Chapter 2

Review of Literature

Introduction

Phytoplankton occupy the pivotal role of marine primary production, the foundation of all the consequent biological, chemical, many geological and ecological processes. The gamut of climate variability, cloud condensation processes, atmospheric exchanges of many gases, including those of greenhouse ones is within the ambit of the biological activities of phytoplankton. In this regard, determination of time-varying plankton distribution and dynamics in the world ocean has been one of the main goals of biological oceanographers from beginning the mid-19th century (Barber and Hilting, 2002). Early studies in the late 1800s (starting with the expeditions of the Challenger from 1872-1876) and early 1900s focused on the distribution of regional phytoplankton species, and led to the concept of plankton elements. Initially proposed by H.H. Gran (vide Semina, 1997), a plankton element is a phytoplankton assemblage characterized by an indicator species whose presence signified a distinct water mass upon which the plankton-types were dependent. As knowledge of local and regional distribution of phytoplankton taxa gradually increased during the twentieth century, their global distribution pattern began to emerge. Smayda (1958) was among the first researchers to compile and map the global distribution of diatom species, and speculate on the underlying factors controlling their distribution. Margalef (1961) proposed a method to classify the distribution of phytoplankton, also based on indicator species. The method was
based on known relationship between species and abiotic factors such as temperature, salinity and nutrients. Around the same time, researchers from the Soviet Union compiled the maps of global pattern of phytoplankton distribution using data from over 2000 stations, and generated global occurrence and distribution picture for numerous species (Semina, 1997).

**Importance of Phytoplankton Community**

It has been of great interest during the past decades, to evaluate the response of phytoplankton to physical forcing and nutrient availability to understand the ecosystem and to establish better links with climate changes (Margalef, 1978; Cloern and Dufford, 2005). Species composition and population density of phytoplankton are sensitive to environmental changes, to the point that certain species, and sometimes entire communities, can serve as bio-indicators of environmental conditions (Margalef, 1978; Gameiro and Brotas, 2010). The continual documentation of phytoplankton population dynamics can provide an invaluable record of water quality, can signal if radical changes have occurred within an estuarine system, and can offer clues to the causes of changes when they do or do not occur (Margalef, 1978; Spatharis et al., 2008).

Planktonic algae affect concentrations of dissolved gases (oxygen and carbon dioxide), concentrations of dissolved inorganic and organic substances, and pH (Cloern and Dufford, 2005). Phytoplankton photosynthesis is reported fix up to 50 Giga ton of carbon per year, contributing nearly half of global primary production (Falkowski et al., 1998).
The composition of the phytoplankton is reported to control the efficiency of the biological carbon pump in the ocean (Dandonneau et al., 2002). Dandonneau et al. (2002) stated that the fraction of marine primary production that sinks to depth (export production) depends on the phytoplankton species that are present in an area. The larger species (diatoms, dinoflagellates) are responsible for the export of carbon, whereas most of the production can be attributed to the smaller species (cyanobacteria), with that production being rapidly recycled in place. Therefore, knowing the species composition of the phytoplankton community can be a useful tool for determining the carbon fluxes in an area. Schlitzer et al. (2002) also described how the dominance of diatoms in certain areas can be associated with higher export efficiency of carbon and nitrogen than dinoflagellate-dominated regions. All these findings confirm that floristic composition, even defined at the species level, is an important state variable to determine surface fluxes and its variation in response to climate change.

Finally, the photosynthetic fixation of inorganic carbon by phytoplankton offers a source of organic carbon and energy for higher trophic levels and ultimately determines the success of fisheries (Cloern, 1979). The understanding of phytoplankton dynamics is, therefore, central to the comprehension of how estuarine ecosystems work and respond to stresses imposed by man and nature. Understanding the dynamics and structural composition of the phytoplankton communities over annual and decadal time-scales in estuarine and coastal areas can offer a better insight of the influence and effects the variations in biological oceanographic processes. In these environments, diatoms followed by
dinoflagellates are the most important groups dominate the phytoplankton community.

**Studies on Phytoplankton in the Estuaries**

Estuaries are transition zones linking freshwater and marine systems, and are therefore characterized by gradients of chemical, physical and biological components in the water column. They act as filters that trap both natural and anthropogenic materials transferring from the continents to the open sea. Salinity is one of the major abiotic factors which is be highly variable in estuarine ecosystems (Remane, 1958; Gasiunaite, 2000). In typical estuaries, changes in salinity within short time periods are usually considerable due to tidal actions. Phytoplankton dynamics are also dependent on physico-chemical and biological factors which have been shown to exhibit pronounced seasonal variations due to climatic factors (Schiewer, 1998).

Estuaries are one of the ecosystems most influenced by human activities in recent time. Anthropogenic inputs of nutrients, qualitative and quantitative changes in freshwater input and modification of shoreline possibly affect the balance of the ecosystem, sometimes referred to as the ecosystem health. Increased nutrient concentrations and loadings, particularly in the form of dissolved organic and inorganic nitrogen, can facilitate phytoplankton growth in nitrogen limited estuaries. Community structure and trophic transfer of carbon and energy derived from phytoplankton can mediate or amplify the effect of nutrient-loading and eutrophication as the material is exported or remineralized, respectively. Whether a
phytoplankton cell is exported or remineralized within the microbial loop (Azam, 1983) is, to a large extent, driven by the size-structure of the phytoplankton community (Legendre and Rassoulzadegan, 1995).

Seasonal and interannual variability of phytoplankton biomass, community composition, and distribution can differ markedly among estuarine habitat types (e.g. Subramanyan, 1959; Devassy and Goes, 1988; Cloern, 1991; Cloern and Dufford, 2005; Mitbavkar and Anil, 2008; Patil and Anil, 2008). These authors found strong relationships between phytoplankton dynamics and species composition, and the physicochemical, hydrographic, and morphological characteristics of an area. Variables like temperature, salinity, nutrient availability, turbulence, tides, and river inflow can define the type of phytoplankton community and the production of phytoplankton biomass are reported. For example, Cloern (1991) and Shiah et al. (1996) reported that in coastal and estuarine environments, the inter-annual variability of the phytoplankton communities and primary production in the locations they studied were driven by the variability of annual precipitation, delivery of nutrients, and river discharge. Cloern and Dufford (2005) affirm that changes in temperature and salinity combined increase stratification in the water column and cause slow circulation that controlled nutrient gradient patterns and primary production. Garibotti et al. (2005) emphasized that vertical mixing in the water column was one of the major factors regulating phytoplankton stocks. Wawrik et al. (2003) stated that the seasonal cycle of phytoplankton abundance was mainly controlled by the availability of sunlight and by nutrients supplied from below or laterally by currents or river discharge. Agawin et al. (2000)
suggested that population sizes and types of phytoplankton resulted from the combined effects of temperature and nutrients. Dandonneau et al. (2002) observed that yearly changes in local dynamics shaped the phytoplankton community structure and biomass.

On the other hand, various studies on phytoplankton communities in estuaries have concluded that diatoms are the most important taxonomic groups, either in terms of abundance or in terms of diversity or both (Adolf et al., 2006; Gameiro et al., 2007). Diatoms can survive in systems with a high turbidity and shorter water retention times (Lionard et al., 2008). These communities are composed of dynamic multi-species assemblages characterized by high diversity and rapid successional shifts in species composition in response to environmental changes. Identifying the ecological variables that regulate the seasonal succession of diatom communities is essential to understand the consequences of eutrophication and climate change (Mendes et al., 2009). The distribution of diatoms has been found to reflect the average ecological conditions of water (Cholnoky, 1968). Further, diatoms have been shown to be positively correlated with nitrogenous compounds (Cholnoky, 1968), particularly nitrate nitrogen (Blum, 1957). The pattern of diatom distribution could thus be a useful guide to the status and degree of pollution of sewage in any water body. Beyond that, phytoplankton composition and abundance are intimately linked to higher trophic levels through grazing by herbivores and cascading effects on ecosystem trophodynamics (Urrutxurtu et al., 2003).

In many estuaries and coastal areas, seasonal patterns of phytoplankton community-composition are characterized by a spring diatom bloom (Domingues and Galvão,
2007) or a late autumn/winter spring diatom bloom (Adolf et al., 2006; Lopes et al., 2007). Diatom abundances usually decreases in the summer, due to Nitrogen (N) and/or Silicate (Si) limitation (Domingues and Galvão, 2007), and pelagic and benthic grazing (Domingues and Galvão, 2007; Mendes et al., 2009).

**Studies on Phytoplankton and Nutrients**

Different phytoplankton types require different nutrients in different amounts. In order to photosynthesize, phytoplankton must have macronutrients such as carbon (C), nitrogen (N), phosphorus (P), silica (Si; for diatoms only), and micronutrients such as Fe, among a host of other mineral salts. Thus, the growth rates of phytoplankton are often limited by several nutrients, not just one, and this is based on species composition and abundance (Brand, 1991). One of the important theories involving phytoplankton growth is Liebig's Law of the Minimum, which states that organisms will become limited by whatever resource is in the lowest supply compared to their needs (Von Liebig, 1840). The nutrient stock being transformed into biomass significantly decreases when a phytoplankton bloom occurs. In the same way, nutrient availability usually limits species growth at the end of the bloom (Howarth et al., 1988; Roelke et al., 1999; Popovich et al., 2008).

Nutrient deficiency results in either morphological or physiological changes that reduce the overall performance of the phytoplankton as primary producers. Phytoplankton respond to nutrient deficiency through increased uptake of additional nutrients (compensation), and the development of more efficient uptake systems for the limiting nutrient (acquisition). Severe nutrient deficiency will result in a
complete shutdown of physiological processes; and cell death. Indicators of nutrient deficiency can be used to determine which nutrient or combination of nutrients is limiting the phytoplankton communities. Nutrient limitation indicators are based on the premise that the cellular constituents, nutrient uptake, and certain enzymatic activities will vary in predictable ways depending on the nutritional state of the phytoplankton cell (Healey and Hendzel, 1979). Hecky and Kilham (1988) proposed that elemental analysis cannot adequately report on the availability of nutrients. Thus, the uses of alternative strategies to assay nutrient bioavailability as sensed by the cells are essential.

Most studies on multiple nutrient limitation focus on two elements (e.g., N and P) being simultaneously limiting, however, it is possible that three or more elements can be simultaneously limiting. Phytoplankton growth requires nitrogen (N) and phosphorus (P) in an approximate molar ratio of 16:1 (the Redfield ratio; Redfield, 1958). N or P limitation in an aquatic system is considered to occur when the availability of N relative to P is well below or above this ratio of 16:1, respectively (Howarth et al., 1988). Past studies have shown that marine systems of moderate to high productivity are typically N limited (Howarth and Marino, 2006), while similarly productive freshwater systems are most often P limited (Hecky and Kilham, 1988; Howarth et al., 1988). However, relatively little is known about impacts of low-salinity estuaries. The Baltic Sea is perhaps the best-studied estuary of this type where productivity has been shown to be limited by P at salinities lower than 3 to 4 psu and by N at higher salinities (Weber et al., 2002). Recent studies suggest that Fe and P co-limit N2-fixation in the N-limited eastern tropical North
Atlantic (Mills et al., 2004). In fact, co-limitation by Fe and light best describes the high nutrient low chlorophyll (HNLC) regions in 40% of the world oceans (De Baar et al., 2005). Although the concept of co-limitation has recently been accepted in marine systems (De Baar et al., 2005), it is yet to be embraced by the freshwater community and the potential for co-limiting nutrients and the different types of co-limitation are often not discussed in the literature.

Salinity

Factors that affect phytoplankton growth and assemblage in estuaries and coastal areas include salinity, temperature, turbidity, light and nutrients (Morais et al., 2003; Gasiunaite et al., 2005). The abiotic factor salinity may be highly variable in coastal and estuarine ecosystems (Gasiunaite, 2000). Many reports have related salinity changes to the population dynamics of phytoplankton (e.g. Hallegraeff et al., 1995; Weise et al., 2002; Honjo, 2003), and in the laboratory, the effects of salinity on the growth of phytoplankters have been examined both with acclimation to a desired salinity for a certain period of time (e.g. Nagasoe et al., 2006; Matsubara et al., 2007) and without acclimation (Mahoney and McLaughlin, 1979). Results from such analyses suggest from none/mild to drastic changes in the growth, chlorophyll $a$ as well as cell abundances.

Distribution of phytoplankton along estuarine gradients tends to imply increasing concentration of cyanobacteria and chlorophytes in brackish water zone (Nakanishi and Monsi, 1965; Muylaert and Sabbe, 1999), dinoflagellates, and diatoms in the zones of mid-to-high salinities (>10 psu; Kies, 1997). Species diversity is known to
be the lowest at ~5 psu, the approximate lethal limit for many truly marine phytoplankton in estuaries (Rijstenbil, 1988). Salinity change can result in osmotic stress on cells, uptake or loss of ions and effects on the cellular ionic ratio (Guillard, 1962). Although brackish and estuarine phytoplankton are somewhat more halotolerant over specific ranges, phytoplankton taxa vary greatly in their ability to osmoregulate in response to salinity changes (Barron et al., 2002; Thessen et al., 2005). Inhibitory effects on physiological processes of phytoplankton can follow changes in salinity (Khomutov et al., 1990). Alterations in salinity levels result frequently in increased respiratory activity to maintain osmotic balance (Qasim et al., 1972).

**Temperature**

Temperature is another very important ecological parameter that affects almost every aspect of aquatic life. Temperature affects the rate of metabolism and equilibrium of biochemical reactions (Eppley, 1972) and, consequently, plays a role in determining the geographic distributions of organisms (Hochachka and Somero, 1984). Phytoplankton occupy divergent thermal environments both on geographic and seasonal scales. Previous studies on phytoplankton have shown that whole-cell physiological responses, such as growth rate and changes in community structure through succession, are influenced by temperature (e.g. Eppley, 1972; Anderson et al., 1994). In addition, the thermal dependence of cellular biochemical composition, photosynthetic performance, respiration, and inorganic nitrogen uptake in
microalgae have been characterized in a broad range of species from diverse habitats (Thompson et al., 1992).

**Harmful Algae Blooms (HAB)**

Coinciding with recent increases in anthropogenic nutrient-loading to coastal estuaries worldwide, there has been an increase in phytoplankton biomass and consequently, increases in the occurrence, distribution, and species diversity of phytoplankton (Smayda, 1990; Hodgkiss and Ho, 1997; Babin et al., 2008). In particular, widespread attention and dedicated research have been on to the study of bloom forming species that have deleterious consequences on the biological and chemical dynamics of estuaries.

The first effect of phytoplankton blooms on coastal areas and estuarine ecosystems include light attenuation caused by the accumulation of algal biomass in the water column. The reducing light levels reaching benthic environments are reported to have decimated the populations of benthic macroalgae and sea grasses, as they require high light levels for growth (Duarte, 1995). As a result, phytoplankton have out-competed the other two major estuarine plant types, the benthic macroalgae and the seagrasses, in many estuaries (Duarte, 1995).

Another effect of phytoplankton blooms involves the ability of some species to produce potent neurotoxins. These toxins directly poison shellfish and finfish as they are released into the water column by the phytoplankton or as the fish and shellfish consume the toxic phytoplankton species (Hodgkiss and Ho, 1997). These
toxins can accumulate in the tissues of shellfish and can eventually be conveyed to humans upon consumption of the infected fauna.

One of the first reported fatal human intoxications following ingestion of shellfish affected the crew members of George Vancouver’s expedition to British Colombia in 1793 (Dale and Yentsch, 1978). Captain Vancouver noted that it was taboo for the local Indian tribes not to consume shellfish when the waters became phosphorescent such phosphorence can be caused by dinoflagellate blooms (Hallegraeff, 1995). The causative alkaloid toxins, now called paralytic shellfish poisons (PSP). There have been increasing numbers of reports in the recent times on the frequent occurrence of HAB from various coastal regions of the world oceans.

**Toxic Dinoflagellates**

While harmful algal blooms, in a strict sense, are completely natural phenomena which have occurred throughout recorded history, during the preceding couple of decades, the public health and economic impacts of such events appear to have increased in frequency, intensity and geographic distribution (Anderson et al., 2008; Kudela et al., 2008; Shipe et al., 2008). For example, until 1970, toxic dinoflagellate blooms of *Alexandrium tamarense* and *Alexandrium catenella* were the only known ones from temperate waters of Europe, North America and Japan (Dale and Yentsch, 1978). By 1990 however, this phenomenon was well documented from South Africa, Australia, New Zealand, India, Thailand, Brunei, Sabah, Philippines and Papua New Guinea (Hallegraeff, 1993; Hansen et al., 2001).
Other species, *Alexandrium minutum*, until 1988 was known from Egypt but now has been reported from Australia, Ireland, France, Spain, Portugal, Italy, Turkey, the east coast of North America, Thailand, New Zealand, Taiwan, Japan and India (Hallegraeff, 1995; Hansen et al., 2001; D’Costa et al., 2008).

It is difficult to define a cell concentration that constitutes a HAB as some species are so toxic that their presence, even in relatively low numbers, they may be harmful. Thus, the recommended concentration limits for species like *Dinophysis acuminata* and *Alexandrium* spp. are only 500 cells L\(^{-1}\) in Danish waters (Andersen, 1996). Thus the HAB may instead be defined as “events where the concentration of one or several harmful algae reach a level, which can cause harm to other organisms in the sea e.g. by killing fish and shellfish” (Andersen, 1996).

In the coastal areas of the Indian Ocean, red tide is typically dominated by such species as *Noctiluca scintillans*, *Peridinium quinquecorne* and *Trichodesmium erythraeum* (e.g. Devassy et al., 1978; Devassy, 1989; Hansen et al., 2001; Sahayak et al., 2005.). However in September 2004 an incident of fish mortality accompanied by the release of an obnoxious gas (presumably H\(_2\)S) from the sea that caused sickness to children led to a public health alarm around Trivandrum (Lat. 8.5° N). Investigations carried out just after this incident suggested a bloom of holococolithophore and oxygen (O\(_2\)) depletion in the upwelled water to be the cause of fish mortality (Ramaiah et al., 2005). Such blooms, not recorded previously, are quite significant in the context of coastal ecosystem dynamics and biogeochemical processes. Karunasagar and Segar (1990) reported a case of PSP related death from
Mangalore, west coast of India. Godhe et al. (2008) have also documented the prevalence of toxic dinoflagellates in Indian coastal waters.

**Toxic Diatoms**

So far, only marine pennate diatoms have been confirmed as being toxic and all except two belong to the genus *Pseudo-nitzschia* (Table 2.1). Species within the genus *Pseudo-nitzschia* can produce domoic acid (DA), a potent neurotoxin that causes amnesic shellfish poisoning (ASP). *Pseudo-nitzschia* was until recently the only toxic diatom genus known. However, a benthic *Nitzschia, N. navis-varingica* has recently been demonstrated to produce domoic acid (Kotaki et al. 2000, Lundholm and Moestrup 2000). A culture of *Amphora coffeaeformis* was also shown to produce domoic acid (Shimizu et al., 1989). Approximately 12 species of *Pseudo-nitzschia* are documented as DA producers (Table 2.1). On the west coast of the United States, the major DA producers are *P. australis, P. multiseries* and *P. cf. pseudodelicatissima* (could be *P. cuspidata*; Adams et al. 2000; Bates and Trainer 2006). *Pseudo-nitzschia pseudodelicatissima, P. seriata* and *P. calliantha* have caused DA contamination in shellfish in Atlantic Canada (Bates et al. 1998, Bates and Trainer 2006). In Europe, the toxigenic species are *P. seriata, P. australis* and *P. multiseries* (Bates and Trainer, 2006). In New Zealand, *P. australis* is the main source of DA related HAB hazards (Rhodes et al. 1998).

Notwithstanding the causes of HAB, efficient monitoring systems have to be established in order to minimize public health risks and damage to aquaculture. Most developed countries do have programmes in place for monitoring and
Table 2.1. Species of diatoms reported to possess the ability of domoic acid production

<table>
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<tr>
<th>Species</th>
<th>Reference</th>
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<tr>
<td><em>Amphora coffeaeformis</em></td>
<td>Shimizu et al. (1989)</td>
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<tr>
<td><em>Nitzschia navisvaringica</em></td>
<td>Kotaki et al. (2000)</td>
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<tr>
<td><em>Pseudo-nitzschia australis</em></td>
<td>Fritz et al. (1992)</td>
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<tr>
<td><em>P. calliantha</em></td>
<td>Martin et al. (1990)</td>
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<tr>
<td><em>P. cuspidata</em></td>
<td>Bill et al. (2005)</td>
</tr>
<tr>
<td><em>P. delicatissima</em></td>
<td>Smith et al. (1991)</td>
</tr>
<tr>
<td><em>P. fraudulenta</em></td>
<td>Rhodes et al. (1998)</td>
</tr>
<tr>
<td><em>P. galaxiae</em></td>
<td>Cerino et al. (2005)</td>
</tr>
<tr>
<td><em>P. multiseries</em></td>
<td>Bates et al. (1989)</td>
</tr>
<tr>
<td><em>P. multistriata</em></td>
<td>Rhodes et al. (2000)</td>
</tr>
<tr>
<td><em>P. psuedodelicatissima</em></td>
<td>Lundholm et al. (1997)</td>
</tr>
<tr>
<td><em>P. pungens</em></td>
<td>Rhodes et al. (1996)</td>
</tr>
<tr>
<td><em>P. seriata</em></td>
<td>Lundholm et al. (1994)</td>
</tr>
<tr>
<td><em>P. turgidula</em></td>
<td>Rhodes et al. (1996)</td>
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forewarning of the coming HAB. However, in many third world countries the existing capacity and funding are too limited to allow establishment of monitoring programmes, which is particularly true of the Indian Ocean-rim countries. This is critical, as the people of coastal areas in this region are highly dependent on artisanal fishery and are thus vulnerable to any incident that may affect seafood availability.

**Phytoplankton Studies in the Arabian Sea**

The Arabian Sea is a highly complex oceanic basin, encompassing eutrophic upwelling, downwelling and oligotrophic stratified environments (Burkill et al., 1993 and references therein). These environments are strongly influenced by biannual monsoon winds that blow from the southwest (SW) between June and September and the northeast (NE) during December-February. During the SW monsoon, wind-driven upwelling occurs along a broad (ca 800 km wide) region parallel to the Oman coast. Upwelling brings cooler but nutrient rich water into the euphotic zone. The SW monsoon also creates downwelling of water further offshore towards the centre of the northern Arabian Sea. The downwelling, caused by localized negative wind stress curl, creates an area of deepened (~100 m) mixed layer (Burkill, 1999; Tarran et al., 1999). These oscillations in physical forcing and periodic nutrient enhancement result in dramatic seasonal and spatial variations in biological communities and processes. As a consequence, the Arabian Sea is a natural laboratory for studying variability in microbial community structure and its link to production and physical forcing mechanisms (Brown et al., 2002).
The combination of a wide range of physical conditions and warm water temperatures throughout the year result in phytoplankton communities which are highly diverse (Taylor, 1976; Kleinje, 1991). At different times and locations, phytoplankton community structure may be dominated by any of several taxonomic groups. For example, various diatoms and *Phaeocystis* spp. were abundant in and near upwelling areas during the late SW Monsoon (Latasa and Bidigare, 1998). Veldhuis et al. (1997) found that picophytoplankton (cells < 2 μm) were important in the western Arabian Sea, but more so during the NE monsoon (Dec-Feb) as compared to the SW monsoon (June-Sept). Extremely high *Prochlorococcus* concentrations were observed during the spring intermonsoon (Mar-May) and at oligotrophic, offshore stations throughout the year (Campbell et al., 1998; Latasa and Bidigare, 1998) Chlorophytes were dominant in coastal upwelling areas during the SW monsoon (Latasa and Bidigare, 1998). *Synechococcus* was numerically dominant at various locations and seasons in the northwestern Indian Ocean (Burkill et al., 1993; Campbell et al., 1998). In addition, of the major taxonomic groups of phytoplankton, the coccolithophorids have been shown to be particularly diverse in the Arabian Sea basin (Kleinje, 1991; Tarran et al., 1999).

The west coast of India experiences monsoon forced hydrographic changes that clearly mirror those observable on a basin-wide scale in the northern Arabian Sea (Shetye et al., 1990; Madhupratap et al., 1996; Prasanna Kumar et al., 2001; Parab et al., 2006). The studies of phytoplankton diversity along the west coast of India began in the early 1940s (Chacko, 1942; John and Menon, 1945; Subramanyan, 1959; Subramanyan and Sarma, 1961; Devassy et al., 1978, 1979; Devassy and
Goes, 1988; Sawant and Madhupratap, 1996; Parab et al., 2006; Ramaiah et al., 2007). However, the information on phytoplankton ecology of central west coast of India is still limited (Devassy and Goes, 1988). Further, only a few studies are available from Mandovi-Zuari estuaries on phytoplankton pigments (Bhargava, 1973; Bhargava and Dwivedi, 1974; Bhargava and Dwivedi, 1976; Roy et al., 2006 and some recent works cited below), biomass and productivity (Devassy and Goes, 1989; Krishna and John, 2003; Qasim, 2005). More recently, field observations (Mitbavkar and Anil, 2002; Mitbavkar and Anil, 2008; Patil and Anil, 2008) were carried out to measure phytoplankton composition and chlorophyll from these estuaries. In general, phytoplankton have been found to be most abundant during the upwelling period that lasts from May-June to October-November. Diatoms constitute the bulk of microplankton exhibiting rich diversity (Roy et al., 2006). Dinoflagellates are the next abundant group (Alkawri and Ramaiah, 2010) occasionally forming blooms sometimes associated with fish kills (Naqvi et al., 1998 and references therein), and in rare cases resulting in paralytic shellfish poisoning (Karunyasagar et al., 1984).

Biochemical Composition Studies in Phytoplankton

The biochemical composition of phytoplankton is one of the most important studies of planktonic communities as nutritional status of primary producers could be a useful prediction to account for the energetic dependability in the trophic food web (Sicko-Goad and Andersen, 1991). One of the most important issues for furthering our mechanistic understanding of the functioning of marine ecosystems is resolving...
and parameterization of the processes regulating the transfer of autotrophic production to higher trophic levels (Diekmann et al., 2009). Changes in transfer efficiency due to food chain length and biochemical constraints on growth and reproduction have the potential to regulate the dynamics of higher trophic levels as well as the flow of materials within marine food webs.

The biochemical composition of phytoplankton varies with species. Light, temperature, and/or environmental conditions and growth stage are other parameters that affect the composition (Pahl. et al., 2010). Variations in biochemical composition due to growth stage is frequently related to culture age and nutrient depletion, particularly if an organism is grown in batch culture (Morris et al. 1983). Typically, phytoplankton cultures become depleted in nutrients, as they enter stationary phase of growth, and total lipid and carbohydrates increase while protein declines (Lourenco et al. 1997). The fluctuating nutrient supply could affect nutritional status of the phytoplankton (Suttle and Harrison, 1988; Fong et al., 1993). The nutritional status is reflected in the major cellular constituents of lipids, proteins and carbohydrates (Leonardos and Geider, 2004). Biochemical composition is also related to cellular elemental compositions: protein is the major macromolecular pool of intracellular nitrogen and has crucial functions in all the biological processes (Geider and La Roche, 2002). Lipids comprise a functionally-diverse group of compounds (Gurr, 1991), such as triacylglycerols and some other neutral lipids are associated with energy and carbon storage (Parrish and Wangersky, 1987, Lombardi and Wangersky, 1991). Many polar lipids (especially phospholipids) are associated with various types of membranes (Parrish and
Carbohydrates serve as food sources and storage, or as cell wall constituents (structural polysaccharides; Barlow, 1982). However many of them still have certain functions yet to be understood (Percival, 1979; Zhao, et al., 2009). Studies have shown that cellular glucan content accumulates markedly under nutrient deficiency (Myklestad and Haug, 1972; Myklestad, 1974; Zhao, et al., 2009).

One of the key biological features in many oceans is the annual spring bloom. This bloom typically commences at the onset of stratification, leading to large standing stocks and high rates of primary production by diatoms in the euphotic zone. These blooms subsequently deplete the major nutrients limiting phytoplankton production thereby influencing not only phytoplankton growth rates but also the biochemical composition (Hayakawa et al., 1996). During these blooms, protein synthesis appears to dominate during the initial exponential growth phase, while amounts of cellular protein decrease with increasing nutrient reduction (Mayzaud et al., 1990). Arts et al. (1997) indicated that periods of nutrient deficiency, typically concurrent with the senescent phase, may intensify lipid synthesis in some phytoplankton species and thereby enhance the rate of lipid biomass that is transferred from phytoplankton to zooplankton. However, changes in lipid content due to nitrogen depletion in diatoms appear to be species-specific (Shifrin and Chisholm, 1981). Tonon et al. (2002) reported increases in the total fatty acid content, in particular triacylglycerols after the onset of a stationary growth phase of the diatom species *Thalassiosira pseudonana*. These biochemical changes influence both the quantity and quality of unicellular marine algae which subsequently affect the reproductive
success of copepods and other herbivores via changes in both the rate of egg production and hatching success (Broglio et al., 2003; Koski et al., 2006). The timing of the bloom and overlap with the early life stages of fish stocks have been proposed to be critical for the recruitment of key fish species (Cushing, 1974). As changes in the nutritional quality of microalgae influence copepod populations (i.e. secondary production, e.g. Badylak and Philips, 2008) and given the importance of copepods as prey items for early larval stages of many marine fish species (Morote et al., 2008), biochemical changes in marine unicellular algae can cascade up a food web, eventually influencing top predators including humans (Claustre and Gostan, 1987).

Light is essential for growth and for synthesis of biochemical composition in photosynthetic organisms (Thompson, et al., 1993; Brown, et al., 1996; Leonardos, et al., 2004; De la Peña, 2007). Variations in light regimes affect some aspects of cellular ultrastructure, activity of photosynthetic apparatus and biochemical composition in different species of phytoplankton (Zhukova and Titlyanov, 2006). Under low light conditions, cells of phytoplankton are characterized by a large relative volume of chloroplast, high surface density of thylakoid membranes and low relative volume of carbohydrate and lipid storage bodies (Sukenik et al., 1989). Phytoplankton grown at various irradiance levels are reported by several researchers (Thompson, et al., 1993; Zhukova and Titlyanov, 2006) to display a significant change in their gross biochemical composition, pigment content and/or photosynthetic activity. In addition as Floreto and Teshima (1998) note lipid class compositions of algae are affected by the light environment. Under high light, an
excess of energy is used by the cells for producing storage as triacylglycerols, whereas shade adaptation is achieved by increasing the surface area of thylakoid membranes and their structural components (glycolipids) in order to increase light absorption and light utilization efficiency (Napolitano, 1994). A few studies have examined the influence of light intensity on fatty acid composition of diatoms (Thompson et al., 1990; Brown et al., 1996). There is very sparse information on the effect of light on the lipid production in dinoflagellates (Parrish et al., 1994). Some reports contain contradictory results, especially on changes in proportions of polyunsaturated fatty acids (PUFA) under different light conditions. The inconsistent results led Thompson et al. (1990) to propose that the response of PUFA production to light intensity appears to be species-specific.

**Statistical Analyses**

The effects of various ecological parameters on the phytoplankton at its species, and/or assemblage/community level(s) are not directly discernible from the numerical data sets from an ecological investigation. In order that plausible insights can be obtained, statistical treatment of data is necessary. The most commonly employed statistical analyses are correlation matrices, principal and/or canonical component analyses, diversity indices. The following description is to provide a general perspective of the uses of a few of these analyses on certain data obtained for this study.
Diversity Index

Diversity (or biodiversity) is typically measured by species count (richness) and sometimes with an evenness index; it may also be measured by a proportional statistic that combines both measures, e.g. Shannon–Wiener index (Stirling and Wilsey, 2001). Shannon–Wiener index is one of the most widely used diversity indices for measuring diversity, even though its performance and meaning are often controversial (Gao and Song, 2005).

Shannon’s index is based on information theory, which postulates that the measure of a system’s disorganization degree is the amount of information in that system (Wiener, 1948). As pointed out by Washington (1984), this index ‘has become a magic bullet among ecologists’.

Many researches indicate that correlation between evenness and Shannon–Wiener index is positive and strong; moreover, diversity can change with key ecological processes such as competition, predation and succession, each of which can alter Shannon–Wiener index through changes in evenness without any change in species richness (e.g. Stirling and Wilsey, 2001, and references therein).

Canonical Correspondence Analysis

Canonical correspondence analysis (CCA, Ter Braak, 1986) is a multivariate gradient technique that allows for the interpretation of direct relationship of community data to the known environmental variables by constraining the species ordination to a pattern that best correlates with designated environmental variables. The environmental variables can be quantitative or nominal. As many axes can be
generated as there are environmental variables. The CCA leads to an ordination
diagram in which points represent modes of species distribution along the gradient,
and vectors represent strengths and directions of environmental variables. Resultant
plots portray patterns of variation in community composition that can be explained
best by the environmental variables. They also show approximately the 'centers' of
the species distributions along each of the environmental variables (Ter Braak
1986). According to Rakocinski et al. (1997), CCA is a well-suited technique for
the analysis of estuarine data, to identify indicator species and assemblages, and to
evaluate the influence of natural and contaminant conditions on the community
structure. Many authors used CCA to identify the environmental variables
governing the composition and structure of phytoplankton assemblages (Resende et
al., 2005, and references therein)

Principal Component Analysis

Principal component analysis (PCA) is used to reduce the dimensionality of
multivariate data by linearly transforming them into a new data projection with
minimal loss of information. The main concern of the PCA is to understand the
mode of action or behavior of components of a system and its subsystems (Petersen
et al., 2001; Bengraine and Marhaba, 2003). The power of PCA is in data reduction
by revealing the significant modes of variance within the data, and altering the axes
(the eigenvectors) to orient along these modes. The new orientation is such that the
first principal component (PC) is a directed axis along the vector that accounts for
most of the variance. The axes of the following PCs are orthogonal to one another,
and account for sequentially decreasing amounts of variance. A p-dimension data set can have a maximum of $p$ principal components. As Johnson and Wichern (1998) propose, the principal components represent a more parsimonious description of the covariance structure of the original data.