatio-temporal variations in dinoflagellate communities, but also phytoplankton communities in general. Such data represents decisive data in environmental monitoring, food-web studies and ecosystem modeling.

Phytoplankton diversity dynamics varies from coastal to oceanic environments based on the governing factors in the respective environments (Margalef 1978).

Generally, large phytoplankton tend to be abundant in turbulent, high nutrient, coastal waters, while smaller cells are prominent in stratified and/or oceanic waters (Chisholm 1992, Cullen et al. 2002). In this manner, coastal and oceanic environments represent varied environmental settings and thus support different communities. One of the processes which interlink these communities is offshore-seeding HAB-forming organisms. For example, transport of offshore-seeded *Prorocentrum* and *Ceratium* blooms to inshore waters (Pitcher and Boyd 1996). Thus, understanding phytoplankton community structure and its spatio-temporal variations in both - coastal and oceanic environments will provide the basis for interlinking studies.

In the context of the seas around India, several studies have illustrated the diversity and dynamics of phytoplankton communities (Subrahmanyam 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 the micro-phytoplankton group (diatoms and dinoflagellates) in near-shore coastal waters. 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 to analyze the spatio-temporal variations in phytoplankton communities in the open ocean using the 'ships-

of-opportunity' programme. Considering the limitations of microscopic methods, pigment analysis was included along with routine microscopy to quantify the contribution of smaller phytoplankton groups in oceanic waters. Efforts were also made to elucidate the characteristics of the phytoplankton communities in coastal habitats by evaluating two diverse habitats of Mormugao and Vishakhapatnam ports.

In the process of such evaluation, the effect of preservation on morphology of dinoflagellates and on their quantification was carried out with *Karlodinium veneficum*, a newly reported, non-thecate dinoflagellate from Indian waters. Subsequently, the influence of its culture filtrate and cell extracts on the growth of *Skeletonema costatum*, a key stone species in Indian waters (Patil and Anil 2008, D'Costa and Anil 2010) was evaluated.

The studies carried out are presented in the following chapters:

- *Spatio-temporal variation in surface water dinoflagellates in the Bay of Bengal*
- *Primary description of surface water phytoplankton pigment patterns in the Bay of Bengal*
- *Micro-phytoplankton community structure at Mormugao and Visakhapatnam ports*
- *Effect of preservation on the morphology of Karlodinium veneficum, a non-thecate, potentially harmful dinoflagellate and allelopathy in relation to Skeletonema costatum*