# Microphytoplankton Distribution and Diversity in the Intertidal Zones of Goa

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By

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Under the guidance

of

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# CERTIFICATE

This is to certify that **Mr. Abdulsalam Abdullah Sultan Alkawri** has duly completed the thesis entitled "**Microphytoplankton Distribution and Diversity in the Intertidal Zones of Goa**" under my supervision for the award of the degree of Doctor of Philosophy.

This thesis being submitted to the Goa University, Taleigao Plateau, Goa for the award of the degree of Doctor of Philosophy in Marine Science is based on original studies carried out by him.

This thesis or any part thereof has not been previously submitted for any other degree or diploma in any University or institutions.

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**Date: September 10, 2010**
**Place: Dona Paula**

All corrections suggested by the referees have been incorporated

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8/4/11

# DECLARATION

As required under the University Ordinance 0.19.8 (iv), I hereby declare that the present thesis entitled "**Microphytoplankton Distribution and Diversity in the Intertidal Zones of Goa**" is my original work carried out in the National Institute of Oceanography, Dona Paula, Goa and the same has not been submitted in part or in full elsewhere for any other degree or diploma. To the best of my knowledge, the present research is the first comprehensive work of its kind from the area studied.

The literature related to the problems analyzed and investigated has been appropriately cited. Due acknowledgements has been made wherever facilities and suggestions has been availed of.

**Abdulsalam A. S. Alkawri**

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*Abdulsalam A. S. Alkawri*

*DEDICATED*

*TO MY BELOVED MOTHER*

*LOLA ABDO MOHAMMED*

*AND*

*MY BELOVED FATHER*

*LATE ABDULLAH S. ALKAWRI*

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# Chapter 1

## General Introduction

Dwelling the well-lit surface layers of all oceans, seas, or lakes, the autotrophic component of the plankton community, the phytoplankton, obtain their energy through photosynthesis. Together with terrestrial plants, the phytoplankton are responsible for much of the oxygen present in the Earth's atmosphere. Their cumulative energy fixation in carbon compounds (primary production) is the basis for the vast majority of oceanic and some freshwater food chains. The name "Phytoplankton" comes from the Greek words *phyton* (meaning "plant"), and *planktos* meaning "wanderer" or "drifter". They are microscopic, and usually single-celled. Sournia et al. (1991) estimated that there are about 500 genera and over 5,000 species. They live in wide range of water bodies, from small puddles to large oceans. This means the cells encounter a wide range of environmental variables such as temperature, salinity, nutrient concentrations and light, and also experience a wide range of scales, both temporal and spatial.

The cells range in size from ca. 2 μm to 2 mm, commonly being 10-50 μm (Horner, 2002). The phytoplankton community can be divided into three functional groups based on cell size; picophytoplankton (<2 μm), nanophytoplankton (2-20 μm) and microphytoplankton (>20 μm). Microphytoplankton sized cells are of more universal taxonomic distribution, although the largest species are found among Bacillariophyta (diatoms) and Dinophyta (dinoflagellates) (Lee, 1999). Picophytoplankton and nanophytoplankton are primarily consumed within the

microbial loop (Sandergaard et al., 1991) while the larger cells are grazed by herbivores within the classical food web.

The growth rate of phytoplankton is influenced by the environmental conditions, with ambient nutrient concentrations, light availability and temperature as the most important in estuaries (Anderson et al., 1994; Fisher et al 1999). The phytoplankton growth rate increases with increased temperature (Montagnes and Franklin, 2001) within certain range. The seasonality in temperature and light effects on phytoplankton growth in sup-tropical and tropical regions may be small (Pennock et al., 1999) compared to the seasonality in temperate regions (Fisher et al., 1999). In addition to latitudinal variations, the turbidity of estuarine and coastal waters further complicates the qualitative and quantitative impacts of light on phytoplankton growth (Cloern, 1996). The temperature, light and nutrient limitation on phytoplankton growth are interrelated as growth is only restrained by environmental parameter at a time. In general, the distribution of phytoplankton biomass and productivity are governed by the availability of nutrients on larger spatial scales, and on medium and/or small scales, due to biophysical processes like the light environment, water column stratification/turbulence, temperature, and grazing may also modulate and biomass levels (Pennock and Sharp, 1994). For instance, in highly turbid coastal waters where nutrients are present at moderate to high concentrations, light availability is usually the limiting factor for primary production (Underwood and Kromkamp, 1999).

Phytoplankton grow by absorbing light energy via photopigments (chlorophylls and carotenoides) and utilize it to reduce carbonate ($CO_3$) and produce high energy

carbon compounds (Marshall, 2009). A major bi-product through this process is oxygen, which is released into the water as another essential commodity for biota in these habitats. The rate of photosynthesis, measured as oxygen production or inorganic carbon uptake, is light dependent (Falkowski and Raven, 1997). Phytoplankton respond to the ambient light environment and acclimate to the average daily light exposure (Post at al., 1984). When exposed to the average light environment, phytoplankton utilize light energy at the maximum rate (light-saturated conditions). The light intensity at which saturated photosynthesis rate is reached is dependent on the past light history of the phytoplankton. Thus, light adapted cells have a higher saturation level and a higher rate of photosynthesis (Kirk, 1994). Below and above average light exposures, rates of photosynthesis are sub-optimal due to light limitation and photoinhibition respectively.

From the burgeoning literature it is clear that phytoplankton studies open up new windows to elucidate on how different phytoplankton communities respond to their own environmental conditions. Sustained investigations in estuaries and coastal systems, such as west coast of India, are required to be continued for developing more specific insights into the mechanisms through which physico-chemical variations determines biological variability in estuarine ecosystems. However there is no systematic annual or seasonal collections and analysis of microphytoplankton for understanding of their natural variability in terms of abundance, type, species diversity and seasonal succession. This is especially so in the case of coastal regions of Goa, west coast of India. Keeping these aspects in the fore, the following objectives are formulated with the view to conduct a systematic study:

- To describe phytoplankton species composition, their monthly differences in ecologically different location in Goa, central west coast of India.

  One of the rationales in planning this objective was for the fact that, in spite of a large number of studies available on the composition of Mandovi-Zuari phytoplankton particularly in the vicinity of Dona Paula, it is surprising that the other estuary, namely Chapora, never was investigated. While the variability of physical parameters with in the short distances are expected and proven, a comparative analysis of phytoplankton characteristics in the salinity gradient zone was missing

- To understand the relationship between nutrient concentrations and dynamics of phytoplankton community in terms of monthly shifts, predominance, species diversity and succession.

  By assessing these characteristics, it was hypothesized that an assessment of inter-relationships between hydrographic parameters and the planned biological variables could be possible

- To quantify chlorophyll concentration from different locations for a comparative assessment of its spatio-temporal variability in relation to variations in nutrient concentrations in the sampling spots.

  Chlorophyll *a* being the most immediate sensor of the dynamic changes in inorganic nutrient concentrations, an insight would be possible on the inter-play of nutrients and phytoplankton abundance by analyzing the datasets obtained through regular sampling survey along the salinity gradient zone planned for this study.

- To evaluate changes in lipid and protein patterns from a select set of phytoplankton species grown under different light and nutrient regimes.

  Experimental analyses can provide useful/reliable cues as to how the growth and cellular composition of phytoplankton in natural settings could change. Thus experiments were set up by varying light and nutrient levels to observe differences if any in growth and biochemical composition between experimental treatments.

# 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. Antropogenic 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 Galva˜o,

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 Galva˜o, 2007), and pelagic and benthic grazing (Domingues and Galva˜o, 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 $N_2$-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 to 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 accuminata* 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 $H_2S$) 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 poisoining (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

| **Species** | **Reference** |
|---|---|
| *Amphora coffeaeformis* | Shimizu et al. (1989) |
| *Nitzschia navisvaringica* | Kotaki et al. (2000) |
| *Pseudo-nitzschia australis* | Fritz et al. (1992) |
| *P. calliantha* | Martin et al. (1990) |
| *P. cuspidata* | Bill et al. (2005) |
| *P. delicatissima* | Smith et al. (1991) |
| *P. fraudulenta* | Rhodes et al. (1998) |
| *P. galaxiae* | Cerino et al. (2005) |
| *P. multiseries* | Bates et al. (1989) |
| *P. multistriata* | Rhodes et al. (2000) |
| *P. psuedodelicatissima* | Lundholm et al. (1997) |
| *P. pungens* | Rhodes et al. (1996) |
| *P. seriata* | Lundholm et al. (1994) |
| *P. turgidula* | Rhodes et al. (1996) |

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 (Karunasagar 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

Wangersky, 1987). 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 Wiechern (1998) propose, the principal components represent a more parsimonious description of the covariance structure of the original data.

# Chapter 3

## Hydrography, Chlorophyll *a* and Phytoplankton Abundance

### Introduction

The influence of environmental factors on the spatial and temporal patterns of phytoplankton abundance in estuaries has been a focus of marine research for many years (Smayda, 1980; Kirk, 1994; Cloern, 1996; Lucas et al., 1999). Among the most frequently discussed factors are those that control phytoplankton growth, principally the availability of nutrients and, light, right regimes of temperature, as well as those that contribute to biomass loss, like tidal flushing and grazing (Rijstenbil, 1987; Morais et al., 2003; Gasiunaite et al., 2005). In many cases the impacts of one factor are dependent on other factors. For example, the effect of nutrient loading on phytoplankton abundance in coastal ecosystems is dependent on other factors that affect biomass gains and losses, including: light availability (Cole and Cloern, 1984; Bledsoe and Phlips, 2000), sedimentation, senility and death, hydraulic flushing (Richardson and Jorgensen, 1996) and grazing (Frost, 1980).

The variability in rainfall also affects a wide range of water column conditions related to direct atmospheric and watershed influences, such as nutrient loads, salinity regimes, suspended sediment levels, and general hydrologic conditions (e.g., water residence times and circulation patterns Weise et al., 2002; Phlips et al., 2007). All of these factors can affect the abundance, composition, and distribution of phytoplankton.

Salinity fluctuations in estuaries and coastal areas are large, both spatially and temporally, and reflect relative inputs from watersheds and tidal water intrusion, circulation patterns and vertical and horizontal mixing processes (Redden and Rukminasari, 2008). In vertically stratified shallow waters, phytoplankton cells in surface waters can experience relatively rapid increases in salinity when there is strong vertical mixing. Where salinities fluctuate, inter-specific differences in salinity tolerances of phytoplankton play a major role in structuring phytoplankton communities (Kirst, 1989).

An understanding of the spatial and temporal variations in chlorophyll *a* (chl *a*), phytoplankton abundance and distribution in relation to the varying hydrographic conditions in different salinity regimes would be useful to discern the inter-relationships between physical, chemical and biological components in the ecosystem. In order to elucidate such influences details of different hydrographic parameters studied and their influences on the observed annual variations in the concentrations of chl *a* and cell counts of diatoms and dinoflagellates along the coast of Goa are described in this chapter.

## Material and Methods

### *Study Area and Sampling Procedure*

Lying within the swath of monsoonal region, the central west coast of India experiences intense rainfall, in particular during June-September. During the summer season (Mar-May), both air (28-36 °C) and water (24-32 °C) are higher. The temperature (air: 17-26 °C; water: 21-25°C) is lower during November-February the 'mild winter'. This study was carried out in the estuarine and coastal locations off Goa. Based on salinity gradient, four different locations (Fig. 3.1) were decided for sampling. Sampling location off Anjuna (15°35'5.3"N, 73°44'12.9"E) is truly marine with no direct influence of river discharges within its 7-8 Km radius. Locations in Chapora estuary (15°36'30.8"N, 73°44'18.6"E) and in Dona Paula Bay (15° 27'4.9" N, 73° 48'11.8"E) were chosen to represent true estuarine conditions. The location off Siridao (15° 25'53.1"N, 73° 51'43.8"E), usually with lower salinities, is ~6 Km east of the location in Dona Paula Bay. The substrate is rocky off Anjuna, sandy-silt off Siridao and mostly sandy off Chapora and Dona Paula. As a consequence of monsoonal discharges and intensified turbulence the load of suspended particles is more during monsoon, moderate during post-monsoon and lower/least during pre-monsoon at all these sampling locations.

Since the tidal amplitude off Goa is ~ 1.5 m, to be consistent, surface samples were collected during the lowest low tide-times every month (September 2007 - September 2008) from these locations. The sampling depths during the low tide-times were 3-5 m at different locations. On all sampling days, replicates of two 5-

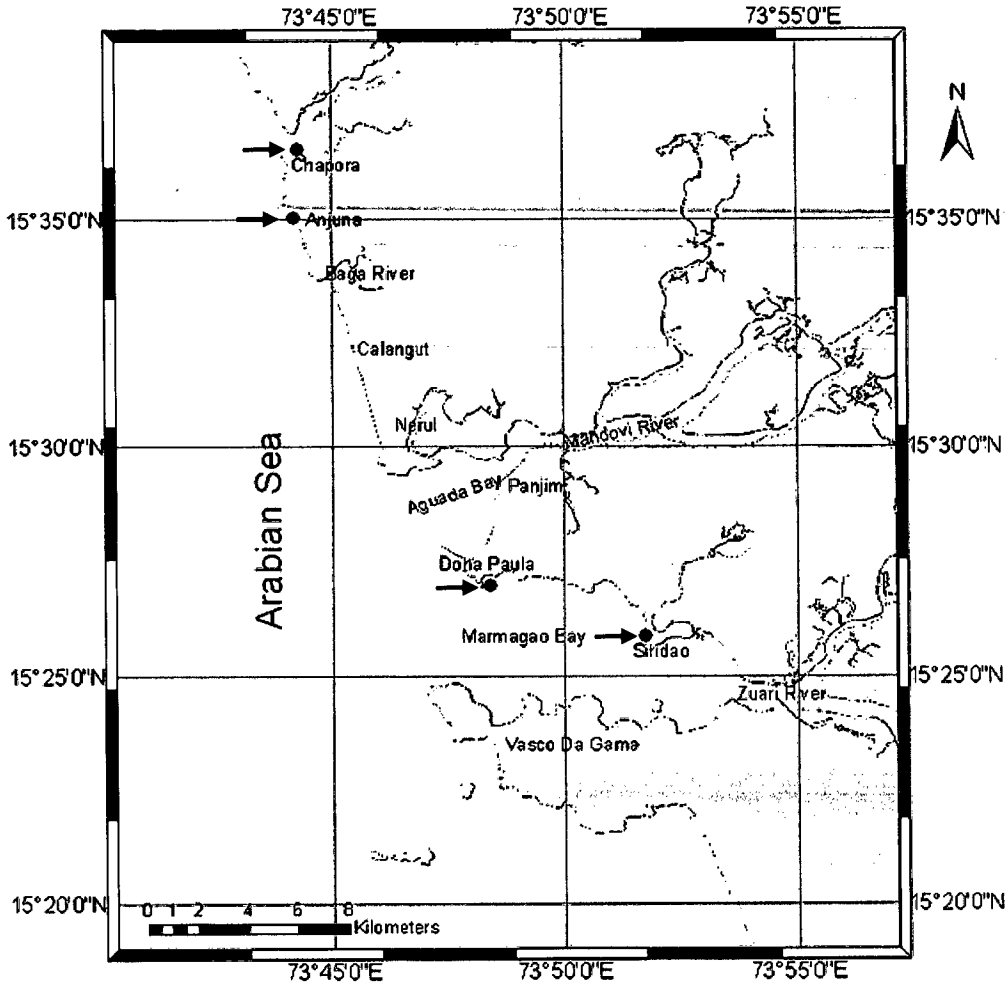



Fig. 3.1. Map showing the sampling locations along the coast off Goa, west coast of India

litre samples were collected from each location using pre-washed Niskin samplers from ~50 cm below the surface. Immediately after their collection the samples were held in a darkened container and transported to the laboratory within 3 hours of their collection.

***Measurement of Environmental Parameters***

Various hydrographical aspects such as temperature, salinity, dissolved oxygen and pH, were analyzed by following standard oceanographic techniques which have been briefly described below:

Temperature

Water temperature was measured in situ with the help of a mercury thermometer calibrated to 0.1 °C and the values are expressed as °C.

pH

Digital pH meter (DPH 504, Global Electronic) was used to estimate pH values in the water samples.

Salinity

The salinity was analyzed by measuring the electrical conductivity ratio, using Guildline "Autosal" model 8400A salinometer (measurement range: 0.005-42 psu). The readings were then, converted from conductivity ratio to salinity. The values have been expressed in psu.

Dissolved Oxygen (DO)

Dissolved oxygen concentrations were estimated by Winkler's method. This method involves fixation of dissolved oxygen using Winkler reagents A and B, immediately after collection of the water sample in a dark bottle, followed by titration against standard sodium thiosulfate solution using starch as indicator. The results were expressed in μmol.

***Nutrients***

Determination of concentrations of dissolved inorganic nutrients such as nitrate ($NO_3$-N), nitrite ($NO_2$-N), phosphate ($PO_4$-P) and silicate ($SiO_4$-S) was carried out by following standard methods described in Parsons et al. (1984) and the JGOFS protocol (UNESCO 1994). Water samples were transported immediately in an ice box to the laboratory. The samples were then analyzed after bringing it to room temperature. Nutrient concentrations were determined from fresh, unfiltered samples.

Nitrate-Nitrogen (NO3-N) was analyzed by reducing it quantitatively to nitrite by passing through a column containing cadmium filings coated with metallic copper (copper sulfate). Nitrite thus produced was then determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine to form highly colored azo dye, which was measured spectrophotometrically at wave length 543 nm, using spectrophotometer Systronics 2202, USA. Concentrations of $NO_3$-N were expressed in μmol.

Nitrite-Nitrogen (NO2-N) was measured by allowing the nitrite to react with sulfanilamide to form diazo compound, which is reacted with N-(1-naphthyl) ethylenediamine and form highly colored azo dye, as described above in nitrate procedure. This dye is measured spectrophotometerically at 543 nm. Nitrite concentrations were expressed in μmol.

Phosphate-Phosphorus (PO4-P) was measured by allowing the water to react with a composite reagent containing ammonium molybdate tetrahydrate solution, sulfuric acid, ascorbic acid and potassium antimonyl-tartrate solution. The resulting complex is reduced to give a blue solution, which is measured spectrophotometerically at wavelength of 885 nm. Phosphate values have been expressed in μmol.

Silicate ($SiO_4$-S) was measured by allowing the seawater sample to react with ammonium molybdate under conditions which result in the formation of silicomolybdate, phosphomolybdate and arsenomolybdate complexes. A reducing agent of metol and oxalic acid is added and silicomolybdate is reduced to a silicomolybdous acid with a blue color, the absorbance of which is measured spectrophotometrically at 810 nm.

***Chlorophyll a (chl a)***

Two replicates of 1 liter water samples from each sampling location were filtered onto 47mm glass fiber filters (Whatman GF/F) and the chl *a* was extracted from them in 10ml of 90% acetone under cold and dark conditions overnight. The chl *a* concentrations were measured flourometrically (Turner Designs, USA, 10-AU-005-

CE) by following the JGOFS protocol (UNESCO 1994). Pheophytin (pheo) concentrations were also measured after acidifying the extracts.

***Phytoplankton Enumeration***

For quantitative and qualitative analyses of phytoplankton-cell counts and their composition, water sample from the each sampling location were fixed with Lugol's solution (1% w/v) and 3% formaldehyde and stored in dark until analyzed, usually within a fortnight of their collection. A settling and siphoning procedure was followed to concentrate samples from 4L to 100 ml for counting phytoplankton cells and identification of genera and wherever possible, to the species level (Utermohl, 1958). Three 1-ml replicates of concentrated samples were transferred to Sedgwick-Rafter plankton counting chamber and examined microscopically at 200-400X magnification. All 1000 squares on the chamber were screened. When uncertain, cover-slipped wet mounts of the specimens were examined at 1000X using oil immersion objective to confirm their identity to specific/generic level. Phytoplankton were identified by consulting numerous earlier descriptions (Subramanyan 1946; Subramanyan and Sarma 1961; Taylor 1976; Dodge, 1982; Constance et al. 1985a, b; Desikachary and Ranjithadevi 1986; Desikachary and Prema 1987; Desikachary et al. 1987; Hasle and Syvertsen 1996; Steidinger and Tangen, 1997; Faust and Gulledge, 2002; Horner, 2002).

### *Statistical Analyses*

Correlation coefficient (R) was performed to evaluate the relationships between the phytoplankton abundance, chl *a* and various environmental parameters that may be regulating their populations.

Linear regression analysis was performed on the log ($x$ +1) transformed values for phytoplankton abundance and chlorophyll *a* concentration at all the locations.

## Results

### *Hydrographic Features*

Water temperatures at all sampling stations exhibited a predictable seasonal fluctuation with the minima during the 'winter' month of February and monsoonal month of August (25°C) and the maximum of 33°C was recorded during the summer month of May (Fig.3.2).

The salinity was influenced by the annual cycle of precipitation: spatially, the maximum salinity of 35.92 psu was found off Anjuna, the truly marine location in June. Also on the annual scale, the salinities at this location did not fluctuate widely unlike either off Dona Paula, Siridao or Chapora. The minimum salinity of 0.44 psu was recorded during the rainy month, August 2008 at Chapora, the location most impacted by freshwater discharges throughout the year. As a consequence of reduced discharges, the salinity increased from October to reach the highest value in June. In general, the salinity was lower during the monsoon months (Fig.3.2).

Dissolved oxygen concentrations ranged from 2.05 to 7.11ml $L^{-1}$ (≡91.5-317.6 μmol) with the maximum off Anjuna and the minimum off Siridao. The

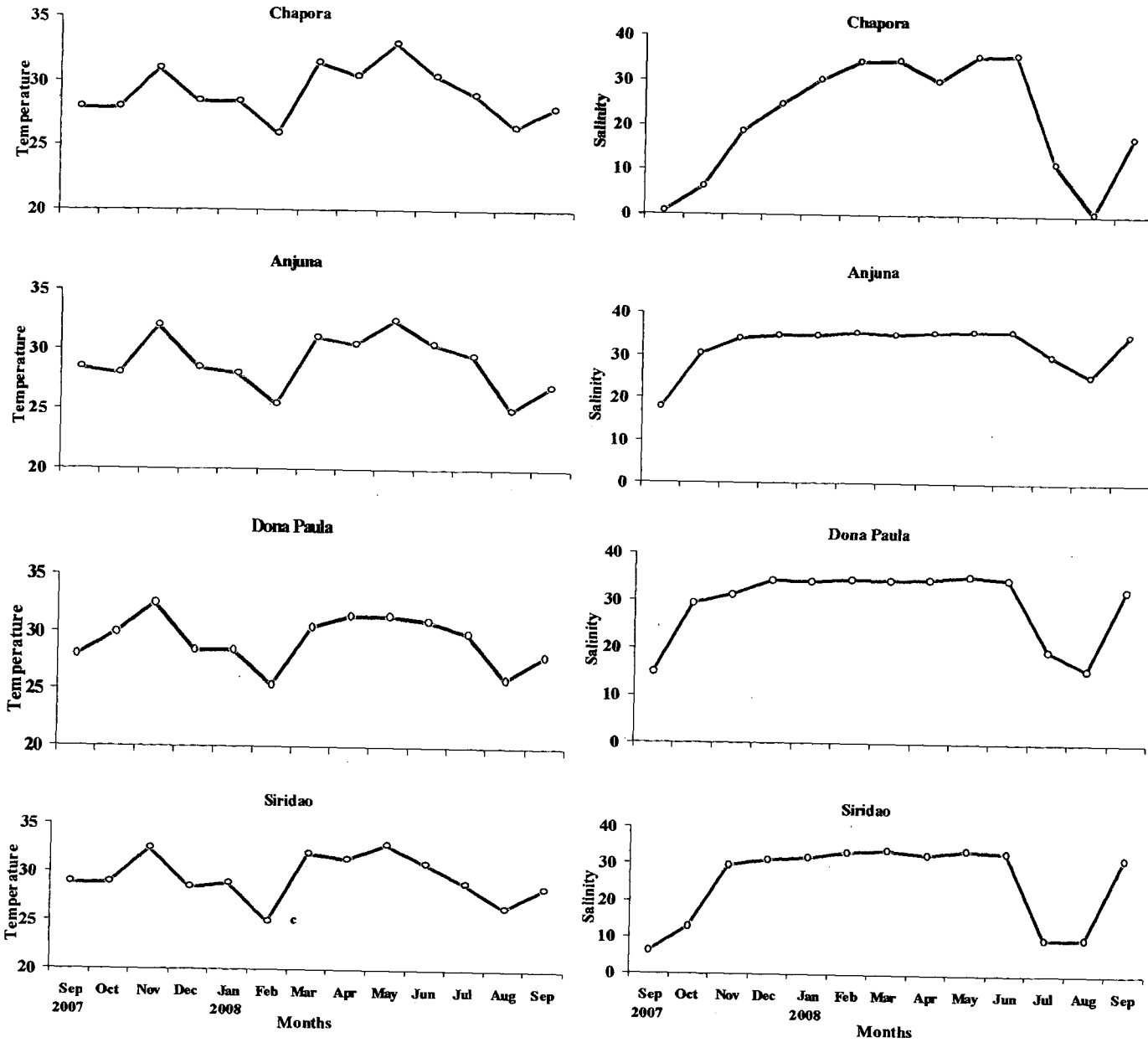



Fig.3.2. Temporal variations in temperature and salinity measured at different sampling locations off Goa, during September 2007-September 2008

concentration was low only during March 2008 off Dona Paula and Siridao (Fig. 3.3). The pH ranged between 7.52 and 8.48 during the study period Fig. 3.3).

***Dissolved Inorganic Nutrients***

Dissolved inorganic nitrogen (nitrate and nitrite) concentrations in the study area displayed a clear seasonal cycle. Concentrations of nitrate were maximum (16.35 μmol) during September 2007 off Chapora and, decreased to 0.004 μmol in January at this location (Fig.3.4). Nitrate concentrations in the study area fluctuated throughout the year. Generally, its concentrations were high during monsoon and low during post and pre-monsoon at all the stations. Nitrite concentrations were usually below 1.0 μmol throughout the sampling period except off Siridao during November (2.35 μmol; Fig.3.4).

Phosphate increased from its low concentrations during pre-monsoon (0.06 μmol off Chapora; 0.14 μmol: Anjuna; 0.23 μmol: Dona Paula and 0.33 μmol: Siridao) to its highs during the monsoon months (Fig.3.5).

Similarly, silicate concentrations were very high during monsoon at all the locations (Fig.3.5). Its concentrations peaked at all locations in September 2007 with the highest concentration at the location off Chapora (105.88 μmol). Following this, its concentrations decreased rather abruptly. The second peak of silicate concentrations were during August 2008 with the highest concentrations off Siridao (128.22 μmol) and Dona Paula (122.67 μmol).

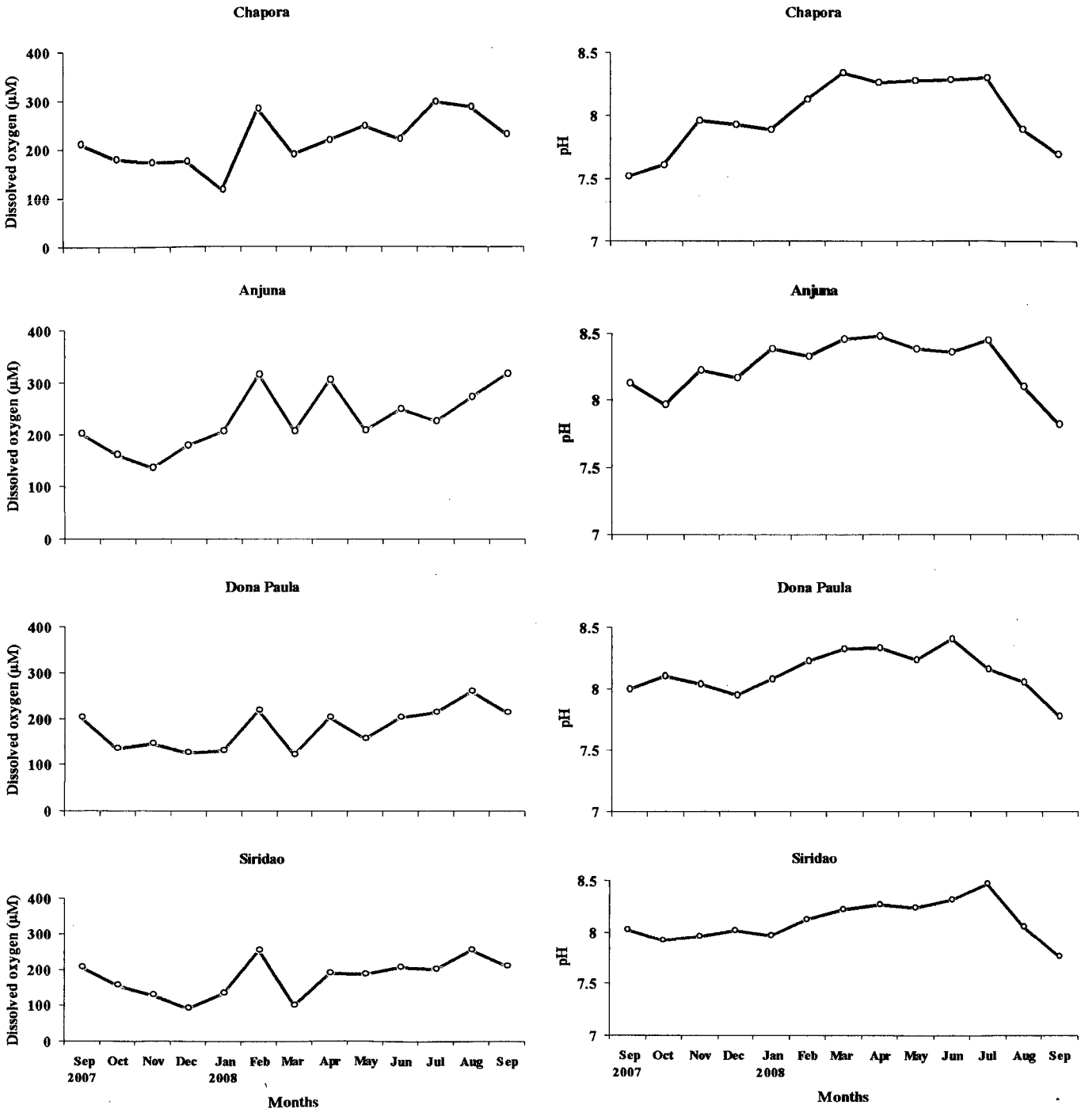



Fig.3.3. Temporal variations in dissolved oxygen and pH measured at different sampling locations off Goa, during September 2007-September 2008

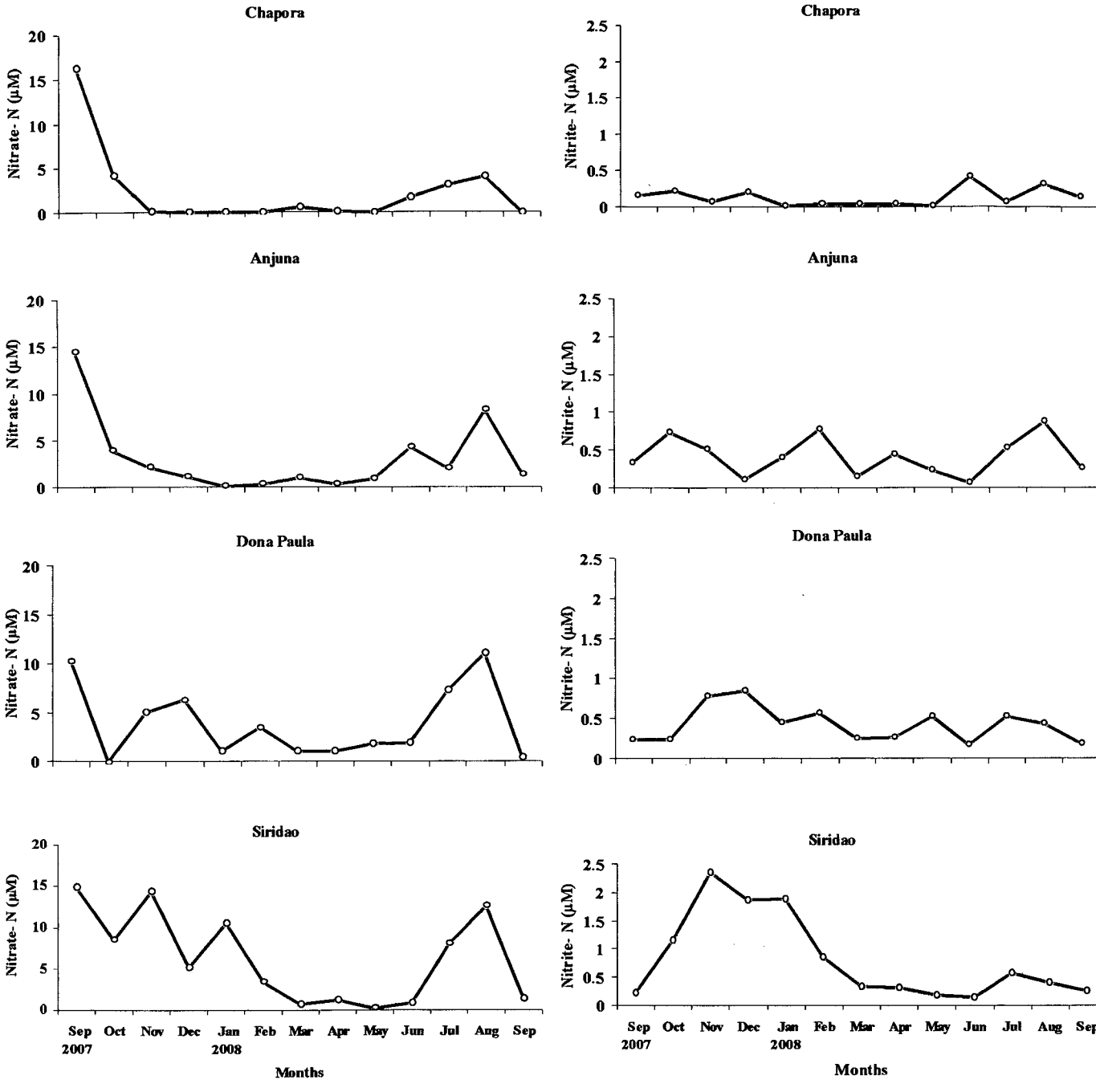



Fig.3.4. Temporal variations in nitrate and nitrite concentrations measured at different sampling locations off Goa, during September 2007-September 2008

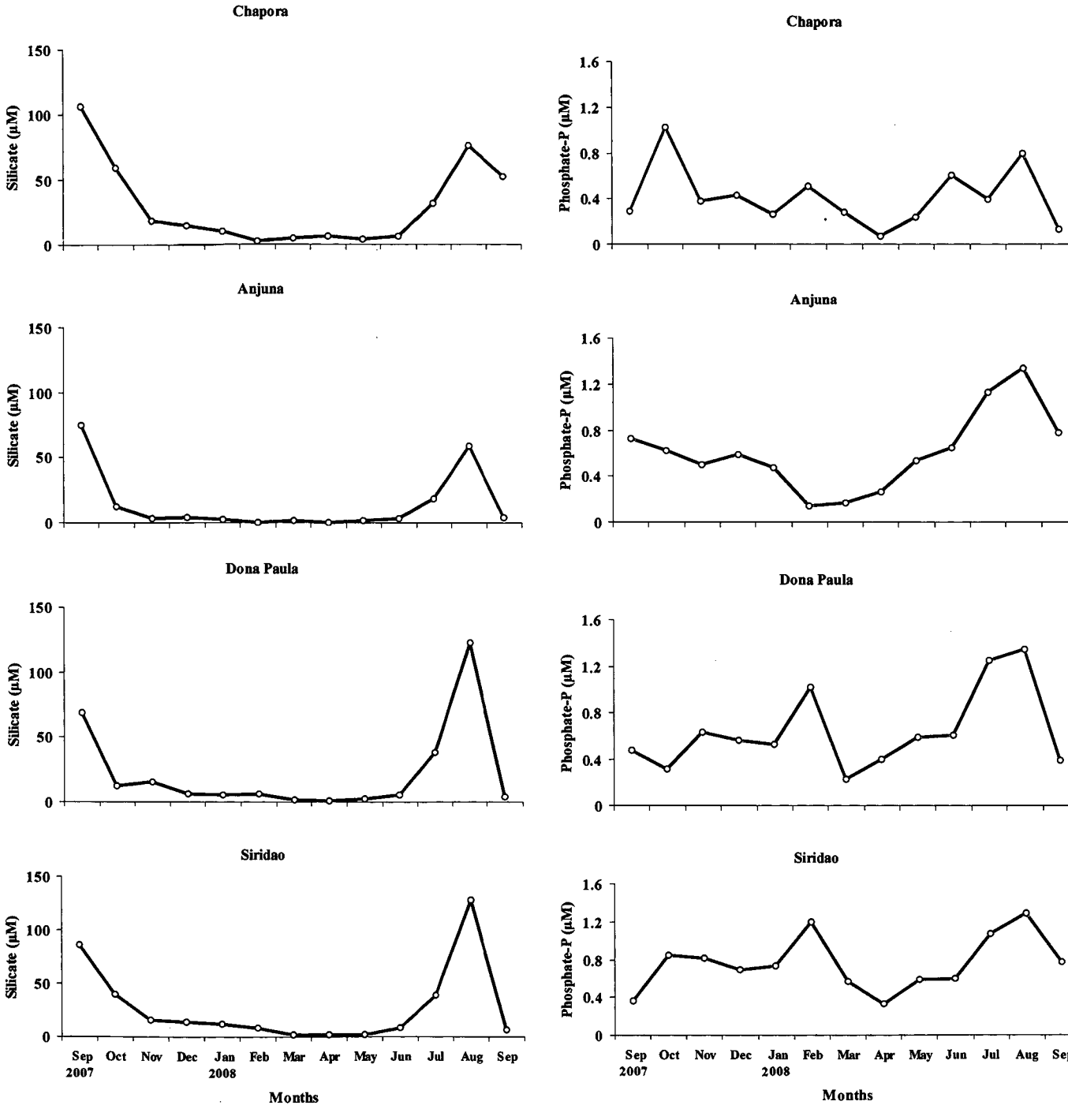



Fig.3.5. Temporal variations in silicate and phosphate concentrations measured at different sampling locations off Goa, during September 2007-September 2008

***Chlorophyll a (Chl a)***

The concentrations of chl *a* (Fig.3.6) varied from 0.42 to 4.19 μg $L^{-1}$ in Chapora and the maximum at this station was in May 2008. At the location off Anjuna, it varied between 0.29 and 2.1 μg $L^{-1}$. Off Dona Paula it varied from 0.44 to 2.54 μg $L^{-1}$. The highest concentration of 7.50 μg $L^{-1}$ was observed off Siridao during September 2008. Its annual range at this location was 1.19 - 7.50 μg $L^{-1}$.

***Phytoplankton Abundance***

The annual variation in total phytoplankton abundance is given in Figure 3. 6. The highest phytoplankton abundance ($7.7\times10^5$ cells $l^{-1}$) was observed in the surface water of the location off Siridao during September 2008, whereas the location of Anjuna had the lowest abundance (868 cells $l^{-1}$) among all locations during June 2008. In general the abundance of phytoplankton at the location of Anjuna was appeared very low (from 868 to $2.4\times10^4$ cells $l^{-1}$). The highest peak of abundance at the location of Chapora ($1.9\times10^5$ cells $l^{-1}$) occurred in May 2008 followed by September 2008 ($1.4\times10^5$ cells $l^{-1}$). At the location of Dona Paula a major peak of phytoplankton abundance ($7.5\times10^4$ cells $l^{-1}$) was observed during September 2008. The abundance of phytoplankton at the location of Siridao was generally high during the study period.

The dominant phytoplankton groups in this study were diatoms, and then dinoflagellates. The other phytoplankton including silicoflagellates, chlorophyceae, and chrysophyceae, were minor groups at all the locations (Fig.3.7).

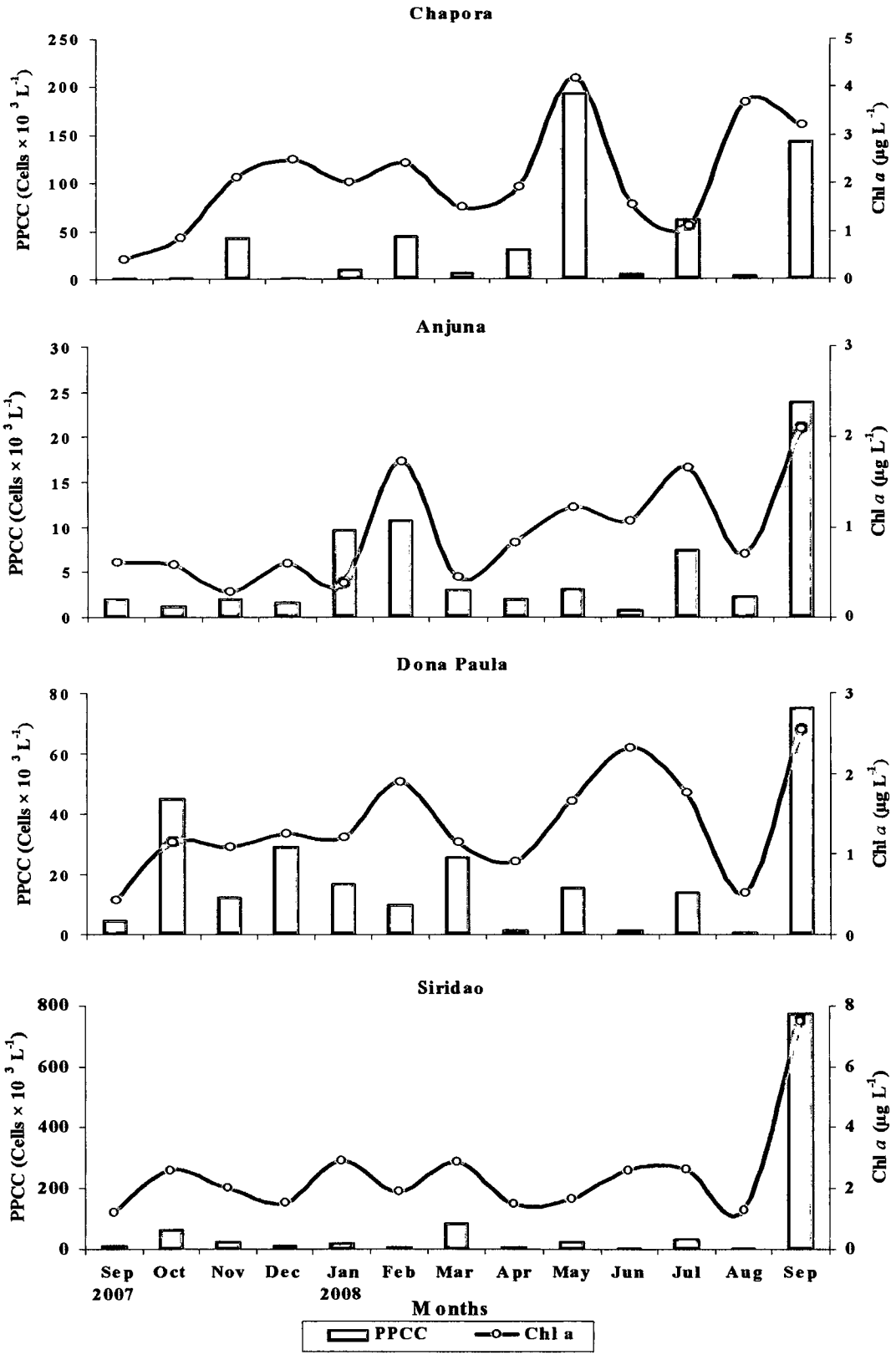



Fig.3.6. Variations in phytoplankton cell counts (PPCC) and chlorophyll *a* (chl *a*) concentrations from September 2007 to September 2008 at different sampling locations off Goa

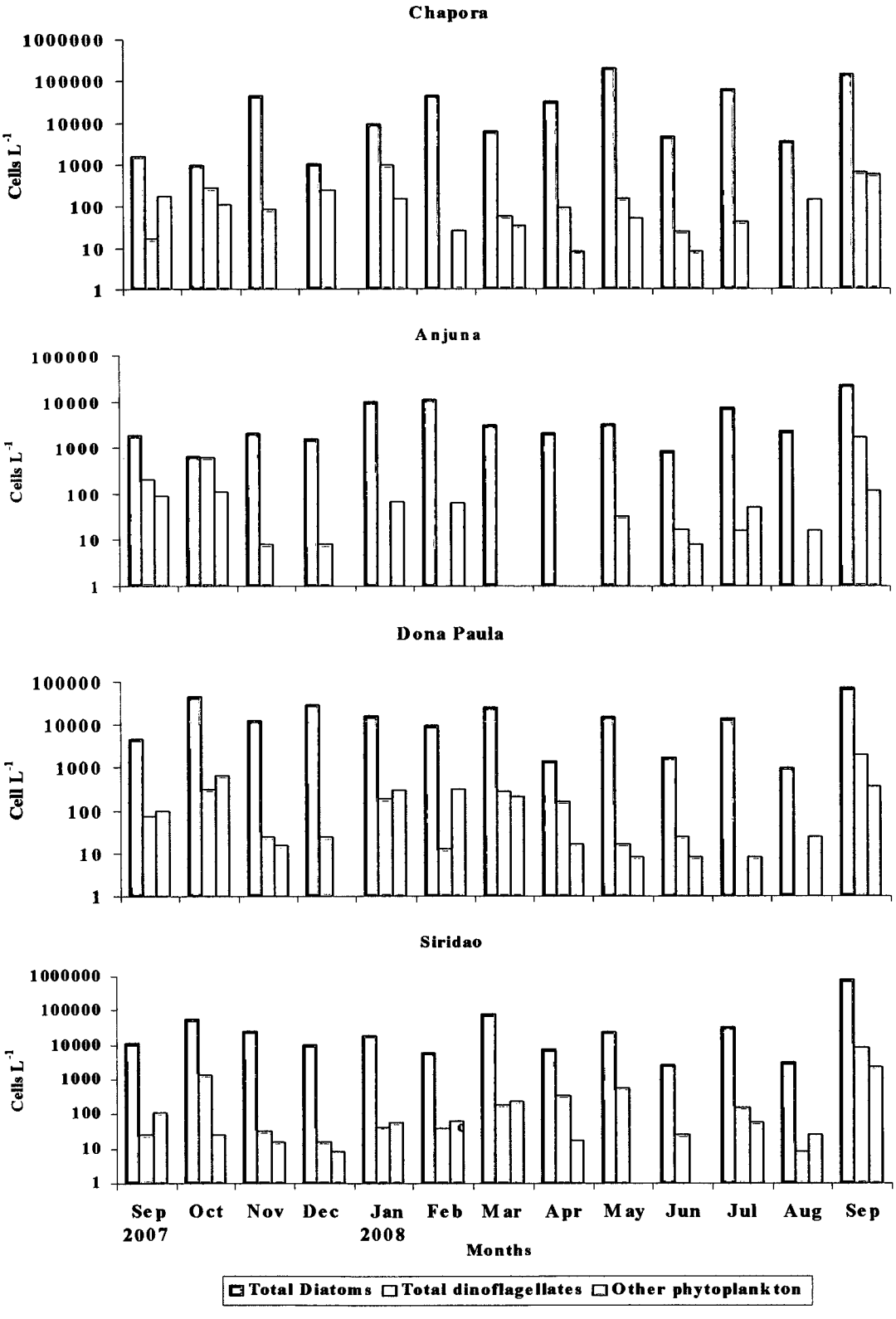



Fig.3.7. Monthly variations in the abundances of total diatoms, dinoflagellates and other phytoplankton at different sampling locations off Goa, during September 2007-September 2008

***Statistical Analyses***

Statistically significant ($p< 0.05$) negative relationships between phytoplankton cell abundance and nitrite R= -0.57, as well as between their abundance and nitrate (R=-0.54); and phosphate (R=-0.56) were observed in the data from the location off Chapora. Their relationship with silicate was not significant at this location (Table 3.1). A significant positive correlation was observed between cell abundance and concentrations of nitrate (R=0.54; $p<0.05$) at the location off Anjuna (Table 3.2). Off Dona Paula (Table 3.3), it was negative between cell abundance and nitrate (R= -0.58; $p< 0.039$); and phosphate (R= 0.56; $p<0.046$). However, none of the ecological parameters in this study showed significant relationship with the phytoplankton cell abundance at the location off Siridao (Table 3.4). Although the phytoplankton abundance showed a positive correlation with salinity at all four locations the temperature showed a negative relationship at the location off Anjuna and positive relationship at all other locations.

With exception the concentration of nitrate (R= 0.67; $p< 0.012$) at the location off Chapora, dissolved oxygen (R= 0.71; $p<0.006$) at the location off Anjuna and salinity (R=0.60; $p<0.03$) at the location off Dona Paula (Table 3.3), none of the other parameters had any significant relationship with chl *a* in this study. On the other hand, concentrations of nitrate and silicate showed highly significant relationship ($p<0.001$) with salinity at all the locations.

Linear regression analysis between total abundance and chl *a* concentration suggests a significant relationship off Siridao (R=0.79; $p<0.001$) followed by

Table 3.1. Correlation matrix (R) for the data on phytoplankton abundance (PPCC), temperature (Temp), salinity (Sal), dissolved oxygen (DO), pH, chlorophyll *a* (Chl *a*), nitrite ($NO_2$), nitrate ($NO_3$), phosphate ($PO_4$) and silicate ($SiO_4$) from water samples analyzed from the location off Chapora

| **Parameters** | **PPCC** | **Temp** | **Sal** | **DO** | **pH** | **Chl *a*** | **$NO_2$** | **$NO_3$** | **$PO_4$** | **$SiO_4$** |
|---|---|---|---|---|---|---|---|---|---|---|
| **PPCC** | 1.00 | | | | | | | | | |
| **Temp** | 0.31 | 1.00 | | | | | | | | |
| **Sal** | 0.44 | 0.51 | 1.00 | | | | | | | |
| **DO** | 0.36 | -0.21 | -0.25 | 1.00 | | | | | | |
| **pH** | 0.41 | 0.54* | 0.63* | 0.30 | 1.00 | | | | | |
| **Chl *a*** | 0.59* | 0.08 | 0.29 | 0.20 | 0.28 | 1.00 | | | | |
| **$NO_2$** | -0.57* | -0.28 | -0.41 | 0.18 | -0.23 | -0.09 | 1.00 | | | |
| **$NO_3$** | -0.54* | -0.32 | -0.84* | 0.24 | -0.44 | -0.67* | 0.45 | 1.00 | | |
| **$PO_4$** | -0.56* | -0.43 | -0.40 | 0.12 | -0.24 | -0.17 | 0.63* | 0.44 | 1.00 | |
| **$SiO_4$** | -0.35 | -0.46 | -0.86* | 0.06 | -0.77* | -0.33 | 0.39 | 0.70* | 0.29 | 1.00 |

* $p<0.05$

Table 3.2. Correlation matrix (R) for the data on phytoplankton abundance (PPCC), temperature (Temp), salinity (Sal), dissolved oxygen (DO), pH, chlorophyll *a* (Chl *a*), nitrite ($NO_2$), nitrate ($NO_3$), phosphate ($PO_4$) and silicate ($SiO_4$) from water samples analyzed from the location off Anjuna

| Parameters | PPCC | Temp | Sal | DO | pH | Chl *a* | $NO_2$ | $NO_3$ | $PO_4$ | $SiO_4$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **PPCC** | 1.00 | | | | | | | | | |
| **Temp** | -0.39 | 1.00 | | | | | | | | |
| **Sal** | 0.19 | 0.29 | 1.00 | | | | | | | |
| **DO** | 0.47 | -0.48 | 0.12 | 1.00 | | | | | | |
| **pH** | -0.13 | 0.50 | 0.31 | 0.03 | 1.00 | | | | | |
| **Chl *a*** | 0.57* | -0.27 | 0.19 | 0.71* | -0.12 | 1.00 | | | | |
| **$NO_2$** | 0.18 | -0.56* | -0.26 | 0.11 | -0.17 | 0.02 | 1.00 | | | |
| **$NO_3$** | -0.54* | -0.17 | -0.83* | -0.20 | -0.44 | -0.16 | 0.13 | 1.00 | | |
| **$PO_4$** | -0.04 | -0.28 | -0.49 | -0.03 | -0.42 | 0.15 | 0.19 | 0.64* | 1.00 | |
| **$SiO_4$** | -0.19 | -0.33 | -0.87* | -0.21 | -0.45 | -0.14 | 0.26 | 0.88* | 0.82* | 1.00 |

* $p<0.05$

Table 3.3. Correlation matrix (R) for the data on phytoplankton abundance (PPCC), temperature (Temp), salinity (Sal), dissolved oxygen (DO), pH, chlorophyll *a* (Chl *a*), nitrite ($NO_2$), nitrate ($NO_3$), phosphate ($PO_4$) and silicate ($SiO_4$) from water samples analyzed from the location off Dona Paula

| **Parameters** | **PPCC** | **Temp** | **Sal** | **DO** | **pH** | **Chl *a*** | **$NO_2$** | **$NO_3$** | **$PO_4$** | **$SiO_4$** |
|---|---|---|---|---|---|---|---|---|---|---|
| **PPCC** | 1.00 | | | | | | | | | |
| **Temp** | 0.07 | 1.00 | | | | | | | | |
| **Sal** | 0.38 | 0.38 | 1.00 | | | | | | | |
| **DO** | -0.59* | -0.45 | -0.53 | 1.00 | | | | | | |
| **pH** | -0.61* | 0.38 | 0.28 | -0.02 | 1.00 | | | | | |
| **Chl *a*** | 0.41 | 0.12 | 0.60* | 0.03 | 0.08 | 1.00 | | | | |
| **$NO_2$** | 0.13 | -0.05 | 0.09 | -0.24 | -0.20 | -0.06 | 1.00 | | | |
| **$NO_3$** | -0.58* | -0.33 | -0.84* | 0.40 | -0.17 | -0.44 | 0.50 | 1.00 | | |
| **$PO_4$** | -0.56* | -0.46 | -0.54* | 0.63* | -0.01 | -0.01 | 0.45 | 0.70* | 1.00 | |
| **$SiO_4$** | -0.33 | -0.41 | -0.90* | 0.45 | -0.36 | -0.51 | 0.14 | 0.74* | 0.63* | 1.00 |

* $p<0.05$

Table 3.4. Correlation matrix (R) for the data on phytoplankton abundance (PPCC), temperature (Temp), salinity (Sal), dissolved oxygen (DO), pH, chlorophyll *a* (Chl *a*), nitrite ($NO_2$), nitrate ($NO_3$), phosphate ($PO_4$) and silicate ($SiO_4$) from water samples analyzed from the location off Siridao

| **Parameters** | **PPCC** | **Temp** | **Sal** | **DO** | **pH** | **Chl *a*** | **$NO_2$** | **$NO_3$** | **$PO_4$** | **$SiO_4$** |
|---|---|---|---|---|---|---|---|---|---|---|
| **PPCC** | 1.00 | | | | | | | | | |
| **Temp** | 0.18 | 1.00 | | | | | | | | |
| **Sal** | 0.12 | 0.32 | 1.00 | | | | | | | |
| **DO** | -0.25 | -0.46 | -0.35 | 1.00 | | | | | | |
| **pH** | -0.45 | 0.27 | -0.02 | 0.14 | 1.00 | | | | | |
| **Chl *a*** | 0.79* | 0.02 | 0.31 | -0.04 | -0.35 | 1.00 | | | | |
| **$NO_2$** | -0.00 | -0.11 | 0.17 | -0.58* | -0.39 | -0.07 | 1.00 | | | |
| **$NO_3$** | -0.19 | -0.41 | -0.63* | -0.09 | -0.35 | -0.32 | 0.62* | 1.00 | | |
| **$PO_4$** | -0.05 | -0.66* | -0.18 | 0.31 | -0.10 | 0.12 | 0.30 | 0.35 | 1.00 | |
| **$SiO_4$** | -0.23 | -0.52* | -0.87* | 0.26 | -0.22 | -0.27 | 0.17 | 0.84* | 0.45 | 1.00 |

* $p<0.05$

Chapora (R=0.59; $p<0.034$) and Anjuna (R=0.57; $p<0.04$) (Fig.3.8). However, this relationship was positive but not significant ($p> 0.05$) off Dona Paula.

## Discussion

On the whole the fluctuations in physico-chemical parameters in the study region are not very different from monsoon affected west coast of India. While a large scale differences in these parameters are documented for the monsoon months, the premonsoon period is usually more saline throughout salt intrusions lengths of all estuaries along the west coast of India (Qasim et al., 1972). Seasonal and spatial dynamics of phytoplankton biomass at four chosen locations for the study was very complex and driven by a variety of physical, chemical and biological factors. Cell abundance exhibited wide fluctuations from as lower as 886 cells $L^{-1}$ to as high as $7.7 \times 10^5$ cells $L^{-1}$, the maximum density occurring during September 2008 followed by the post-monsoon months. It is evident that during this period conducive conditions prevail for the proliferation of both diatoms and dinoflagellates. From the Cochin Backwater, in the south-west coast of India, Devassy and Bhattathiri (1974) observed surface densities of phytoplankton varying from 22.2 to $299.7 \times 10^3$ cells $L^{-1}$. In the Vellar estuary on the east coast of India, the maximum surface phytoplankton density was $15 \times 10^3$ cells $L^{-1}$ (Santhanam et al., 1975). Thus, compared to these estuarine systems, the locations in this study area apparently sustain relatively higher phytoplankton populations. These results also agree with an earlier study done by Kumari and John (2003) who reported similar cell abundance from Mandovi-Zuari estuarine regions. However, the results

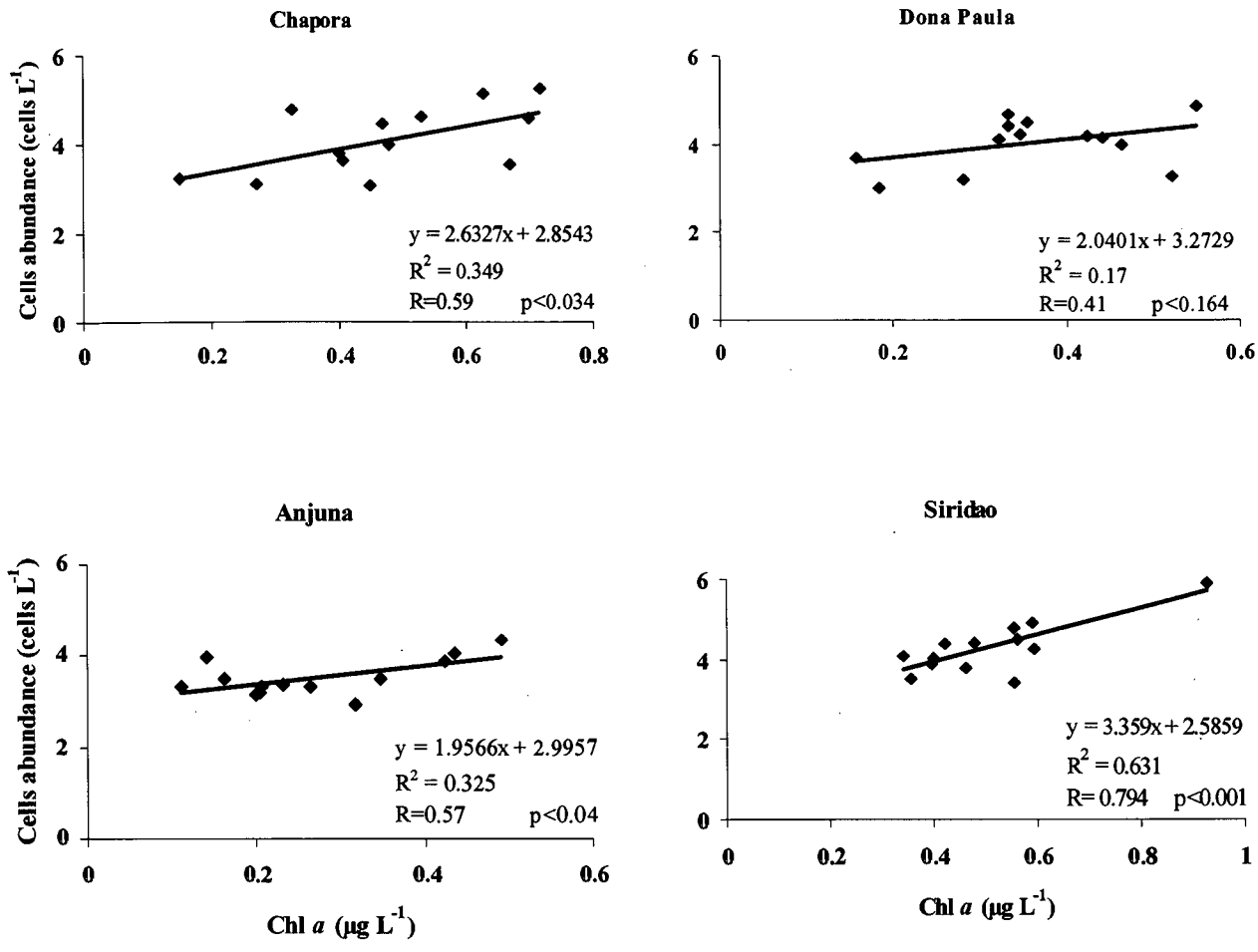



Fig.3.8. Linear regression relationship between log($x$ + 1) transformed values of phytoplankton abundance and chlorophyll *a* (µg $L^{-1}$) during the study period at different sampling locations off Goa

from this study indicate lower cell density compared to the earlier reports of Devassy and Goes (1988) from Mandovi-Zuari estuaries.

It is well known that rainfall patterns impact a variety of factors such as nutrient load, salinity variation and water flushing rates that directly or indirectly affect phytoplankton productivity and standing crop in estuarine ecosystems, (Weise et al., 2002). All of these factors also play an important role in guiding species composition and succession and impact strongly on the abundance of phytoplankton (Smayda, 1980; Smayda and Reynolds, 2001; Anderson and Rengefors, 2006; Reynolds, 2006; Phlips et al., 2010). Spatharis et al. (2008) found that maximum phytoplankton biomass values were usually found in coastal areas influenced by runoff. From the annual variations observed in the phytoplankton abundances, and those in chl *a* concentrations, it is implicit that results of this study corroborate well with those reported earlier.

In monsoon months (June-September), both physical and chemical processes including salinity, lower water temperature and high nutrient input from the Zuari and Chapora rivers could be discerned at the locations off Siridao, Dona Paula and Chapora. During this period nutrients in particular, silicate increased to the highest level off Siridao 128.2 μmol $L^{-1}$and Dona Paula 122.67 μmol $L^{-1}$ and salinity dropped to the lowest level (0.44 psu) off Chapora. Unlike off Siridao, Dona Paula and Chapora having low saline surface waters, the salinity off Anjuna remained higher.

During this study, the environmental conditions in the coast of Goa were characterized by a period of low freshwater input during pre-monsoon months

(February-May), followed by a period of increased freshwater flow during the monsoon season (June-September). The freshwater inflow was reflected in the salinity regimes observed in the study area and enhanced concentrations of inorganic nutrients. The concentrations of nutrient particularly nitrate and silicate correlated significantly with salinity at all locations (Tables 3.1-3.4). This suggests that $NO_3$ and $SiO_4$ influx are strongly correlated with the amount of the rivers discharge entering Goa waters. Observation of this study corroborate with the reports of Devassy and Goes, (1989) and Mitbavkar and Anil (2008) who also noticed strong relationship between salinity and concentration of nitrate and silicate from the Mandovi-Zuari estuaries.

The salinity fluctuations are high in the study area almost throughout the year owing to tidal flow, mixing of fresh water and marine water by wind and water current. Many earlier studies report that the growth of phytoplankton population is governed, albeit slightly, due to salinity variations (Lionard et al., 2005; Putland et al., 2007). Although there was no significant correlation between the phytoplankton abundance and salinity, it is inferrable from the linear regression analysis (Fig. 3.9) that increasing salinity facilitates the growth of phytoplankton.

During August 2008, the cell abundance and phytoplankton biomass (chl *a*) were the lowest at all locations. This depletion in population coincided with the heavy freshwater runoff which probably resulted in flushing of phytoplankton populations (Devassy and Goes, 1988). On the other hand, major peaks of phytoplankton abundance coincided with salinity <32psu. Some of these peaks particularly off Chapora and Siridao occurred when salinities were <20 psu. Studies in the regions

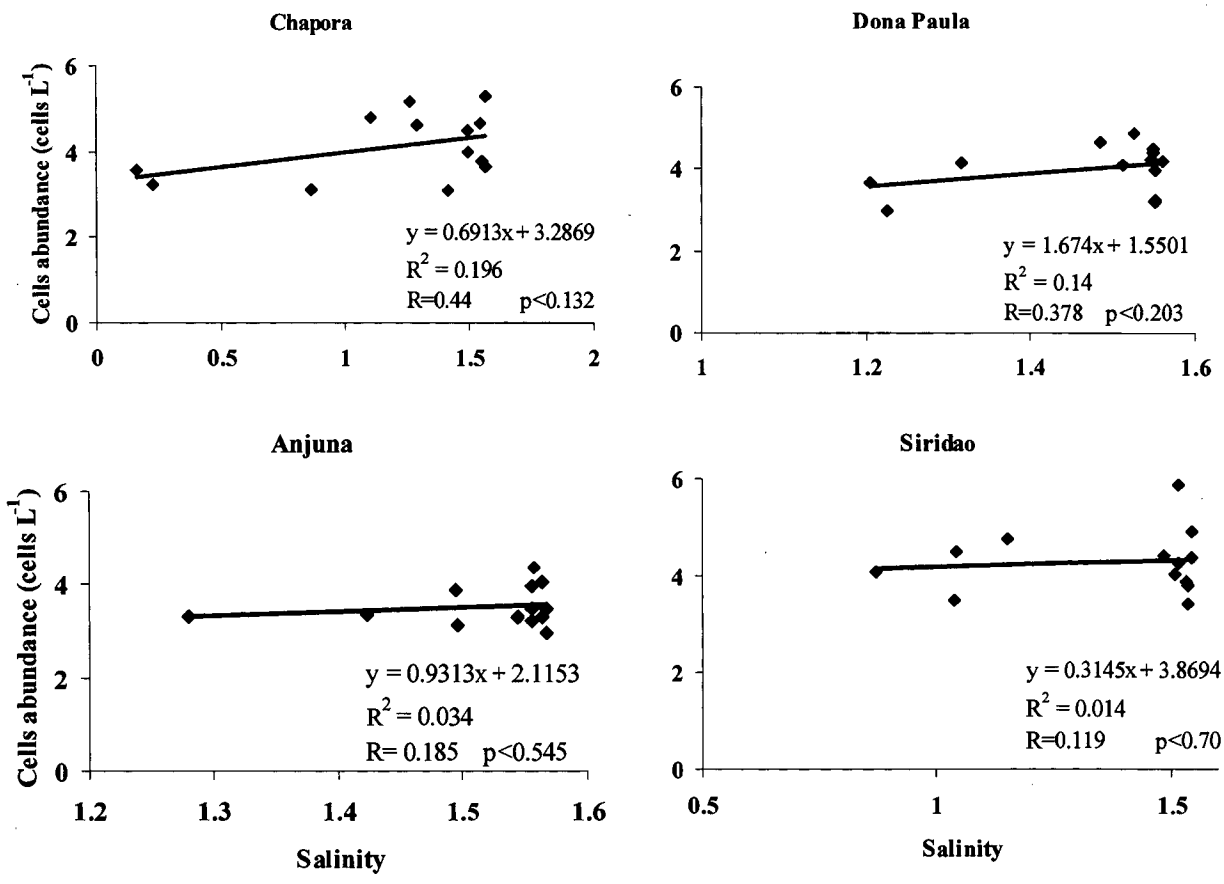



Fig.3.9. Linear regression relationship between log($x$ + 1) transformed values of phytoplankton abundance and salinity during the study period at different sampling locations off Goa

of New England and Bermuda where the salinity changes are very small (31.68 to 36.53 psu) have indicated that many species of phytoplankton preferred lower salinities (Hulburt and Rodman 1963). In a study carried out for five years along the west coast of India showed that almost all peaks in phytoplankton production coincided with periods of low salinity (Subramanyan, 1959), thus exhibiting a preference to low saline waters than high saline waters. Qasim et al. (1972) reported that the rate of photosynthesis and growth of the phytoplankton in the estuaries is affected by salinity fluctuations. Many species of phytoplankton from other parts of the world are also reported to have their maximum growth at lowered salinities (Nakanishi and Monsi, 1965). Results from this study confirm the views of Devassy and Goes (1988; 1989) who suggested that the cell abundance and phytoplankton biomass (chl *a*) is influenced by fluctuating salinity.

Nutrients have been considered as the major factors controlling the composition and abundance of phytoplankton community, and also for the occurrence of the blooms. Silicate and nitrate are implied to play important roles in regulating population dynamics and blooms of diatoms (Eppley, 1977; Hodgkiss and Lu, 2004). While, phosphate and N/P ratio to be regulators of dinoflagellates (Hodgkiss and Ho, 1997; Yutaka et al., 1998). Studies have also shown that diatoms have high growth rates under nutrient-rich conditions (Eppley, 1977) compared to the generally lower growth rates of dinoflagellates (Hodgkiss and Lu, 2004). From the annual data sets from four disparate locations chosen for this study, it is clearly seen that the phytoplankton community in the coastal waters of Goa is dominated by diatoms due to the high nutrient and adequate silicate concentrations. One explanation for higher

diatom abundance, >90% of total cell counts all through the year, and lower abundance of other algal groups is that diatoms are more sensitive to nutrient enrichment than the others and proliferate under ideal nutrient regimes though other governing factors do affect their growth and physiology.

The highest abundance of dinoflagellates were observed September 2008 followed by October 2007 during the periods of calmer sea conditions at all locations. The maximum cells abundance was seen off Siridao ($8.6 \times 10^3$) during September 2008. Their lowest abundance was observed off Anjuna. However, their higher counts were recorded from this location under the conditions of high to moderate nutrient concentrations. According to Margalef (1978), marine environments with low turbulence and high nutrient inputs favor dinoflagellate growth. Dinoflagellates are known to have slower growth rates at low nutrient concentrations compared with many other algal groups. Faust et al. (2005) had suggested that in stable waters with relatively higher nutrient concentrations, dinoflagellates have a competitive growth advantage. It is therefore possible to suggest that the dinoflagellates proliferate during the periods of calmer sea conditions and moderate nutrient concentrations.

With exception of the location off Dona Paula, the linear regression analyses between total abundance and chl *a* concentration indicated a significant positive relationship between these parameters at all other locations (Fig.3.8). Chlorophyll *a* concentration peaked during September 2008 at all locations with the highest concentration (7.5 $\mu g\ L^{-1}$) off Siridao. Maximum phytoplankton abundance coincided with peak values of chl *a* at all four locations during this month. Two major peaks of chl *a* were observed off Chapora during May and August (4.19 and

3.69 μg $L^{-1}$). The highest concentration of chl *a* off Chapora during May 2008 coincided with the bloom proportions of some diatom species. In contrast, phytoplankton cell abundance was quite less during August 2008. Similar results were observed from off Dona Paula. Strangely, maximum chl *a* concentration was observed when cell abundance was quite low during June and February 2008. Such a large gap between high chlorophyll concentration and low cell abundance could imply that the phytoplankton assemblage was mainly composed of pico- and nano-plankton in these periods. Tarran et al. (1999) found that the picoplanktonic autotrophs, less than 2 μm in size, dominate the phytoplankton communities of the Arabian Sea during monsoon months. Roy et al. (2006) reported a greater abundance of picoplankton in the west coast of India. Picoplankton communities are generally dominated by prochlorophyte and cyanobacteria, which are common in tropical oceans (Claustre, 1994) and most likely, represent systems associated with regenerated production.

The lowest chl *a* concentration was observed off Anjuna during the study period. The low chl *a* and cell abundance at this location may be attributed to the generally lower concentrations of nutrients. The high salinity at this location during the study period indicates it to be a truly marine regime. On the other hand, the higher levels of pheophytin at this location might be an indication of some stress/es to phytoplankton cells. As also reported by Jacob and Zarba (1981) and Ram et al (1998), their ratios in general were lower in this coastal location than those locations in the Zuari and Chapora estuarine realms.

In conclusion, variability among environmental hydrographic parameters are dynamically impacted by the monsoonal effects. The general picture of phytoplankton abundance and biomass (chl *a*) along the coast off Goa appears to be governed more by rainfall which impacts the nutrient inputs and salinity fluctuations. This reflection is further supported by the observed decline in phytoplankton abundance during the monsoon season, when high rainfall may limit water residence time and the time available for the buildup of phytoplankton biomass, which increased during the post-monsoon months. Although the other groups of phytoplankton < 20μm were not counted during this study, the results of regression analyses suggest that microphytoplankton -particularly diatoms- made the major contribution to chl *a* concentrations. As is clear in the proceeding chapter, there is a very diverse assemblage of diatom species in the near shore estuarine and upstream regions. The dinoflagellate contribution to the abundance was significant only during a few months that too without a seasonal or successional trend.

# Chapter 4

## Phytoplankton Biovolume and Cell Carbon Estimations

### Introduction

Biovolume, the three dimensional inner space, of marine phytoplankton cells is an important characteristic in the study of phytoplankton ecology, physiology and biochemistry. Phytoplankton cells demonstrate a wide range of shapes and vary over several orders of magnitude in size, from submicron species such as the picoplanktonic to diatoms measuring more than 1 mm in diameter (Reynolds, 1984). In mixed-species samples, high numbers of small-sized species might actually contribute to only a minor fraction of the overall biomass, whereas other, larger-sized species that are much less abundant in numbers might dominate the overall biomass (Smayda, 1978, Wetzel and Likens, 1991). A standard biomass estimate is essential for comparing the relative contribution of different phytoplankton in mixed- taxa samples or between samples and systems (Hillebrand, 1997), to study cell cycle processes (Potapova and Snoeijs, 1997), or to convert phytoplankton biovolume to carbon (Rocha and Duncan, 1985).

Carbon is the principal structural component of both heterotrophic and phototrophic organisms and is the basis for community-wide as well as group-specific comparisons of biomass and bioenergetics. Estimates of carbon biomass of planktonic organisms are usually made by converting microscopic size measurements to cell volumes, which are then converted to carbon biomass using empirically or theoretically derived carbon to volume ratios (Menden-Deuer and

Lessard, 2000). Calculating phytoplankton carbon from biovolume, rather than from particulate organic carbon, eliminates the error due to detrital particulate matter contained in particulate organic carbon (Mullin et al., 1966, Rocha and Duncan, 1985, Montagnes et al., 1994). Several relationships between carbon and biovolume have been established in literature (Mullin et al., 1966; Eppley et al., 1970; Taguchi, 1976; Rocha and Duncan, 1985; Montagnes et al., 1994; Menden-Deuer and Lessard, 2000).

Cell volumes can be calculated from cell-size and shape by use of appropriate geometric formulae. As it is impossible to measure and calculate every individual cell in routine counting, the same shape and a mean size was originally assumed for each species. This simplification, however, introduces error into the biovolume calculation (Olenina et al., 2006). The consensus method for calculating phytoplankton cell biovolume is based on geometric assignation (Smayda, 1978; Edler, 1979; Rott, 1981; Kononen et al., 1984; Vilicic, 1985; Hillebrand et al., 1999; Sun and Liu, 2003; Olenina et al., 2006; Vadrucci et al., 2007). Microscopic observation is a direct, convenient way to obtain species level information on phytoplankton taxa, whereas biovolume calculation based on geometric models of phytoplankton cells and their related conversion biomass are popular in phytoplankton ecology (Hillebrand, 1997; Young and Ziveri, 2000). Though such conversions are widely acceptable and pragmatic, it is indeed necessary to make microscopic measurements of sizes of phytoplankton assemblages in order rivet or fine tune the biovolume calculations. With this view, size measurements of at least 25 cells of all dominant species of phytoplankton were made. Using these

statistically dependable measurements, the size classes of each dominant phytoplankton species and its mean biovolume were worked out.

## Material and Methods

### *Phytoplankton Samples*

Water sample from all four locations (Fig 3.1) were fixed in Lugol's iodine (1% w/v) and 3% formaldehyde and stored in dark until taken up for analyses as per the details already described in Chapter 3.

### *Measurements of Geometric Shapes and Equations*

Lengths, breadths and/or circumferences of individual cells of phytoplankton species from all four sampling locations were performed on as many as 52 water samples during thirteen sampling months. Sixteen basic geometric shapes and equations were used for estimating the biovolume and surface area of phytoplankton cells (Table 4.1). For each taxon, the best fitting geometric shape, and appropriate equation as per the guidelines of Sun and Liu (2003) was used. Biovolumes of *Asterionella glacialis* and *Licmophora* spp were estimated according to Olenina et al. (2006). Other phytoplankton species were estimated using the shapes and equations from Sun and Liu (2003).

The length and width of the cells were measured with the help of an occulometer using light microscope under 400X magnification. Wherever applicable, the height/depth/sideward dimension of the target cell was measured after rolling the

Table 4.1. Geometric shapes and equations used for calculating biovolume and surface area of phytoplankton species from the nearshore/estuarine water off Goa

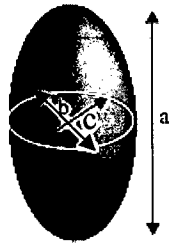
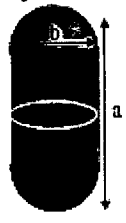
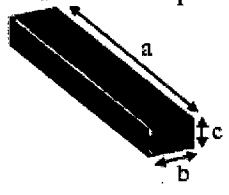
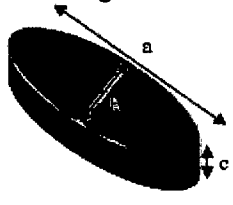
| Shape code | Surface area (A) | Volume (V) | Geometric shape |
|---|---|---|---|
| 1 | $A = \pi \times a^2$ | $V = \pi/6 \times a^2$ |  Sphere |
| 2 | $A = \pi/4 \times (b+c) \times \left[\left(\frac{b+c}{2}\right) + \frac{2a^2}{\sqrt{4a^2-(b+c)^2}} \sin^{-1} \frac{\sqrt{4a^2-(b+c)^2}}{2a}\right]$ | $V = \pi/6 \times a \times b \times c$ |  Ellipsoid |
| 3 | $A = \pi \times a \times \left(\frac{a}{2} + c\right)$ | $V = \pi/4 \times a^2 \times c$ |  Cylinder |
| 4 | $A = \pi \times a \times b$ | $V = \pi \times b^2 \times \left(\frac{a}{4} - \frac{b}{12}\right)$ |  Cylinder+2 half spheres |
| 5 | $A = 2 \times a \times b + 2 \times b \times c + 2 \times a \times c$ | $V = a \times b \times c$ |  Rectangular box |
| 6 | $A = \pi/2 \times (a \times b + b \times c + a \times c)$ | $V = \pi/4 \times a \times b \times c$ |  Prism on elliptic base |

Table 4.1. (Continued)

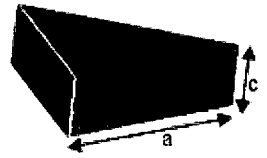
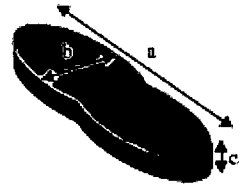
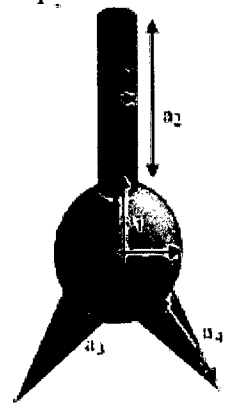
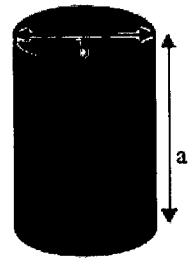
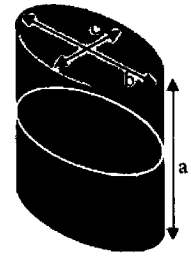
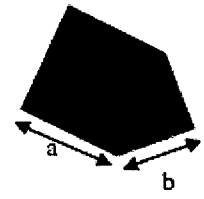
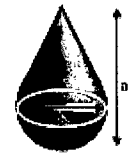
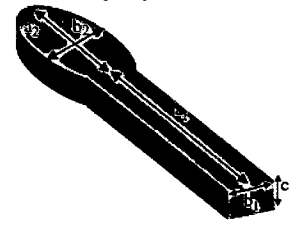
| Shape code | Surface area | Volume | Geometric shape |
|---|---|---|---|
| 7 | $A = a \times b + \frac{\sqrt{a^2 + b^2}}{4} \times c$ | $V = ½ \times a \times b \times c$ |   Prism on parallelogram-base |
| 8 | $A = 3a \times c + \frac{\sqrt{3}}{2} \times a^2$ | $V = \frac{\sqrt{3}}{4} \times c \times a^2$ |   Prism on triangle-base |
| 9 | $A = \pi/2 \times (a \times b + b \times c + a \times c)$ | $V = \pi/4 \times a \times b \times c$ |   Elliptic prism with transapical constriction |
| 10 | $A = \pi/4 \times (b_1 + b_2) \times \left[ \frac{b_1 + b_2}{2} + \frac{a_1^2}{\sqrt{a_1^2 - (\frac{b_1 + b_2}{2})^2}} \times \sin^{-1} \frac{\sqrt{a_1^2 - \left(\frac{b_1 + b_2}{2}\right)^2}}{a_1} \right] + \pi/2 \times b_2 \times \left( 2a_2 + \sqrt{a_3^2 + \frac{b_2^2}{4}} + \sqrt{a_4^2 + \frac{b_2^2}{4}} - b_2 \right)$ | $V = \pi/4 \times a_2 \times b^2_2 + \pi/12 \times (a_3 + a_4) \times b^2_2 + \pi/6 \times a_1 \times b_1 \times b_2$ |  Ellipsoid + 2 cones +cylinder |
| 11 | $A = \pi \times b \times \left( \frac{b}{2} + a \right)$ | $V = \pi/4 \times a \times b^2$ |   Cylinder girdle view |

Table 4.1. (Continued)

| Shape code | Surface area | Volume | Geometric shape |
|---|---|---|---|
| 12 | $A = \pi/2 \times (a \times b + b \times c + a \times c)$ | $V = \pi/4 \times a \times b \times c$ |   Prism on elliptic base girdle view |
| 13 | $A = 3a \times b + \frac{\sqrt{3}}{2} \times b^2$ | $V = \frac{\sqrt{3}}{4} \times a \times b^2$ |   Prism on triangle-base girdle view |
| 14 | $A = \pi/2 \times b^2 \times \left( b + \sqrt{\frac{2a^2 - ab + b^2}{2}} \right)$ | $V = \pi/12 \times a \times b^2$ |   Cone with half Sphere |
| 15 | $A = c \times (2\,a_1 + b_1 + \pi/2 \times a_2 + \pi/2 \times b_2) + 2a_1 \times b_1 + \pi/2 \times a_2 \times b_2$ | $V = c\,(a_1 \times b_1 + \pi/4 \times a_2 \times b_2)$ |   Box + elliptic prism |
| 16 | $A = \pi/2 \times b \times \sqrt{a^2 + b^2}$ | $V = \pi/12 \times a \times b^2$ |   Double cone |

cell by gently touching the cover-slip with a pointed needle under routine examination.

At least 25 cells (10 cells in cases of rarely occurring species) or more individual cells were measured to avoid biasing the results (Sun and Liu, 2003). Cell biovolume and surface areas were calculated from the information of taxa and linear dimensions. To facilitate differentiation of different cell-sizes within a species, they were size-classified. In principle, taxa with large size variations were assigned into more size-classes than those with lower variations.

***Estimation of Per Cell Carbon and Phytoplankton Carbon Biomass***

Biovolumes were converted to carbon units using carbon-biovolume relationships recommended by Menden-Deuer and Lessard (2000) for all groups using the following formulae:

Pg C cell $^{-1}$ = 0.760 × volume$^{0.819}$ for dinoflagellate species

Pg C cell $^{-1}$ = 0.288 × volume$^{0.811}$ for diatoms and silicoflagellates species

Total biomass was then calculated by multiplying cell carbon by cell abundance of major species observed in the samples by following Gosselain et al. (2000) and Putland and Iverson (2007)

## Results

### *Size Classes of Dominant Phytoplankton Species*

The size classes, in the 5 or 10 micron ranges, of the most dominant diatom species (Fig. 4.1) and dinoflagellates (Fig. 4.2) observed during this study imply much larger variations in the cell sizes with in a species. Further, the number of size-classes depended on the size variations in each taxon. *Thalassionema nitzschioides* and *Coscinodiscus marginatus* followed by *Ditylum brightwellii* showed the large variations in their sizes. *T. nitzschioides* was one of the most dominant species and occurred all through the year. Size details of as many as 228 cells of this species were recorded which facilitated to group these cells into 7 size- classes. The length ranged from 20 to 115 μm. The average length was 40.33 μm with the standard deviation of 18.68. *Skeletonema costatum* is also one of the most important diatoms in the west coast of India which occurs all through the year and forms blooms during monsoon and post monsoon months. From the morphometry of 235 cells, four size classes could be discerned and most of the cells clustered between 5 and 10 μm size-class. The average cell length was 11.45 μm and the standard deviation 5.75. It is apparent from the morphometry of other diatoms species that the size variations were, in general, lower.

*Ceratium furca* is one of the most common dinoflagellates in the study area. The length of 32 measured cells ranged between 95 and 215 μm and the length of most cells varied between 95 and 105 μm. While the length of *Prorocentrum micans* ranged only between 30- 50 μm averaging 42.37 μm.

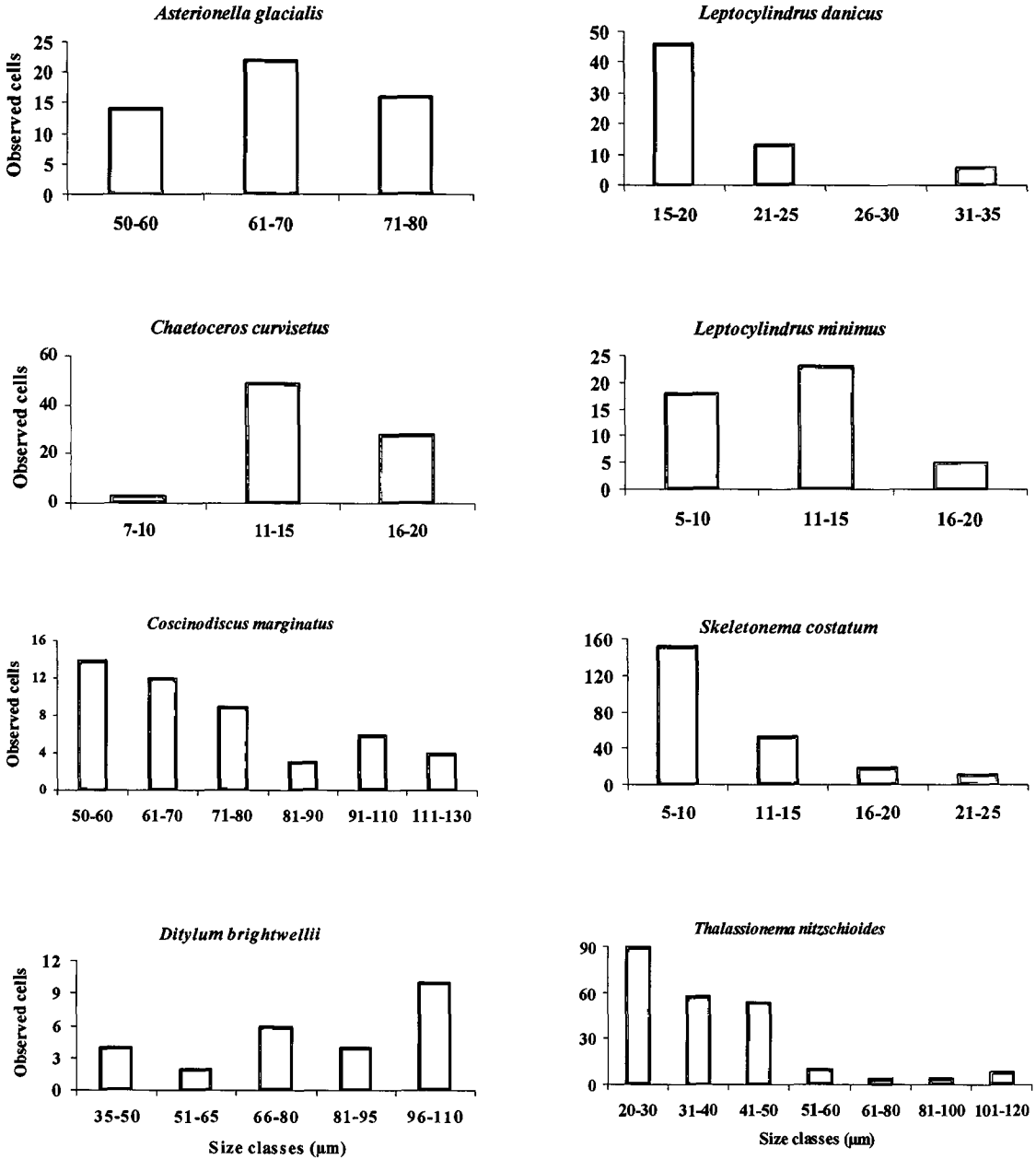



Fig.4.1. Distribution of size classes (in term of cell length or cell diameter) of dominant diatom species nearshore/estuarine water off Goa

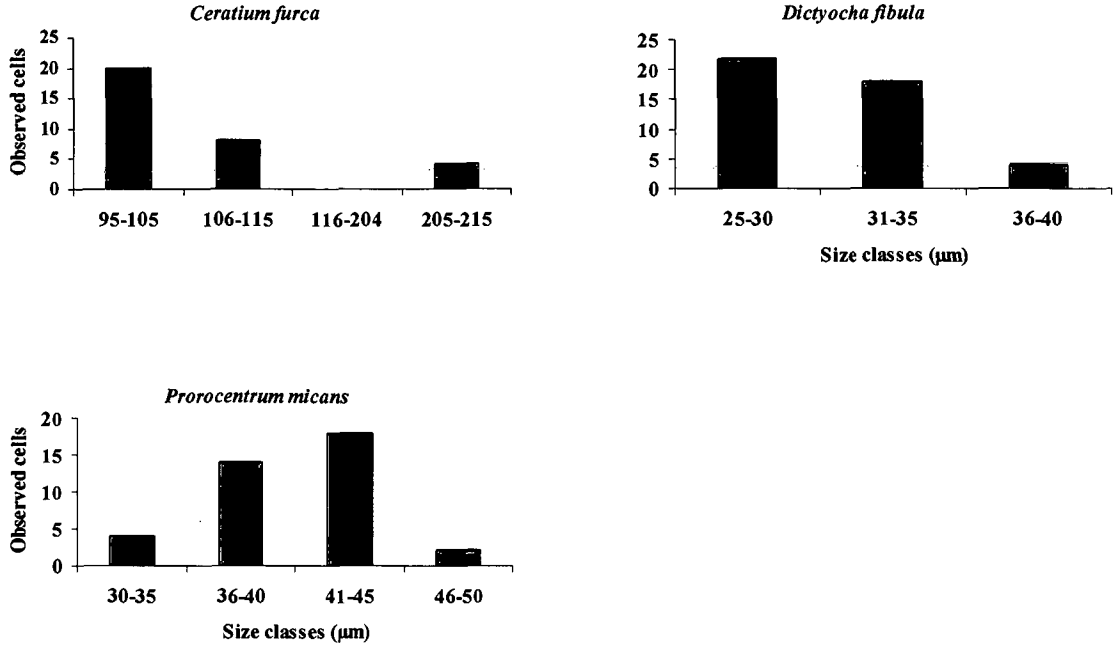



Fig. 4.2. Distribution of size classes (in term of cell length or cell diameter) of dominant dinoflagellate species (*Ceratium furca* and *Prorocentrum micans*) and *Dictyocha fibula* nearshore/estuarine water off Goa

Variations in the diameter of 44 cells of silicoflagellate, *Dictyocha fibula* were quite low in the range of 25-40 μm. Thus, they were aligned into just two size classes.

***Biovolumes and Surface Areas***

During this study, the biovolumes and surface areas of 85 different phytoplankton species were measured. All these species present in the coastal waters of Goa aligned within 16 basic geometric shapes (Table 4.1). Based on these shapes, the determination of surface area and the biovolumes of cells were achieved.

The cell-volumes of all the species studied in this investigation ranged from 251 to 912030 $\mu m^3$. The largest biovolume was observed in *Odontella sinensis* (912030 $\mu m^3$) followed by the dinoflagellate species *Protoperidinium depressum* (677598 $\mu m^3$; Table 4.2). In general, most species of *Biddulphia* and *Coscinodiscus* possess larger biovolume. In contrast, all of *Leptocylindrus* spp have smaller biovolumes. The least biovolume during this study was observed in *Leptocylindrus minimus* (251 $\mu m^3$) followed by Navicula sp (335 $\mu m^3$) *Chaetoceros diversus (*540 $\mu m^3$), *C. laciniosus* (541 $\mu m^3$) and *Skeletonema costatum* (599 $\mu m^3$). Similar to biovolume, the cells of *Odontella sinensis* had the greatest surface area (222717 $\mu m^2$) followed by O. *mobiliensis* (45465 $\mu m^2$). While that of *L. minimus* (230 $\mu m^2$) was the least.

***Carbon Content***

Cellular carbon content for diatoms ranged from 25 to 19632 pg C cell $^{-1}$ (Table 4.2). The lowest carbon content was observed in *Leptocylindrus minimus* (25 pg C cell $^{-1}$) followed by *Navicula* sp (33 pg C cell $^{-1}$) and *Chaetoceros laciniosus* and *C.*

Table 4. 2. Cell-volume, surface area, and mean of carbon content in different phytoplankton species recorded from the nearshore/estuarine water off Goa. Shape codes of phytoplankton species are according to the geometric models in Table 4.1

| | Taxa | Shpe Code | Biovolume ($\mu m^3$) ± S.D | Surface area ($\mu m^2$) ± S.D | Carbon content (Pg cell $^{-1}$) |
|---|---|---|---|---|---|
| | **Diatoms** | | | | |
| 1 | *Achnanthes brevipes* | 9 | 6426± 274 | 465± 091 | 353 |
| 2 | *Amphiprora alata* | 6 | 80970 ± 884 | 11022± 555 | 2755 |
| 3 | *Amphiprora sp1* | 6 | 43473± 113 | 7462± 135 | 1663 |
| 4 | *Amphiprora sp2* | 6 | 117224± 406 | 14251± 338 | 3718 |
| 5 | *Asterionella glacialis* | 14 | 1731± 20 | -- | 257 |
| 6 | *Bacillaria paxillifera* | 5 | 1977± 80 | 1530± 63 | 136 |
| 7 | *Biddulphia biddulphiana* | 12 | 149035±1476 | 31437±1806 | 4518 |
| 8 | *Biddulphia heteroceros* | 12 | 254340±2923 | 22608±2234 | 6969 |
| 9 | *Biddulphia laevis* | 12 | 70295± 777 | 19483± 962 | 2456 |
| 10 | *Biddulphia rhombus* | 12 | 29509± 732 | 11127± 923 | 1215 |
| 11 | *Cerataulina bicornis* | 11 | 9559± 135 | 3134± 185 | 487 |
| 12 | *Chaetoceros coarctatus* | 12 | 4120± 121 | 1452± 96 | 246 |
| 13 | *Chaetoceros curvisetus* | 12 | 1878± 20 | 851± 41 | 130 |
| 14 | *Chaetoceros diversus* | 12 | 540± 21 | 367± 19 | 47 |
| 15 | *Chaetoceros laciniosus* | 12 | 541± 49 | 375± 33 | 47 |
| 16 | *Chaetoceros lorenzianus* | 12 | 23864± 236 | 4898± 324 | 1023 |
| 17 | *Chaetoceros mitra* | 12 | 11876± 312 | 2977± 222 | 581 |
| 18 | *Climacosphenia moniligera* | 15 | 87804±1717 | 16480±1782 | 2942 |
| 19 | *Cocconeis pseudomarginata* | 6 | 10030± 52 | 2651± 80 | 506 |
| 20 | *Corethron criophilum* | 4 | 41497± 195 | 6664± 171 | 1602 |
| 21 | *Coscinodiscus asteromphalus* | 3 | 452160±7692 | 37680±2859 | 11113 |
| 22 | *Coscinodiscus granii* | 3 | 104423± 155 | 12555± 162 | 3386 |
| 23 | *Coscinodiscus jonesianus* | 3 | 178833±1190 | 17751± 635 | 5238 |
| 24 | *Coscinodiscus marginatus* | 3 | 211015±4832 | 20178±1613 | 5990 |
| 25 | *Coscinodiscus radiatus* | 3 | 23138± 643 | 7647± 656 | 997 |
| 26 | *Cyclotella striata* | 3 | 8210± 95 | 2658± 130 | 430 |
| 27 | *Ditylum brightwellii* | 13 | 102757±8341 | 16349±2794 | 3342 |
| 28 | *Eucampia zodiacus* | 12 | 5955± 60 | 1855± 88 | 332 |
| 29 | *Grammatophora marina* | 5 | 7710± 118 | 2768± 171 | 409 |
| 30 | *Guinardia striata* | 11 | 20045± 221 | 5063± 296 | 888 |
| 31 | *Gyrosigma balticum* | 7 | 88197±1539 | 6810±1985 | 2952 |
| 32 | *Gyrosigma fasciola* | 7 | 19651± 90 | 2812± 82 | 874 |
| 33 | *Hemiaulus hauckii* | 12 | 19387± 523 | 4358± 429 | 533 |
| 34 | *Lauderia annulata* | 11 | 18506±2644 | 3785±1004 | 832 |
| 35 | *Leptocylindrus danicus* | 11 | 993± 21 | 583± 43 | 78 |
| 36 | *Leptocylindrus minimus* | 11 | 251± 9 | 230± 23 | 25 |
| 37 | *Licmophora gracile* | 8 | 25619± 895 | -- | 1083 |
| 38 | *Licmophora paradoxa* | 8 | 6234± 120 | -- | 344 |
| 39 | *Melosira nummuloides* | 11 | 4121± 170 | 1368± 162 | 360 |
| 40 | *Meuniera membranacea* | 12 | 75066± 100 | 10215± 170 | 2590 |
| 41 | *Navicula cancellata* | 6 | 12069± 421 | 3669± 463 | 588 |
| 42 | *Navicula directa* | 6 | 2817± 25 | 1387± 56 | 181 |

Table 4.2 (continued)

| | Taxa | Shpe Code | Biovolume ($\mu m^3$) ± S.D | Surface area ($\mu m^2$) ± S.D | Carbon content (Pg cell $^{-1}$) |
|---|---|---|---|---|---|
| 43 | *Navicula disclusa* | 6 | 35325± 1754 | 6123± 340 | 1406 |
| 44 | *Navicula fusca* | 6 | 29438± 2430 | 5691± 264 | 1212 |
| 45 | *Navicula lyra* | 6 | 21251± 3091 | 4980± 1225 | 931 |
| 46 | *Navicula maculosa* | 6 | 6280± 214 | 2198± 357 | 346 |
| 47 | *Navicula simulans* | 6 | 7065± 749 | 2237± 153 | 381 |
| 48 | *Navicula* sp | 6 | 353± 12 | 353± 60 | 33 |
| 49 | *Nitzschia closterium* | 7 | 1676± 15 | 629± 21 | 119 |
| 50 | *Nitzschia longissima* | 7 | 1516± 87 | 718± 111 | 109 |
| 51 | *Nitzschia sigma* | 7 | 10111± 166 | 2455± 144 | 510 |
| 52 | *Nitzschia rectilonga* | 7 | 30234± 557 | 5041± 341 | 1239 |
| 53 | *Odontella mobiliensis* | 12 | 84447± 1895 | 45465± 3626 | 2850 |
| 54 | *Odontella sinensis* | 12 | 912030±80159 | 222717±43915 | 19632 |
| 55 | *Paralia sulcata* | 3 | 18840± 231 | 4396± 89 | 844 |
| 56 | *Pleurosigma aestuarii* | 7 | 19940± 90 | 2429± 53 | 884 |
| 57 | *Pleurosigma angulatum* | 7 | 61256± 1138 | 5081± 383 | 2197 |
| 58 | *Pleurosigma galapagense* | 7 | 56815± 36 | 5837± 52 | 2067 |
| 59 | *Pseudo-nitzschia pungens* | 7 | 1281± 49 | 561± 65 | 95 |
| 60 | *Pseudo-nitzschia sp* | 7 | 1388± 22 | 694± 28 | 102 |
| 61 | *Rhizosolenia imbricata* | 11 | 34316± 5250 | 9908± 2440 | 1373 |
| 62 | *Rhizosolenia setigera* | 11 | 10110± 113 | 4544± 317 | 510 |
| 63 | *Skeletonema costatum* | 4 | 599± 72 | 346± 85 | 52 |
| 64 | *Surirella ovalis* | 6 | 23952± 69 | 4752± 87 | 711 |
| 65 | *Surirella* sp | 6 | 18398± 52 | 3925± 95 | 574 |
| 66 | *Thalassionema nitzschioides* | 5 | 1031± 168 | 867± 242 | 80 |
| 67 | *Thalassiosira eccentrica* | 3 | 14893± 265 | 4142± 244 | 698 |
| 68 | *Thalassiosira* sp | 3 | 14169± 987 | 3471± 575 | 670 |
| | **Dinoflagellates** | | | | |
| 69 | *Alexandrium minutum* | 2 | 15698± 130 | 6013± 265 | 2076 |
| 70 | *Ceratium breve* | 10 | 14828± 379 | 4272± 587 | 1981 |
| 71 | *Ceratium furca* | 10 | 6327± 46 | 1409± 106 | 986 |
| 72 | *Ceratium fusus* | 10 | 8964± 169 | 3291± 284 | 1312 |
| 73 | *Ceratium lineatum* | 10 | 4063± 69 | 1144± 192 | 686 |
| 74 | *Ceratium tripos* | 10 | 10447± 587 | 5675± 432 | 1487 |
| 75 | *Dinophysis acuminata* | 2 | 40718± 239 | 9439± 353 | 4531 |
| 76 | *Dinophysis caudata* | 2 | 46849± 256 | 8377± 739 | 5083 |
| 77 | *Dinophysis* sp | 2 | 14748± 294 | 4198± 99 | 1972 |
| 78 | *Prorocentrum gracile* | 2 | 19055± 330 | 3157± 125 | 2433 |
| 79 | *Prorocentrum micans* | 2 | 14159± 6 | 4073± 76 | 1907 |
| 80 | *Protoperidinium conicum* | 16 | 70272± 2798 | 9117± 1072 | 7085 |
| 81 | *Protoperidinium depressum* | 16 | 677598± 1132 | 41246± 270 | 45329 |
| 82 | *Protoperidinium oceanicum* | 16 | 125011± 467 | 13191± 880 | 11356 |
| 83 | *Protoperidinium steinii* | 16 | 15968± 170 | 3400± 37 | 2105 |
| | **Silicoflagellate** | | | | |
| 84 | *Dictyocha fibula* | 1 | 16829± 56 | 3175± 71 | 770 |
| 85 | *Dictyocha octonaria* | 1 | 13137± 49 | 2692± 11 | 630 |

*diversus* (47 pg C cell $^{-1}$). Among the diatoms, the highest cell carbon was observed in *Odontella sinensis*. Ranging from 686 to 45329 pg C cell $^{-1}$, dinoflagellates have more cellular carbon compared to other phytoplankton groups (Table 4.2). The highest carbon content was observed in *Protoperidinium depressum* and the lowest in *Ceratium lineatum*.

***Carbon Biomass***

Phytoplankton carbon biomass varied significantly both spatially and temporally in the coast off Goa (Fig 4.3). The highest carbon biomass was observed during September 2008 at all the locations except off Chapora. A peak in standing stock was recorded at the location off Chapora in May 2008 when *Chaetoceros laciniosus* was the dominant taxon and formed bloom during this month. Spatially, Siridao showed the highest carbon biomass. The lowest was observed off Anjuna. It was clear from this study that the main carbon contributors were diatom at the all four locations (Fig.4.3). Most of diatoms carbon was contributed by the larger species (particularly, *Coscinodiscus marginatus and Ditylum brightwellii*). However, dinoflagellates were the dominant group in terms of carbon content off Anjuna during October 2007 only.

It is interesting to note that off Siridao, most of the dominant species contributed substantially to the carbon biomass including the dinoflagellate species, *Ceratium furca* (Fig 4.7). *Coscinodiscus marginatus* was the most contributing species to the standing stocks off Chapora (Fig 4.4). However, *Ditylum brightwellii* contributed the highest biomass at the location off Anjuna (Fig 4.5). Cell carbon from samples

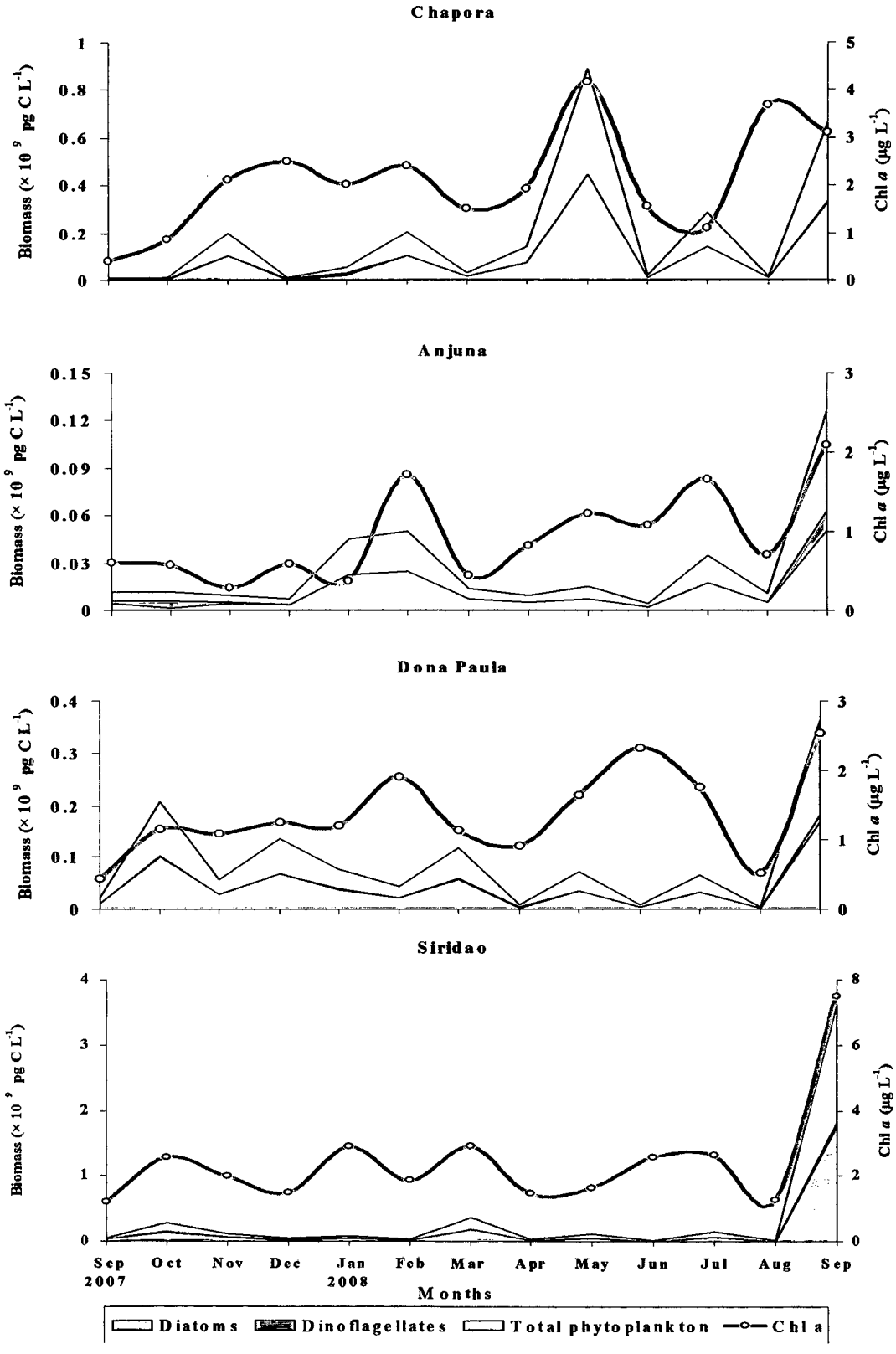



Fig. 4.3. Carbon biomass of total phytoplankton, diatoms and dinoflagellates calculated from the biovolume of taxa at different sampling locations off Goa, during September 2007-September 2008

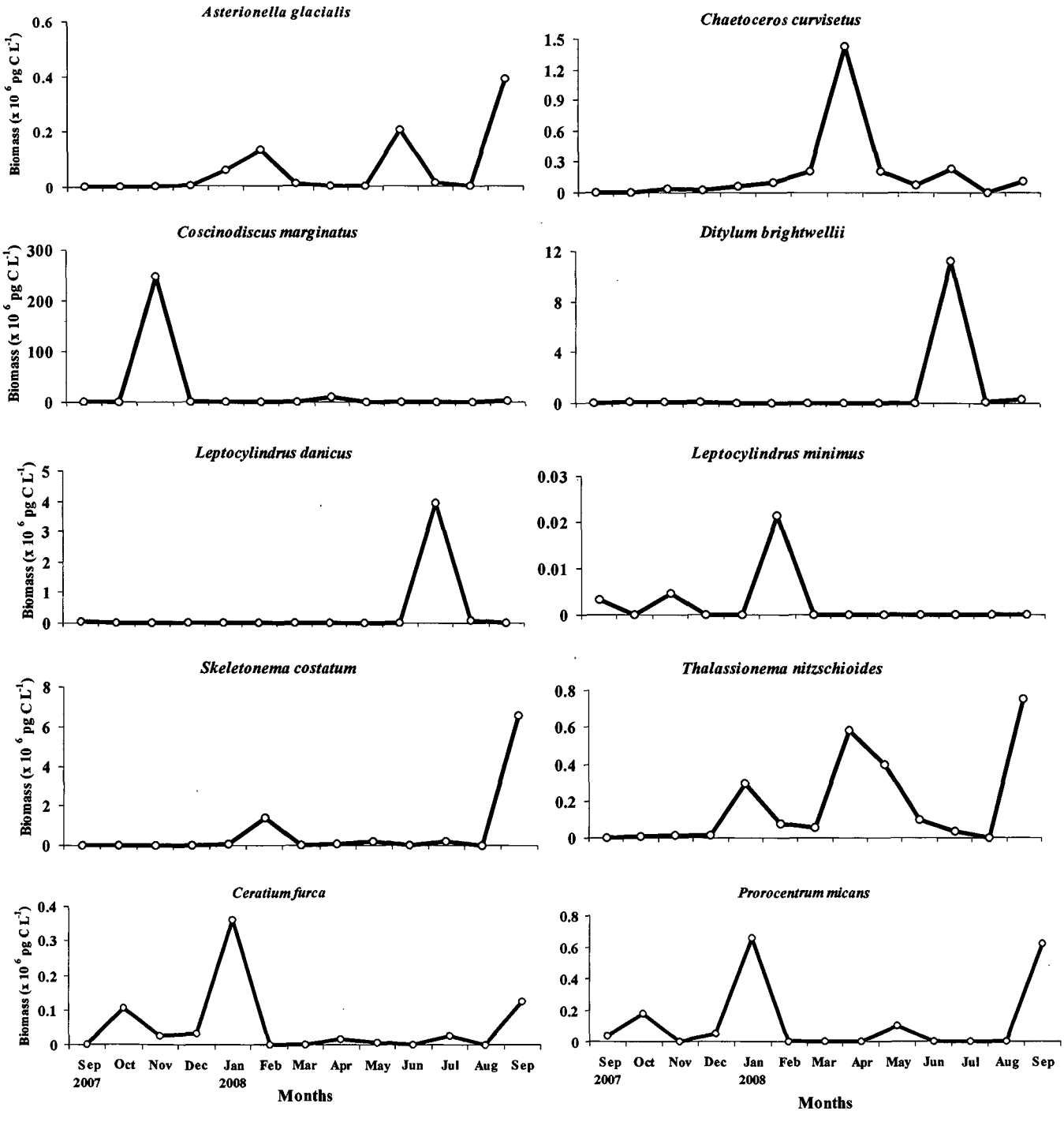



Fig 4.4. Carbon biomass of the most dominant species from the sampling location off Chapora

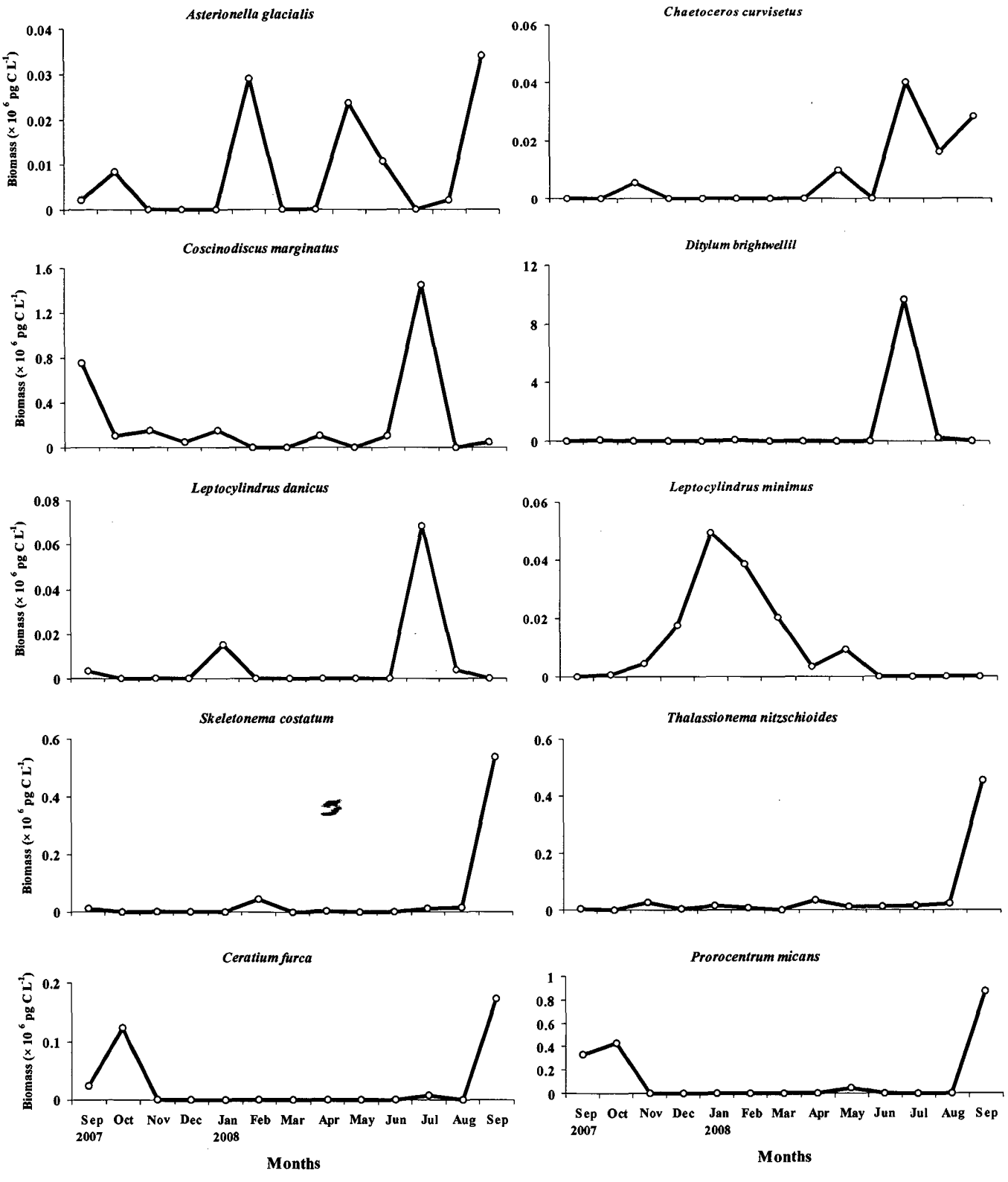



Fig 4.5. Carbon biomass of the most dominant species from the sampling location off Anjuna

collected from off Dona Paula is attributable mainly to diatom, *D. brightwellii* which dominated the phytoplankton biomass, followed by *Chaetoceros curvisetus* and *Skeletonema costatum* (Fig 4.6).

The chl *a* data showed trends very similar to those of the estimated carbon values (Fig 4.3).

## Discussion

Biovolume is one of the most important and basic morphometric descriptors of phytoplankton. To measure the biovolume of phytoplankton, different sets of equations and geometric shapes have been used by different researchers (Smayda, 1978; Edler, 1979; Rott, 1981; Kononen et al., 1984; Vilicic, 1985; Hillebrand et al., 1999; Sun and Liu, 2003; Olenina et al., 2006; Vadrucci et al., 2007). The selection of the equivalent geometric shapes requires careful attention (Smayda 1978). In general, the use of morphometric parameters of phytoplankton for defining the health of transitional ecosystems status appears to be validated by the quantity of experimental evidence highlighting the sensitivity and symptomatic responses of these parameters to environmental forcing and pollution (Vadrucci et al., 2007). However, a good descriptor needs to be not only sensitive and symptomatic of environmental risk but also comparable and reliable. This is possible only if there exists a standardized procedure for its determination.

It is clear from this study that both biovolume and surface area of the most dominant species are smaller. *Skeletonema costatum* is one of the most dominant species in the west coast of India (Patil and Anil 2008; Kumar et al 2009, D'Costa

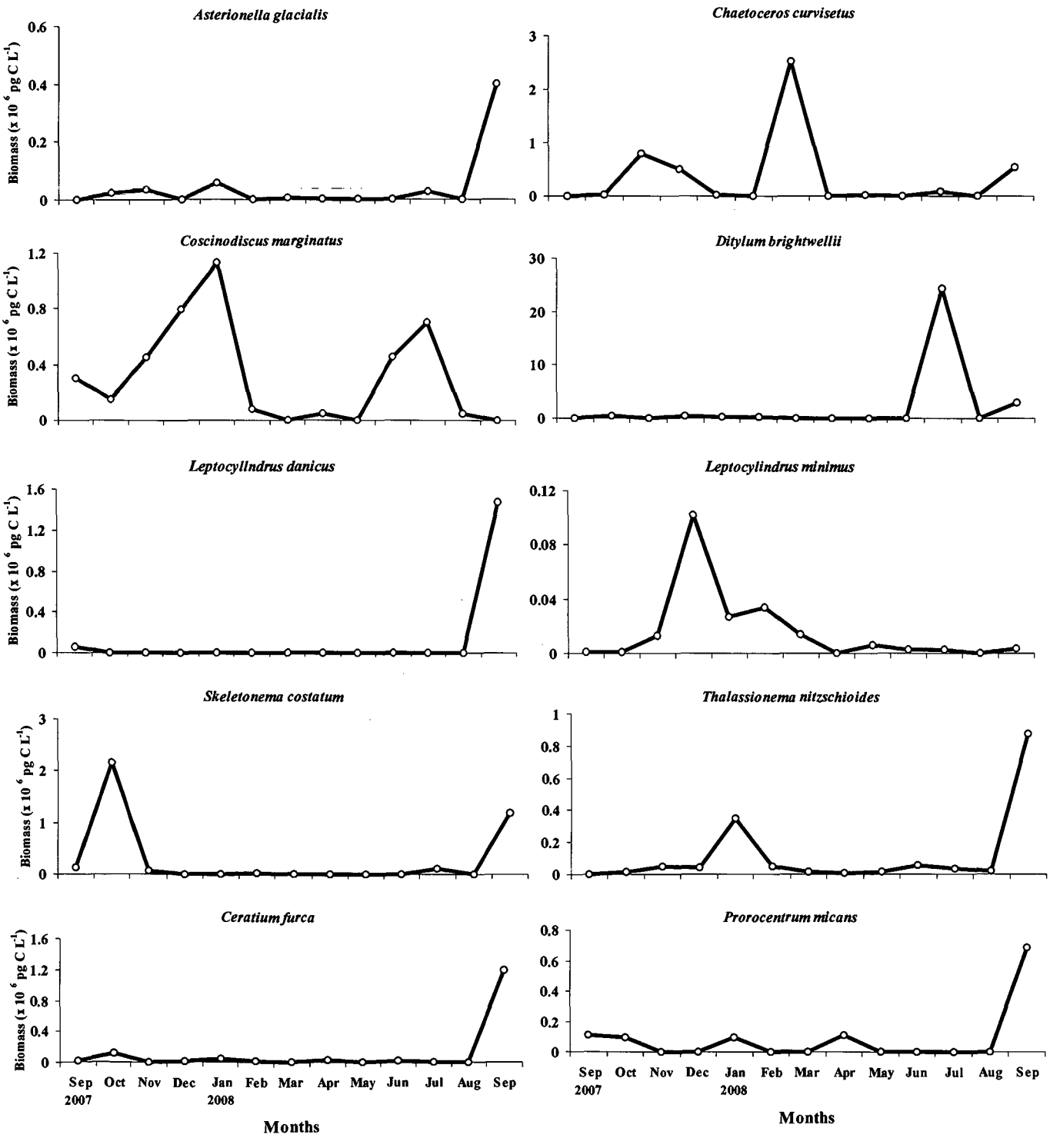



Fig 4.6. Carbon biomass of the most dominant species from the sampling location off Dona Paula

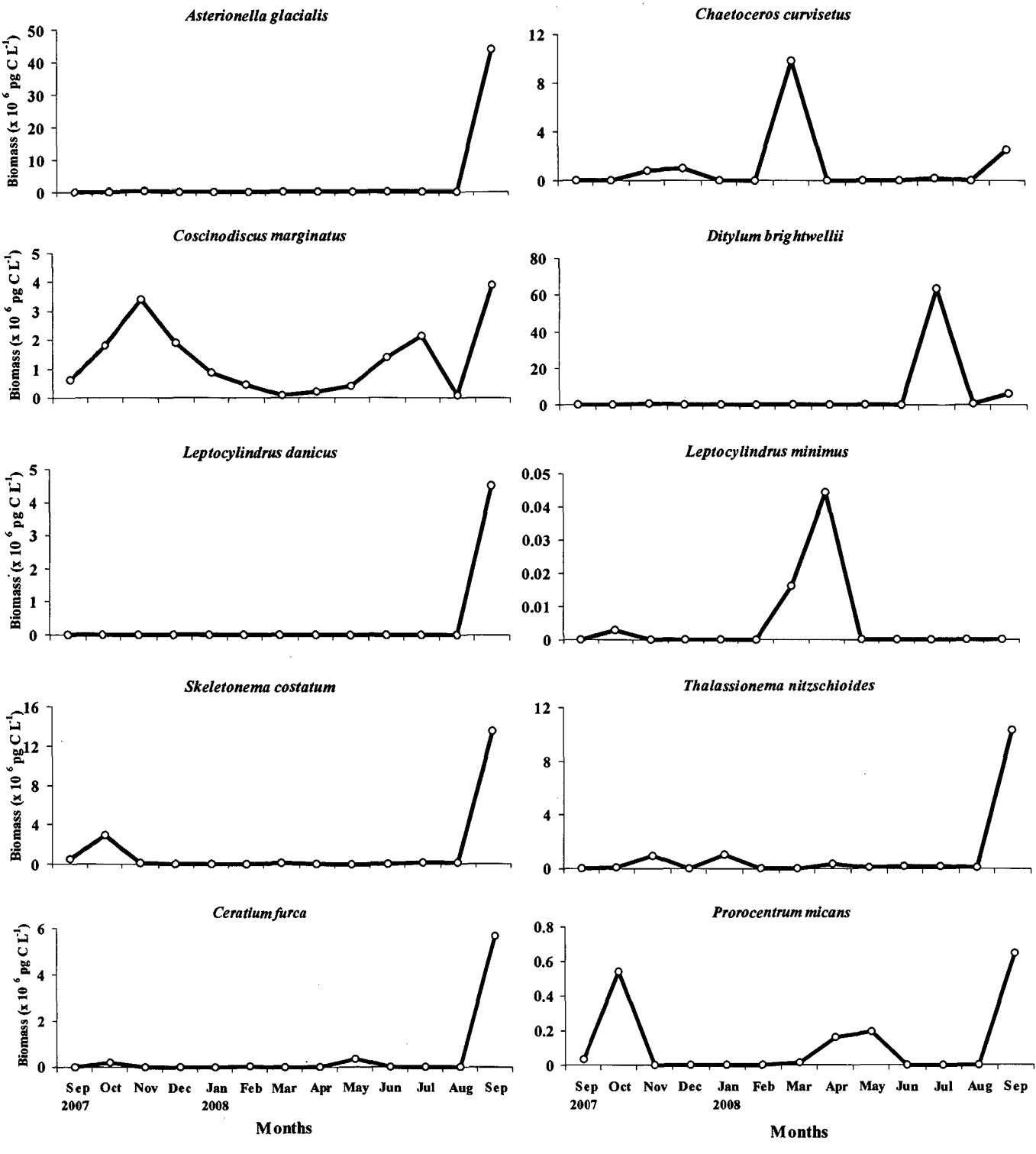



Fig 4.7. Carbon biomass of the most dominant species from the sampling location off Siridao

and Anil, 2010). The average biovolume and surface area of this species were 599 $\mu m^3$ and 346 $\mu m^2$ (Table 4.2). Similar results have been reported for this species in the Baltic Sea (Olenina et al., 2006). On the contrary, Hegarty and Villareal (1998) found that the biovolume of live cells of *S. costatum* was very low (197 $\mu m^3$). *Thalassionema nitzschioides* also is one of the most common diatoms that formed blooms many times during this study. Mitbavkar and Anil (2008) reported blooms of this species especially during monsoon months in Dona Paula Bay. The average biovolume (1031 $\mu m^3$) and surface area (867 $\mu m^2$) of *T. nitzschioides* observed during this study are consistent with the earlier studies of Olenina et al. (2006) and Kumar et al. (2009). *Coscinodiscus marginatus* is another dominant species in the study area which had greater biovolume. This species was particularly dominant off Chapora. The larger cell diameter and large biovolume (an average 211015 $\mu m^3$) of this diatom might be making them an important source of living carbon biomass in the estuary. Earlier studies also reported that most *Coscinodiscus* spp possess larger diameter and biovolumes (Olenina et al., 2006; Kumar et al., 2009).

Biovolumes of *Odontella sinensis* (912030 $\mu m^3$) and *Protoperidinium depressum* (677598 $\mu m^3$) were the greatest observed during this study. These results agree with those of Olenina et al. (2006) who also found that the biovolumes of these species in the Baltic Sea (1 000 875 $\mu m^3$ and 546 766 $\mu m^3$ for these species respectively) are substantial. Menden-Deuer and Lessard (2000) reported that the biovolume of *Protoperidinium depressum* was 278883 $\mu m^3$ under laboratory conditions. Smayda (1978) found that the large species which often occurred less abundant in numbers might dominate the overall biomass.

Cellular carbon content of phytoplankton species varied directly with cell size. Other factors which can affect the carbon content are the nutritional state and the light level. Cells, in general, will begin to lower overall carbon content in high light environments (Cullen and Lewis, 1988), or when nutritionally deplete (Menden-Deuer and Lessard, 2000). It has been suggested that the cell carbon and cell volume relationships demonstrated for phytoplankton cells may be adopted for bacteria and protozoa (Ishizaka and Ishikawa, 1995). From the overall lower/smaller cell carbon content in most of the preponderant diatom species in the study area, it is inferable that the excess light levels may be responsible for such smaller carbon content though the biovolume were relatively greater.

The cellular carbon content in dinoflagellates species was quite high. Many species of dinoflagellates are covered with cellulosic plates, which comprise the theca (e.g., Dodge, 1985). Species lacking these plates are referred to as athecate, or naked. Owing to this cellulosic coating, thecate dinoflagellates have long been hypothesized to be more carbon dense than other plankton (Smetacek, 1975; Edler, 1979; Menden-Deuer and Lessard, 2000). On the other hand, the cellular carbon content of diatoms was low in the most species. Diatoms are traditionally known to have large vacuoles; there may be less carbon per volume than in other phytoplankton organisms of comparable size (Sicko-Goad et al., 1984; Moal et al., 1987; Menden-Deuer and Lessard, 2000; Gosselain, et al 2000). Montagnes et al. (1994) did not find any difference among the diatom taxa although it was emphasized that cell carbon may not be representative of all the species encountered in natural communities. These authors also suggest that since only a

few species of diatoms are used, the large number of other species included in the regression may have eclipsed possible differences. Menden-Deuer and Lessard (2000) found differences between diatoms and non-diatom taxa only for species with volumes greater than 3000 $\mu m^3$.

Diatoms dominate the phytoplankton communities in the study area (Fig 4.3). In general, phytoplankton standing stocks were higher at all the locations except off Anjuna. The dominance of diatoms at these locations was similar to that found in the coastal regions of other oceanic basins, such as the approaches to the English Channel (Holligan et al., 1984) and the Northeast Pacific Ocean (Hobson and Ketcham, 1974). Larger phytoplankton carbon biomass at most stations were similar to that found in areas in the subtropical Pacific Ocean around Hawaii (Takahashi and Bienfang, 1983), the North Atlantic Ocean during the spring phytoplankton bloom (Weeks et al., 1993) and from the Crozet Basin (Kopczynska and Fiala, 2003). On the other hand, Tarran et al. (1999) reported carbon biomass from the coastal regions of Arabian Sea similar to that found in our study off Anjuna.

A point to be noted is that measuring the height of phytoplankton cells under the microscope can be difficult for some species. As Sun and Liu (2003) point out, the algal cell usually keeps a definite position on the slide when the centre of gravity is low, making it difficult to measure the cell height. In most instances, an algal species will keep a fixed position, but on rare occasions the side view of the cell is visible, providing the opportunity to measure the height. If the cell is rotated, it will increase the chances of getting a side view. By tapping the cover slip with a pin-like

object, algal cells will roll with the movement of the surrounding medium. Such rolling/rotation is not possible using a pin, when the sample is examined using Sedgwick–Rafter. There are two ways to solve the problem as suggested by Sun and Liu (2003): one is to concentrate the sample after observation and follow up with a standard compound microscope: the other is to estimate the height from the width of the cell, because the height of small algal cells is usually approximately equal to the width. All such effects were tried to obtain the height diameter as far as possible.

Determination of biovolume and surface area of marine phytoplankton cells is very important for many related ecological parameters. From the detailed analyses as described above, it is possible to suggest that these measurements would prove useful in providing information on cellular carbon content and then calculation of phytoplankton biomass. In addition, it is providing the opportunity of differentiating between the contributions of different taxonomic groups which cannot be calculated through 'bulk measurements'.

# Chapter 5

## Distribution Pattern of Diatom Assemblages

### Introduction

About one-fifth of the photosynthesis on Earth is carried out by microscopic, eukaryotic phytoplankton known as diatoms (Nelson et al., 1995). These photosynthetic workhorses are found in waters worldwide, wherever there is sufficient light and nutrients. Their name is derived from the Greek term diatomos, meaning 'cut in half', a reference to their distinctive two-part cell walls made of silica. They 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. Their abundance makes them important food sources in aquatic ecosystems. When diatoms die, their cell walls are left behind and sink to the bottom of bodies of water. Massive accumulations of diatom-rich sediments compact and solidify over long periods of time to form rock rich in fossilized diatoms that is mined for use in abrasives and filters.

Diatoms range between 20-200 microns in diameter or length, although sometimes they can be up to 2 millimeters long. The cell may be solitary or colonial (attached by mucous filaments or by bands into long chains). They may occur in such large numbers and be well preserved enough to form sediments composed almost entirely of diatom frustules (diatomites), these deposits are of economic benefit being used in filters, paints, toothpaste, and many other applications.

Each year, diatom photosynthesis in the sea generates about as much organic carbon as all the terrestrial rainforests combined (Nelson et al., 1995; Field et al., 1998). But unlike much of the carbon generated by trees, the organic carbon produced by diatoms is consumed rapidly and serves as a base for marine food webs (Armbrust, 2009). In estuarine and shallow coastal environments, diatoms are considered as the most important component of the phytoplankton assemblage. They are responsible for more than 50% of marine primary production (Nelson et al., 1995). They are amongst the major representatives of the seasonal coastal blooms and, have traditionally been considered the main source of food for herbivorous zooplankton. The diatom assemblages in a given locale respond rapidly to environmental changes and, can thus provide highly informative assessments of the biotic integrity or impairment of aquatic systems. Further, some species tolerant to wider variations in their environment would prove useful for a reliable characterization of environmental variability (Stevenson and Pan, 1999). In the open ocean, a relatively large proportion of diatom organic matter sinks rapidly from the surface, becoming food for deep-water organisms (Sarthou et al., 2005). A small fraction of this sinking organic matter escapes consumption and settles on the sea floor, where it is sequestered over geological timescales in sediments and rocks and contributes to petroleum reserves.

The Indian summer monsoon alters salinity and nutrient regimes rather drastically along the central west coast of India (Shetye et al., 2007). This leads to seasonal shifts in phytoplankton assemblages on the annual scale. An understanding of spatial and annual compositional variabilities in phytoplankton assemblages would

be useful for realizing the ecological effects in monsoon affected regions. Ecology and hydrodynamics of estuarine and coastal areas are strongly influenced by land/riverine runoff. In addition, the perpetual exchanges from the adjacent open sea do bear a strong effect on their physical, chemical and biological characteristics resulting in pronounced ecological gradients, which in turn influence the composition and abundance of phytoplankton communities (Smayda, 1983) in such habitats. Owing to nutrient enrichment from rivers, organic [sewage] pollution from on shore settlements and from the entrainment of coastal waters, these environments frequently experience high fertility and, bloom proportions of certain phytoplankton populations (Ketchum, 1967; Alkawri and Ramaiah, 2010).

We aimed to investigate as to how the diatom assemblages respond to the dynamics of monsoon affected estuarine and near-coastal regions. Delineation of the effect of nutrient concentrations -and their ratios- was done to recognize their influence on the abundance and distribution of diatoms. In addition, through canonical correspondence analysis (CCA), we aimed to decipher the environmental variable(s) responsible for seasonal variations in the abundance of major diatom species extant in these locations with disparate salinities.

## Material and Methods

### *Analyses of Diatom Community Composition*

For quantitative and qualitative analyses of diatom-cell counts and their composition, water samples from the each sampling location were fixed with Lugol's solution (1% w/v) and 3% formaldehyde and stored in dark until analyzed,

usually within a fortnight of their collection. A settling and siphoning procedure was followed to concentrate samples from 4L to 100 ml for counting diatom cells and identification of genera and wherever possible, to the species level. Three 1-ml replicates of concentrated samples were transferred to Sedgwick-Rafter plankton counting chamber and examined microscopically at 200-400X magnification. All 1000 squares on the chamber were screened. When uncertain, cover-slipped wet mounts of the specimens were examined at 1000X using oil immersion objective to confirm their identity to specific/generic level. For confirmation the identification of some diatom species; sample (max. 10 ml) is poured into a 100 ml Erlenmayer flask. One ml 10 % HC1 was added, then 2 ml of 30% $H_2SO_4$ and 10 ml saturated $KMnO_4$, were added. The mixture was left for 24h, during which the sample was shaken a few times. Then, 10 ml saturated oxalic acid was added in drops until the solution was colourless. The sample is then washed 3-4 times in distilled water (gentle centrifugation 1000 x *g*, 12 min), after air drying, the preparation was mounted in Naphrax (Woelfel et al., 2007). Generic and species identification of diatoms was done following keys (Subramanyan, 1946; Subramanyan and Sarma, 1961; Constance et al., 1985a, b; Desikachary and Ranjithadevi, 1986; Desikachary and Prema, 1987; Desikachary et al., 1987; Hasle and Syvertsen, 1996; Horner, 2002).

***Nutrient Concentrations and other Environmental Parameters***

Nutrient concentrations and other parameters were measured as described in Chapter 3. The nutrient concentrations were used for calculating the N: P: Si ratios.

***Statistical Analyses***

Diversity Indices

The diversity indices of diatoms in the study area were calculated by using the Shannon Weaver formula:

$$H^{`} = - \sum_{i=1}^{S} Pi \log_2 Pi$$

where, S is total number of species and Pi is the proportion of the numbers of individuals of species i to the total number of individuals Pi = ni/N (Omori and Ikeda, 1984).

Species richness (SR), the number of species recorded from a region, was calculated by Margalef's formula (Margalef, 1951):

$SR = (S - 1)/\log_n N$,

where, S = total number of species and, N = total individuals present in the sample.

Species evenness ($J'$) was calculated according to Pielou (1966):

$J' = H' / \text{Log}_2 S$,

where, S is total number of species and $H'$ is the Shannon diversity index.

Spearman's Rank Correlation Test

Spearman's rank correlation test was performed to evaluate the relationships between the diatom species with various environmental parameters that may be regulating their populations. This test was performed using the software Statistica version 6.0 (Statsoft, USA).

Canonical Correspondence Analysis

Canonical correspondence analysis (CCA) was carried out to elucidate the relationship between the environmental parameters and major diatom species by following Ter Braak (1986). The environmental parameters included in the CCA are nitrate, phosphate, silicate, dissolved oxygen, temperature and salinity. The CCA was performed using Multi-Variate Statistical Package Program Version 3.1 (Kovach, 1998). To avoid the influence of rare species, individual species contributing <5% to the total abundance in any sample were excluded for CCA as suggested by Jongman et al. (1987).

## Results

### *Nutrient Ratios*

The N:Si ratio was in general <1 with spatial variation during the study period. This ratio was much lower at the locations off Chapora (Fig.5.1) followed by Siridao (Fig. 5.2) suggesting higher silicate concentrations relative to nitrate at these locations. Higher N:Si ratios (3 and 2.58) were recorded during February and April 2008 at the location off Anjuna (Fig. 5.1). The Si:P ratios fluctuated rather widely and were often more than the 16:1. They were much larger off Chapora followed by Siridao. Their ratios were generally smaller off Anjuna and Dona Paula, indicating lower silicate concentrations at these locations. In any case, the Si concentrations at these locations were higher than 3 $\mu mol\ L^{-1}$ throughout the study period.

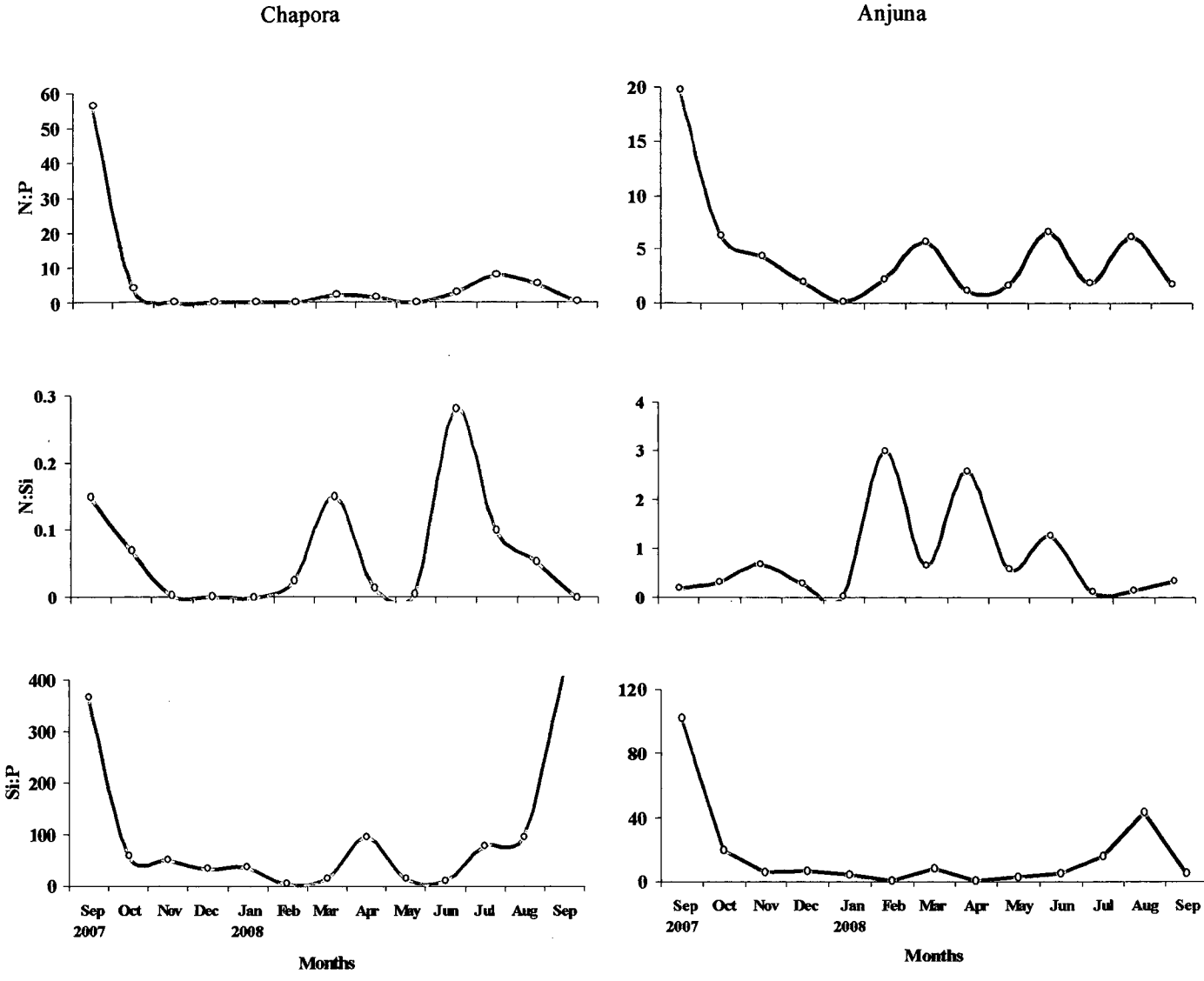



Fig.5.1. Temporal variations of N:P, N:Si and Si:P ratios from different sampling locations off Anjuna and Chapora

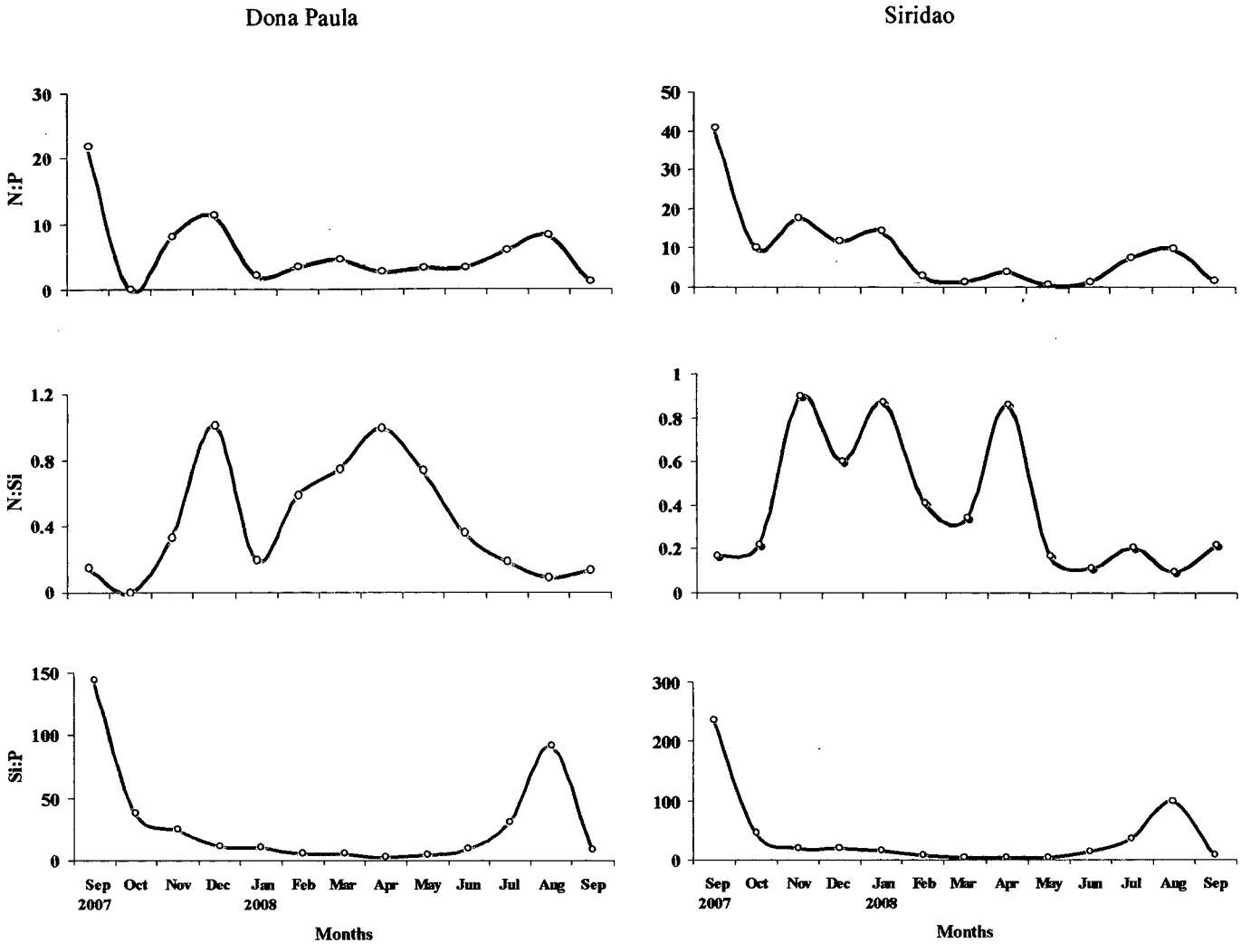



Fig.5.2. Temporal variations of N:P, N:Si and Si:P ratios from different sampling locations off Dona Paula and Siridao

***Cell Abundances***

In general, the counts of diatom cells off Anjuna were low except during February ($10.8\times 10^3$ cells $L^{-1}$) and September 2008 ($22\times10^3$ cells $L^{-1}$) when bloom proportions of *Rhabdonema punctatum* and *Skeletonema costatum* were observed (Fig.5.3). Off Chapora, where the cell counts fluctuated rather irregularly without any seasonal trend, the higher abundances were during May 2008 ($19.3 \times 10^4$ cells $L^{-1}$) mainly due to a bloom of *Chaetoceros laciniosus* and, during September 2008 ($14.2 \times 10^4$ cells $L^{-1}$) this time by *S. costatum*. The total diatom counts in Dona Paula Bay ranged between 974 and $7.3 \times 10^4$ cells $L^{-1}$ with their maxima during September 2008 ($7.3 \times 10^4$ cells $L^{-1}$) and October 2007 ($4.42 \times 10^4$ cells $L^{-1}$). Off Siridao, the highest abundance was observed during September 2008 ($7.6 \times 10^5$ cells $L^{-1}$; Fig. 5.3). The counts were low off Anjuna, when the chl *a* was also low (November 2007). The higher diatom counts off Chapora and Siridao generally coincided with higher chl *a* concentrations except during June and August 2008. During these two months, the chl *a* concentrations were high but the diatom counts were low.

***Species Assemblages and Diversity Indices***

A total of 150 species belonging to 56 different genera were identified from the samples collected during this study. While numbers of species of Pennales (P) were more, the cell abundance of the Centrales (C) were far more than those of Pennales (Fig. 5.3). The number of diatom species off Anjuna, Chapora, Dona Paula and

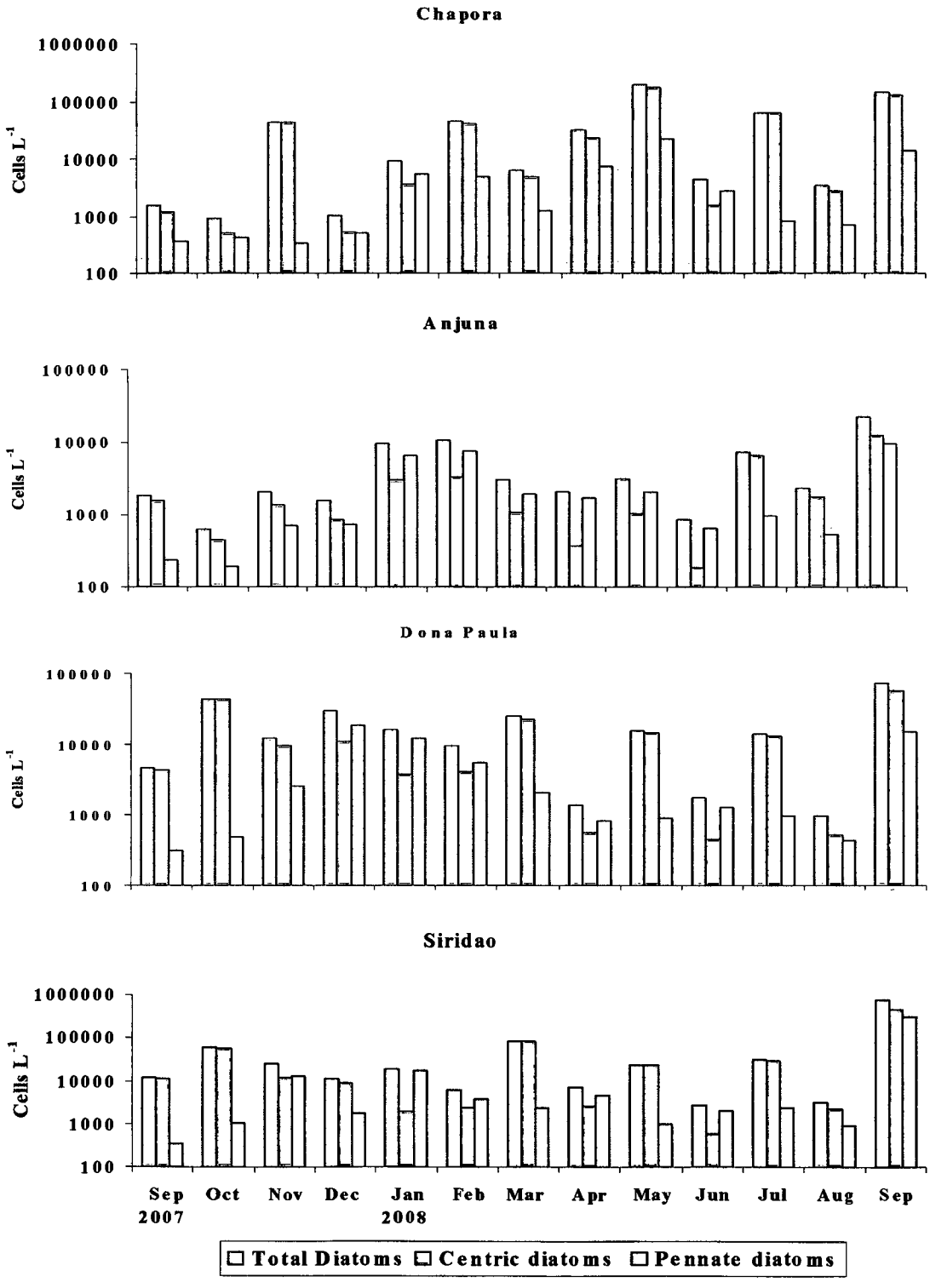



Fig. 5.3. Monthly variations in the abundances of total, centric and pennate diatoms at different sampling locations off Goa, during September 2007-September 2008

Siridao were 104 (46C, 58P), 98 (48C, 50P), 130 (57C, 73 P) and 112 (51C, 61P) respectively (Tables 5.1 and 5.2).

Off Anjuna, the maximum cell abundances were of *Rhabdonema punctatum, Skeletonema costatum, Thalassionema nitzschioides* and *Leptocylindrus minimus* (Fig. 5.4). Off Chapora however, *Chaetoceros laciniosus, Skeletonema costatum, Leptocylindrus danicus, Coscinodiscus marginatus,* and *Thalassionema nitzschioides* (Fig. 5.4) contributed maximally to the total diatom counts. In the Dona Paula Bay, *Skeletonema costatum, Chaetoceros curvisetus, Leptocylindrus danicus, Thalassionema nitzschioides* and *Corethron criophilum* were the most dominant (Fig. 5.5). The most abundant species in Siridao were *Skeletonema costatum, Asterionella glacialis, Thalassionema nitzschioides, Chaetoceros curvisetus* and *Guinardia striata* (Fig. 5.5).

Thus, *Skeletonema costatum* and *Thalassionema nitzschioides* were common at all the locations and, were present throughout the sampling period with occasional exception off Chapora. Cell abundances of *S. costatum* fluctuated rather widely which was generally preponderant during monsoon and post-monsoon seasons. The species of *Rhabdonema punctatum*, most abundant off Anjuna, was recorded only during post- and pre- monsoon with higher numbers during January and February. Off Chapora and Dona Paula, *L. danicus* was abundant only during monsoon and, *Amphiprora sp*-1 was recorded only off Chapora during that season.

In the overall, both the number of species (130) and diversity indices (H`: 0.57 to 4.84) were higher in the Dona Paula Bay (Fig. 5.6). The diversity indices off

Table 5.1. Centric diatom species, ranges of their cell counts $l^{-1}$ (mean ± SD) recorded from the surface waters at different sampling locations off Goa. The taxa codes of the major species used in the canonical correspondence analysis are indicated. ND denotes species not detected

| Taxa Code | Taxa | Sampling location | | | |
|---|---|---|---|---|---|
| | | Anjuna | Chapora | Dona Paula | Siridao |
| | **Centric Diatoms** | | | | |
| | *Actinocyclus senarius* | 0-42 (9±16) | 0-8 (2±3.5) | 0-83 (9±23.4) | 0-38 (9±14) |
| | *Asterolampra marylandica* | ND | ND | 0-17 (1.3±4.7) | ND |
| | *Asteromphalus cleveanus* | 0-8 (1.2±3) | 0-8 (0.6±2.2) | 0-25 (3.8±8) | 0-8 (0.6±2) |
| | *Asteromphalus elengans* | 0-8 (1.2±3) | ND | 0-13 (1.6±4) | ND |
| | *Asteromphalus heptactis* | ND | ND | 0-25 (2±7) | ND |
| | *Asteromphalus hiltonianus* | ND | ND | 0-8 (0.6±2) | 0-8 (0.6±2) |
| | *Auliscus sculptus* | ND | 0-13 (1±3.6) | ND | ND |
| | *Bacteriastrum delicatulum* | ND | ND | 0-67 (11.5±23.7) | 0-100 (11.53±30) |
| | *Bacteriastrum furcatum* | ND | 0-300 (31±84) | 0-75 (6.4±20.7) | 0-225 (17±62) |
| | *Bacteriastrum hyalinum* | 0-33 (3±9) | 0-58 (4.5±14) | 0-17 (1.3±4.7) | 0-17 (2.6±6.4) |
| BIBID | *Biddulphia biddulphiana* | 0-517 (84±158) | 0-50 (3.84±14) | 0-50 (11.5±18) | ND |
| | *Biddulphia granulata* | ND | ND | 0-33 (2.5±9) | 0-17 (1.3±4.7) |
| | *Biddulphia heteroceros* | 0-8 (1.2±3) | 0-8 (0.6±2.2) | ND | 0-13 (1±3.6) |
| BILAE | *Biddulphia laevis* | 0-8 (2.5±3.84) | 0-33 (4.8±10) | 0-225 (50±66) | 0-200 (44±57) |
| | *Biddulphia longicruris* | ND | ND | ND | 0-8 (0.6±2) |
| BIRHO | *Biddulphia rhombus* | 0-767 (113.5±243) | 0-33 (6.4±10) | 0-233 (41±62) | 0-150 (51±39) |
| | *Campylodiscus hibernicus* | 0-25 (2±7) | 0-25 (2±7) | 0-13 (2±4.4) | 0-38 (3±10.53) |
| | *Campylodiscus latus* | ND | ND | 0-50 (4.5±14) | ND |
| | *Cerataulina bicornis* | 0-117 (23±38) | 0-25 (5.4±7.5) | 0-92 (12±28) | 0-75 (18±24) |
| | *Cerataulus turgidus* | ND | 0-13 (1±3.6) | 0-8 (0.6±2) | 0-42 (3±12) |
| | *Chaetoceros affinis* | ND | 0-58 (4.5±16) | 0-42 (3±11.6) | 0-175 (13.46±49) |
| | *Chaetoceros coarctatus* | 0-58 (4.5±16) | 0-108 (26±42) | 0-375 (37.5±103) | 0-3350 (258±929) |
| CHCUR | *Chaetoceros curvisetus* | 0-308 (59±100) | 0-10942 (1463±2917) | 0-19442(2660±5438) | 0-75275 (8466±20818) |
| | *Chaetoceros didymus* | ND | ND | 0-58 (4.4±16) | ND |
| | *Chaetoceros diversus* | 0-25 (2±7) | 0-1433 (164±412) | 0-8 (0.6±2) | 0-313 (34±86) |
| CHLAC | *Chaetoceros laciniosus* | 0-200 (27.5±60) | 0-161350 (13301±44566) | 0-1100 (109±302) | 0-438 (34±121) |
| CHLOR | *Chaetoceros lorenzianus* | 0-225 (32±64) | 0-6800 (908±1915) | 0-1217 (325±479) | 0-1875 (428±668) |
| | *Chaetoceros mitra* | 0-25 (4-8) | 0-13 (1±3.6) | 0-25 (2±7) | 0-8 (1.2±3) |
| CHSUB | *Chaetoceros subtilis* | 0-992 (76±275 | ND | ND | ND |
| COCRI | *Corethron criophilum* | 0-25 (3±8) | 0-375 (47±103) | 0-13533(1051±3751) | 0-21875 (1728±6054) |
| | *Coscinodiscus asteromphalus* | 0-13 (2±4.4) | 0-13 (2.8±4.6) | 0-138 (13±38) | 0-67 (6±19) |
| | *Coscinodiscus centralis* | 0-67 (5±19) | 0-17 (1.3±4.7) | 0-75 (6±21) | 0-550 (47±152) |
| | *Coscinodiscus gigas* | ND | ND | 0-13 (1±3.6) | 0-25 (3.53±7.61) |
| | *Coscinodiscus granii* | 0-83(8±23) | 0-33(4.5±11) | 0-42(5±13) | 0-375(36±104) |
| | *Coscinodiscus jonesianus* | ND | 0-263(20±73) | ND | ND |
| COMAR | *Coscinodiscus marginatus* | 0-242 (37±70) | 0-41258 (3373±11390) | 0-188 (53±61) | 17-650 (221±209) |
| CORAD | *Coscinodiscus radiatus* | 0-242 (35±66) | 0-650 (76±177) | 0-100 (42±31) | 0-313 (68±82) |
| CYSTR | *Cyclotella striata* | 0-233 (46.5±59.4) | 0-138 (29±41) | 0-352 (105±103) | 0-363 (114±107) |
| DIBRI | *Ditylum brightwellii* | 0-2900 (234±801) | 0-3342 (276±922) | 0-7258 (653±1998) | 8-18942 (1634±5221) |
| | *Eucampia zodiacus* | ND | ND | 0-17 (1.3±4.7) | 0-450 (37±124) |
| GUSTR | *Guinardia striata* | 0-483 (38±134) | 0-850 (85±237) | 0-7142 (569±1976) | 0-89000 (6900±24668) |

Table 5.1 (continued)

| Taxa Code | Taxa | Anjuna | Chapora | Dona Paula | Siridao |
|---|---|---|---|---|---|
| | *Hemiaulus hauckii* | 0-17 (2.6±6.4) | 0-175 (19±51) | 0-92 (11.5±25) | 0-325 (25±90) |
| HEHAR | *Hemidiscus hardmanianus* | 0-25 (4.46±9.4) | 0-25 (2±7) | 0-700 (77±192) | ND |
| | *Lauderia annulata* | ND | ND | 0-25(2±7) | ND |
| LEDAN | *Leptocylindrus danicus* | 0-875 (89±242) | 0-50733 (4038±14033) | 0-18958 (1512±5245) | 0-57850 (4465±16040) |
| LEMIN | *Leptocylindrus minimus* | 0-1975 (444±652) | 0-850 (89±236) | 0-4083 (631±1121) | 1766 (194±504) |
| MENUM | *Melosira nummuloides* | 0-808 (90±219) | 0-413 (39±113) | 0-1700 (404±555) | 0-775 (84±212) |
| | *Odontella aurita* | ND | 0-8 (0.6±2.2) | 0-8 (2±3.5) | 0-17 (1.3±4.7) |
| ODMOB | *Odontella mobiliensis* | 0-113 (26.6±40.7) | 0-292 (54±87) | 0-125 (29±35) | 0-175 (69±56) |
| ODSIN | *Odontella sinensis* | 0-350 (32±97) | 0-117 (22±32.4) | 0-1242 (112±340) | 0-3683 (346±1018) |
| | *Paralia sulcata* | 0-42 (13.46±66.4) | 0-92 (11±26.7) | 0-75 (17±27) | 0-113 (16.4±34) |
| | *Planktoniella sol* | 0-17 (2.5±5.3) | 0-38 (5±10.6) | 0-38 (6.9±13) | 0-88 (7±24) |
| | *Rhizosolenia imbricata* | 0-108 (14±32) | 0-63 (10±18) | 0-38 (7±13) | 0-1388 (109±384) |
| | *Rhizosolenia setigera* | 0-38 (5.46±12) | 0-38 (7±10.6) | 0-583 (47±161) | 0-6700 (519±1857) |
| | *Rhizosolenia styliformis* | ND | 0-58 (4.5±16) | ND | 0-92 (12±30) |
| SKCOS | *Skeletonema costatum* | 0-10317 (921±2833) | 0-126333 (12605±34918) | 0-41317 (5440±12443) | 0-261600 (26007±72387) |
| | *Stephanodiscus* sp | 0-8 (0.60±2) | ND | ND | ND |
| | *Striatella* sp | 0-8 (0.60±2) | ND | 0-213 (27±62) | ND |
| | *Symbolophora trinitatis* | 0-8 (0.60±2) | 0-25 (2.5±7) | 0-13 (2±5) | 0-8 (2±3.5) |
| THECC | *Thalassiosira eccentrica* | 0-208 (42.6±69.6) | 0-38 (11±13) | 0-150 (41±44) | 0-425 (79±119) |
| | *Thalassiosira lineata* | 0-75 (22±27) | 0-63 (7.4±17.5) | 0-75 (24±30) | 0-109 (32±33) |
| THSP | *Thalassiosira* sp | 0-250 (46±76) | 0-1600 (199±433) | 0-350 (90±118) | 0-3175 (320±865) |
| | *Triceratium dubium* | 0-25 (4.46±8) | 0-33 (3±9) | ND | 0-8 (0.60±2) |
| | *Triceratium favus* | 0-13 (1.6±4) | ND | 0-8 (0.60±2) | ND |
| | *Triceratium weissei* | ND | ND | 0-13 (2.2±4.4) | 0-25 (4.46±8) |

Table 5.2. Pennate diatom species, ranges of their cell counts $l^{-1}$ (mean ± SD) recorded from the surface waters at different sampling locations off Goa. The taxa codes of the major species used in the canonical correspondence analysis are indicated. ND denotes species not detected

| Taxa code | Taxa | Anjuna | Chapora | Dona Paula | Siridao |
|---|---|---|---|---|---|
| | **Pennate Diatoms** | | | | |
| | *Achnanthes brevipes* | 0-42 (9±16) | 0-50 (4.46±13.9) | 0-1325 (175±372) | 0-100 (27±37) |
| | *Achnanthes exilis* | ND | ND | 0-113 (18±37.6) | 0-142 (20±46) |
| | *Achnanthes longipes* | ND | ND | 0-300 (23±83) | ND |
| Amala | *Amphiprora alata* | 0-363 (37±100) | 0-175 (42±60.7) | 0-563 (61.5±152) | 0-788 (91±212) |
| | *Amphiprora ornata* | 0-8 (0.6±2) | 0-8 (0.6±2.2) | ND | ND |
| Amsp1 | *Amphiprora sp1* | ND | 0-283 (49±98) | ND | ND |
| | *Amphiprora sp2* | 0-17 (2.6±6.4) | ND | 0-17 (1.3±4.7) | 0-8 (0.60±2) |
| | *Amphora bigibba* | ND | ND | 0-17 (2.3±5.7) | ND |
| | *Amphora coffeaeformis* | 0-25 (4.5±9.4) | 0-42 (7.7±13.9) | 0-63 (11±22) | 0-42 (5±12) |
| | *Amphora crassa* | 0-17 (1.3±4.7) | ND | ND | ND |
| | *Amphora monilifera* | ND | ND | ND | 0-25 (2.53±7) |
| | *Amphora proteus* | ND | 0-17 (2.6±6) | 0-50 (4.46±14) | ND |
| | *Amphora turgida* | 0-33 (5±10.2) | 0-125 (15±36) | 0-250 (34.6±76) | 0-138 (14±38) |
| Asgla | *Asterionella glacialis* | 0-133 (33±48) | 0-1525 (240±459) | 0-1567 (165±427) | 0-171350 (13303±47488) |
| Bapax | *Bacillaria paxillifera* | 0-108 (15.4±35) | ND | 0-10558 (885±2908) | 0-192 (30±61) |
| Clmon | *Climacosphenia moniligera* | 0-83 (20±28) | 0-75 (14±27.3) | 0-38 (7±11.5) | 0-58 (8±18) |
| | *Cocconeis pellucida* | 0-25 (5±10) | ND | 0-313 (33±88.5) | 0-13 (1±3.6) |
| | *Cocconeis placentula* | 0-25 (3±8) | ND | 0-25 (2±7) | ND |
| | *Cocconeis pseudomarginata* | 0-142 (13.5±40) | 0-17 (1.3±4.7) | 0-408 (62±140) | ND |
| | *Cocconeis scutellum* | 0-67 (6±18.5) | 0-8 (0.6±2) | 0-225 (33±4.67) | 0-33 (4±10) |
| | *Cymbella cymbiformis* | ND | ND | ND | 0-17 (1.3±4.7) |
| | *Cymbella yarrensis* | 0-8 (0.60±2) | ND | 0-25 (4±9) | ND |
| | *Diploneis crabro* | 0-25 (2.5±7) | ND | 0-100 (12±28) | 0-16 (3±5) |
| | *Diploneis smithii* | 0-34 (2.6±9.4) | 0-25 (7.7±11) | 0-8 (0.602) | 0-25 (4±8) |
| | *Diploneis weissflogii* | 0-25 (2.5±7) | 0-8 (0.6±2) | 0-25 (3-8) | ND |
| | *Fragilaria striatula* | ND | 0-83 (6.4±23) | 0-108 (10-30) | 0-17 (1.3±4.7) |
| | *Fragilaria oceanica* | 0-233 (20±64.4) | 0-163 (12.5±45) | ND | ND |
| Grmar | *Grammatophora marina* | 0-413 (135±151) | 0-75 (22±32) | 0-308 (76±92) | 0-83 (18±24) |
| | *Gyrosigma balticum* | ND | 0-113 (14±31) | 0-100 (13±28) | 0-25 (8±11) |
| | *Gyrosigma fasciola* | 0-38 (3±10.54) | 0-50 (5±14) | 0-13 (2±5) | 0-50 (7±17) |
| | *Licmophora flabellata* | 0-58(4.46±16) | ND | 0-75 (6±21) | ND |
| | *Licmophora abbreviata* | ND | ND | 0-308 (24±85) | 0-8 (0.60±2) |
| Ligra | *Licmophora gracilis* | 0-117 (19.6±33.7) | 0-13 (1.6±4) | 0-263 (63±91) | 0-83 (11±24) |
| Lipar | *Licmophora paradoxa* | 0-1917 (211±532) | 0-50 (4±14) | 0-1183 (104±325) | 0-50 (6±15) |
| | *Meuniera membranacea* | 0-58 (4.46±16) | 0-8 (0.6±2) | ND | 0-213 (20±59) |
| | *Navicula bacillum* | ND | ND | 0-25 (2±7) | 0-100 (8±28) |
| | *Navicula cancellata* | 0-42 (5±13) | 0-38 (6.7±11.3) | 0-125 (12±35) | 0-67 (7±19) |
| | *Navicula clavata* | ND | ND | 0-50 (6.4±15) | ND |
| | *Navicula delicatula* | ND | ND | 0-33 (4±10) | ND |
| Nadir | *Navicula directa* | 0-717 (101±196.5) | 0-483 (46.5±132) | 0-375 (110±131) | 0-1025 (99±280) |
| | *Navicula disclusa* | 0-8 (1.2±3) | 0-50 (6±15) | ND | 0-17 (1.3±4.7) |
| | *Navicula elliptica* | 0-8 (0.60±2) | ND | 0-8 (0.60±2) | ND |

Table 5.2 (continued)

| Taxa code | Taxa | Anjuna | Chapora | Dona Paula | Siridao |
|---|---|---|---|---|---|
| | *Navicula fusca* | 0-13 (1±3.6) | 0-200 (15.4±55.5) | 0-25 (2±7) | 0-267 (22±74) |
| | *Navicula granii* | 0-8 (0.60±2) | ND | 0-108 (19±40) | 0-75 (7±21) |
| | *Navicula granulata* | ND | 0-42 (3±11.6) | 0-50 (4±14) | ND |
| | *Navicula humerosa* | ND | 0-66 (9.5±22) | 0-100 (12±29) | 0-50 (4±14) |
| Nalyr | *Navicula lyra* | 0-58 (9±16.4) | 0-250 (47±74) | 0-88 (20±26) | 0-50 (12±15) |
| | *Navicula maculosa* | 0-13 (1±3.6) | ND | 0-8 (0.60±2) | ND |
| | *Navicula monilifera* | ND | ND | 0-83 (6±23) | 0-17 (1.3±5) |
| | *Navicula rhombica* | 0-208 (39.4±55) | 0-25 (2.5±7) | 0-233 (71±88) | 0-117 (19±33) |
| | *Navicula simulans* | 0-37 (2.84±10.3) | 0-50 (5±14) | 0-25 (4.46±9) | 0-25 (2.5±7) |
| | *Navicula* sp1 | ND | ND | 0-67 (5±19) | 0-492 (49±139) |
| | *Navicula* sp2 | ND | ND | 0-50 (4±14) | ND |
| | *Navicula* sp3 | ND | ND | ND | 0-192 (20±55) |
| Niclo | *Nitzschia closterium* | 0-42 (8.3±14) | 0-67 (16.4±22) | 0-475 (76±154) | 0-158 (16±43) |
| | *Nitzschia longissima* | 0-33 (5±11) | 0-258 (31.5±89.6) | 0-138 (24±50) | 0-1025 (83±283) |
| | *Nitzschia lorenziana* | ND | ND | 0-17(2.3±6) | ND |
| | *Nitzschia obtusa* | 0-175 (24.4±57) | 0-25 (4±9.4) | 0-25 (6.4±11) | 0-17 (4±7) |
| | *Nitzschia panduriformis* | 0-8 (0.60±2) | ND | 0-8(0.60±2) | ND |
| | *Nitzschia rectilonga* | 0-42 (4±11.7) | 0-8 (1.2±3) | 0-175 (39±63) | 0-42 (4±12) |
| Nisig | *Nitzschia sigma* | 0-138 (30.5±40) | 0-288 (33±78) | 0-525 (138±525) | 0-324 (62±92) |
| | *Nitzschia* sp | 0-8 (0.60±2) | ND | 0-183 (14±51) | 0-25 (4±9) |
| | *Nitzschia vitrea* | 0-125 (24±36) | 0-92 (16±30) | 0-342 (60±102) | 0-25 (4±8.4) |
| | *Pleurosigma aestuarii* | 0-213 (46.53±66.4) | 0-88 (11.3±26) | 0-38 (7±13) | 0-17 (5±7) |
| Plang | *Pleurosigma angulatum* | 0-163 (40±47.5) | 0-638 (84±170) | 0-1475 (174±408) | 0-450 (57±120) |
| | *Pleurosigma directum* | ND | 0-8 (0.6±2) | 0-25 (2.53±7) | ND |
| | *Pleurosigma elongatum* | ND | 0-8 (0.6±2) | ND | ND |
| | *Pleurosigma formosum* | 0-8 (0.60±2) | 0-88 (11±30) | ND | 0-58 (5±16) |
| | *Pleurosigma galapagense* | 0-108 (18±33) | ND | 0-638 (82±176) | 0-63 (7±18) |
| | *Pleurosigma normanii* | 0-8 (0.60±2) | ND | 0-8 (0.60±2) | 0-25 (2±7) |
| | *Pleurosigma speciosum* | ND | 0-25 (2±7) | ND | ND |
| | *Podocystis spathulata* | ND | ND | 0-17 (1.3±4.7) | ND |
| Pspun | *Pseudo-nitzschia pungens* | 0-100 (18.6±35) | 0-16208(1450±4461) | 0-733 (65±202) | 0-9363 (723±2596) |
| Pssp | *Pseudo-nitzschia* sp | ND | 0-1075(108±297) | 0-342(26±95) | 0-575(48±159) |
| Rhpun | *Rhabdonema punctatum* | 0-5538 (1007±1955) | ND | 0-233 (24±65) | 0-250 (53±93) |
| | *Surirella fastuosa* | ND | 0-25 (2±7) | 0-50 (5±14) | 0-76 (10±21) |
| Suova | *Surirella ovalis* | 0-142 (34±40.4) | 0-100 (18±32) | 0-192 (45±59) | 0-117 (48±45) |
| | *Surirella* sp | 0-13 (1±3.6) | 0-13 (2±4.4) | 0-33 (6±11) | 0-25 (2±7) |
| | *Synedra affinis* | 0-33 (2.5±9) | 0-38 (3±10.5) | 0-200 (15±55) | 0-8 (0.60±2) |
| Syfor | *Synedra formosa* | 0-13 (1±3.6) | ND | 0-1488 (114±413) | 0-975 (115±295) |
| | *Synedra* sp1 | 0-42 (3±11.65) | ND | 0-767 (117±285) | 0-25 (2±7) |
| | *Synedra* sp2 | 0-17 (1.3±4.7) | ND | 0-388 (30±108) | 0-33 (3±9) |
| Thnit | *Thalassionema nitzschioides* | 0-5692 (578±1542) | 17-9442(2222±3134) | 25-10933 (1462±3058) | 50-129100 (12657±35238 |
| Penna1 | *Diatom1* | ND | ND | 0-350 (32±97) | 0-1017(180±365) |
| | *Diatom2* | 0-33 (7.6±12) | 0-58 (11±22) | 0-8 (0.60±2) | 0-100 (13±30) |

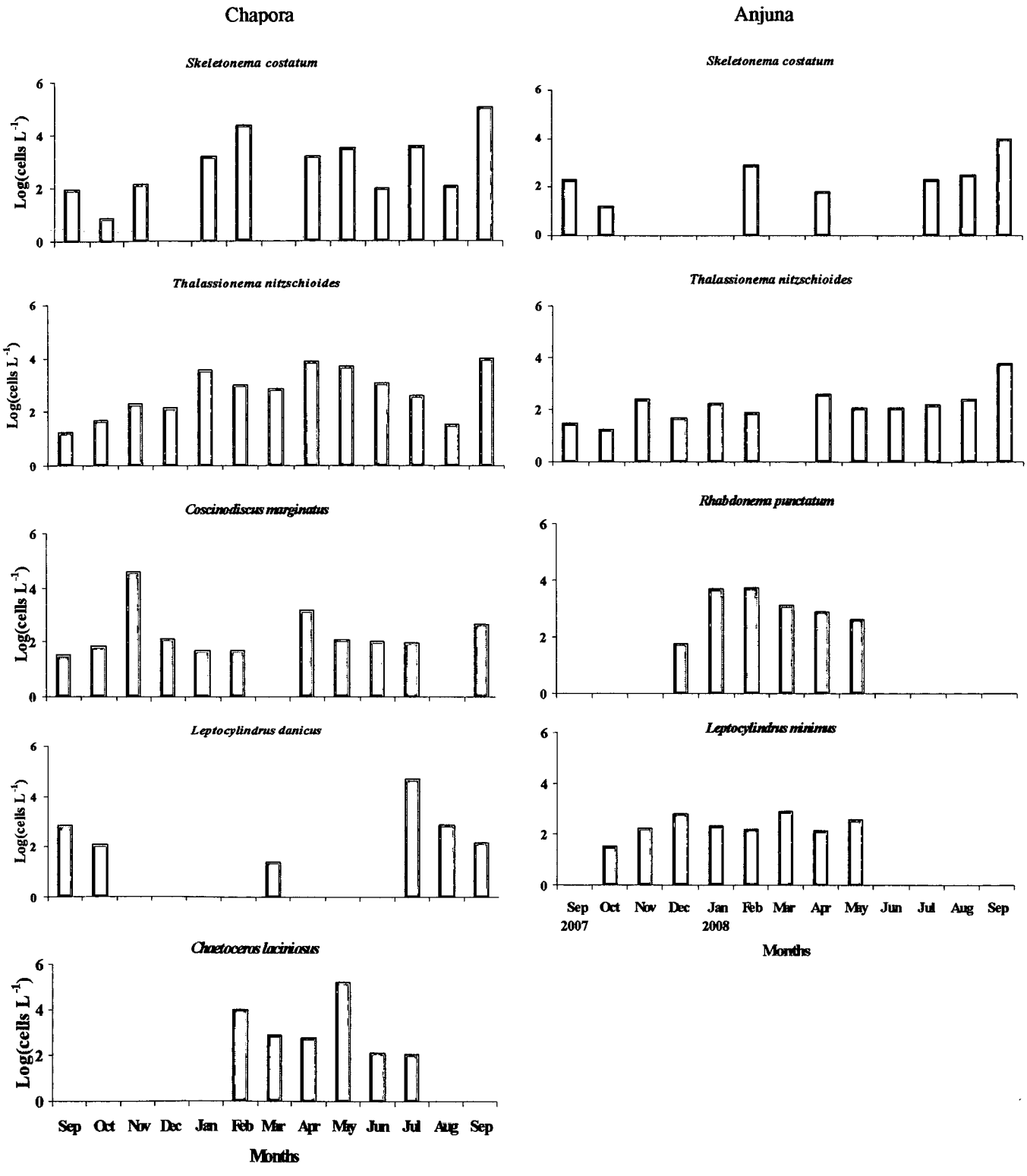



Fig. 5.4. Distribution patterns of the most dominant diatom species from the sampling locations off Chapora and Anjuna

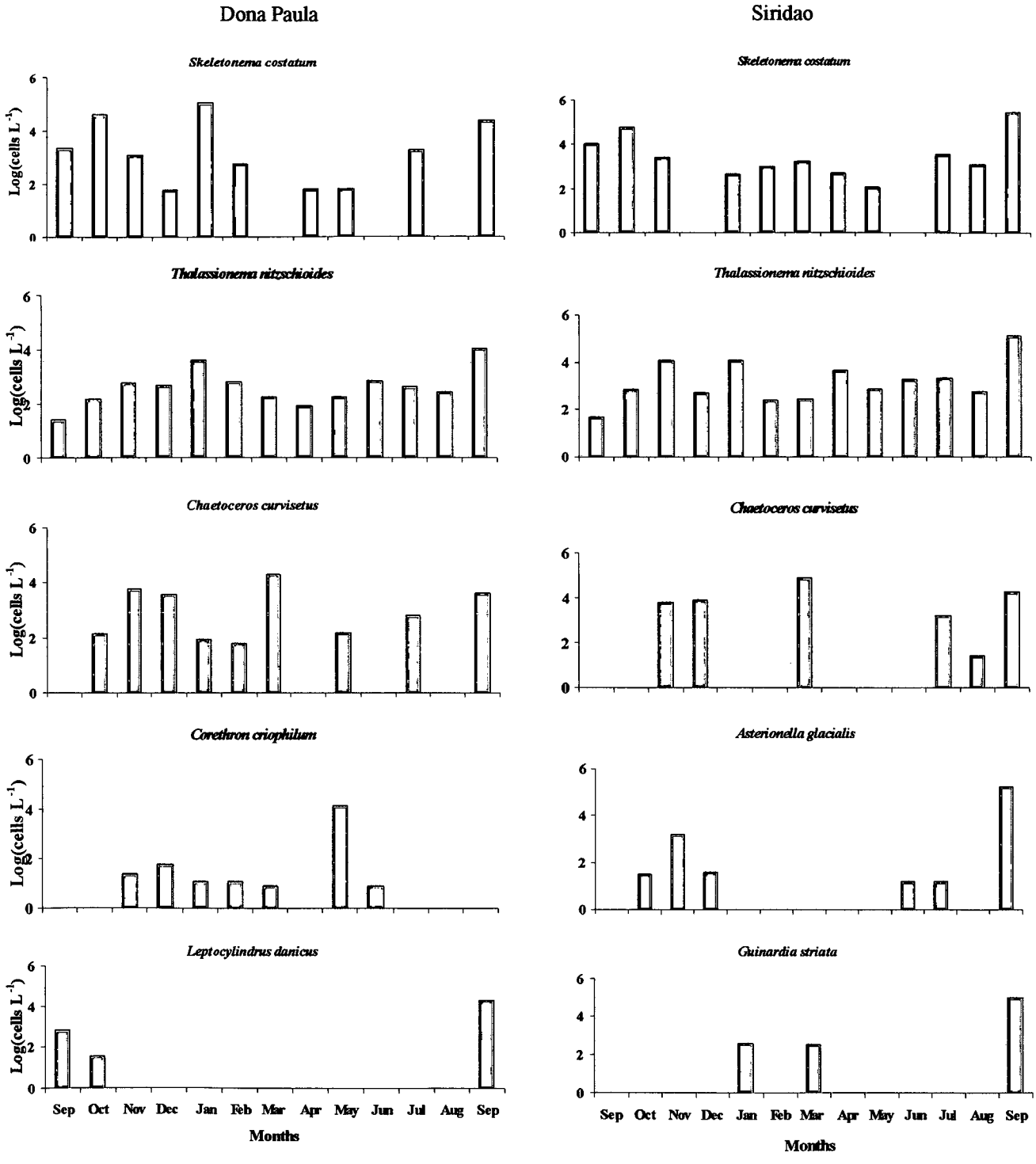



Fig. 5.5. Distribution patterns of the most dominant diatom species from the sampling locations off Dona Paula and Siridao

Chapora, Siridao and Anjuna were 0.39-3.59, 0.53-3.93 and 2.48-4.23 respectively. Both $H^{`}$ and evenness were generally low off Chapora followed by Siridao.

***Spearman's Rank Correlation Coefficients***

Between different, dominant species and dissolved inorganic nutrients, the following relationships were statistically significant (Table 5.3). Off Anjuna, *Rhabdonema* sp showed a significant negative relationship with nitrate ($NO_3$: R= - 0.89, $p \leq 0.01$), Phosphate ($PO_4$: R=-0.85, $p \leq 0.01$) and silicate ($SiO_4$: R= - 0.83, $p \leq 0.01$). Also, *L. minimus* was negatively correlated with nitrate (R= -0.8, $p \leq 0.01$), phosphate (R= - 0.86, $p \leq 0.01$) and silicate (R= - 0.75, $p \leq 0.01$). *L. danicus* correlated positively with silicate (R= 0.7, $p \leq 0.01$). Off Dona Paula however, it showed positive correlation only with nitrite (R= 0.59, $p \leq 0.01$). The other significant correlation coefficients were the following. *S. costatum* showed negative correlation with water temperature (R= - 0.69, $p \leq 0.01$), and positive correlation with nitrite (R= 0.57 and $p \leq 0.05$) respectively at the location off Anjuna.

The dominant species off Chapora, *C. laciniosus* and *T. nitzschioides*, correlated positively with salinity (R=0.7, 0.63 and $p \leq 0.01, 0.05$) and negatively with silicate (R=- 0.84, -0.59 and $p \leq 0.01, 0.05$). *Leptocylindrus danicus* showed negative correlation with salinity (R= - 0.71, $p \leq 0.01$) and positive correlation with silicate (R= 0.7, $p \leq 0.01$) at the location off Chapora. In Dona Paula Bay, *C. criophilum* showed positive correlation with salinity and nitrite (R=0.59, 0.66 and $p \leq 0.05, 0.01$).

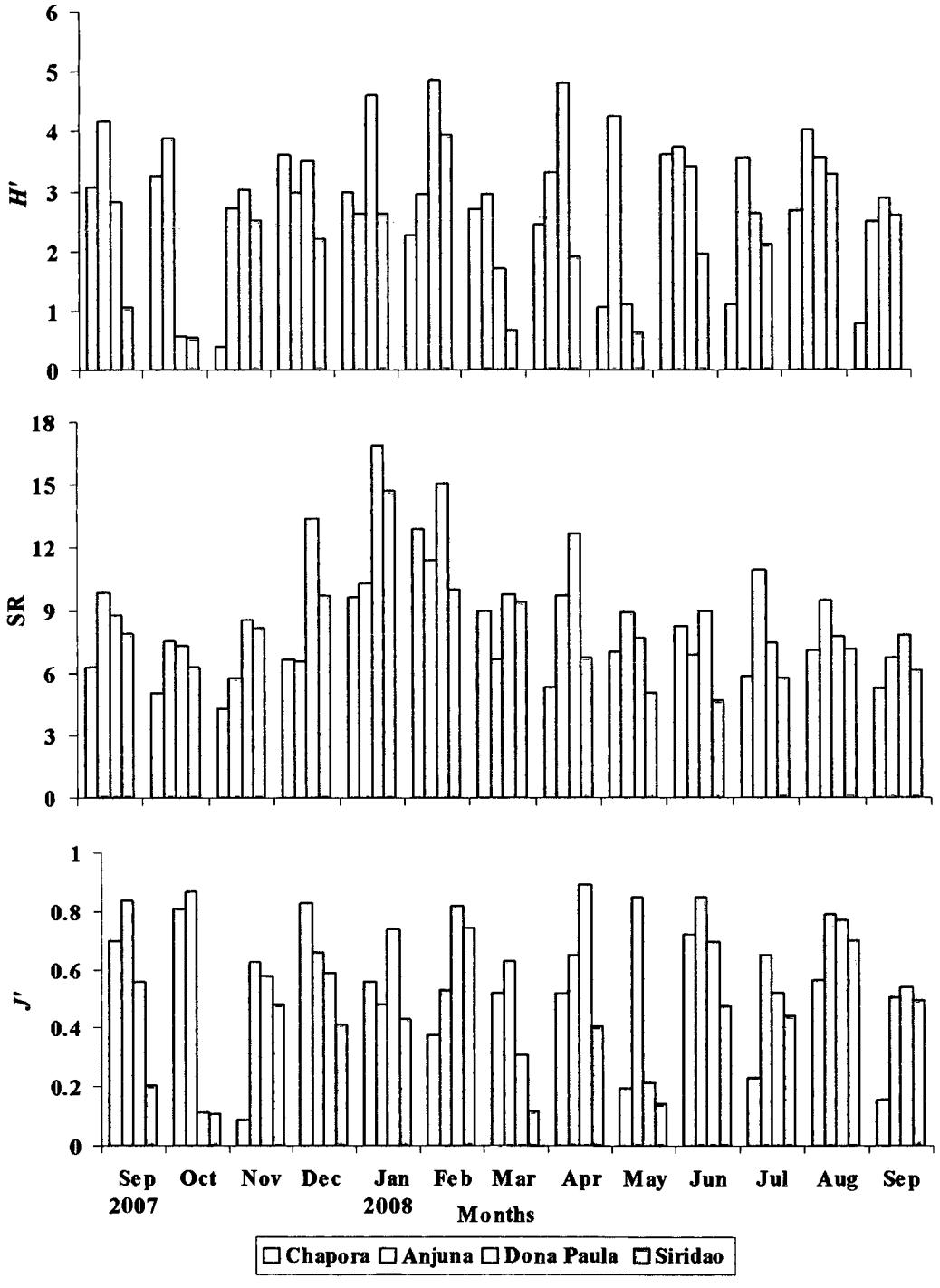



Fig 5.6. Species diversity ($H'$), richness (SR) and evenness ($J'$) indices of diatoms at different sampling locations off Goa, during September 2007-September 2008

Table 5.3 Spearman's rank correlation coefficients (R) between different environmental variables and total diatom cells count (TDC) as well as the most dominant species from different sampling locations off Goa

| Parameters | TDC | *R. punctatum* | *S. costatum* | *T. nitzschioides* | *L. minimus* |
|---|---|---|---|---|---|
| **Anjuna** | | | | | |
| Temp | -0.26 | 0.04 | -0.70* | 0.06 | 0.13 |
| Sal | 0.19 | 0.46 | -0.24 | 0.13 | 0.32 |
| $NO_2$ | 0.20 | -0.07 | 0.57* | 0.07 | -0.08 |
| $NO_3$ | -0.58* | -0.90* | 0.17 | -0.25 | -0.81* |
| $PO_4$ | -0.15 | -0.85* | 0.38 | 0.007 | -0.86* |
| $SiO_4$ | -0.31 | -0.83* | 0.31 | -0.22 | -0.75* |

| **Chapora** | | | | | | |
|---|---|---|---|---|---|---|
| | TDC | *C. laciniosus* | *S. costatum* | *L. danicus* | *C. marginatus* | *T. nitzschioides* |
| Temp | 0.30 | 0.44 | -0.16 | -0.39 | 0.38 | 0.35 |
| Sal | 0.34 | 0.72* | 0.07 | -0.75* | 0.16 | 0.63 |
| $NO_2$ | -0.63* | -0.51 | -0.41 | 0.41 | -0.14 | -0.56* |
| $NO_3$ | -0.56* | -0.19 | -0.46 | 0.63* | -0.42 | -0.70* |
| $PO_4$ | -0.58* | -0.23 | -0.38 | 0.20 | -0.37 | -0.68* |
| $SiO_4$ | -0.43 | -0.84* | -0.19 | 0.73* | -0.12 | -0.59 |

| **Dona Paula** | | | | | | |
|---|---|---|---|---|---|---|
| | TDC | *S. costatum* | *C. curvisetus* | *L. danicus* | *T. nitzschioides* | *C. criophilum* |
| Temp | -0.03 | -0.24 | 0.26 | -0.34 | -0.21 | 0.29 |
| Sal | 0.01 | -0.35 | 0.03 | -0.47 | 0.24 | 0.59* |
| $NO_2$ | -0.01 | -0.17 | 0.30 | -0.59* | 0.10 | 0.66* |
| $NO_3$ | -0.58* | 0.33 | -0.30 | -0.28 | -0.07 | 0.05 |
| $PO_4$ | -0.56* | -0.42 | -0.26 | -0.52* | 0.30 | 0.20 |
| $SiO_4$ | -0.26 | 0.13 | -0.2 | 0.16 | -0.09 | -0.25 |

| **Siridao** | | | | | | |
|---|---|---|---|---|---|---|
| | TDC | *S. costatum* | *A. glacialis* | *T. nitzschioides* | *C. curvisetus* | *G. striata* |
| Temp | 0.26 | -0.15 | -0.07 | 0.21 | 0.02 | -0.06 |
| Sal | 0.02 | -0.47 | -0.19 | 0.03 | 0.14 | 0.30 |
| $NO_2$ | 0.16 | 0.07 | 0.27 | 0.17 | 0.07 | 0.03 |
| $NO_3$ | -0.09 | 0.36 | 0.14 | -0.03 | -0.16 | -0.17 |
| $PO_4$ | -0.01 | 0.27 | 0.27 | 0.09 | 0.06 | -0.11 |
| $SiO_4$ | -0.09 | 0.38 | 0.15 | -0.24 | -0.08 | -0.33 |

* $p \leq 0.05$

***Relationship between Environmental Parameters and Diatom Species***

The forward selection of environmental parameters by the CCA retained seven variables that significantly explained the species distribution (Fig. 5.7). The first two axes explained 57.68% of the relationship between environmental variables and major diatom species. Salinity, silicate followed by phosphate and nitrate were the most important environmental variables influencing in the distribution of the major diatom species. In general, the ordination by the CCA grouped most of the centric diatoms in to the right quadrants apparently signifying the influences of higher concentrations of $NO_2$ and, $NO_3$. Most pennate species aligned in the left quadrant with lower nutrient concentrations but higher salinity (Fig. 5.7). As discernible from the Fig. 5.8, nitrate and phosphate in particular were the most important variables affecting the species composition during pre-monsoon (February–May) unlike salinity and silicate during post-monsoon (October-January) or, all nutrients and salinity during monsoon (June-September) period.

## Discussion

Taxonomic analyses of phytoplankton yield valuable insights on how their natural assemblages coexist in any given ecotype. Besides, the relative contribution of each species to primary production can be discerned through such efforts. The diatom abundances were high in the locations with relatively higher concentrations of nitrate/nutrients but wide fluctuations in salinity. On the annual scale, the observed cell abundance was the highest off Siridao followed by Chapora and, Dona Paula. Since the early days of phytoplankton ecology, nutrients have been known for

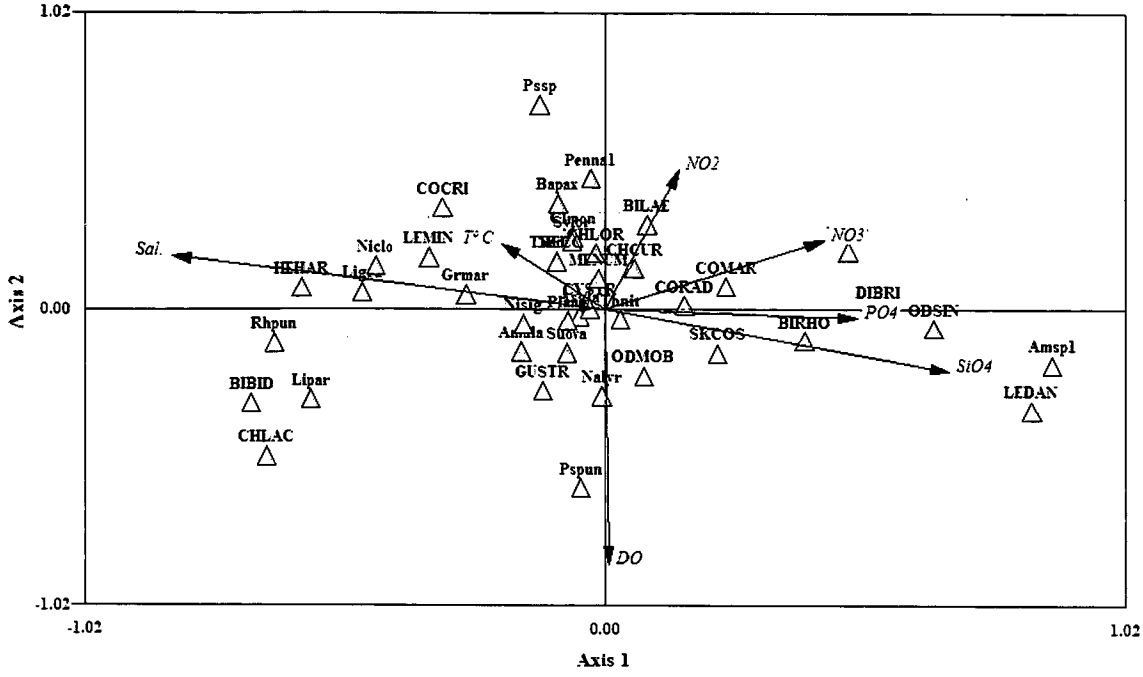



Fig. 5.7. Canonical correspondence analysis (CCA) based ordination of dominant diatom taxa against selected environmental variables observed during September 2007-September 2008 from four different locations off Goa. Codes of diatom species used in this analysis are listed in tables 5.1 and 5.2

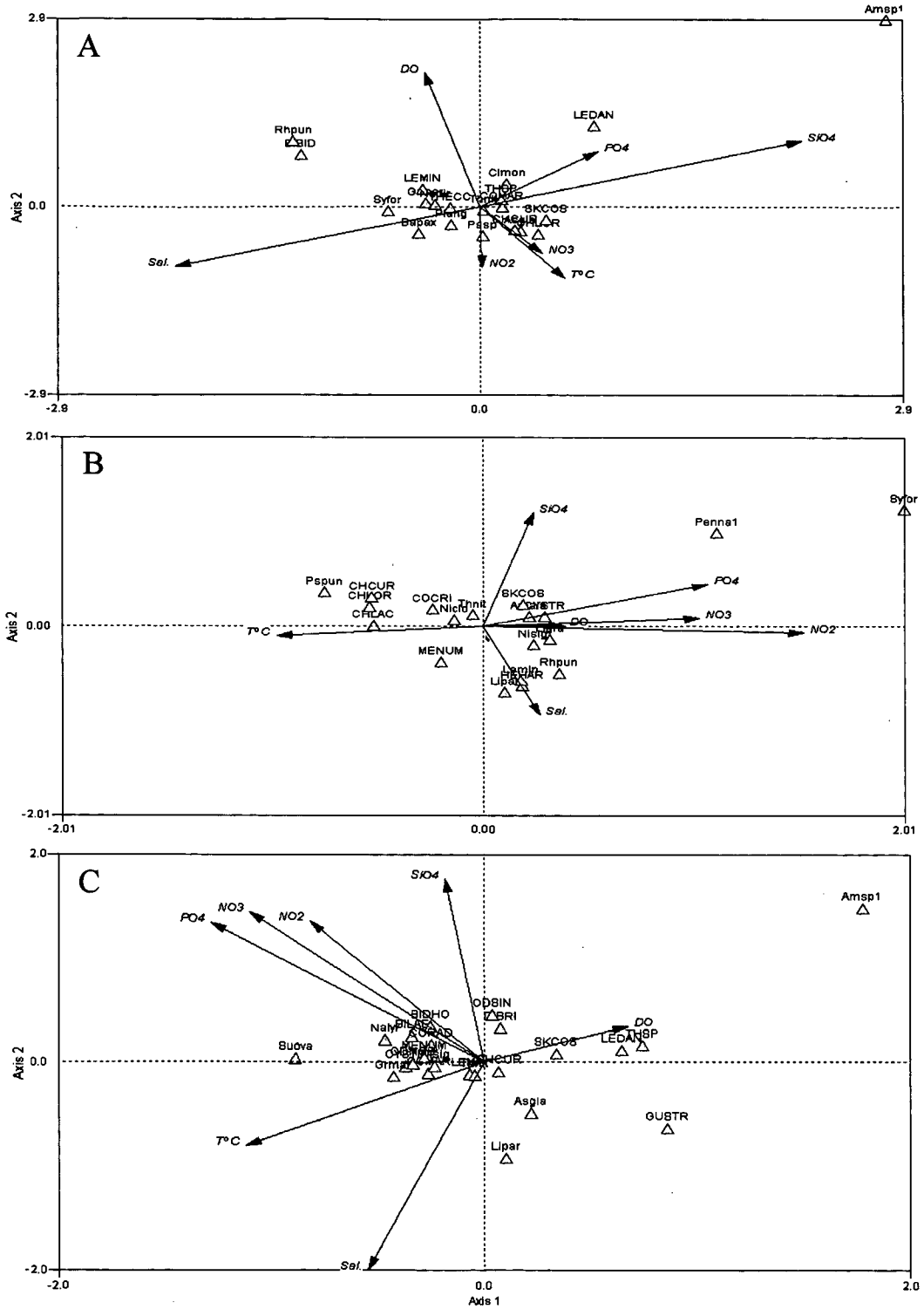



Fig. 5.8. Canonical correspondence analysis (CCA) ordination of dominant diatom taxa, against selected environmental variables observed during post-monsoon (A), pre-monsoon (B) and monsoon (C) seasons during September 2007-September 2008 from four different locations off Goa. Codes of diatom species used in this analysis are listed in tables 5.1 and 5.2

controlling the phytoplankton community structure and biomass (Tilman, 1982). That nutrients bore the greatest influence on diatom abundance is clear from the strong negative correlation between diatom abundance and nutrient concentrations at most locations. Though phosphate concentrations were critical off Dona Paula, the nitrate concentrations did seem to affect diatom abundances off Anjuna, Siridao as well as Dona Paula.

Redfield et al. (1963) were the first to propose that the elemental N:Si:P ratio of 16:16:1 is the ideal for phytoplankton under optimal conditions. Howarth (1988) suggested that deviations from this molar ratio are useful to infer which of these nutrients could be potentially limiting for phytoplankton. Despite this approach having risen criticisms (Howarth, 1988), it is used widely (see Brzezinski, 1985, Paul et al., 2008) for inferring the inorganic nutrient requirements.

In order to define potential nutrient control better, the N:Si; N:P and Si:P were derived using observed data on concentrations of nitrate-N, silicate-Si and phosphate-P. These ratios were compared with Redfield ratios of N:Si (16:16); N:P (16:1) and Si:P (16:1). It was evidenced that the N:P ratios were mostly lower than the Redfield ratios (16:1) at all sampled locations except during September 2007. Although these ratios are used to merely define resource availability as a consequence of loading and biotic activity, there was clear indication of nitrate deficiency relative to phosphate. Concentrations of ammonium were not measured in any of the samples. However, it is possible to suppose that ammonium would be in non-detectable concentrations, if at all, in the sampling locations that are shallow, well mixed and oxygenated.

During the sampling period, the N:Si ratios were lower (< 1) unlike those reported by Brzezinski (1985). But the high abundance of diatoms is suggestive that the prevailing nitrate concentrations did facilitate the diatom abundances throughout the year. Results of the present study agree with the observations of Leblanc et al. (2004), who found a deficiency of N over Si in the photic layer, while a shift towards a systematic Si deficiency below the photic layer in the Almeria-Oran front. On the other hand, the high Si:P ratios especially off Chapora and Siridao are attributable to the active coastal silica pump. The high abundances of diatoms at these locations could therefore be due to the high Si:P ratios. Such realms are reported to support diatom dominated systems (Bethoux et al., 2002).

The peak abundances of diatoms were during the post-monsoon, in particular during September 2008, at all locations when water temperature was in the range of 27- 28.5 °C and salinity, 17- 35. Based on experimental evidences, Qasim et al. (1972) had confirmed that, within limits, waters with reduced salinity supported a greater abundance of phytoplankton in the estuaries. Gopinathan (1974) reported the dominance of *S. costatum* immediately after or following a break in the monsoon in the Cochin Backwaters. Subramanyan (1959) remarked that a fall in temperature, from 32 to 25°C, to optimum levels, a slight lowering of salinity (35 to 32) and a plentiful supply of nutrients during the early monsoon period lead to intense phytoplankton blooms. Thus, the reduction in salinity by itself is not a precondition for the proliferation of phytoplankton, but rather it is due to the adaptability of certain phytoplankters to moderate changes in salinity brought about by the south-west monsoon showers coupled with a large input of nutrients.

Beginning the mid 1940s, planktonic diatoms from the Indian waters have been investigated by Subramanyan (1946), Chennubhotla (1969), and Desikachary (1977). There are many recent studies along the west coast of India (D'Costa et al., 2008; Harnstrom et al., 2009, and references therein) mostly on the composition of phytoplankton. There are sporadic reports of unusual blooms of phytoplankton often leading to undesirable ecological consequences (Naqvi et al., 1998; Ramaiah et al., 2007). All the species observed during this study have been previously reported in Indian waters from the analyses by Menon (1945), Subramanyan (1946), Subramanyan and Sarma (1961), Desikachary and Ranjithadevi (1986) or, Desikachary and Prema (1987; Plates 5.1-5.5). In the present study, *S. costatum* followed by *T. nitzschioides* were the two most dominant species from the disparate salinity gradient locations chosen for this study.

The highest abundance of diatoms observed in September 2008 at all four locations was due to *S. costatum* blooms. Mallin (1994) and Muylaert and Sabbe (1999) point out *S. costatum* as a common taxon in the phytoplankton of temperate estuaries. In our study, its blooms coincided with low salinity and abundances negatively correlated with salinity at the all locations except Chapora. As has been reported by many researchers along the Indian coasts (Babu et al., 2001; Paul et al., 2007; Patil and Anil, 2008) it is very likely that this species prefers lower salinity.

In spite of highly varying nutrient regimes at the sampling locations, bloom proportions of *T. nitzschioides* (>1000 cells $L^{-1}$), the other dominant and common species were prevalent throughout the year. This cosmopolitan species (Hasle and Syvertsen, 1996) is reported to be surviving wide nutrient regimes (Abrantes, 1988)

from the highly productive to very low nutrient regimes (Kobayashi and Takahashi, 2002). Observations of the present study corroborate with these earlier reports.

The occurrence of oligohaline *Amphiprora* spl, during monsoon and, only off Chapora, is attributable to the generally low salinity prevalent at this location. Similarly *L. danicus* was numerous at all locations during the monsoon especially off Chapora and Dona Paula. Its preponderance coincided with decrease in salinity due to freshwater discharges from the Zuari River and Chapora River during the monsoon months.

The sampling locations off Chapora, Siridao and Dona Paula are comparatively richer in nutrients than the truly marine location off Anjuna. From the significant relationship between cell abundances and nutrient concentrations at the first three locations, it can be suggested that the preponderance and blooming of *Chaetoceros curvisetus* followed by *C. lorenzianus* are governed by the higher nutrient concentrations. Kobayashi and Takahashi (2002) attributed increased occurrence of *Chaetoceros* spp to higher nutrient concentrations in the western and central Pacific.

Off Anjuna, with sizable counts of *Rhabdonema punctatum* and *T. nitzschioides* the pennate diatoms were more abundant during most sampling months than the centric diatoms Pennate diatoms, with higher surface to volume ratio, would efficiently assimilate nutrients even when their concentrations are very low or, are limiting and therefore, thrive in such locations. Further, as only Pennales attained bloom proportions at most locations, it is possible to suggest that wind and/or tidal mixing

at the shallow locations introduce these mostly-benthic forms into the water column.

The diversity (or biodiversity) is typically measured by counts/numerical abundance of a species count (richness) and sometimes with its evenness index. It is useful for inferring a number of ecosystem processes (Telesh, 2004). It is mostly measured by the widely used Shannon–Wiener index *H'*, a proportional statistic that combines both these measures (Stirling and Wilsey, 2001). In essence, 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. No clear relationship between species richness and diversity indices was found as was also noted by Reed (1978) and Gao and Song (2005). These authors also found that diversity indices were closely related to evenness, whereas species numbers (richness) were unimportant in determining species diversity. However, the *H'* indices observed during the study are useful to point out that they are low when there is rapid growth by a few species in some locations.

The CCA results clearly indicated that salinity was the most important environmental variable in explaining diatom distribution in the studied estuaries. This is in agreement with previous works that pointed salinity as the most important factor in controlling the distribution of diatoms in estuarine environments (Oppenheim, 1991; Juggins, 1992; Hassan et al., 2007; Hassan, et al., 2009).

Also from the CCA, it is evident that the diatom species present in the right quadrants are those profiting mostly from pulses of nutrient enrichment such as *S.*

*costatum* and *C. curvisetus*. Species tolerating low salinity, such as *L. danicus* and *Amphiprora* spl, aligned into the lower right quadrant and species favored by higher salinity aligned into the left quadrants (Fig. 5.7). These were *Rhabdonema punctatum* and *Licmophora paradox* (Pennales) as well as *Chaetoceros laciniosus* and *Biddulphia biddulphiana* (Centrales). Diatom species dominant during post and pre-monsoon months in general followed the nutrient gradients and aligned into the right hand side quadrants explaining >65% of their cumulative variability (Fig 5.8 A and B). Similarly, species that became dominant during monsoon months, as a consequence of increased nutrient concentrations, aligned into the upper left quadrant (Fig. 5.8C).

It can be inferred from this investigation that while the diversity of diatoms is quite high from the coastal waters of Goa, though only a few species are predominant. Devassy and Goes (1988) had reported 63 diatom species from the Mandovi-Zuari estuarine complex. Many of these species were suggested to be thriving even at very low salinities. Later, Redekar and Wagh (2000) recorded 66 species of planktonic diatoms from the Zuari estuary. Recently, Patil and Anil (2008) also recorded 89 species of benthic diatom propagules from the Zuari estuary. While the diversity indices observed during this investigation are similar to the ones reported earlier from this region by Mitbavkar and Anil (2008), they are for instance, much higher than those reported from mesotrophic Changjiang Estuary (Gao and Song, 2005) or, from tropical costal waters (Abid, et al., 2008). That the species of pennate diatoms are far more numerous and widely distributed is an indication that their larger surface-to-volume ratios facilitate their survival during low nutrient

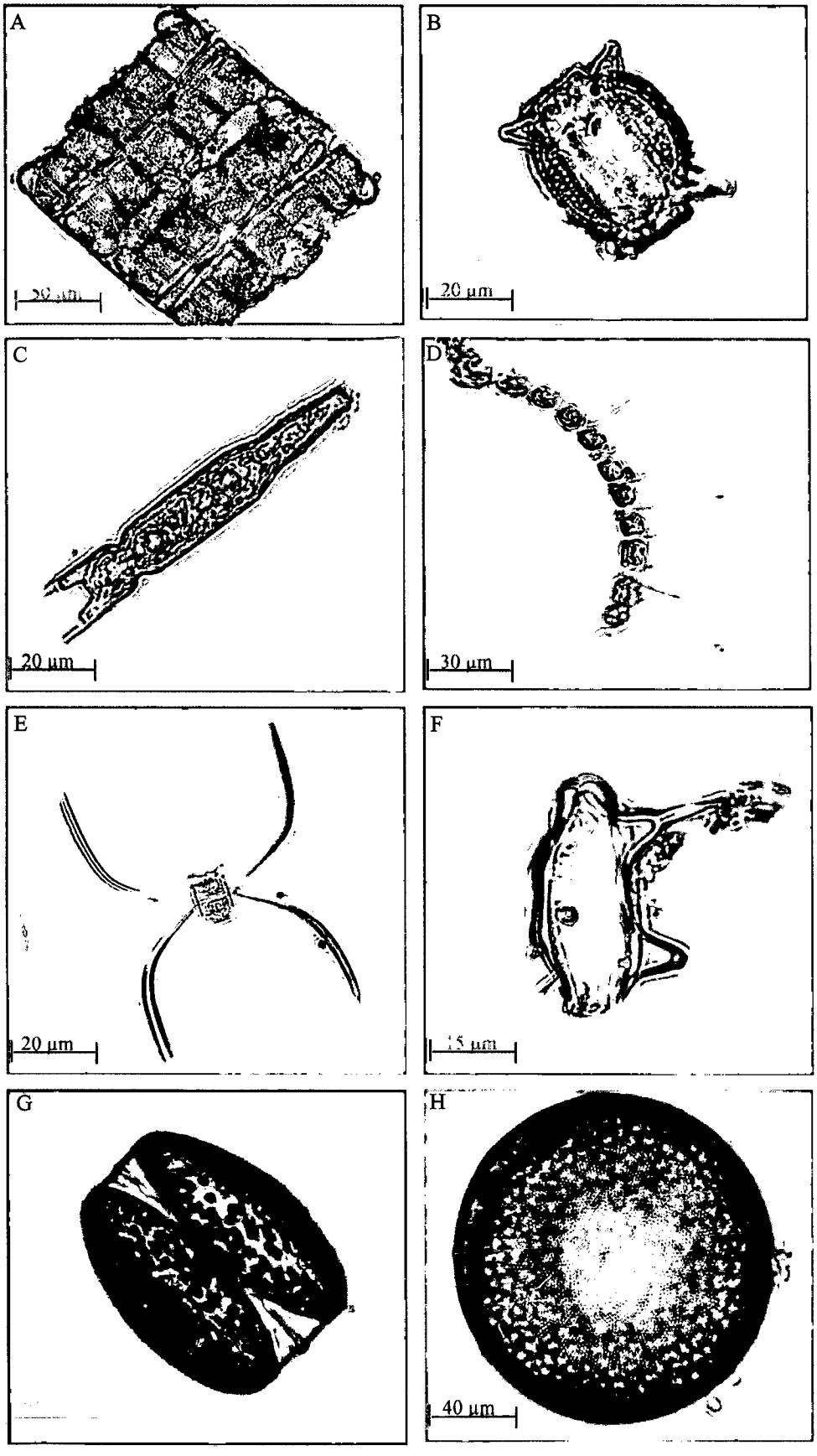
and/or unfavorable salinities. The numerical abundance of some Centrales though high, many species showed conspicuous absence in particular off Anjuna (the truly marine location) and off Chapora (one of the two estuarine locations sampled) on the annual scale. Besides, the bloom proportions of some centric diatoms at many sampling locations during different periods of the year might also imply that the Centrales proliferate when the nutrient concentrations and salinities are favorable. The 'tolerant' (therefore diverse) Pennales persist in these shallow regions and, thrive through the dynamic shifts the physico-chemical parameters undergo in the ecosystems regulated by salinity gradients.

Light microscope photographs (Plates 5.1, 5.2 and 5.3) of a few centric diatom species from different sampling locations off Goa

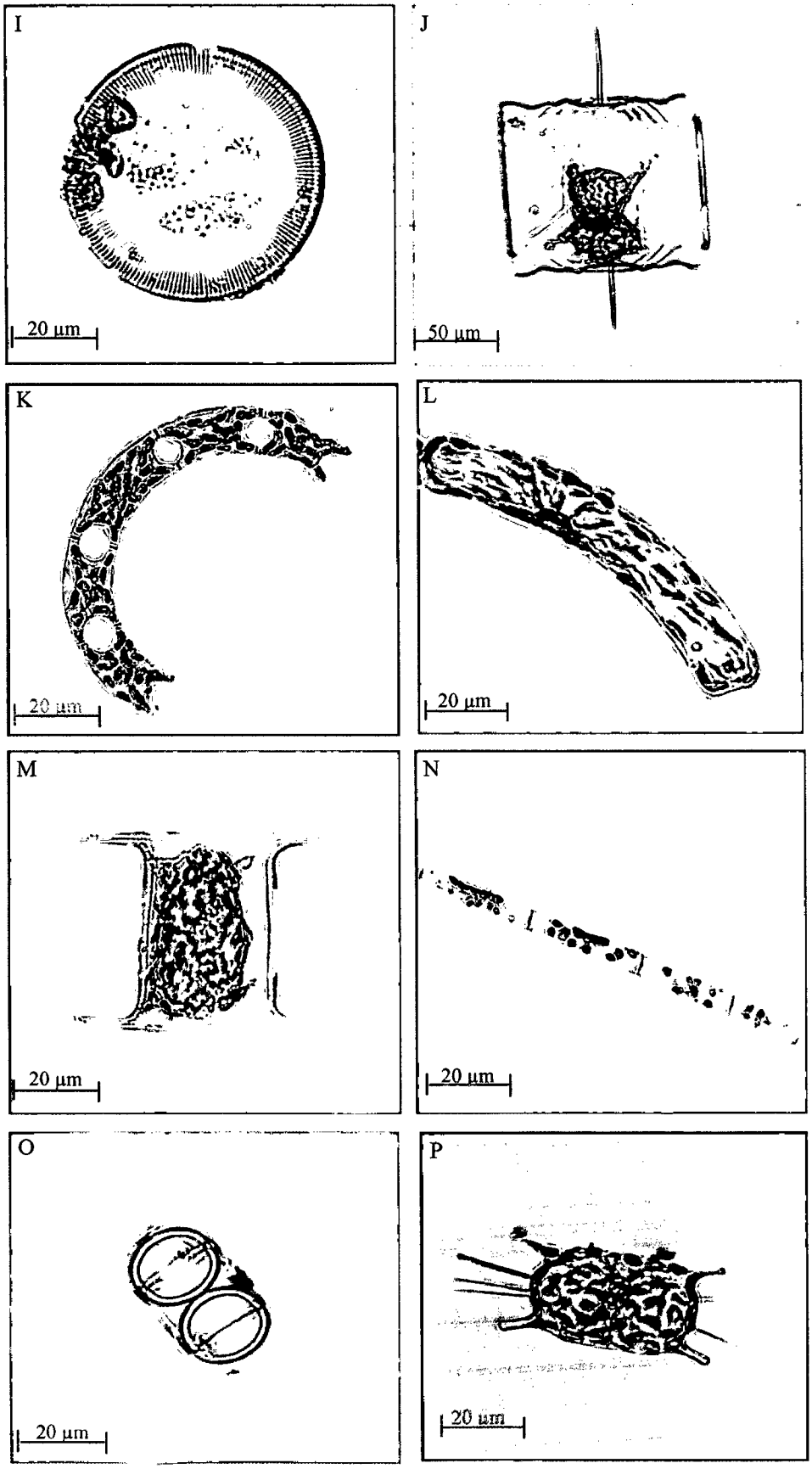
Legend:

A) *Biddulphia biddulphiana*
B) *Biddulphia rhombus*
C) *Cerataulina bicornis*
D) *Chaetoceros curvisetus*
E) *Chaetoceros diversus*
F) *Chaetoceros mitra*
G) *Coscinodiscus granii*
H) *Coscinodiscus marginatus*
I) *Cyclotella striata*
J) *Ditylum brightwellii*
K) *Eucampia zodiacus*
L) *Guinardia striata*
M) *Hemiaulus hauckii*
N) *Leptocylindrus danicus*
O) *Melosira nummuloides*
P) *Odontella mobiliensis*
Q) *Odontella sinensis*
R) *Planktoniella sol*
S) *Rhizosolenia imbricata*
T) *Skeletonema costatum*
U) *Striatella* sp
V)*Symbolophora trinitatis*
W) *Thalassiosira* sp
X) *Triceratium dubium*

# Plate 5.1



# Plate 5.2



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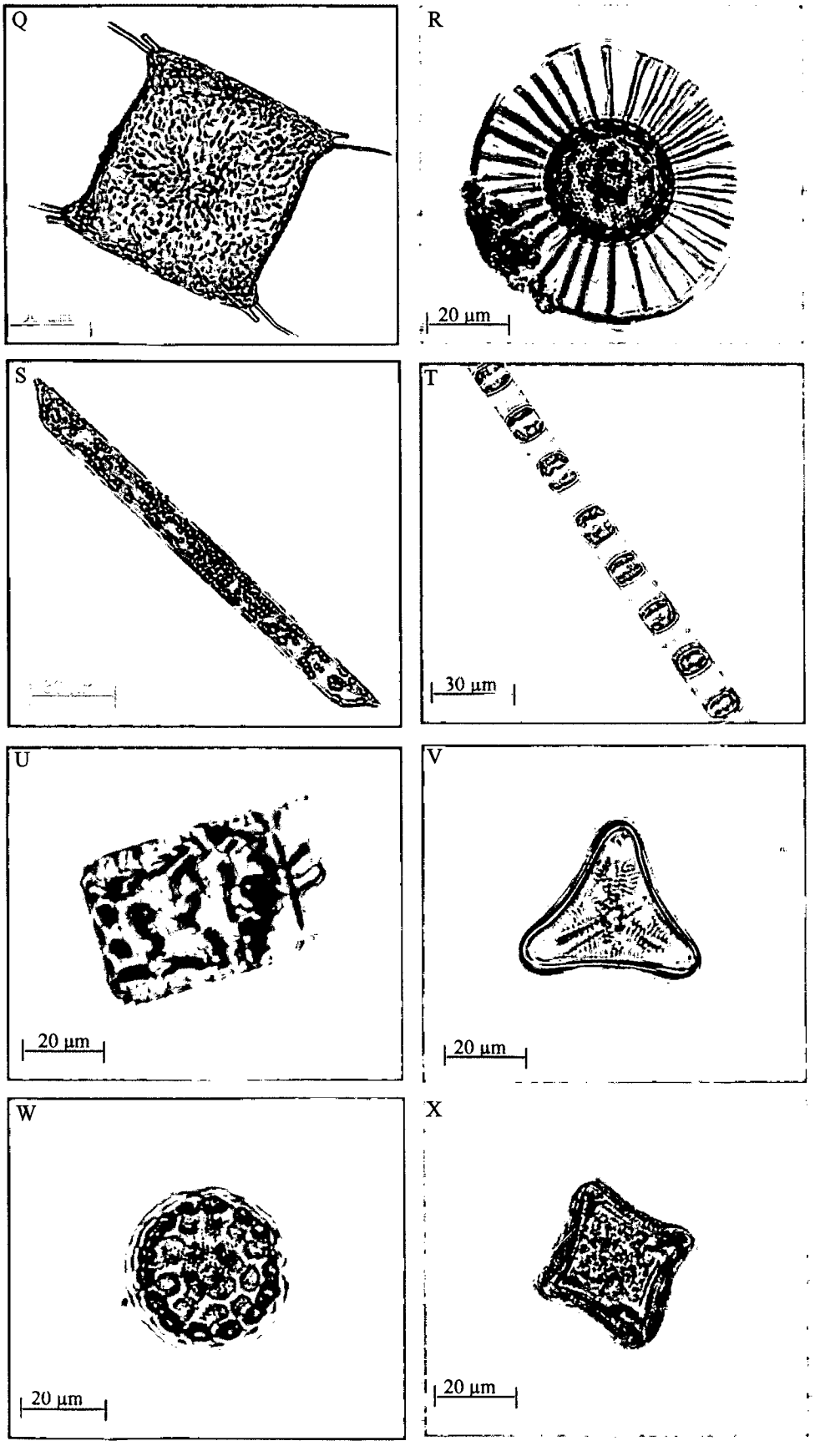
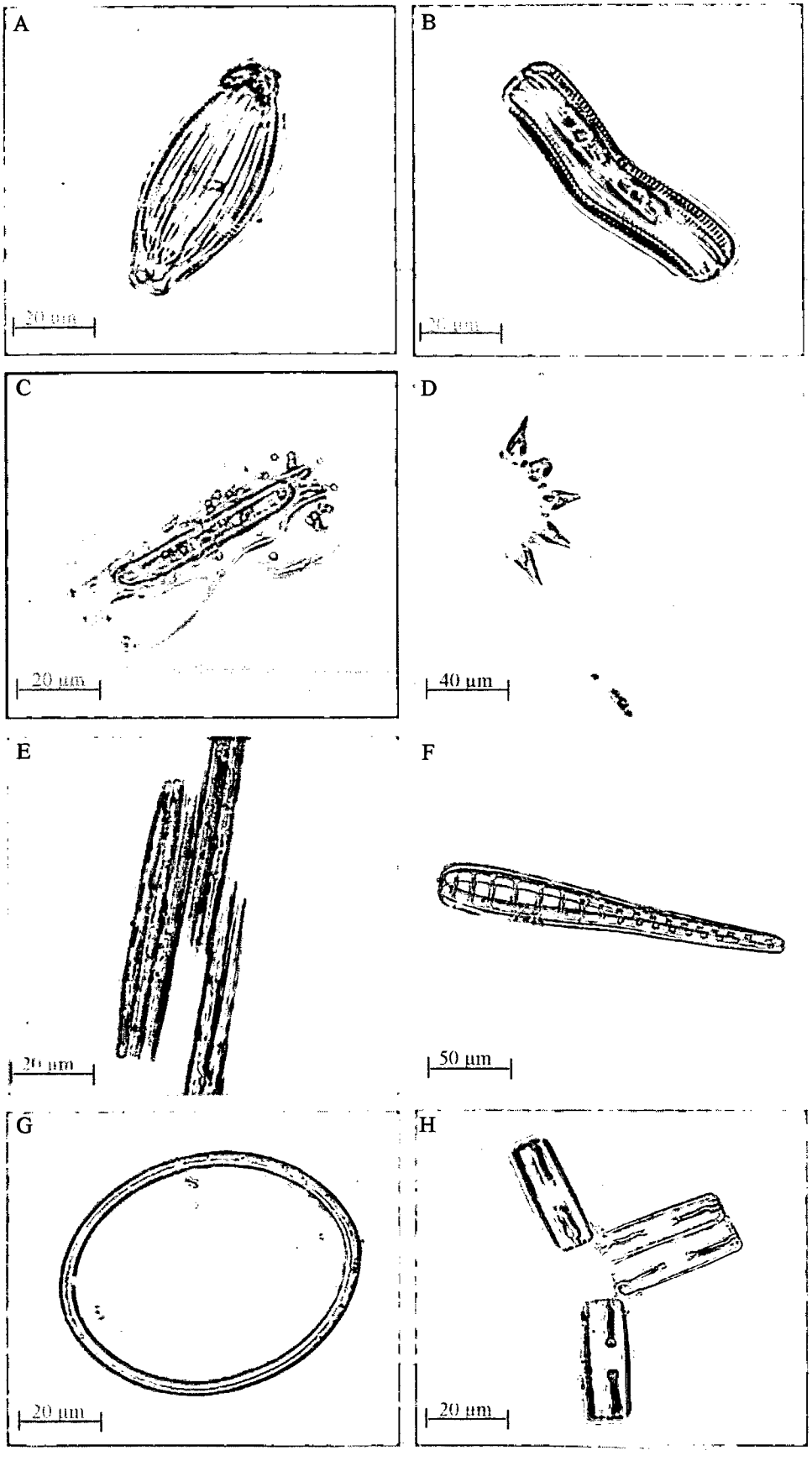


Light microscope photographs (Plates 5.4 and 5.5) of a few pennate diatom species from different sampling locations off Goa

Legend:

A) *Amphora coffeaeformis*
B) *Achnanthes brevipes*
C) *Amphiprora* sp
D) *Asterionella glacialis*
E) *Bacillaria paxillifera*
F) *Climacosphenia moniligera*
G) *Cocconeis pellucida*
H) *Grammatophora marina*
I) *Gyrosigma balticum*
J) *Navicula* sp
K) *Nitzschia closterium*
L)*Pleurosigma aestuarii*
M) *Pleurosigma angulatum*
N) *Rhapdonema punctatum*
O) *Synedra* sp
P) *Thalassionema nitzschioides*

# Plate 5.4



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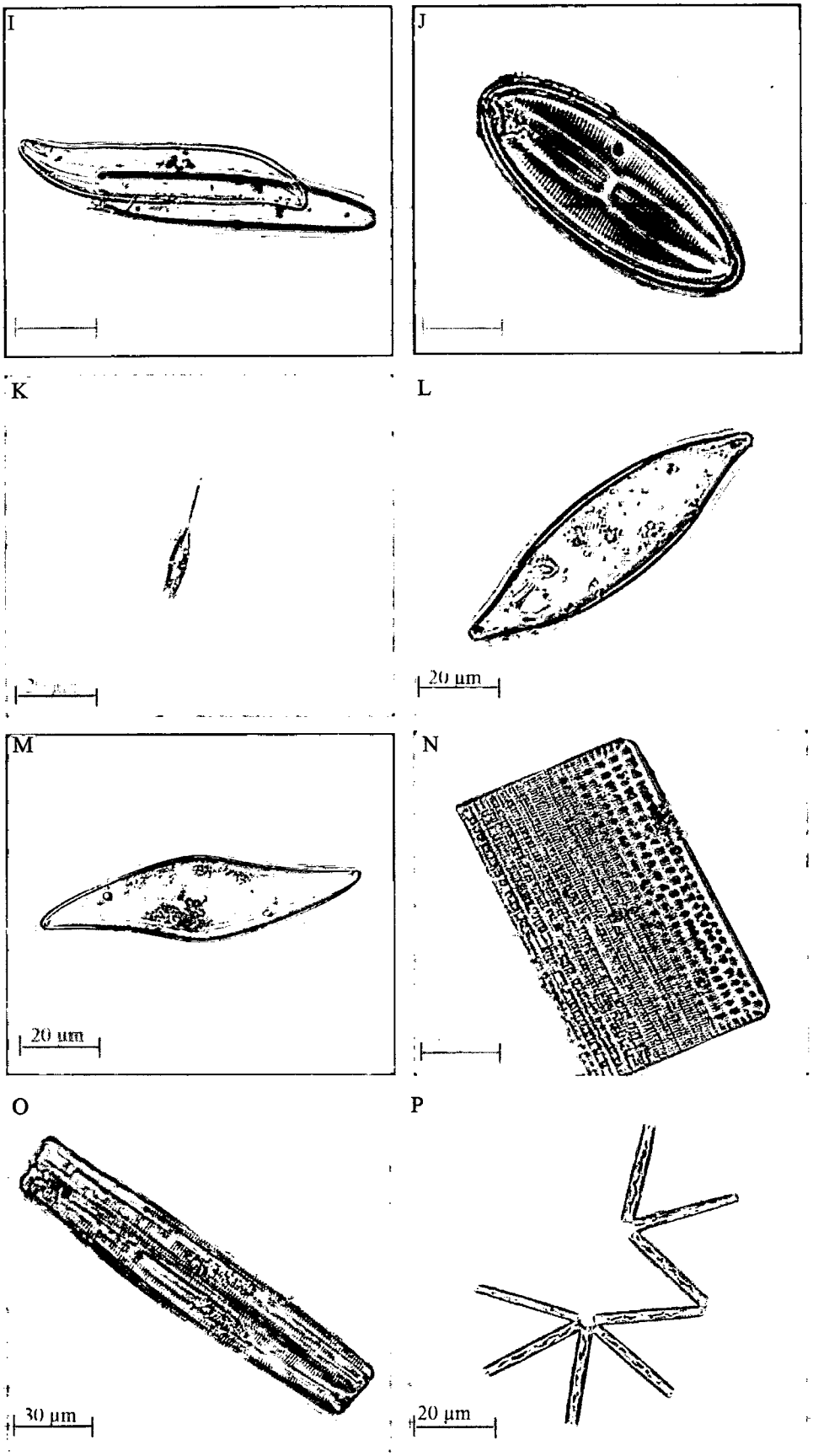


# Chapter 6

## Abundance and Assemblage of Dinoflagellates

### Introduction

After diatoms, the 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. Many of the photosynthetic members are mixotrophs, and the heterotrophs feed by a wide variety of mechanisms (Schnepf and Elbrächter, 1992).

While most dinoflagellates are free-living in marine and freshwater environments, others, such as the "zooxanthellae" of reef-building corals, are beneficial endosymbionts and still others are parasites of many protist, invertebrate and vertebrate hosts. Some are luminescent and some are toxin producers. Their greatest diversity is in the marine plankton where they can produce "red tides" and other monospecific blooms (Taylor, et al., 2008). Dinoflagellate species are adapted to a variety of habitats: from pelagic to benthic, from temperate to tropical seas, and from estuaries to freshwater. Many species are cosmopolitan and can survive in variety of habitats: in the plankton, or attached to sediments, sand, corals, or macroalgal surfaces (Faust and Gulledge, 2002).

Dinoflagellates are important components of the phytoplankton assemblages in the near-shore and continental shelf environments. Some species however, are primary causal agents of paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning

(DSP) and related toxic phenomena (red tides). Such toxic species are of serious economic considerations. Often, the red tide phenomenon has been ascribed to harmful algal blooms (HAB). Over the years, there has been marked rises in the occurrence of HAB along with their spread to new geographical regions (Anderson et al., 2002a). The apparent global expansion of HAB is linked to intensified anthropogenic eutrophication of coastal waters and to increased load of nutrients (Hallegraeff, 1993; Anderson et al., 2002a and 2002b). Many dinoflagellate species have been implicated in fish kills and consequent economic losses in many regions of the world oceans (Adams et al., 1968; Hoagland et al., 2002). Human fatalities due to PSP, DSP and/or other dinoflagellate related toxicities have been of serious public health concerns. In view of the intra- and inter- annual variability in the species composition of dinoflagellates, they are regularly monitored in most European countries, the Americas and Japan. The most countries, however, have not yet set effective monitoring programs, though there are reports of human health concerns as a consequence of HAB (Ramaiah et al., 2005).

In the monsoon affected west coast of India, many studies have documented the occurrence of different genera of dinoflagellates (Devassy and Bhattathiri, 1981; Lingadhal et al., 1998; Tiwari and Nair, 1998; Mendon et al., 2002; Alkershi, 2004). Most of these studies recorded 6 to 8 genera of dinoflagellates to be prevalent with *Protoperidinium* and *Ceratium* as common genera along the central west coast of India. Cases of *Noctiluca* blooms (Sahayak et al., 2005) and fish kills due to dinoflagellate blooms (Naqvi et al., 1998) are also reported in particular from the southern regions. Godhe et al. (2008) have also documented the prevalence of

toxic dinoflagellates in Indian coastal waters. Cysts/resting stages of over 30 dinoflagellate species in 10 genera from the coast of Mumbai are reported from an exhaustive recent study by D'Costa et al. (2008). Karunasagar and Segar (1990) reported a case of PSP related death.

Most non-parasitic and/or symbiotic dinoflagellate species are marine, numerous in temperate waters, and flourish during summer months (Taylor, 1987). Nitrogen and phosphorus concentrations bear a strong influence on stimulation of phytoplankton blooms, including HAB forming species in coastal areas (Harrison, 1976; Paerl, 1988, 1997; Baek et al., 2008). Some dinoflagellate species may respond well to increased nutrient additions by proliferating in numbers. However, it would be relevant to elucidate their variability in their natural ecosystems subjected to varying physico-chemical characteristics, for instance, during monsoon and non-monsoon periods. In addition, from the perspectives of public health-related issues, it would be necessary to elucidate the differences in the species composition of toxic dinoflagellates from the coastal regions. In view of this, we planned a year-round systematic survey from four locations off Goa where salinity differences/variations are large. We also aimed to document the variations in dinoflagellates in relation to environmental variability from this sparsely investigated region.

## Material and Methods

### *Dinoflagellate Assemblage*

For quantitative and qualitative analyses of dinoflagellates, cell counts and composition, water samples from each of the four stations (Chapora, Anjuna, Dona Paula and Siridao) were fixed with Lugol's solution (1% wt/vol) and 3% formaldehyde and stored in the dark until analyzed. Further, the dinoflagellates were identified to their generic/specific level based on the available literature (Taylor 1976; Dodge, 1982; Steidinger and Tangen, 1997; Faust and Gulledge, 2002; Horner, 2002).

### *Statistical Analyses*

Shannon-Weaver (H'), species richness (SR) and evenness (J') indices were calculated according to Omori and Ikeda (1984) in order to obtain more information on the dinoflagellate community, using the data on species and cell numbers.

Spearman's rank correlation test was performed to assess the relationships between the dinoflagellate species and various observed environmental parameters that may be regulating their population. This test was performed using the software Statistica, version 6.0 (Statsoft, Oklahoma, USA).

Canonical Correspondence Analysis (CCA) was employed to investigate the relationship between the environmental parameters and dinoflagellate abundance (Ter Braak, 1986). The CCA integrated 6 environmental variables and 25 taxa recorded from the study area. The environmental parameters included in the CCA are nitrite, nitrate, phosphate, dissolved oxygen, temperature and salinity. A total of

43 samples collected at four sampling locations were entered into the CCA, leaving nine samples out of the analysis based on the absence of dinoflagellates. The CCA was performed using Multi-Variate Statistical Package Program Version 3.1 (Kovach 1998).

## Results

### *Dinoflagellate Assemblages*

The abundance of dinoflagellates was lower than that of diatoms (Chapter 5) throughout the year at all sampled locations. Distinct temporal and spatial variations in dinoflagellate cell densities were clearly seen (Fig.6.1). Some or the other species occurred throughout the period of study in the nearshore waters of Goa. Except during September 2008 (8604, 1924 and 1666 cells $L^{-1}$ off Siridao, Dona Paula and Anjuna, respectively), the abundance of dinoflagellates was mostly $<10^3$ cells $L^{-1}$. Twenty-five species of planktonic dinoflagellates belonging to seven genera were recorded during this study (Table 6.1). The number of heterotrophic dinoflagellate species was quite less compared to that of autotrophic ones.

The cell abundances of *Dinophysis* and *Protoperidinium* with seven species each and *Ceratium* with six species were greater than the other genera/species recorded during the present study. The most dominant species in the study area were *Ceratium furca* and the red tide-forming species, *Prorocentrum micans*. The cell counts of *C. furca* were the highest during September 2008 off Siridao and those of *P. micans* during September 2008 off Anjuna (Fig 6.2). The lowest numbers of

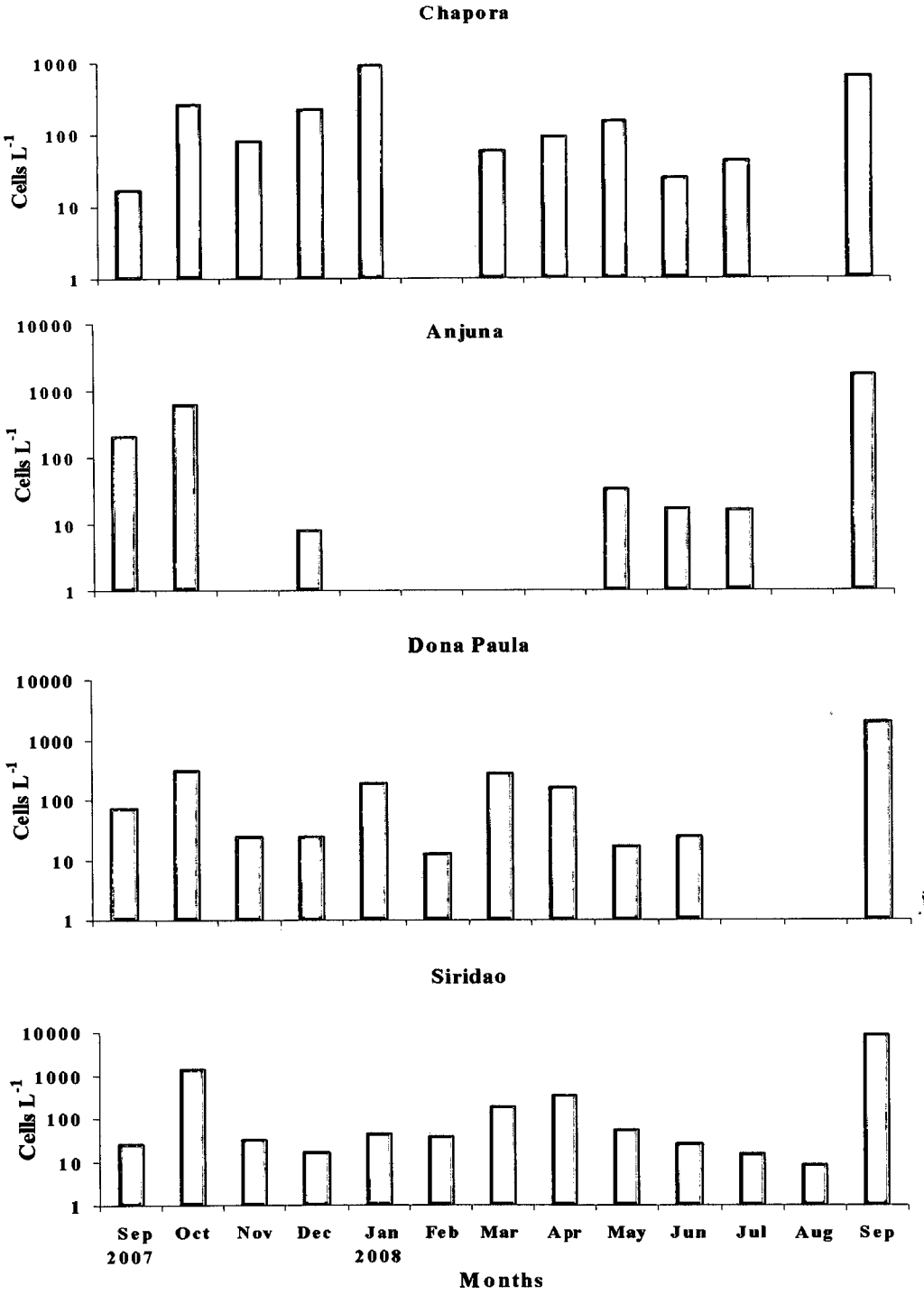



Fig.6.1 Variations in dinoflagellate cell counts in the surface waters from September 2007 to September 2008 at different sampling locations off Goa

Table 6.1. List of dinoflagellates species, ranges of their cell counts $L^{-1}$ (mean ±SD) recorded from the surface waters at different sampling locations off Goa. The taxa codes of the species used in the canonical correspondence analysis are indicated. ND denotes species not detected at a given location

| Taxa code | Taxon | Sampling location | | | |
|---|---|---|---|---|---|
| | | Chapora | Anjuna | Dona Paula | Siridao |
| | **Autotrophic** | | | | |
| ALMIN | *Alexandrium minutum* | 0-133 (13±38) | ND | 0-63 (5±18) | 0-875(72±242) |
| CEFUR | *Ceratium breve* | 0-17(1±5) | 0-8 (1±2) | ND | ND |
| CEFUS | *Ceratium furca* | 0-367(54±103) | 0-175 (26±57) | 0-1208 (113±331) | 0-5763 (492±1587 |
| CELIN | *Ceratium fusus* | 0-42(6±14) | 0-117 (10±32) | 0-58 (5±16) | 0-350 (28±97) |
| CEBRE | *Ceratium lineatum* | ND | ND | 0-42 (3±12) | 0-1713 (136±474) |
| CETRI | *Ceratium tripos* | 0-8 (1±2) | 0-25 (2±7) | 0-17(1±5) | 0-50 (4±14) |
| CEVUL | *Ceratium vultur* | 0-8 (1±2) | ND | ND | ND |
| DIACU | *Dinophysis acuminata* | 0-25 (4±8) | 0-392 (30±109) | 0-125 (12±35) | 0-238 (19±66) |
| DICAU | *Dinophysis brevisulcus* | ND | ND | 0-83 (6±23) | ND |
| DIMIL | *Dinophysis caudata* | 0-33 (3± 9) | 0-258 (22±71) | ND | ND |
| DIHAS | *Dinophysis fortii* | ND | ND | 0-8 (1±2) | ND |
| DIBRE | *Dinophysis hastata* | ND | ND | 0-8 (1±2) | ND |
| DIFOR | *Dinophysis miles* | ND | 0-17 (1±2) | ND | ND |
| DISP | *Dinophysis* sp | ND | ND | 0-183 (20±52) | 0-175 (19±49) |
| GOSP | *Gonyaulax* sp | 0-8(1±2) | 0-25 (3±2) | 0-8 (1±2) | ND |
| PRGRA | *Prorocentrum gracile* | 0-150 (14±41) | 0-83 (6±23) | 0-17 (3±6) | 0-17 (3±6) |
| PRMIC | *Prorocentrum micans* | 0-342 (7±122) | 0-458 (68±139) | 0-358 (44±98) | 0-338 (64±115) |
| PYHOR | *Pyrophacus horologicum* | 0-58 (6±16) | 0- 50 (4±14) | 0-50 (6±15) | 0-142 (17±39) |
| | **Heterotrophic** | | | | |
| Prcon | *Protoperidinium conicum* | 0-17(3±7) | 0-50 (5±14) | ND | ND |
| Prdep | *Protoperidinium depressum* | 0-25(5±8) | 0-8 (1±3) | 0-50 (5±14) | 0-38 (3±6) |
| Proce | *Protoperidinium oceanicum* | 0-25 (2±7) | 0-25 (2±7) | 0-25 (2.5±7) | 0-63 (5±17) |
| Prste | *Protoperidinium steinii* | 0-67 (15±24) | 0-183 (17±51) | 0-33 (6±11) | 0-117 (13±32) |
| Prsub | *Protoperidinium subinerme* | ND | ND | 0-8 (1±2) | ND |
| Prten | *Protoperidinium tenuissimum* | ND | ND | ND | 0-17 (1±5) |
| Prsp | *Protoperidinium* sp | ND | ND | ND | 0-25 (3 ± 7 |

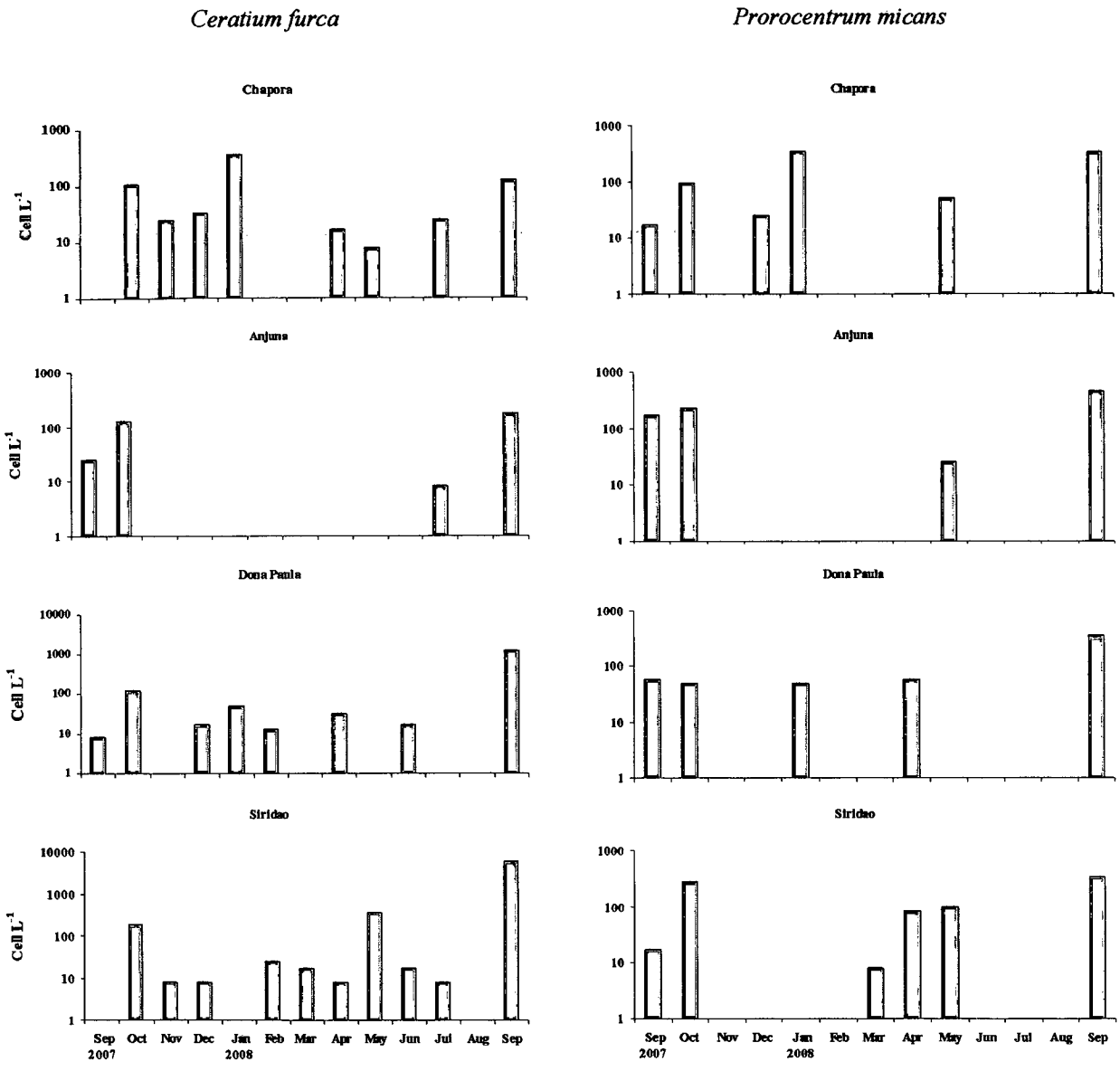



Fig.6.2 Distribution patterns of the most dominant dinoflagellate species at different sampling locations off Goa, during September 2007-September 2008

species were observed off Anjuna from where the dinoflagellates were not detected during many sampling months (Table 6.1).

Of the known toxic species, *Alexandrium minutum, Dinophysis acuminata, D. caudata* and *Prorocentrum micans* were more abundant in the study area. Cells of *D. hastata, D. brevisulcus, D. fortii* and *D. miles* though detected at all locations were rare. With its highest numbers in October 2007 (875 cells $L^{-1}$), *Alexandrium minutum* was generally quite preponderant off Siridao, the low salinity sampling location of this study. In general, before and during this species attained its peak abundance, an increase in nutrient concentrations was observed. Cell counts of *Dinophysis acuminata* and *D. caudata* showed wide fluctuations except during September 2008 when they occurred in high numbers at all locations. Abundance of *D. acuminata* was maximum off Anjuna (392 cells $L^{-1}$) and Siridao (238 cells $L^{-1}$) when the salinity was 35 and 31.8 psu respectively at these locations. Nitrate and phosphate concentrations were more before this species attained its peak abundance. On the other hand, *Prorocentrum micans* was recorded at all locations and almost throughout the year.

The dinoflagellate species diversity indices were usually <2.00 (Fig 6.3). The highest *H'* was recorded from off Anjuna followed by Dona Paula during the post-monsoon period. Commensurating with cell abundances and the number of species enumerated, the evenness varied from 0.0 (poor) to 1.99 (high) and the species richness from 0.0 (single species dominance) to 3.58 (multispecies proliferates).

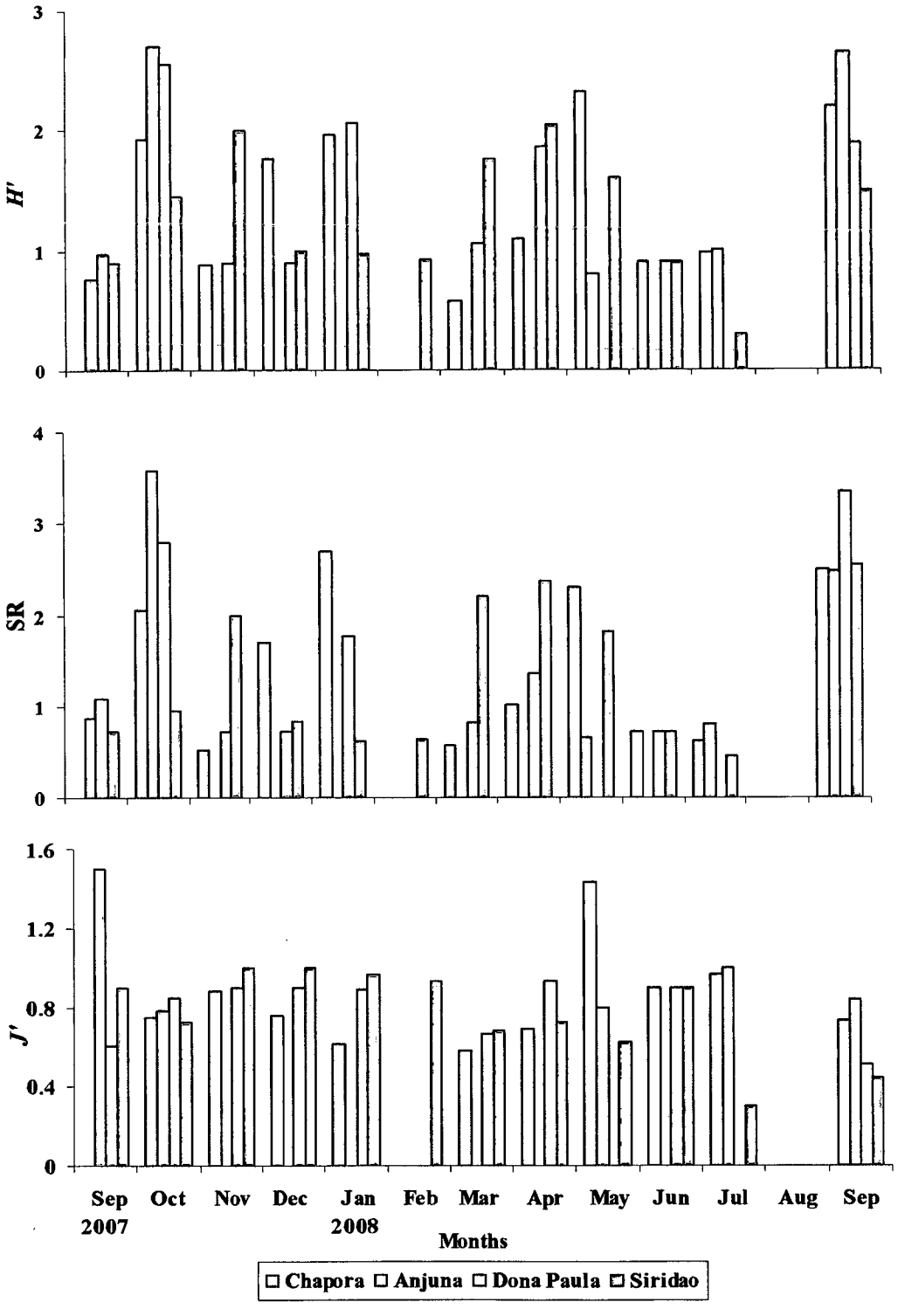



Fig. 6.3 Diversity indices (*H'*), species richness (SR) and evenness (*J'*) of dinoflagellates at different sampling locations off Goa, during September 2007-September 2008

***Statistical Analyses***

Spearman's rank correlation analysis (Table 6.2) indicated that nutrients, in particular nitrate and phosphate, affect the abundance of dinoflagellates at most locations. Generally, significant negative relationships ($R=-0.57$; $p< 0.043$) between dinoflagellate cell abundance and nitrate, as well as between their abundance and dissolved oxygen ($R=-0.56$; $p< 0.044$) were observed in the data from the location off Chapora (Table 6.2). A significant positive correlation was observed between cell abundance and concentrations of phosphate ($R=0.54$; $p<0.05$) and negative relationship with pH ($R=-0.54$; $p<0.05$) at the location off Siridao (Table 6.2). At the location of Dona Paula, it was negative between cell abundance and nitrate ($R= -0.796$; $p< 0.001$), nitrite ($R= -0.56$; $p< 0.047$) and phosphate ($R= 0.93$; $p<0.000$). However, none of the ecological parameters in this study showed a significant correlation with the dinoflagellate cell abundance at the location off Anjuna (Table 6.2).

The CCA employed to discern the relationship between environment variables and dinoflagellates composition highlighted that the first two axes explained 40% of the variability in dinoflagellate composition due to variations in environmental parameters. As indicated by the CCA bi-plot (Fig 6.4), many of the dinoflagellate species were located near the centre of the ordination indicating that these species were not affected much as consequence of changing environmental variable. However, temperature and salinity play important roles in the distribution of dinoflagellate species. Dinoflagellate species aligning into the left quadrants (e.g. *Dinophysis caudata, D. accuminata, Ceratium fuses* and *Protoperidinium conicum*)

Table 6.2. Spearman's rank correlation coefficients between different environmental variables and total dinoflagellate cell counts from different sampling locations off Goa. Statistically significant values are asterisked

| Parameters | Chapora | | Siridao | | Dona Paula | | Anjuna | |
|---|---|---|---|---|---|---|---|---|
| | R | p | R | p | R | p | R | p |
| Temperature | 0.227 | 0.455 | 0.064 | 0.835 | 0.101 | 0.741 | 0.308 | 0.304 |
| Salinity | 0.096 | 0.754 | -0.062 | 0.839 | -0.008 | 0.978 | 0.374 | 0.207 |
| pH | 0.283 | 0.348 | -0.544 | 0.05* | -0.146 | 0.632 | -0.132 | 0.667 |
| Dissolved oxygen | -0.564 | 0.044* | -0.153 | 0.617 | -0.534 | 0.059 | -0.093 | 0.760 |
| Nitrate | -0.566 | 0.043* | 0.489 | 0.089 | -0.796 | 0.001* | -0.495 | 0.085 |
| Nitrite | -0.305 | 0.310 | -0.251 | 0.406 | -0.558 | 0.047* | -0.159 | 0.602 |
| Phosphate | -0.434 | 0.137 | 0.543 | 0.05* | -0.932 | 0.000* | -0.156 | 0.609 |

seen to suggest a preference to lower salinity, lower temperature, and higher levels of dissolved oxygen. As discernible from the Fig. 6.5C, nitrite was the most important variable affecting the species composition during monsoon (June-September) and salinity during pre-monsoon (February–May; Fig. 6.5B). On the other hand, most species during the post-monsoon occupy lower left quadrant, in association with higher of salinities and lower nutrients concentrations (Fig. 6.5A).

***Distribution of other Phytoplankton Species***

Four species belonging to three genera were recorded during this study (*Dictyocha fibula, D. octonaria, Staurastrum* sp and *Dinobryon* sp; Fig 6.6). The most dominant species in the study area were the silicoflagellate species *D. fibula* followed by *D. octonaria.* In deed *D. fibula* occurred throughout the year at all the sampling locations. *Staurastrum* sp occurred only during monsoon months at most locations.

## Discussion

As already highlighted in chapter 3, seasonal variability in the study area is largely due to the intense rainfall during the monsoon months of June to September. This variability apparently leads to increased nutrient inputs through upwelling and terrigenous sources. Since the early days of phytoplankton ecology, nutrients have been known for controlling the phytoplankton community structure and biomass (Raymont, 1980; Tilman, 1982; Gouda and Panigrahy, 1996, Sawant et al., 2007).

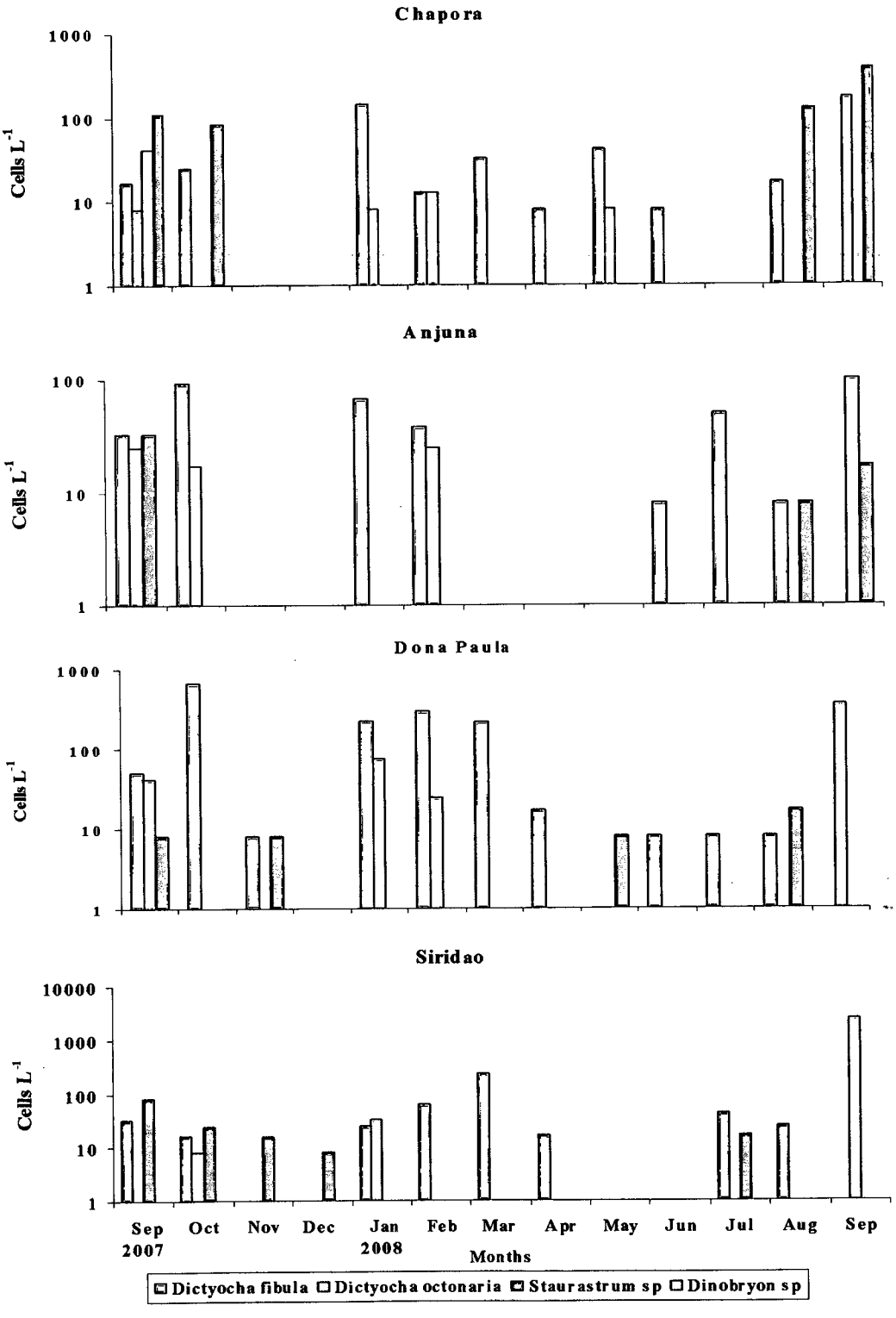



Fig. 6.6. Distribution patterns of other phytoplankton species at different sampling locations off Goa, during September 2007 - September 2008

As proposed by Bakun and Broad (2003), the alterations in physical and/or chemical factors disrupt biological settings in which some species can proliferate.

In terms of generic composition, the observations from this study suggest some similarities with those from a few other studies along the west coast of India (Tiwari and Nair, 1998; Alkershi, 2004; D'Costa et al., 2008). Mendon et al. (2002) recorded six genera of dinoflagellates off Mangalore, where *Ceratium furca* was generally more abundant as was the case during this study. Further, several investigators have noted the regular occurrence of *Ceratium* spp (Devassy and Bhattathiri, 1981; Lingadhal, et al., 1998). In the present study, the highest abundance of dinoflagellates at the all locations was during September 2008 followed by October 2007. High temperature and salinity are favorable for the growth of dinoflagellates (Qasim et al., 1972; Taylor, 1973; Joseph and Pillai, 1975). Yoo (1991) reported that changes in salinity, pH, nitrogen and phosphate cause variations in the abundance and composition of dinoflagellates. The low-euryhaline species (occurring exclusively when salinity was <15) was *Alexandrium minutum*, the highly-euryhaline (15-30 psu) species were: *Ceratium furca, Prorocentrum micans* and *Pyrophacus horologicum* and an unidentified stenohaline *Dinophysis* species occurred exclusively when salinity was >30. The letter species was mostly in the higher salinity periods in particular off Siridao and Dona Paula.

The most dominant species was *Ceratium furca.* Its highest abundance was recorded from 'estuarine' locations off Siridao, Dona Paula and Chapora that are enriched by land-based nutrient inputs. This observation corroborates with the proposition by Licea et al. (2004) who concluded that nutrients contribute to the

bloom formation in the Gulf of Mexico. Apparently, the allochthonous nutrient addition seems to contribute to the increased cell densities and types of dinoflagellates.

While cell densities of *C. furca* were more abundant when the nutrient concentrations were high, it was found rather throughout the year, albeit in low abundances and/or when concentrations of nutrients were lower. As Baek et al. (2008) suggest, this species seems to thrive in the low nutrient conditions through physiological acclimation of its specific characteristics for nutrient uptake.

That the nutrients bear great influence on dinoflagellates abundance in the study area is clear from the strong negative correlation between their abundance and nitrite, nitrate as well as phosphate. It is thus inferable that the wide variation in the abundance of dinoflagellates on an annual scale is largely due to variations in nutrient concentrations. It is possible that the temporal differences in their abundance explain the observed variance fitting into the first coordinate. The diversity indices from this study are useful to suggest the existence of a thriving assemblage of dinoflagellates in the locations subjected to salinity variations. Indeed, the $H'$ are generally higher from the estuarine regions when compared with the truly marine, higher salinity location off Anjuna.

Although cysts were not looked for in this study, it is clear from the extensive observations of D'Costa et al. (2008) that there exists a seed population of many of the dinoflagellate species in the shallow coastal sediments, including the known, potentially toxic forms. Similar to the earlier report of Ciminiello et al. (2000) such studies demonstrate the value of cyst sampling to confirm that some species can be

missed because they either occur at very low concentration or bloom for very short periods. It is believed however that our concentrating as much as 4 liters of seawater and examining three 1 ml replicates of 40X concentrates, helped count even the rarely occurring planktonic dinoflagellates in the water samples.

The genera *Prorocentrum* and *Protoperidinium* (plates 6.1 and 6.2), in particular, cells of *Prorocentrum micans* were observed at all the stations over wide ranges of water temperatures, salinities and nutrient concentrations. Although *P. micans* is capable of forming extensive blooms (Dodge, 1982; Steidinger and Tangen, 1997), it is usually considered harmless (Taylor, 1979; Anderson et al., 1985; Graneli et al., 1990). Such preponderance can be attributed to the fact that their active swimming-cell stages can adapt to the ecological variations.

The CCA multivariate analysis implied clear temporal variations in dinoflagellates, mainly governed by temperature, salinity, and then nutrients. The highest levels of nutrients and low salinity characterize the monsoon season. Thus, the species with low temperature optima are associated with these variables, being located on the left side plots, opposite to the temperature vector. Pre-monsoon period is characterized by higher levels of temperature, high salinity and low concentrations of nutrient (Fig 6.5B). Therefore, on the right side plots, associated with these variables, are the species aligning with high temperature optima. Kremp and Anderson (2000) reported that temperature and salinity affect the germination of dinoflagellates. Also Pospelova et al. (2004) found that the main factor affect the distribution of dinoflagellates in the southern New England was temperature. In some cases, a species is characterized by an individual behaviour in one system, while in another

aquatic system it behaves differently, thus leading to different optima and tolerances (Resende et al., 2005). These differences are probably a consequence of the existence, within several species, of two or more strains with different tolerances and environmental response.

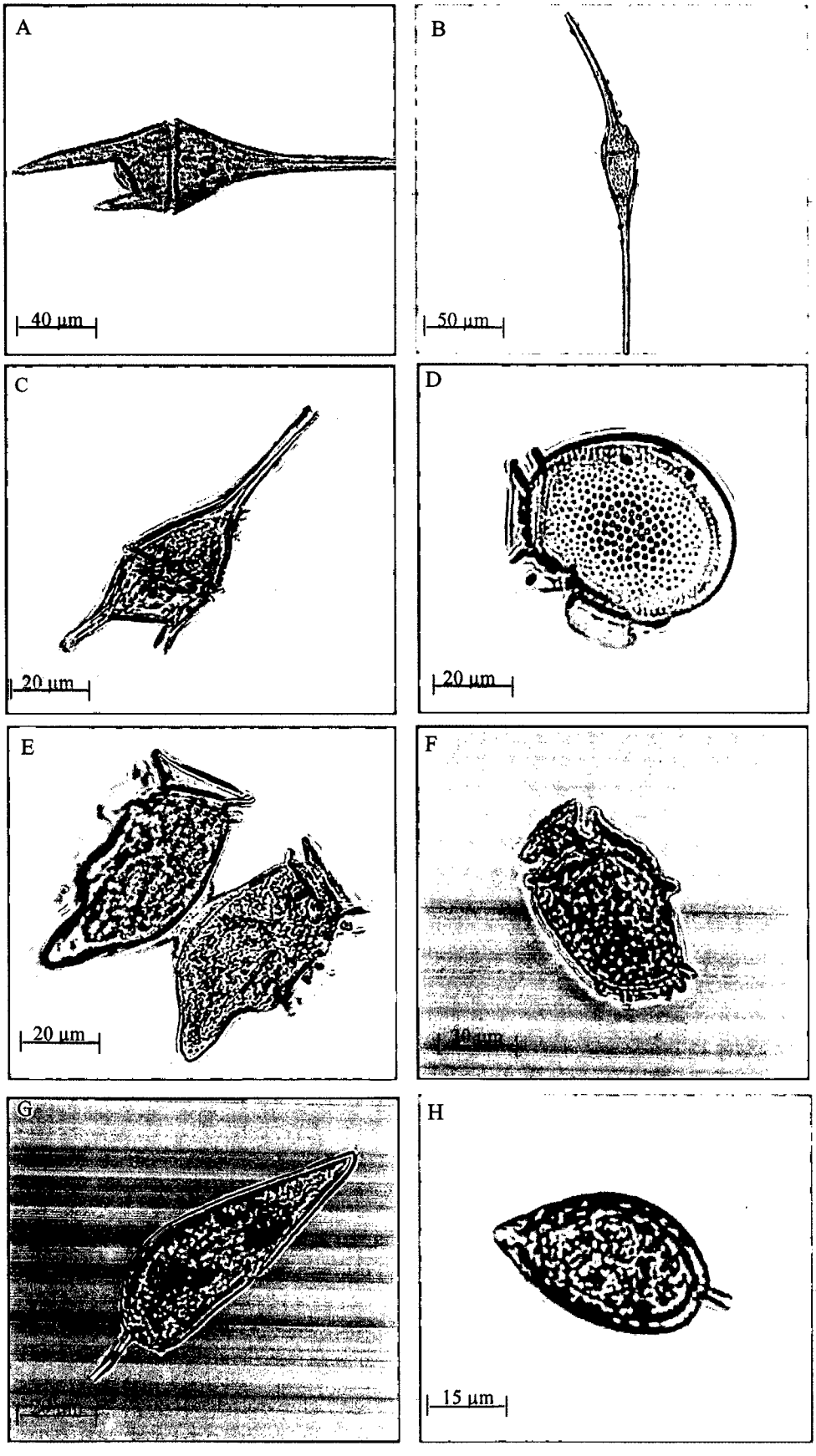
The ecophysiology and toxin production of certain dinoflagellate species have been investigated for decades. Unfortunately, these works have been mainly on the species occurring in temperate waters. The effect of environmental changes on toxin production in tropical dinoflagellates species is becoming increasingly evident (Hansen, 1989; Tomas and Baden, 1993; Godhe et al., 2008). Results from this study are useful to ascertain the effects of salinity on the dinoflagellates. More investigations on other environmental factors are essential to give a holistic interpretation on growth and toxin production of these tropical species. A better view of salinity–toxicity relationship can only be realized with further examination of natural population of these species.

Light microscope photographs (Plate 6.1) of some autotrophic dinoflagellates from different sampling locations off Goa

Legend:

A) *Ceratium furca*
B) *Ceratium fusus*
C) *Ceratium lineatum*
D) *Dinophysis acuminata*
E) *Dinophysis caudata*
F) *Dinophysis* sp
G) *Prorocentrum gracile*
H) *Prorocentrum micans*

# Plate 6.1

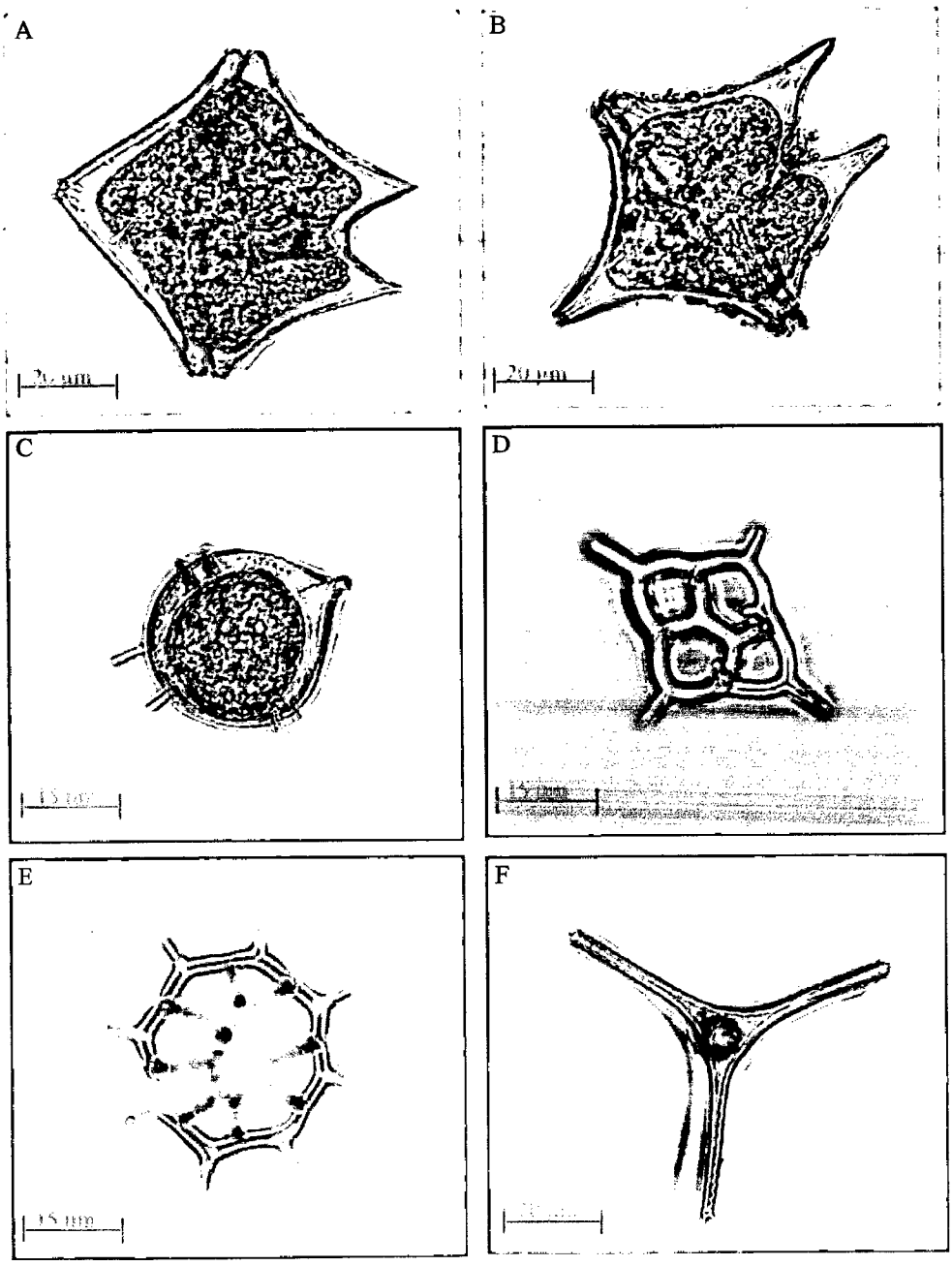


Light microscope photographs (Plate 6.2) of some heterotrophic dinoflagellates and other phytoplankton species from different sampling locations off Goa

Legend:

A) *Protoperidinium conicum*
B) *Protoperidinium oceanicum*
C) *Protoperidinium steinii*
D) *Dictyocha fibula*
E) *Dictyocha octonaria*
F) *Staurastrum* sp

# Plate 6.2



contaminated fish (Bates et al., 1998). To date, observations on *Pseudo-nitzschia pungens* have received little attention in the Indian waters, despite of it being one of the most commonly reported, potentially toxic representatives of the genus worldwide (Hasle, 2002). Clones of *P. pungens* isolated from various geographic areas exhibited different abilities to produce DA. Its toxic clones have been reported from New Zealand (Rhodes et al., 1996), Washington State and Monterey Bay, California (Trainer et al., 1998).

Although there is no report on ASP, many studies have reported the occurrence of harmful algal blooms in the Indian waters (Naqvi et al., 1998; Sahayak et al., 2005; Alkawri and Ramaiah, 2010). Since 1981, cases of paralytic shellfish poisoning (PSP) from coastal Tamil Nadu, Karnataka and Maharashtra are reported with adverse effects (Devassy and Bhat, 1991). In 1981, a PSP outbreak resulted in the hospitalization of 85 people and death of three persons due to the consumption of bloom-affected mussel, *Meretrix casta* in Tamil Nadu. A similar incidence reported from Mangalore in 1983, but in both the causative species were not identified (Bhat and Prabhu Matondkar, 2004). Godhe et al. (1996) reported *Gymnodinium catenatum*, a potent PSP species in both planktonic cells and cysts in the sediment, in the coastal waters of Karnataka (off Mangalore). They also noted that the low number of cells would not bring about toxic effects. In September 1997, an outbreak of PSP was reported in three villages of Kerala, resulting in the death of seven persons and hospitalization of over 500, following consumption of mussel, *Perna indica* (Karunasagar et al., 1997).

Reports on the occurrence of toxic phytoplankton from this region are rather scanty (Devassy and Goes, 1988; D'Costa et al., 2008). In order to highlight the prevalence of quite a number of toxic species, we report here the occurrence, abundance and annual variability of diatoms and dinoflagellates known form other regions of the world oceans as potentially toxic to human health.

## Material and Methods

Detailed description of phytoplankton cell counts and identification are provided in Chapters 3 and 5. Generic and species identification was done according to the various keys mentioned therein.

Also the details of statistical analyses (Spearman's rank correlation test and Canonical correspondence analysis) provided in Chapters 5 and 6.

## Results

### *Species Assemblages and Cell Abundance*

A total of 12 known toxic phytoplankton species belonging to 6 genera were recorded during this study (Table 7.1). The most dominant among these was the diatom, *Pseudo-nitzschia pungens*. Its percentage contribution to total diatom counts during each month ranged from none to 4.3 % off Anjuna; none to 8.4% off Chapora; none to 1.7 % off Dona Paula and, from none to 1.2 % off Siridao (Fig. 7.1). It was in high abundance off Chapora almost throughout the year. Its bloom proportions, $1.6 \times 10^4$ cells $L^{-1}$, occurred at this location during May 2008. At other locations, it was either in non-detectable limits or in quite low densities though its

Table 7. 1. Occurrence of harmful algae in the coastal waters off Goa, most probable period of their high abundance and, as per literature, their possible harmful effects. The taxa codes of the species used in the canonical correspondence analysis are indicated

| Taxa code | Toxic phytoplankton | Seasonality | Toxins reported | Impact |
|---|---|---|---|---|
| AMCOF | *Amphora coffeaeformis* | Dec-Jun, Aug | DA | ASP |
| PSPUN | *Pseudo-nitzschia pungens* | Dec-May, Jul- Sep. | DA | ASP |
| ALMIN | *Alexandrium minutum* | Oct, Dec, Jan, Sep. | GTXs, NSTX | PSP |
| CEFUS | *Ceratium fusus* | Oct, May, Sep. | ?? | Toxic to marine invertebrate larvae |
| DIACU | *Dinophysis acuminata* | Oct, Jan, Aug-Sep. | OA, DTX-1, PTX-2 | DSP |
| DIBRE | *Dinophysis brevisulcus* | Mar | ?? | DSP |
| DICAU | *Dinophysis caudata* | Oct, May, Sep. | OA, PTX-2 | DSP |
| DIFOR | *Dinophysis fortii* | Sept | OA, DTX-1, PTX-2 | DSP |
| DIHAS | *Dinophysis hastata* | Sep | ?? | DSP |
| DIMIL | *Dinophysis miles* | Jun | OA, DTX-1 | DSP |
| DISP | *Dinophysis sp* | Mar - Jun | ?? | ?? |
| PRMIC | *Prorocentrum micans* | Oct, Dec - Jan, May-Mar, Sep. | ?? | Toxic to shellfish |

Abbreviations are: DTX-1: dinophysistoxin-1; GTX: gonyautoxin; NSTX: neosaxitoxin; ASP: amnesic shellfish poisoning; PSP: paralytic shellfish poisoning; DSP: diarrhetic shellfish poisoning; OA: okadaic acid and PTX-2: pectenotoxin-2

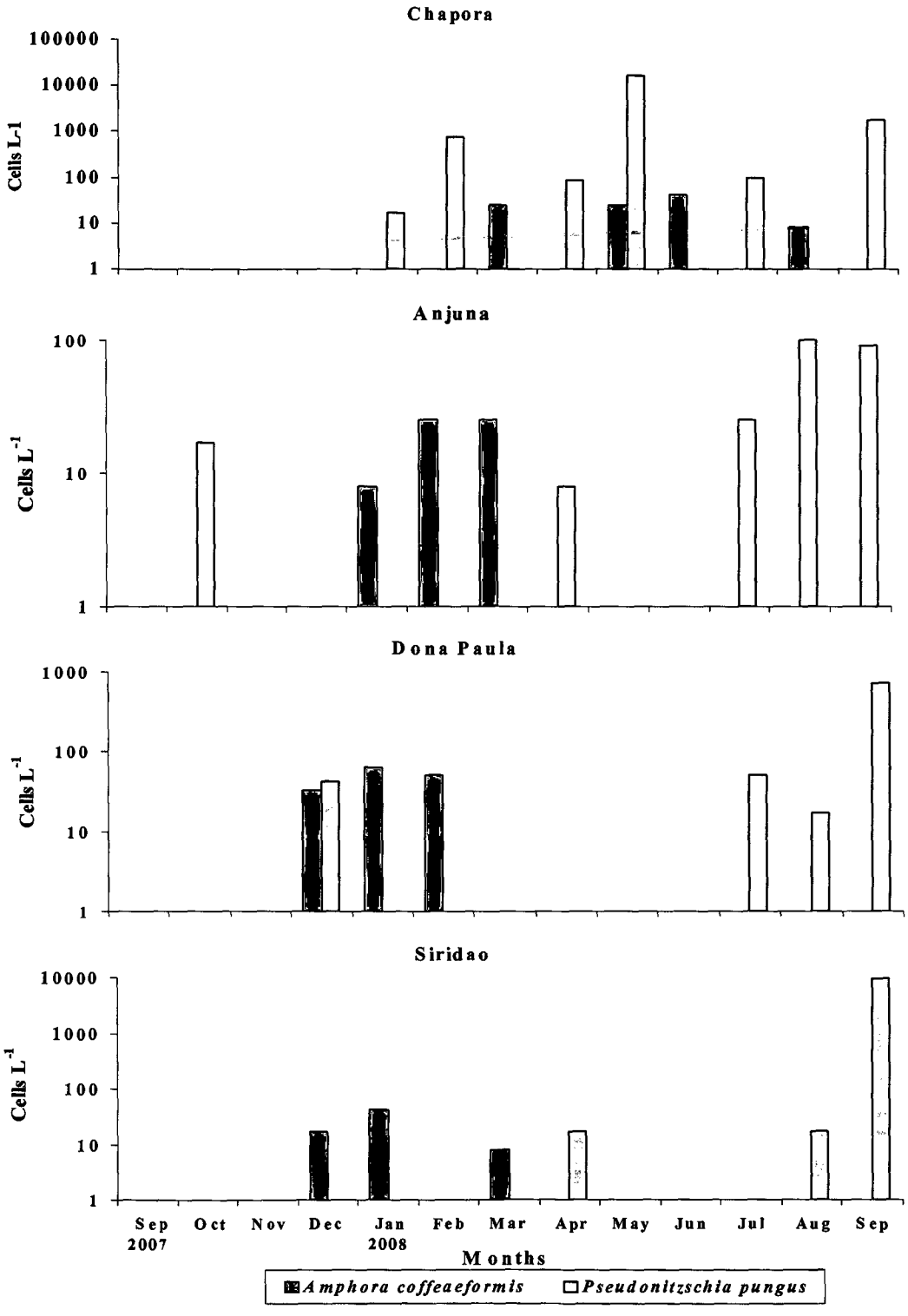



Fig.7.1. Abundance and pattern of monthly variations of toxic diatom species off Goa during September 2007 to September 2008

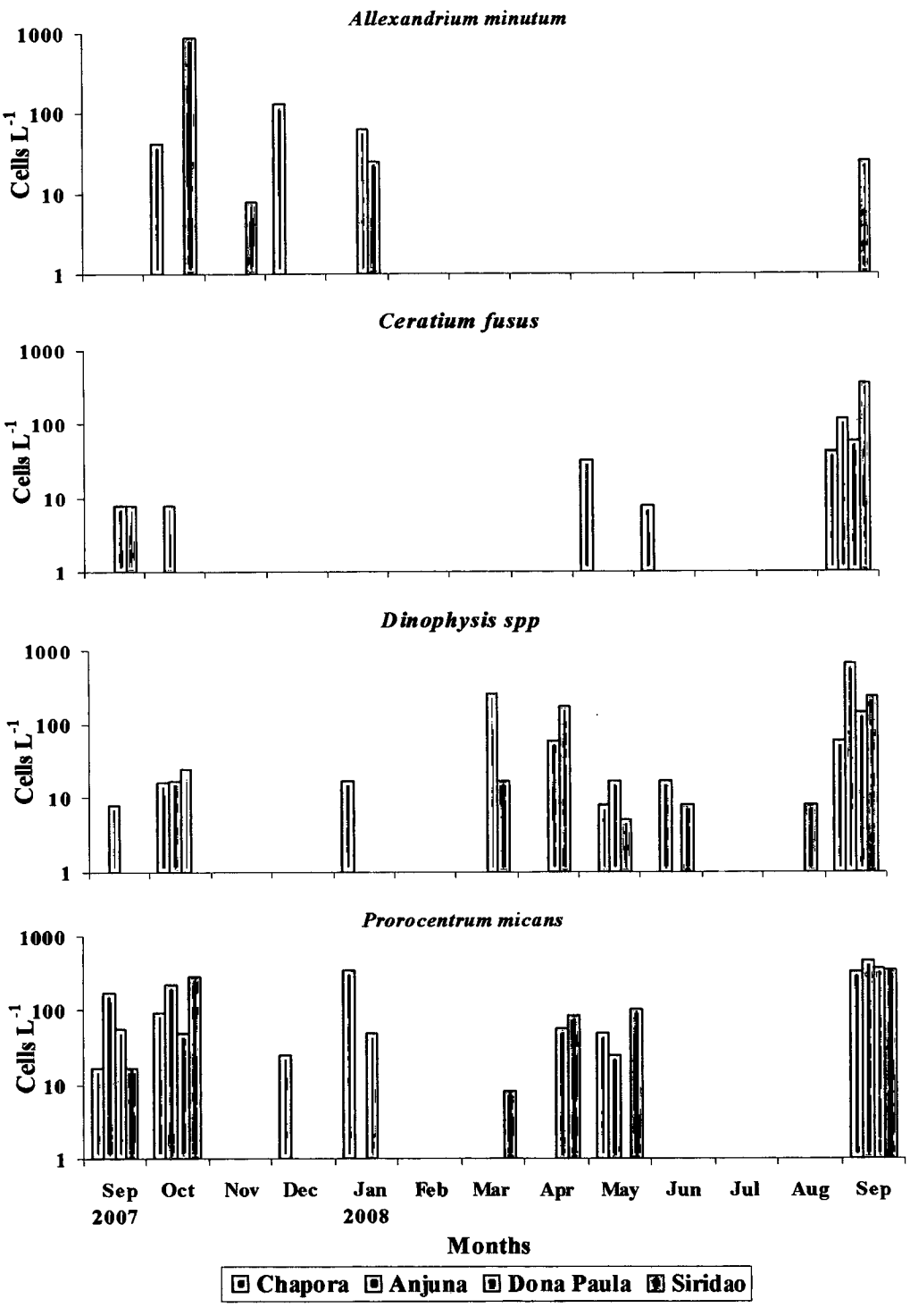



Fig 7.2. Abundance and patterns of monthly variations of toxic dinoflagellate species off Goa during September 2007 to September 2008

***Statistical Analysis***

Between different, harmful algae and dissolved inorganic nutrients, the following relationships were statistically significant (Table 7.2). Off Chapora, *Pseudo-nitzschia pungens* showed a significant negative relationship with nitrite ($NO_2$: R= - 0.6, $p \leq 0.03$), nitrate ($PO_4$: R=-0.58, $p \leq 0.038$) and Phosphate ($SiO_4$: R= - 0.54, $p \leq 0.05$). *Amphora coffeaeformis* showed negative correlation with nitrate (R= - 0.6, $p \leq 0.03$), phosphate (R= - 0.7, $p \leq 0.008$) and silicate (R= - 0.6, $p \leq 0.03$) at the location off Anjuna. Also, *P. pungens* was positively correlated with phosphate (R= 0.63, $p \leq 0.02$). However, *Prorocentrum micans* showed a negative correlation with nitrite (R= - 0.54, $p \leq 0.05$) and phosphate (R= -0.62, $p \leq 0.023$), *Dinophysis acuminata* only correlated negatively with nitrate at the location off Dona Paula. *Dinophysis sp* showed a negative correlation with all the nutrient concentrations and positive correlation with water temperature and salinity off Siridao (Table 7.2).

***Relationship between Environmental Parameters and Harmful Algae***

Ordination resulting from the canonical corresponding analysis (CCA) produced the biplots presented in Fig. 7.3. Forward selection of environmental parameters retained seven variables (salinity, temperature, nitrate, nitrite, phosphate, silicate and dissolved oxygen) that significantly explained the species distribution. The first two axes explained 47.4 % of the relationship between environmental variables and harmful algae. Most of the species, particularly *Dinophysis* spp, associated with low temperature and moderate salinity and nutrient concentrations. *Alexandrium minutum* from the most important species observed in the study area was associated

Table 7.2. Spearman's rank correlation coefficients between different environmental variables and most harmful algae from different sampling locations off Goa. Statistically significant values are asterisked

| Parameters | Temp | | Sal | | $NO_2$ | | $NO_3$ | | $PO_4$ | | $SiO_4$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | p | R | p | R | p | R | p | R | p | R | p |
| **Chapora** | | | | | | | | | | | | |
| *A. coffeaeformis* | 0.45 | 0.12 | 0.51 | 0.07 | 0.09 | 0.75 | 0.07 | 0.82 | 0.12 | 0.69 | -0.38 | 0.19 |
| *P. pungens* | 0.03 | 0.93 | 0.27 | 0.37 | -0.6 | 0.03* | -0.58 | 0.04* | -0.54 | 0.05* | -0.40 | 0.19 |
| *A. minutum* | -0.19 | 0.53 | -0.21 | 0.49 | 0.39 | 0.18 | 0.08 | 0.79 | 0.44 | 0.14 | | |
| *C. fusus* | 0.23 | 0.46 | 0.38 | 0.21 | 0.01 | 0.97 | -0.38 | 0.20 | -0.31 | 0.31 | | |
| *D. acuminata* | -0.32 | 0.29 | -0.18 | 0.56 | -0.02 | 0.94 | -0.31 | 0.31 | -0.18 | 0.56 | | |
| *D. caudata* | -0.34 | 0.25 | -0.33 | 0.27 | 0.27 | 0.37 | 0.02 | 0.95 | 0.01 | 0.97 | | |
| *P. micans* | -0.17 | 0.58 | -0.09 | 0.76 | -0.13 | 0.67 | -0.43 | 0.14 | -0.26 | 0.39 | | |
| **Anjuna** | | | | | | | | | | | | |
| *A. coffeaeformis* | -0.16 | 0.61 | 0.22 | 0.47 | 0.05 | 0.86 | -0.6 | 0.03* | -0.70 | 0.008* | -0.60 | 0.03* |
| *P. pungens* | -0.48 | 0.09 | -0.35 | 0.23 | 0.51 | 0.07 | 0.3 | 0.32 | 0.63 | 0.020* | 0.48 | 0.09 |
| *C. fusus* | -0.38 | 0.20 | -0.03 | 0.91 | 0.09 | 0.77 | 0.16 | 0.61 | 0.29 | 0.320 | | |
| *D. acuminata* | -0.31 | 0.30 | 0.15 | 0.61 | -0.15 | 0.61 | 0.00 | 1.00 | 0.31 | 0.300 | | |
| *D. caudata* | -0.13 | 0.67 | -0.07 | 0.82 | -0.07 | 0.82 | 0.25 | 0.40 | 0.34 | 0.260 | | |
| *P. micans* | -0.15 | 0.62 | -0.11 | 0.72 | -0.06 | 0.84 | 0.28 | 0.35 | 0.35 | 0.240 | | |
| **Dona Paula** | | | | | | | | | | | | |
| *A. coffeaeformis* | -0.44 | 0.14 | 0.23 | 0.44 | 0.49 | 0.08 | 0.06 | 0.85 | 0.14 | 0.65 | -0.07 | 0.82 |
| *P. pungens* | -0.39 | 0.18 | -0.37 | 0.21 | 0.07 | 0.83 | 0.24 | 0.43 | 0.22 | 0.47 | 0.27 | 0.37 |
| *A. minutum* | -0.12 | 0.70 | 0.00 | 1.00 | 0.08 | 0.80 | -0.15 | 0.61 | -0.08 | 0.80 | | |
| *C. fusus* | -0.40 | 0.17 | -0.37 | 0.20 | -0.46 | 0.11 | -0.04 | 0.89 | -0.35 | 0.24 | | |
| *D. acuminata* | -0.19 | 0.54 | -0.22 | 0.47 | -0.51 | 0.07 | -0.62 | 0.02* | -0.50 | 0.08 | | |
| *Dinophysis sp* | 0.51 | 0.07 | 0.50 | 0.08 | -0.04 | 0.90 | -0.41 | 0.16 | -0.44 | 0.13 | | |
| *P. micans* | -0.17 | 0.56 | -0.28 | 0.35 | -0.54 | 0.05* | -0.43 | 0.14 | -0.62 | 0.02* | | |
| **Siridao** | | | | | | | | | | | | |
| *A. coffeaeformis* | -0.03 | 0.91 | 0.19 | 0.520 | 0.43 | 0.140 | 0.01 | 0.970 | -0.20 | 0.490 | -0.12 | 0.71 |
| *P. pungens* | -0.29 | 0.34 | -0.08 | 0.800 | -0.26 | 0.390 | -0.07 | 0.830 | 0.06 | 0.860 | -0.17 | 0.58 |
| *A. minutum* | -0.03 | 0.91 | -0.18 | 0.540 | 0.45 | 0.120 | 0.32 | 0.290 | 0.27 | 0.370 | | |
| *C. fusus* | -0.24 | 0.42 | -0.25 | 0.400 | -0.39 | 0.180 | 0.19 | 0.530 | -0.20 | 0.510 | | |
| *D. acuminata* | -0.48 | 0.09 | -0.20 | 0.510 | -0.18 | 0.550 | 0.08 | 0.770 | 0.37 | 0.200 | | |
| *Dinophysis sp* | 0.68 | 0.01 | 0.72 | 0.006* | -0.58 | 0.036* | -0.78 | 0.002* | -0.69 | 0.009* | | |
| *P. micans* | 0.23 | 0.45 | 0.15 | 0.610 | -0.44 | 0.130 | -0.31 | 0.300 | -0.39 | 0.190 | | |

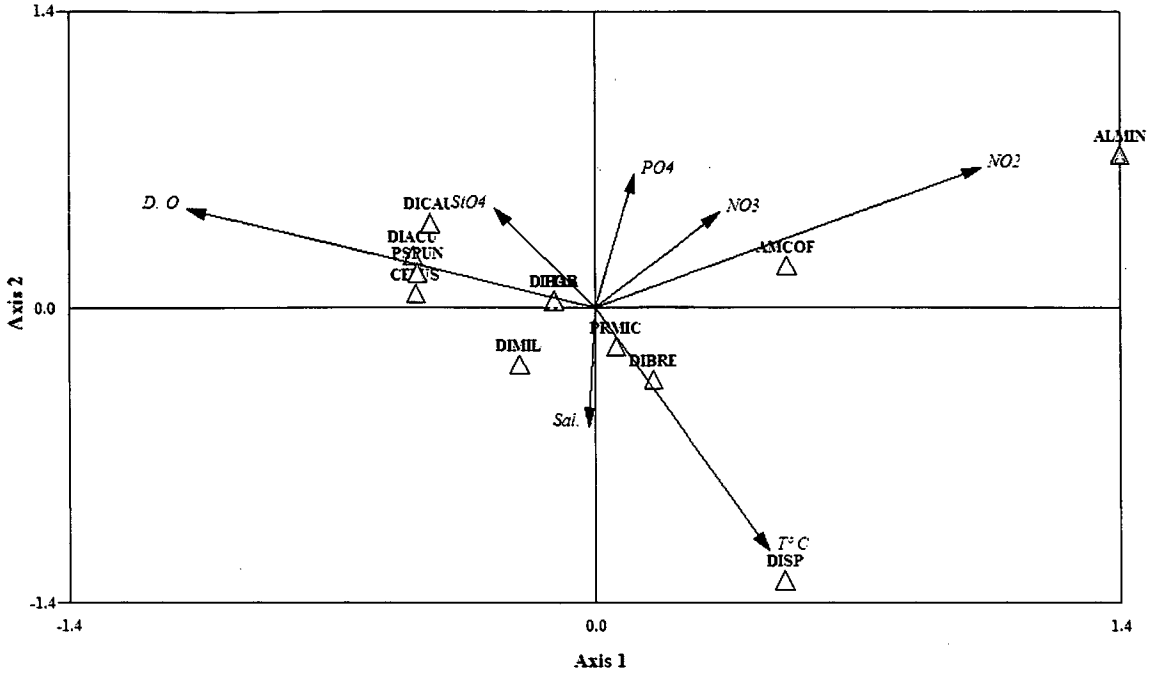



**Fig. 7.3.** Canonical correspondence analysis (CCA) based ordination of harmful algae against selected environmental variables observed during September 2007-September 2008 from four different locations off Goa. Codes of harmful algae used in this analysis are listed in Table 7.1

with low salinity and high nutrient concentrations. However *Dinophysis* sp, was observed only during pre-monsoon months, associated with high level of temperature. Although the diatom species pseudo-nitzschia pungens showed preference for low nutrient concentrations, *Amphora coffeaeformis* associated with high pulse of nutrients.

## Discussion

From this comprehensive analysis, it is evident that the toxic phytoplankton species are rather common in the Indian waters. The fact that as many as 12 species known to be potentially toxic were found in the nearshore waters is an indication that any or all of them can be of serious concern when the environmental factors become favorable for their proliferation (Naqvi et al., 1998; Ramaiah et al., 2007; Alkawri and Ramaiah, 2010). Sampling of different salinity regimes off Goa was helpful in bringing-forth not only the spatio-temporal differences in the occurrence, abundance and species variations among toxic phytoplankton. From this study it was also clear that the *Pseudo-nitzschia pungens* is observed and reported for the first time from the nearshore waters of Goa. Additionally, it was perennially preponderant in the low-saline waters off Chapora. Interestingly, its higher abundance and even the bloom densities, in particular off Chapora, coincided with low nitrate, phosphate and silicate concentrations in the ambient waters; this occurrence is implying that low inorganic nutrients might be favorable for its growth and proliferation. Apparently, *P. pungens* is a cosmopolitan species (Hasle, 2002), widely distributed along Atlantic coasts and Pacific rims. Further, several

strains isolated from New Zealand, Washington and Monterey Bay were observed to produce the DA (Bates et al, 1998; Rhodes et al., 1996; Trainer et al., 1998), which causes amnesic shellfish poisoning (ASP). It is a rare and naturally occurring amino acid, which is toxic to marine mammals, seabirds and human beings alike (Bhat, 2008). It was not attempted in this study to examine whether this species is capable of DA production. Of the 14 known toxic diatom species, as many as twelve *Pseudo-nitzschia* spp are capable of producing DA. This is because, earlier studies reported significant negative correlations between both DA and abundances of *Pseudo-nitzschia* and between its counts and the ambient concentrations of silicate, Nitrate and nitrite or phosphate (Anderson et al., 2006; Schnetzer et al., 2007). This is to be taken as a forewarning of a very high probability of *Pseudo-nitzschia* spp (and their strains) with DA production capability of occurring in the waters of Goa. This could be important in particular when nutrient concentrations low but their abundances are near and/or at bloom proportions and, when their cell numbers correlate negatively with ambient nutrient concentrations (for instance, for data from off Chapora, R=-0.58 and p=0.038 between counts of *Pseudo-nitzschia pungens* (*P-n p*) and nitrate; R=-0.54 and p= 0.05 between *P-n p* and phosphate and, R= -0.4 and p=0.19 between *P-n p* and silicate. In fact, it appears that concentrations of DA increase during this diatom's senility and/or following its death.

A culture of the other pennate diatom, *Amphora coffeaeformis,* was also shown to produce DA (Maranda et al. 1990), though this has not been confirmed in other isolates (Bates et al. 1989).

Among the dinoflagellates recorded during the present study, *Alexandrium minutum*, known to be potentially toxic (Moestrup et al., 2004), is being observed/reported for the first time in the near shorewaters of Goa. Known for paralytic shellfish poisoning (PSP) production, *A. minutum* was most prevalent off Siridao. It was recorded in the planktonic form off Chapora and Dona Paula as well. In addition to PSP-toxins, Cembella et al. (2000) and Maclean et al. (2003) suggest that some *Alexandrium* spp can also produce the spirolides, another class of toxins whose effects on humans have not been clarified yet.

The diarrhetic shellfish poisoning (DSP) caused mainly due to *Dinophysis* spp has been reported to be the main toxin-related problem in several countries adjoining the Mediterranean Sea, from where mussel contamination due to okadaic acid and dinophysistoxins has been reported (Delgado et al., 1996; Marasovic´ et al., 1998; Koukaras and Nikolaidis, 2004). Further, bioassays with mussels collected from regions where *Dinophysis* are numerous resulted in the death of the mice (Siano et al., 2002). All seven species of *Dinophysis* recorded during this study are reported to be potential causative agents of DSP. The most abundant species in the waters off Goa is *D. acuminata* (838 cells $L^{-1}$) followed by *Dinophysis* sp (508 cells $L^{-1}$). While recognition of the actual strain(s) producing the toxins is essential, the cell counts of *Dinophysis* spp recorded during this study ought to be considered to denote the presence of potential DSP producing strains.

*Ceratium fusus*, found in this study at all the locations, can tolerate a great range of salinities (5-70 psu; Taylor et al., 2004). This species can cause harm to invertebrate larvae by an unknown mechanism (Taylor et al., 2004). Rensel and Prentice (1980)

and Cardwell et al. (1979) have reported on the adverse effects of *C. fusus* on oyster larvae and shrimps.

The cells of *Prorocentrum micans* were also observed at all the locations over wide ranges of salinity, temperature and nutrient concentrations. This could be attributed to the fact that their active swimming-cell stages can adapt to the ecological variations as suggested by Dodge (1982). Although *P. micans* is capable of forming extensive blooms, it is usually considered harmless (Graneli et al., 1990). However, there are reports of *P. micans* having caused problems of shellfish kills in Portugal (Pinto and Silva, 1956) and South Africa (Horstman, 1981). It is suspected to excrete metabolites inhibitory to diatoms growth, but they are thought to not to enter the food chain or, affect higher trophic level organisms (Uchida, 1977).

All the potential PSP-toxin producers were rather low in numbers in the study area. Besides, there are reports that a cell abundance of ~400 cells $L^{-1}$ is sufficient to cause DSP (Dr Luis Proenca, personal communication). Results from the present study suggest that salinity effects are very strong on the cell densities of both general and potentially toxic species of dinoflagellates. Although PSP contamination of shellfish has never been reported along the Goa coast so far, attainment of cell densities >1000 cells $L^{-1}$ of some known toxic dinoflagellates at least during some period in an annual cycle is to be taken as an indication of the possibility of PSP in this tourism driven economy.

Outbreaks of shellfish poisoning cases have caused serious economic losses in the fishery industries of the affected countries. Public health concerns arise with increasing poisoning cases due to the consumption of contaminated bivalves. This

has been particularly pronounced in countries where seafood is the main source of protein. The perception of dinoflagellate caused-poisoning would change when there are accurate reporting of PSP and DSP as well as there are documentations of toxic species in the near shore waters.

To the best of my knowledge, despite recording a variety of potentially toxic species and their wide seasonal ranges (Table 7.1), no human health problems have been ever recorded in Goa, nor were fish kills observed during algal blooms. This apparent paradox has several possible explanations that could act in isolation or synergistically. For a harmful bloom to develop at a given site, three conditions must coincide with the harmful species must be present at that site. Also such species must reach a threshold concentration, which varies from species to species and within the same species in relation to its toxicity and, the bloom must hit the target organism (s) either directly or through vectors (Zingone and Wyatt, 2005). From our analyses, the first condition, the presence of toxic species, is fulfilled in several places and times of the year in coastal waters off Goa. Therefore it is reasonable to caution that the toxic species do prevail in these waters and their contact with target organisms fortunately, so far, may be missing or hitherto unreported.

# Chapter 8

## Effect of Light Intensity and Nutrients on Biochemical Components

### Introduction

Phytoplankton are the primary food source for at least some stages in the life histories of most animals both in open-ocean and in animal rearing or mariculture systems. Ecological variations, and nutrient regimes are known to affect the growth, chlorophyll, cell constituents as well as primary productivity potential of these autotrophic life-forms (Brown et al., 1996; Stryer, 1988). Most microalgae that have been tested as food for reared animals have differed significantly in their nutritional value, frequently because of differences in their biochemical composition (Brown et al., 1996; De la Peña, 2007; Diekmann, et al., 2009). It is therefore relevant to assess the biochemical variability in the phytoplankton species. Such studies would also lead to evaluating the autotrophic microflora as food for reared marine animal species. Contextually, in spite of the significant investments to date, the consistent supply of highly nutritious, live microalgae remains a major 'bottleneck' and can affect the productivity and profitability of many commercial hatchery and nursery operations. Furthermore, the cultivation of microalgae is likely to remain a vital process within most aquaculture facilities as microalgae are the biological starting point for energy flow through aquatic ecosystems (De Pauw et al., 1984), and live microalgae are an essential food source for molluscs, crustaceans and fish species, for at least during the early part of their life cycle (Pahl et al., 2010).

The nutritional value of the microalgal biomass depends on several physiological and biochemical attributes including size and shape, digestibility, non-toxicity and biochemical composition. Furthermore, the nutritional value of the microalgae should match the nutritional requirements of the target organisms (Webb and Chu, 1983; Brown et al., 1989). In general, diatoms appear to be a good food for many species, which may be because of high concentrations of proteins, lipids and carbohydrates (Lynn, et al., 2000). Their lipids are rich in fractions of - C = O – bonds, providing much more energy during their oxidation processes than other biological compounds. Mainly owing to their high reduction levels, they constitute a convenient storage material for living organisms. In algae they are widely distributed, especially in several resistance stages (Miller, 1962). Proteins have crucial functions in all the biological processes. Their activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation, growth and cellular differentiation control (Stryer, 1988). Some carbohydrates serve as food sources and storages, while other ones are cell wall constituents (Percival, 1979).

Considering the importance of these biochemical constituents in cellular physiology, one of the objectives for this study was to evaluate the effect of altered nutrient concentrations and light regimes on the growth and chlorophyll among different species of diatoms. Experiments were set up such that the combined effects of varying nutrient concentrations and light regimes could be measured. This set up was therefore envisaged to evaluate a hypothesis that environmental variability not only affects the growth but also the cellular constituents. The changes in biochemical composition in five tested diatom species (*Asterionella*

*glacialis, Skeletonema costatum, Thalassionema nitzschioides, Melosira nummuloides and Navicula* sp) under varying nutrient treatments may help to provide some evidence to explain the temporal characteristic of the blooms caused by them. These species are widely distributed throughout the west coast of India and as has been seen already some of them attained bloom proportions in the study area.

## Material and Methods

### *Source and Culture Conditions of Diatoms*

Five species of diatom belonging to five different genera (*Asterionella glacialis, Skeletonema costatum, Thalassionema nitzschioides, Melosira nummuloides and Navicula* sp) were used in this study. The cultures were originally isolated from Dona Paula Bay. Cultures of these species were maintained in the exponential growth phase by weekly re-inoculation into fresh culture media prepared in autoclaved seawater (34 psu) enriched with f/2 media (Guillard, 1975). Cells were grown in Erlenmeyer flasks (100 ml) containing 50 ml culture medium and were maintained at 28 ± 1 °C under a light regime of a 12h light: 12h dark photocycle.

### *Effects of Light Intensity and Culture Medium*

To attain axenic status, the cultures were further treated with a mixture of antibiotics (1 mg $ml^{-1}$ of penicillin G, 0.5 mg $ml^{-1}$ of streptomycin, and 0.2 mg $ml^{-1}$ of chloramphenicol) by following Patil and Anil (2005). This axenic status was maintained during the experimental period. The addition of antibiotics does not

affect photosynthesis or C fixation by the algae (Goto et al., 1999, Wolfstein et al., 2002). The cultures were then transferred to antibiotic-free medium in autoclaved Erlenmeyer flasks (100 ml) and were maintained for one week under the same conditions as described above.

Two separate experiments for each species were performed to test the effect of light and nutrients on the growth and gross biochemical constituents of these species. Mass cultures of each species were raised in 1 L flasks by inoculating cultures volume 5–10% of the final volume of 500 ml.

The flasks for mass cultures were held under two different light regimens. One set was incubated under the light intensity of 4500 lux (High intensity light: HL) and the other set under 1500 lux (Low intensity light: LL). Under each light level, three nutrients regimes namely, normal strength (N-f/2), half strength (H-f/2), and double strength (D-f/2) were set up for all five species. Replicates of two flasks for each species were maintained for each nutrient regime.

When diatom cultures attained the exponential growth phase, about 6 days after inoculation (Correa-Reyes et al., 2001), samples from each treatment were filtered onto 47mm glass fiber filters (Whatman GF/F) for estimating (protein, carbohydrates and lipids). The filters with algal cells were stored at - 20 °C until taken up for biochemical analyses.

***Cell Counts and Growth Rate Estimation***

In order to follow the attainment of maximum cell abundance, and specific growth rate, cells were enumerated from subsamples drawn once every two days.

Triplicates of 1 ml samples each were transferred on to Sedgwick-Rafter plankton counting chamber, and examined microscopically at 200X and 400X magnification. Growth rates were determined by regression of *ln* cell number vs. time during exponential phase using the formula:

$\mu = ln\ (N_1/N_0)/\ t_1 - t_0$,

where $N_1$ is the cell number at time $t_1$ , $N_0$ is the cell number at time 0 ($t_0$), and $\mu$ is the specific growth rate.

***Chlorophyll a***

Concentration of chlorophyll *a* was determined once every two days for all the species, by drawing subsamples from the mass culture flasks and by filtering two replicates of 10 ml culture volume onto 25mm glass fiber filters (Whatman GF/F) and extracting the pigment in 90% acetone solution under cold and dark conditions overnight. Fluorescence was then determined in the extract with a Turner Designs, 10-AU-005-CE fluorometer, by following the JGOFS protocol (UNESCO 1994). The concentrations of chlorophyll *a* per cell were also calculated.

***Estimation of Total Protein***

For the protein analyses, diatom samples were collected onto a glass fiber filters (Whatman GF/F) and immediately frozen at – 20 °C. A minimum of 500000 cells were sampled to ensure sufficient biomass for analyses (Diekmann, et al., 2009). A Sigma-Aldrich protein assay kit (no. BCA-1 and B 9643) was used to measure protein content according to Smith et al. (1985). In this method, proteins reduce

alkaline Cu (II) to Cu (I) in a concentration-dependent manner (Lowry et al., 1951). Bicinchoninic acid (BCA) is a highly specific chromogenic reagent for Cu (I), forming a purple complex with an absorbance maximum at 562 nm (Smith et al., 1985).

In brief, four ml 0.1 M NaOH was added to the filter in test tube and kept in boiling water bath for 30 minutes for extraction of protein contents. Two ml BCA were added to the extracts. Absorbance of the solution was measured at 562 nm using a spectrophotometer (Systronics 2202, USA). Bovine serum albumin was used as the protein standard.

***Estimation of Total Carbohydrate***

Samples for carbohydrate analyses were taken as described above. The determinations were made based on the method of Dubois et al. (1956) and Myklestad and Haug (1972). In essence, 5 ml of 80 % sulphuric acid was added to the sample and was allowed to digest for about 20 - 21 hours at room temperature. Two ml of 5 % phenol reagent followed by 5 ml of concentrated sulphuric acid were added to the digested sample. The contents were allowed to cool. Glucose was used as a primary standard and the absorbance in the samples were measured photometrically at 490 nm.

***Estimation of Total Lipid***

Diatom cultures were collected onto a glass fiber filter (GF/F) as described above. The glass fiber filter was cut into small pieces and transferred together with glass

beads into a 25ml homogenizer glass. A mixture of a chloroform: methanol: water (2: 1: 0.8, v/v) were added for extraction according to Bligh and Dyer (1959). The suspension was homogenized under cooling conditions in a cell homogenizer. Separation of phases was subsequently induced by the addition of chloroform and water. The organic phase was collected and the water-containing material was re-extracted until no residual dye could be visualized. Subsequently the organic extracts were evaporated and analyzed by the lipid oxidation technique (Parsons et al., 1984) using stearic acid as standard. Following these techniques, two ml of 0.15 % acid dichromate solution was added to the organic extracts in the test tubes and kept in a boiling water bath for 15 minutes and then the solution were allowed to cool in running tap water. After that 4.5 ml distilled water was added and the absorbance of the solution was measured photometrically at 440 nm.

***Structural Elucidation of Methanol Extracts***

*Asterionella glacialis* and *Skeletonema costatum* were grown in f/2 medium according to Guillard, (1975). Both diatoms were growth on 12:12 h light:dark cycle with the light intensity of 4500 lux at 28 ± 1°C. Diatom cultures were collected on a glass fiber filter (GF/F) and then extracted by a modified version of Bligh and Dyer (1959) as described under "lipid analysis".

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) has emerged as a powerful tool in the structural elucidation of metabolites in crude mixtures, especially at very low levels encountered in biological samples and without any prior purification. Hence, this technique has been used to identify some of the

metabolites of these diatoms. For the purpose mass spectrometry (MS) on crude methanol extract was performed to study the structural characteristics of the metabolites of *Asterionella glacialis* and *Skeletonema costatum.* The entire analyses were carried out at the ESI-MS facility in the National institute of Oceanography with the assistance from facility management.

Mass spectra were recorded, in the positive mode, on a quadrupole time-of-flight (QTOF)-XL MS/MS, Applied Biosystems, Switzerland, equipped with MDS Sciex Analyst Software. In addition to full scan mass spectra, collision induced dissociation (CID) spectra were undertaken in the MS/MS mode. The declustering potential and the collision energy were optimized for MS/MS experiments so as to cause fragmentation of the selected molecular ion species as evident by the appearance of fragment ions and decrease in the intensity of the molecular ion. Samples after dilution with 1:1 ($MeOH:H_2O$) were directly infused, at a constant flow rate of 10 µl/min into the ion spray source using an integrated syringe pump. Since the instrument is not calibrated each time a discrepancy of +1/+2 is observed.

***Statistical Analysis***

Data sets obtained from all experimental results were subjected to statistical analyses. Differences in growth rates, cellular chlorophyll *a* and biochemical constituents under different nutrient regimens and light intensities were deciphered by applying one-way analysis of variance (ANOVA) and least significant difference (LSD) multiple-range analyses (Sokal and Rohlf, 1981).

## Results

### Growth Characteristics of Diatoms Under Low Light Intensity (LLI)

#### *Cell Abundance*

From the cell abundance data, it is discernible that the growth patterns of all five species in (H-f/2) under LLI were very different (Fig. 8.1). For instance, *Navicula* sp attained the highest cell numbers ($1.5 \times 10^6$ cells ml $^{-1}$) by day, while *Thalassionema nitzschioides* exhibited the lowest cell abundance. The exponential growth phase of *Melosira nummuloides* began from day 2 itself and continued until the end of the experiment. However, the exponential growth phase of *T. nitzschioides* and *Navicula* sp continued until day 8, after which time, the cell abundance began to decrease as stationary phase was reached. *Asterionella glacialis* began growing rapidly from the second day, and the stationary growth phase occurred after day 4 which continued until day 8. Growth of *Skeletonema costatum* exhibited a typical growth, and the maximum cell abundance was observed on day 6 after that, the cell abundance decreased.

Similar to H-f/2 treatment, the cell abundance of *Navicula* sp in N-f/2 LLI attained the highest cell abundance ($1.6 \times 10^6$ cells ml$^{-1}$). Exponential growth phase of *Navicula* sp and *M. nummuloides* continued until day 8. Whereas, the stationary phase of *S. costatum* and *A. glacialis* started from day 4, and day 6 respectively. The cell abundance of *T. nitzschioides* increased with the time and reached the maximum on the sixth day ($4.2 \times 10^4$ cells ml$^{-1}$).

With the exception of *S. costatum*, cell abundance of other four species in D-f/2 medium under LLI attained high counts compared to the other nutrient treatments.

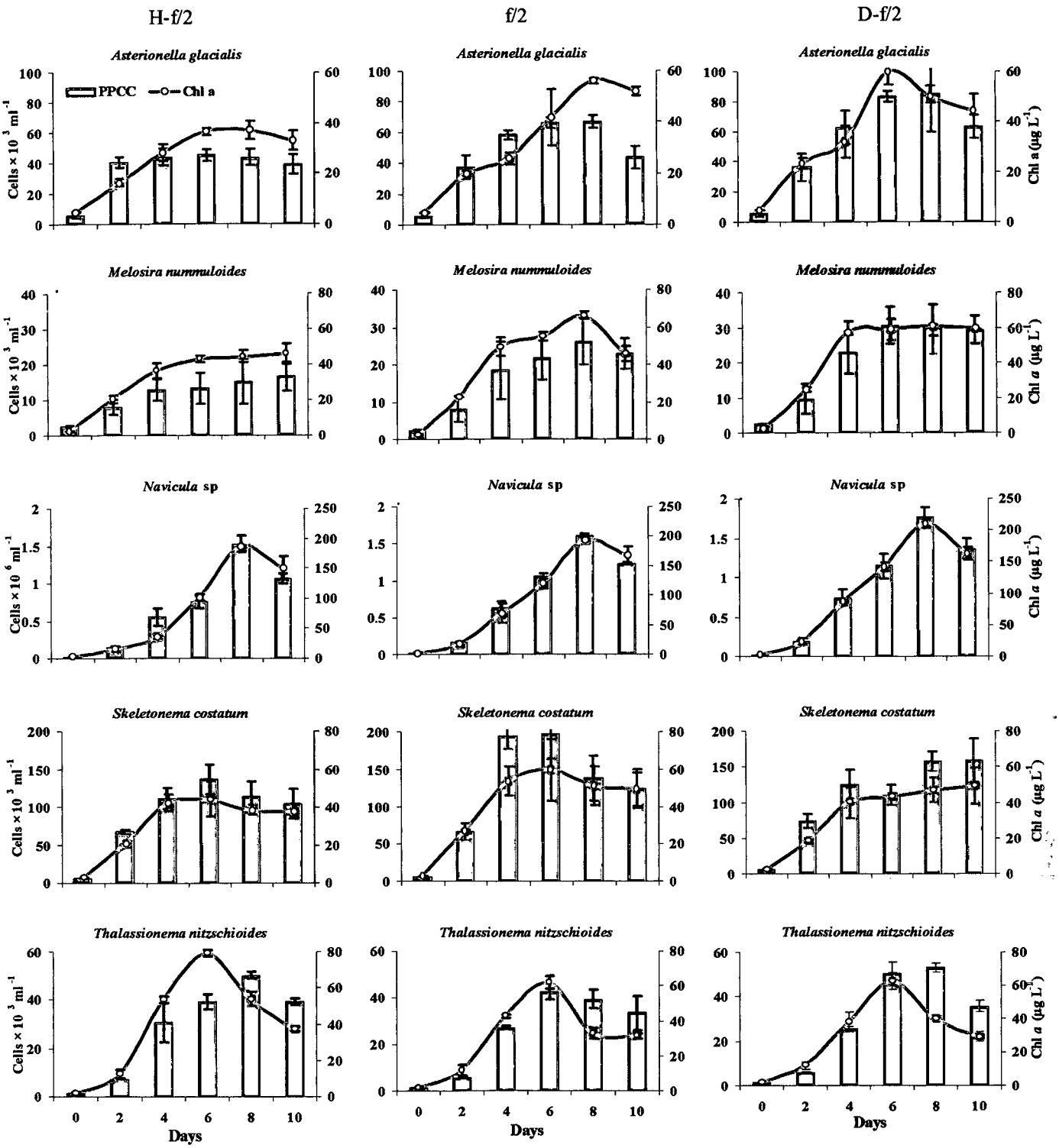



Fig. 8.1. Variations in cell abundance, and chl *a* concentrations in different diatom species (*Asterionella glacialis, Melosira nummuloides, Navicula* sp *Skeletonema costatum and Thalassionema nitzschioides,*) cultured under different treatments of nutrient medium (H-f/2: half strength of f/2 media; f/2: normal f/2 media; D-f/2: Double strength of f/2 media) at low intensity of light

The highest abundance was observed on day 8 for all the species except *M. nummuloides*. The stationary growth phase of *M. nummuloides* began on day 6 which continued until the end of the experiment.

The growth rates under different nutrient treatments under LLI did not show much variations among the species tested (Table 8.1). In general, the highest growth rate (0.73 $\mu$ day$^{-1}$) was observed in *Navicula* sp in D-f/2 under LLI while the lowest growth rate (0.28 $\mu$ day$^{-1}$) was observed in *M. nummuloides* under H-f/2 nutrient treatments under LLI.

***Total and Per Cell Chlorophyll a (chl a)***

The concentrations of chlorophyll *a* exhibited a similar trend as of cell abundance in all five species in H-f/2 medium under LLI (Fig. 8.1). The highest chl *a* was observed in the culture flasks of *Navicula* sp (186.7 $\mu$g L$^{-1}$) on day 8 followed by *T. nitzschioides* (79.5 $\mu$g L$^{-1}$) on day 6. The chl *a* concentrations of *A. glacialis, S. costatum* and *M. nummuloides* were similar.

There was very little variability in per cell chl *a* content of all the species in H-f/2 under LLI (Fig. 8.2). The highest cellular chlorophyll *a* was observed in the pennate diatoms, *T. nitzschioides* (3 pg cell$^{-1}$) followed by *A. glacialis* and the lowest in *Navicula* sp (0.06 pg cell $^{-1}$).

Excepting in the cultures of *Navicula* sp, the chl *a* concentrations were lower in other cultures in N-f/2 under LLI compared with the same treatment under high light intensity of (Fig. 8.1). The chl *a* concentrations were the highest on day 8 in the cultures of *Navicula* sp (193.1 $\mu$g L$^{-1}$), *M. nummuloides* (66.2 $\mu$g L$^{-1}$) and *A.*

Table 8.1. The growth rate of different diatom species in different treatments of nutrient medium (H-f/2: half strength of f/2 media; f/2: normal f/2 media and D-f/2: Double strength of f/2 media) under different light intensities (LLI: low light intensity; HLI: high light intensity)

| **Species** | **Light intensity** | **Nutrient treatments** | | |
|---|---|---|---|---|
| | | **H-f/2** | **f/2** | **D-f/2** |
| *Asterionella glacialis* | LLI | 0.31 | 0.35 | 0.37 |
| | HLI | 0.51 | 0.52 | 0.51 |
| *Melosira nummuloides* | LLI | 0.28 | 0.31 | 0.36 |
| | HLI | 0.51 | 0.53 | 0.55 |
| *Navicula sp* | LLI | 0.67 | 0.70 | 0.73 |
| | HLI | 0.56 | 0.59 | 0.62 |
| *Skeletonema costatum* | LLI | 0.53 | 0.56 | 0.59 |
| | HLI | 0.62 | 0.63 | 0.64 |
| *Thalassionema nitzschioides* | LLI | 0.59 | 0.59 | 0.60 |
| | HLI | 0.64 | 0.64 | 0.65 |

*glacialis* (56 μg $L^{-1}$). In the cultures of *T. nitzschioides* (61.85 μg $L^{-1}$) and *S. costatum* (59.75μg $L^{-1}$) such highs were on day 6 itself.

With exception of *M. nummuloides* the cellular chl *a* in other species was high on day 8 and/or 10 in N-f/2 compared those either in H-f/2 or D-f/2 under LLI. Similar to H-f/2 nutrient treatment, the highest per cell chl *a* was observed in *T. nitzschioides* and the lowest in *Navicula* sp.

In the D-f/2 treatments, the chl *a* concentrations of all species exhibited trend similar to cell abundances. The highest chl *a* concentration was seen in the culture flasks of *Navicula* sp (211 μg $L^{-1}$) on day 8.

The cellular chl *a* was the lowest in D-f/2 specially during the last two days compared with other treatments at low light intensity in all the species (Fig. 8.2).

## Growth Characteristics of Diatoms Under High Light Intensity (HLI)

### *Cell Abundance*

Except for *Navicula* sp, the cell abundance of other four diatoms was comparatively higher under HLI in all nutrient strengths (Fig. 8.3). In H-f/2, the highest cell abundance was achieved by *Navicula sp*, while the lowest by *T. nitzschioides*. However, the exponential growth of *A. glacialis, M. nummuloides,* and *Navicula* sp that occurred from day 1 itself lasted until day 6, but continued in cases of *S. costatum* and *T. nitzschioides* until day 8.

From the cell abundance data of all five species, it is discernible that the growth patterns in N-f/2 under HLI were very different. The highest cell abundance of

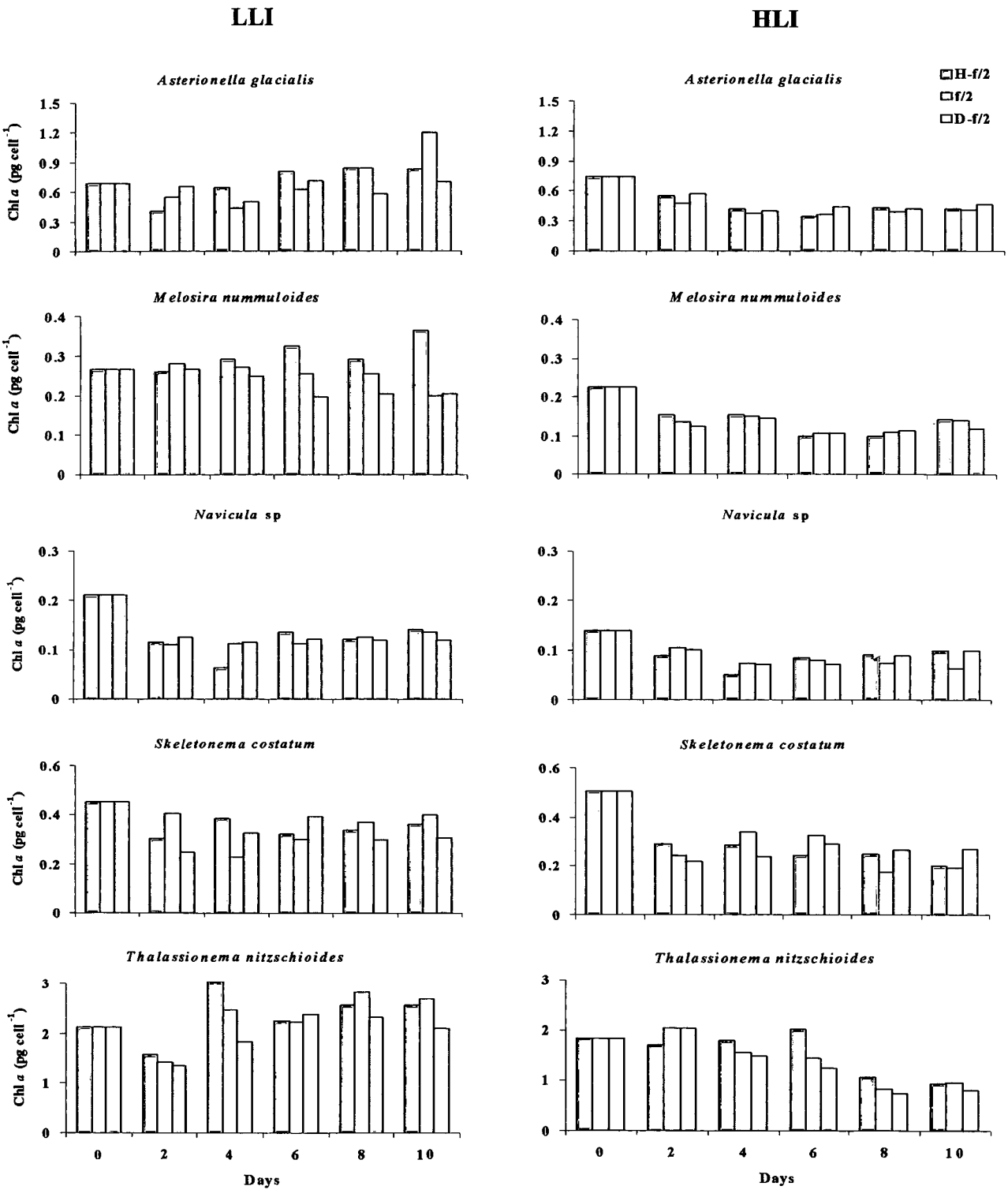



Fig. 8.2. Variations in chl *a* per cell in different diatom species (*Asterionella glacialis, Melosira nummuloides, Navicula* sp *Skeletonema costatum and Thalassionema nitzschioides,*) cultured under different treatments of nutrient medium (H-f/2: half strength of f/2 media; f/2: normal f/2 media; D-f/2: Double strength of f/2 media) at different light intensity (LLI: low light intensity; HLI high light intensity)

*Skeletonema costatum* ($4.9 \times 10^5$ cells $ml^{-1}$) was observed on day 8. Other diatoms species attained their cell number maxima on day 6 itself.

Similar to those in N-f/2, maximum cell numbers of *Navicula* sp ($1.3 \times 10^6$ $ml^{-1}$) and *M. nummuloides* ($72.5 \times 10^3$ $ml^{-1}$) were observed on day 6 and, of *A. glacialis* ($1.6 \times 10^6$ $ml^{-1}$), on day 4 in D-f/2 under HLI. The exponential growth of *T. nitzschioides* continued until the end. The cell abundance of *S. costatum* did not change much.

Growth rates of diatom species other than *Navicula* sp cultured under HLI were, in general, higher in all the nutrients strengths than under LLI (Table 8.1). The highest growth rate was seen in *T. nitzschioides* (0.65) followed by *S. costatum* (0.64).

***Total and Per Cell Chl a***

With the exception of *Navicula* sp, chl *a* concentrations were more in other diatoms under HLI *vis a vis* LLI. The highest concentration was observed on the day 4 in *A. glacialis* (62.1 $\mu g\ L^{-1}$), on day 6 in *M. nummuloides* (51.7 $\mu g\ L^{-1}$) and *Navicula* sp (75.25 $\mu g\ L^{-1}$), and on day 8 in *T. nitzschioides* (146.5 $\mu g\ L^{-1}$; Fig.8.3).

The differences in per cell chl *a* between the species grown in different strengths of f/2 under HLI were imminent (Fig.8.2) than under LLI. The lowest per cell chl *a* in H-f/2 was observed in *S. costatum* (0.2 pg $cell^{-1}$) followed by *Navicula* sp (0.05 pg $cell^{-1}$).

The concentrations of chl *a* increased with time and reached the highest value on day 6 in all diatom species cultured in N-f/2 under HLI (Fig.8.3). Overall, cultures

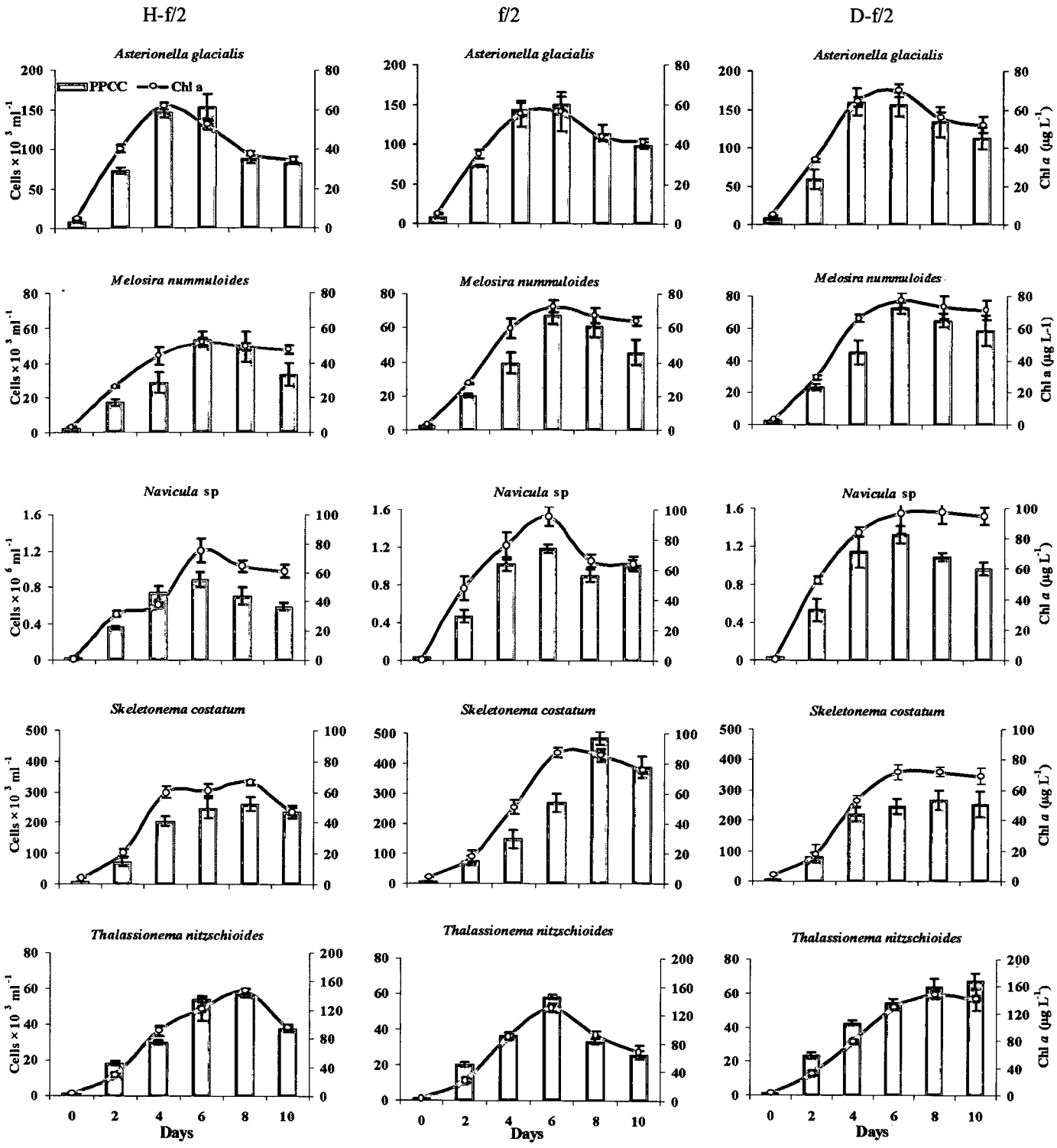



Fig. 8.3. Variations in cell abundance, and chl *a* concentrations in different diatom species (*Asterionella glacialis, Melosira nummuloides, Navicula* sp *Skeletonema costatum and Thalassionema nitzschioides,*) cultured under different treatments of nutrient medium (H-f/2: half strength of f/2 media; f/2: normal f/2 media; D-f/2: Double strength of f/2 media) at high intensity of light

of *T. nitzschioides* (130.4 μg $L^{-1}$) followed by *Navicula* sp (95.65 μg $L^{-1}$) attained the highest concentrations.

Per cell chl *a* fluctuated in all species and the lowest concentration was observed in *Navicula* sp (0.06 pg cells $^{-1}$).

The exponential growth of all species growing in D-f/2 under HLI occurred from day 1 to day 6, after that the chl *a* concentrations remained fairly stable in all species except *A. glacialis*. The highest concentrations of 70.1 μg $L^{-1}$ were observed on day 6 in *A. glacialis* flasks after which a decrease was seen.

Similar to other nutrient treatments, *T. nitzschioides* culture under HLI had the highest per cell chl *a*, and *Navicula* sp, the lowest.

## Biochemical Composition of Diatom Species Grown Under LLI

### *Variations in Protein Contents*

The total cellular protein contents of *Asterionella glacialis, M. nummuloides* and *S. costatum* grown in H-f/2 were lower (Fig. 8.4) compared to those in the other media treatments under LLI. The highest protein content was observed in *T. nitzschioides* (261.2 pg $cell^{-1}$) and the lowest in *Navicula* sp (20.9 pg $cell^{-1}$).

The total protein contents between the species in N-f/2 differed. The highest protein content was in *Thalassionema nitzschioides* (222.15 pg cell $^{-1}$), followed by *A. glacialis* (82.4 pg $cell^{-1}$) and *S. costatum* (78.9 pg $cell^{-1}$). However, the highest protein content in *M. nummuloides* (65.3 pg $cell^{-1}$) was only in the N-f/2 under LLI unlike under high light and/or other strengths of f/2 medium.

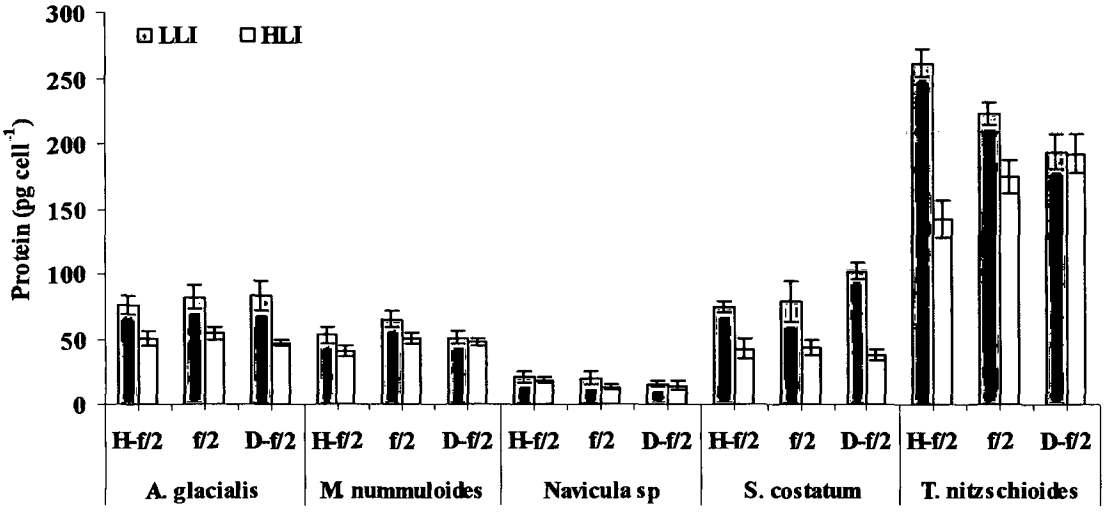



Fig. 8.4. Cellular protein contents in *Asterionella glacialis, Melosira nummuloides, Navicula* sp, *Skeletonema costatum,* and *Thalassionema nitzschioides* under different treatments of nutrients (H-f/2: half strength of f/2 media; f/2: f/2 media and D-f/2: Double strength of f/2 media) at different intensities of light (LLI: low light intensity and HLI: high light intensity)

Similar with other nutrient treatments, the lowest content in D-f/2 medium under LLI was observed in *Navicula* sp (15.7 pg cell$^{-1}$) and the highest in *T. nitzschioides* (193.5 pg cell$^{-1}$; Fig.8.4). Cellular protein was lower in D-f/2 medium compared to N-f/2 and/or H-f/2 treatments. Protein contents were higher in *S. costatum* (102 pg cells $^{-1}$) and *A. glacialis* (83.5 pg cells $^{-1}$) grown in D-f/2 medium under LLI.

***Variations in Carbohydrate Contents***

The highest total cellular carbohydrates were observed in H-f/2 under LLI in most tested diatoms species (Fig.8.5). *Thalassionema nitzschioides* and *M. nummuloides* had the highest contents (39.4 and 36.4 pg cells $^{-1}$ respectively), while *Navicula* sp had the lowest (5.7 pg cells $^{-1}$) cellular total carbohydrates

Under the LLI, the highest total carbohydrate was observed in *T. nitzschioides* (37.3 pg cells $^{-1}$) followed by *M. nummuloides* (23.8 pg cells $^{-1}$). *Skeletonema costatum* (11.8 pg cells $^{-1}$) and *Asterionella glacialis* (11.3 pg cells $^{-1}$) had comparable carbohydrate contents. However, the lowest contents were in *Navicula* sp (3.45 pg cells $^{-1}$).

With the exception of *T. nitzschioides,* there was no difference in the cellular total carbohydrates at low intensity of light between D-f/2 and N-f/2 nutrient treatment in all the species studied (Fig.8.5).

***Variations in Lipid Contents***

In general, total cellular lipid content decreased with increasing strengths of f/2 medium (Fig. 8.6). The highest lipid content in H-f/2 under LLI was observed in all

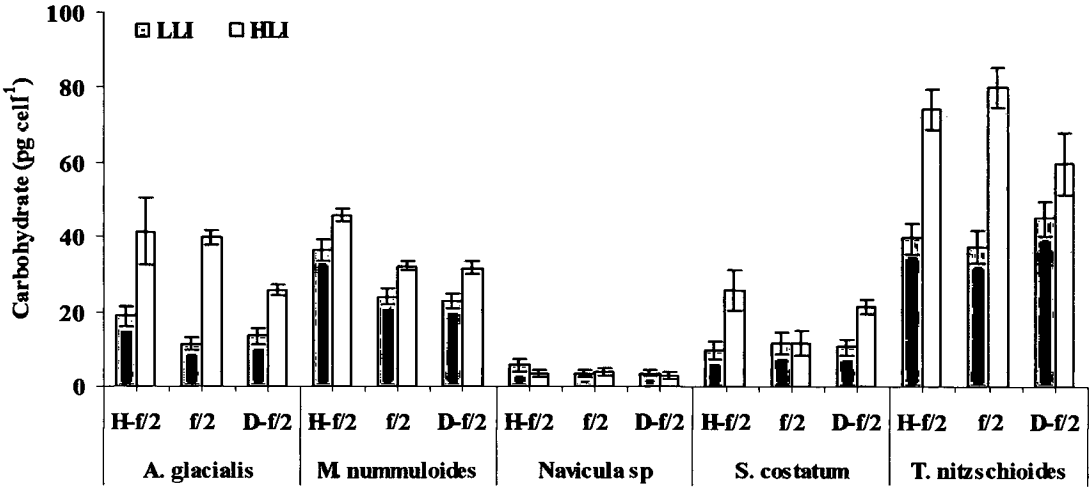



Fig. 8.5. Cellular carbohydrate contents in *Asterionella glacialis, Melosira nummuloides, Navicula* sp, *Skeletonema costatum,* and *Thalassionema nitzschioides* under different treatments of nutrients (H-f/2: half strength of f/2 media; f/2: f/2 media and D-f/2: Double strength of f/2 media) at different intensities of light (LLI: low light intensity and HLI: high light intensity)

species except *Navicula* sp. But *Thalassionema nitzschioides* had the highest content (86 pg cell$^{-1}$).

The highest lipid content was observed in *T. nitzschioides* followed by *M. nummuloides* and the lowest in *S. costatum* (4.3 pg cell$^{-1}$) followed by *A. glacialis* (5.3 pg cell $^{-1}$) in f/2 medium under LLI. Overall, the lipid content in *Navicula* sp (7.45 pg cells $^{-1}$) in the N-f/2 strength was higher than that in other two nutrient strengths under the same LLI.

The cellular total lipid content in cultures grown in D-f/2 under LLI was poorer in all the diatoms species with its lowest in *A. glacialis* (3.65 pg cell$^{-1}$).

## Biochemical Composition of Diatom Species Grown Under HLI

### *Variations in Protein Contents*

All the diatom species grown in H-f/2 under HLI had low protein content (Fig. 8.4). The cellular protein content was 50.9 pg cell$^{-1}$ in *A. glacialis*, 41.25 pg cell$^{-1}$ in *M. nummuloides*, 18.8 pg cell$^{-1}$ in *Navicula* sp, 42.5 pg cell$^{-1}$ in *S. costatum* and 142.35 pg cell$^{-1}$ in *T. nitzschioides*.

Most diatom species cultured in N-f/2 had slightly higher protein content compared to other treatments (Fig.8.4) with *Navicula* sp having the lowest (13.2 pg cell$^{-1}$) and *T. nitzschioides* (175 pg cells $^{-1}$), the highest.

Similar to the ones observed in other nutrient strengths, the highest protein content was observed in *T. nitzschioides* (192.25 pg cells $^{-1}$) and the lowest in *Navicula* sp (Fig.8.4).

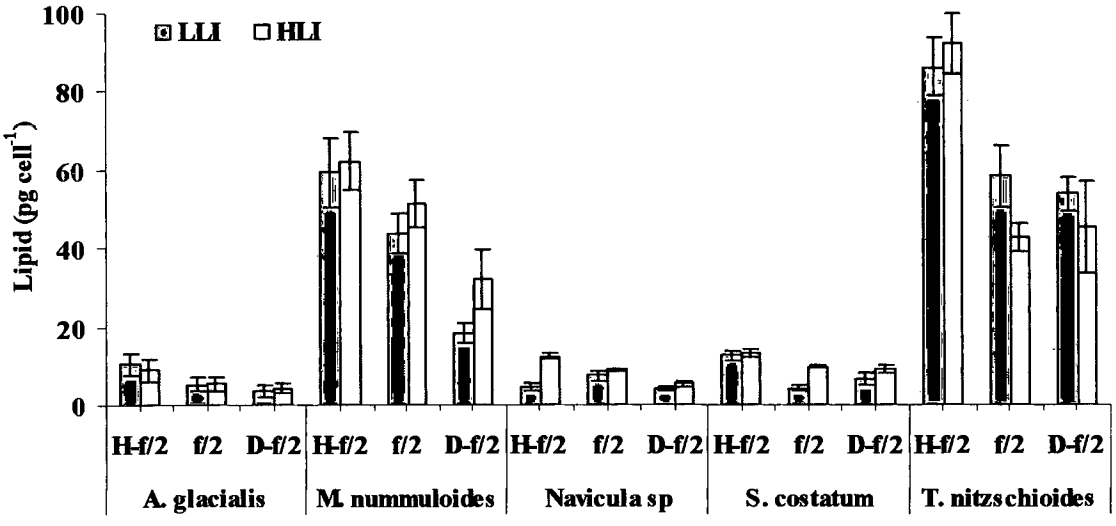



Fig. 8.6. Cellular lipid contents in *Asterionella glacialis, Melosira nummuloides, Navicula* sp, *Skeletonema costatum,* and *Thalassionema nitzschioides* under different treatments of nutrients (H-f/2: half strength of f/2 media; f/2: f/2 media and D-f/2: Double strength of f/2 media) at different intensities of light (LLI: low light intensity and HLI: high light intensity)

***Variations in Carbohydrate Contents***

In general, cells grown under HLI accumulated more carbohydrate than under LLI (Fig. 8.5). The highest concentration was observed in cultures of *T. nitzschioides* in H-f/2.

Carbohydrate content of diatoms cultured in N-f/2 under HLI varied between the species (Fig 8.5) with the highest in *T. nitzschioides* (79.7 pg cells $^{-1}$) and the lowest in *Navicula* sp (3.7 pg cells $^{-1}$).

In general, the total carbohydrate content was low in all diatoms species cultured in D-f/2 (Fig. 8.5). It was much lower in *Navicula* sp than in *T. nitzschioides.* Total carbohydrate content was similar in *A. glacialis, S. costatum* and *M. nummuloides.*

***Variations in Lipid Contents***

The change in cellular total lipid content in H-f/2 under HLI reached a maximum in all diatoms species. Similar with protein and/or carbohydrate contents, total lipid in *T. nitzschioides* was much higher than that of other species. Whereas, it was lower in *A. glacialis* (8.9 pg cell$^{-1}$), *Navicula* sp (12.4 pg cell$^{-1}$) and *S. costatum* (13.2 pg cell$^{-1}$).

A clear decrease in lipid contents in all species was observed in N-f/2 under HLI (Fig. 8.6). The centric *M. nummuloides* (51.3 pg cell$^{-1}$) contained the highest lipid content, and the pennate diatom *A. glacialis* (5.5 pg cell$^{-1}$), the lowest.

With the exception of *T. nitzschioides,* the cellular lipid contents of all the diatoms species in D-f/2 under HLI were much lower than those of H and N treatments

(Fig.8.6). The highest lipid content was in *T. nitzschioides* (45.2 pg cell $^{-1}$) followed by *M. nummuloides* (32 pg cell$^{-1}$) in D-f/2.

**Statistical Analysis**

The results from ANOVA suggest that the growth rates of all five species were strongly affected by light intensity. The species of *Melosira nummuloides* (p= 0.00006), *Asterionella glacialis* (p= 0.0002), *Skeletonema costatum* (p= 0.0005) and *Navicula* sp (p= 0.0005) followed by *Thalassionema nitzschioides* (p= 0.05) showed significant differences in growth rates with changing of light intensity (Table 8.2). There was no significant differences (p>0.05) between growth rates and differing nutrient concentrations (Table 8.3).

With the exception of per cell chl *a* of *S. costatum* (p= 0.068), the result from ANOVA suggest that per cell chl *a* contents in other species was strongly affected by light intensity (Table 8.2), than due to varying nutrient strengths (Table 8.3).

The species of *M. nummuloides* followed by *T. nitzschioides* were the most affected species by the light intensity. The per cell chl *a* in *Asterionella glacialis* and *Navicula* sp also exhibited similar response.

Although there was no significant differences in protein contents of diatoms (p >0.05) cultured in different nutrient concentrations, the changing of light intensity affected protein content in all the diatoms (p<0.05; Table 8.2). Such differences were also discernable in carbohydrate concentrations (Table 8.2).

Compared with total protein and/or carbohydrates, the total lipids in all the diatom species except *Navicula* sp (p= 0.14) was strongly affected by changing of nutrient

Table 8.2. Results of ANOVA (p-value) on the influence of light intensity on growth rate, chl *a*, protein, carbohydrate and lipid per cell of different diatoms species studied in the laboratory

| **Species** | **growth rate** | **Chl *a* per cell** | **Protein** | **Carbohydrate** | **Lipid** |
|---|---|---|---|---|---|
| *Asterionella glacialis* | 0.0002 | 0.0005 | 0.000 | 0.000 | 0.880 |
| *Melosira nummuloides* | 0.0000 | 0.0000 | 0.013 | 0.017 | 0.408 |
| *Navicula* sp | 0.0005 | 0.0006 | 0.046 | 0.042 | 0.036 |
| *Skeletonema costatum* | 0.0004 | 0.0680 | 0.000 | 0.009 | 0.144 |
| *Thalassionema nitzschioides* | 0.0550 | 0.0000 | 0.005 | 0.000 | 0.633 |

Table 8.3. Result of ANOVA (p-value) on the influence of nutrients variation on growth rate, chl *a*, protein, carbohydrate and lipid per cell of different diatoms species studied in the laboratory.

| **Species** | **growth rate** | **Chl *a* per cell** | **Protein** | **Carbohydrate** | **Lipid** |
|---|---|---|---|---|---|
| *Asterionella glacialis* | 0.736 | 0.960 | 0.920 | 0.340 | 0.0000 |
| *Melosira nummuloides* | 0.498 | 0.459 | 0.093 | 0.000 | 0.0000 |
| *Navicula* sp | 0.347 | 0.969 | 0.065 | 0.011 | 0.1360 |
| *Skeletonema costatum* | 0.424 | 0.953 | 0.847 | 0.440 | 0.0090 |
| *Thalassionema nitzschioides* | 0.769 | 0.548 | 0.956 | 0.920 | 0.0001 |

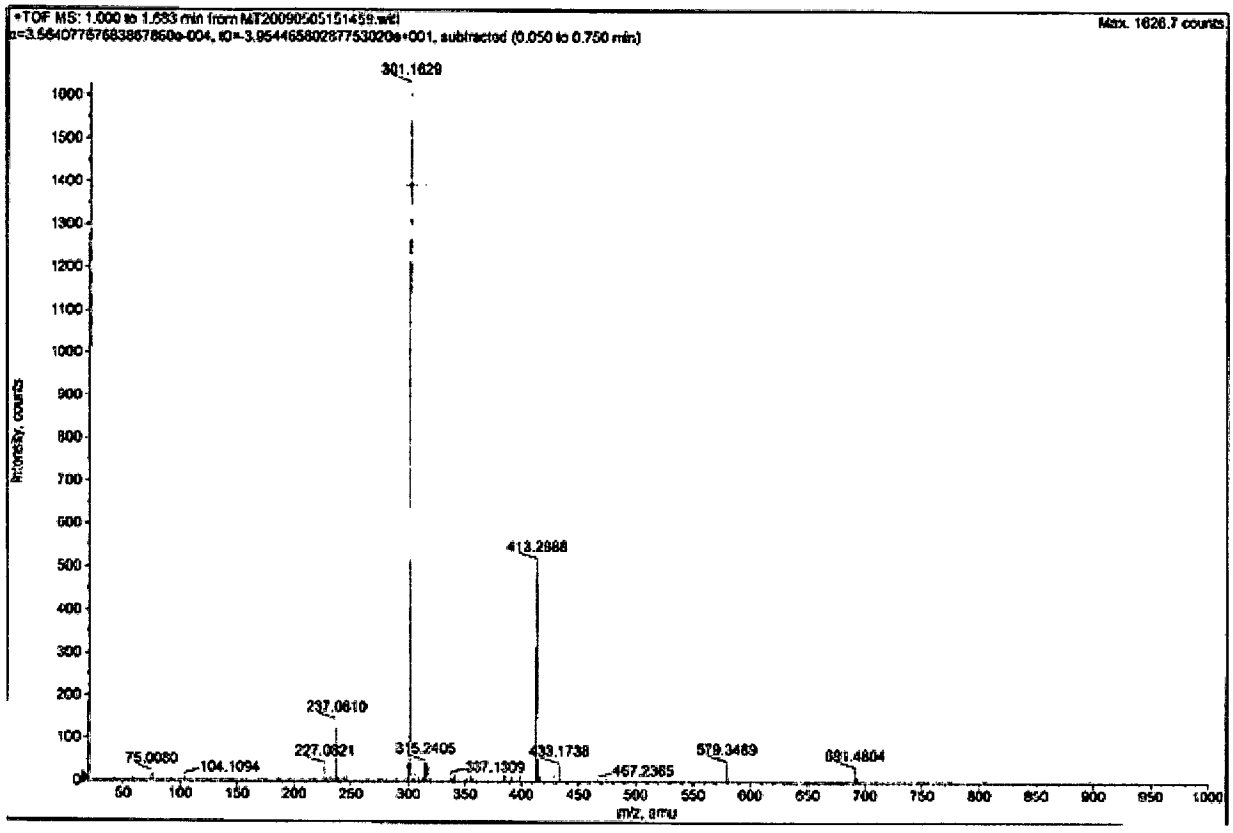



Fig.8.7. Positive ESI-MS profile of the crude methanol extract of *Asterionella glacialis*

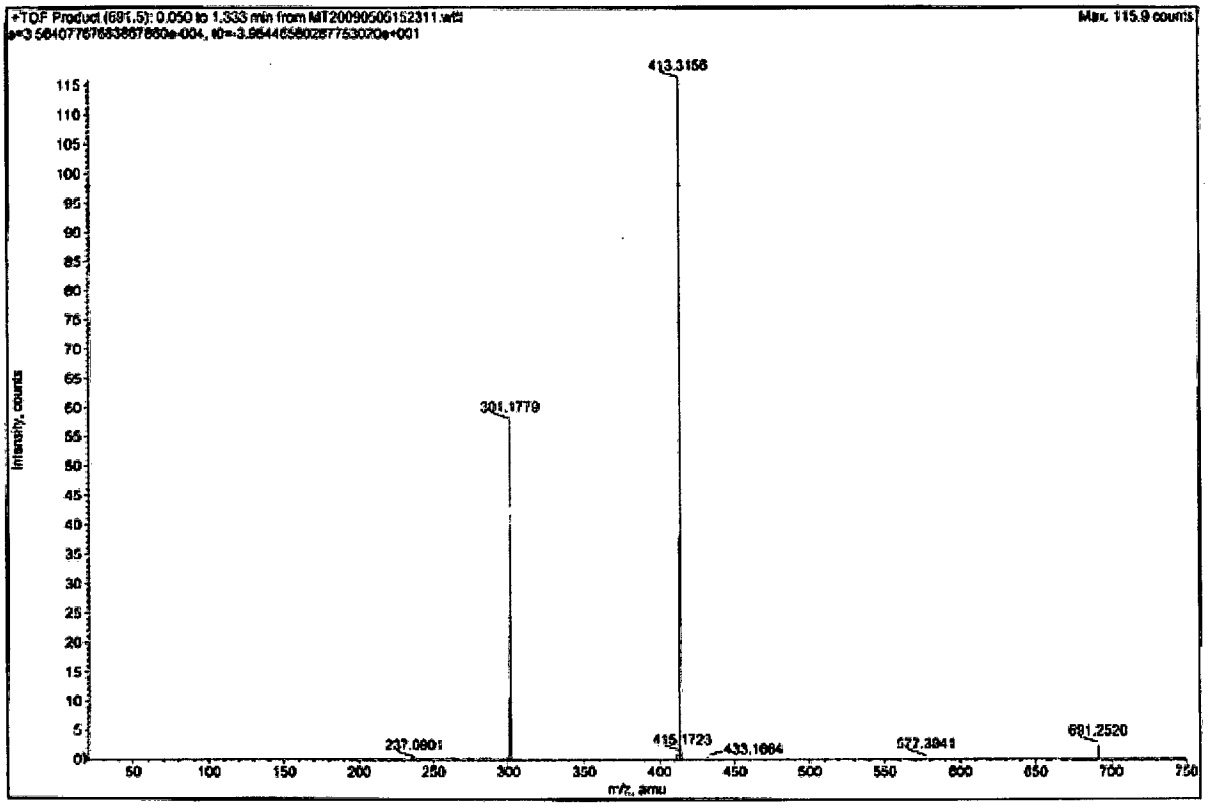



Fig.8.8. Positive MS/MS spectrum of the $[M+Na]^+$ ion at m/z 691

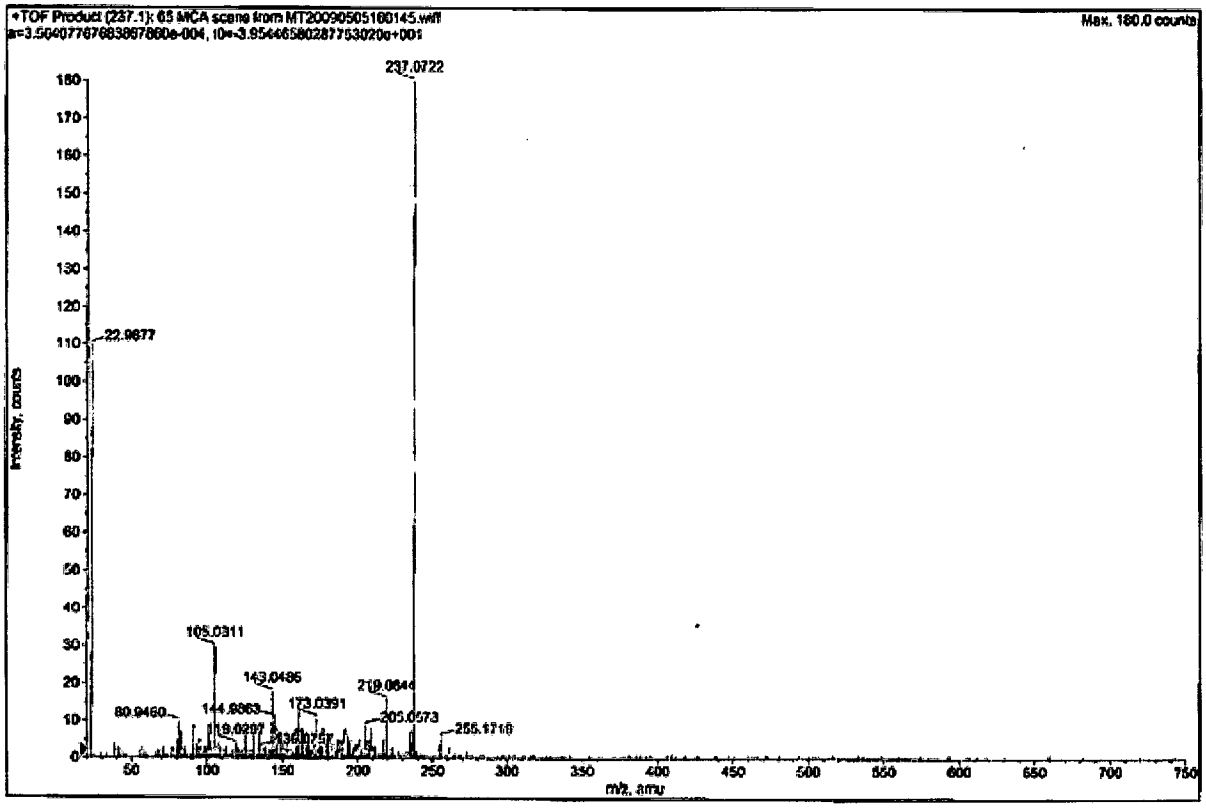



Fig.8.9. Positive MS/MS spectrum of the $[M+H]^+$ ion at m/z 255

due to the presence of a unique monounsaturated fatty acid $\Delta^3$- trans-hexadecenoic acid [16:1], also known as palmitoleic acid. Phosphatidyl glycerol of chloroplast, in particular, is characterized by presence of $\Delta^3$- trans-hexadecenoic acid [16:1].

Fragmentation pattern for the pseudomolecular ion $[M+H]^+$ (Fig 8.10) with the molecular mass of 473 amu led to the identification of a 3.*O*-(6'-sulfoquinovopyranosyl)-1-phophatidyl glycerol. Thus MS/MS of the pseudomolecular ion at *m/z* 474 exhibited the positive ions at m/z 81 and 171 that has a mass corresponding to likely loss of phosphatidyl glycerol moiety. Based on that the glycolipid with pseudomolecular ion at *m/z* 474 was characterized as 2- *O* – palmitoyl-3.*O*-(6'-sulfoquinovopyranosyl)-1-phophatidyl glycerol. A similar fragmentation pattern observed for the sulfonoquinovosyl molecular species with pseudomolecular ion at *m/z* 579 (Fig.8.11) led to its identified as 2-*O*-linolenyl-3-*O*-(6'-sulfoquinovopyranosyl)-sn-glycerol.

The presence of fatty acids, octadecatrienoic at *m/z* 317 (18:3) and octadecatetraenoic acids at *m/z* 315 (18:4) as potassium adducts could also be detected (Fig 8.7 and 8.12).

Molecular species with pdeudomolecular ion $[M+H]^+$ at m/z 104 (Fig. 8.13) was identified as N,N-dimethyl glycine.

**Metabolites from *Skeletonema costatum***

In the present context it was tried to tentatively assign structures to some of the selected metabolism of *Skeletonema costatum*, on the basis of ESI-MS/MS or tandem mass spectrometry.

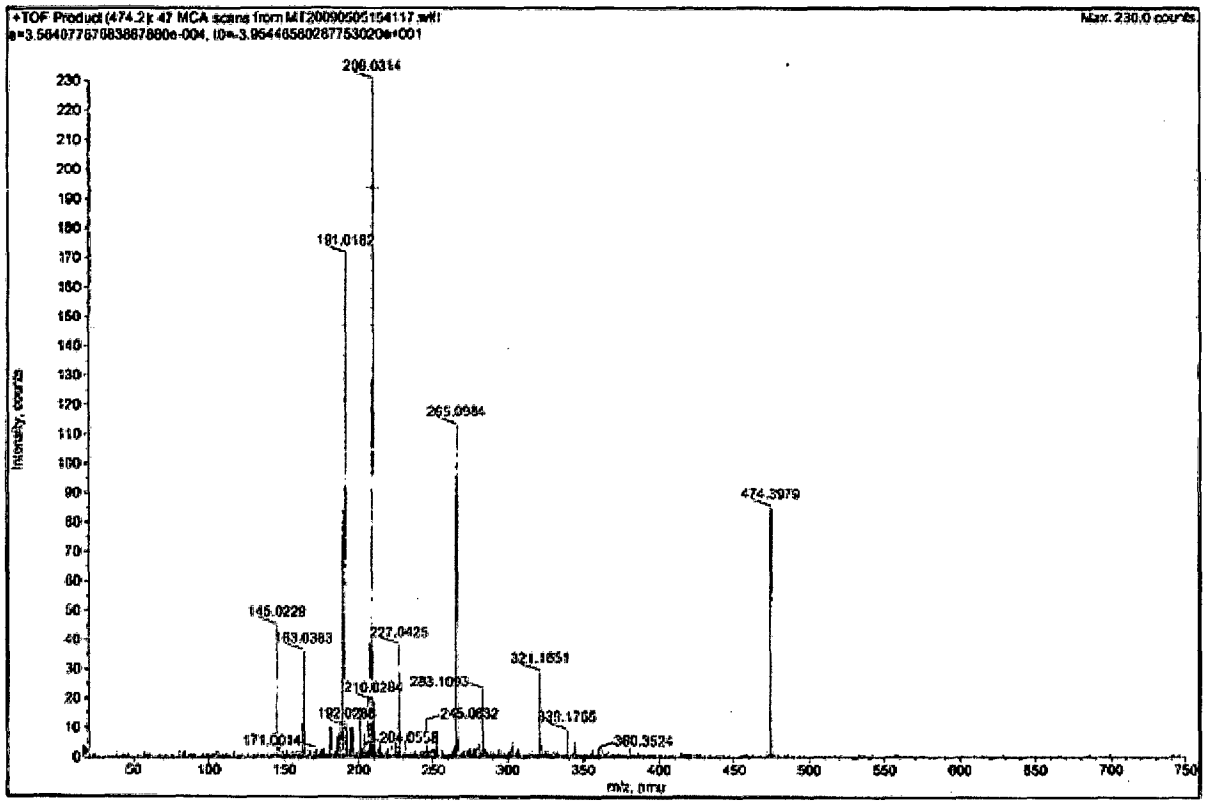



Fig.8.10. Positive MS/MS spectrum of the phospholipid with $[M+H]^+$ ion at m/z 473

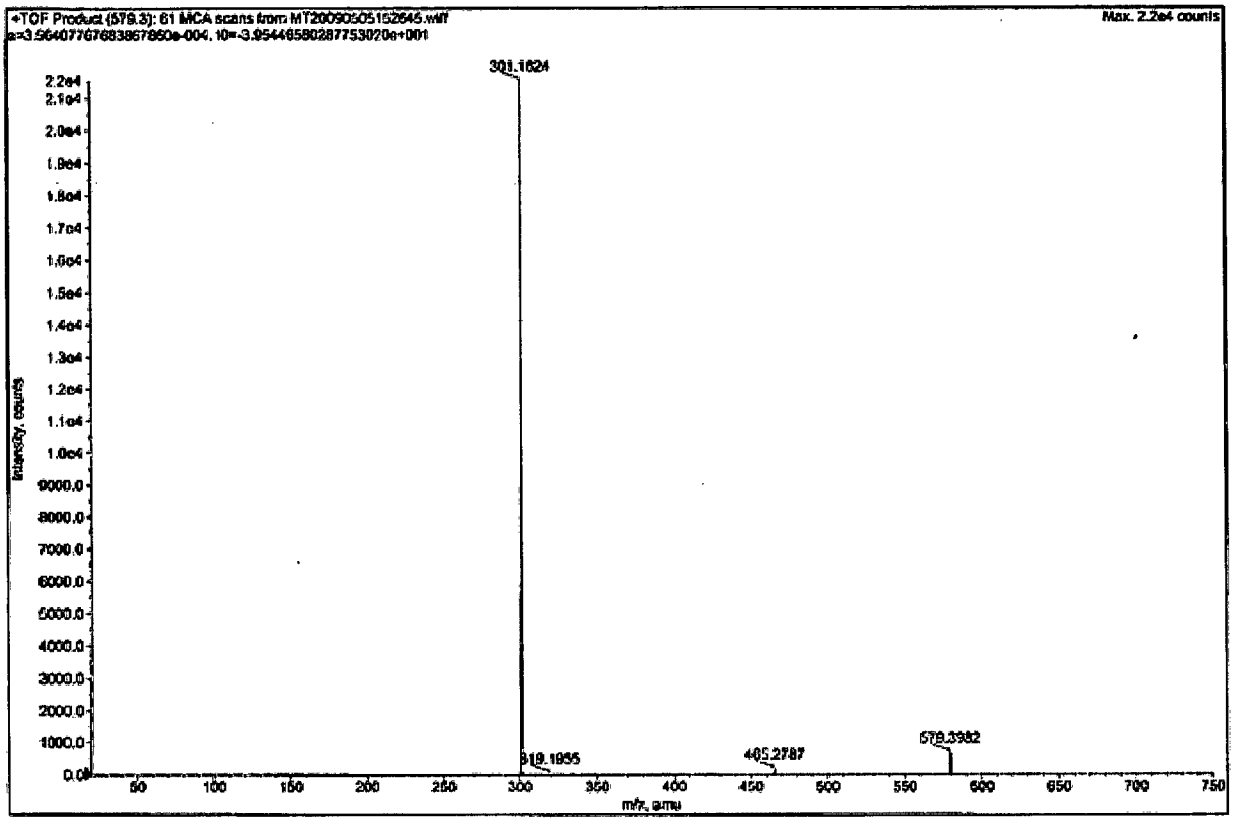



Fig.8.11. Positive MS/MS spectrum of the glycolipid with $[M+H]^+$ ion at m/z 579

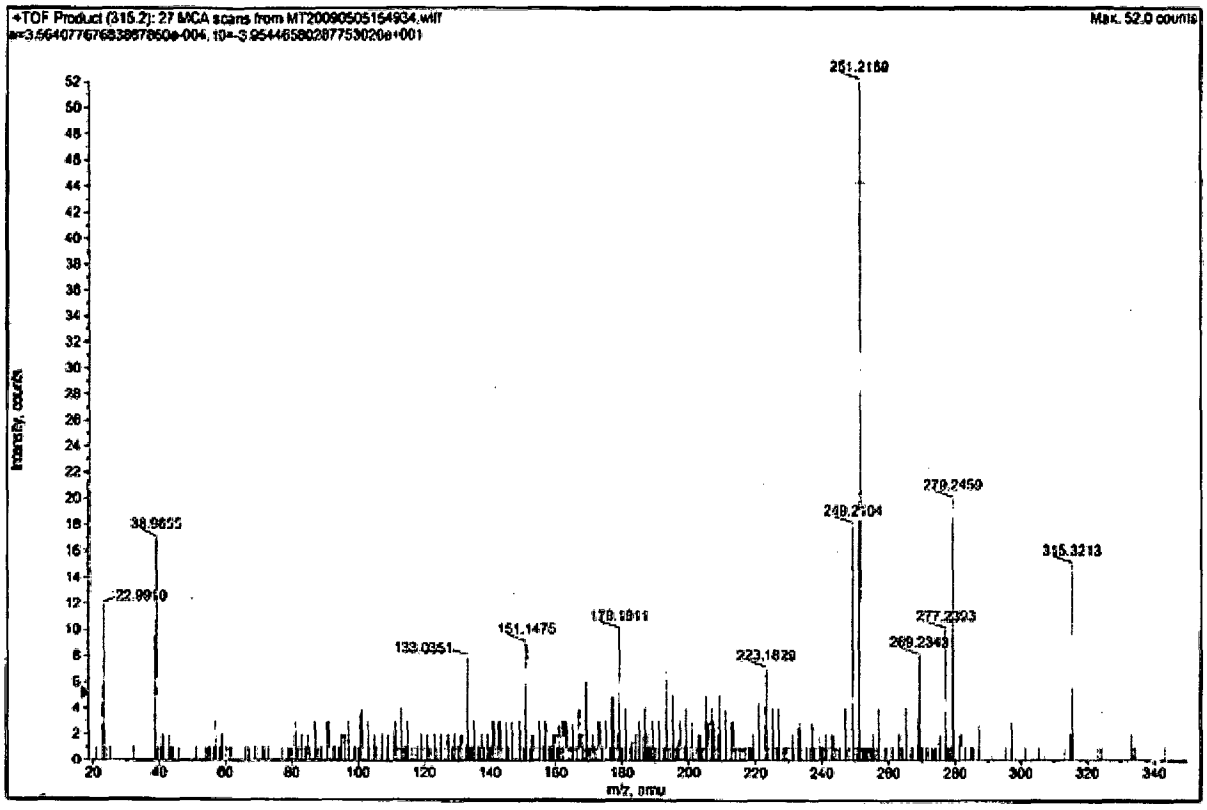



Fig.8.12. Positive MS/MS spectrum of the $[M+K]^+$ ion of octadecatetraenoic acid at m/z 315

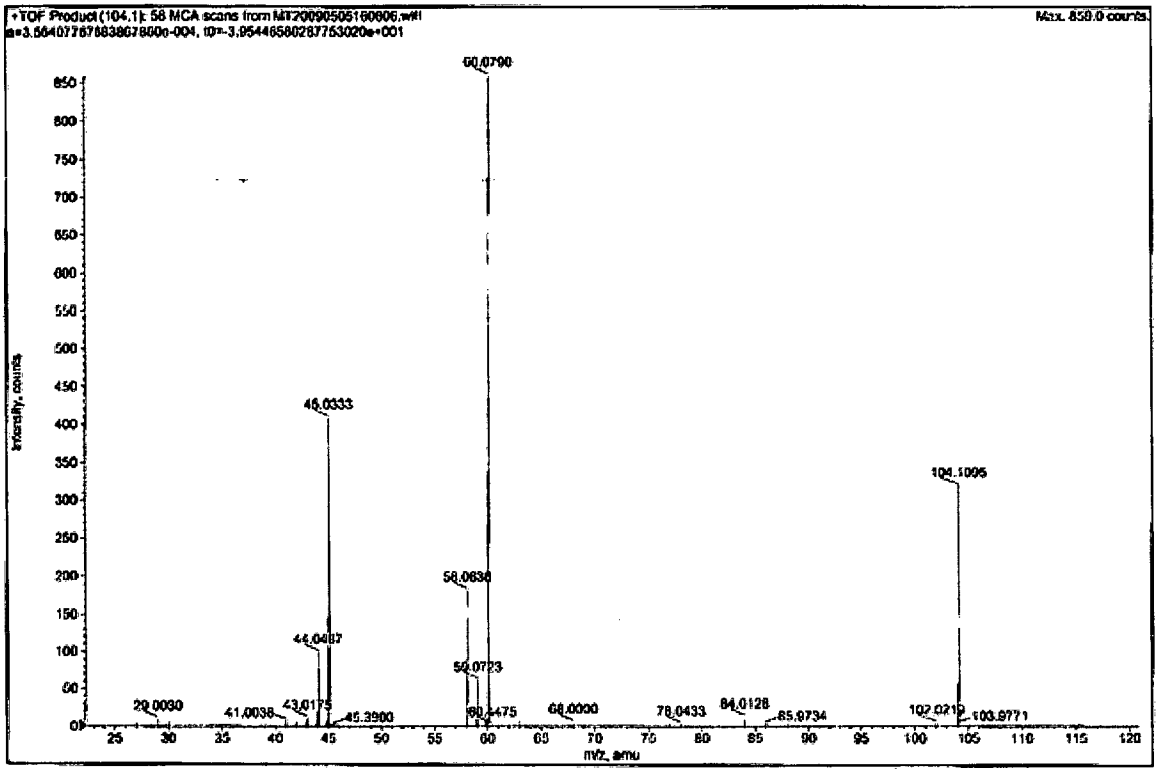



Fig.8.13. Positive MS/MS spectrum of the amino acid N,N-dimethyl glycine.with pseudomolecular ion $[M+H]^+$ at m/z 104

ESI-MS profile of the crude methanolic extract (Fig 8.14) of this species shows the presence of unsaturated fatty acids as evidenced by the presence of signals at $[M+H]^+$ at *m/z* 281 and m/z 283 due to C18:2 (linoleic acid) and C18:1 (oleic acid) respectively. A most prominent signal was observed at m/z 149 which corresponded to $[M+Na]^+$ sodium adduct of carboxylic acid of 2E,4Z heptadienal (aldehydes are easily oxidized to the corresponding carboxylic acids at room temperature and in the presence of light). The signal at m/z 341 was assigned to sodium adduct of C-14-hydroxy EPA (eicosapentaenoic acid) which is known as precursor of 2E,4Z heptadienal. Besides these polyunsaturated carboxylic acids the following glycerolipids were also identified from the crude methanol extract.

Collision induced dissociation (CID) of the pseudomolecular ion at *m/z* 535 resulted in the elimination of palmitoleic acid moiety from the molecule to yield the most intense signal at *m/z* 281 (Fig. 8.15). Elimination of ethanolamine together with palmitoleic acid resulted in the formation of the fragment at *m/z* 221 which in fact corresponds to protonated ion involving glactose with the glycerol backbone. Based on fragmentation pattern observed the molecule was identified as 1 galactosyl-2palmitoleyl- -3 ethanolamine-sn-glycerol.

The molecular species with mass of 796.45 has been identified as 1-glycophosphoethanolamine-2-palmitoley-3-phosphatidylglycerol (Fig. 8.16). This molecule on CID gave the most intense signal at *m/z* 81 indicative of the presence of phosphatidyl moiety. The next most intense signal resulted from the elimination of glycophosphoethanolamine moiety resulting in the formation of fragment at *m/z*

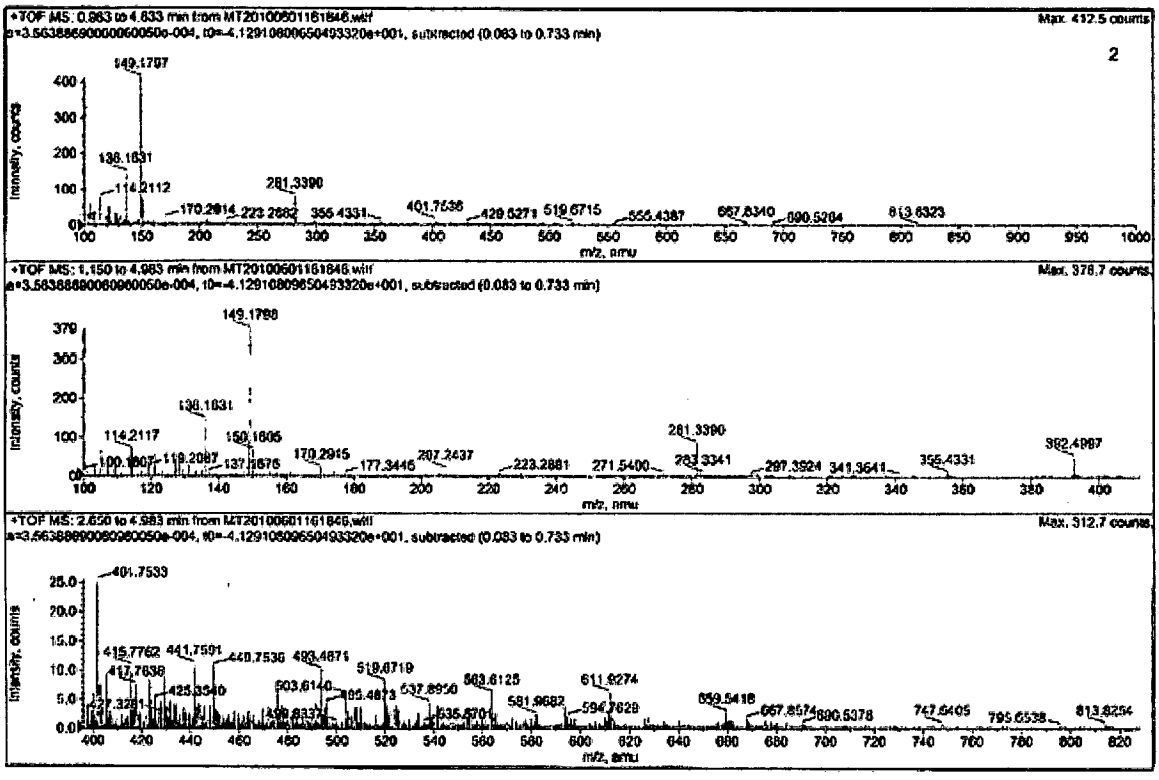



Fig.8.14. Positive ESI-MS profile of the crude methanol extract of *Skeletonema costatum*

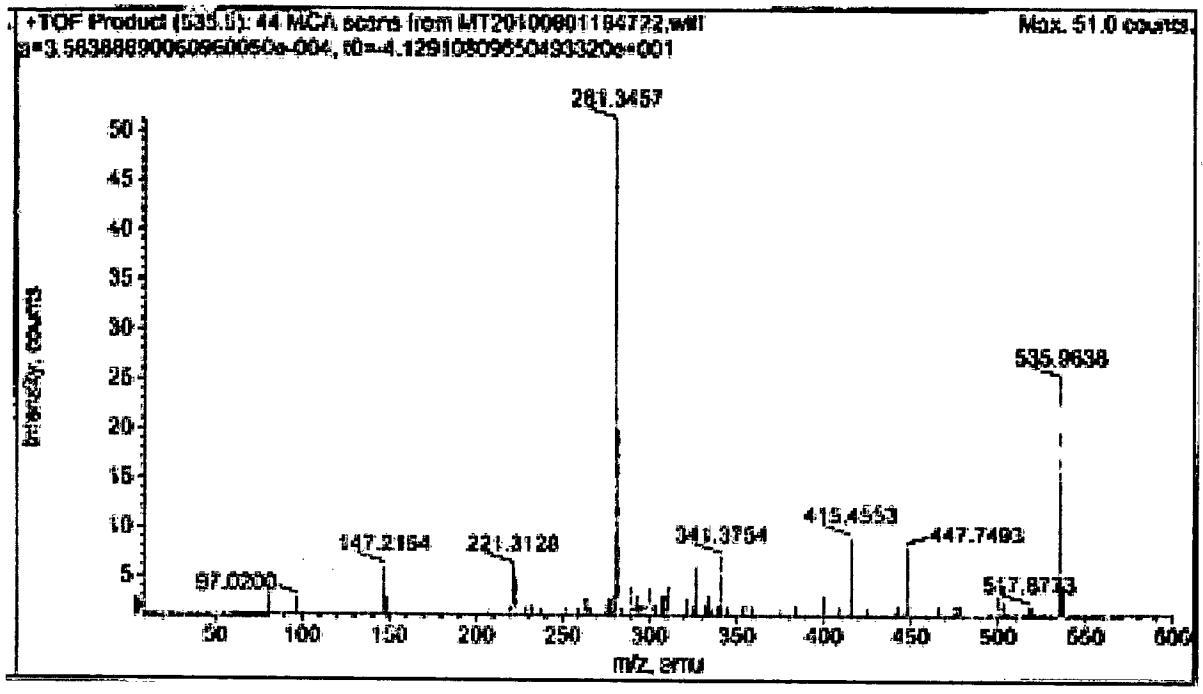



Fig.8.15. Positive MS/MS spectrum of the glycerolipid with $[M+H]^+$ ion at m/z 535

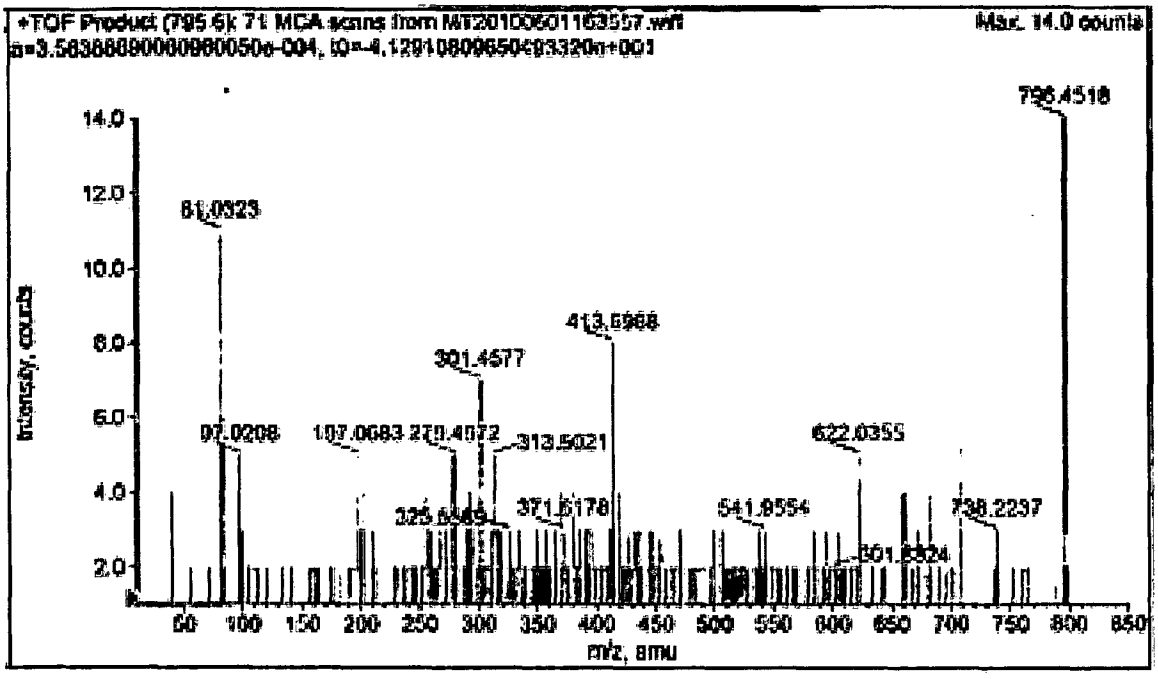



Fig.8.16. Positive MS/MS spectrum of $[M+H]^+$ ion at m/z 796

413. The presence of palmitoleoyl group was evident from signal at *m/z* 542 resulting from the elimination of palmitoleic acid (C16:1) from the molecule.

The product ion spectrum of the molecular species at *m/z* 521 is illustrated in Figure 8.17. It represents lyso-2-oleoyl -3phosphatidyl choline-sn-glycerol. The main fragmentation pathway observed here is the formation of ion at *m/z* 416 originating from the loss of 105 amu, corresponding to the loss of choline moiety as N,N,N-trimethylethanolammonium cation. The intense fragment at *m/z* 282 is consistent with the molecular mass of oleoyl group placed at the sn-2 position being stericaly more favourable. Its elimination from the molecule results in the formation of fragment *m/z* 239. The loss of trimethyl amine group leads to the fragment ion at *m/z* 460.

Tandem MS scanning experiment of protonated molecular ion $[M+H]^+$ at *m/z* 505 (Fig. 8.18) showed the base peak at *m/z* 322 due to the elimination of dimetylamino phosphoethanolamine moiety along with the glycerol carbon from the molecule. Fragmentation of the ester bond led to the ion at *m/z* 239 indicating the presence of palmitoyl group. Structure, 1-phosphatidyl-2-acyl (C16:2) -3-phosphoglycerol was assigned to this phospholipids with molecular mass of 505 amu. Thus, *Skeletonema costatum* seems to be rich in phospholipids.

## Discussion

### *Cell Abundance, Growth Rate and Chlorophyll a Concentrations*

Bloom characteristics of phytoplankton in the sea are determined by complex relationships of several biological and physico-chemical factors. Since most of

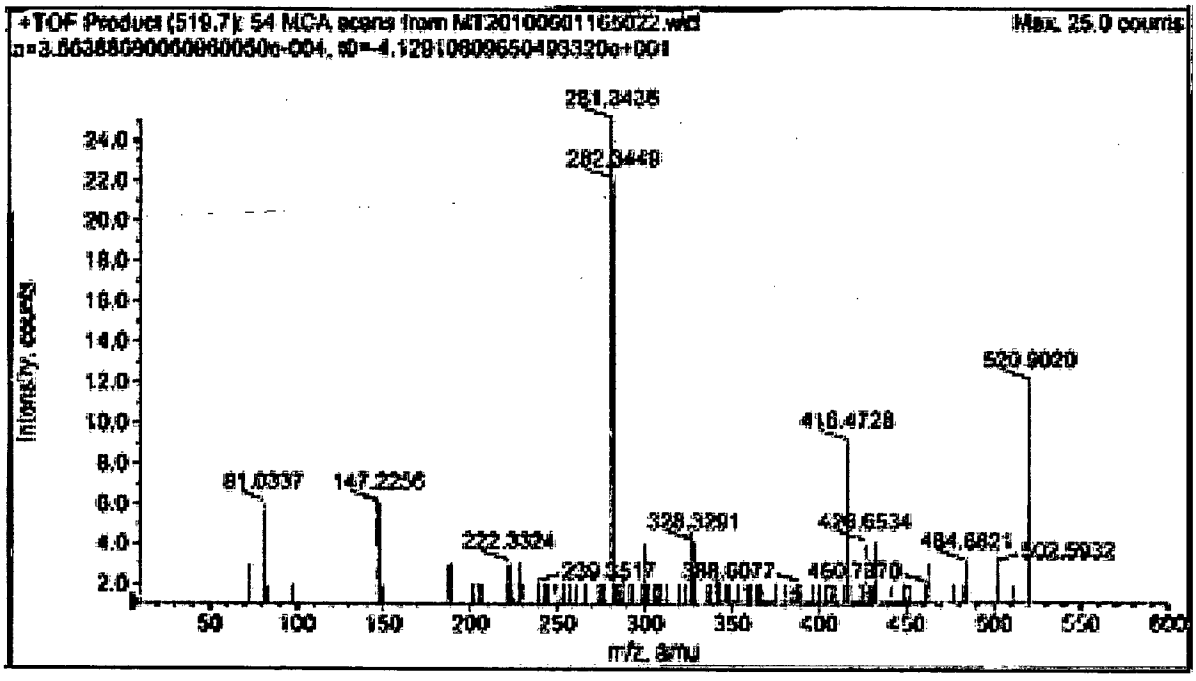



Fig.8.17. Positive MS/MS spectrum of the glycerolipid with $[M+H]^+$ ion at m/z 521

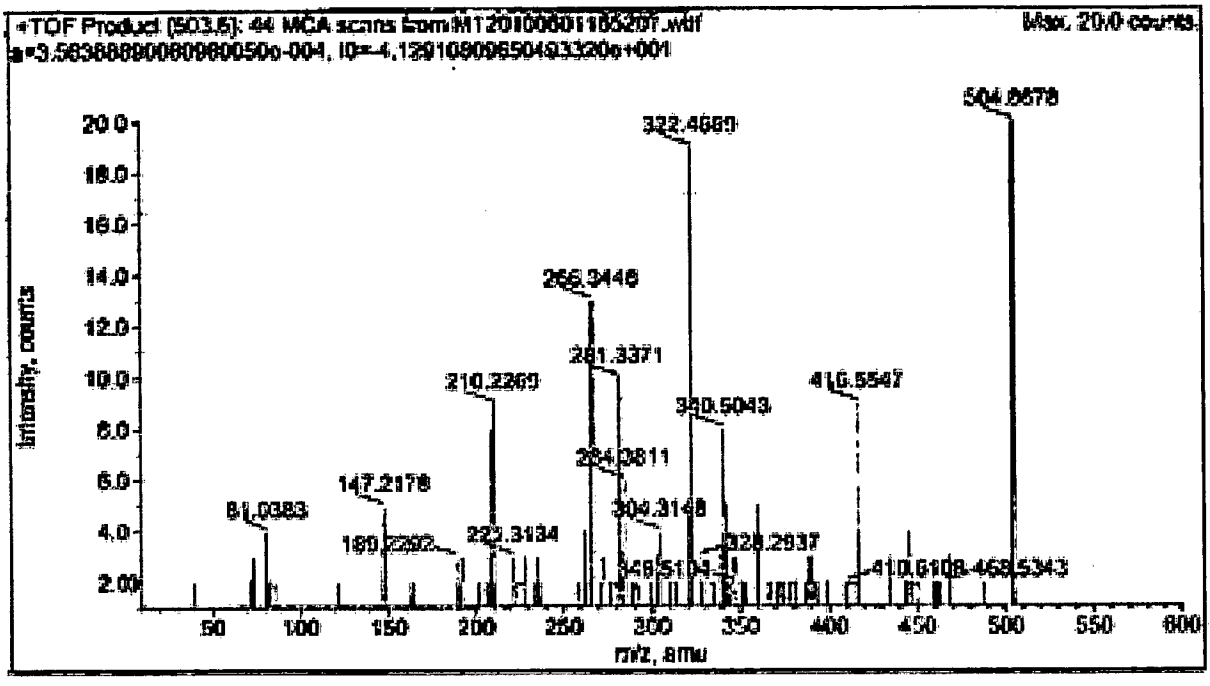



Fig.8.18. Positive MS/MS spectrum of the phospholipids with $[M+H]^+$ ion at m/z 505

these factors can be controlled in laboratory experiments, evaluation of the influence of specific parameter(s) is possible. Although the laboratory conditions differ substantially from nature, laboratory studies do provide important insights for understanding the functioning of biological systems, including effects of nutrients on bloom dynamics (total abundance, chl *a* etc.) of phytoplankton.

In the present study, the culture of all diatoms species (except *Navicula* sp) grown at high light intensity grew faster, attaining high cell abundance than all four other of the cultures grown at low light ($P < 0.05$). This observation is in accordance with other studies (Gilstad and Sakshaug, 1990; Hegarty and Villareal, 1998), results of which prove that the growth rates of specific phytoplanktonic algae are better under higher light intensity and prolonged photoperiod. Such enhanced growth in low light could corroboratively attributable to the fact that benthic diatoms are efficient in utilizing low irradiances for inorganic assimilation (Kronkamp et al., 1998; De la Peña., 2007). Benthic microalgae exposed to relatively lower irradiances are reported by several authors (Morris, 1981; Rivkin and De Laca, 1990; De la Peña, 2007) to have distinct low level photo adaptations to enhance their growth. With higher cell density and growth rate it is apparent that *Navicula* sp grew better, faster under low light intensity than when exposed to higher light.

The chl *a*, being the most dominant photosynthetic pigment in phytoplankton, has a special importance since it is often used to estimate algal biomass and production from *in situ* and remote sensing studies in nature (Develi et al., 2006). Most of these studies assume that there is a linear relationship between phytoplankton biomass and chl *a* concentration (Mitbavkar and Anil, 2008). However, as observed in this

study, there seen to be several intrinsic factors affecting chl *a* contents of phytoplankton.

It is apparent from the present observations that chl *a* content per cell differs among species as well as under different concentrations of nutrients and light intensities. Although chl *a* is often used as a universal biomass marker, fluctuations in chl *a* concentrations have been observed among various classes of algae (Reynolds, 2006). Cell size has also been suggested as a reason for such differences among different algae, and therefore one would expect that total chl *a* increase with increasing cell volume. From the biovolumes of the diatoms used for culture experiments, it is discerned that per cell chl *a* is affected also by the varying biovolums within the species.

It was clear in this study that chl *a* content per cell decreases under high light intensities. Results of this study are similar to the ones reported by Cullen (1982); Mocka and Kroona (2002) and Develi et al. (2006) who highlighted that the chl *a* content per cell is affected by changing light intensity. As also noted by Geider et al. (1998) and Mocka and Kroona (2002) it is appear that at low light, almost all carbon and organic nitrogen produced in the cell are converted into pigments and protein for photosynthesis.

***Biochemical Composition***

It has been known that nitrogen availability affects synthesis and storage of cellular compounds such as pigments, proteins, carbohydrates, amino acids, nucleic acids and lipids (Lourenço et al., 1997). Phosphorus is also an essential component for

the algal metabolism, so its deficiency in algal cells could also affect the synthesis of biochemical molecules and compounds (Kilham et al., 1997). In addition, it has been well documented that silicate limitation leads to increased lipid production in diatoms, more than any other macronutrient limitation (Shifrin and Chisholm, 1981; Roessler, 1988; Diekmann et al., 2009). There is much evidence that lipid storage could occur as a response to reductions of the nitrogen and phosphorus in the environment (Siron et al., 1989; Kilham et al., 1997; Li et al., 2005. etc). In the present study, the total lipid was significantly affected by changing the nutrient concentrations ($P < 0.05$) in four of the five diatoms species studied. In *Navicula* sp however, the variation of its concentration was not statistically significant ($P= 0.136$). The highest value of lipids in the experimental sets with low nutrient concentrations (H-f/2) could be attributed to nutrient depletion. The increase in the lipid content in the low nutrient concentrations agrees with the findings of Shifrin and Chisholm (1981), who observed that the mass of lipid per cell doubled under silicate depletion. Under nutrient stress, microalgae generally produce substantially less polar lipids and more of neutral lipids (Ben-Amotz and Avron, 1985) and neutral lipids were mostly associated with energy and carbon storage (Smith et al., 1993). Roessler (1988) found that the increase in lipids appeared to be because of an increase in the neutral lipids, particularly triglycerides, for the diatom *Cyclotella cryptica.*

The protein concentration did not commensurately increase in media with higher nutrient concentrations. Sarthou et al. (2005) and Uriarte et al. (2006) reported that diatoms vary in their ability to acclimate to high nutrient availability. Generally,

protein is the major macromolecular pool of intracellular nitrogen (Geider and La Roche, 2002), thus nitrogen consumption from the medium has an impact on protein synthesis (Smit et al., 1997). Harrison et al. (1990) and Kilham et al. (1997) found lower protein content in a variety of N-limited algae than in N non-limited control cells. It was the case in this study for *Skeletonema costatum* at low light intensity and *Thalassionema nitzschioides* at high light intensity. In these two species, total protein contents in the low nutrient concentration treatments (H-f/2) were significantly lower than those of the higher nutrient concentration treatments (D-f/2; Fig.8.4). In contrast, the highest accumulation of protein in the species of *Navicula* sp was observed under H-f/2 treatments at both light intensities. This may attributed to the availability, especially of nitrate in the culture media, and this was clear from the continued exponential phase of this species until day 8.

Sterner and Robinson (1994) found no difference in the protein content of *Scenedesmus* sp cultured under moderate N limitation or severe P limitation. Similarly, the present results also highlighted that the amount of protein *Asterionella glacialis* at both high and low light intensities, and *Skeletonema costatum* at high light intensity, exhibited no obvious change under various nutrient treatments. For these two species, the changes in total protein content per cell were much lower than those of both total lipid and carbohydrate content as Zhao et al. (2009) proposed, this may be due to the fact that protein is a significant structural and metabolic component of all algal cells.

The increases in the quantities of carbohydrates under low nutrient concentrations (H-f/2) treatments and their decreases in the high nutrients concentrations (D- f/2)

in most diatom species studied in this experiment could be ascribed to depletion of nutrients on one hand to the possible alteration of Redfield ratio as a consequence of growth and metabolic maintenance on the other. Several studies reported that the carbohydrate levels were observed to increase under silicate limitation (Lynn et al., 2000; Diekmann et al., 2009). Other studies have also shown an increase in carbohydrate of diatoms under phosphate limitation (Darley, 1977; Lynn et al., 2000). Besides a structural function, carbohydrate accumulation has been suggested for long-term storage of excess energy under nitrogen or phosphorus limitation (Myklestad and Haug, 1972; Myklestad, 1974; Verity et al., 1988; Fernandez et al., 1992; Van Rijssel et al., 2000). Nutrient limitation can cause an increase of polysaccharides release by cultured diatoms, possibly because of a higher photosynthetic rate under P limited conditions (Alcoverro et al., 2000; Urbani et al., 2005). Guerrini et al. (2000), from their work with the benthic diatom *Achnantes brevipes*, hypothesised that accumulation and release of excess photosynthetic products under P-limitation may occur due to a slow down of carbohydrates catabolism.

Our results confirm the relevance of nutrient limitation in determining an increase of carbohydrates production and highlight species-specific differences in the amount of these organic compounds.

Often, changing light intensity is shown to bear a dramatic effect on the biochemical profiles of microalgae (Thompson et al., 1990; Brown et al., 1996). In the present study, total cellular carbohydrate content and total protein in cells from cultures grown under the different light regimes showed greater differences than

lipid. At low irradiance, carbohydrate was utilized and protein synthesized (Terry et al., 1987; Abid et al., 2008).

It was clear from this study that all the species grown under HLI contained less protein compared to ones grown under LLI. The protein content increased in low light in large part because of the increased of cell pigment content. At low light, almost all carbon and organic nitrogen produced in the cell is converted into pigments and proteins for photosynthesis (Geider et al., 1998; Mocka and Kroona, 2002; Leonardos' and Geider, 2004). Chan (1978) reported variability of the cellular total protein content for many diatoms and dinoflagellates without significant correlations with irradiance levels. Leonardos and Geider (2005) found that the total protein of the cryptophyceae *Rhinomonas reticulate* was more under low light than high light level. Result of the present study agree with those of Leonardos'and Geider (2004) who observed more than double of cellular protein in the species of *Chaetoceros muelleri* under low light condition.

Studies of Thompson et al. (1991) and Mocka and Kroona (2002) suggested that high-light grown cells frequently contain more carbon and carbohydrates than low light grown cells. This was as well the case in the present study. All diatoms species accumulated higher carbohydrate under HLI. Culture experiments with *Skeletonema costatum* have shown that the production of storage carbohydrates is most pronounced in cells exposed to saturating irradiance, that is, when photosynthetic rates are higher than metabolic rates (Hitchcock, 1980). Claustre and Gostan (1987) reported similar results with the haptophytes *Hymenornonas elongata* and *Isochrysis galbana*, cultured under different light levels. Lower concentrations of

carbohydrate in the cultures receiving low light are probably associated with polymeric glucose breaking down during the dark period to provide material and energy for metabolism, cell division, and nitrogen uptake (Brown et al., 1996). Diurnal rhythms in carbohydrate metabolism have been observed in the diatom *Skeletonema costatum* (Virum et al., 1986): levels of polymeric glucan increased throughout the light period, then decreased during the night. Similar to culture experiments, Van Oijen et al. (2003) attributed low carbohydrate production at the Antarctic Polar Front south of South Africa in deeper water due to low light conditions.

Although the cellular total lipid content in the diatoms species except *Thalassionema nitzschioides* decreased with decreasing light intensity, the changing of light intensity had significant effect only on the cellular lipid of *Navicula* sp. Al-Hasan et al. (1989) observed that the total lipid content of photosynthetic eukaryotes decreases in the dark. In addition, Mocka and Kroona (2002) found that the pool of lipids, particularly the storage lipid triacylglycerol remained constant in three Antarctic sea ice diatoms cultured under different light levels.

Various studies conducted either in aquatic ecosystems or nutrient-saturated cultures demonstrated that the protein synthesis was higher than accumulation of carbohydrates and/or lipids in the diatoms species (Terry et al., 1987; Araujo and Garcia, 2005; Abid et al., 2008). In this study also it was found that protein synthesis was higher than accumulation of carbohydrates and lipids in all diatoms species.

Over all, the capability of accumulation of total protein, lipid and carbohydrate is different between the examined diatom species. The species of *Thalassionema nitzschioides* had the highest biochemical contents and *Navicula* sp. the lowest. Cell size has been suggested as a reason for difference in concentration among different algae (Reynolds, 2006). Therefore, one would expect that total biochemical components increase with increasing cell volume

In order to see if the biovolume of the diatom species used in the culture experiment had any impact on cellular constitutes, these parameters were compared amongst themselves. It was clear that the cellular biochemical compositions on the whole were affected by the varying biovolume (Table 8.4). Among these species *Navicula* sp possess the smaller biovolume (mean: 335 $\mu m^3$) and lower concentrations of biochemical constituents. In general, the biochemical constituents were higher concentrations when the biovolumes were larger. The variations in protein, carbohydrate and lipid content were affected due to changing nutrient regimes and light intensities thus, inturn, due to the mean biovolume of the examined species. In essence, there appears to be a linear relationship between biovolume and biochemical composition.

***Metabolites from Asterionella glacialis and Skeletonema costatum***

To the best of my knowledge no explicit data on lipid composition of *Asterionella glacialis* exist. In this study it was found that 1,2 acyl phosphatidyl glycerol from the major lipid compounds in this species. Phosphatidyl glycerol in particular is found in all photosynthetic organisms (Benning 1998). The monounsaturated fatty

Table 8.4. Mean biovolumes and overall cellular protein, carbohydrate and lipid contents in diatoms grown in f/2 media at different intensities of light (LLI: low light intensity and HLI: high light intensity)

| **Species** | **Biovolume ($\mu m^3$)*** | **Protein (Pg cell $^{-1}$)** | | **Carbohydrate (Pg cell $^{-1}$)** | | **Lipid (Pg cell $^{-1}$)** | |
|---|---|---|---|---|---|---|---|
| | | **LLI** | **HLI** | **LLI** | **HLI** | **LLI** | **HLI** |
| *Asterionella glacialis* | 1731 | 82.35 | 54.35 | 11.30 | 39.80 | 5.30 | 5.45 |
| *Melosira nummuloides* | 4121 | 65.30 | 50.70 | 23.80 | 31.90 | 43.50 | 51.30 |
| *Navicula* sp | 335 | 20.15 | 13.20 | 3.45 | 3.70 | 7.45 | 8.90 |
| *Skeletonema costatum* | 599 | 78.90 | 43.65 | 11.80 | 11.45 | 4.25 | 9.85 |
| *Thalassionema nitzschioides* | 1031 | 222.20 | 174.90 | 37.20 | 79.70 | 58.30 | 42.70 |

* Mean biovolume calculated from the field samples

acid $\Delta^3$- trans-hexadecenoic acid [16:1] (3t) was also seen from *Asterionella glacialis.* De Roeck-Holtzhauer et al. (1993) found that 16:1 (palmitoleic acid) was the major monosaturated acid in *Chaetoceros calcitrans* and *Skeletonema costatum.* Moreover, Clarke et al. (2007) have shown that the injection of free fatty acid, cis-6-hexadecanoic acid (C16:1 n-10), is effective for treating systemic *Staphylococcus aureus* infection in a mouse model.

The polyunsaturated aldehydes (PUAs) 2E, 4Z-heptadienal was the major compound identified from the diatom *Skeletonema costatum* using NMR complemented with mass spectrometry (D'Ippolito et al., 2004).

*Skeletonema costatum* is known to produce oxilipins i.e. polyunsaturated aldehydes (PUAs) 2E,4Z octadienal and 2E,4Z heptadienal derived from chloroplast glactolipids/plasma membrane phospholipids(D'Ippolito et al., 2004; Fontana et al., 2007). By keeping this in mind these molecules were looked for in the ESI-MS profile (Fig.8.14) of the crude extract not as aldehydes but as the corresponding carboxylic acids as they are unstable as aldehydes. These molecules were derivatized with carbethoxyethylidene-triphenylphosphorane (CET-TTP) for their easy detection before the analysis by LC-MS. Instead of signals for these molecules, the most prominent signal at m/z 149 was looked for which corresponded to $[M+Na]^+$ sodium adduct of carboxylic aci of 2E, 4Z heptadienal. The signal at m/z 341 was assigned to sodium adduct of C-14- hydroxy EPA (eicosapentaenoic acid) which is reported as precursor of 2E, 4Z heptadienal

Wichard et al. (2005) reported that several diatom species produce a wide range of secondary metabolites, including volatile polyunsaturated aldehydes (PUAs), such

as 2E,4E/Z-heptadienal, 2E,4E/Z-octadienal, and 2E,4E/Z-decadienal. Diatom *Skeletonema marinoi* [formerly *S. costatum*- (Sarno et al., 2005)], is reported produce C7 aldehyde 2*E*-4E/*Z*- heptadienal, as well as C8 aldehydes 2*E*-4E/*Z*-octadienal and 2*E*-4E/*Z*,7Z-octatrienal, which are derived from eicosapentaenoic, hexadecatrienoic, and hexadecatetraenoic acid, respectively (D'Ippolito et al., 2004). It is well documented that these compounds are produced upon the mechanical wounding of diatom cells, thus representing a wound-activated defence of the unicellular algae (Pohnert, 2000). PUA production is initiated by the release of unsaturated fatty acids from galacto- and phospholipids that occurs immediately after cell disruption (Pohnert, 2002; D'Ippolito et al., 2004).

This species also contained interesting quantities of 18:1 (oleic acid) among the monosaturated acids and 18:2 (linoleic acid). Many studies reported these compounds from *Skeletonema costatum* (Berge, et al., 1995; Yan et al., in press). De Roeck-Holtzhauer et al. (1993) found a high content of oleic acid and linoleic acid from the marine green algae species *Tetraselmis suecica.*

Diatoms are among the richest primary sources of the polyunsaturated C-16 acids particularly, 6,9,12 hexadecatrienoic acid (C16:3) and 6,9,12,15 hexadecatrienoic acid. These are two major fatty acid components of diatom glycolipids (Fontana et al., 2007). From the crude extract of *Skeletonema costatum*, a signal at m/z 271 corresponding to sodium adduct of C16:4 fatty acids was observed, while C16:3 was not detected as there was no corresponding signal at m/z 273.

D'Ippolito et al. (2004) identified a glycerolipid, from *Skeletonema costatum,* whose sodium adduct is having molecular mass of 795 $[M+Na]^+$. A molecular

species having nearly the same molecular mass i.e. *m/z* 796 but the fragmentation that was observed in this study does not agree with the fragmentation of the reported glycerolipid. The reported glycerolipid is expected to display peaks at *m/z* 493 and 543 due to the elimination of fatty acids eicosapentaenoic acid (C20:5; EPA). and hexadecadienoic acid (C16:2). However, these signals were not observed in the molecule identified in this study suggesting that the two molecules are not identical.

An understanding of the growth characteristics of marine phytoplankton is of greater pragmatic relevance in the current scenario of rapid decline in many fish and other seafood stocks as they are currently exploited above the maximum sustainable level. As such, many stocks could be vulnerable to extinction if current practices of overfishing persist. For instance, the Food and Agriculture Organization reported that ~ 75% of the native fish stocks were fully exploited, overexploited, depleted or recovering from depletion (FAO, 2007). It has been widely suggested that the increased demand for seafood may be met by expanding the aquaculture activity. In this context, all experiments helping to realize the rearing potential of autotrophic phytoplankton, the primary food organisms are of great significance. The ease of growing all five species selected for this study seems to serve as good candidates for mass culture.

# Chapter 9

## Summary

Marine Planktonology is a subject pursued globally for over 150 years, though its systematic analysis exploded with the tasks of segregating and classifying the faunal components of the biological collections during the Challenger Expedition (1872-1876). With the advent of compound microscopy techniques, the microscopic floral and faunal taxonomy achieved greater strides and brought out an exciting array of much desired portrayal of morphologic differentiation and, as a consequence, publicizing the existence of microscopic, myriad, and munificent life forms. The present day planktonology has made deep inroads, much to the delight of ecobiologists. Region/season/column specific descriptions of their assemblages keep gaining attention globally. This is because, such information based knowledge generation would help the mankind to appreciate the impacts of Global Change at large.

The Arabian Sea is a highly complex oceanic basin, encompassing one end of the spectrum, eutrophic upwelling, downwelling and oligotrophic stratified environments on the other end. All these environments are strongly influenced by biannual monsoon winds that blow from southwest (SW) between June and September, and northeast (NE) during December-February. 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. A period of low freshwater input during pre-monsoon months (February-May), followed by a period

of increased freshwater flow during the monsoon season (June-September) is typical of the west coast of India. The rainfall patterns impact a variety of features such as nutrient load, salinity variation and water flushing rates that directly or indirectly affect phytoplankton standing crop and its productivity in this area. All of these factors also play an important role in guiding or regulating phytoplankton species composition, abundance and succession.

For this study, monthly sampling was carried out to describe the spatio-temporal variations in some of the important aspects of phytoplankton along the coast of Goa from four locations selected on the basis of their disparate salinity regime. Standard protocols were followed for sampling and the analyses of the samples. The main parameters analyzed were chl *a,* phytoplankton abundance and species composition in addition to the most essential chemical parameters (nitrate, phosphate, silicate, dissolved oxygen). This study covered four different locations from the coast of Goa, among these sampling from Chapora and Anjuna for phytoplankton related investigations is done for the first time.

Species composition and monthly differences of phytoplankton were studied to understand their responses to the variations of environmental parameters and the effects of monsoon in this area. Relationships between nutrient concentrations and dynamics of phytoplankton community in terms of their monthly shift, predominance, species diversity and succession were assessed. Experiments were set up to observe the differences of growth and biochemical composition of diatoms to understand the effect of varying concentration of nutrients and light on these aspects.

Following are some of the major observations from this study

- From the monthly data sets from four disparate locations chosen for this study, cell abundance and biomass (chl *a*) exhibited wide fluctuations; with the highest cell abundance and chl *a* values occurring during September 2008 followed by the post-monsoon months. Spatially, the low saline location (Siridao) exhibited high phytoplankton abundance.
- The general picture of phytoplankton abundance and biomass (chl *a*) is impacted rather strongly by rainfall which is the cause of alterations to the nutrient inputs and salinity fluctuations. This reflection is further supported by the observed decline in phytoplankton abundance during the monsoon season, when high rainfall limit the water residence time and the time available for the buildup of phytoplankton biomass, which appeared to be ideal during the post-monsoon months.
- The most dominant species observed in the study area, have smaller surface areas, as a consequence smaller biovolumes, and lower per cell carbon. *Coscinodiscus marginatus* was the dominant species in the study area had greater biovolume. This species was particularly dominant off Chapora. Its larger cell diameter and large biovolume qualify it to be an important source of living carbon biomass in the estuary.
- A total of 150 species of diatoms belonging to 56 genera, and 25 species of dinoflagellates belonging to 7 genera were identified during this study. Being the most abundant with the greatest number of species observed, diatom community is apparently the most pivotal autotrophic biota in the

study area. Such dominance is aptly enabled due to the high nutrient and adequate silicate concentrations as can be discerned from the observations. The most important species were the centric diatom *Skeletonema costatum* followed by the pennate diatom *Thalassionema nitzschioides*.

- Among diatoms, number of pennales species were higher while the cell abundance of the centrales was more than those of pennales. Bloom proportions of some centric diatoms at many sampling locations during different periods of the year might also imply that the centrales proliferate when the nutrient concentrations and salinities are favorable. In the overall, both the number of species and diversity indices were higher in the Dona Paula Bay.
- The canonical component analysis results clearly indicate that salinity is the most important environmental variable for explaining diatom distribution in the study area.
- The highest abundance of dinoflagellates at the all locations was during September 2008 followed by October 2007. In the overall, higher temperature and salinity appear to favorable the growth of dinoflagellates. Among these, *Ceratium furca* and *Prorocentrum micans* were the most dominant ones.
- Among the total of 12 known harmful algae recorded during this study, two are of diatom species and 10 of dinoflagellate species belonging to 6 genera. From this comprehensive analysis, it is evident that the toxic phytoplankton species are rather common in the Indian waters. The fact that as many as 12

species known to be potentially toxic were found in the near-shore waters is an indication that any or all of them can be of serious human/environmental health concerns when the natural factors become favorable for their proliferation.

- Among the dinoflagellates recorded during this study, *Alexandrium minutum*, known to be potentially toxic is being observed for the first time in the near-shore waters of Goa. Known for paralytic shellfish poisoning (PSP), it was most prevalent off Siridao.
- From the experimental analysis of the growth and biochemical composition of five different diatom species, *Navicula* sp attained the highest cell density and growth rate under low light intensity (LLI) than under high light intensity (HLI).
- The effect of light intensity on the variations in protein and carbohydrates was more than that due to the effects as a consequence of alternations in nutrient concentrations in the diatom species studied in the experiment. Total cellular carbohydrate and protein contents in cultures grown under the different light regimes showed greater differences than the lipid contents did. All the species grown under HLI contained less protein compared to the ones grown under LLI. The protein content increase in low light is ascribable in large part to the increased cell pigment contents. Also, all diatoms species accumulated higher carbohydrate under HLI. This could be attributed to the utilization of carbohydrate and synthesis of protein at low

irradiance levels. In contrast, the total lipid was affected significantly by changing the nutrient concentrations.

- The capability of accumulation of total protein, lipid and carbohydrate was different between the examined diatom species. Among them, *Thalassionema nitzschioides* had the highest biochemical contents and *Navicula* sp. the lowest. Also, it was found that protein synthesis was higher than accumulation of carbohydrates and lipids in all five diatoms species.
- From the ESI-MS profile of the methanol extract of the diatom *Asterionella glacialis,* it was found that 1,2 acyl phosphatidyl glycerol is the major lipid compound. The monounsaturated fatty acid $\Delta^3$- trans-hexadecenoic acid [16:1] (3t) was also found. The polyunsaturated aldehydes (PUAs) 2E, 4Z-heptadienal was the major compound identified from *Skeletonema costatum* using NMR complemented with mass spectrometry. *Skeletonema costatum* is known to produce oxilipins i.e. polyunsaturated aldehydes (PUAs) 2E, 4Z octadienal and 2E,4Z heptadienal derived from chloroplast glactolipids/plasma membrane phospholipids.

## A few Suggestions for Future Studies

To my mind, the results from this work do provide many important insights on phytoplankton ecology and diversity in the nearshore/estuarine water off Goa. Questions on the prevalent relationship between different ecological parameters and phytoplankton distribution are answered. Yet many more questions have arisen as an outcome of this extensive study.

Building upon this work, it is clear that continuous study of time series of the phytoplankton assemblage will certainly help to discern the dynamics, if any, in the patterns observed either temporal (monthly, seasonal, annual) or geographical/regional variability. In fact there are hardly any longer term observations from this part of the world on the trends of compositional shifts in the phytoplankton communities in particular.

While my efforts on compositional analyses are solely through microscopy, modern methods of HPLC based chemotaxonomy through quantification of a variety of phytoplankton pigments from the whole sample would prove useful in a discernment of the dominant flora in a reliable, quicker and less laborious manner. In principle, a data set that comes from different complementary methods of analyzing phytoplankton species composition is better. Thus inclusion of analyses of pigments using HPLC would prove pivotal to the biodiversity confirmation.

Another important issue addressed in this study was the measurement and gathering of extensive data on phytoplankton size and biovolume from many cells of all the dominant species extant in the waters off Goa. These characteristics are essential for details on phytoplankton ecology. It would be of great ecological importance if the carbon contents of dominant, individual species are measured for most -if not feasible for all- of the species of phytoplankton occurring in the region. In this regard, axenic cultures of phytoplankton species are needed to be made and the carbon content determined.

From this study, it is evident that the harmful algae are common in the waters off Goa. Future research in the region should focus on qualitative assessment of toxic

compounds each of the harmful species produces, and on the analyses of temporal variability in the production of such compounds. It is also worthwhile to look for low-volume high-value molecules in the phytoplankton inhabiting the low saline *vis a vis* those inhabiting the truly marine waters.

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## Publications

- **Alkawri, A.A.S.**, Ramaiah, N., 2010. Spatio-temporal variability of dinoflagellate assemblages in different salinity regimes in the west coast of India. **Harmful Algae** 9, 153-162.

- **Alkawri, A.A.S.**, Ramaiah, N., 2011. Distribution of diatom *Pseudo-nitzschia* and dinoflagellates of *Dinophysis* spp along coast off Goa. **Journal of environmental biology** 32, 65-70.

- **Alkawri, A.A.S.**, Ramaiah, N. Influence of salinity and inorganic nutrients on diatom-assemblages in a monsoon affected tropical coastal region. **Estuaries and coasts** (revision requested).