Chapter 1

General Introduction
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Phytoplankton are single celled microscopic algae that make up the floating pastures of the aquatic system (phyton-plant; planktos-wandering). They provide the food base, which supports, either directly or indirectly, the entire animal population of the aquatic ecosystem and they also contribute significantly to climatic processes. Phytoplankton are represented by diverse algal groups with size ranging from 0.2 microns to several millimetres. They include diatoms, dinoflagellates, phytoflagellates, coccolithophorids, red algae, green algae and bluegreen algae (Cyanobacteria). In the coastal marine environment, diatoms dominate the phytoplankton community.

Diatoms are one of the most beautiful organisms to look at under a microscope! They are unicellular and eukaryotic microorganisms that form an important component of the aquatic ecosystem. Diatoms are protists belonging to phylum Bacillariophyta and class Bacillariophyceae. Their cell walls are shaped like tiny glass pillboxes, with an amazing array of sizes, shapes and ornamentation. The rigid silicate frustule's encases the vegetative protoplast. There are two basic body shapes of diatoms based on symmetry, centric (round with radial symmetry, Order Centrales) and pennate (thin ellipse with bilateral symmetry, Order Pennales) (Fig. 1) and either may be found in the plankton or on the benthos. While the pennate diatoms are solitary cells (although often living in dense assemblages or even forming tubes together), the centric diatoms may be solitary or chain forming, linked by projections from their cell wall or membrane. These two major taxonomic divisions also reflect a major ecological difference (Round 1971). Centric diatoms are mainly holoplanktonic or meroplanktonic, with only a few genera that are associated with substrates throughout their life cycles. Araphidineae (pennate diatoms without a raphe system) and Monoraphidineae (pennate diatoms with one raphe system on one valve) are attached to sand grains, rocks and biological substrates,
Fig. 1. Schematic diagrams of centric and pennate diatoms with main features (Hasle and Syvertsen 1997).

whereas genera of the Biraphidinae (pennate diatoms with two raphe systems on both valves) are almost completely attached to mud with only a few planktonic species. They are the major primary producers (both pelagic and benthic environment) and are thought to be responsible for up to 25% of the world’s net primary productivity (Jeffery and Hallegraeff 1990) whereas total phytoplankton account for up to 40% of the global primary production (Falkowski 1994). At least some can live heterotrophically in the dark, if supplied with a suitable source of carbon (Round et al. 1990). Dinoflagellates form the other important component of the marine and freshwater phytoplankton.
Dinoflagellates (dinos-whirling) are a remarkably diverse and complex group of unicellular flagellates. About half the species are photosynthetic; while others have heterotrophic nutrition. Some species have attracted attention because of their beautiful bioluminescent properties or their role in producing either toxic or non-toxic blooms (Hallegraeff, 1993). Till now, considerable amount of work on modern dinoflagellate cysts taxonomy (Matsuoka & Fukuyo 2000) and viable banks of cysts have been reported from marine sediments all over the world (Blanco 1990, Nehring 1996, Anderson 1997, Sonneman and Hill 1997, Godhe et al. 2000, Persson et al. 2000)

Diatoms are ubiquitous in the aquatic habitats. They are abundant in the phytoplankton and phytobenthos of marine and freshwaters, whatever the latitude. Within aquatic habitat diatoms further extend their habitats from the pelagic to benthic environment and are also found to colonize various submerged living and nonliving surfaces. In the pelagic and benthic environment the growth and behaviour of the diatom community structure is controlled by various environmental conditions.

Planktonic diatoms are invariably as dense as or denser than water; they also lack any form of propulsion. In non-turbulent water, they will usually sink quickly out of the photic zone and be deposited onto the sediment. Sinking rates are related to cell size and shape, colony size and physiological conditions. In general, sinking rates increase with cell and colony size and are greater in senescent populations. Diatom life cycle includes vegetative, sexual and resting stages. Normally, diatoms reproduce by vegetative division. Most diatoms undergo size reduction during vegetative division, and to restore maximum cell size they must occasionally interrupt vegetative growth with a sexual cycle, which restores maximum cell size. The restoration of cell size by sexual reproduction is a unique feature of diatoms (Drebes 1977). Centric and pennate diatoms
differ somewhat in the details of sexual reproduction, but in both groups, zygote formation is followed by the formation of an enlarged cell called an auxospore, which then develops into a vegetative cell whose dimensions are near the maximum size characteristics of the species.

Many diatoms undergo dormancy by forming specialized resting stages. The formation of resting stages as survival alternatives in coastal diatoms is now a well-known phenomenon, although research in this area is sparse. There are two types of diatom resting stages, resting spores and resting cells. Resting spores are heavily silicified stages that are morphologically distinct from the vegetative cells, whereas resting cells are similar to the vegetative cells with altered physiological and cytoplasmic characteristics. Resting spore formation is more common in marine centric diatoms and is rare in pennates. But resting cell formation is observed more often in pennates and freshwater diatoms (Sick-Goad et al. 1986, Round et al. 1990). Among the resting spores three types of spores (Fig. 2) can be distinguished following Ross et al. 1979: 1. Exogenous — mature resting spore not enclosed by its parent frustule, 2. Semiendogenous — one valve of the mature spore enclosed within the parent cell and the other valve free, and 3. Endogenous — mature spore completely enclosed within the parent cell. A number of external factors have been found to induce spore formation or to exert effects upon spore development. These include the availability of various nutrients (N, P, Fe, Si), temperature, light, and pH. In almost every species, nitrogen deficiency appears to be an effective and usually, the most effective or even the sole inducer. Not all clones of spore forming species seem to be capable of spore formation (Hargraves and French 1983, Hargraves 1984) or perhaps different clones are induced to produce spores by different
Fig. 2. Formation of vegetative cells, resting cells and resting spores from a vegetative parent cell. *Thalassiosira nordenskioeldii* is an example of a species forming all three types of resting spores (Hasle and Syvertsen 1997).

Environmental condition. Furthermore, not all cells of a clone are necessarily able to form spores. Only cells of a restricted size range that have the potential to become gametangia and in some centric diatoms, only particular stages in the size reduction cycle can produce spores. Once formed, the resting stages behave as fine silt particles, often accumulating in high numbers on the sediment bottom. These resting stages can withstand periods of unfavourable conditions on annual and decadal scales to survive mass extinction and have been regarded as benthic. They are traditionally considered as overwintering forms, which could provide stocks for subsequent and seasonal blooms as well as for species dispersal (Lund 1954, French and Hargraves 1980, Garrison 1981, Hollibaugh et al. 1981). Wind velocity, currents and tides are the other important
physical forcings that resuspend the benthic phytoplankton along with sediments thereby influencing the phytoplankton community (de Jonge 1995, de Jonge and van Beusekom 1995). In order to understand the bloom and the resting stage formation in diatoms in general, it is important to investigate the transitions between the planktonic and resting stages of the species, and the factors influencing these transitions. In situ evaluation of these factors will further enhance understanding of their role in diatom life cycles like survival strategies, prediction of blooms and species succession. Phytoplankton community structure has an important role in determining the ecosystem functioning and trophic dynamics. Studies to this effect have already shown in other regions of the world and its importance (Chiba and Saino 2002, Nielsen and Hansen 1999, Devassy and Goes 1988)

In addition to free-floating mode of life, the diatoms are also known to colonize a variety of living and nonliving surfaces submerged in aquatic environments and these are mostly benthic diatoms. Benthic diatoms are much less understood ecologically as compared to planktonic diatoms. The benthos is more diverse than the plankton, both in terms of number of species and the life forms present (Round et al. 1990). Unfortunately, the benthic diatom communities and their environments are far more difficult to sample and quantify than those of the plankton, so ecologists have largely ignored them.

There are various modes of attachments to the substrates but these fall in two categories:
1. the adnate i.e. closely appressed to the substratum, as in Cocconeis, Amphora, Epithemia, and 2. the cells are pedunculate i.e attached to the substrata by stalks or pads e.g. Cymbella, Grammatophora, Licmophora, Achnanthes. The adnate forms are rarely colonial whereas the pedunculate often form colonies. The development of pedunculate forms on the surface transforms the structure of the community from two-dimensional to
three-dimensional. Within the microscopic forests produced, some species can be seen to be canopy formers, whereas others form the shrub and field layers (Round et al. 1990). These communities are probably the most complex in which the diatoms play a major role.

The attached communities may be classified according to their substrata; thus epipelic, epipsamnic, epilithic, epibiotic and fouling communities may be distinguished, growing on mud, sand grains, rocks, biological substrates (such as aquatic plants, molluscs, crustaceans, marine reptiles, marine mammals, marine birds) and the objects placed in the sea respectively. There are overlaps between these communities in the species they contain and, except for the epipsammon, all contain very similar life forms. Raphid diatoms, being abundant in most benthic habitats, are very often the early algal colonizers of natural and artificial substrata, where they adhere and produce copious quantities of adhesive mucilage during the construction of microfilms i.e. primary film. Microfilm formation is an important phenomenon occurring on surfaces immersed in the aquatic environment. The term microfilm, also referred to as biofilm, is highly variable in time and heterogenous in composition. Microfilm mainly comprises of adsorbed macromolecules, attached bacteria and diatoms, with all of these components enmeshed in a matrix of extracellular polymers. Microfilm formation on a marine substratum modifies its surface chemistry (Characklis and Cooksey 1983) and strongly influences the development of a macrofouling community. Earlier studies revealed that large numbers of diatoms are present in microfilms on wetted and illuminated surfaces but only few studies have focussed on the occurrence of diatoms in the microfilm and their role in subsequent colonization of macrofoulers in the marine environment (Rao 1990). In Indian waters, the work related to biofilm has been done by Daniel (1955), Mathew and Nair (1981), Karande (1987 & 1989), Kelkar (1989), Bhosle et al. (1990 a & b),

Diatoms growing attached to surfaces of living organisms i.e. plants and animals are termed as epibiotic diatoms. A variety of organisms form hard substrata in the benthic marine environment. When free space is not available, one organism (epibiont) grows on the other (basibiont). This process is defined as epibiosis. Adaptation to epibiosis by basibionts arises from three ways: tolerance, avoidance and defence (Wahl 1989). Many sessile marine organisms such as sponges, corals, bryozoans, holothurians, ascidians and mobile organisms such as most of the crustaceans, sea weeds and turtles keep an essentially clean body surface in the face of competition for space by epibionts (Davis et al. 1989; Wahl 1989; Caine 1986, Clare et al. 1992). Horseshoe crab is also one such animal which acts as a moving substrata for simple to complex communities of small marine organisms like green algae, diatoms, coelenterates, mussels, oysters, barnacles, bryozoans etc. in a densely populated marine environment. *Tachypleus gigas* (Müller) is the most abundant horseshoe crab species available along the Orissa coast (India). Adults reach terminal anecdysis once sexually mature and live with their carapace for 4 to 9 years. Mikkelsen (1988) reported that horseshoe crabs probably keep their surfaces clean from ectocommensals and epiphytes. He also reported that females carry less number of epibionts than males indicating a potential gender difference. Epibiont overgrowth is controlled by employing either one or a combination of ecological, physical and chemical defence mechanisms (Wahl et al. 1994). Studies unravelling the epibiotic chemical defence of marine organisms can provide insights into the development of novel, ecofriendly antifouling compounds and strategies.
Attachment of diatoms to toxic and non-toxic surfaces immersed in illuminated environments is invariably associated with the production of sticky exopolymers/mucus (Chamberlian 1976, Cooksey et al. 1984, Daniel et al. 1987, Hoagland et al. 1993). It has been shown that this polymer is synthesized in the Golgi apparatus, packaged into vesicles (Daniel et al. 1980) and secreted from the plasma membrane at the raphe slit (Webster et al. 1985). Exuded polymers are assembled into a variety of structures such as trails (material left behind motility), sheaths (organic matrices tightly associated with the cell wall), capsules (organic matrices loosely attached with the cell walls), and stalks (permanent attachment structures) (Hoagland et al. 1993). Once attached, the cells divide rapidly giving rise to colonies that eventually coalesce to form a compact biofilm, which may achieve 500 µm thickness (Callow and Callow 2002). Previous studies have shown that the biofilm and exopolymers act as a source of positive or negative cues for larvae of fouling organisms and can thus play an important role in the macrofouling cycle. Understanding the role of the diatoms and their exopolymers in the settlement and metamorphosis of fouling invertebrate larvae will give new dimensions to biofouling studies.

In this study on the ecology of diatoms, the following aspects have been addressed and are presented as

➢ Diatoms in the pelagic and benthic environment

➢ Diatoms in the microfilm

➢ Diatoms in the epibiotic community

➢ Influence of diatoms on cyprid metamorphosis of *Balanus amphitrite*