Chapter 6
Summary
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Diatoms constitute an important part of the microphytobenthic community in the intertidal sand flat. Intertidal sandflats are dynamic environments, where the tidally generated water movement and the associated processes of deposition and resuspension of sediment affect the composition and distribution of diatoms. In many studies, diatoms were only investigated in the top few centimeters of the sediment (Rizynk et al. 1978; Colijn and Dijkema 1981; Varela and Penas 1985; Lukatelich and McComb 1986). However, the presence of diatoms at a depth of 20 cm has also been reported (Steele and Baird 1968; Colijn and Dijkema 1981; de Jonge and Colijn 1994), based on chlorophyll a estimations. In intertidal sandflats, a number of factors may be responsible for displacing the diatoms from the surface sediment layers to the deeper layers. Temporal and spatial variations in the viable diatom population of the microphytobenthic community revealed the presence of diatoms up to a depth of 15 cm all along the intertidal zone, from the low tide to the high tide zone. Their rejuvenation in culture revealed that their viability was not affected by the conditions prevailing at this depth. This depth harbored not only the pennate (epipsammic and epipelic) diatoms, some of which are permanent residents of this area, but also the centric diatoms of planktonic origin. The occurrence of diatoms such as *Amphora* and *Navicula* throughout the year in the sediments indicated that the two pennate forms were natives of this area. The appearance of the centric diatom, *Thalassiosira* only upon incubation indicated the presence of their resting stages. These resting stages must have been carried to the study area with coastal sediments and redeposited in intertidal sediments.

A number of physical, biological and chemical factors may be responsible for the temporal and spatial variation in the diatom abundance, diversity, diatom richness and
evenness. Grain size fractions which, served as predictors of some diatoms, differed with depths at the low, mid and high tide zones. This depicts that factors other than grain size have a role to play in the temporal and vertical distribution of diatoms.

Wind stimulated the resuspension of the sediment along with pennate diatoms up to 5 cm depth only at the low tide zone. The depth up to which chlorophyll $a$ could act as indicators of both, the pennate and centric diatom abundance reduced from 10 cm depth at the low tide zone to 5 cm at the mid tide zone and 0 cm at the high tide zone.

In the 0-5 cm depth, the ability of the pennate diatoms to remain attached to the substrate may play an important role in this contribution whereas in centric diatoms they may be the ones brought onto the sediments by the incoming tides. In the 5 to 10 cm depth at the low tide zone, the positive correlation with chlorophyll $a$ revealed that resuspension was not effective up to this depth and the stock was securely placed.
However, a negative correlation of chlorophyll $a$ with diatoms in the 10-15 cm depth and below 5 cm depth at the low and mid tide zones respectively, even when viable diatoms were found in appreciable numbers, suggests survival of these diatoms below physical disturbance level through the adoption of survival strategies such as heterotrophy or resting stage formation. The non-significant relationship at the high tide zone throughout the 15 cm depth may be due to the effects of desiccation when exposed for longer durations. However, they were viable as primary producers, when resurfaced and could play an important role in the benthic community (Fig. 6.1).

Vertical migratory behavior of benthic diatoms is one of the adaptive strategies employed for a life in intertidal habitats. This self-initiated migration helps diatoms to move to the surface during low tides for photosynthesis and move down during high tides (Round and Palmer 1966; Round 1979; Joint et al. 1982; Paterson 1989). There exists a controversy regarding the reason for the vertical migration and the factors affecting it. Irradiance and tidal rhythm are the two variables considered to be governing the vertical migration (Round and Palmer 1966). Experiments carried out to delineate the influence of these factors in a tropical intertidal sand flat revealed that rising to the sediment surface for fulfilment of their light requirements for photosynthesis was the first priority. If not fulfilled during the low tide exposure, diatoms could withstand the tidal effects and stay up at the surface even during the high tide coverage. In summer, the surface cell abundance of epipelic diatoms was high only during the morning low tide whereas in winter it was found to be the highest during the mid-morning high tide and continued to be so even during the following evening low tide. This ability of microphytobenthos to migrate vertically within the surface sediment when their requirements are fulfilled may be considered as a form of behavioral photoacclimation, allowing cells to avoid potentially
damaging irradiance and temperature conditions (Kromkamp et al. 1998; Perkins et al. 2001). In the laboratory experiments wherein the effects of tides were removed, the endogenous clock continued to operate in a similar fashion as that in the field when provided with 12 h light: 12 h dark condition whereas continuous darkness brought in a tidal rhythm. Expression of a tidal rhythm may be attributed to an innate behavior. This behavior is otherwise superimposed by the diel rhythm in the presence of light. In continuous light, diatoms preferred to stay up at the surface longer than that observed in field. This indicates that there is an optimum duration up to which the diatoms can remain exposed to light. The above observations reveal that irradiance has an overriding effect over tides. Temporal differences in the irradiance and the resulting changes in diatom migration can have implications in the littoral primary productivity.

In this entire process of vertical migration, an important factor that needs consideration is the impact of physical forcing on the sediment caused by wind or tidal currents. Turbulence and shear stress generated by the incoming tide lead to suspension of the diatom cells into the overlying waters (Baillie and Welsh 1980; Delgado et al. 1991; de Jonge and Van Beusekom 1992, 1995; de Jonge and Van den Bergs 1987). This may be an additional factor responsible for the lowering of the surface diatom population, other than their positive geotrophic movement during immersion. However, the entire suspended population will not be lost to water column, since a part of it will start resettling at the beginning of emersion. This process will in turn contribute to the rise in cell numbers at the sediment surface during subsequent exposure, along with the upward migration from the deeper sediment layers.
Presence of diatoms in the water column is also reflected in the microfouling population of different types of substrata immersed in the sub-surface estuarine waters of the present study area. Diatoms, the early autotrophic colonizers, are an important constituent of the biofouling community in the marine environment. The diatom populations in the surrounding environment and that in the fouling community revealed that the diversity is not evenly reflected. Pennate diatoms were abundant in the fouling film than centric diatoms while the reverse was evident in case of the water column. This difference is attributed to the capability of the pennate diatoms to attach to surfaces with the help of a raphe. The diatom populations, both in the water and the biofilm were dominated by the pennate diatom, *Navicula delicatula*. The distribution of *N. delicatula* in the water column and the biofilm was found to be independent of the distribution of the other diatoms. In the surrounding waters, 32 genera (20 centrics, 12 pennates) including 50 species (28 centrics, 22 pennates) were encountered. The abundance and diversity changed with the substratum. It was found to be higher on polystyrene than on stainless steel. Species such as *Coscinodiscus concinnus* and *cymbella* sp. were found exclusively on polystyrene whereas *Pinnularia* sp. was encountered only on stainless steel (Fig. 6.2). Such substratum influences can differ with organisms and would need careful consideration in determining the factors that govern the diversity of microbial films.

Although, a dominant pennate benthic diatom of the microphytobenthic community, *Navicula delicatula* representing the water column as the most abundant form indicates that it can extend its niche from the intertidal habitat to the ambient waters. Most of the pennate diatoms which are primarily encountered on the bottom sediments, are often found in the water column (de Jonge et al 1992; Tomas 1997).
through resuspension and it seems reasonable to assume that their primary production in the water column is as effective as it is on the tidal flats (de Jonge et al. 1995).

![Diagram of fouling diatoms on polystyrene and stainless steel](image)

**Fig. 6.2 Variations in the fouling diatom community over polystyrene and stainless steel**

However, the other dominant diatom, *Amphora coffeaeformis* restricted its distribution to the intertidal sediments. Such a distribution reveals species-specific differences in habitat selection.

Space as a resource is important to periphytic diatoms. However, limited availability of space as compared to the vast diversity of species, leads to intense competition for this resource. Thus, diversity is controlled by the competitive strategies employed by each member of the community, whether a pioneer or a late arrival. In this struggle for existence, the fittest species can carve a better niche for itself, by exhibiting competitive traits. The community structure is influenced by gain and loss processes
and competitive ability of each member. In field, the gain factors are immigration of fresh recruit and their multiplication while the loss factors are grazing, death and sloughing off. The study carried out to evaluate variations in the marine periphytic diatom diversity by eliminating fresh recruitment and grazing, components of the gain and loss processes respectively revealed three cases. These cases illustrated the influences of intergeneric competition (case I), simultaneous inter and intrageneric competition (case II) and competitive exclusion or co-existence (case III) (Fig. 6.3).

In case I, where *Navicula delicatula* and *Amphora coffeaeformis* were the major players, exclusion of *N. delicatula* was observed below the substratum carrying capacity levels, indicating a role of nutrient adequacy or allelopathy. Laboratory
experiments revealed that the competitive influence of the population of *A. coffeaeformis* over *N. delicatula* is independent of the initial cell density, with capability to overtake its competitor species even at 1% initial inoculum. Time required by *A. coffeaeformis* to overtake *N. delicatula* reduced from 7 days (1% initial inoculum) to 3 days (20% initial inoculum). In case II, intrageneric competition was observed among three species of *Amphora* i.e., *A. turgida, A. hyalina* and *A. coffeaeformis* wherein the success of *A. turgida* was not influenced by the nutrient availability whereas that of other two species was nutrient dependent. *A. turgida, A. hyalina* and *A. coffeaeformis* can co-exist in nutrient enriched conditions, where the common nutrient supply is sufficient, whereas in nutrient limiting conditions, according to the resource competition theory, only one of the three species of *Amphora*, i.e., *A. turgida* proved to be a successful competitor. This species could possibly sequester the limiting nutrients at a faster rate thus making it unavailable to the other species of *Amphora* i.e., *A. hyalina and A. coffeaeformis*. This species was found to co-exist with *N. delicatula*, which could be due to a difference in nutrient requirements. A simultaneous intergeneric competition between *N. delicatula* and three species of *Amphora* was not nutrient dependent. In case III, capability of *Nitzschia longissima* to eliminate the other components was positively influenced by nutrient availability. Paucity of nutrients supported richer diversity. These three cases also illustrated that, competitive traits of a periphytic diatom species is switched on at an appropriate cell density ratio of the competitor and target species. Such traits will determine the community variations in oligo, meso and eutrophic conditions.

Studies also showed that exposure to low temperature can result in morphological changes in diatoms. Low temperature influences the survival capabilities, which differed with species. *A. coffeaeformis* turned out to be a better survivor to low
temperature than *N. delicatula*. This study has implications in cryopreservation of these diatoms (Fig. 6.4).

![Diagram showing influence of low temperature on N. delicatula and A. coffeaeformis](image)

**Fig. 6.4 Influence of low temperature on *N. delicatula* and *A. coffeaeformis***

Study of life cycle in *Amphora coffeaeformis* and *Navicula delicatula* revealed that sexual reproduction other than a mode of regaining normal cell size could be induced on sudden exposure to stress conditions such as salinity variations in pennate diatoms. This may be a type of survival strategy adopted to overcome stress conditions.

Generally morphological characters are used to classify diatoms to species level. Frustule morphology, however, can change with environmental and culture conditions. Also, considerable time and effort are required to identify a particular species when different morphological characteristics are difficult to distinguish under the light microscope. An alternative to microscopy identification is the use of molecular probes, which can bind to either internal or external sites on the target species and be visualized using fluorescence techniques. The specificity of the antigen-antibody reaction provides a powerful tool for the study of individual microorganisms in their natural environment. *Navicula delicatula* is a pioneer and most dominant diatom encountered in the biofilms. Antibodies directed against cell surface antigens of *N. delicatula* were developed for an easier and quicker identification and to trace the relative abundance of this pennate diatom.

The antiserum could successfully label 100% of *N. delicatula* cells from culture, which was used to develop antisera. No cross reactivity was observed with unialgal
cultures of the same genera. However, on an average 50% of *N. delicatula* cells were labelled by the antisera among the field populations observed (Fig. 6.5). It is evident that this method is not completely reliable for population studies and needs further validation. However, an important and interesting fact, which surfaced, is the occurrence of more than one 'serotype' of a particular species in the natural population. This indicates genetic diversity among the species and gives scope for population genetic studies and needs further attention.