

**Spatio-temporal variation of
dinoflagellates along estuarine regions of
Goa, west coast of India**

Thesis submitted to Goa University for the award of the degree of

DOCTOR OF PHILOSOPHY

In School of Earth, Ocean and Atmospheric Sciences

By

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II Shree Ganeshay Namah II

**When You Journey With God,
Nothing Is Impossible**

Dedicated to my Family

STATEMENT

As required by the Goa University Ordinance OA-19, I state that the present thesis entitled “**Spatio-temporal variation of dinoflagellates along estuarine regions of Goa, west coast of India**” is my original research work and has not been submitted previously. The present study carried out is first of its kind from the study area mentioned.

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

Samiksha Sharad Prabhudessai

CERTIFICATE

This is to certify that the thesis entitled "**Spatio-temporal variation of dinoflagellates along estuarine regions of Goa, west coast of India**", submitted by **Ms. Samiksha Sharad Prabhudessai** for the award of Doctor of Philosophy in School of Earth, Ocean and Atmospheric Sciences is based on her original studies carried out by her under my supervision. The thesis or any part therein has not been previously submitted for any degree or diploma in any Universities or Institutions.

Prof. C. U. Rivonker

Research Guide

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Chapter-1

General Introduction

1.1. Background information

Phytoplankton are key components of the marine food chain, responsible for nearly 40% of global primary production (Falkowski et al., 1998). Among 5000 species of phytoplankton that are found, nearly 7% are known to be responsible for Harmful Algal Blooms (HABs) (Sournia, 1995). These include the eukaryotic green algae or Chlorophytes, Dinoflagellates, Cryptophytes, Chrysophytes, Diatoms, and the prokaryotic blue-green algae or Cyanobacteria (Round, 1965; Sournia, 1978; Reynolds, 1984).

Dinoflagellates are a highly diverse group of flagellates, found to occur in freshwater, oceanic, estuarine as well as continental shelf environments. A schematic morphology of a typical dinoflagellate is illustrated in Fig. 1.1. Based on the nutritional modes they are classified as phototrophic (autotrophic, mixotrophic) and heterotrophic (Narale et al., 2013). They are known to be one of the most important groups involved in the formation of HABs and toxic red tides (Anderson, 1989; Hallegraeff, 1993), a causative agent of paralytic shellfish poisoning (PSP) (Hurley et al., 2014).

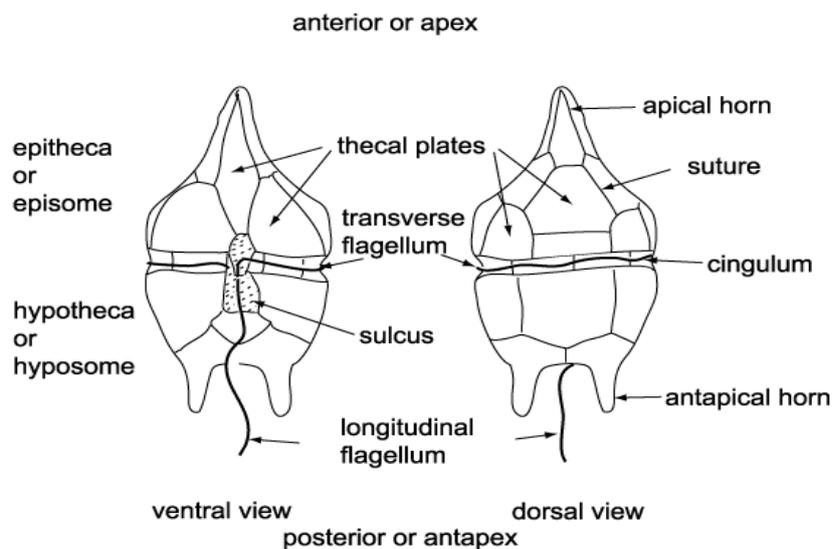


Fig. 1.1. Thecate, motile peridiniacean dinoflagellate (Fensome et al., 1996)

The life cycle of many of these dinoflagellates is characterised by a planktonic vegetative stage, and a benthic stage (Fig. 1.2). Adverse environmental conditions (high salinity, low nutrients, low light penetration) triggers dinoflagellates to undergo gamete formation (sexual reproduction). The gametes then fuse to form the planozygote and go through encystment leading to the formation of resting cysts. Resting cysts can be temporary

resting state-pellicle cyst, temporary or ecdysial cyst, or dormant resting cysts or hypnozygotes. Among these dormant resting cysts or hypnozygotes rapidly sink into the sediment and remain viable for a longer period hence are studied widely and are termed as dinoflagellate cysts. It is known that about 10% of the 2000 identified dinoflagellate species (Taylor and Pollinger, 1987) produce dinoflagellate cysts (Head, 1996) as part of their life cycle.

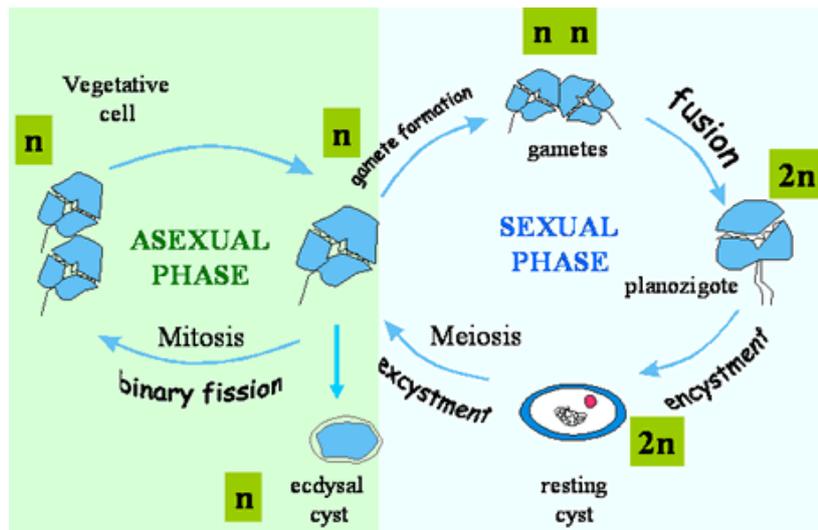


Fig. 1.2. Life cycle of dinoflagellates (Warns et al., 2013)

Christian Gottfried Ehrenberg was the first person to recognize fossilised dinoflagellate and reported his discovery in July, 1836 (Fig. 1.3). The dinoflagellate cysts are non-motile (Walker, 1984; Figueroa et al., 2008) and are typically 15 to 100 μm in diameter. These cysts are cylindrical with rounded ends and the wall is clear and is usually covered with mucous substance. Internally, the cell contains granular material, an orange-red accumulation body (that forms the colour of red tide) and numerous lipid globules.

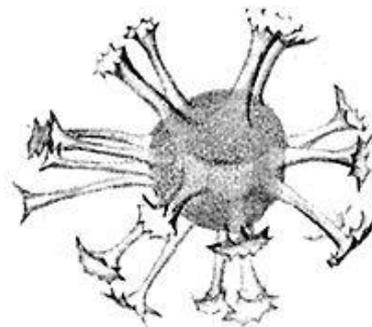


Fig. 1.3. Dinoflagellate cyst drawn by Ehrenberg in 1837

Based on the morphology, dinoflagellate cysts are classified into three major groups. The proximate cyst, the chorate cyst and the cavate cyst. Proximate cysts may develop their wall directly within the theca. They vary from spherical to peridinoid and sometimes have numerous projections on the surface. The chorate cyst is characterized by various ornaments rising from the surface of the cyst. These ornaments functionally support the cell wall of the planozygote from the cyst surface and are morphologically variable. Cyst of this type is usually spherical, subspherical or ovoidal. Cavate cysts are generally described with two or more wall layers that define a cavity.

Dinoflagellate cysts are composed of dinosporin, a resistant biopolymer that is refractory towards degradation (Fensome et al., 1993). This resistance provides high preservation potential towards physical, chemical and biological degradation (D' Silva et al., 2012). Hence it is well preserved in sediments for several years (Dale, 1983) and even up to century (Rebeiro et al., 2011).

Therefore, dinoflagellate cysts have immense ecological significance and act as “seed beds”. (Dale, 2001). Thus, they can be useful in elucidating past bloom incidences (D' Silva et al., 2012), facilitates the reoccurrence of blooms, promotes dispersion (Dale, 1983; Angles et al., 2012), important in initiating and terminating blooms (Azanza et al., 2004; Anderson et al., 2005) and can serve as proxies for anthropogenic eutrophication (Pospelova et al., 2005; Shin et al., 2011).

Many species of dinoflagellates are known to involve in the formation of Harmful Algal Blooms (red tides) (Alkawri and Ramaiah, 2010). However, only a few species among them are known to be toxic and are primary causal agents of Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), Ciguatera Fish Poisoning (CFP) (Hallegraeff, 1993). Toxic dinoflagellates produce a potent toxin (saxitoxin, brevetoxin, okadaic acid, ciguatoxin) and can eventually find a way to humans through consumption of fish or shellfish (Fig. 1.4) (Munday and Reeve, 2013). This can lead to intoxication in human causing serious health effects (Diarrhoea, vomiting, nausea, respiratory failure, abdominal cramps and pain) (Hallegraeff, 1993). Some dinoflagellate species however are only involved in the bloom formation, which can lead to the conditions such as hypoxia/ anoxia (low oxygen level) leading to detrimental effects on marine life (Heisler et al., 2008).

Harmful Algal Blooms and toxic events can be a natural phenomenon (Anderson et al., 2002). However, in past decades, ongoing eutrophication due to various anthropogenic activities (domestic and industrial discharge, agricultural runoffs, aquaculture farms, marine traffic, economic transportations, ballast water discharge and trade routes) (Mochemadkar et al., 2013; Narale et al., 2015), has led to an increase in nutrient inputs (mostly nitrate and phosphate) (Anderson et al., 2008; Heisler et al., 2008). This eventually has altered physico-chemical and biological characteristics of the coastal environments leading to effects such as HABs (Narale et al., 2015), which in recent past decades has increased in frequency and intensity worldwide (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993; Paerl et al., 2001; Anderson et al., 2002; Sellner et al., 2003; Backer et al., 2015). Hence, the study of phytoplankton dynamics, including dinoflagellates and their cysts, has gained importance.

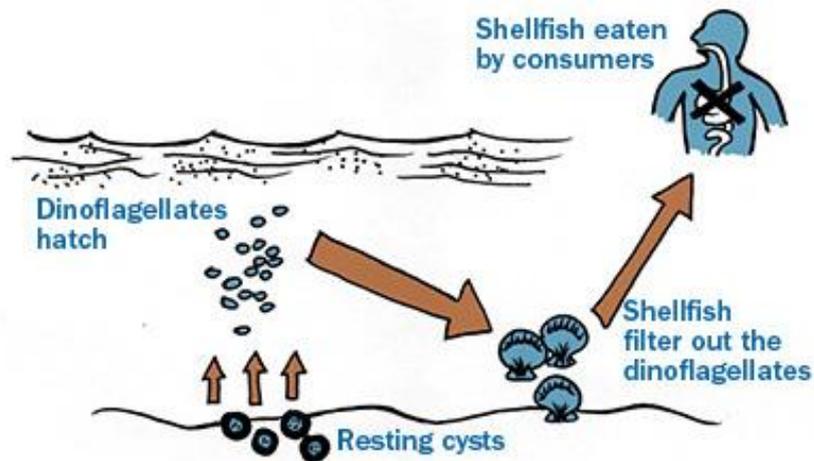


Fig. 1.4. Human intoxication due to consumption of shellfish (Munday and Reeve, 2013)

1.2. Literature review

Several studies on phytoplankton distribution (D' Costa et al., 2008; Madhu et al., 2010; George et al., 2012; Parab et al., 2013; Ahmed et al., 2015; Nassar and Fahamy, 2016; Majewska et al., 2017) suggest that, phytoplankton abundance and distribution is affected by fluctuating monsoonal regimes, particularly due to variation in physico-chemical parameters (DO, pH, temperature, nutrient concentration and salinity), and showed its peak values during monsoons. Madhu et al. (2010) reported nutrient enrichment to be favourable factor for phytoplankton proliferation. Many studies (Madhu et al., 2007; Patil and Anil, 2011; Mochemadkar et al., 2013; Pednekar et al., 2014) have highlighted

nutrient inputs (mainly nitrate and phosphate) to be important in the proliferation of phytoplankton.

Several other studies (Ahel et al., 1996; Huang et al., 2004; Chakraborty et al., 2011; Pednekar et al., 2011; Ahmed et al., 2015; Aquino et al., 2015; Fambo et al., 2015) have highlighted salinity to be the prime regulating factor in the distribution of phytoplankton, and showed its positive correlation with phytoplankton abundance.

Madhu et al. (2007) reported that decrease in salinity and pH during monsoons resulted in low phytoplankton diversity. In another study, high pH and high water residence found to be positively correlated with phytoplankton composition and biomass (Costa et al., 2009). Nazneen (1980) reported water temperature to be one of the vital factors that control the phytoplankton growth and abundance. Bharadwaja (1940) stated that both temperature and light are responsible factors for higher phytoplankton density. Few studies have reported high alkalinity to be associated with high phytoplankton density (Radwan, 1994; Anyinkeng et al., 2016).

A review on the distribution of phytoplankton in coastal, shelf and estuarine waters (Boyle and Silke, 2010), highlighted vertical water column stability and horizontal transport process to be influential on the abundance and composition of phytoplankton.

Although phytoplankton occurs naturally and supports the aquatic food web, they also pose harmful effects on the aquatic system. As primary producers, they spontaneously respond to changes in nutrient concentration occurring naturally or due to anthropogenic activities (Korpinen and Bonsdorff, 2015), which ultimately alters species composition and biomass (Moncheva et al., 2001). Anthropogenic nutrient enrichment of coastal waters overtime have given rise to issues like eutrophication and HABs worldwide (Anderson et al., 2002) which has lead to a significant economic (shellfish closures, wild or farmed fish mortalities,), public health impacts (human sickness and death from eating tainted seafood), and ecological effects (produce extremely dangerous toxins that can sicken or kill people and animals, create dead zones in the water, raise treatment costs for drinking water), on coastal and estuarine regions around the world (Anderson, 1989; Hallegraeff, 1993; Anderson et al., 2012).

The first case of human poisoning due to shellfish consumption contaminated with dinoflagellate toxins happened in 1793, in an area now known as Poison Cove

(Hallegraeff, 1993). Until 1970, PSP-producing dinoflagellate blooms of (*Alexandrium (Gonyaulax) tamarense* and *Alexandrium (Gonyaulax) catenella*) were only known from temperate waters of Europe, North America and Japan (Dale and Yentsch, 1978). Until 1988, the type species *A. minutum* was only known from Egypt (Halim, 1960), it has now been reported from Australia, France, Spain, Portugal, Italy, Turkey and the east coast of North America (Hallegraeff et al., 1991). By 1990, PSP was familiar throughout the Southern Hemisphere, in South Africa, Australia, India, Thailand, Brunei, Sabah, the Philippines and Papua New Guinea (Hallegraeff, 1993).

The first observations on blooms in Indian waters were reported by James Hornell in 1908 along the Malabar Coast to Laccadive Island (Hornell, 1908). Along the east coast of India, 33 cases of algal blooms were reported. There was also a reporting of PSP outbreak in Tamil Nadu coast in 1981. Along the west coast of India, 68 cases of algal bloom occurrences were reported and among them three were Paralytic Shellfish Poisoning (PSP) cases.

Over the last several decades coastal countries throughout the world have experienced a dramatic increase and geographic spread in HAB events (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993; Paerl et al., 2001; Sellner et al., 2003; GEOHAB, 2010). Largely due to water quality deterioration as a result of nutrient enrichment. Other factors such as increased scientific awareness of toxic species, utilisation of coastal waters for aquaculture purpose, domestic and industrial sewage disposal, and transport of dinoflagellate resting cysts through ships ballast water or associated with the movement of shellfish stocks from one area to the other. As well as due to natural processes such as, oceanic and estuarine circulation, river flow; the combined physical (e.g., currents, upwelling, etc.) chemical (e.g., salinity, nutrients, etc.) factors, coupled with unique life cycles and behaviors of some HAB forming species, result in blooms that impact coastal ecosystems and populations (Sellner et al., 2003). Basin scale circulation also act as effective vectors for distributing bloom taxa, leading to coastal blooms (Sellner et al., 2003). In addition unusual climatological conditions, dust deposition (Hallegraeff, 1993). Wind-induced upwelling, Spring tide-induced destratification and introduction of bottom recycled nutrients can promote growth of HAB species leading to blooms (Haas et al., 1981; Sellner and Brownlee, 1990).

Anderson (1997) reported the transport of *Alexandrium* spp. due to coastal currents in the Gulf of Maine from the Bay of Fundy along the New England coast. A hurricane led the introduction of the PSP producing *Alexandrium* population from Scotian shelf into the Gulf of Maine in 1972 (Anderson, 1994).

Wind-delivered iron-rich dust from the Sahara forms blooms of *Cyanobacteria*, *Trichodesmium* spp., in the west Florida shelf, (Walsh and Steidinger, 2001). Basin Circulation led to a devastating impact on locally and afar in 1987, as *K. brevis* originating in the eastern Gulf of Mexico were transported to North Carolina, with total losses estimated at US \$25 million (Tester and Fowler, 1990; Tester et al., 1991).

Along the Benguela region of the South African coast, *K. mikimotoi* is found to be dominant in upwelling regions (Pitcher et al., 2002). Chang (1999) has reported subsequent mass mortalities caused by *Gymnodinium brevisulcatum* and upwelling induced bloom formation.

Hydrological events, such as rain-induced buoyant plume formation or delivery of micronutrients, also favoured HAB formation (Sellner et al., 2003). In the Chesapeake Bay Loftus et al. (1972) reported the increase of dinoflagellate biomass following a heavy rainfall. Graneli et al. (1993) in his study reported that selenium and cobalt from soil during heavy rains might be partially responsible for blooms of *Chrysochromulina polylepis* in the Skagerrak and Kattegat. El Niño driven lower-than-normal sea temperatures of New Zealand's northeast coast is a cause of recurrent spring *Mesodinium/Noctiluca* blooms (Rhodes et al., 2001). Maclean (1989) reported a conditional evidence for a coincidence between *Pyrodinium* blooms and El Niño-Southern Oscillation (ENSO).

Increased incidences of PSP and CFP in the Indo-Pacific were noted as a result of El Niño events (Maclean, 1989). North Atlantic Oscillations (NAO) is known to be implicated as drivers for upwelling-induced blooms along the coast in Spain (Fraga and Bakun, 1993). In estuarine waters, apart from seasonality which is found to be important in broad terms, phytoplankton population structure is affected by local factors such as the periodic rise and fall of the tide and episodic changes in river flow (Boyle and Silke, 2010). Banerjee and Kumar (2014) reported dust deposition due to dust storms enhance blooms. Reduction in predation pressure, a novel behaviour of phytoplankton is also found to be a mechanism for

HAB proliferation (Harvey and Deuer, 2012). Warmer temperature is known to be a key factor affecting phytoplankton bloom dynamics (Trombetta et al., 2019). Escalera et al. (2010) found that physically-driven accumulation due to long-shore transport along with high temperature leads to blooms.

Several studies (Banse et al., 1996; Paerl et al., 2001; Anderson et al., 2002; Imai et al., 2006, Mishra et al., 2009; Li et al., 2009; Davidson et al., 2014; Al-Azri et al., 2015) have highlighted that eutrophic conditions resulted due to anthropogenic nutrient supply (mainly nitrate and phosphate) is responsible in proliferation of phytoplankton eventually leading to HABs. Experimental studies carried out (Chapellea et al., 2010; Anderson et al., 2012) also revealed nitrate and phosphate to be the crucial nutrients in the proliferation of toxic species.

High nutrient loadings during monsoon season lead to proliferation of potentially harmful phytoplankton species (Banse et al., 1996; Anderson et al., 2002; Sellner et al., 2003; Al-Azri et al., 2015). Lewandowska (2015) reported that high growth rates, photo-adaptation to low light condition, nutrient kinetics as well as grazing resistance might also be important for bloom formation.

The 1988 bloom in the Kattegat has been related to a change in the nutrient status from nitrogen to phosphorus limitation (Maestrini and Graneti, 1991). Experimental studies from Sweden indicates that river water draining from agricultural soils, rich in humic and fulvic acids stimulate dinoflagellate blooms (Graneli and Moreira, 1990). In the Baltic Sea and in the Peel Harvey Estuary, Australia an agricultural run-off (phosphorus) found to stimulate cyanobacterial blooms (Hallegraeff, 1993).

Anthropogenic emissions of carbon dioxide (CO₂) and the ongoing accumulation in the surface ocean together with decreasing pH and calcium carbonate saturation found to impact phytoplankton community composition leading to blooms (Schulz et al., 2017). Between 1965 and 1976 a 7-fold increase of red tide outbreaks concurrent with a 2-fold increase in the COD (Chemical Oxygen Demand) loading from untreated sewage and industrial waste was seen (Hallegraeff, 1993).

A complex 'cultural eutrophication' scenario had emerged in Long Island waters, where an unusual 'brown tide' had been related to the discharge of chelators (such as citric acid) in

detergents and lawn treatments, with a suppression of zooplankton grazing due to pesticides (Casper et al., 1989; 1991).

The first harmful *Pyrodinium* blooms became evident in 1972 in Papua New Guinea. Red-brown water discolorations lead in fatal food poisoning of three children (Maclean, 1977). Since then, toxic *Pyrodinium* blooms have apparently spread to Brunei and Sabah (1976), the central Philippines (1983) and the northern Philippines (1987).

Fossilised 'dinoflagellate cysts' are known from sedimentary deposits as far back as the Triassic (230 million years ago) (Bolch and Hallegraeff, 1990). The capacity of dinoflagellates to encyst is known from the early 20th century. Later, dinoflagellate cyst formation from gamete fusion was reported, which concluded that encystment is associated with sexual reproduction (Von Stosch, 1967; 1973).

However, in 1960s and 1970s, formation of dinoflagellate cysts was regarded as a response to unfavourable conditions (Wall, 1965; Anderson and Wall, 1978).

Identification of dinoflagellate cysts is the first crucial step in many ecological studies. Taxonomic studies (Bolch and Hallegraeff, 1990; Godhe et al., 2000; Orlova et al., 2004; Aimei et al., 2007; Uzar et al., 2010) on recent dinoflagellate cysts were carried out and highlighted the presence of toxic species. At the present time, dinoflagellate cyst identification with the help of molecular methods has gained importance due to limited taxonomic keys (Gao et al., 2017).

In recent years the formation of dinoflagellate cysts has been detected under various conditions, in both culture and in nature (Bravo et al., 2010). The dormancy features of dinoflagellate cysts is found to vary according to environmental conditions, mostly temperature and nutrient levels (Anderson, 1998; Figueroa et al., 2005) and its duration is affected by the amounts of intracellular storage products (Steidinger and Haddad, 1981). In addition, temperature, salinity and SPM are also found to be the crucial parameters that are known to affect the dinoflagellate cysts distribution in sediments (D' Costa et al., 2008; Price et al., 2016).

A nutrient concentration in surface waters is found to exert strong control on the distribution of dinoflagellate cysts (Marret and Zonneveld, 2003). Mainly phosphate concentration is found to positively correlate with the distribution of dinoflagellate cysts

(Bokelmann and Zonneveld, 2007). A study carried out by Kim et al. (2009) have stated that increase in nutrient loading from domestic sewage and effluent, and industrial pollution leads to a shift in autotrophic to heterotrophic dinoflagellate cyst composition. Hence, they can also be used as proxies for anthropogenic eutrophication. Some studies (Shin et al., 2010; Sildever et al., 2015) have mentioned the effect of salinity on dinoflagellate cyst distribution and stated a positive correlation of dinoflagellate cyst abundance with salinity.

In the Eastern South Atlantic, dinoflagellate cysts of the genus *Echinidinium* are observed in the upwelling waters (Zonneveld et al., 2001a) and in the western equatorial Atlantic corresponding with high nutrient input from river discharge (Vink et al., 2000). Figueroa et al. (2005) reported dinoflagellate cyst formation by the *Alexandrium catenella* in response to phosphorous limitation. The effects of temperature and salinity on the growth of *A. insuetum* were also investigated (Shin et al., 2014). Grzebyk and Berland (1996) in his study on *Prorocentrum minimum*, reported high number of dinoflagellate cysts due to temperature stress. Shin et al. (2013) revealed that pH affects the morphology and viability of *Scrippsiella trochoidea* dinoflagellate cysts in the hypoxic zone of a eutrophied area.

The study on the dinoflagellate cysts from New Bedford Harbor and Apponagansett Bay exhibit effect of environmental change due to human activities in the watersheds over the period of 500 years (Pospelova et al., 2002). Analysis carried out from 3 Buzzards Bay (Massachusetts, USA) embayments revealed capability of dinoflagellate cysts as biological indicators of environmental conditions (Pospelova et al., 2005).

Dinoflagellate cysts are buried below the sediment, which are exposed to both darkness and low oxygen conditions. This has made to carry out the studies on the effects of anoxia and light conditions on dinoflagellate cysts survival. Anderson et al. (1987) demonstrated that dinoflagellate cyst germination was inhibited by anoxia in six dinoflagellate species. Dinoflagellate cyst germination in response to darkness differ according to species type (Anderson et al., 1987; Kremp and Anderson, 2000) whereas anoxia may be the vital factor that prevents germination as it results in complete inhibition (Anderson et al., 1987). Zonneveld et al. (2007) reported high dinoflagellate cyst abundance in oxygen-deficient zones compared to well oxygenated sediments.

Few other studies (Pospelova et al., 2002; Richerol et al., 2008; Aydin et al., 2015) have highlighted eutrophication due to pollution by mines effluents, due to ballast water transport leads to a decline in species richness and total cyst abundance.

A single ballast tank was estimated to contain more than 300 million toxic dinoflagellate cysts which could be germinated into toxic cultures (Hallegraeff and Bolch, 1991). Hallegraeff and Bolch (1992) reported the presence of *G. catenatum* dinoflagellate cysts in four ballast water samples in Australian ports from both Korea and Japan. In 1986, 1987 and 1991 a dense dinoflagellate blooms lead in the closure of 15 shellfish farms (Hallegraeff and Sumner, 1986). Similarly, a toxic dinoflagellate *Alexandrium catenella*, caused the closure of shellfish farms in Port Phillip Bay, Melbourne (Hallegraeff, 1993).

Another vector for the dispersal of dinoflagellate cysts and vegetative cells from one area to another is by the transfer of shellfish stocks as the faeces and digestive tracts of bivalves carries dinoflagellate cysts or viable cells (Bricelj et al., 1998).

Baldwin (1987) and Matsuoka et al. (2002) in his study on dinoflagellate cysts stated that physical process such as sedimentation and tidal flow pattern may influence sinking rates and hence the dinoflagellate cyst distribution. Few other studies (Kawamura, 2004; D' Silva et al., 2011; Narale et al., 2013; Wijethilake et al., 2015) have mentioned the effect of grain size on the distribution of dinoflagellate cysts in sediments and revealed high dinoflagellate cyst abundance in fine grain-sized (silty-clayey) sediments. Further, studies have explained the lower rates of dinoflagellate cyst germination in the presence of potential grazers (Rengefors and Anderson, 1998).

Some studies have implicated them as the source of toxins (Yentsch and Mague, 1979; Cembella et al., 1988) whereas other studies have highlighted their role in species distribution and expansion (Hallegraeff and Bolch, 1991; Hallegraeff, 1998). As dinoflagellate cysts are also regarded as microfossils they are used extensively in oil exploration (biostratigraphy) (Persson and Rosenberg., 2003).

Thus a baseline data of dinoflagellate cysts will be useful in designing models for prediction, control and mitigation of HAB events.

The coastal zone is characterized by a variety of landforms like the estuaries, lagoons, beaches and islands. Estuaries are one of the most important environments of the coastal

zone. They serve as nursery grounds for marine life and also support the economy of local people due to fishing and shellfish collection activities. They are also vital sites of ports and harbours, shipping, transportation and industry. Various anthropogenic activities (agricultural, transportation, dredging, dumping, shoreline reconstruction to accommodate human housing, and industries) are carried out along the estuaries that affect the health of the estuaries threatening their integrity. Hence, estuaries are known to be the most heavily affected coastal ecosystems around the globe (Kennish, 2002). Nutrient inputs due to various anthropogenic activities carried out along the estuaries lead to eutrophic conditions that can further give rise to HABs or proliferation of toxic species in such areas. Hence, this would lead to a serious risk on marine life as well as human health.

Goa is among one of the states of India. It is located on the west coast of India (140° 53' 57" to 150° 47' 59" North latitudes and 73° 40' 54" to 74° 20' 11" East longitudes). It is a small state but known all over the world as "The tropical paradise of tourists". The coastal zone of the state is traversed by nine major dynamic estuaries. Majority of the estuaries in Goa have encouraged the development of civilization and is largely responsible for the anthropogenic output. However, excessive load of anthropogenic activities (such as mining, tourism, sewage disposal, sand mining, human habitation, shrimp farming) have been affecting these estuaries from the past few decades. Hence, the recognition of such impacts is one of the critical issues in modern times.

Till date, few studies on HABs have been carried out in the coastal waters off Goa (Ramamurthy et al., 1972; Devassy et al., 1978; O' Herald, 2001; Prabhu Matondkar et al., 2004; Sanilkumar et al., 2009) which have highlighted bloom cases in the coastal waters off Goa. Devassy and Nair (1987); Patil and Anil (2011); Pednekar et al. (2012) made an attempt to carry out the study along Mandovi and Zuari estuaries of Goa with regard to HABs and concluded that salinity and nutrient inputs are the prime cause for the proliferation of blooms and toxic species. However, only two studies have attempted to document dinoflagellate cyst abundance and distribution in the recent sediments, one from the coastal waters off Goa (D' Silva et al., 2012), and another study (Patil, unpublished) along the Zuari estuary, Goa (D' Costa et al., 2008). However, no information on distribution of HAB species and the dinoflagellate cysts, along the other estuaries in Goa is available.

The present study includes Chapora (North Goa) and Sal (South Goa) estuaries of Goa. Fishing and shellfish collection is an important activity carried out in these regions that supports the livelihood of the families which are dependent on the fishing business. Hence, bloom formation by toxic species in such areas can pose a potential risk on marine life as well as human health. Hence, in the present study, an attempt has been made to interpret the distribution of HAB forming phytoplankton and dinoflagellate cysts along the selected study area.

The present study was carried out along these two estuaries considering the following objectives.

1.3.Objectives

- 1) Spatial and temporal variation of dinoflagellates along the salinity gradient in relation to physico-chemical parameters along the study area.
- 2) Spatial and temporal variation of dinoflagellate cysts along the salinity gradient along the study area.
- 3) Distribution and diversity of dinoflagellate cysts based on the habitat heterogeneity along the study area.

Chapter-2

Materials and Methods

2.1. Study area

The study area comprises coastal waters up to 5 m depth off the central west coast of India. The continental drainage along the central west coast of India is characterised by small rivers and streams that drain into the Arabian Sea (D' Silva et al., 2012). Goa is located on the west coast of India (140° 53' 57" to 150° 47' 59" North latitudes and 73° 40' 54" to 74° 20' 11" East longitudes). The coastal zone of Goa is traversed by nine major dynamic estuaries. Land use pattern and other anthropogenic activities dramatically transform and alter estuarine and coastal marine environmental conditions, and recognition of these impacts is, therefore, one of the critical issues in modern times (Pradhan and Shirodkar, 2011). The present study area includes Chapora and Sal estuary in North and South Goa respectively (Fig. 2.1). River Chapora is located in the Bardez taluka and is 30 km in length (Untawale et al., 1982). Chapora estuary is lined by patchy mangrove vegetation (upstream), with sparse distribution near the mouth. An array of anthropogenic activities (fishing, sand mining, shrimp farming ponds, discharge of domestic wastes, and human settlements along the river banks) are being carried out. Sal river (16 km) is located in the Salcete taluka. Along the Sal estuary, mangroves occur throughout the length, although their density is less downstream due to dense human habitation and agricultural fields. Fishing grounds off the mouth of Sal estuary comprise of two different habitats (silty substratum towards the north of the mouth and submerged rock towards the south). Numerous anthropogenic activities (fishing, shellfish collection, shrimp farms, discharge of domestic wastes, mega-housing projects construction, and agricultural runoffs) are carried out in this estuary.

Chapora and Sal estuaries are a nursery grounds and dwelling place for juveniles and various aquatic life, including fish. In addition, many families directly or indirectly are dependent on fishing activities carried out along this river, thus generating income due to fishing business, and hence are able to support their livelihood. However, several anthropogenic activities carried out along these estuaries have been affecting the water quality, aquatic life and humans. Continuous anthropogenic activities might completely deteriorate the environment of these estuaries leading to eutrophic conditions. This eventually could lead to HABs by toxic species, which can pose serious effects on marine life as well as human health. Therefore, recognition of such impacts is one of the critical issues in these estuaries.

In view of the available information, it is pertinent that systematic studies on baseline information about the water quality, dynamics of phytoplankton and dinoflagellate cysts will be useful in detecting the future possibilities of blooms.

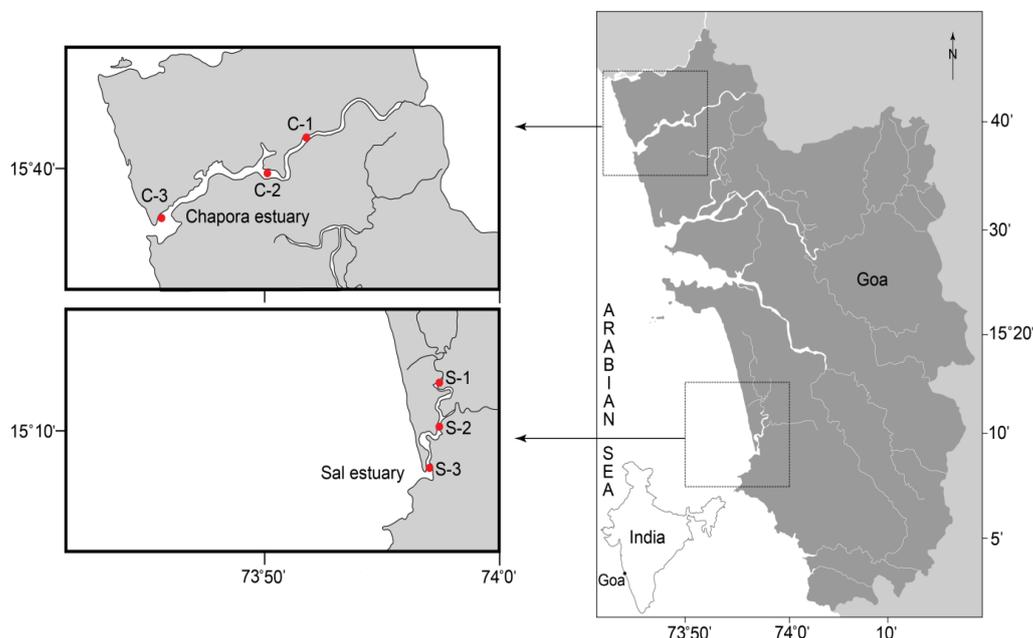


Fig. 2.1. Map indicating sampling locations

2.2. Sampling sites

Three stations were selected in each estuary based on the salinity gradient. In Chapora estuary, station C-1 (upper estuary, $15^{\circ} 39.072'$ and $E 073^{\circ} 47.995'$), C-2 (middle estuary, $N 15^{\circ} 38.396'$ and $E 073^{\circ} 45.848'$) and C-3 (lower estuary, $N 15^{\circ} 38.137'$ and $E 73^{\circ} 45.640'$) were selected and in Sal estuary, three stations namely, S-1 (upper estuary, $N 15^{\circ} 10.095'$ and $E 073^{\circ} 56.781'$), S-2 (middle estuary, $N 15^{\circ} 10.494'$ and $E 73^{\circ} 57.502'$) and S-3 (lower estuary, $N 15^{\circ} 11.842'$ and $E 073^{\circ} 58.240'$) were selected (Fig. 2.1). Variation in salinity gradient in Chapora estuary was found to be larger due to its longer length whereas, a small variation in salinity gradient was noticed in Sal estuary due to its shorter length.

2.3. Sample collection

Sampling was carried out during February, 2016 to March, 2017 at the above mentioned sampling sites (Table 2.1). Surface water samples (phytoplankton taxonomy and estimation of physico-chemical parameters) and sediment samples (for sediment texture analysis and dinoflagellate cyst enumeration) were collected during low tide at monthly intervals during February, 2016 to March, 2017.

Surface water samples were collected with a bucket. Water temperature was measured with a mercury thermometer onboard. For dissolved oxygen (DO) concentration estimation, samples were collected in 60 mL stoppered glass bottles and fixed with Winkler's A and B reagents on board. Samples for estimating salinity and pH were collected in 60 mL plastic bottles, and those for estimating suspended particulate matter (SPM) and nutrient concentrations (nitrate, nitrite, phosphate, silicate) were collected in 250 mL plastic bottles.

Surface sediment samples for dinoflagellate cysts enumeration was collected using sediment cores (PVC cores, 8 cm long, an inner diameter of 3.5 cm) at each station in triplicates. All sediment cores were sectioned at 2 cm intervals, mixed well and stored at 4°C in the dark for further dinoflagellate cyst enumeration. For sediment texture analysis (sand: silt: clay) approximately 30 g of sediment samples were collected and preserved on ice.

Table 2.1. Details of the sampling carried out along the salinity gradient in Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuaries of Goa

Chapora estuary	Sampling Date	Time(h)	Tidal amplitude (m)	Air Temperature (°C)	Sal estuary	Sampling Date	Time (h)	Tidal amplitude (m)	Air temperature (°C)
C-1	16/02/16	10:00	0.4	26	S-1	18/02/16	13:45	0.4	34
C-2	16/02/16	11:50	0.4	33	S-2	18/02/16	12:35	0.4	36
C-3	16/02/16	13:10	0.4	32	S-3	18/02/16	10:35	0.4	32
C-1	17/03/16	09:35	0.4	29	S-1	31/03/16	11:45	0.5	37
C-2	17/03/16	10:55	0.4	30	S-2	31/03/16	11:10	0.5	34
C-3	17/03/16	12:00	0.4	30	S-3	31/03/16	09:05	0.5	32
C-1	13/04/16	08:15	0.3	28	S-1	14/04/16	08:45	0.4	30
C-2	13/04/16	09:05	0.3	29	S-2	14/04/16	11:50	0.4	35
C-3	13/04/16	11:00	0.3	28	S-3	14/04/16	09:45	0.4	33
C-1	13/05/16	07:55	0.5	31	S-1	14/05/16	09:50	0.6	29
C-2	13/05/16	08:30	0.5	31	S-2	14/05/16	13:25	0.6	35
C-3	13/05/16	10:45	0.5	32	S-3	14/05/16	10:45	0.6	30
C-1	10/06/16	12:10	0.3	28	S-1	11/06/16	12:20	0.5	26
C-2	10/06/16	11:35	0.3	29	S-2	11/06/16	09:55	0.5	26
C-3	10/06/16	09:45	0.3	31	S-3	11/06/16	08:50	0.5	26
C-1	27/07/16	08:10	0.7	26	S-1	11/07/16	08:30	0.8	29
C-2	27/07/16	09:00	0.7	26	S-2	11/07/16	11:00	0.8	28
C-3	27/07/16	11:05	0.7	26	S-3	11/07/16	09:20	0.8	27
C-1	10/08/16	07:40	0.8	26	S-1	09/08/16	07:40	0.6	25
C-2	10/08/16	08:30	0.8	26	S-2	09/08/16	10:30	0.6	32

Chapora estuary	Sampling Date	Time(h)	Tidal amplitude (m)	Air Temperature (°C)	Sal estuary	Sampling Date	Time (h)	Tidal amplitude (m)	Air temperature (°C)
C-3	10/08/16	10:35	0.8	27	S-3	09/08/16	08:25	0.6	26
C-1	08/09/16	07:30	0.8	24	S-1	07/09/16	08:05	0.7	27
C-2	08/09/16	08:10	0.8	26	S-2	07/09/16	11:05	0.7	30
C-3	08/09/16	10:15	0.8	31	S-3	07/09/16	08:55	0.7	28
C-1	07/10/16	07:20	0.8	24	S-1	08/10/16	07:30	0.8	26
C-2	07/10/16	08:00	0.8	25	S-2	08/10/16	11:00	0.8	29
C-3	07/10/16	10:15	0.8	29	S-3	08/10/16	08:20	0.8	26
C-1	20/11/16	07:30	0.7	21	S-1	21/11/16	07:35	0.7	22
C-2	20/11/16	08:15	0.7	20	S-2	21/11/16	11:15	0.7	32
C-3	20/11/16	11:05	0.7	35	S-3	21/11/16	08:30	0.7	23
C-1	07/12/16	07:30	0.7	21	S-1	06/12/16	07:35	0.7	24
C-2	07/12/16	08:20	0.7	20	S-2	06/12/16	11:20	0.7	29
C-3	07/12/16	10:50	0.7	31	S-3	06/12/16	08:50	0.7	26
C-1	05/01/17	07:30	0.5	19	S-1	04/01/17	07:40	0.6	21
C-2	05/01/17	08:15	0.5	19	S-2	04/01/17	11:20	0.6	30
C-3	05/01/17	11:00	0.5	28	S-3	04/01/17	08:25	0.6	19
C-1	04/02/17	07:30	0.4	21	S-1	03/02/17	07:50	0.4	24
C-2	04/02/17	08:15	0.4	23	S-2	03/02/17	11:35	0.4	32
C-3	04/02/17	11:20	0.4	30	S-3	03/02/17	08:45	0.4	28
C-1	05/03/17	07:25	0.3	22	S-1	04/03/17	07:45	0.2	25
C-2	05/03/17	08:15	0.3	23	S-2	04/03/17	11.45	0.2	36
C-3	05/03/17	12:10	0.3	29	S-3	04/03/17	08:40	0.2	26

2.4. Laboratory procedure

A) Dissolved oxygen (DO) concentration

DO concentration was estimated by Winkler's method (Griffiths and Jackman, 1957). DO samples were fixed on board with Winkler's A and B reagents. At the laboratory, the fixed samples were treated with 50% HCL and titrated against sodium thiosulphate solution. Burette value was noted and DO concentration was expressed as mg.L^{-1} .

B) Salinity, pH and Temperature

Salinity was measured in psu using digital salinometer and estimation of pH was done using pH meter probe after returning to the laboratory. The temperature measurements were carried out onboard with a mercury thermometer.

C) Suspended particulate matter (SPM) concentration

200 mL of water sample was filtered through 0.45 μm Millipore filter paper and the SPM retained on the filter paper was dried in the oven and weighed (Zao et al., 2018). SPM concentration was expressed as mg.L^{-1} .

D) Nutrient estimation

The quantitative estimation of major nutrients like nitrite, nitrate, phosphate and silicate was carried out in the laboratory by following the method suggested by Grasshoff et al. (1999).

i) Nitrite-Nitrogen ($\text{NO}_2\text{-N}$)

Nitrite ions in water were allowed to react with sulphanilamide in an acid solution. The resulting diazo compound then coupled with an aromatic amine N (1-naphthyl)-ethylene-diamine dihydrochloride to form highly coloured azo dye and its absorption was measured spectrophotometrically at a particular wavelength.

To carry out nitrite estimation, 25 mL of water sample was taken in a graduated tube and 0.5 mL of sulphanilamide reagent was added to it followed by 0.5 mL N (1-naphthyl)-ethylene-diamine dihydrochloride to form a coloured dye. Its light absorption was measured at a wavelength of 540 nm spectrophotometrically. Nitrite concentration was expressed as μM .

ii) Nitrate-Nitrogen (NO_3-N)

Determination of nitrate in water sample involved its reduction to nitrite and subsequent photometric determination based on the formation of azo dye. Among several reducing agents proposed, amalgamated cadmium was found to be the most suitable for this estimation. A reduction column packed with amalgamated cadmium granules was made and the efficiency of the column (95%) was periodically tested with standards.

To estimate nitrate concentration, 50 mL of water sample was treated with 1 mL of ammonium chloride reagent. The treated sample was allowed to pass through the reduction column at a particular flow rate (6.2 mL per minute). 25 mL sample was collected in a graduated tube and 0.5 mL of sulphanilamide and 0.5 mL N (1-naphthyl)-ethylene-diamine dihydrochloride was added. The absorbance of the resulting coloured solution was measured at a wavelength of 543 nm. Nitrate concentration was expressed as μM .

iii) Phosphate-Phosphorus (PO_4-P)

This method involved the reaction of ammonium molybdate reagent with water, forming yellow coloured phosphomolybdic acid. This acid was reduced by ascorbic acid to a blue coloured complex. Reduction reaction was accelerated by adding antimony tartrate catalyst.

To carry out an estimation of phosphate in the water sample, 25 mL of water sample was taken in a graduated tube and 0.5 mL of ascorbic acid followed by 0.5 mL of mixed reagent was added. After 10 minutes, the absorbance of the coloured solution was measured at a wavelength of 880 nm spectrophotometrically. Phosphate concentration was expressed as μM .

iv) Silicate-silicon (SiO_4-Si)

The method for estimation of silicates depends on the formation of silicomolybdic complex within a wide limit of reagent concentration and acidity. When the water sample was treated with an acid molybdate reagent, yellow coloured silicomolybdic complex was formed in the presence of a reducing agent, ascorbic acid. Here, oxalic acid was added to avoid the reduction of excess molybdate and eliminate the influence of phosphate arsenate present in the sample. As the silico complex has limited stability in the presence of oxalic

acid, reductant ascorbic acid was added immediately. The blue silicomolybdate complex was formed within 30 minutes and remained stable for hours.

To carry out silicate estimation in the water sample, 25 mL of water sample was taken in plastic graduated tubes and 1 mL of mixed reagent was added to it. After 10 minutes, 1 mL of oxalic acid was added followed by 0.5 mL of ascorbic acid. The absorption of the coloured compound was measured after 20 minutes at a wavelength of 810 nm spectrophotometrically. Silicate concentration was expressed as μM .

E) Sediment texture analysis (sand: silt: clay)

15 g of sediment sample was oven dried (60°C), transferred to 1000 mL beaker containing distilled water and stirred vigorously. The sediment sample was allowed to settle for a few days, and supernatant water was decanted. This was repeated 3-4 times to remove salt content. After decanting, 10 mL of 10 % sodium hexametaphosphate solution was added to the sample. Sodium hexametaphosphate is used to dissociate clay particles.

The following day, 5 mL of 30% hydrogen peroxide was added to the sample to oxidize the organic matter. Contents of the beaker were then poured over 63 μm mesh size sieve, and the filtrate was collected in a 1000 mL measuring cylinder. The contents on the mesh were washed with distilled water, till clear filtrate passed through the mesh. Volume in the cylinder was made up to 1000 mL with distilled water. Contents on the mesh were transferred to the pre-weighed beaker (sand content) and dried in an oven at 60°C (Lewis, 1984).

The filtrate collected in the cylinder was used to determine clay content in the sample. This filtrate was homogenized by stirring for 2 minutes with a stirrer and allowed to settle at room temperature. 25 mL of this filtrate was pipetted out at 8 ϕ from measuring cylinder by inserting a pipette at a depth of 10 cm from the 1000 mL mark (according to the standard table) (Folk, 1968).

The pipetted solution (clay content) was transferred to the pre-weighed beaker and dried at 60°C . The dried contents in the beaker were weighed and percentages of sand, silt and clay fractions were determined.

F) Taxonomy of phytoplankton

For qualitative and quantitative analysis of phytoplankton, sample collection was carried out from July - December, 2016. Water samples were collected in 1L plastic bottles in triplicates and were fixed with 0.2% Lugol's iodine. Samples were allowed to settle for 48 h and were concentrated to 100 mL by carefully siphoning the top layer of the sample with tubing, one end of which was covered with a 10 μm mesh, to avoid loss of small-sized phytoplankton (Willen, 1976). 1 mL of concentrated sub-sample was analysed under an inverted microscope (Nikon Ti-S) at 200x, 400x and 600x magnification. Images were captured in Nikon imaging software (NIS elements BR) equipped with colour digital camera Ri2 (16.25 megapixel) and taxonomic identification of phytoplankton was carried out following published identification manual (Cupp, 1943; Tomas, 1997; Van, 2006; Sahu, 2013). Further the phytoplankton cell density was expressed as nos. $\times 10^3 \text{ cells.L}^{-1}$.

G) Taxonomy of dinoflagellate cysts

For qualitative and quantitative analysis of dinoflagellate cysts, sample collection was carried out from February, 2016 - March, 2017. Sediment samples from the upper (0-2 cm) section of the core were treated using the palynological method (Matsuoka and Fukuyo, 2000) with some modifications (Narale et al., 2013). To isolate the dinoflagellate cysts, each sediment sample was weighed (3-6 g), sonicated and sieved through 100 μm and 10 μm nylon meshes. Subsequently, the sample was digested with hydrochloric (10%) and hydrofluoric acids (30%) in a wooden fume hood. The treated samples were rinsed 4-5 times with distilled water and sieved through 100 μm and 10 μm nylon meshes. The residue retained on 10 μm mesh was transferred to a vial and final volume was made up to 10 mL with distilled water. The final treated sample was preserved with 4-5% formalin and stored in dark at 4°C for further identification.

Aliquot of 1 mL of the pre-treated sample was observed under Nikon Ti-S inverted microscope at 200x, 400x and 600x magnifications. Photographs of dinoflagellate cysts were captured in Nikon imaging software (NIS elements BR) equipped with colour digital camera Ri2.

Dinoflagellate cysts were identified based on published descriptions (mentioned in Table no. 4.1). The dinoflagellate cyst abundance was expressed as cysts.g⁻¹ dry sediment and the water content was calculated according to the formula given by Matsuoka and Fukuyo (2000).

2.5. Statistical methods

Statistical analyses were performed using PRIMER 6 and CANOCO 4.5 software. A constrained ordination method, Redundancy Analysis (RDA) was performed using CANOCO 4.5 to evaluate the relationships between the species composition and environmental variables, based on forward selection and Monte Carlo permutation test. RDA is a multivariate analysis technique for two sets of variables (Israels, 1992). It summarises linear relationships between components of response variables with a set of explanatory variables (Borcard, 2006). The most practical situation is the one in which these are sets of explanatory (X) and dependent (Y) variables (Israels, 1992). In RDA, the ordination process is directly influenced by a set of explanatory variables (Borcard, 2006).

Multi-dimensional Scaling (MDS) plots were produced using PRIMER 6 to visualise seasonal differences between sites based on cell density and S-17 Bray- Curtis similarity measure was calculated.

MDS is a means of visualising the level of similarity of individual cases of a dataset. MDS is used to translate 'information about the pairwise 'distances' among a set of 'n' objects. Objects can be colours, faces, coordinate, political persuasion, or any kind of real or conceptual stimuli (Kruskal and Wish, 1978). Objects that are more similar (or have shorter distances) are closer together on the graph than objects that are less similar (or have longer distances). Bray-Curtis is a coefficient used as a measure of resemblance between samples in analyses of multivariate data (Bray and Curtis, 1957).

Shannon- Weiner diversity indices were computed using PRIMER 6. A diversity index is a mathematical measure of species diversity in a community. It provides more information about community composition. The Shannon diversity (H') index that is commonly used to characterize species diversity in a community and accounts for both abundance and evenness of the species present.

Multivariate Multiple Regression (MMR) was carried out using STATISTICA 8.0 software. MMR is an extension of standard multiple linear regression model. It is the method of modelling multiple responses, or dependent variables with a single set of predictor variables.

Chapter-3

**Spatial and temporal variation
of dinoflagellates in relation to
physico-chemical parameters
along the study area**

3.1. Introduction

Dinoflagellates and diatoms are known to be the major groups among the phytoplankton community, which are mostly involved in HAB's. During recent years, Harmful algal blooms (HABs) are considered as a matter of concern globally; particularly in the coastal habitats, influenced by biogeochemical processes by means of abrupt and unpredictable interactions (Glibert et al., 2005) as well as anthropogenic activities carried out along the coast. Among these, few species of dinoflagellates are known to produce powerful biotoxins, responsible for so-called 'red tides' (Lindsey and Scott, 2010), and can cause serious impact on marine life as well as human health (Hallegraeff, 1993). Published literature (Hallegraeff, 1993) reveals a noticeable rise in the occurrence of HABs, often globally and by more than one harmful or toxic species with emphasis on the more frequent and widespread occurrence of the toxic dinoflagellate, *Alexandrium*. In this genus, allelopathic, bio-active effects have been studied. It was noted that the release of secondary metabolites (Ogata and Kodama, 1986; Rizvi and Rizvi, 1992) was found to affect the growth of other competitive species which thrive in the same environment. Hence leaving more resources and space for the species with allelopathic ability to bloom (Lelong et al., 2011).

Estuaries are known to be dynamic ecosystems due to their constantly changing physicochemical surroundings. As they receive freshwater influx during monsoon, which leads to regular alteration in the salinity as well as the concentration of nutrients (Parab et al., 2013). Hence this leads in the formation of salinity gradient along the estuary. These gradients are known to affect the temporal dynamics of HAB forming and toxic species as a result of tidal and monsoonal influence in the estuaries (Alkawri and Ramaiah, 2010). In addition, an array of anthropogenic activities such as construction of jetties, shipbuilding yards and cruising, mining, agricultural runoff, sewage and industrial effluent discharge, shrimp farming activities carried along the estuaries (Pradhan and Shirodkar, 2009; Maya et al., 2011) result in increased nutrient inputs (nitrate and phosphate). All these activities affect the quality of the coastal water (Pednekar et al., 2012; Narale and Anil, 2016) and in turn, can lead to detrimental effects on human and marine life.

Continuous nutrient input consecutively creates favourable conditions for certain HAB species to bloom in that particular environment (Anderson et al., 2002). Among the HAB species, some are known to be toxic (*Alexandrium* species) bloom formers (Franco et al.,

1994) possessing the allelopathic ability. As a result, the release of secondary metabolites due to allelopathy may outcompete other species for nutrients (Fistarol et al., 2004). Hence can lead to blooms of toxic species which can pose a serious risk on the life forms dwelling in that particular environment.

Considering the above facts, an attempt has been made to study the distribution of HAB forming diatoms and dinoflagellates from the Chapora (North Goa) and Sal estuary (South Goa). The objectives of the present study were (1) to know the influence of salinity in the proliferation of HABs along the salinity gradient in an estuary (2) influence of environmental parameters on HABs and (3) to reveal the biotic interaction between toxic and non-toxic HABs.

3.2. Materials and Methods

3.2.1. Sampling sites

In each estuary, three stations were selected based on the salinity gradient. In Chapora estuary, station C-1, was selected towards the upstream of the estuary (15°39.072' and E 073°47.995'), C-2, in the middle of the estuary (N 15°38.396' and E 073°45.848'), C-3, towards the downstream of the estuary (N 15°38.137' and E 73°45.640') and in Sal estuary, three stations namely, S-1, was towards the upstream of the estuary (N 15°10.095' and E 073°56.781'), S-2, in the middle of estuary (N 15°10.494' and E 73°57.502'), and S-3, towards the downstream of the estuary (N 15°11.842' and E 073°58.240') were selected (Fig. 2.1). Variation in the salinity gradient was noted in both the estuaries. In Chapora estuary variation was found to be larger due to its longer length whereas, small variation in salinity gradient was noticed in Sal estuary due to its shorter length. Sampling was carried out during July-December, 2016, which comprised of monsoon (July-September) and non-monsoon (October-December) season. Surface water samples (phytoplankton taxonomy and estimation of physico-chemical parameters) were collected during low tide at monthly intervals during the sampling period.

3.2.2. Physico-chemical parameters

The temperature measurements were carried out onboard with a mercury thermometer. Dissolved oxygen (DO) estimation was carried out in the laboratory by Winkler's method (Griffiths and Jackman, 1957). Digital salinometer was used for salinity measurements and

the nutrient (nitrate, nitrite, phosphate, and silicate) concentration was estimated using standard procedure (Grasshoff et al., 1999).

3.2.3. Taxonomy of HAB forming diatom and dinoflagellate species

Surface water samples for phytoplankton identification were collected in triplicates in 1 L plastic bottles and were fixed with 0.2% Lugol's iodine. Samples were allowed to settle for 48 h and were concentrated to 100 mL by carefully siphoning the top layer of the sample with tubing, one end of which was covered with 10 μ m mesh, to avoid loss of small-sized phytoplankton (Willen, 1976). 1 mL of concentrated sub-sample was analysed under an inverted microscope (Nikon Ti-S) at 200x, 400x and 600x magnification and taxonomic identification of phytoplankton was carried out following published identification manual (Tomas, 1997; Cupp, 1943).

3.2.4. Statistical analyses

Statistical analyses were performed using PRIMER 6 and CANOCO 4.5 software. To relate the distribution of HAB species with environmental variables, Redundancy analysis (RDA) was performed with the help of CANOCO 4.5 software. Based on forward selection and Monte Carlo permutation test of the nine environmental variables; salinity, pH, DO, and phosphate concentration were statistically significant ($p < 0.05$). Shannon-Weiner diversity indices were computed using PRIMER 6.

3.3. Results

3.3.1. Physico-chemical parameters

During the sampling period, a small variation in temperature was noted between Chapora and Sal estuary. The temperature variation in Chapora estuary was between 21- 29°C, whereas, in Sal estuary, it was between 22-30°C. The recorded temperature in both Chapora and Sal estuaries during the sampling period were found to be similar to the previous studies carried out from Chapora (Alkawri and Ramaiah, 2010) and Sal estuary (Fernandes and Achuthankutty, 2010). Precipitation and freshwater runoff during monsoon season was found to affect salinity. Hence in both the estuaries, at all three stations, a noteworthy decline in salinity was observed during monsoon season. Along Chapora estuary it ranged between 0-2 psu (C-1), 1-3 psu (C-2), 1-6 psu (C-3) during monsoon and during non-monsoon it was noted to be 1-4 psu (C-1), 2-15 psu (C-2) and 6-26 psu (C-3). Whereas along Sal, salinity varied between 0-3 psu (S-1), 1-4 psu (S-2), 4-15 psu (S-3)

during monsoon and between 1-26 psu (S-1), 7-30 psu (S-2) and 31-36 psu (S-3) during non-monsoon.

In the estuaries, the concentration of dissolved inorganic nutrients (nitrate, nitrite, phosphate, and silicate), showed variation at all the sampling sites along the salinity gradient. Considerable variation in silicate concentration was noted during a non-monsoon season in the two estuaries. Silicate concentration was found to be higher throughout the study period as compared to other nutrients. The highest silicate concentration (179.96 μM) was noted in Chapora estuary (C-2) during monsoon season. High nitrate concentration was recorded during monsoon season as compared to a non-monsoon season in both the estuaries. Significant variation in nitrate concentration was noted between the estuaries during both monsoon and non-monsoon season, whereas significant variation between the stations was noted only during the non-monsoon season. Highest nitrate concentration (22.39 μM) was recorded in Sal estuary (S-2) during monsoon. In Sal estuary, high phosphate concentration was recorded at all three stations, whereas it varied between the stations in Chapora estuary. Highest phosphate concentration (7.54 μM) was recorded in Chapora estuary (C-3) during non-monsoon. However, high phosphate and nitrate concentration were recorded in Sal estuary at all three stations.

3.3.2. Diatom and dinoflagellate assemblages

Total of fifty eight diatom species and fifteen dinoflagellate species was recorded in the present study (Table 3.1). In Chapora estuary, forty eight diatom species and four dinoflagellate species were recorded. Whereas, in Sal estuary, thirty five diatom species and fourteen dinoflagellate species were noted. The cell density of diatoms dominated at all three stations in both the estuaries (Fig. 3.1).

Table 3.1. List of diatoms and dinoflagellates encountered along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary

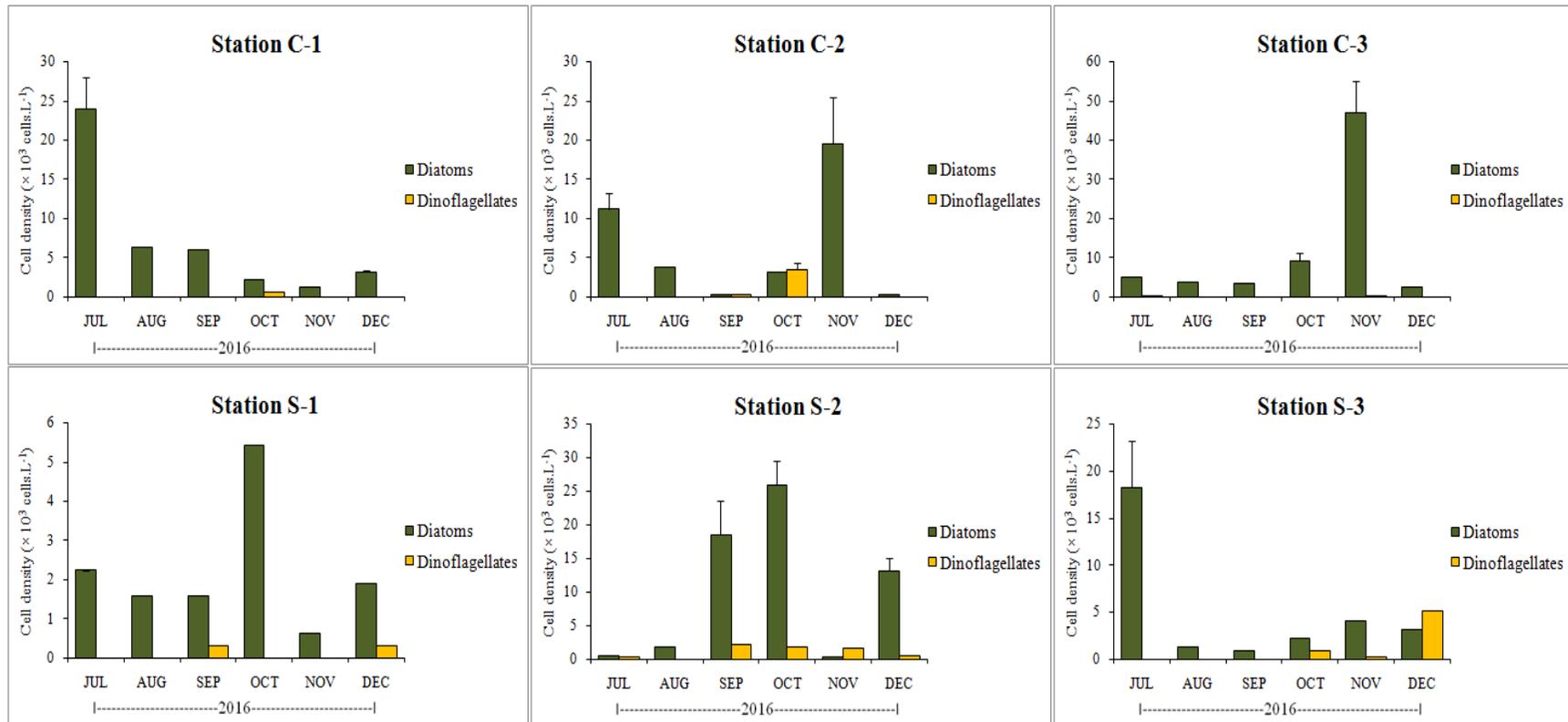
Estuary	Chapora			Sal		
Stations	C-1	C-2	C-3	S-1	S-2	S-3
Diatoms						
<i>Asterionellopsis glacialis</i>	+	+	+	-	-	+
<i>Bacillaria paradoxa</i>	-	+	-	-	-	+
<i>Biddulphia mobiliensis</i> *	+	-	-	-	-	+
<i>Biddulphia sinensis</i>	+	-	+	-	-	-
<i>Ceratulina dentata</i> *	+	-	-	-	-	-
<i>Chaetoceros lorenzianus</i> *	-	-	-	-	-	+
<i>Chaetoceros convolutes</i> *	-	-	-	-	-	+
<i>Chaetoceros curvisetus</i> *	+	-	-	-	-	+
<i>Chaetoceros danicus</i> *	-	-	-	-	-	+
<i>Chaetoceros affinis</i>	+	-	-	-	-	+
<i>Chaetoceros decipiens</i>	+	-	+	-	-	-
<i>Chaetoceros simplex</i>	-	-	+	-	-	-
<i>Coscinodiscus radiatus</i> *	+	+	+	-	+	+
<i>Coscinodiscus centralis</i> *	-	+	-	-	-	+
<i>Coscinodiscus granii</i> *	-	+	-	-	-	+
<i>Ditylum brightwellii</i>	-	-	-	-	-	+
<i>Entomoneis sulcata</i>	+	-	-	-	-	-
<i>Gyrosigma balticum</i>	+	-	-	-	-	+
<i>Gyrosigma fasciola</i>	-	-	+	-	-	-
<i>Lauderia annulata</i>	+	-	-	-	-	+
<i>Leptocylindrus danicus</i>	+	+	-	-	-	-
<i>Melosira granulata</i>	+	+	+	-	+	-
<i>Melosira nummuloides</i>	-	-	+	-	+	+
<i>Navicula .transitrans var.derasa</i>	-	-	+	-	+	+

<i>Navicula amphirhynchus</i>	-	-	+	-	-	-
<i>Navicula crptocephala</i>	-	+	-	-	-	-
<i>Navicula delicatula</i>	-	-	+	-	-	-
<i>Navicula directa</i>	-	-	+	-	-	-
<i>Navicula distans</i>	-	-	+	-	-	-
<i>Navicula f.delicatula</i>	-	-	+	-	+	-
<i>Navicula microspora</i>	-	-	+	-	-	-
<i>Navicula nitzschioides</i>	-	-	-	-	-	+
<i>Navicula pupula</i>	-	+	+	-	-	-
<i>Navicula tuscula</i>	-	-	+	-	-	-
<i>Nitzschia accicularis</i>	+	-	-	+	-	+
<i>Nitzschia closterium</i>	-	+	+	+	+	+
<i>Nitzschia coarctata*</i>	-	-	+	-	-	-
<i>Nitzschia longissima</i>	-	-	+	+	+	+
<i>Nitzschia palea</i>	-	-	+	+	-	-
<i>Nitzschia sigma</i>	-	-	+	-	-	-
<i>Nitzschia sigmoidea*</i>	+	+	+	-	+	-
<i>Pinnularia rectangulata</i>	-	-	+	-	-	-
<i>Pinnularia viridis</i>	-	-	+	-	-	-
<i>Planktoniella sol</i>	-	+	+	-	-	+
<i>Pleurosigma directum</i>	+	-	-	-	+	+
<i>Pleurosigma normanii</i>	-	+	-	-	-	-
<i>Pleurosigma delicatula</i>	-	-	-	+	-	-
<i>Pleurosigma elongatum</i>	-	-	-	+	-	-
<i>Pseudo-nitzschia delicatissima*</i>	-	-	-	-	-	+
<i>Pseudo-nitzschia pungens*</i>	-	-	-	-	-	+
<i>Rhizosolenia eriensis*</i>	+	+	-	-	-	-
<i>Rhizosolenia setigera*</i>	+	-	-	-	+	+
<i>Skeletonema costatum*</i>	+	+	+	+	+	+
<i>Synedra acus</i>	-	+	+	+	+	-

<i>Synedra ulna</i>	+	+	-	+	-	-
<i>Thalassionema nitzschioides</i>	-	-	-	-	-	+
<i>Thalassiosira Oestrupii</i>	-	+	-	-	-	-
<i>Thalassiosira punctigera</i> *	-	+	-	-	-	-
Dinoflagellates						
<i>Alexandrium minutum</i> **	-	+	-	+	+	-
<i>Alexandrium pseudogonyaulax</i> **	-	-	-	-	+	-
<i>Alexandrium tameranense</i> **	-	-	-	-	+	+
<i>Ceratium furca</i> *	-	-	-	-	-	+
<i>Ceratium fusus</i> *	-	-	+	-	-	+
<i>Cochlodinium polykrikoides</i> **	-	-	-	-	-	+
<i>Gonyaulax spinifera</i> **	-	-	-	-	-	+
<i>Peridiniopsis polonicum</i> *	-	-	-	-	+	-
<i>Peridiniopsis cunningtonii</i> *	+	-	-	-	-	-
<i>Peridinium goslaviense</i>	-	-	-	-	+	-
<i>Peridinium quinquecorne</i> *	-	+	-	+	+	-
<i>Prorocentrum gracile</i> *	-	-	-	-	+	-
<i>Prorocentrum micans</i> **	-	-	-	-	-	+
<i>Protoperidinium pellucidum</i> *	-	-	-	-	+	-
<i>Pyrophacus steinii</i>	-	-	-	-	-	+

*HAB forming species; ** Toxic (red tide forming species)

Fig. 3.1. Spatio-temporal variations in the cell density (cells. L⁻¹) of diatoms and dinoflagellates along salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary



High diversity of diatoms was noted in both the estuaries in comparison with dinoflagellates. Diatom diversity was found to be high in Chapora estuary ($H' = 4.06$) as compared to Sal ($H' = 3.16$). Whereas, diversity of dinoflagellates was found to be high in Sal ($H' = 2.63$) as compared to Chapora estuary ($H' = 1.38$).

3.3.3. HAB forming diatom and dinoflagellate assemblages

During the present study, in both the estuaries distinct variation in the cell density and distribution of HAB forming diatoms and dinoflagellates (hereafter mentioned as HAB species) were noted. Among 58 diatom species recorded, seventeen were found to be HAB forming and out of seventeen dinoflagellate species, thirteen were HAB forming, among which six were known to be toxic red tide forming species (Table 3.2). Twelve species of diatoms and four species of dinoflagellates were observed in Chapora estuary, and in Sal estuary, fourteen species of diatoms and twelve species of dinoflagellates were recorded. HAB forming diatom cell density was found to be higher than that of the HAB forming dinoflagellates at most of the stations in Chapora estuary. Whereas, an cell density of HAB forming dinoflagellates was found to be higher in comparison with HAB forming diatoms at most of the stations in Sal estuary (Fig. 3.2). The diversity of HAB forming diatoms ($H' = 1.75$) and dinoflagellates ($H' = 1.54$) in Sal estuary was found to be high as compared to the diversity of diatoms ($H' = 1.21$) and dinoflagellates ($H' = 1.21$) in Chapora estuary.

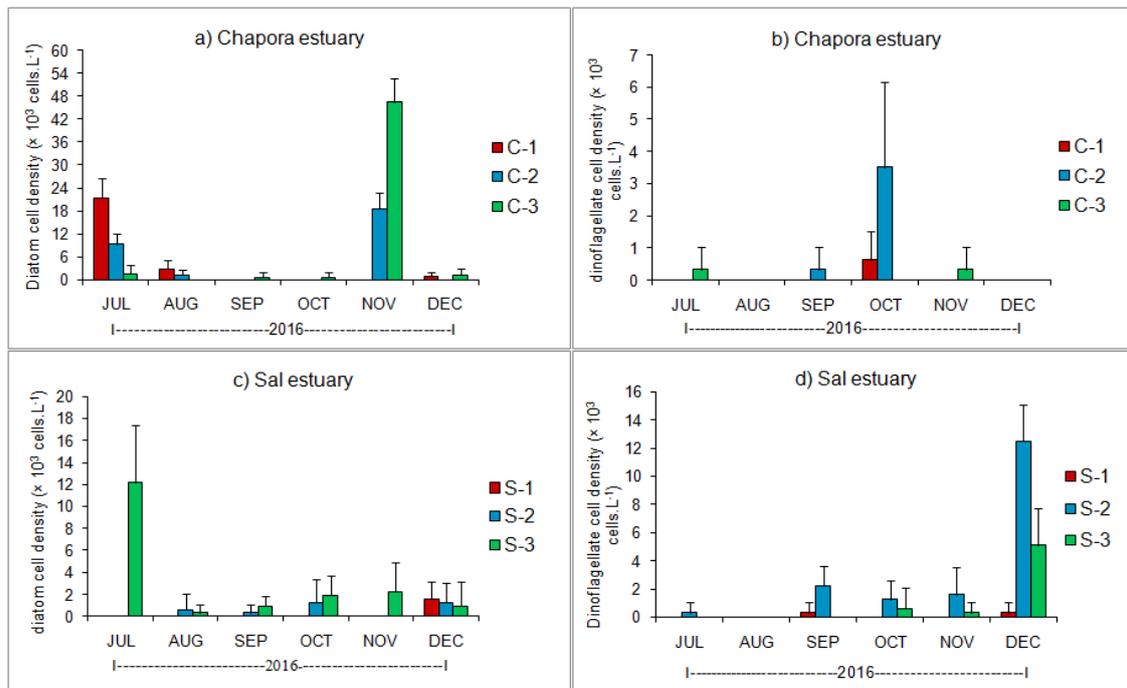
Table 3.2. List of HAB forming diatoms and dinoflagellates encountered along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary

Taxon	Taxon Abbreviation	Chapora estuary			Sal estuary		
		C-1	C-2	C-3	S-1	S-2	S-3
Diatoms							
<i>Biddulphia mobiliensis</i>	<i>Bd mb</i>	+	-	-	-	-	+
<i>Ceratulina dentata</i>	<i>Cr dn</i>	+	-	-	-	-	-
<i>Chaetoceros curvisetus</i>	<i>Ch cr</i>	+	-	-	-	-	+
<i>Nitzschia sigmaidea</i>	<i>Nz sg</i>	+	+	+	-	+	-
<i>Rhizosolenia eriensis</i>	<i>Rh er</i>	+	+	-	-	-	-
<i>Rhizosolenia setigera</i>	<i>Rh st</i>	+	-	-	-	+	+

<i>Skeletonema costatum</i>	<i>Sk cs</i>	+	+	+	+	+	+
<i>Coscinodiscus centralis</i>	<i>Co cn</i>	-	+	-	-	-	+
<i>Coscinodiscus granii</i>	<i>Co gr</i>	-	+	-	-	-	+
<i>Coscinodiscus radiatus</i>	<i>Co rd</i>	-	+	+	-	+	+
<i>Thalassiosira punctigera</i>	<i>Th pn</i>	-	+	+	-	-	-
<i>Nitzschia coarctata</i>	<i>Nz cr</i>	-	-	+	-	-	-
<i>Chaetoceros lorenzianus</i>	<i>Ch lz</i>	-	-	-	-	-	+
<i>Chaetoceros convolutes</i>	<i>Ch cn</i>	-	-	-	-	-	+
<i>Chaetoceros danicus</i>	<i>Ch dn</i>	-	-	-	-	-	+
<i>Pseudo-nitzschia delicatissima</i>	<i>Pnz dl</i>	-	-	-	-	-	+
<i>Pseudo-nitzschia pungens</i>	<i>Pnz pn</i>	-	-	-	-	-	+
Dinoflagellates							
<i>Peridiniopsis cunningtonii</i>	<i>Pds cn</i>	+	-	-	-	-	-
<i>Alexandrium minutum</i> **	<i>Al mn</i>	-	+	-	+	+	-
<i>Peridinium quinquecorne</i>	<i>Pr qn</i>	-	+	-	-	+	-
<i>Ceratium furca</i>	<i>Cm fr</i>	-	-	+	-	-	+
<i>Ceratium fusus</i>	<i>Cm fu</i>	-	-	+	-	-	+
<i>Prorocentrum gracile</i>	<i>Pm gr</i>	-	-	-	+	+	-
<i>Alexandrium pseudogonyaulax</i> **	<i>Al psg</i>	-	-	-	-	+	-
<i>Alexandrium tamarense</i> **	<i>Al tm</i>	-	-	-	-	+	+
<i>Peridiniopsis polonicum</i>	<i>Pds po</i>	-	-	-	-	+	-
<i>Protoperidinium pellucidum</i>	<i>Prd pl</i>	-	-	-	-	+	-
<i>Cochlodinium polykrikoides</i> **	<i>Chl pk</i>	-	-	-	-	-	+
<i>Gonyaulax spinifera</i> **	<i>Gn sp</i>	-	-	-	-	-	+
<i>Prorocentrum micans</i> **	<i>Pm mc</i>	-	-	-	-	-	+

+ Present, - Absent, ** Toxic species

Fig 3.2. Spatio-temporal variations in the cell density (cells. L⁻¹) of HAB forming diatoms and dinoflagellates along salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary. (a) HAB forming diatoms cell density in Chapora estuary (b) HAB forming dinoflagellate cell density in Chapora estuary (c) HAB forming diatom cell density in Sal estuary (d) HAB forming dinoflagellate cell density in Sal estuary



A seasonal variation in the composition and distribution of HAB species was noted along the salinity gradient in both the estuaries. (Table 3.3). *Coscinodiscus radiatus*, *Nitzschia sigmoidea*, *Skeletonema costatum*, *Rhizosolenia eriensis*, *Peridinium quinquecorne*, *Ceratium furca*, *Alexandrium minutum*, *Alexandrium tamarense* are the HAB forming species recorded at a wide salinity range. Further, it was also observed that among the six toxic red tide forming dinoflagellate species, *Alexandrium minutum*, *Alexandrium tamarense*, *Alexandrium pseudogonyaulax*, *Cochlodinium polykrikoides*, *Prorocentrum micans*, and *Gonyaulax spinifera*, *Alexandrium minutum* (12×10^3 cells.L⁻¹) dominated during a non-monsoon season in the Sal estuary.

Table 3.3. Seasonal variation in the distribution of HAB forming diatoms and dinoflagellates along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary

Taxon	Chapora estuary						Sal estuary					
	C-1		C-2		C-3		S-1		S-2		S-3	
Diatoms	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM
<i>Biddulphia mobiliensis</i>	+	-	-	-	-	-	-	-	-	-	+	-
<i>Ceratulina dentata</i>	-	+	-	-	-	-	-	-	-	-	-	-
<i>Chaetoceros curvisetus</i>	+	-	-	-	-	-	-	-	-	-	+	-
<i>Nitzschia sigmaidea</i>	-	+	+	-	+	+	-	-	-	+	-	-
<i>Rhizosolenia eriensis</i>	+	-	+	-	-	-	-	-	-	-	-	-
<i>Rhizosolenia setigera</i>	+	-	-	-	-	-	-	-	-	+	-	+
<i>Skeletonema costatum</i>	+	-	+	-	+	-	-	+	+	+	+	+
<i>Coscinodiscus centralis</i>	-	-	-	+	-	-	-	-	-	-	+	-
<i>Coscinodiscus granii</i>	-	-	-	+	-	-	-	-	-	-	-	+
<i>Coscinodiscus radiatus</i>	+	-	+	+	-	+	-	-	-	+	+	-
<i>Thalassiosira punctigera</i>	-	-	-	+	-	-	-	-	-	-	-	-
<i>Nitzschia coarctata</i>	-	-	-	-	-	+	-	-	-	-	-	-
<i>Chaetoceros lorenzianus</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Chaetoceros convolutes</i>	-	-	-	-	-	-	-	-	-	-	+	-

<i>Chaetoceros danicus</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Pseudo-nitzschia delicatissima</i>	-	-	-	-	-	-	-	-	-	-	-	+
<i>Pseudo-nitzschia pungens</i>	-	-	-	-	-	-	-	-	-	-	-	+
Dinoflagellates												
<i>Peridiniopsis cunningtonii</i>	-	+	-	-	-	-	-	-	-	-	-	-
<i>Alexandrium minutum</i> **	-	-	-	+	-	-	+	-	+	+	-	-
<i>Peridinium quinquecorne</i>	-	-	+	+	-	-	-	+	-	+	-	-
<i>Ceratium furca</i>	-	-	-	-	-	-	-	-	-	-	-	+
<i>Ceratium fusus</i>	-	-	-	-	+	+	-	-	-	-	-	+
<i>Prorocentrum gracile</i>	-	-	-	-	-	-	-	-	-	+	-	-
<i>Alexandrium pseudogonyaulax</i> **	-	-	-	-	-	-	-	-	-	+	-	-
<i>Alexandrium tamarense</i> **	-	-	-	-	-	-	-	-	+	+	-	+
<i>Peridiniopsis polonicum</i>	-	-	-	-	-	-	-	-	+	-	-	-
<i>Proto-peridinium pellucidum</i>	-	-	-	-	-	-	-	-	+	-	-	-
<i>Cochlodinium polykrikoides</i> **	-	-	-	-	-	-	-	-	-	-	-	+
<i>Gonyaulax spinifera</i> **	-	-	-	-	-	-	-	-	-	-	-	+
<i>Prorocentrum micans</i> **	-	-	-	-	-	-	-	-	-	-	-	+

** Toxic species, M-Monsoon, NM-Non-monsoon

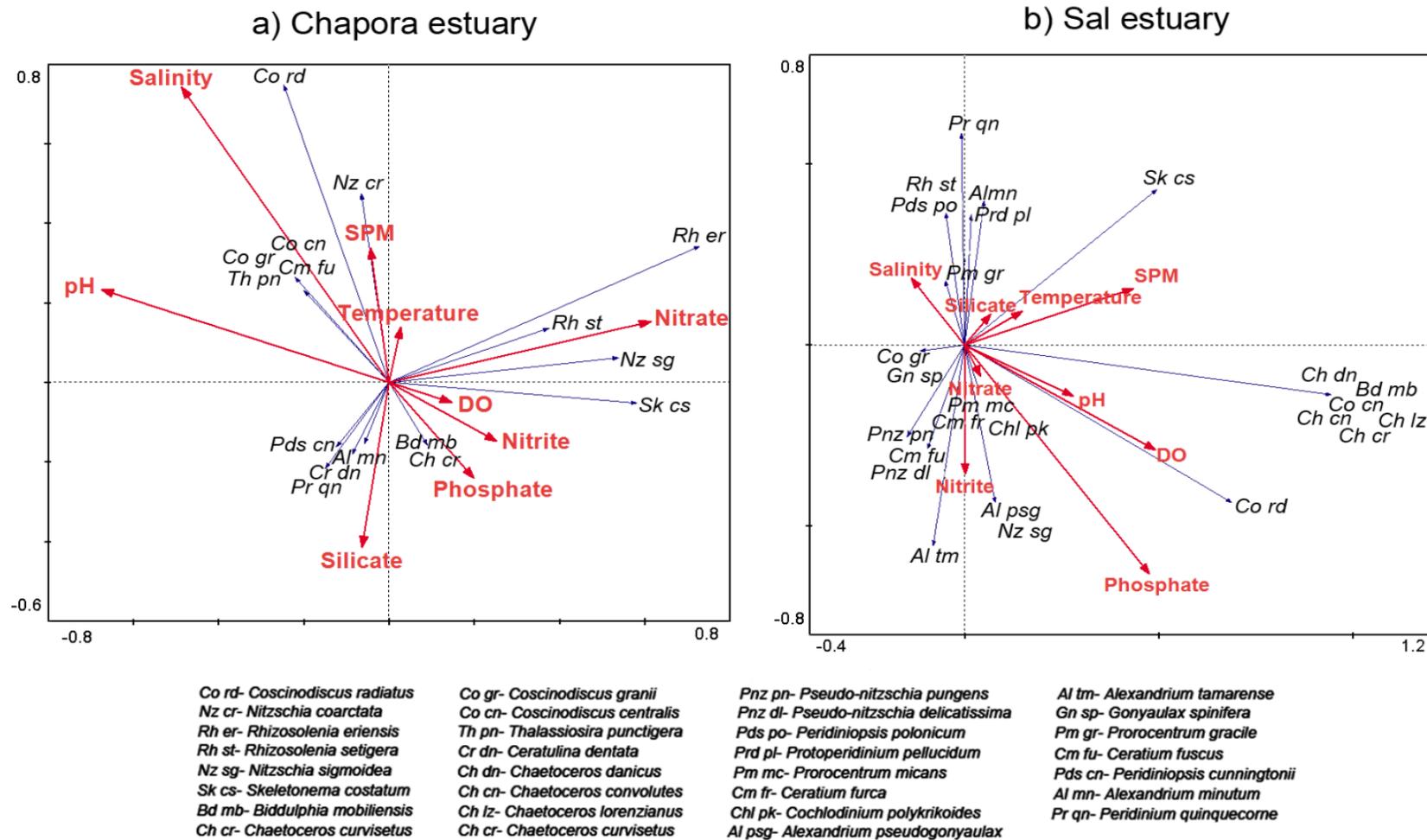
3.3.4. Toxic dinoflagellates and its biotic interaction

In Chapora estuary only one toxic red tide forming species, *Alexandrium minutum* was reported, whereas in Sal estuary six toxic species, *Alexandrium minutum*, *Alexandrium tamarense*, *Alexandrium pseudogonyaulax*, *Cochlodinium polykrikoides*, *Prorocentrum micans*, and *Gonyaulax spinifera* were reported.

In Chapora estuary (C-1) and in Sal estuary (S-3), highest diatom peak was noted in the month of July. This peak was found to be dominated by freshwater species (Fig. 3.2). Toxic red tide forming dinoflagellate species, *Alexandrium minutum* recorded in both the estuaries revealed an allelopathic effect on the diatom cell density.

In Chapora estuary, the highest dinoflagellate peak noted in the month of October (C-2) comprised of *A. minutum*. The presence *A. minutum* has found to suppress diatom cell density in the same month. Whereas the absence of *A. minutum* species in the month of November, noted to lead a proliferation of diatom cell density in the same month. This was noted by the highest peak in the same month (Fig. 3.2). Similar observations were recorded in Sal estuary in the month of December (S-2). Wherein the highest dinoflagellate peak during the month of December, comprised of *A. minutum* revealed an inhibitory effect on diatom cell density in the same month. Further, RDA bi-plots revealed a positive correlation of genus *Alexandrium* with phosphate concentration (Fig. 3.3).

Fig. 3.3. RDA (Redundancy Analysis) bi-plots depicting the relationship between HAB species and environmental parameters in (a) Chapora and (b) Sal estuary



3.4. Discussion

Studies carried out on the distribution of HABs worldwide (Hallegraeff, 1993; D' Silva et al., 2012) as well as along the coastal waters off Goa (Devassy et al., 1979; Alkawri and Ramaiah, 2010) as well as in estuarine complex of Goa (Pednekar et al., 2012) revealed that, the variations in environmental parameters affect the distribution of phytoplankton including HAB species. Variations in physico-chemical conditions in a particular area can occur either naturally (monsoonal runoff, precipitation, upwelling) or due to anthropogenic activities (agricultural runoffs, domestic or industrial sewage disposal, sand mining activities).

The present study carried out in Chapora and Sal estuaries elucidates that, seasonal variations in the distribution of phytoplankton including HAB species are noticeably affected by the physico-chemical parameters. Monsoonal runoff and anthropogenic input due to various activities such as agricultural runoff; discharge of domestic and industrial wastes are found to alter the physico-chemical properties along these estuarine environments. Noticeable seasonal changes in salinity were observed during the present study; although it is well-known that, in the tropical estuaries, freshwater incursion during monsoon leads to the decline in salinity (Shetye et al., 2007). The variation in nutrient concentration in the study area is generally as a result of intense rainfall along with anthropogenic input. Higher silicate values noted can be probably due to the sand mining and other anthropogenic activities carried out. Domestic and agricultural runoff must be the primary cause for the contribution of high phosphate concentration. Further, shrimp farming ponds located along these estuaries could also be a major contributor. High nitrate concentration noted during monsoon can be mostly due to agricultural runoff due to heavy rainfall as well as the contribution from anthropogenic activities. High nitrate concentration was noted at all three stations during the monsoons as compared to a non-monsoon season in both the estuaries. Pednekar et al. (2011, 2014) also recorded similar observations from the studies carried out along the Mandovi - Zuari estuaries of Goa.

High cell density of diatoms noted in Chapora estuary as compared to the cell density of dinoflagellates, was found to be in concurrent with the observations that were reported earlier from various other parts of Indian coasts (Sawant and Madhupratap, 1996; Sarojini and Sarma, 2001). However, high dinoflagellate cell density recorded at most of the stations in Sal estuary might be probably due to high salinity and temperature values,

which are known to be favourable for the growth of dinoflagellates (Joseph and Pillai, 1975). In addition, high phosphate concentration in Sal estuary might have also supported high dinoflagellate cell density. As it is known that, a decline in phosphate concentration results in a decline of dinoflagellates in surface waters (Patil and Anil, 2011). Studies carried out by, Ciotti et al. (1995) reported that as dinoflagellates are known to be more mobile and buoyant than diatoms, they have nutrient retrieval strategy. Hence this ability leads in diel migration of nutrient-depleted dinoflagellates in the rich nutrient layers (Smayda and Reynolds, 2003).

As the estuaries are known as transitional zones between freshwater and marine environments, the HAB species recorded were found to be brackish, euryhaline and eurythermal. The euryhaline species reported during the study might be due to the tidal influx. As it is known from the previous study that, the tidal current has a role in the transport of coastal euryhaline species (Bazin et al., 2014). Therefore, these species then proliferate in these estuarine conditions (Pednekar et al., 2014) as they have the ability to survive at a wide salinity range (Pednekar et al., 2011). The seasonal variation of HAB species along the salinity gradient was discernible as a result of a change in salinity, temperature, and nutrient values in these estuarine systems (Quinlan and Philips, 2007; Matos et al., 2011). Further, the effect of physico-chemical factors (salinity, DO, phosphate) on the composition and distribution of HAB species (Huang et al., 2004; Sahu et al., 2012) was also revealed based on Redundancy Analysis (RDA) plots.

The apparent difference in the composition and cell density of HAB forming and toxic dinoflagellates was noted and was found to be high during the non-monsoon season. This suggests salinity to be a vital factor that is mainly influencing the cell density of toxic dinoflagellates (Alkawri and Ramaiah, 2010). In addition, presence of grazers, allelopathy (Smayda, 1998; Graneli and Johansson, 2003), may also contribute to the distribution and composition of species in the specific study area at a particular time (Anderson et al., 2002).

In the present study, based on high nitrate and phosphate concentration recorded at all three stations, Sal is found to be more polluted as compared to Chapora. Also, the high diversity of toxic species recorded in Sal estuary indicates the presence of favourable conditions for their growth and proliferation. Among the toxic dinoflagellate species reported in the present study, six toxic dinoflagellate species, *Alexandrium minutum*,

Alexandrium tamarense, *Alexandrium pseudogonyaulax*, *Cochlodinium polykrikoides*, *Prorocentrum micans*, and *Gonyaulax spinifera* were reported in the Sal estuary.

Alexandrium minutum and *Alexandrium tamarense* are known to be a red tide forming Paralytic shellfish poisoning (PSP) producers. Toxic blooms of *Alexandrium tamarense* is generally reported in Japan (Fukuyo et al., 1985), in Europe (Moestrup and Hansen, 1988), and are also common along the NE coast of North America (New England and Canada) (Loeblich and Loeblich, 1975). Production of potent PSP neurotoxins (Hallegraeff, 1991) by *A. minutum*, which is known to affect humans, other mammals, birds and fishes (Hallegraeff et al., 1988) via transmission through contaminated shellfish, is a matter of concern.

Alexandrium pseudogonyaulax produces a unique phycotoxin, goniodomin A (GA) with antifungal effects (Murakami et al., 1988). This toxin GA is known to target the liver and thymus (Terao et al., 1990). *A. pseudogonyaulax* is a coastal species which has been reported from several localities in Europe.

Another red tide causing dinoflagellate is *Cochlodinium polykrikoides*. It is a cosmopolitan species found in warm temperate and tropical waters (Steidinger and Tangen, 1996). It is associated with immense fish kills (Patil and Anil, 2011), and is known for a great economic loss in Japanese and Korean waters (Yuki and Yoshimatsu, 1989; Fukuyo et al., 1990).

Prorocentrum micans is commonly found in neritic and estuarine waters but also found in oceanic environments. *P. micans* is considered harmless even though it is capable of forming extensive blooms, (Anderson et al., 1985; Graneli et al., 1990). Although, there are few reports of *P. micans* known to cause shellfish kills in Portugal (Pinto and Silva, 1956) and South Africa (Horstman, 1981).

Gonyaulax spinifera is reported to be a prolific yessotoxin producer (Rhodes et al., 2006). The toxin bioaccumulates in the tissues of bivalve molluscs (mussels, scallops, and clams), allowing its entry into the food chain (Mudadu et al., 2017). *G. spinifera* is a widespread species in the coastal waters of New Zealand and has distribution worldwide (Steidinger and Tangen, 1996).

Among the six potentially toxic red tide forming dinoflagellate species reported in the present study, PSP producer of genus *Alexandrium* revealed biotic interaction between toxic and non-toxic HAB species. Allelopathy is known as one of the biotic interaction involved in the release of secondary metabolites (Rizvi and Rizvi, 1992). The effect of these secondary metabolites found to suppress the growth of competitive species that thrive in the same environment. This results in leaving more resources and space for the allelopathic species to bloom (Lelong et al., 2011).

Different *Alexandrium* species may produce different allelochemicals (Lelong et al., 2011) which help in out-competing other species for nutrients as well as space (Tillmann and John, 2002; Fistarol et al., 2004). Hence, it can be inferred that, if nutrient enrichment prevails in the specific environment, the species of this genus can lead to the formation of toxic blooms (Richardson and Jorgensen, 1996; Richardson, 1997).

The prime sources involved in nutrient enrichment of coastal waters are, agricultural and sewage effluents, atmospheric deposition, groundwater inflow, as well as increasing aquaculture systems (Vitousek et al., 1997; Anderson et al., 2002), which in turn leads in eutrophication conditions. Published reports (Paerl, 1988; Anderson et al., 2002; Baek et al., 2008) suggests that, in coastal areas, eutrophication chiefly by nitrate and phosphate derived from anthropogenic input supports bloom formation, including HAB forming species.

Similar observations were recorded in the present study, wherein Redundancy Analysis (RDA) bi-plots showed a positive correlation of phosphate concentration with genus *Alexandrium*. From this observation, it can be inferred that phosphate input might lead to the proliferation of *Alexandrium* species. This, in turn, can lead *Alexandrium* species to bloom by out-competing other species due to its allelopathic ability (Lelong et al., 2011). While it is known that, allelopathic ability supports in the proliferation of a specific species, and the species that is able to compete effectively for the available growth limiting nutrients is likely to bloom. Labry et al. (2008) in an experimental study also concluded that *Alexandrium* cell density increases in corresponding with phosphate supply outcompeting competitor species.

Although there was no significant correlation of nitrate concentration with *Alexandrium*, it might have posed an effect on the distribution and diversity of this genus. In the previous

study, it has been reported, nitrate as the triggering factor in the formation of *Alexandrium* blooms (Collos et al., 2007). From the previous and the present study, it can be assumed that the nutrient enrichment is a principal factor which sustains blooms of allelopathic algae by altering the N:P balance (Graneli et al., 2008). Further, the dominance of potent PSP neurotoxin producer species *Alexandrium minutum* (12×10^3 cells.L⁻¹) in the Sal estuary is of great concern as this species is known to affect fishes, birds and human beings via consumption of contaminated shellfish (Larsen and Moestrup, 1989). In addition, as shellfish collection and fishing are the regular activities carried out along this estuary, high cell density of this toxic species, as well as a high diversity of other toxic dinoflagellate species can cause an immense health risk to the people and marine organisms along this region.

It is imperative from the observations made in the present study; a noticeable increase in HABs due to an increase in eutrophication of coastal waters as a result of anthropogenic activities is of great concern (Anderson et al., 2002). It is pertinent that reductions in nutrient input might mitigate blooms, but may not completely eradicate HAB species. Also, other factors such as allelopathy, the presence of grazers should be considered in elucidating the reason for the development of HABs. In order to reduce the risk of HABs, people should be made aware about the effects of pollutant loadings on coastal waters through training programs that a possible effect of increased nutrient loading will result in HABs. Other effective measures would be to avoid sewage discharge, human interference through tourism and release of effluents from aquaculture farms and agricultural lands.

Chapter-4
Qualitative and Quantitative
analysis of dinoflagellate cysts

4.1. Introduction

Dinoflagellates are a globally well-known group of phytoplankton due to their role in the formation of Harmful algal blooms (HABs) and its toxic effects (D' Silva et al., 2011) on aquatic life and human health (Burkholder, 1998). About 10% of the 2000 identified dinoflagellates produce cysts as a part of their life cycle (Bravo and Fiueroa, 2014). Dinoflagellate cysts can be well preserved in sediment for several years (Lewis et al., 1999), as they are composed of resistant biopolymer called 'dinosporin' which offers a resistance towards degradation (Fensome et al., 1993; D' Silva et al., 2012). Hence, they serve as potential "seed banks" (Anderson et al., 1995) and are known to have important roles in species dispersal, survival under unfavourable conditions, termination as well as initiation of blooms and in providing past bloom incidences (Heiskanen, 1993; Anderson et al., 1995; Ishikawa and Taniguchi, 1996; Dale, 2001; D' Silva et al., 2012).

In recent decades, various anthropogenic activities (industrialisation, sewage and agricultural runoffs) along coastal regions have led in nutrient enrichment, eventually leading to the formation of HABs (Gowen et al., 2012). This has adversely affected coastal regions, posing a threat to aquatic life and human health and has also lead to economic loss to fishing industries (D' Silva et al., 2011). Estuaries are the aquatic environments which are known to be highly affected by anthropogenic activities and hence are susceptible to eutrophication (Price et al., 2016). Dinoflagellate cysts have received great attention as biological indicators of eutrophication (Matsuoka, 1999; Radi and de Vernal, 2008). Published literature (Orlova et al., 2004; Rubino et al., 2010; Baula et al., 2011; Furio et al., 2012; D'Silva et al., 2012; Narale et al., 2013) suggest the role of such dinoflagellate cysts in the marine ecosystems. Recent studies (Su-Myat et al., 2012; Matsuoka et al., 2017; 2018) have been focused in the coastal regions, however, few studies have been carried out in estuarine environments (Godhe et al., 2000; Wang et al., 2004; Price et al., 2016; Matsuoka et al., 2017).

Goa is developing at a rapid rate due to tourism and is largely responsible for anthropogenic output making estuaries highly susceptible to anthropogenic influence. Much of the studies carried out along the coast of Goa on the dinoflagellate cysts are from the coastal waters (D' Costa et al., 2008; D' Silva et al., 2011; 2012), however, studies on the cyst assemblages along the estuaries of Goa is lacking. The present study on the distribution of dinoflagellate cysts along the salinity gradient in Chapora (North Goa) and

Sal estuary (South Goa) is first of its kind. The objectives of the present study are 1) To elucidate the seasonal distribution of dinoflagellate cysts along the salinity gradient 2) to reveal the influence of physico-chemical parameters on the distribution of dinoflagellate cysts 3) nutrient limitation as a triggering factor for dinoflagellate cyst formation. Further, the present information provided here may act as the baseline data on the distribution and composition of dinoflagellate cysts that may be useful to predict, control and mitigate the formation of future bloom events.

4.2. Materials and methods

4.2.1 Sampling sites

Three stations were selected in each estuary based on salinity gradient. In Chapora estuary, station C-1, upper estuary ($15^{\circ}39.072'$ and E $073^{\circ}47.995'$), C-2, middle estuary (N $15^{\circ}38.396'$ and E $073^{\circ}45.848'$), C-3, lower estuary (N $15^{\circ}38.137'$ and E $73^{\circ}45.640'$), were selected and in Sal estuary, three stations namely, S-1, upper estuary (N $15^{\circ}10.095'$ and E $073^{\circ}56.781'$), S-2, middle estuary (N $15^{\circ}10.494'$ and E $73^{\circ}57.502'$), S-3, lower estuary (N $15^{\circ}11.842'$ and E $073^{\circ}58.240'$), were selected (Fig. 2.1). Sampling was carried out during February 2016 to March 2017. Surface water samples (estimation of physico-chemical parameters) were collected during low tide at monthly intervals during pre-monsoon (February-May, 2016; February-March, 2017) monsoon (June-September, 2016) and post-monsoon (October-December, 2016; January, 2017) season.

4.2.2. Physico-chemical parameters

The temperature was measured on-board with a mercury thermometer. Dissolved oxygen (DO) estimation was carried out by Winkler's method (Griffiths and Jackman, 1957); pH was estimated using pH meter probe, digital salinometer was used for salinity measurements, the nutrient concentration was estimated (nitrate, nitrite, phosphate, and silicate) using standard procedure (Grasshoff et al., 1999).

4.2.3. Sediment sampling

Surface sediment sample at each station was collected using sediment cores (PVC cores, 8 cm long, an inner diameter of 3.5 cm) in triplicates. All sediment cores were sectioned at 2 cm intervals, mixed well and stored at 4°C in the dark for further dinoflagellate cyst enumeration.

4.2.4. Sediment processing and analysis

Sediment samples from the upper (0-2 cm) section of the core were treated using the palynological method (Matsuoka and Fukuyo, 2000) with some modifications (Narale et al., 2013). Each sediment sample (3-6 g) was weighed in a beaker and washed with distilled water to remove the salt content. The samples were then sonicated and acid-digested (10% HCL and 30% HF). After rinsing the sample 3-4 times with distilled water, the acid-free sample was sieved through 100 and 10 μm mesh size to remove coarse and fine matter respectively. The residue retained on a 10 μm mesh was then transferred into a vial and a final volume was made upto 10 ml with distilled water. These vials were then stored at 4°C in the dark for dinoflagellate cyst identification.

Aliquot of 1 mL of the pre-treated sample was observed under Nikon Ti-S inverted microscope at 200x, 400x and 600x magnifications. Photographs of dinoflagellate cysts were captured in Nikon imaging software (NIS elements BR) equipped with colour digital camera Ri2. Dinoflagellate cysts were identified based on published descriptions (mentioned in Table 4.1). The dinoflagellate cyst abundance was expressed as cyst.g⁻¹ dry sediment. The water content was calculated according to the formula given by Matsuoka and Fukuyo (Matsuoka and Fukuyo, 2000).

4.2.5. Statistical methods

Statistical analyses were performed using PRIMER 6, STATISTICA 8.0 and CANOCO 4.5 software. To evaluate the relationships between the dinoflagellate cyst composition and environmental variables, a constrained ordination method, Redundancy Analysis (RDA) was used. Shannon-Weiner diversity indices were computed using PRIMER 6. Multivariate Multiple Regression (MMR) was carried out using STATISTICA 8.0.

4.3. Results

4.3.1. Seasonal distribution of dinoflagellate cysts along the salinity gradient

The observations made based on the data collected on the seasonal distribution of dinoflagellate cysts suggest that there was no similarity in terms of occurrence and abundance of dinoflagellate cyst at a particular season among the estuaries. However, high dinoflagellate cyst abundance was noted during the post-monsoon season (Fig. 4.1a) in Chapora, whereas in Sal it was observed during pre-monsoon season (Fig. 4.1b).

Fig. 4.1. Seasonal variation in (a, b) dinoflagellate cyst abundance; (c, d) salinity values and (e-j) percentage of sand, silt and clay fraction in Chapora and Sal estuary



A total of sixteen dinoflagellate cysts belonging to the order, Gymnodiniales, Gonyaulacales and Peridiniales were observed in both the estuaries (Plate 4.1.). In Chapora estuary, among sixteen dinoflagellate cyst species recorded, five were autotrophic and eleven were heterotrophic. On the contrary, in Sal estuary eleven species were recorded, wherein four were autotrophic and seven were heterotrophic. It is pertinent from the above observations that the heterotrophic dinoflagellate cysts dominated the assemblage in both the estuaries (Table 4.1).

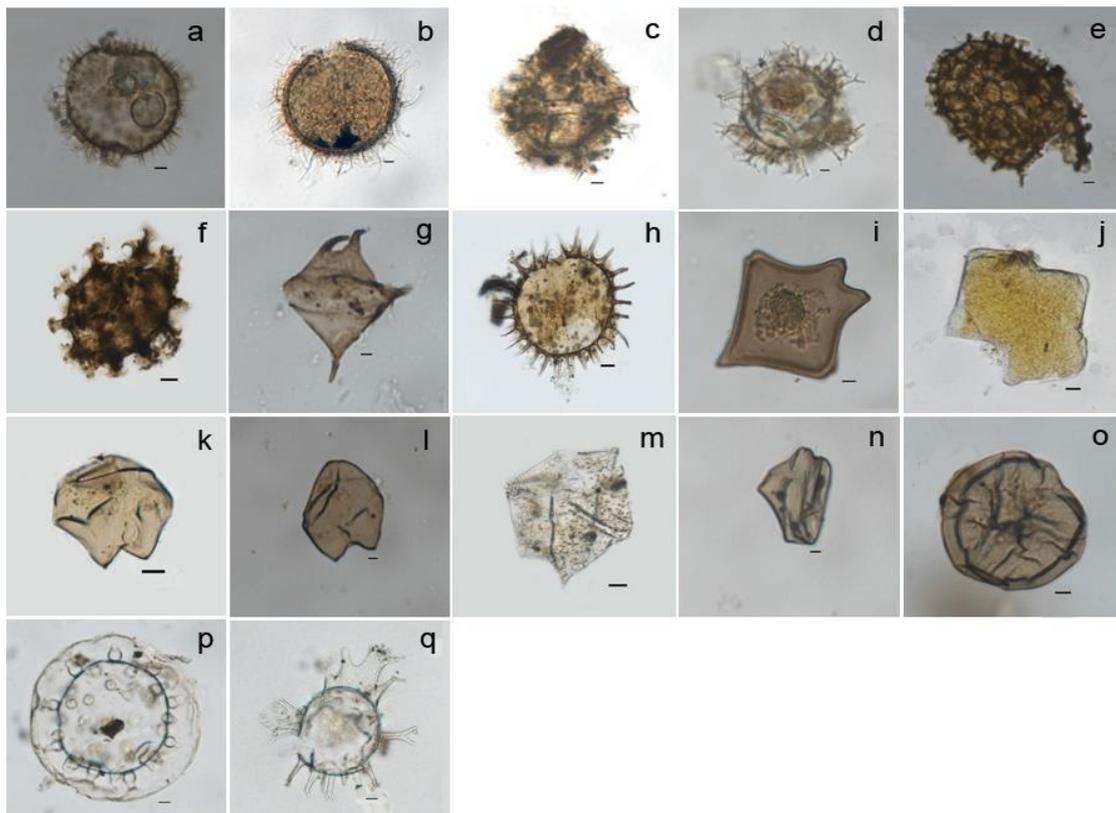


Plate 4.1. Dinoflagellate cysts encountered in the present study: (a) *Archaeoperidinium* sp., (b) *Bitectatodinium spongium* (c) *Gonyaulax scrippsae*, (d) *Gonyaulax spinifera* complex, (e) *Polykrikos kofoidii*, (f) *Polykrikos schwartzii*, (g) *Protooperidinium compressum*, (h) *Protooperidinium conicum*, (i) *Protooperidinium latissimum*, (j) *Protooperidinium leonis*, (k) *Protooperidinium oblongum*, (l) *Protooperidinium obtusum*, (m) *Protooperidinium pentagonum*, (n) *Protooperidinium subinerme* (lateral view), (o) *Protooperidinium subinerme* (ventral view), (p) *Pyrophacus steinii*, (q) *Spiniferites mirabilis*. All scale bars 10 μ

Table 4.1. List of dinoflagellate cysts encountered in the present study along with the source of identification

Biological name	Taxon	Paleontological name	Nutritional type	Toxicity	References
<i>Archaeoperidinium</i> sp.	<i>Ar</i> sp.	-	Heterotrophic	-	Mertens et al., 2012
<i>Bitectatodinium spongium</i>	<i>Bt sm</i>	-	Autotrophic	-	Zonneveld, 1997; Zonneveld and Jurkschat, 1999
<i>Gonyaulax scrippsae</i>	<i>Gn sc</i>	<i>Spiniferites bulloideus</i>	Autotrophic	-	Nehring, 1997a; Wall and Dale, 1968a
<i>Gonyaulax spinifera</i> complex*	<i>Gn sp cp</i>	<i>Spiniferites ramosus</i>	Autotrophic	HAB	Wall and Dale, 1970; Zonneveld and Pospelova, 2015
<i>Polykrikos kofoidii</i>	<i>Pl kf</i>	-	Heterotrophic	-	Shin et al., 2010; Zonneveld and Pospelova, 2015
<i>Polykrikos schwartzii</i>	<i>Pl sz</i>	-	Heterotrophic	-	Shin et al., 2010; Zonneveld and Pospelova, 2015
<i>Protoperidinium compressum</i>	<i>Prd cm</i>	<i>Stelladinium stellatum</i>	Heterotrophic	-	Wall and Dale, 1968a; Mcminn, 1991; Nehring, 1997a
<i>Protoperidinium conicum</i>	<i>Prd cn</i>	<i>Selenopemphix quanta</i>	Heterotrophic	-	Wall and Dale, 1968a; Bolch and Hallegraeff, 1990
<i>Protoperidinium latissimum</i>	<i>Prd lm</i>	-	Heterotrophic	-	Wall and Dale, 1968a
<i>Protoperidinium leonis</i>	<i>Prd ls</i>	<i>Quinquecuspis concretum</i>	Heterotrophic	-	Bolch and Hallegraeff, 1990; Wall and Dale, 1968a

<i>Protoperidinium oblongum</i>	<i>Prd obm</i>	-	Heterotrophic	-	Wall and Dale, 1968a; Bolch and Hallegraeff, 1990
<i>Protoperidinium obtusum</i>	<i>Prd obt</i>	-	Heterotrophic	-	Uzar et al., 2010
<i>Protoperidinium pentagonum</i>	<i>Prd pn</i>	<i>Trivantedinium capitatum</i>	Heterotrophic		Wall and Dale, 1968a; Bolch and Hallegraeff, 1990; Mcminn, 1991
<i>Protoperidinium subinerme</i>	<i>Prd sb</i>	<i>Selenopemphix nephroides</i>	Heterotrophic	-	Bolch and Hallegraeff, 1990; Wall and Dale, 1968a
<i>Pyrophacus steinii</i>	<i>Py st</i>	<i>Tuberculodinium vancampoae</i>	Autotrophic	-	Wall and Dale, 1971
<i>Spiniferites mirabilis</i>	<i>Sp mr</i>	<i>Spiniferites mirabilis</i>	Autotrophic	-	Bolch and Hallegraeff, 1990

* Toxic species

The diversity and abundance of dinoflagellate cysts were found to be high in the Chapora estuary ($H' = 2.73$; 227 cysts.g⁻¹ dry sediment) as compared to the Sal estuary ($H' = 2.27$; 18 cysts.g⁻¹ dry sediment). The observations made on the diversity and abundance with regard to the salinity gradient reveal that at station C-3 (towards downstream) of Chapora estuary and S-1 (towards upstream) of Sal estuary was found to have highest dinoflagellate cyst diversity.

It was noted that in Sal estuary, the dinoflagellate cysts were not observed during October - December, 2016 and January, 2017 at station S-1 (Fig. 4.1b). Further, the two species namely *Gonyaulax scrippsae* and *Gonyaulax spinifera* complex were observed at a wide salinity and temperature range in both the estuaries (Table 4.2).

Table 4.2 Seasonal variation of dinoflagellate cysts along salinity gradient encountered in the present study along the stations in Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary

Estuary	CHAPORA									SAL								
	C-1 (0-8 psu)*			C-2 (1-16 psu)*			C-3 (1-26 psu)*			S-1 (0-27 psu)*			S-2 (1-31 psu)*			S-3 (4-36 psu)*		
Season	Pr M	M	Po M	Pr M	M	Po M	Pr M	M	Po M	Pr M	M	Po M	Pr M	M	Po M	Pr M	M	Po M
<i>Archaeperidinium</i> sp.	+	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-
<i>Bitectatodinium</i> <i>spongium</i>	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-
<i>Gonyaulax scrippsae</i>	-	-	-	+	+	-	+	+	+	+	+	-	-	+	+	-	-	-
<i>Gonyaulax spinifera</i> complex**	+	-	-	+	+	-	+	-	+	+	+	-	-	+	+	-	-	-
<i>Polykrikos kofoidii</i>	-	-	-	+	-	-	-	+	+	+	-	-	-	-	+	-	-	-
<i>Polykrikos</i> <i>schwartzii</i>	-	-	-	+	-	-	+	+	+	-	+	-	-	-	-	-	-	-
<i>Protoperidinium</i> <i>compressum</i>	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
<i>Protoperidinium</i> <i>conicum</i>	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-

<i>Protoperidinium latissimum</i>	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
<i>Protoperidinium leonis</i>	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-
<i>Protoperidinium oblongum</i>	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
<i>Protoperidinium obtusum</i>	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Protoperidinium pentagonum</i>	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
<i>Protoperidinium subinerme</i>	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Pyrophacus steinii</i>	+	-	-	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-
<i>Spiniferites mirabilis</i>	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-

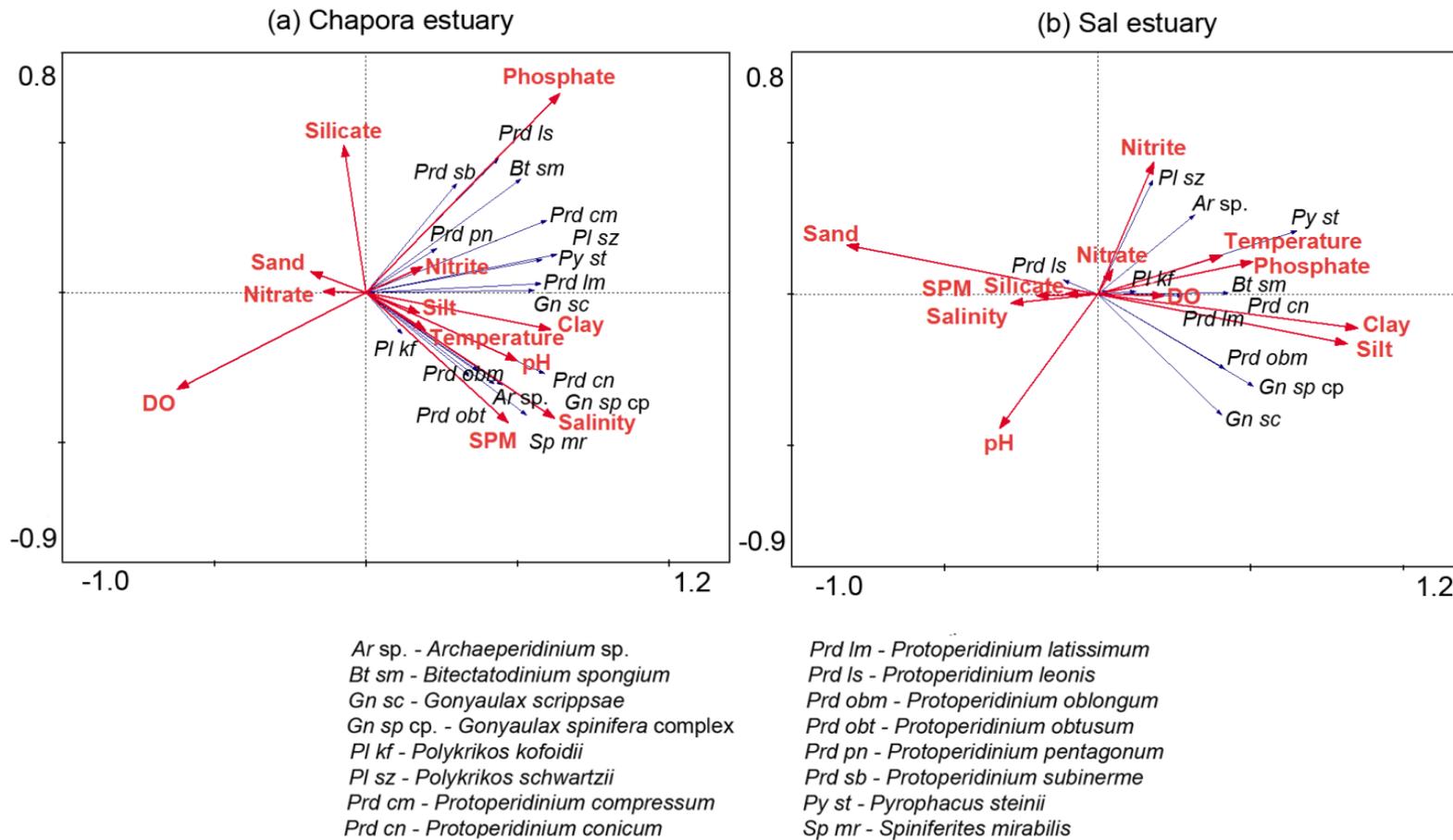
**Toxic species; + Present; - Absent; PrM-Pre-monsoon; M-Monsoon; PoM-Post monsoon

*Values in the parenthesis indicate the salinity range at the stations.

4.3.2. Influence of physico-chemical parameters on the distribution of dinoflagellate cysts

The observations made in the present study indicated that a wider salinity gradient (0-36 psu) was observed in Sal as compared to Chapora (0-26 psu). Redundancy Analysis (RDA) bi-plots (Fig. 4.2) reveal that, both the estuarine systems in spite of being tropical show different ecosystem functions. The variations in the physico-chemical parameters were found to affect the dinoflagellate cyst composition and distribution in both the estuaries. It is obvious from the RDA plot that in Chapora estuary, salinity, pH, DO, and phosphate concentration influenced the distribution and composition of dinoflagellate cyst whereas, in Sal estuary, grain size, water temperature, pH and phosphate concentration were found to be the influencing parameters.

Fig. 4.2. Redundancy Analysis (RDA) bi-plots depicting the relationship between dinoflagellate cysts and environmental parameters in (a) Chapora and (b) Sal estuary



4.3.3. Nutrient limitation as a triggering factor for dinoflagellate cyst formation

In the present study, high dinoflagellate cyst abundance was noted in Chapora estuary which coincided with low nitrate values. Whereas low dinoflagellate cyst abundance in Sal estuary coincided with high nitrate concentration. Only one toxic species namely *Gonyaulax spinifera*, that is capable of forming blooms was reported in both the estuaries. Further, based on the RDA bi-plots (Fig. 4.2) *Gonyaulax spinifera* abundance showed an inverse relationship with nitrate concentration in both the estuaries.

4.4. Discussion

4.4.1. Seasonal distribution of dinoflagellate cysts along the salinity gradient

Based on the seasonal distribution of dinoflagellate cysts in Chapora and Sal estuary, high dinoflagellate cyst abundance in Chapora was noted during post-monsoon season whereas in Sal estuary high abundance was recorded during pre-monsoon season. These results were found to positively correlate with salinity in Chapora estuary, whereas in Sal it was seen to be correlating with temperature and salinity values. Published studies (D' Silva et al., 2013; Satta et al., 2013; Price et al., 2016) reported salinity and temperature to be the crucial factors influencing the distribution and abundance of dinoflagellate cysts. In addition, it is also probable that other factors such as tidal amplitude, turbidity and water residence time (Price et al. 2016) also might have affected the abundance of dinoflagellate cysts.

In the present study, the dominance of heterotrophic dinoflagellate cysts indicates nutrient loadings due to monsoonal runoff as well as due to anthropogenic inputs and also due to high concentration of suspended particulate matter (SPM) noted in both the estuaries (Table 4.3) that have resulted in low light penetration. Hence, it is explicit that reduced light intensity can possibly influence the high abundance of heterotrophic cysts (Dale, 1983; Dale, 2001). Further, it is also necessary to mention the shrimp farming activities carried out along these areas also release a significant quantity of used water with high organic load and less dissolved oxygen (Boyd, 2000; Hernandez et al., 2007). Hence this might have also lead in making the ecosystem favourable for the increased abundance of heterotrophic forms.

Table 4.3 Variations of physico-chemical parameters in Chapora and Sal estuary

Parameters	Chapora		
Stations	C-1	C-2	C-3
pH	6.32- 7.83	6.59- 8.24	7.21- 8.41
Dissolved oxygen (mg.L ⁻¹)	5.99- 8.90	5.54- 8.10	4.22- 6.16
SPM (mg.L ⁻¹)	1.04- 42	2.2- 91	10.26- 196.6
Phosphate (µM)	0.24- 0.75	0.35- 1.33	0.52- 7.54
Nitrate (µM)	0.56- 6.71	0.31- 8.28	0.43- 6.26
% sand	49.61- 74.04	81.73- 99.32	56.81- 91.70
% silt	25.57- 50.00	0.47- 17.97	7.98- 42.45
% clay	0.31- 0.56	0.20- 0.57	0.22- 0.74
Parameters	Sal		
Stations	S-1	S-2	S-3
pH	6.39- 7.88	6.58- 7.79	6.8- 8.25
Dissolved oxygen (mg.L ⁻¹)	3.63- 8.50	4.43- 6.83	3.12- 6.88
SPM (mg.L ⁻¹)	5.5- 229.34	3.26- 536	7.7- 175.34
Phosphate (µM)	0.89- 2.89	0.64- 2.06	0.76- 3.00
Nitrate (µM)	0.51- 18.30	0.41- 22.39	0.53- 12.18
% sand	51.76- 81.27	83.23- 97.53	96.38- 98.85
% silt	18.03- 47.09	2.17- 16.44	1.08- 3.47
% clay	0.27- 1.15	0.23- 0.90	0.02- 0.16

In Chapora estuary the observed high diversity and abundance values could be attributed to lower levels of eutrophication, as evidenced by reduced nitrate content in the Chapora (8.28 µM) as compared to Sal (22.39 µM) estuary. High dinoflagellate cyst abundance in Chapora estuary could also be attributed to fine sediment size (silty-clayey). As dinoflagellate cysts are known to settle out in fine silt fraction with rich organic matter in such protected areas largely due to the reduced turbulence enhancing the sedimentation rate. Further, the size of the dinoflagellate cyst is almost in similar size fraction as that of

the slit particle ($<63 \mu\text{M}$) (Narale et al., 2013, Dale, 1979; Goodman, 1987). However, the low diversity and abundance of dinoflagellate cysts in Sal estuary could also be due to the dominance of sandy sediments as observed in the present study. Earlier work carried out along the west coast of India (D' Silva et al., 2011) also reported a positive correlation of fine silty sediment and dinoflagellate cyst diversity.

Along the salinity gradient, high diversity and abundance of dinoflagellate cysts in Chapora (at station C-3) and Sal (at station S-1) estuary suggests that such high values coincided with high salinity and silt fraction values (Fig. 4.1). These observations were also confirmed by performing MMR analysis. Based on MMR analysis it was noted that the silt concentration and salinity were found to have higher predictive power on the total dinoflagellate cyst abundance in Chapora estuary ($R= 0.62$, $P< 0.00035$). In contrast, silt concentration was found to have higher predictive power on the total dinoflagellate cyst abundance in Sal estuary ($R= 0.66$, $P< 0.00007$) suggesting significant correlation ($P< 0.05$) of dinoflagellate cyst abundance with silt and salinity. Earlier studies (Shin et al., 2010; Alkawri and Ramaiah, 2010; Sildever et al., 2015; Price et al., 2016) carried out suggest that salinity is known to be a crucial parameter that affects the growth of dinoflagellates and a triggering factor in the cyst formation.

Absence of dinoflagellate cysts in Sal estuary during October - December, 2016 and January 2017 at station S-1 (Fig. 4.1b) might be due to the excystment of cyst in the overlying water column due to favourable conditions (nutrient, temperature and salinity) as observed in the present study. Hence these physico-chemical factors might have controlled the distribution of dinoflagellates and the cysts (Alkawari and Ramaiah, 2010). Presence of two species namely *Gonyaulax scrippsae* and *Gonyaulax spinifera* complex at a wide salinity and temperature range in both the estuaries reveal its euryhaline and eurythermal nature (Hand et al., 1965).

4.4.2. Influence of physico-chemical parameters on the distribution of dinoflagellate cysts

In the present study, RDA bi-plots (Fig. 4.2) suggests that the distribution of dinoflagellate cysts in sediments and their formation depends on the physico-chemical parameters (Zonneveld et al., 2013). Earlier studies (Pospelova et al., 2008; D' Silva et al., 2013) have also reported that the dinoflagellate cyst assemblages are regulated by various physico-

chemical parameters (salinity, DO, pH, nutrient concentration and water temperature) coupled with sediment accumulation and encystment rates. In addition, habitat preference or competitive ability (nutrient) between the same or different set of taxa may also lead to changes in the cyst composition (Smayda and Reynolds, 2003).

4.4.3. Nutrient limitation as a triggering factor for dinoflagellate cyst formation

Nitrate is known to be an important nutrient in the distribution of phytoplankton (Xu et al., 2010). Resting cysts are known to be the fate of sexuality (Anderson and Wall, 1978) which are formed as a response to stress or unfavourable conditions (Bravo and Figuero, 2014). One of such unfavourable condition is a nutrient limitation (nitrate) and is one of the common triggering factors for resting cyst formation (Regenfors and Anderson, 1998; Figueiredo et al., 2006). Experimental studies carried out, also concluded nitrate depleted medium to be the most common and successful method to induce sexuality that leads to the formation of resting cysts in the cultures containing vegetative cells of phytoplankton (Pfiester and Anderson, 1987; McQuoid and Hobson, 2008).

High dinoflagellate cyst abundance in Chapora and low dinoflagellate cyst abundance in Sal which coincided with low and high nitrate concentration respectively, indicates that low nitrate concentration in Chapora has to lead to stress on the dinoflagellates in the overlying water column eventually leading to encystment. Hence a high number of dinoflagellate cysts were noted. Whereas in Sal estuary, comparatively higher nitrate concentration was recorded which created favourable habitat for dinoflagellates in the water column. Hence lower dinoflagellate cysts were noted in Sal estuary.

Further, it is known that germination occurs when favourable environmental conditions persist (Bravo and Figuero, 2014). In the present study, based on RDA bi-plots an inverse relationship between *G. spinifera* and nitrate concentration was noted (Fig. 4.2). *G. spinifera* is known to be a bloom forming and a prolific yessotoxin producer (Rhodes et al., 2006), and was reported in both the estuaries. Inverse relationship of this species with nitrate indicates that high nitrate input in these estuaries might lead to excystment of this species, eventually leading to blooms.

Toxic species namely *Gonyaulax spinifera* reported in both the estuaries is known to be a prolific producer of yessotoxin (Rhodes et al., 2006). Inverse relationship of the abundance of *Gonyaulax spinifera* with nitrate in both the estuaries suggests that high

nitrate input in these estuaries might lead to excystment of *Gonyaulax spinifera* species, eventually leading to blooms. It is imperious that encystment is enhanced during nutrient depletion (Anderson et al., 1984, Ellegaard et al., 1998), as indicated earlier that high abundance of dinoflagellate cysts occurs at low nitrate concentrations (Marret and Zonneveld, 2003). Thus in the present study, nutrient dynamics especially nitrates in the overlying waters could be one of the potential reason to enable excystment leading to seasonal bloom formation (Anderson et al., 1987; Figueroa et al., 2005).

In view of the observations made in the present study, the presence of toxic species in these estuarine areas is a matter of great concern, which probably has a potential risk to form HABs. Further, once established in such tropical habitats there is also a possibility of repeated occurrences of such blooms that are largely responsible for water quality deterioration, with its detrimental effect and can play a vital role in altering the biodiversity and ecosystem function (Riccardi et al., 2009; Mudadu et al., 2017). This situation is further synergised by the anthropogenic loading in extremely vulnerable habitats largely influenced by the land use pattern (human settlement, tourism, sewage disposal, agricultural runoff, shrimp farming ponds) in the coastal habitats (personal communication). Hence, it may be necessary that such fragile environs need regular monitoring to assess health status.

Chapter-5
Antonymous nature of
phytoplankton groups
(Chlorophyceae and
Cyanophyceae) in the tropical
estuarine environments of Goa,
south west coast of India

5.1. Introduction

Estuarine ecosystems are one of the complex habitats due to their frequently changing physico-chemical surroundings (Parab et al., 2013) largely influenced by the land-sea interactions and the coastal processes. Published literature (Tittensor et al., 2010; Moyle et al., 2010) suggests that these habitats in tropics are highly productive and support a diversified invertebrate population. This unique feature makes these habitats as the potential sites for spawning and nursery of variety of juvenile marine fishes, Crustaceans, and other planktonic organisms (Whitefield, 1998; Sheaves et al., 2014). In recent times, much of these habitats are largely influenced by the anthropogenic input (Kennish, 2005) mainly derived from the land use pattern and the coastal developmental activities (Kennish, 2002) thus causing a deleterious effect on the functioning of these ecosystems.

Phytoplankton community in these habitats, being primary producers form an important entity and establish themselves as an essential link in the food chain through the provision of organic matter (Sridhar et al., 2006). However, a sizable group of these communities are also known to form blooms during favourable conditions (Sanilkumar et al., 2009) and also can be detrimental due to their toxicity (Hallegraeff et al., 1995). Phytoplankton blooms are now known to be one of the important issues that threaten most of the aquatic habitat functioning. Among the environmental variables, salinity (Lancelot and Muylaert, 2011) and anthropogenic nutrient input can cause algal bloom and affect ecosystem function (Zaias et al., 2010; Stewart et al., 2011). Therefore, it is imperative that better understanding of these vulnerable habitats is essential to understand their functioning and to generate decision-making system for efficient management (Beck et al., 2001). Several studies were carried out on diatoms and dinoflagellate blooms (Alkawri and Ramaiah, 2010; Patil and Anil, 2011; Pednekar et al., 2012). However few studies have been carried out on the Chlorophyceae and Cyanophyceae phytoplankton blooms (Paerl et al., 2001; Mishra et al., 2009; Figueiredo et al., 2006).

Here, an attempt has been made to provide information on the distribution of Chlorophyceae and Cyanophyceae with the following objectives (1) to elucidate the distribution of Chlorophyceae and Cyanophyceae along the salinity gradient (2) to highlight the effect of physico-chemical parameters on the distribution of Chlorophyceae and Cyanophyceae (3) to reveal the presence of toxic bloom forming Cyanobacteria.

5.2. Materials and Methods

5.2.1. Sampling sites

Three stations were selected in each estuary based on salinity gradient. In Chapora estuary, station C-1, upper estuary (15°39.072' and E 073°47.995'), C-2, middle estuary (N 15°38.396' and E 073°45.848'), C-3, lower estuary (N 15°38.137' and E 73°45.640'), were selected and in Sal estuary, three stations namely, S-1, upper estuary (N 15°10.095' and E 073°56.781'), S-2, middle estuary (N 15°10.494' and E 73°57.502'), S-3, lower estuary (N 15°11.842' and E 073°58.240'), were selected (Fig. 2.1). Sampling was carried out during July-December, 2016. Surface water samples (phytoplankton taxonomy and estimation of physico-chemical parameters) were collected during low tide at monthly intervals during monsoon (July-September) and non-monsoon (October-December) season.

5.2.2. Physico-chemical parameters

Temperature was measured onboard with a mercury thermometer. Dissolved oxygen (DO) estimation was carried out by Winkler's method (Griffiths and Jackman, 1957), pH was estimated using pH meter probe, Digital salinometer was used for salinity measurements, nutrient concentration was estimated (nitrate, nitrite, phosphate and silicate) using standard procedure (Grasshoff et al., 1999).

5.2.3. Taxonomy of HAB forming Chlorophyceae and Cyanophyceae species

Water samples for phytoplankton identification were collected in 1 L plastic bottles in triplicates and were fixed with 0.2% Lugols's iodine. Samples were allowed to settle for 48 h and were concentrated to 100 ml by carefully siphoning the top layer of the sample with tubing, one end of which was covered with 10 µm mesh, to avoid loss of small-sized phytoplankton (Willen, 1976). 1 mL of concentrated sub-sample was analysed in inverted microscope (Nikon Ti-S) at 200x, 400x and 600x magnifications and taxonomic identification of Chlorophyceae and Cyanophyceae group was carried out following published identification manual (Van, 2006; Sahu, 2013).

5.2.4. Statistical methods

Statistical analyses were performed using PRIMER 6 and CANOCO 4.5 software. To evaluate the relationships between the phytoplankton species composition and environmental variables, a constrained ordination method, RDA was used. Multidimensional Scaling (MDS) plots were produced using PRIMER 6 to visualise

seasonal differences between sites based on cell density and S-17 Bray- Curtis similarity measure was calculated. Shannon- Weiner diversity indices were computed using PRIMER 6.

5.3. Results

5.3.1. Distribution of Chlorophyceae and Cyanophyceae along the salinity gradient

A total of twenty five species of Chlorophyceae and three species of Cyanophyceae, were recorded in the present study (Table 5.1). In Chapora, twenty three species of Chlorophyceae and three species of Cyanophyceae were reported, however, in the Sal, only ten species of Chlorophyceae and one species of Cyanophyceae was noted. It is clear from the observations made in the present study that the Chlorophyceae was found to be dominant in both the estuaries.

Table 5.1.List of freshwater phytoplankton (Chlorophyceae and Cyanophyceae) encountered along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary during the present study

Taxon	Taxon Abbreviation	Chapora estuary			Sal estuary		
		C-1	C-2	C-3	S-1	S-2	S-3
Chlorophyceae							
<i>Actinastrum</i> sp.	<i>Ac</i> sp.	+	+	+	-	+	+
<i>Chlamydomonas</i> sp.	<i>Ch</i> sp.	+	+	-	-	-	-
<i>Coelastrum</i> sp.	<i>Cl</i> sp.	-	-	-	+	+	+
<i>Cosmarium depressum</i>	<i>Cs dp</i>	-	-	-	+	-	-
<i>Cosmarium obsoletum</i>	<i>Cs ob</i>	-	+	-	-	-	-
<i>Cosmarium</i> sp.	<i>Cs</i> sp.	+	+	-	-	+	-
<i>Cosmarium subcostatum</i>	<i>Cs sb</i>	-	+	-	-	-	-
<i>Golenkinia</i> sp.	<i>Gl</i> sp.	+	+	-	-	-	-
<i>Oocystis</i> sp.	<i>Os</i> sp.	+	+	+	-	+	-
<i>Pediastrum</i> sp.	<i>Pd</i> sp.	-	+	-	-	-	+
<i>Scenedesmus acuminatus</i>	<i>Sc ac</i>	-	+	-	-	+	-
<i>Scenedesmus quadricaudata</i>	<i>Sc qd</i>	+	+	-	+	+	-
<i>Scenedesmus</i> sp.	<i>Sc</i> sp.	-	-	+	+	+	-

<i>Spirogyra</i> sp.	<i>Sr</i> sp.	+	-	-	-	-	-
<i>Staurastrum anatinum</i>	<i>St an</i>	-	+	-	-	-	-
<i>Staurastrum avicula</i>	<i>St av</i>	+	+	-	-	-	-
<i>Staurastrum chaetoceros</i>	<i>St ch</i>	-	+	-	-	-	-
<i>Staurastrum clevei</i>	<i>St cl</i>	+	+	+	-	-	-
<i>Staurastrum elongatum</i>	<i>St el</i>	-	+	-	-	-	-
<i>Staurastrum gracile</i>	<i>St gr</i>	+	+	-	-	-	-
<i>Staurastrum leptocladum</i>	<i>St lp</i>	+	+	-	-	-	-
<i>Staurastrum longibrachiatum</i>	<i>St lg</i>	+	-	-	-	-	-
<i>Staurastrum sexangulare</i>	<i>St sn</i>	+	-	-	-	-	-
<i>Staurastrum</i> sp.	<i>St</i> sp.	+	+	+	+	+	-
<i>Xanthidium</i> sp.	<i>Xn</i> sp.	-	+	-	-	-	-
Cyanophyceae							
<i>Merismopedia</i> sp.	<i>Mp</i> sp.	-	+	-	-	-	-
<i>Microcystis</i> sp.	<i>Ms</i> sp.	-	+	-	-	-	-
<i>Oscillatoria</i> sp.	<i>Oc</i> sp.	+	-	-	+	+	-

+ Present, – Absent

It has been also noted that the diversity of these groups were found to be of lesser magnitude in the Sal (Chlorophyceae $H' = 2.28$, Cyanophyceae $H' = 0$) compared to Chapora (Chlorophyceae $H' = 3.12$, Cyanophyceae $H' = 1.09$) estuary.

With respect to Chlorophyceae, the significant seasonal difference in the composition and distribution was observed in both the estuaries (Table 5.2), and cell density of Chlorophyceae was found to be higher during monsoon season (Fig. 5.1). In contrast, no significant seasonal difference in the cell density of Cyanophyceae was observed along the salinity gradient in both the estuaries (Fig. 5.1).

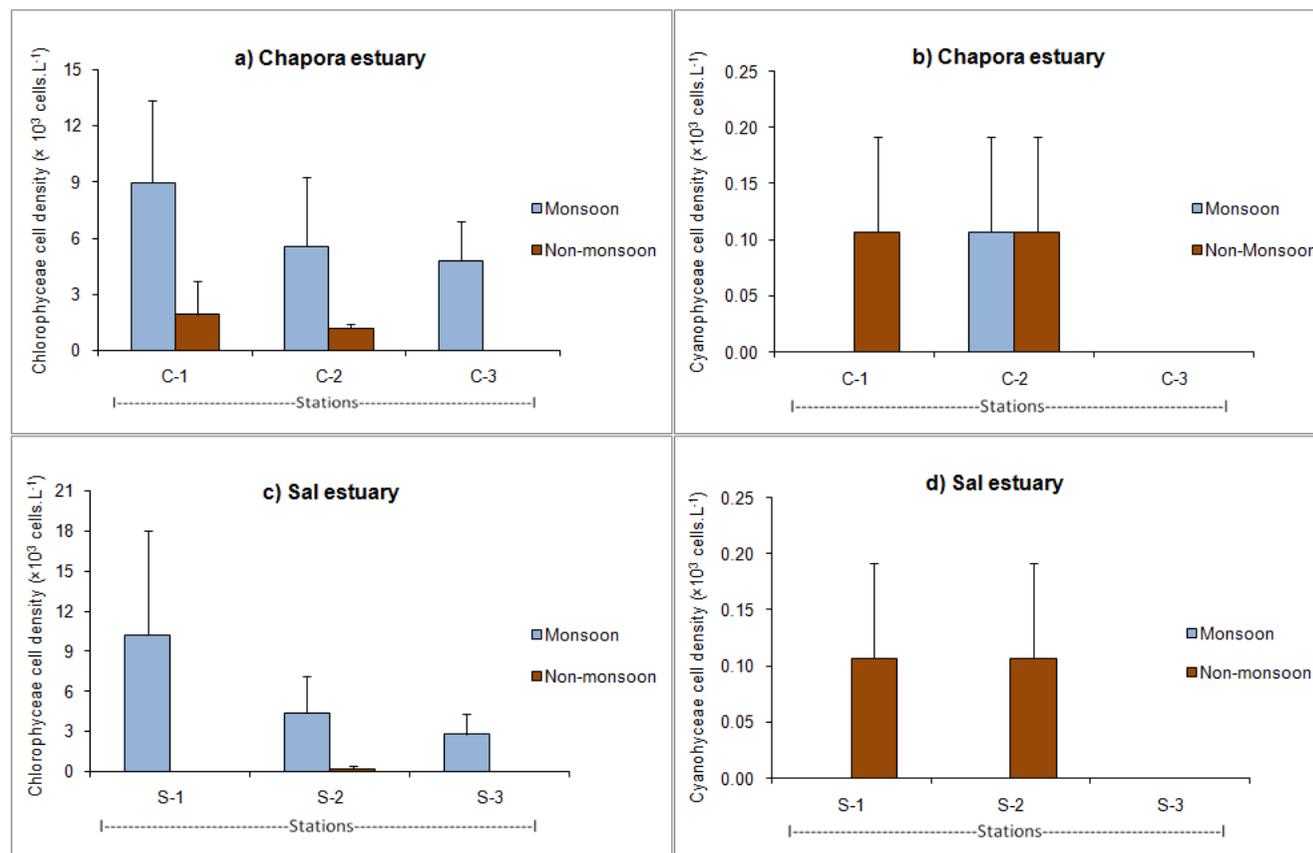
Table 5.2. Seasonal variation in the distribution of Chlorophyceae and Cyanophyceae along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary during the present study

Taxon	Chapora estuary						Sal estuary					
	C-1		C-2		C-3		S-1		S-2		S-3	
Chlorophyceae	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM
<i>Actinastrum</i> sp.	+	+	+	-	+	-	-	-	+	-	+	-
<i>Chlamydomonas</i> sp.	+	-	+	+	-	-	-	-	-	-	-	-
<i>Coelastrum</i> sp.	-	-	-	-	-	-	+	-	+	-	+	-
<i>Cosmarium depressum</i>	-	-	-	-	-	-	+	-	-	-	-	-
<i>Cosmarium obsoletum</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Cosmarium</i> sp.	+	+	+	-	-	-	-	-	-	+	-	-
<i>Cosmarium subcostatum</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Golenkinia</i> sp.	+	-	+	-	-	-	-	-	-	-	-	-
<i>Oocystis</i> sp.	+	-	+	-	+	-	-	-	+	-	-	-
<i>Pediastrum</i> sp.	-	-	+	-	-	-	-	-	-	-	+	-
<i>Scenedesmus acuminatus</i>	-	-	-	+	-	-	-	-	+	-	-	-
<i>Scenedesmus quadricaudata</i>	-	+	-	+	-	-	+	-	+	-	-	-
<i>Scenedesmus</i> sp.	-	-	-	-	+	-	+	-	+	-	-	-
<i>Spirogyra</i> sp.	+	-	-	-	-	-	-	-	-	-	-	-

<i>Staurastrum anatinum</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum avicula</i>	+	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum chaetoceros</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum clevei</i>	+	-	+	-	+	-	-	-	-	-	-	-
<i>Staurastrum elongatum</i>	-	-	-	+	-	-	-	-	-	-	-	-
<i>Staurastrum gracile</i>	+	+	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum leptocladum</i>	+	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum longibrachiatum</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>Staurastrum sexangulare</i>	-	+	-	-	-	-	-	-	-	-	-	-
<i>Staurastrum</i> sp.	+	+	+	+	+	-	+	-	+	-	-	-
<i>Xanthidium</i> sp.	-	-	+	-	-	-	-	-	-	-	-	-
Cyanophyceae												
<i>Merismopedia</i> sp.	-	-	-	+	-	-	-	-	-	-	-	-
<i>Microcystis</i> sp.	-	-	+	-	-	-	-	-	-	-	-	-
<i>Oscillatoria</i> sp.	-	+	-	-	-	-	-	+	-	+	-	-

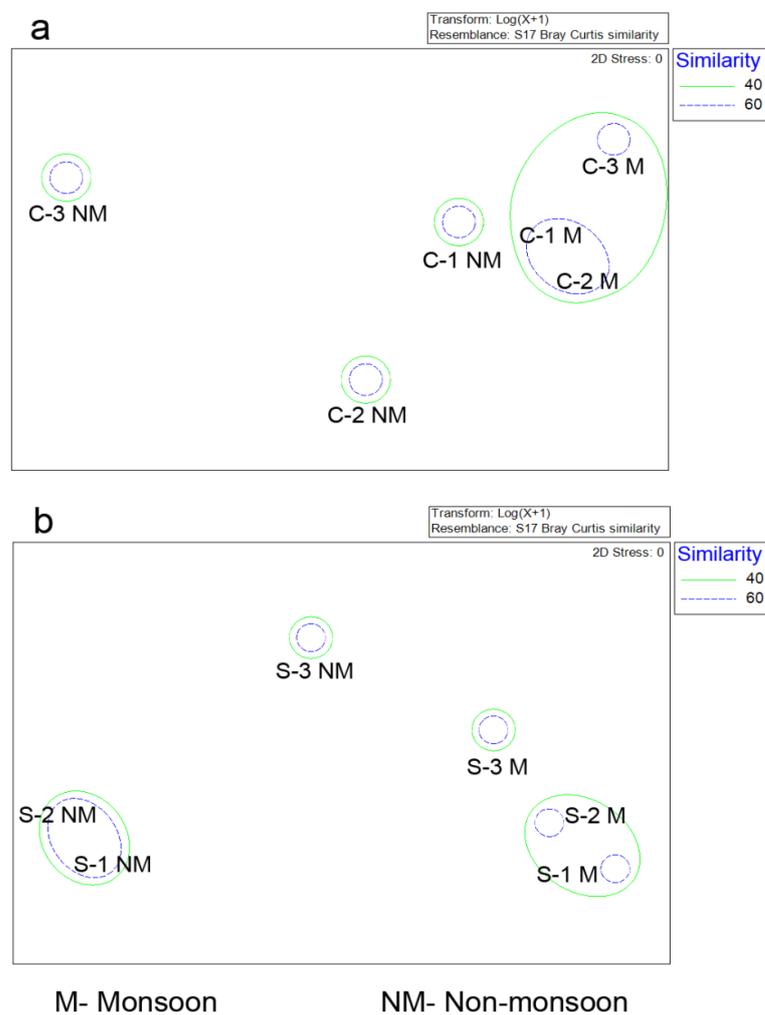
+ Present, – Absent

Fig. 5.1. Spatio-temporal variations in the cell density (cells. L⁻¹) of Chlorophyceae and Cyanophyceae along salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary. (a) Chlorophyceae cell density in Chapora estuary (b) Cyanophyceae cell density in Chapora estuary (c) Chlorophyceae cell density in Sal estuary (d) Cyanophyceae cell density in Sal estuary



Further, in order to reveal the seasonal distribution of these phytoplankton groups along the salinity gradient based on its cell density, MDS analysis (Fig. 5.2) was carried out. The results of MDS analysis indicated a seasonal difference among the stations with respect to these phytoplankton groups.

Fig. 5.2. Multidimensional scaling (MDS) ordination for (a) Chapora and (b) Sal estuary based on Bray-Curtis similarity of stations during the study period based on freshwater phytoplankton cell density season-wise along the salinity gradient



5.3.2. Effect of physico-chemical parameters on the distribution of Chlorophyceae and Cyanophyceae

During both, monsoon and non-monsoon season, salinity variations along the gradient were found to be large in Chapora estuary whereas small variation in salinity values along

the gradient was seen in Sal estuary (Table 5.3). It was noted that the concentration of dissolved inorganic nutrients (nitrate, nitrite, phosphate and silicate) was higher in monsoon as compared to non-monsoon season. Among other nutrients estimated, the concentration of nitrate along the gradient was recorded to be highest during monsoon season in both Chapora (8.28 μM) and Sal (22.39 μM) estuary. There was no significant difference in the water temperature between Chapora (26°C - 29°C) and Sal (28°C - 30°C) estuary during monsoon. Dissolved oxygen (DO) concentration is an important constituent of water and its concentration in water is an indicator of prevailing water quality (George et al., 2012a). DO concentration was found to be higher in Chapora (8.90 mg.L^{-1}) as compared to Sal (6.88 mg.L^{-1}) estuary in monsoon season and showed remarkable seasonal variations with a range of 5.30 mg.L^{-1} - 8.90 mg.L^{-1} in Chapora estuary and 3.74 mg.L^{-1} - 6.88 mg.L^{-1} in Sal estuary. Significant changes in pH were noted in Chapora (6.59 - 8.04) and Sal (6.70 - 8.15) estuary. SPM value was found to be towards a higher range in Sal estuary (5.5 - 536 mg.L^{-1}) as compared to Chapora estuary (1.04 - 197 mg.L^{-1}).

Table 5.3. Seasonal variation of salinity along the salinity gradient in Chapora and Sal estuary

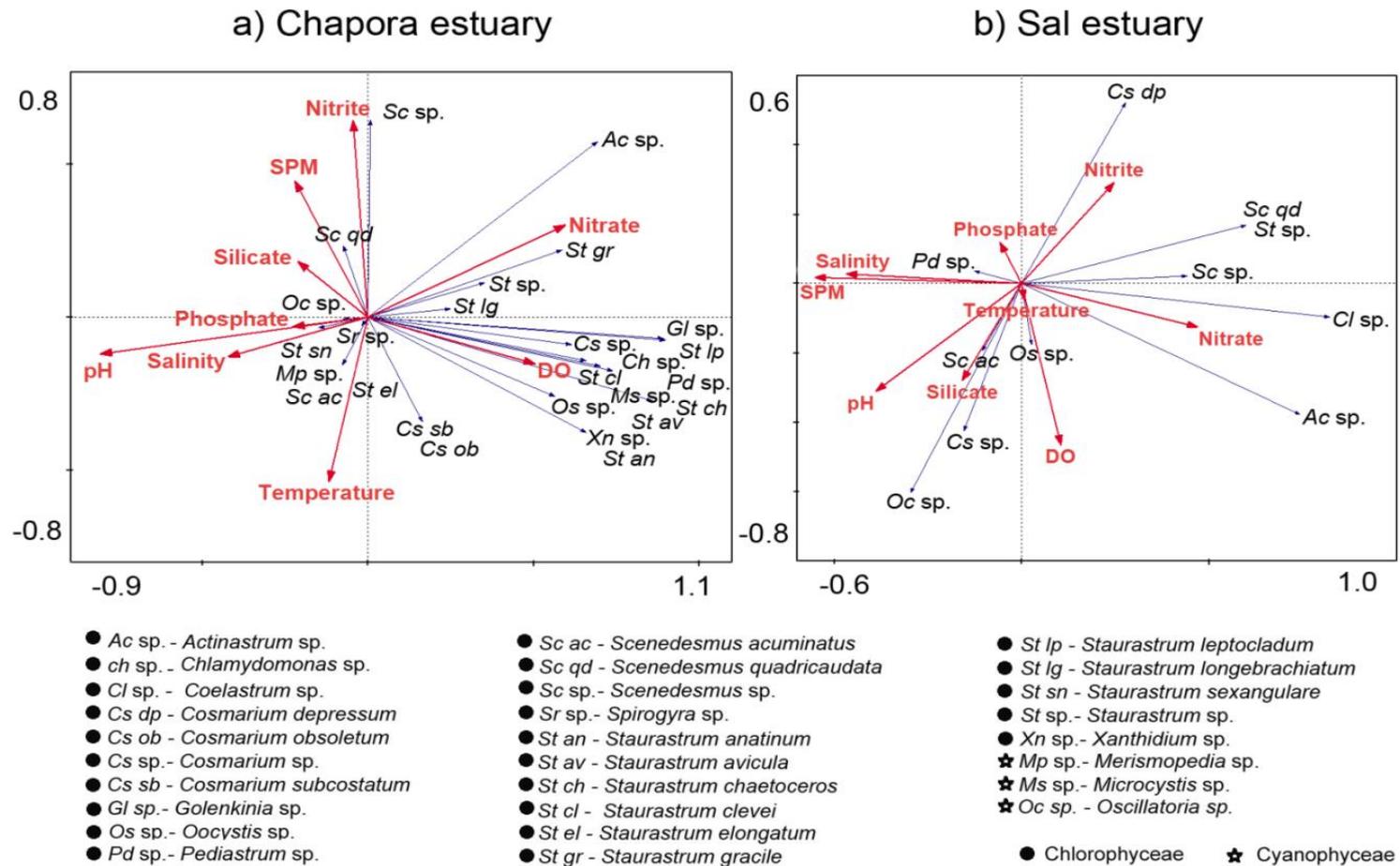
Estuary	CHAPORA			SAL		
Station	C-1	C-2	C-3	S-1	S-2	S-3
Monsoon	0-2	1-3	1-6	0-3	1-4	4-15
Non-monsoon	1-4	2-15	6-26	1-26	7-30	31-36

Based on Redundancy Analysis (RDA) plots it is revealed that, nitrate concentration including other physico-chemical parameters (salinity, temperature, DO, pH) influenced the distribution of Chlorophyceae group (Fig. 5.3).

5.3.3. Presence of toxic bloom forming Cyanobacteria

In the present study, two toxic bloom forming Cyanobacterial genus (*Microcystis* and *Oscillatoria*) were reported. In Chapora estuary both *Microcystis* (0.3×10^3 cells.L⁻¹) and *Oscillatoria* (0.32×10^3 cells.L⁻¹) were reported in monsoon and non-monsoon season respectively. Whereas in Sal, *Oscillatoria* was reported (0.64×10^3 cells.L⁻¹) only during non-monsoon season.

Fig. 5.3. Redundancy Analysis (RDA) bi-plots depicting the relationship between freshwater phytoplankton (Chlorophyceae and Cyanophyceae) and environmental parameters in (a) Chapora and (b) Sal estuary.



5.4. Discussion

5.4.1. *Distribution of Chlorophyceae and Cyanophyceae along the salinity gradient*

Salinity is the indicator of freshwater incursion into the estuary during monsoon season and helps to explain the spatial and temporal distribution of phytoplankton along the gradient (Madhavi et al., 2015). In both estuaries, salinity was affected by precipitation during monsoon and a noteworthy decline in salinity was observed at all three stations.

As the tide is one of the most important parameters controlling the dynamics of Chapora estuary, the tidal range is low and freshwater flow during monsoon may reduce the salinity (Pathak and Kotnala, 2002). It is evident from the present observations that the Sal estuary experiences a significantly more saline condition as compared to Chapora during both the seasons. It is apparent from the geomorphology of the Sal estuary as a small narrow shallow estuary and the tide upstream gets progressively flood dominant, a feature common in narrow, shallow estuaries (Parulekar and Chatterji, 1988).

In the present study, the observations made revealed that the distribution and composition of Chlorophyceae and Cyanophyceae were strongly affected based on salinity gradient.

This chapter deals with only two groups of phytoplankton (Chlorophyceae and Cyanophyceae), that are representatives of freshwater forms. Hence high diversity was found in the Chapora estuary which coincided with the low saline waters, forming a favourable habitat to support freshwater species (Rao and Pragada, 2010; Madhavi et al., 2015).

The high Cell density of Chlorophyceae during monsoon season signifies that these species are known to thrive well during the low salinity periods. Similar observations were also reported by Marshall and Alden (1990); George et al. (2012a); Madhavi et al. (2015). Significant seasonal variation of Chlorophyceae group along the salinity gradient could be due to the variations in salinity values along with the changes in physico-chemical parameters (Devassy and Bhattathiri, 1974, Huang et al., 1997a). This probably suggests that these environs, although within near proximity display different and unique ecosystem function.

Whereas, no significant seasonal difference in the cell density of Cyanophyceae along the salinity gradient indicates that they are known to be mostly confined to the freshwater zones (George et al., 2012a). The succession of phytoplankton along the salinity gradient

has been attributed to the fact that most phytoplankton species are stenohaline and undergo osmotic stress upon exposure to salinity changes (Bisson and Kirst, 1995). Thus from the present study, it is noted that salinity gradients strongly influence the distribution and abundance of Chlorophyceae and Cyanophyceae groups in estuaries (Rao and Pragada, 2010; Evagelopoulos et al., 2009).

Further, MDS analysis carried out showed groupings of monsoonal stations in Chapora (C-1 M, C-2 M and C-3 M) and Sal (S-1 M and S-2 M) based on 40% similarity of phytoplankton composition along the salinity gradient. In Sal estuary, it is seen that station S-3 M was not grouped along with S-1 M and S-2 M. This is due to comparatively higher salinity values recorded at S-3 M station during monsoons. This observation suggests the monsoonal effect on the distribution of these dominant species in Chapora (*Actinastrum* sp., *Oocystis* sp., *Staurastrum clevei*, *Staurastrum* sp.) and Sal (*Scenedesmus quadricaudata*, *Scenedesmus* sp., *Staurastrum* sp.) estuaries along the salinity gradient (Fig. 5.2).

5.4.2. Effect of physico-chemical parameters on the distribution of Chlorophyceae and Cyanophyceae

In the present study, the selected estuarine systems are dominated by freshwater influx leading to a significant reduction in the salinity during monsoonal months. In such dynamic systems, it was found that Chlorophyceae dominated over Cyanophyceae, suggesting the former group can tolerate and withstand dynamic environments of estuaries with reduced salinities as compared to Cyanophyceae (Annalakshmi and Amsath, 2012; Panigrahi and Patra, 2013). Published literature (Paerl and Ustach, 1982, Figueiredo et al., 2006) suggested that in freshwater systems, Cyanophyceae dominated the cell count over Chlorophyceae, and lead to bloom formation.

Estuarine environments are subjected to various changes in physico-chemical properties due to natural (freshwater runoff during monsoon) as well as anthropogenic activities (sewage disposal, agricultural runoffs, sand mining, human settlements). In the present study, RDA was carried out in order to understand the effect of physico-chemical parameters on the distribution of Chlorophyceae and Cyanophyceae. RDA revealed that nitrate concentration including other physico-chemical parameters (salinity, temperature, DO, pH) affected the distribution of Chlorophyceae group.

Previous studies by Garnier et al. (1995) reported that Chlorophyceae develop in an N rich environment. Mishra et al. (2009), in his study on Chlorophyceae reported nitrate as the regulating factor in the growth of phytoplankton. This suggests that nitrate is one of the prime regulating factors in the proliferation of Chlorophyceae species. Based on the observations from the present study it was noted that, high nitrate concentration coupled with high DO concentration and low salinity values might have led Chlorophyceae to outcompete Cyanophyceae in these estuarine environments. Similar observations reported by Rajagopal et al. (2010) suggests that, besides salinity and nutrients, other physico-chemical parameters (DO, temperature, pH) also strongly affect Chlorophycean members in freshwater zones. Eventually, the combination of all these factors shapes the seasonal and spatial changes in the composition and distribution of species (Cloern and Dufford, 2005). It is not likely that any one organism will have the ability to excel under all circumstances. However, appearance of a dominant group suggests its ability to maximise under the prevailing environmental conditions (Oliver and Ganf, 2000).

Published reports (Paerl et al., 2014; Cartensens et al., 2015) suggest that Chlorophyceae bloom formation occurs in the estuarine systems in low-salinity upper reaches of Scheldt estuary, responding positively to elevated freshwater input. Formation of blooms in estuaries is of great concern as estuaries are highly productive regions and serve as the breeding and nursery grounds for a variety of marine fishes and many other marine organisms, like several shrimp species especially during their early stages (Kannappan and Karthiketan, 2013). Hence, it is imperative that the anthropogenic input largely influenced by the land use pattern act as one of the driving force towards the enrichment of nutrients especially nitrogen in the tropical estuarine systems.

5.4.3. Presence of toxic bloom forming cyanobacteria

The cyanobacteria (Blue-Green Algae) are the most prevalent and nuisance freshwater algal taxa in nutrient enriched (N and P) freshwater and brackish ecosystems (Paerl, 1988; Sellner, 1997) causing serious water quality deterioration (Codd and Bell, 1996; Carmichael, 1998) through hypoxia and anoxia of underlying waters, which may lead to fish and bottom fauna mortalities (Pearl and Tucker, 1995). Historically, genera *Anabaena*, *Microcystis*, *Nodularia*, and *Oscillatoria* are the most notorious which are traditionally confined to heavily nutrient enriched impoundments (Paerl, 1988; Paerl, 1995). In the present study two toxic bloom forming cyanobacterial genus, *Microcystis*

and *Oscillatoria* were reported. In recent times, the distribution and frequency of occurrence of *Microcystis* and other freshwater cyanobacteria blooms are increasing and have become a worldwide concern (Fristachi et al., 2008). At higher trophic levels, *Microcystis* blooms affect fish health through impacts on the growth rate, histopathology, delayed hatching, feeding rate and behaviour (Malbrouck and Kestemont, 2006). The reporting of these toxic bloom forming genera in the present study is an alarming threat to the health and ecosystem function, which probably can pose a serious risk in future to water quality and public health affecting the fish diversity.

Chapter-6

Habitat heterogeneity and diversity of dinoflagellate cysts

6.1. Introduction

Dinoflagellates are one of the major phytoplankton groups, which are present in continental shelf environments, freshwater and marine systems (Kim et al., 2009). The life cycle of many of these is characterised by a planktonic vegetative stage, and at least 10% of the species have a known non-motile benthic stage (Head, 1996). At present, more than seventy species of marine and twenty species of freshwater dinoflagellates are known to produce resting cysts (Nehring, 1993a). These cysts are morphologically distinctive and differ from the motile planktonic stage (Anderson et al., 1995). Cyst morphology is increasingly regarded as an important character in the classification of modern dinoflagellates (Dale, 1977; Dodge, 1982).

The morphology of resting cysts was well defined in the 1970s and 1980s, in earlier paleontological studies of fossil cysts (Bravo and Figueroa, 2014). Generally, cysts are defined on the basis of their shape and colour, the structure and surface ornamentation of the wall, and the features of the archeopyle, paratabulation, and cell contents (Bravo and Figueroa, 2014).

The cell wall of dinoflagellate cysts is composed of dinosporin which is extremely resistant to physical, chemical, and biological breakdown (Wall et al., 1977; Taylor, 1987). Hence once settled, they accumulate in the sediments and remain viable for extended periods (from months up to a century) (Lundholm et al., 2011). Therefore, a survey on dinoflagellate cysts in sediments is useful to obtain integrated records of the planktonic population, over time and space (Kim et al., 2009).

Several studies on dinoflagellate cyst assemblages were carried out around the world (Matsuoka, 1976a; Davey, 1971; Lewis et al., 1984; Baldwin, 1987; Hallegraeff, 1998; Matsuoka et al., 1999; Devillers et al., 2000; Godhe et al., 2000; Mizushima, 2007; D' Costa et al., 2008; Kim et al., 2009; Uzar et al., 2010; Baula et al., 2011; D' Silva et al., 2011; 2012; Narale et al., 2013; Aydin et al., 2015). Goa is known to be one of the rapidly developing states due to tourism and hence is responsible for a huge amount of anthropogenic output. Till date, D' Silva et al. (2012) and Patil, unpublished (D' Costa et al., 2008) have made an attempt to carry out a study on dinoflagellate cysts from coastal waters off Goa and in Zuari estuary respectively. However, no studies have been conducted along the other estuarine regions of Goa. Hence the present study conducted along the salinity gradient in Chapora (North Goa) and Sal estuary (South Goa) is first of

its kind. The primary objective was to carry out taxonomic identification of dinoflagellate cysts types observed in recent sediments and to understand the distribution and diversity of these dinoflagellate cysts based on the habitat heterogeneity among the selected study areas.

6.2. Materials and methods

6.2.1. Sampling sites

Three stations were selected in each estuary based on salinity gradient. In Chapora estuary, station C-1, upper estuary (15°39.072' and E 073°47.995'), C-2, middle estuary (N 15°38.396' and E 073°45.848'), C-3, lower estuary (N 15°38.137' and E 73°45.640'), were selected and in Sal estuary, three stations namely, S-1, upper estuary (N 15°10.095' and E 073°56.781'), S-2, middle estuary (N 15°10.494' and E 73°57.502'), S-3, lower estuary (N 15°11.842' and E 073°58.240'), were selected (Fig. 2.1). Sampling was carried out during February 2016 to March 2017. Surface water samples (estimation of physico-chemical parameters) were collected during low tide at monthly intervals during pre-monsoon (February-May, 2016; February-March, 2017) monsoon (June-September, 2016) and post-monsoon (October-December, 2016; January, 2017) season.

6.2.2. Sediment sampling

Surface sediment sample at each station was collected using sediment cores (PVC cores, 8 cm long, an inner diameter of 3.5 cm) in triplicates. All sediment cores were sectioned at 2 cm intervals, mixed well and stored at 4°C in the dark for further dinoflagellate cyst enumeration.

6.2.3. Sediment processing and analysis

Sediment samples from the upper (0-2 cm) section of the core were treated using the palynological method (Matsuoka and Fukuyo, 2000) with some modifications (Narale et al., 2013). Each sediment sample (3-6 g) was weighed in a beaker and washed with distilled water to remove the salt content. The samples were then sonicated and acid-digested (10% HCL and 30% HF). After rinsing the sample 3- 4 times with distilled water, the acid-free sample was sieved through 100 and 10 µm mesh size to remove coarse and fine matter respectively. The residue retained on a 10 µm mesh was then transferred into a vial and a final volume was made upto 10 ml with distilled water. These vials were then stored at 4°C in the dark for dinoflagellate cyst identification.

Aliquot of 1 mL of the pre-treated sample was observed under Nikon Ti-S inverted microscope at 200x, 400x and 600x magnifications. Photographs of dinoflagellate cysts were captured in Nikon imaging software (NIS elements BR) equipped with colour digital camera Ri2. Dinoflagellate cysts were identified based on published descriptions (mentioned in Table 4.1). The dinoflagellate cyst abundance was expressed as cyst.g^{-1} dry sediment. The water content was calculated according to the formula given by Matsuoka and Fukuyo (Matsuoka and Fukuyo, 2000).

6.3. Results

The dinoflagellate cysts morphotypes reported from the present study are described below. The Number of dinoflagellate cysts encountered along the salinity gradient in Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary is mentioned in Table 6.1.

Based on the species distribution among the two estuaries, low species diversity was noted in Sal ($H' = 2.27$) whereas comparatively higher diversity was noted in Chapora ($H' = 2.73$) estuary (Fig. 6.1).

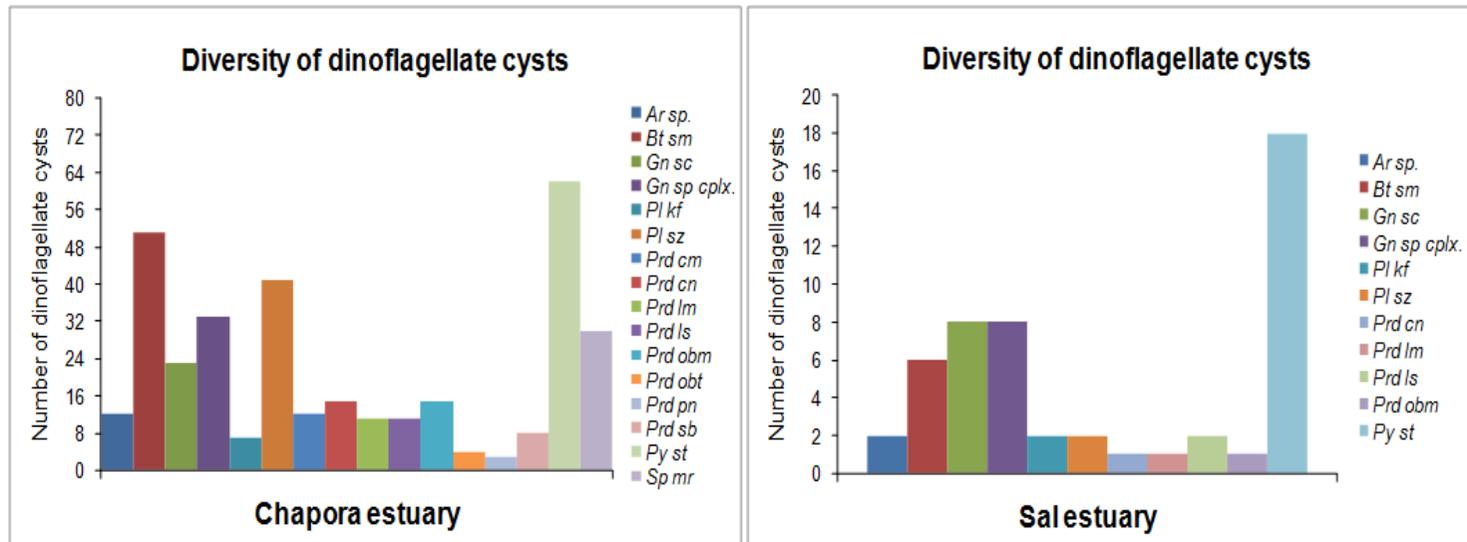
Table 6.1. Number of dinoflagellate cysts encountered along the salinity gradient in the present study in Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary

Estuary	CHAPORA			SAL		
Stations	C-1 (0-8 psu)*	C-2 (1-16 psu)*	C-3 (1-26 psu)*	S-1 (0-27 psu)*	S-2 (1-31 psu)*	S-3 (4-36 psu)*
<i>Archaeoperidinium</i> sp.	3	1	8	2	0	0
<i>Bitectatodinium spongium</i>	0	2	49	6	0	0
<i>Gonyaulax scrippsae</i>	0	6	17	5	3	0
<i>Gonyaulax spinifera</i> complex**	4	7	22	5	3	0
<i>Polykrikos kofoidii</i>	0	1	40	2	0	0
<i>Polykrikos schwartzii</i>	0	1	6	1	1	0
<i>Protoperidinium compressum</i>	0	0	12	0	0	0
<i>Protoperidinium conicum</i>	0	0	15	1	0	0
<i>Protoperidinium latissimum</i>	0	0	11	1	0	0
<i>Protoperidinium leonis</i>	0	2	9	0	2	0
<i>Protoperidinium oblongum</i>	0	0	15	1	0	0
<i>Protoperidinium obtusum</i>	0	0	4	0	0	0
<i>Protoperidinium pentagonum</i>	0	1	2	0	0	0
<i>Protoperidinium subinerme</i>	0	3	5	0	0	0

<i>Pyrophacus steinii</i>	1	3	58	13	5	0
<i>Spiniferites mirabilis</i>	0	0	30	0	0	0

**Toxic species; *Values in the parenthesis indicate the salinity range at the stations

Fig. 6.1. Diversity of dinoflagellate cysts in Chapora and Sal estuary



6.3.1. Dinoflagellate cyst morphotype composition

A total of sixteen dinoflagellate cyst morphotypes were identified in the sediments from the two estuaries, belonging to the order, Gymnodiniales, Gonyaulacales and Peridinales. A total of sixteen dinoflagellate cyst morphotypes were noted in Chapora estuary and eleven in Sal estuary. Among, sixteen dinoflagellate cysts reported in Chapora estuary, five were autotrophic and eleven were heterotrophic and among eleven dinoflagellate cysts reported in Sal estuary, four were autotrophic and seven were heterotrophic (Table 4.1).

6.3.1.1. *Archaeperidinium* species Jorgensen, 1912 (Plate 4.1, Fig. a)

The cyst is spherical to sub-spherical in shape (50.10 µm long, 42.19 µm wide), brown in colour and with a smooth to finely granulate wall. Several equidistant capitates processes (4.94 µm wide) are present, which have circular broad bases but are not branched.

6.3.1.2. *Bitectatodinium spongium* (Zonneveld) Zonneveld & Jurkschat 1999 (Plate 4.1, Fig. b)

The cyst is spherical to sub-spherical (57 µm long, 52.17 µm wide), brown in colour with a spongy wall. Some larger fibrous filaments are present and some filaments are capitates.

6.3.1.3. *Gonyaulax scrippsae* Kofoid 1911 (Plate 4.1, Fig. c)

Palynological name: *Spiniferites bulloideus*

Ovoidal cysts (68.15 µm long, 40.12 µm wide), brown in colour ornamented with simple sutural processes and septa with smooth cyst wall. Trifurcate or sometimes bifurcate processes (9.93 µm long) are present.

6.3.1.4. *Polykrikos kofoidii* Chatton 1914 (Plate 4.1, Fig. e)

The proximate cysts are elongated in shape (60.10 µm long, 48.68 µm wide), pale brown in colour. The processes are hollow and cylindrical or infundibular (11.45 µm).

6.3.1.5. *Polykrikos schwartzii* Bütschli 1873 (Plate 4.1, Fig. f)

The elongated, ovoidal proximate cysts (89.11 µm long, 54.09 µm wide) are brown in colour. The outer cyst membrane may be micro-granulate or micro-reticulate. The processes are fibrous and flare distally to form a reticulate structure (6.19 µm long).

6.3.1.6. *Protoperidinium compressum* (Abé) Balech 1974 (Plate 4.1, Fig. g)

Palynological name: *Stelladinium stellatum*

Cysts are dorso-ventrally compressed with unique stellate morphology (60 µm long, 46.21µm wide), and are brownish in colour. It has a long spine at each corner lying in the dorsoventral plane. They carry one apical, two antapical and two lateral horns.

6.3.1.7. *Protoperidinium conicum* (Gran) Balech 1974 (Plate 4.1, Fig. h)

Palynological name: *Selenopemphix quanta*

Pale brown, Ovoidal to kidney-shaped cysts (67.14 µm long, 63.16 µm wide), apically/antapically compressed with a pentagonal outline. The cyst wall is smooth and ornamented by several parallel rows of long needle-shaped spines. Two rows of spines outline the girdle zone. Numerous processes are present along two circular rims (16.11 µm long).

6.3.1.8. *Protoperidinium latissimum* (Kofoid) Balech 1974 (Plate 4.1, Fig. i)

These brown cysts are compressed in a dorsoventral plane (88.20 µm long, 68.89 µm wide), concavo-convex to hemispherical in polar view, and elliptical in lateral view.

6.3.1.9. *Protoperidinium leonis* (Pavillard) Balech 1974 (Plate 4.1, Fig. j)

Palynological name: *Quinquecuspis concretum*

Pentagonal to ellipsoidal cysts (78 µm long, 86.67 µm wide), pale brown in colour with a smooth or granular surface. Apical and antapical protrusions have thickened wall.

6.3.1.10. *Protoperidinium oblongum* (Aurivillius) Parke & Dodge 1976 (Plate 4.1, Fig. k)

Pale brown heart-shaped, rhomboidal to cordate and dorso-ventrally compressed cysts (42.16 µm long, 51.21 µm wide) with thin and smooth olive-brown cyst wall. Rounded apical and antapical 'horns' are present.

6.3.1.11. *Protoperidinium obtusum* (Karsten) Parke & J. D. Dodge 1976 (Plate 4.1, Fig. l)

Pale brown, peridinoïd or heart-shaped cysts without ornaments (53.13 µm long, 50 µm wide). Antapical horns were short with brownish cystwall.

6.3.1.12. *Protoperidinium pentagonum* (Gran) Balech 1974 (Plate 4.1, Fig. m)

Palynological name: *Trivantedinium capitatum*

The cysts are peridinoid (74.12 µm long, 59.54 µm wide), pentagonal in shape, colourless, dorso-ventrally compressed. Cysts are ornamented with numerous randomly distributed short, rigid, needle-shaped processes.

6.3.1.13. *Protoperidinium subinerme*(Paulsen) A. R. Loeblich III 1969 (Plate 4.1, Fig. n,o)

Palynological name: *Selenopemphix nephroides*

Ovoidal to kidney-shaped cysts (40 µm long, 51.80 µm wide), pale brown in colour with a smooth surface and no spines. In polar view, the cysts show weak dorso-ventral compression.

6.3.1.14. *Pyrophacus steinii* (Schiller) Wall & Dale 1971 (Plate 4.1, Fig. p)

Palynological name: *Tuberculodinium vancampoae*

Cysts are colourless (89.32 µm long, 73.14 µm wide), discoidal with large barrel-shaped processes (7.14 µm long) which can be distally connected.

6.3.1.15. *Spiniferites mirabilis* (M. R. Rossignol) K. Matsuoka 1974 (Plate 4.1, Fig. q)

Palynological name: *Spiniferites mirabilis*

Spherical to ovoidal, colourless cyst with smooth cyst wall (52.23 µm long, 41.42 µm wide).

Bifurcate and trifurcate processes (13.34 µm long) are present; the processes are tubular and conical, branching into fairly broad distal tips with Y-shaped extremities. Antapical processes connected forming a crown-shape.

6.3.2. Harmful species

In the present study, only one toxic dinoflagellate cyst morphotype, *Gonyaulax spinifera* was recorded in both the estuaries. In Chapora estuary this species was encountered at all three stations (C-1, C-2 and C-3). However, higher abundance was noted at station C-3 (56 cysts.g⁻¹ dry sediment) as compared to other two stations C-1 (6 cysts.g⁻¹ dry sediment) and C-2 (14 cysts.g⁻¹ dry sediment). In Sal estuary, this species was noted only at only two stations (S-1 and S-2). Among the two stations, high abundance was noted at S-1 (11 cysts.g⁻¹ dry sediment) as compared to station S-2 (3 cysts.g⁻¹ dry sediment).

6.3.2.1. *Gonyaulax spinifera* (Claparède & Lachmann) Diesing complex 1866 (Plate 4.1, Fig. d)

Palynological name: *Spiniferites ramosus*

Sub-spherical, colourless cyst (48 µm long, 54.81 µm wide) with a thick, tectate, double-layered cell wall. Trifurcate and sometimes bifurcate processes are present (15.14 µm long), originating at reflected plate-area corners.

6.4. Discussion

The present study on dinoflagellate cysts is the first attempt to elucidate the occurrence and distribution in the selected estuarine systems of Goa, namely Chapora (North Goa) and Sal (South Goa). Living dinoflagellate cysts were found at all the stations in both the estuaries. Among the sixteen dinoflagellate cyst species reported in the present study, the most common type were *Bicatatodinium spongium*, *Polykrikos kofoidii*, *Pyrophacus steinii* and *Spiniferites mirabilis*. All these species were noted at a wide salinity range (1-31 psu). However, their abundance was found to be higher at high salinity values as they are known to be marine species (Vernal et al., 2018; Rochon et al., 2008).

Archaeoperidinium sp. was first reported in coastal waters of British Columbia (Canada) (Mertens et al., 2012). *Bicatatodinium spongium* was previously reported in Gulf of Tehuantepec, south Pacific coast of Mexico (Bedoya et al., 2008), western Bay of Bengal, India (Uddandam et al., 2017). Many dinoflagellate cyst species encountered in the present study (*Gonyaulax scrippsae*, *Polykrikos kofoidii*, *Protoperidinium compressum*, *Protoperidinium latissimum*, *Protoperidinium leonis*, *Protoperidinium oblongum*, *Protoperidinium pentagonum*, *Pyrophacus steinii*, *Spiniferites mirabilis*) were previously reported in the coastal areas in Chonburi province, Thailand (Srivilai et al., 2012). *Protoperidinium pentagonum*, *Pyrophacus steinii*, *Spiniferites mirabilis*, *Protoperidinium oblongum*, *Protoperidinium obtusum* were previously reported in Bolinao Pangasinan, Northern Philippines (Baula et al., 2011). Few species (*Protoperidinium subinerme*, *Spiniferites mirabilis*, *Protoperidinium compressum*, *Bicatatodinium spongium*) were known to be previously reported along Somali Basin northwest Arabian sea (Zonneveld and Brummer, 2000); in Chiang river estuary, Southeastern China (*Polykrikos schwartzii*, *Protoperidinium leonis*, *Pyrophacus steinii*) (Wang et al., 2004); in North Sea (*Gonyaulax scrippsae*, *Polykrikos schwartzii*) (Nehring, 1995), in Sunda shelf, south China sea (*Protoperidinium latissimum*, *Protoperidinium oblongum*, *Protoperidinium subinerme*)

(Kawamura, 2004); along the Swedish west coast (*Gonyaulax scrippsae*, *Protoferidinium pentagonum*, *Protoferidinium subinerme*) (Godhe and McQuoid, 2003); in Izmir Bay, Aegean sea, Eastern Mediterranean (*Protoferidinium obtusum*, *Protoferidinium nudum*) (Uzar et al., 2010).

Protoferidinium obtusum, *Spiniferites mirabilis*, *Polykrikos kofoidii* were previously reported in Marmara, Aegean and eastern seas of Turkey (Aydin and Uzar, 2014); surface sediments of Saldanha Bay, south Africa (*Polykrikos schwartzii*, *Protoferidinium compressum*) (Joyce et al., 2005); in coastal Mediterranean site (*Gonyaulax scrippsae*) (Montessor et al., 1998); in western coast of the Bering sea (*Polykrikos schwartzii*) (Orlova and Morozova, 2013); in surface sediments of Thermaikos Gulf, Greece (*Protoferidinium compressum*) (Giannakourou et al., 2005), Veracruz reef zone, and Gulf of Mexico (Okoldkov et al., 2007); in coastal waters of Tokyo Bay, Japan (*Protoferidinium conicum*) (Matsuoka et al., 2003), Red sea off the coasts of Saudi Arabia (Mohammed and Al-Shehri, 2011), and eastern and north-eastern Atlantic ocean (Dodge and Harland, 1991); in surface sediments of southwestern black sea and Canakkale strait (Dardanelles) (*Protoferidinium nudum*) (Aydin et al., 2015); in Todos Santos Bay, Baja California, Mexico (*Protoferidinium oblongum*) (Manjarrez et al., 2005); in Daya bay, South China sea (*Protoferidinium pentagonum*) (Wang et al., 2004) and east coast of southern Norway (Grosfjeld and Harland, 2001); in Manila Bay, Philippines (*Pyrophacus steinii*) (Siringanan et al., 2008); in Izmir Bay, eastern Aegean sea, the Mediterranean (*Spiniferites mirabilis*) (Aydin et al., 2014); in southern coast of Korea (*Protoferidinium latissimum*) (Shin et al., 2011).

Several species reported in the present study (*Gonyaulax scrippsae*, *Polykrikos kofoidii*, *Polykrikos schwartzii*, *Protoferidinium compressum*, *Protoferidinium latissimum*, *Protoferidinium conicum*, *Protoferidinium leonis*, *Protoferidinium oblongum*, *Protoferidinium pentagonum*, *Protoferidinium subinerme*, *Protoferidinium nudum*, *Pyrophacus steinii*, *Spiniferites mirabilis*) were reported in the west (Godhe et al., 2000; D' Silva et al., 2011; D' Silva et al., 2012), as well as along the east coast of India (*Gonyaulax scrippsae*, *Polykrikos kofoidii*, *Protoferidinium compressum*, *Protoferidinium conicum*, *Protoferidinium latissimum*, *Protoferidinium leonis*, *Protoferidinium nudum*, *Protoferidinium pentagonum*, *Protoferidinium subinerme*, *Protoferidinium oblongum*, *Protoferidinium latissimum*, *Pyrophacus steinii*) (Narale et al., 2013; D' Silva et al.,

2013). The observations made in the present study suggest the range extension of these species, which were noted to be marine and heterotrophic. Most heterotrophic dinoflagellate cyst species indicates high nutrient inputs, which is a rising issue worldwide (Anderson et al., 2002). A majority of Earth's water is regarded as saline waters, and growth of dinoflagellates and cyst formation is mainly triggered by high salinity values (Shin et al., 2010; Price et al., 2016). Hence the occurrence of these species is noted widely.

Among the sixteen dinoflagellate cyst species encountered in this study, *Archaeoperidinium* sp., *Bicatatodinium spongium* and *Protoperidinium obtusum* were found to be reported for the first time along the west coast of India.

Further, the differences in the diversity of dinoflagellate cysts between the two estuaries were noted. Various anthropogenic activities (fishing, shellfish collection, shrimp farms, domestic sewage discharge, agricultural runoffs, mega-housing projects) carried out along the Sal estuary possibly have lead in high nitrate concentration, as noted from the present study (22.39 μM). As a result, the low diversity of dinoflagellate cysts in this estuary was noted (Fig. 6.1). Whereas in Chapora estuary, a high diversity of dinoflagellate cysts were reported (Fig. 6.1). An array of anthropogenic activities (fishing, sand mining, shrimp farming ponds, discharge of domestic wastes, human settlements along the river banks) are also carried out in Chapora estuary. However, low nitrate concentration (8.28 μM) recorded from the present study indicates a low level of anthropogenic inputs in this estuary. From the above observations, it is seen that, high nutrient inputs (nitrate concentration) lead in the decline of dinoflagellate cyst species diversity. Similar observations were also reported in other studies (Pospelova et al., 2002; Richerol et al., 2008; Aydin et al., 2015).

In Chapora estuary, the population density is found to be very less. During the survey carried out during January/February 2019, it was noted that the domestic untreated sewage discharge from the individual house directly into the river was observed on the northern as well as southern bank of the river. (Preparation of Action Plan, 2019). A traditional activity of sand extraction through a manual method in these estuaries was also seen, which is presently stopped as the permits have not been renewed by the state government (Preparation of Action Plan, 2019).

In addition, the observed DO values were noted to be normal and hence indicate well oxygenated water during all three seasons (Preparation of Action Plan, 2019).

Whereas, the river Sal, which was once known as the lifeline of Salcete, is now found to be the most polluted and contaminated river beds in the state (Central Pollution Control Board, 2018). High population density is also noted along this river; around 350 houses in this small village were noted. A high BOD concentration noted in this river indicates increased levels of pollution. Sources stated that the direct release of sewage is the cause of biochemical pollution in rivers. Runoffs containing sewage only add to the problem (Central Pollution Control Board, 2018). Furthermore, large nutrients inputs from agricultural land have made the Sal estuary highly eutrophic (Fernandes et al., 2018). It is also noted that the real estate, with mega-housing projects have substantially destroyed and polluting village environment as well as river Sal (Central Pollution Control Board, 2018). Further, hotels in south Goa produces large dumps of non-biodegradable garbage which along with sewage is discharged in the river Sal (Pradhan, 2016). The Central Pollution Control Board (CPCB) on the basis of BOD concentration, have categorised Sal estuary as priority III and Chapora as priority V and the same has been included in Table 6.2. As per CPCB guideline, a priority I refer to being most severe and priority V the least (Table 6.2).

Table 6.2. Status of indicators of river Pollution in Goa (Central Pollution Board, 2018)

Sr. No	River	BOD Range	Priority
1	Mandovi	3.9	V
2	Assonora	7.0	IV
3	Bicholim	3.9	V
4	Chapora	5.0	V
5	Khandepar	3.9	V
6	Mapusa	6.2	IV
7	Sal	3.2-10.6	III
8	Valvant	3.1	V

It is pertinent to note that both these estuarine habitats are being subjected to increased anthropogenic pressure as mentioned above and the observations made in the present study provide evidence to support the human interference (domestic sewage discharge, sandmining, agricultural runoffs, mega-housing projects, shrimp farming ponds, fishing, shellfish collection) affecting the water quality in these estuarine habitats. Hence, such impacts have found to affect the diversity of dinoflagellate cyst species in these areas. Further, such continuous human activities also could negatively influence other aquatic life. Therefore, the present study elucidates the effect of anthropogenic activities in such fragile habitats and recommends frequent monitoring of the water quality of estuarine areas to support diverse life forms reflecting the health of coastal ecosystems.

Summary

- 1) The present study suggests the effect of physico-chemical parameters on the composition and distribution of HAB species and salinity was noted to be the major influencing factor in the distribution of HAB and toxic dinoflagellate species in both the estuaries.
- 2) The high number of toxic dinoflagellate species in Sal estuary (*Alexandrium minutum*, *Alexandrium tamarense*, *Alexandrium pseudogonyaulax*, *Cochlodinium polykrikoides*, *Prorocentrum micans*, and *Gonyaulax spinifera*) revealed the prevalence of favourable conditions, with an increase in nutrient input, a probable cause for the proliferation of toxic species. Further, the nutrient enrichment due to eutrophication is a principal factor in supporting blooms of allelopathic algae by altering the N: P balance.
- 3) A total of sixteen dinoflagellate cysts belonging to the order, Gymnodiniales, Gonyaulacales, and Peridiniales were recorded in the present study. Among these, eleven species were found to be heterotrophic, and five were autotrophic. Only one prolific yessotoxin producer dinoflagellate cyst species, *Gonyaulax spinifera* was reported in the present study.
- 4) Total dinoflagellate cyst abundance was affected by the variations in the physico-chemical parameters in both the estuaries with significant influence of salinity and sediment texture. In addition, the occurrence of dinoflagellate cysts within the estuaries suggests the risk of forming such blooms is high with consistently in high saline waters and with silt-clayey sediment texture.
- 5) The dominance of heterotrophic dinoflagellate cysts in both the estuaries indicates nutrient (nitrate) loading coupled with organically rich waters with high suspended load, promote the dominance of dinoflagellate cysts.
- 6) Nutrient limitation (nitrate) is noted to be a triggering factor for dinoflagellate cyst formation. Redundancy Analysis (RDA) bi-plots revealed nitrate input as a probable cause for the excystment of toxic species, *Gonyaulax spinifera* in both the estuaries.
- 7) High nitrate concentration coupled with other physico-chemical parameters (DO, temperature, and pH) was found to play an important role in the dominance of Chlorophyceae as compared to Cyanophyceae group in both the estuaries. Further, the presence of two toxic bloom-forming cyanobacterial genus (*Microcystis* and

Oscillatoria) in these estuarine systems is of concern, as nutrient input is known to be the chief cause of bloom formation.

- 8) Since fishing and shellfish collection are important activities carried out in these estuarine regions, presence of HAB and toxic species is of concern, as it can cause a serious risk on marine life as well as human health. Hence, there is a need to frequently monitor the water quality of such estuarine regions and to reduce anthropogenic nutrient inputs.

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Appendix

Appendix 4.1

Table 1. Physico-chemical parameters at station C-1 (upper Chapora estuary)

Sampling Month	Water temperature (°C)	Air temperature (°C)	Dissolved oxygen (mg. L ⁻¹)	Salinity (PSU)	SPM (mg. L ⁻¹)	pH	Nutrient concentration (μM)			
							Nitrite	Nitrate	Phosphate	Silicate
Feb-16	26	26	6.08	6	7.2	7.83	0.30	0.56	0.44	97.34
Mar-16	32	29	6.64	5	42	7.6	0.39	0.69	0.25	91.87
Apr-16	31	28	6.75	6	5.4	7.5	0.36	0.78	0.44	95.64
May-16	31	31	6.85	4	3.62	7.5	0.36	0.64	0.48	101.29
Jun-16	30	28	5.99	6	10.4	6.32	1.43	3.98	0.36	144.40
Jul-16	27	26	8.90	0	7.1	6.74	0.19	6.71	0.30	65.51
Aug-16	26	26	7.55	2	12.3	7.2	0.14	4.04	0.40	59.22
Sep-16	27	24	7.96	2	2.66	7.13	0.10	1.65	0.24	110.77
Oct-16	27	24	8.13	1	3.78	7.03	0.05	0.58	0.38	72.33
Nov-16	26	21	6.38	3	1.04	7.25	0.20	1.50	0.28	138.69
Dec-16	21	21	6.72	4	4.26	7.29	0.53	3.31	0.72	82.25
Jan-17	25	19	6.89	7	36.2	7.3	0.17	2.50	0.50	117.55
Feb-17	27	21	7.24	8	14.3	7.1	1.49	3.13	0.44	36.15
Mar-17	28	22	6.53	5	16.1	7.26	1.07	3.58	0.75	123.22

Table 2. Physico-chemical parameters at station C-2 (middle Chapora estuary)

Sampling Month	Water temperature (°C)	Air temperature (°C)	Dissolved oxygen (mg. L ⁻¹)	Salinity (PSU)	SPM (mg. L ⁻¹)	pH	Nutrient concentration (μM)			
							Nitrite	Nitrate	Phosphate	Silicate
Feb-16	28	33	7.29	15	21.7	8.24	0.35	0.53	0.70	72.60
Mar-16	34	30	6.59	13	91	7.87	0.30	0.31	0.35	75.10
Apr-16	32	29	6.25	14	11.5	7.67	0.50	0.76	0.52	78.23
May-16	32	31	6.55	13	16.2	7.8	0.36	0.45	0.75	117.61
Jun-16	30	29	5.54	15	12.2	7.4	2.90	3.41	0.62	112.22
Jul-16	27	26	8.10	1	2.2	6.59	0.28	8.28	0.52	71.30
Aug-16	27	26	7.42	3	5.92	7.18	0.21	6.55	0.57	39.03
Sep-16	28	26	7.46	1	3.08	7.25	0.18	4.84	0.35	179.96
Oct-16	27	25	7.57	2	6.54	7.24	0.24	3.19	0.55	64.95
Nov-16	26	20	5.90	15	17.02	7.56	0.28	2.87	0.42	106.33
Dec-16	26	20	5.79	12	22	7.55	0.97	4.50	1.33	63.46
Jan-17	25	19	6.11	16	46.7	7.61	0.38	2.45	0.90	108.67
Feb-17	27	23	7.57	16	25	7.67	0.38	3.35	0.58	41.88
Mar-17	28	23	7.55	13	28.04	7.71	0.22	0.80	0.91	59.08

Table 3. Physico-chemical parameters at station C-3 (lower Chapora estuary)

Sampling Month	Water Temperature (°C)	Air temperature (°C)	Dissolved oxygen (mg. L ⁻¹)	Salinity (PSU)	SPM (mg. L ⁻¹)	pH	Nutrient concentration (µM)			
							Nitrite	Nitrate	Phosphate	Silicate
Feb-16	29	32	6.42	22	26.9	8.41	0.31	0.43	0.52	55.83
Mar-16	35	30	6.44	24	196.6	8.2	0.41	0.57	1.85	53.61
Apr-16	34	28	6.68	22	106	7.91	0.67	1.28	5.91	70.56
May-16	33	32	6.70	23	41.4	8.09	0.37	0.60	1.52	54.45
Jun-16	30	31	5.88	19	70.8	7.63	2.50	3.17	1.05	88.64
Jul-16	27	26	4.91	3	71	7.21	4.45	6.26	2.03	114.08
Aug-16	27	27	7.59	1	7.24	7.51	0.32	6.11	1.19	57.29
Sep-16	29	31	4.22	6	10.4	7.56	0.66	0.85	5.14	169.27
Oct-16	29	29	4.65	6	10.26	7.58	0.34	0.59	7.54	145.15
Nov-16	29	35	6.16	26	35.9	8.04	0.22	1.49	0.61	45.22
Dec-16	27	31	5.30	24	47.68	7.87	1.07	3.76	1.59	63.51
Jan-17	26	28	6.44	24	58.34	7.99	0.33	2.96	1.02	64.13
Feb-17	29	30	6.70	24	46.9	7.85	0.30	0.85	1.17	41.22
Mar-17	32	29	7.65	21	57.38	8.13	0.65	0.80	2.26	32.63

Table 4. Physico-chemical parameters at station S-1 (upper Sal estuary)

Sampling Month	Water temperature (°C)	Air temperature (°C)	Dissolved oxygen (mg. L ⁻¹)	Salinity (PSU)	SPM (mg. L ⁻¹)	pH	Nutrient concentration (µM)			
							Nitrite	Nitrate	Phosphate	Silicate
Feb-16	32	34	5.65	25	36.7	7.3	0.49	0.52	1.65	23.40
Mar-16	34	37	6.91	20	70.6	7.48	0.33	0.58	1.94	11.92
Apr-16	32	30	6.27	21	52.2	7.88	0.39	0.86	2.89	9.70
May-16	33	29	8.50	23	61.1	7.66	0.45	0.51	1.86	31.00
Jun-16	30	26	7.20	6	5.5	7.05	0.97	9.68	1.88	55.46
Jul-16	28	29	4.32	0	7.7	6.7	2.51	18.30	2.50	52.14
Aug-16	29	25	3.78	3	8.6	6.84	1.66	11.33	1.98	22.01
Sep-16	29	27	3.63	2	8.2	6.81	1.65	13.99	1.09	57.84
Oct-16	30	26	4.19	1	6.2	6.84	1.15	12.54	1.35	59.66
Nov-16	22	22	4.42	26	31.36	7.41	1.35	4.25	0.89	65.89
Dec-16	29	24	3.74	24	41.14	7.43	0.50	10.03	1.55	40.74
Jan-17	26	21	5.52	26	229.34	7.5	0.70	6.41	1.33	50.59
Feb-17	29	24	5.21	27	41.42	6.39	0.32	2.53	1.41	26.45
Mar-17	29	25	5.34	25	50.92	7.33	0.34	1.92	1.38	29.51

Table 5. Physico-chemical parameters at station S-2 (middle Sal estuary)

Sampling Month	Water temperature (°C)	Air temperature (°C)	Dissolved oxygen (mg. L ⁻¹)	Salinity (PSU)	SPM (mg. L ⁻¹)	pH	Nutrient concentration (μM)			
							Nitrite	Nitrate	Phosphate	Silicate
Feb-16	31	36	6.83	27	40.1	7.65	0.36	0.53	1.23	10.05
Mar-16	33	34	6.36	25	536	7.64	0.31	0.55	1.16	31.77
Apr-16	34	35	5.97	24	40.9	7.58	0.25	0.54	1.67	33.66
May-16	34	35	5.34	31	58	7.62	0.28	0.81	1.00	29.76
Jun-16	29	26	6.51	7	16.8	7.11	1.60	9.74	1.71	67.03
Jul-16	29	28	6.29	1	3.26	7.13	2.12	22.39	1.93	67.57
Aug-16	29	32	5.54	1	5.9	7.1	1.37	20.90	1.44	38.93
Sep-16	30	30	5.82	4	10.4	7.1	0.41	10.98	0.64	100.99
Oct-16	30	29	5.99	7	17.2	7.79	0.63	9.88	2.06	59.30
Nov-16	29	32	4.48	30	41.2	7.6	0.71	5.71	1.17	45.20
Dec-16	29	29	4.43	29	44.5	7.43	0.27	7.08	1.30	11.36
Jan-17	27	30	5.70	28	117.1	7.78	0.55	4.84	1.64	39.60
Feb-17	30	32	6.35	26	40.2	6.58	0.39	4.38	1.07	22.53
Mar-17	30	36	6.40	27	56.5	7.27	0.40	0.41	1.70	34.80

Table 6. Physico-chemical parameters at station S-3 (lower Sal estuary)

Sampling Month	Water Temperature (°C)	Air temperature (°C)	Dissolved oxygen (mg. L ⁻¹)	Salinity (PSU)	SPM (mg. L ⁻¹)	pH	Nutrient concentration (µM)			
							Nitrite	Nitrate	Phosphate	Silicate
Feb-16	29	32	5.97	34	37.1	8.1	0.45	0.53	1.36	4.98
Mar-16	32	32	3.12	32	66.1	7.79	0.48	0.94	1.05	17.82
Apr-16	32	33	5.32	31	38	7.87	0.58	0.96	1.43	14.45
May-16	33	30	4.11	35	56.3	7.94	0.59	1.48	1.66	10.77
Jun-16	29	26	5.15	17	19.6	7.43	0.99	10.08	1.25	49.89
Jul-16	29	27	6.88	4	50.4	7.68	1.49	12.18	3.00	51.32
Aug-16	27	26	6.38	5	7.7	7.42	0.75	10.75	1.16	50.21
Sep-16	29	28	5.84	15	18.58	7.47	0.45	5.71	1.09	74.41
Oct-16	28	26	5.79	31	38.76	7.82	0.16	1.81	2.15	4.32
Nov-16	27	23	4.61	36	37.8	8.13	0.28	2.96	0.90	10.82
Dec-16	29	26	4.22	34	45.84	8.15	0.39	2.50	1.51	9.81
Jan-17	27	19	5.36	34	175.34	8.25	0.26	2.09	1.24	13.09
Feb-17	29	28	5.30	34	48.8	6.8	0.33	2.99	0.76	8.12
Mar-17	29	26	5.30	32	58.82	7.49	0.43	1.65	1.90	17.81

Published articles

Prabhudessai, S., Vishal, C. R. and Rivonker, C. U. (2019). Biotic interaction as the triggering factor for blooms under favourable conditions in tropical estuarine systems. *Environmental Monitoring and Assessment*, **191** (2), 54. <https://doi.org/10.1007/s10661-018-7172-7>.

Prabhudessai, S. S., Vishal, C. R., and Rivonker, C. U. (2019). Antonymous nature of freshwater phytoplankton in the tropical estuarine environments of Goa, southwest coast of India. *Regional Studies in Marine Science*, **32**, 100880. <https://doi.org/10.1016/j.rsma.2019.100880>

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Research papers presented at the conference

Participation in the National Conference on Mangrove Ecosystem, organized by Mangrove Society of India in Association with CSIR-National Institute of Oceanography, Dona Paula, Goa held on 26th-27th July, 2017.

Participation in the “Research Showcase Presentation (RSP)” at the 3rd Annual International Conference Intellectual Property: Prosecution to Litigation, Part II: Protecting Inventions and Copyrights held on November 15-16, 2018 at Goa College of Pharmacy, Panaji-Goa.

Workshop/Conferences attended

Participation in the National Conference of Young Researchers 2017, on New Frontiers in Life Sciences and Environment. Organized by Faculty of Life Sciences and Environment, Goa University held on 16-17th march, 2017 at Goa University.

Participation in a UGC- Sponsored short term course in Research Methodology for Science Students, held from 28th November to 3rd December, 2016.

Participation in Intellectual Property Rights (IPR) Awareness Programme, organized by ASSOCHAM, Goa University and IP Office, at Goa University, Goa on 11th December 2018.

Biotic interaction as the triggering factor for blooms under favourable conditions in tropical estuarine systems

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Abstract The formation of harmful algal blooms (HABs) in the marine environment is detrimental to the ecosystem function affecting the sequence of biological events. Hence, the present study is focused on the seasonal distribution of HAB-forming diatoms and dinoflagellates and their ecological interactions in two tropical estuaries, namely Chapora and Sal in the west coast of India. A total of 17 species of diatoms belonging to nine genera and 13 species of dinoflagellates belonging to eight genera were recorded from the present study (monsoon and non-monsoon season). The redundancy analysis (RDA) revealed that the salinity and phosphate concentration influence the distribution of HAB-forming diatoms and dinoflagellates in both the estuaries. Out of 13 species of dinoflagellates observed, six species are known as potentially toxic such as *Alexandrium minutum*, *A. tamarense*, *A. pseudogonyaulax*, *Cochlodinium polykrikoides*, *Prorocentrum micans* and *Gonyaulax spinifera*. Among these estuaries, Sal was represented with a high diversity of toxic species, associated with high phosphate and nitrate content in the water column. Further, the results indicate that the bloom of *A. minutum* suppressed the growth of other species of diatoms and dinoflagellates, suggesting its possible allelopathic effect.

Keywords Harmful algal blooms · Toxic · Dinoflagellates · Diatoms · Tropical estuary

Introduction

Harmful algal blooms (HABs) in recent times are considered as a matter of concern worldwide, especially in the coastal habitats, primarily influenced by biogeochemical processes with sudden and unpredictable interactions (Glibert et al. 2005). Among these, few species of phytoplankton produce powerful bio-toxins, responsible for so-called red tides (Lindsey and Scott 2010), and can cause serious impact not only on marine life and human health, but also the local and regional economies (Bushaw-Newton and Sellner 1999). Published literature (Hallegraeff 1993) reveals a marked rise in the occurrence of HABs, often globally and by more than one harmful or toxic species with emphasis on more frequent and widespread occurrence of the toxic dinoflagellate, *Alexandrium*. In this genus, allelopathic, bioactive effects have been studied suggesting the release of secondary metabolites (Ogata and Kodama 1986; Rizvi and Rizvi 1992) impeding the growth of competitive species leaving more resources and space for the allelopathic species to bloom (Lelong et al. 2011).

Estuaries are composite ecosystems due to their persistently changing physicochemical surroundings as they receive freshwater influx during monsoon that leads to constant variation in the salinity as well as nutrient concentration (Parab et al. 2013) leading to

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gradient formation along the estuary. These gradients due to tidal and monsoonal influence in estuaries affect the temporal dynamics of HAB forming and toxic species (Alkawri and Ramaiah 2010). In addition, anthropogenic activities (construction of jetties, shipbuilding yards and cruising, mining, agricultural runoff, sewage and industrial effluents discharge), carried along the estuaries (Pradhan and Shirodkar 2009; Maya et al. 2011), result in increased nutrient loading (nitrate and phosphate) which affects the health of the coastal water (Pednekar et al. 2012; Narale and Anil 2017). This nutrient enrichment successively creates favourable environmental conditions for certain HAB species to bloom (Anderson et al. 2002). Among these HAB species, some are known to be toxic (*Alexandrium* species) bloom formers (Franco et al. 1994) with allelopathic ability. As a result, the release of secondary metabolites due to allelopathy may help other species to outcompete for nutrients (Fistarol et al. 2004) and this may in turn lead to blooms of such toxic species that can be detrimental for marine and human life.

Taking the above facts into consideration, an attempt has been made to provide information on the distribution of HAB-forming diatoms and dinoflagellates from the Chapora (North Goa) and Sal estuary (South Goa). The objectives of the present study were (1) to know the influence of salinity in the proliferation of HABs along the salinity gradient in an estuary and (2) the influence of environmental parameters on HABs and (3) to reveal the biotic interaction between toxic and non-toxic HABs.

Materials and methods

Study area

The study area comprises coastal waters up to 5 m depth off the central west coast of India. The continental drainage along the central west coast of India is characterised by small rivers and streams that drain into the Arabian Sea (D'Silva et al. 2012). Goa is located on the west coast of India (140° 53' 57" to 150° 47' 59" north latitudes and 73° 40' 54" to 74° 20' 11" east longitudes). The coastal zone of Goa is traversed by nine major dynamic estuaries. Land use pattern and other anthropogenic activities dramatically transform and alter estuarine and coastal marine environmental conditions, and recognition of these impacts is, therefore, one of the

critical issues in modern times (Pradhan and Shirodkar 2011).

The present study area includes Chapora and Sal estuary in North and South Goa respectively (Fig. 1). Chapora estuary is lined by patchy mangrove vegetation (upstream), with sparse distribution near the mouth. An array of anthropogenic activities (fishing, sand mining, aquaculture ponds, discharge of domestic wastes, industrial effluents, human settlements along the river banks, agricultural runoff) are being carried out. Along the Sal estuary, mangroves occur throughout the length, although their density is less downstream due to dense human habitation and agricultural fields. Fishing grounds off the mouth of Sal estuary comprise of two different habitats (silty substratum towards the north of the mouth and submerged rock towards the south). Numerous anthropogenic activities (fishing, shellfish collection, shrimp farms, discharge of domestic and industrial wastes) are carried out in this estuary.

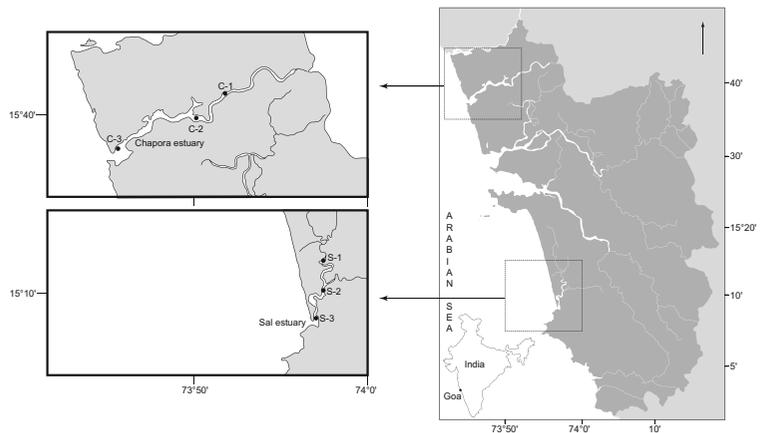
Sampling sites

Three stations were selected in each estuary based on salinity gradient. In Chapora estuary, station C-1 (upper estuary, 15° 39.072' and E 073° 47.995'), C-2 (middle estuary, N 15° 38.396' and E 073° 45.848') and C-3 (lower estuary, N 15° 38.137' and E 73° 45.640') were selected and in Sal estuary, three stations namely, S-1 (upper estuary, N 15° 10.095' and E 073° 56.781'), S-2 (middle estuary, N 15° 10.494' and E 73° 57.502') and S-3 (lower estuary, N 15° 11.842' and E 073° 58.240') were selected (Fig. 1). Variation in salinity gradient in Chapora estuary was found to be larger due to its longer length whereas, a small variation in salinity gradient was noticed in Sal estuary due to its shorter length. Sampling was carried out during July–December, 2016. Surface water samples (phytoplankton taxonomy and estimation of physicochemical parameters) were collected during low tide at monthly intervals during monsoon (July–September) and non-monsoon (October–December) season.

Physicochemical parameters

The temperature was measured onboard with a mercury thermometer. Dissolved oxygen (DO) estimation was carried out by Winkler's method. Digital salinometer was used for salinity measurements; the nutrient (nitrate, nitrite, phosphate and silicate) concentration was estimated using standard procedure (Grasshoff et al. 1999).

Fig. 1 Map indicating sampling locations



Taxonomy of HAB-forming diatom and dinoflagellate species

Water samples for phytoplankton identification were collected in 1-L plastic bottles in triplicates and were fixed with 0.2% Lugol’s iodine. Samples were allowed to settle for 48 h and were concentrated to 100 mL by carefully siphoning the top layer of the sample with tubing, one end of which was covered with a 10- μ m mesh, to avoid loss of small-sized phytoplankton. One millilitre of concentrated sub-sample was analysed under an inverted microscope (Nikon Ti-S) at $\times 200$, $\times 400$ and $\times 600$ magnification and taxonomic identification of phytoplankton was carried out following published identification manual (Tomas 1997; Cupp 1943).

Results

Physicochemical parameters

During the sampling period, the average temperature in Chapora estuary ranged between 21 and 29 °C, whereas, in Sal estuary, it ranged between 22 and 30 °C. Temperatures recorded in both Chapora and Sal estuaries during the sampling period were similar to the previous studies carried out from Chapora (Alkawri and Ramaiah 2010) and Sal estuary (Fernandes and Achutankutty 2010). Salinity was affected by precipitation during monsoon, and a noteworthy decline in salinity was observed in the monsoon season at all three stations in both the estuaries. The concentration of dissolved inorganic nutrients (nitrate, nitrite, phosphate and silicate) showed variation at

all the sampling sites in both the estuaries. Significant variation in silicate concentration was noted during non-monsoon season between the two estuaries. Silicate concentration was higher throughout the study period in comparison with other nutrients, and the highest concentration (179.96 μ M) was noted in Chapora estuary (C-2) during the monsoon season. The highest phosphate concentration (7.54 μ M) was recorded in Chapora estuary (C-3) during non-monsoon. Significant variation in nitrate concentration was noted between the estuaries during both monsoon and non-monsoon season, whereas significant variation between the stations was noted only during the non-monsoon season. The highest nitrate concentration (22.39 μ M) was noted in Sal estuary during monsoon. However, high phosphate and nitrate concentrations were recorded in Sal estuary at all three stations.

HAB-forming diatom and dinoflagellate assemblage

Distinct variations in the abundance and distribution of HAB-forming diatoms and dinoflagellates (hereafter mentioned as HAB species) were noted during the study period in the two estuaries. A total of 17 species of diatoms and 13 species of dinoflagellates were recorded in the present study (Table 1). Twelve species of diatoms and 4 species of dinoflagellates were observed in Chapora estuary, and in Sal estuary, 14 species of diatoms and 12 species of dinoflagellates were recorded. Although the abundance of diatoms was higher than that of dinoflagellates at most of the stations in Chapora estuary, the latter dominated in most of the stations in Sal estuary (Fig. 3).

Table 1 List of HAB-forming diatoms and dinoflagellates encountered along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary

Taxon	Taxon abbreviation	Chapora estuary			Sal estuary		
		C-1	C-2	C-3	S-1	S-2	S-3
Diatoms							
<i>Biddulphia mobiliensis</i>	<i>Bd mb</i>	+	–	–	–	–	+
<i>Cerataulina dentata</i>	<i>Cr dn</i>	+	–	–	–	–	–
<i>Chaetoceros curvisetus</i>	<i>Ch cr</i>	+	–	–	–	–	+
<i>Nitzschia sigmoidea</i>	<i>Nz sg</i>	+	+	+	–	+	–
<i>Rhizosolenia eriensis</i>	<i>Rh er</i>	+	+	–	–	–	–
<i>Rhizosolenia setigera</i>	<i>Rh st</i>	+	–	–	–	+	+
<i>Skeletonema costatum</i>	<i>Sk cs</i>	+	+	+	+	+	+
<i>Coscinodiscus centralis</i>	<i>Co cn</i>	–	+	–	–	–	+
<i>Coscinodiscus granii</i>	<i>Co gr</i>	–	+	–	–	–	+
<i>Coscinodiscus radiatus</i>	<i>Co rd</i>	–	+	+	–	+	+
<i>Thalassiosira punctigera</i>	<i>Th pn</i>	–	+	+	–	–	–
<i>Nitzschia coarctata</i>	<i>Nz cr</i>	–	–	+	–	–	–
<i>Chaetoceros lorenzianus</i>	<i>Ch lz</i>	–	–	–	–	–	+
<i>Chaetoceros convolutes</i>	<i>Ch cn</i>	–	–	–	–	–	+
<i>Chaetoceros danicus</i>	<i>Ch dn</i>	–	–	–	–	–	+
<i>Pseudo-nitzschia delicatissima</i>	<i>Pnz dl</i>	–	–	–	–	–	+
<i>Pseudo-nitzschia pungens</i>	<i>Pnz pn</i>	–	–	–	–	–	+
Dinoflagellates							
<i>Peridiniopsis cunningtonii</i>	<i>Pds cn</i>	+	–	–	–	–	–
<i>Alexandrium minutum*</i>	<i>Al mn</i>	–	+	–	+	+	–
<i>Peridinium quinquecorne</i>	<i>Pr qn</i>	–	+	–	–	+	–
<i>Ceratium furca</i>	<i>Cm fr</i>	–	–	+	–	–	+
<i>Ceratium fusus</i>	<i>Cm fu</i>	–	–	+	–	–	+
<i>Prorocentrum gracile</i>	<i>Pm gr</i>	–	–	–	+	+	–
<i>Alexandrium pseudogonyaulax*</i>	<i>Al psg</i>	–	–	–	–	+	–
<i>Alexandrium tamarense*</i>	<i>Al tm</i>	–	–	–	–	+	+
<i>Peridiniopsis polonicum</i>	<i>Pds po</i>	–	–	–	–	+	–
<i>Protoperidinium pellucidum</i>	<i>Prd pl</i>	–	–	–	–	+	–
<i>Cochlodinium polykrikoides*</i>	<i>Chl pk</i>	–	–	–	–	–	+
<i>Gonyaulax spinifera*</i>	<i>Gn sp</i>	–	–	–	–	–	+
<i>Prorocentrum micans*</i>	<i>Pm mc</i>	–	–	–	–	–	+

+Present, –Absent, *toxic species

The composition and distribution of HAB species varied seasonally in both the estuaries based on salinity gradient (Table 2). *Coscinodiscus radiatus*, *Nitzschia sigmoidea*, *Skeletonema costatum*, *Rhizosolenia eriensis*, *Peridinium quinquecorne*, *Ceratium furca*, *Alexandrium minutum*, *Alexandrium tamarense* are the HAB-forming species recorded at a wide salinity range. Further, it was also observed that among the toxic

dinoflagellates reported, *Alexandrium minutum* (12×10^3 cells L^{-1}) dominated during the non-monsoon season in the Sal estuary.

Statistical analysis

Redundancy analysis (RDA) was performed to relate the distribution of HAB species with environmental

Table 2 Seasonal variation in the distribution of HAB-forming diatoms and dinoflagellates along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary

Taxon	Chapora estuary						Sal estuary					
	C-1		C-2		C-3		S-1		S-2		S-3	
	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM
Diatoms												
<i>Biddulphia mobiliensis</i>	+	-	-	-	-	-	-	-	-	-	+	-
<i>Cerataulina dentata</i>	-	+	-	-	-	-	-	-	-	-	-	-
<i>Chaetoceros curvisetus</i>	+	-	-	-	-	-	-	-	-	-	+	-
<i>Nitzschia sigmoidea</i>	-	+	+	-	+	+	-	-	-	+	-	-
<i>Rhizosolenia eriensis</i>	+	-	+	-	-	-	-	-	-	-	-	-
<i>Rhizosolenia setigera</i>	+	-	-	-	-	-	-	-	-	+	-	+
<i>Skeletonema costatum</i>	+	-	+	-	+	-	-	+	+	+	+	+
<i>Coscinodiscus centralis</i>	-	-	-	+	-	-	-	-	-	-	+	-
<i>Coscinodiscus granii</i>	-	-	-	+	-	-	-	-	-	-	-	+
<i>Coscinodiscus radiatus</i>	+	-	+	+	-	+	-	-	-	+	+	-
<i>Thalassiosira punctigera</i>	-	-	-	+	-	-	-	-	-	-	-	-
<i>Nitzschia coarctata</i>	-	-	-	-	-	+	-	-	-	-	-	-
<i>Chaetoceros lorenzianus</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Chaetoceros convolutes</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Chaetoceros danicus</i>	-	-	-	-	-	-	-	-	-	-	+	-
<i>Pseudo-nitzschia delicatissima</i>	-	-	-	-	-	-	-	-	-	-	-	+
<i>Pseudo-nitzschia pungens</i>	-	-	-	-	-	-	-	-	-	-	-	+
Dinoflagellates												
<i>Peridiniopsis cunningtonii</i>	-	+	-	-	-	-	-	-	-	-	-	-
<i>Alexandrium minutum*</i>	-	-	-	+	-	-	+	-	+	+	-	-
<i>Peridinium quinquecorne</i>	-	-	+	+	-	-	-	+	-	+	-	-
<i>Ceratium furca</i>	-	-	-	-	-	-	-	-	-	-	-	+
<i>Ceratium fusus</i>	-	-	-	-	+	+	-	-	-	-	-	+
<i>Prorocentrum gracile</i>	-	-	-	-	-	-	-	-	-	+	-	-
<i>Alexandrium pseudogonyaulax*</i>	-	-	-	-	-	-	-	-	-	+	-	-
<i>Alexandrium tamarense*</i>	-	-	-	-	-	-	-	-	+	+	-	+
<i>Peridiniopsis polonicum</i>	-	-	-	-	-	-	-	-	+	-	-	-
<i>Protoperidinium pellucidum</i>	-	-	-	-	-	-	-	-	+	-	-	-
<i>Cochlodinium polykrikoides*</i>	-	-	-	-	-	-	-	-	-	-	-	+
<i>Gonyaulax spinifera*</i>	-	-	-	-	-	-	-	-	-	-	-	+
<i>Prorocentrum micans*</i>	-	-	-	-	-	-	-	-	-	-	-	+

*Toxic species; M monsoon, NM non-monsoon

variables (Fig. 2). Based on forward selection and Monte Carlo permutation test of the nine environmental variables, salinity, pH, DO and phosphate concentration were statistically significant ($p < 0.05$). The diversity of

HAB-forming diatoms ($H' = 1.75$) and dinoflagellates ($H' = 1.54$) in Sal estuary was found to be high as compared to the diversity of diatoms ($H' = 1.21$) and dinoflagellates ($H' = 1.21$) in Chapora estuary.

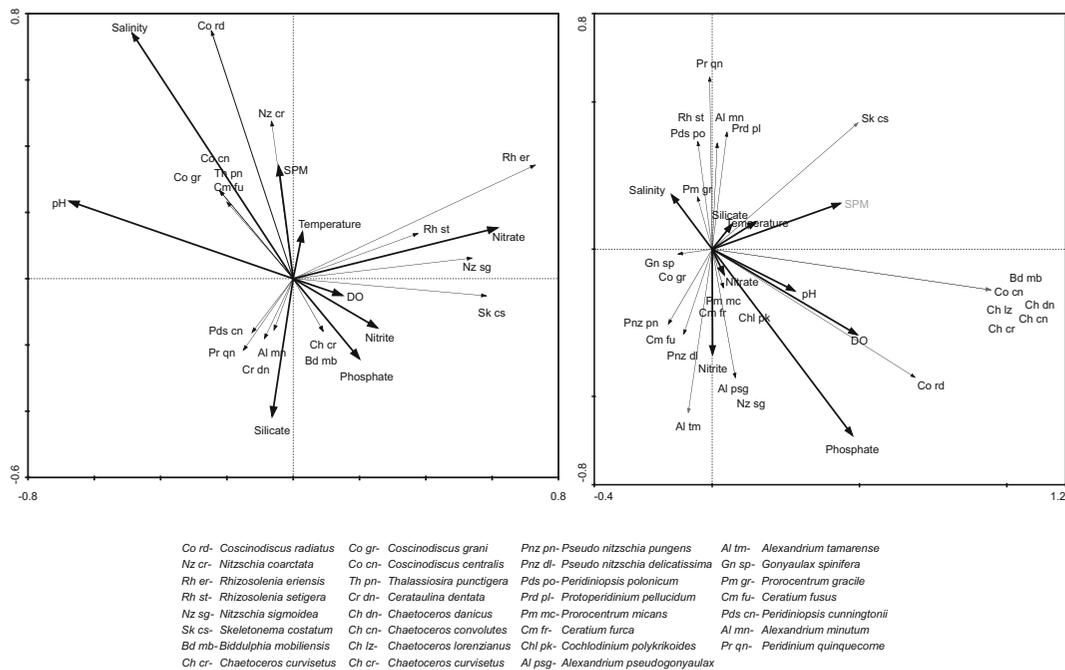


Fig. 2 Redundancy analysis (RDA) bi-plots depicting the relationship between HAB species and environmental parameters in **a** Chapora and **b** Sal estuary

Toxic dinoflagellates and its biotic interaction

In Chapora estuary, only one toxic red tide-forming species, *Alexandrium minutum* was reported, whereas in Sal estuary, six toxic species, *Alexandrium minutum*, *Alexandrium tamarense*, *Alexandrium pseudogonyaulax*, *Cochlodinium polykrikoides*, *Prorocentrum micans* and *Gonyaulax spinifera* were reported.

In Chapora estuary (C-1) and Sal estuary (S-3), the highest diatom peak in July was dominated by freshwater species (Fig. 3). Toxic dinoflagellate *Alexandrium minutum* recorded in both the estuaries revealed the allelopathic effect on diatom cell density. The highest dinoflagellate peak in Chapora estuary, in October (C-2), comprises of *A. minutum* that suppressed diatom cell density in the same month, whereas the absence of *A. minutum* in November leads to a proliferation of diatoms which is noted by the highest peak in the same month. Similar observations were noted in Sal estuary in December with the highest dinoflagellate peak comprised of *A. minutum* and revealed the inhibitory effect on diatom cell density in the same month.

Discussion

Several studies on the distribution of HABs carried out worldwide (Hallegraeff 1993), along the coasts of India (D'Silva et al. 2012) and along the coastal waters off Goa (Devassy et al. 1979; Alkawri and Ramaiah 2010), revealed that the distribution of HAB species was influenced by variations in environmental parameters that occur either naturally or due to anthropogenic activities. However, an estuarine study carried out by Pednekar et al. (2012) in Mandovi-Zuari estuarine complex of Goa, on the occurrence and distribution of HAB species, revealed similar results.

The present study in Chapora and Sal elucidates that seasonal variations in phytoplankton distribution are markedly affected by the environmental parameters with an apparent influence on the distribution of HAB species. Alteration in physicochemical properties along these estuarine environments occurs mainly due to monsoonal runoff and anthropogenic input such as agricultural runoff and discharge of domestic and industrial wastes. During the present study, it was observed that salinity undergoes noticeable changes seasonally, although it is established that the decline in salinity during

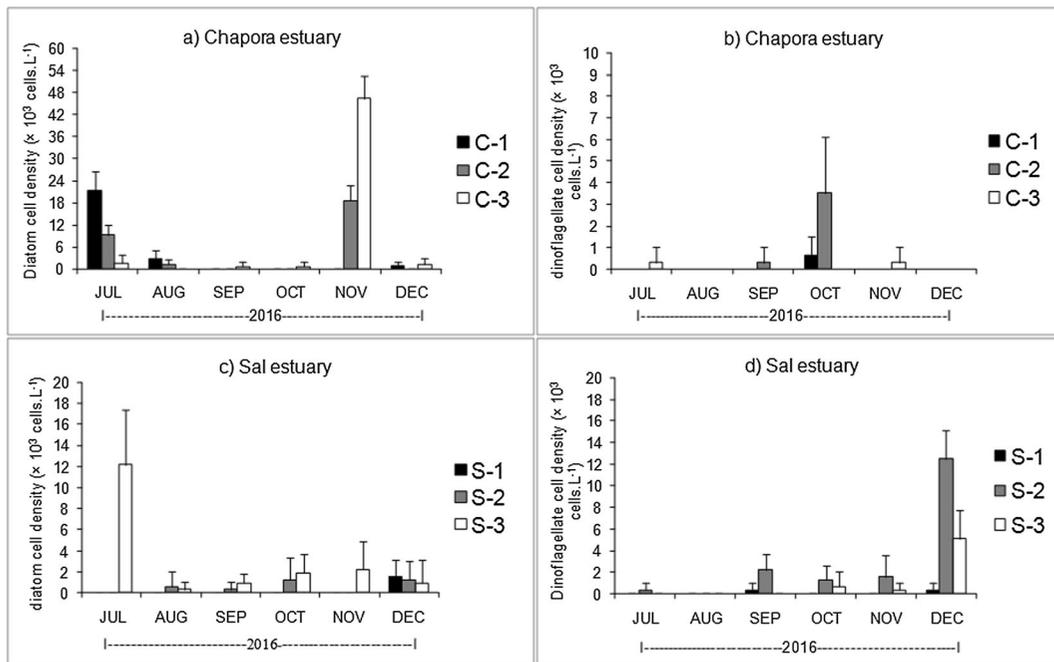


Fig. 3 Spatio-temporal variations in the cell density (cells L⁻¹) of HAB-forming diatoms and dinoflagellates along salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary. **a** HAB-forming diatoms cell density in Chapora estuary, **b** HAB-forming

dinoflagellate cell density in Chapora estuary, **c** HAB-forming diatom cell density in Sal estuary and **d** HAB-forming dinoflagellate cell density in Sal estuary

monsoon in the tropical estuaries is due to the incursion of fresh river water (Shetye et al. 2007). The variation in nutrient concentration in the study area is generally due to the intense rainfall besides anthropogenic activities. Sand mining and other anthropogenic activities are the primary reasons for higher silicate values. Domestic and agricultural runoff must have contributed to high phosphate concentration. In addition, shrimp farming ponds located along these estuaries can be a primary contributor. High nitrate concentration in monsoon is mostly from agricultural runoff due to heavy rainfall and anthropogenic input. Nitrate concentration was found to be higher in the monsoon season as compared to non-monsoon at all three stations in both the estuaries. Similar observations were recorded by Pednekar et al. (2011, 2014) along with other estuaries of Goa.

High diatom density noted from Chapora estuary was found to be similar with the observations that have been reported from different parts of Indian coasts (Sawant and Madhupratap 1996; Sarojini and Sarma 2001). However, high dinoflagellate cell density at most of the stations along Sal is due to high salinity and temperature that were found to be favourable for the growth of dinoflagellates (Joseph and Pillai 1975). Also, high

phosphate concentration along Sal estuary must have supported high dinoflagellate cell density as a decline in phosphate concentration leads to a reduction in dinoflagellates in surface waters (Patil and Anil 2011). Ciotti et al. (1995) reported that dinoflagellates are more mobile and buoyant than diatoms and have nutrient retrieval strategy which in turn leads to diel migration of nutrient-depleted dinoflagellates into nutrient-rich layers (Smayda and Reynolds 2003).

Among the HAB species reported, some were brackish, euryhaline and eurythermal as the estuaries form transitional zones between freshwater and marine environments. Some of the euryhaline species reported during the study were mostly due to the tidal influx. Bazin et al. (2014) found that tidal current plays a role in the transport of coastal euryhaline species and therefore proliferate in these estuarine conditions (Pednekar et al. 2014) due to their ability to withstand a wide salinity range (Pednekar et al. 2011). The seasonal succession of HAB species along the salinity gradient in both the estuaries was apparent due to salinity, temperature and nutrient variation in these estuarine systems (Quinlan and Philips 2007; Matos et al. 2011). Further, the RDA plots also revealed the influence of physicochemical

factors (salinity, DO, phosphate) on the composition and distribution of HAB species (Huang et al. 2004; Sahu et al. 2012).

Noticeable difference in the composition and cell density of HAB forming and toxic dinoflagellates was observed and found to be high in non-monsoon period suggesting salinity as a critical factor affecting the abundance of toxic dinoflagellates (Alkawri and Ramaiah 2010). In addition, other factors such as allelopathy (Smayda 1998; Graneli and Johansson 2003) and the presence of grazers may also contribute to the species composition at that particular time and in that specific environment (Anderson et al. 2002).

In the present study, Sal is found to be more polluted based on high nitrate and phosphate concentration recorded at all stations in comparison to Chapora. Also, the presence of high diversity of toxic species in Sal indicates favourable conditions in this estuary for their proliferation. Among the toxic species reported in the present study, six toxic dinoflagellate species, *Alexandrium minutum*, *Alexandrium tamarense*, *A. pseudogonyaulax*, *Cochlodinium polykrikoides*, *Prorocentrum micans* and *Gonyaulax spinifera*, were reported in Sal estuary. *Alexandrium minutum* and *Alexandrium tamarense* are known to be a red tide-forming Paralytic shellfish poisoning (PSP) producers. Toxic blooms of *Alexandrium tamarense* is commonly reported in Japan (Fukuyo et al. 1985) and in Europe (Moestrup and Hansen 1988) and are common along the NE coast of North America (New England and Canada) (Loeblich and Loeblich 1975). *A. minutum* associated with toxic PSP blooms (red tides) (Hallegraeff 1991) which in turn affect humans, other mammals, birds and possibly fish (Hallegraeff et al. 1988) is of great concern.

A. pseudogonyaulax has been reported from several localities in Europe. Another red tide-causing dinoflagellate is *Cochlodinium polykrikoides* and is known to kill fishes (Patil and Anil 2011). It is associated with large fish kills and significant economic loss in Japanese and Korean waters (Fukuyo et al. 1990). There are few reports of *Prorocentrum micans* known to cause shellfish kills in Portugal (Pinto and Silva 1956) and South Africa (Horstman 1981). *Gonyaulax spinifera* is reported to be a prolific yessotoxin producer (Rhodes et al. 2006). The toxin bioaccumulates in the tissues of bivalve molluscs (mussels, scallops and clams), allowing its entry into the food chain (Mudadu et al. 2017).

Among the six potentially toxic species reported in the present study, potent red tide-forming PSP producer

of genus *Alexandrium* revealed biotic interaction between toxic and non-toxic HAB species in these selected estuarine environments. Allelopathy is one of the biotic interaction described by the release of secondary metabolites (Rizvi and Rizvi 1992) which impede the growth of competitive species leaving more resources and space for the allelopathic species to bloom (Lelong et al. 2011). Different *Alexandrium* species may produce different allelochemicals (Lelong et al. 2011) which facilitate to outcompete other species for nutrients (Tillmann and John 2002; Fistarol et al. 2004). Hence, it can be inferred that the species of this genus can lead to toxic blooms in the particular environment if nutrient enrichment prevails (Richardson and Jorgensen 1996; Richardson 1997).

The massive and increasing quantities of agricultural and sewage effluents (Vitousek et al. 1997), atmospheric deposition and groundwater inflow and the increasing aquaculture systems (Anderson et al. 2002) are the major primary sources which lead to eutrophication due to nutrient enrichment in coastal waters. Published reports (Paerl 1988; Anderson et al. 2002; Baek et al. 2008) suggest that eutrophication primarily by nitrate and phosphate derived from anthropogenic input supports bloom formation, including HAB-forming species in coastal areas.

Similar observations were noted from the present study, wherein genus *Alexandrium* showed a positive correlation with phosphate. From this observation, it can be inferred that phosphate input might lead to the proliferation of *Alexandrium* species which in turn can lead to bloom formation due to its allelopathic ability (Lelong et al. 2011). It is known that allelopathic ability can lead to a proliferation of a specific species and the species can compete successfully for the available growth-limiting nutrient and is likely to form blooms. An experimental study carried out by Labry et al. (2008) concluded that *Alexandrium* abundance increased in parallel with phosphate supply outcompeting competitor species.

Though nitrate did not show any significant correlation with *Alexandrium*, it might have influenced the distribution and diversity of this genus. In the previous study, it has been reported that nitrate triggers the formation of *Alexandrium* blooms (Collos et al. 2007). It can be assumed that the nutrient enrichment is an underlying factor which sustains blooms of allelopathic algae by altering the N:P balance (Graneli et al. 2008). Further, the dominance of *Alexandrium minutum* ($12 \times$

10^3 cells L^{-1}) in the Sal estuary is of great concern as this species is known to produce potent PSP neurotoxin which enters into the food chain and affects fishes, birds and human beings via consumption of contaminated shellfish (Larsen and Moestrup 1989). In addition, as shellfish collection and fishing are the routine activities carried out along this estuary, the high abundance of this toxic species and the high diversity of other toxic dinoflagellate species pose a great health risk to the people and marine organisms along this region.

It is imperative from the observations made in the present study; a noticeable increase in HABs due to an upsurge in eutrophication of coastal waters as a result of human activities is of considerable concern (Anderson et al. 2002). It is pertinent that reductions in nutrient input might mitigate blooms, but may not completely eradicate HAB species. Also, other factors such as allelopathy and the presence of grazers should be considered in elucidating the reason for the development of HABs.

Conclusion

The above observations suggest that physicochemical parameters influence the composition and distribution of HAB species and among these, salinity is the major influencing factor in the distribution of toxic dinoflagellate species. A high number of toxic dinoflagellate species in Sal estuary revealed the prevalence of favourable conditions, with the increase in nutrient input, a probable cause for the proliferation of toxic species. Eventually, we can assume that nutrient enrichment due to eutrophication is a principal factor supporting many blooms of allelopathic algae by altering the N:P balance. Since fishing and shellfish collection is an important activity in these regions, bloom formation by toxic species due to inadvertent disposal of waste lead to detrimental effects, especially in the Sal estuary.

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Antonymous nature of freshwater phytoplankton in the tropical estuarine environments of Goa, southwest coast of India

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ABSTRACT

Estuaries are the most dynamic marine ecosystems serving as potential spawning and nursery grounds for an array of marine fishes and their life stages. Producer community explicitly represented by phytoplankton form essential inertia to propagate the food chain, however on the contrary also could be detrimental due to blooming events. The present study envisages studying the distribution of green algae (Chlorophyceae) and blue-green algae (Cyanophyceae) in two tropical estuaries, namely Chapora and Sal in Goa along the west coast of India. Water samples were collected during July–December, 2016, comprising both monsoon and non-monsoon season. A total of twenty-five species of Chlorophyceae and three species of Cyanophyceae were recorded from these estuaries. Among the two, the Chlorophyceae group was found to be dominant and diverse. These two groups of phytoplankton were strongly influenced by the season in both the estuaries. The Multi-dimensional scaling (MDS) plots revealed salinity as one of the crucial parameters in the composition and distribution of these freshwater groups. Based on Redundancy analysis (RDA) plots, it was noted that, in addition to salinity, other physicochemical parameters (DO, pH, and nitrate concentration) could also affect the distribution of these groups. Two toxic bloom-forming genera (*Microcystis* and *Oscillatoria*) belonging to the Cyanophyceae group were reported. It is imperative that such events would play a role in the proliferation of these toxic species in the higher trophic levels.

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1. Introduction

Estuarine ecosystems are one of the complex habitats due to their frequently changing physicochemical surroundings (Parab et al., 2013) mainly influenced by the land–sea interactions and the coastal processes. Published literature (Tittensor et al., 2010; Moyle et al., 2010) suggests that these habitats in tropics are highly productive and support a diversified invertebrate population. This unique feature makes these habitats as the potential sites for spawning and nursery of variety of juvenile marine fishes, crustaceans, and other planktonic organisms (Whitefield, 1998; Sheaves et al., 2014). In recent times, much of these habitats are generally influenced by the anthropogenic input (Kennish, 2005) mainly derived from the land use pattern and the coastal developmental activities (Kennish, 2002) thus causing a deleterious effect on the functioning of these ecosystems.

Phytoplankton community in these habitats, being primary producers form an vital entity and establish themselves in the food chain through the provision of organic matter (Sridhar et al., 2006). However, a sizable group of these communities is also known to form blooms during favourable conditions (Sanilkumar

et al., 2009) and also can be detrimental due to their toxicity (Hallegraeff et al., 1995). Phytoplankton blooms are now known to be one of the important issues that threaten most of the aquatic habitat functioning. Among the environmental variables, salinity (Lancelot and Muylaert, 2011) and anthropogenic nutrient input can cause algal bloom and affect ecosystem function (Zaias et al., 2010; Stewart et al., 2011). Therefore, it is imperative that better understanding of these vulnerable habitats is essential to understand their functioning and to generate decision-making system for efficient management (Beck et al., 2001). Number of studies were carried out on diatoms and dinoflagellate blooms (Alkawri and Ramaiah, 2010; Patil and Anil, 2011; Pednekar et al., 2012), however, few studies have been carried out on the freshwater phytoplankton blooms (Mishra et al., 2009; Figueiredo et al., 2006).

In the present study, an attempt has been made to provide the information on the distribution of Chlorophyceae and Cyanophyceae (hereafter referred as freshwater phytoplankton groups) with the following objectives (1) to elucidate the distribution of freshwater phytoplankton groups along the salinity gradient (2) to highlight the effect of physicochemical parameters on the distribution of freshwater phytoplankton groups (3) to reveal the presence of toxic bloom-forming Cyanobacteria.

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2. Materials and methods

2.1. Study area

The study area comprises coastal waters up to 5 m depth during low tide off the central west coast of India. The continental drainage along the central west coast of India is characterised by small rivers and streams that drain into the Arabian Sea (D' Silva et al., 2012). Goa is located on the west coast of India (14° 53' 57" to 15° 47' 59" North latitudes and 73° 40' 54" to 74° 20' 11" East longitudes). The coastal zone of Goa is traversed by 9 major dynamic estuarine rivers. Land use pattern and other anthropogenic activities dramatically transform and alter estuarine and coastal marine environmental conditions, recognition of these impacts on the riverine/estuarine or the coastal marine environment is, therefore, one of the important issues in modern times (Pradhan and Shirodkar, 2011).

The present study area includes Chapora and Sal estuary in North and South Goa, respectively (Fig. 1). Chapora estuary is lined by patchy mangrove vegetation (upstream), with sparse distribution near the mouth of the estuary. An array of anthropogenic activities (fishing, sand mining, aquaculture ponds, discharge of domestic wastes, industrial effluents, human settlements along the river banks, agricultural runoff) are being carried out. Along Sal estuary, mangroves occur throughout the length of the estuary, although their density is very less downstream due to dense human habitation and agricultural fields. Fishing grounds off the mouth of Sal estuary comprise of two different habitats (silty substratum towards the North of the mouth and submerged rock towards the South). Numerous anthropogenic activities (fishing, shellfish collection, shrimp farms, discharge of domestic and industrial wastes) are being carried along this estuary.

2.2. Sampling sites

Three stations were selected in each estuary based on the salinity gradient. In Chapora estuary, station C-1, upper estuary (15°39.072' and E 073°47.995'), C-2, middle estuary (N 15°38.396' and E 073°45.848'), C-3, lower estuary (N 15°38.137' and E 73°45.640'), were selected and in Sal estuary, three stations namely, S-1, upper estuary (N 15°10.095' and E 073°56.781'), S-2, middle estuary (N 15°10.494' and E 73°57.502'), S-3, lower estuary (N 15°11.842' and E 073°58.240'), were selected (Fig. 1). Sampling was carried out during July–December, 2016. Surface water samples (phytoplankton taxonomy and estimation of physico-chemical parameters) were collected during low tide at monthly intervals during monsoon (July–September) and non-monsoon (October–December) season. The sampling was carried out at low tide as the purpose was to monitor phytoplankton and the cyst distribution in the water and sediment respectively. Hence, along with the collection of water samples, sediment core samples for cyst abundance was done at low tide to ease the collection of sediment sample.

2.3. Physico chemical parameters

The temperature was measured onboard with a mercury thermometer. Dissolved oxygen (DO) estimation was carried out by Winkler's method, and pH was estimated using a pH metre probe. Digital salinometer was used for salinity measurements, and nutrient concentration was estimated (nitrate, nitrite, phosphate, and silicate) using the standard procedure (Grasshoff et al., 1999).

Table 1

Seasonal variation of salinity along the salinity (PSU) gradient in Chapora and Sal estuary.

	Chapora			Sal		
	C-1	C-2	C-3	S-1	S-2	S-3
Monsoon	0–2	1–3	1–6	0–3	1–4	4–15
Non-monsoon	1–4	2–15	6–26	1–26	7–30	31–36

2.4. Taxonomy of HAB forming Chlorophyceae and Cyanophyceae species

Water samples for phytoplankton identification were collected in 1-L plastic bottles in triplicates and were fixed with 0.2% Lugol's iodine and were kept for settling for 48 h. The same were concentrated to 100 ml by carefully syphoning the top layer of the sample with tubing, one end of which was covered with 10 µm mesh, to avoid loss of small-sized phytoplankton. 1 mL of concentrated sub-sample was analysed using inverted microscope (Nikon Ti-S) at 200X, 400X and 600X magnifications and taxonomic identification of freshwater phytoplankton were carried out following published identification manual (Van, 2006; Sahu et al., 2013).

2.5. Statistical methods

Statistical analyses were performed using PRIMER 6 and CANOCO 4.5 software. To evaluate the relationships between the phytoplankton species composition and environmental variables, a constrained ordination method, RDA was used. MDS plots were produced using PRIMER 6 to visualise seasonal differences between sites based on cell density and S-17 Bray–Curtis similarity measure was calculated. Shannon–Weiner diversity indices were computed using PRIMER 6.

3. Results and discussion

3.1. Distribution of Chlorophyceae and Cyanophyceae along the salinity gradient

Salinity is the indicator of freshwater incursion into the estuary during monsoon season and helps to explain the spatial and temporal distribution of phytoplankton along the gradient (Madhavi et al., 2015). In both estuaries, salinity was affected by precipitation during monsoon, and a noteworthy decline in salinity was observed at all three stations. Salinity variations in Chapora estuary were found to be large during both monsoon and non-monsoon season (Table 1). As the tide is one of the most important parameters controlling the dynamics of Chapora estuary, the tidal range is low, and freshwater flow during monsoon may reduce the salinity. However, comparatively small salinity variations were seen in Sal estuary (Table 1). It is evident from the present observations that the Sal estuary experiences a significantly more saline condition as compared to Chapora during both the seasons. It is apparent from the geomorphology of the Sal estuary as a small narrow, shallow estuary, and the tide upstream gets progressively flood dominant, a feature common in narrow, shallow estuaries.

In the present study, the observations made on phytoplankton composition revealed that freshwater phytoplankton group distribution and composition was strongly affected based on salinity gradient. A total of twenty-five and three species of Chlorophyceae and Cyanophyceae, respectively, were recorded in the present study (Table 2). The observations made reveal that the Chlorophyceae was found to be dominant in both the estuaries. In Chapora, twenty-three species of Chlorophyceae and three

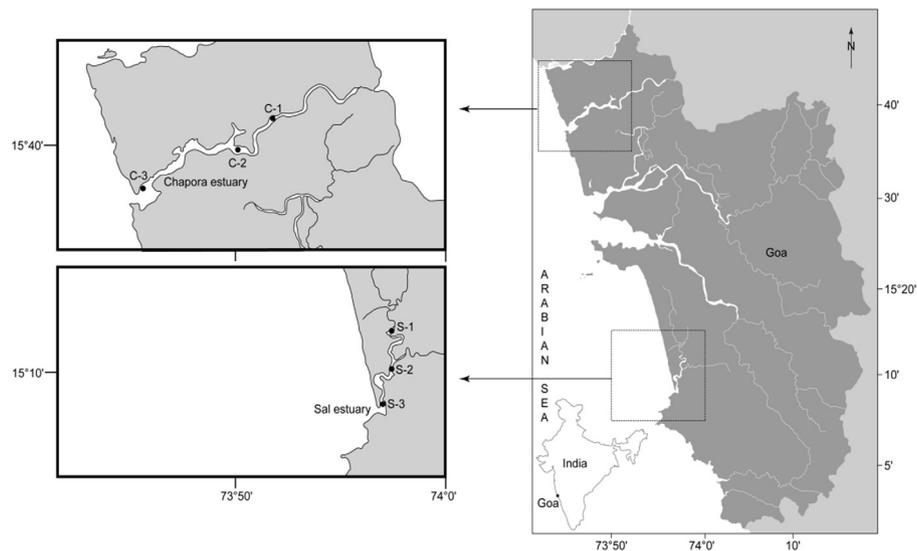


Fig. 1. Map indicating sampling locations.

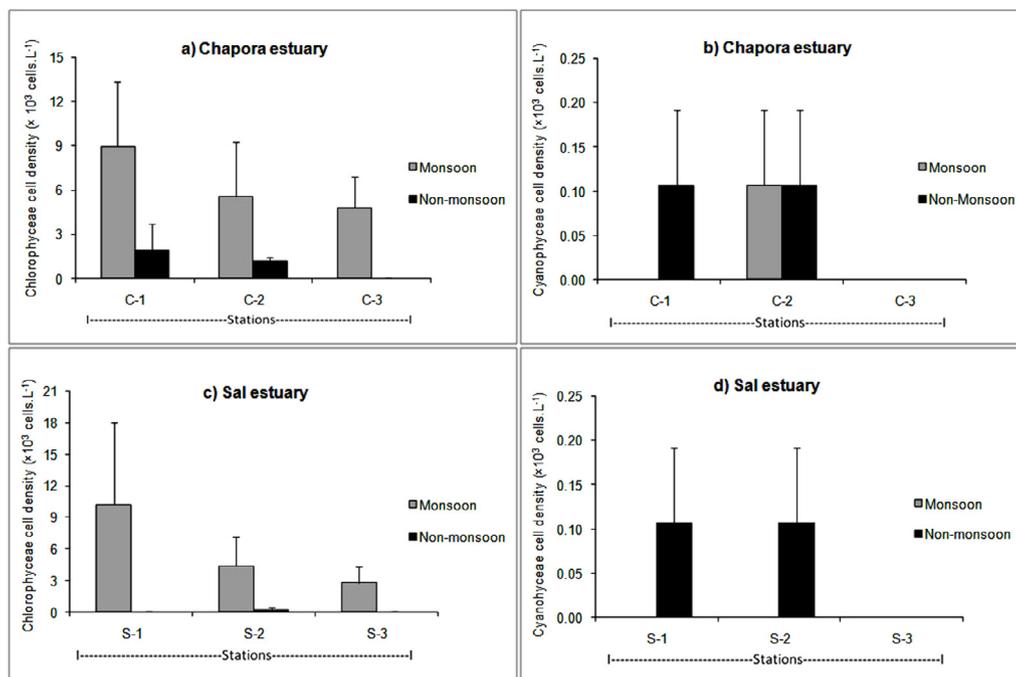


Fig. 2. Spatio-temporal variations in the cell density (cells L⁻¹) of Chlorophyceae and Cyanophyceae along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary. (a) Chlorophyceae cell density in Chapora estuary (b) Cyanophyceae cell density in Chapora estuary (c) Chlorophyceae cell density in Sal estuary (d) Cyanophyceae cell density in Sal estuary.

species of Cyanophyceae were reported, however, in the Sal, only ten species of Chlorophyceae and one species of Cyanophyceae were observed. This suggests that the diversity of these freshwater groups were found to be of lesser magnitude in the Sal (Chlorophyceae $H' = 2.28$, Cyanophyceae $H' = 0$) estuarine system compared to Chapora (Chlorophyceae $H' = 3.12$, Cyanophyceae $H' = 1.09$) as salinity values were high in Sal estuary in comparison with Chapora. As much of the observed phytoplankton species were represented by freshwater forms, high diversity was found in the Chapora estuary which coincides with the low saline waters, forming a favourable habitat to support freshwater species (Rao and Pragada, 2010; Madhavi et al., 2015).

A comparison of the cell density of Chlorophyceae revealed high cell density in Chapora at all the stations during monsoon season, however, no significant variation in the cell density was observed during non-monsoon season at all the stations in both the estuaries. On the other hands, the cell density of Cyanophyceae was found to be very low ($\leq 0.11 \times 10^3$ cells L⁻¹) in both the estuaries at all the stations during both monsoon and non-monsoon months. The analysis of the data (Fig. 2) on cell density (Chlorophyceae and Cyanophyceae) in both the estuaries suggest that there was no significant difference in the cell density among the estuaries.

An MDS analysis was carried out to reveal the seasonal distribution of freshwater phytoplankton along the salinity gradient based on its cell density (Fig. 3). The results of MDS analysis indicated a seasonal difference among the stations based on

Table 2
List of freshwater phytoplankton (Chlorophyceae and Cyanophyceae) encountered along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary during the present study.

Taxon	Taxon Abbreviation	Chapora estuary			Sal estuary		
		C-1	C-2	C-3	S-1	S-2	S-3
Chlorophyceae							
<i>Actinastrum</i> sp.	Ac sp.	+	+	+	–	+	+
<i>Chlamydomonas</i> sp.	Ch sp.	+	+	–	–	–	–
<i>Coelastrum</i> sp.	Cl sp.	–	–	–	+	+	+
<i>Cosmarium depressum</i>	Cs dp	–	–	–	+	–	–
<i>Cosmarium obsoletum</i>	Cs ob	–	+	–	–	–	–
<i>Cosmarium</i> sp.	Cs sp.	+	+	–	–	+	–
<i>Cosmarium subcostatum</i>	Cs sb	–	+	–	–	–	–
<i>Golenkinia</i> sp.	Gl sp.	+	+	–	–	–	–
<i>Oocystis</i> sp.	Os sp.	+	+	+	–	+	–
<i>Pediastrum</i> sp.	Pd sp.	–	+	–	–	–	+
<i>Scenedesmus acuminatus</i>	Sc ac	–	+	–	–	+	–
<i>Scenedesmus quadricaudata</i>	Sc qd	+	+	–	+	+	–
<i>Scenedesmus</i> sp.	Sc sp.	–	–	+	+	+	–
<i>Spirogyra</i> sp.	Sr sp.	+	–	–	–	–	–
<i>Staurastrum anatinum</i>	St an	–	+	–	–	–	–
<i>Staurastrum avicula</i>	St av	+	+	–	–	–	–
<i>Staurastrum chaetoceros</i>	St ch	–	+	–	–	–	–
<i>Staurastrum clevei</i>	St cl	+	+	+	–	–	–
<i>Staurastrum elongatum</i>	St el	–	+	–	–	–	–
<i>Staurastrum gracile</i>	St gr	+	+	–	–	–	–
<i>Staurastrum leptocladum</i>	St lp	+	+	–	–	–	–
<i>Staurastrum longibrachiatum</i>	St lg	+	–	–	–	–	–
<i>Staurastrum sexangulare</i>	St sn	+	–	–	–	–	–
<i>Staurastrum</i> sp.	St sp.	+	+	+	+	+	–
<i>Xanthidium</i> sp.	Xn sp.	–	+	–	–	–	–
Cyanophyceae							
<i>Merismopedia</i> sp.	Mp sp.	–	+	–	–	–	–
<i>Microcystis</i> sp.	Ms sp.	–	+	–	–	–	–
<i>Oscillatoria</i> sp.	Oc sp.	+	–	–	+	+	–

+ Present. – Absent.

phytoplankton composition. The MDS plots showed groupings of monsoonal stations based on 40% similarity of phytoplankton composition along the salinity gradient. This observation suggests the monsoonal effect on the distribution of these dominant freshwater phytoplankton species in Chapora (*Actinastrum* sp., *Oocystis* sp., *Staurastrum clevei*, *Staurastrum* sp.) and Sal (*Scenedesmus quadricaudata*, *Scenedesmus* sp., *Staurastrum* sp.) estuaries along the salinity gradient. Further, it was found that the salinity was comparatively higher at S-3 M coupled with low cell density and diversity of freshwater phytoplankton, hence it was not grouped along with S-1 M and S-2 M (Fig. 3).

The significant seasonal difference in the composition and distribution of Chlorophyceae was observed in both the estuaries (Table 3). The cell density of Chlorophyceae was found to be higher during monsoon season (Fig. 2) signifying that these species are known to thrive well during the low salinity periods. Similar observations were also reported by Marshall and Alden (1990), George et al. (2012), Madhavi et al. (2015). The seasonal variations could be due to the salinity gradient along with changes in physicochemical parameters (Devassy and Bhatathiri, 1974; Huang et al., 1997). These observations probably suggest that these environments, although within proximity, display different and unique ecosystem function.

In contrast, no significant seasonal difference in the cell density of Cyanophyceae was observed along the salinity gradient (Fig. 2) as these are known to be mostly confined to the freshwater zones (George et al., 2012). The succession of phytoplankton along the salinity gradient has been attributed to the fact that most phytoplankton species are stenohaline and undergo osmotic stress upon exposure to salinity changes (Bisson and Kirst, 1995). Thus from the present study, it is noted that salinity gradients strongly influence the distribution and cell density of freshwater phytoplankton groups in estuaries (Rao and Pragada, 2010;

Evangelopoulos et al., 2009). This paper exclusively focus on the freshwater phytoplankton (Chlorophyceae and Cyanophyceae) in estuarine systems. Earlier studies reported that Cyanophyceae to be dominant in freshwater systems (mainly ponds and lakes). However, the present study carried out in the estuarine regions, which happens to be different and dynamic, as compared to freshwater systems, it was found that Chlorophyceae can withstand the dynamic nature of these environments as compared to Cyanophyceae, however only at low salinity conditions.

3.2. Effect of physicochemical parameters in the out competence of Chlorophyceae

The present observations indicate that only three species of Cyanophyceae (*Microcystis* sp., *Merismopedia* sp. and *Oscillatoria* sp.) and twenty-five species of Chlorophyceae group were found. Published literature (Paerl and Ustach, 1982; Figueiredo et al., 2006) suggests that in freshwater systems, Cyanophyceae dominated the cell count over Chlorophyceae, and lead to bloom formation. However, here an estuarine system dominated by freshwater influx with significant reduction in the salinity during monsoonal months, it was found that Chlorophyceae dominated over Cyanophyceae, suggesting the former group can tolerate and withstand dynamic environments of estuaries with reduced salinities as compared to Cyanophyceae (Annalakshmi and Amsath, 2012; Panigrahi and Patra, 2013).

Estuarine environments are subjected to varied changes in physicochemical properties due to natural (freshwater runoff during monsoon) in addition to anthropogenic activities. As it is known that, in estuarine systems, river flow during monsoon is one of the main sources of nutrients (Nche-Fambo et al., 2015) and hence in the present study, it was noted that the concentration of dissolved inorganic nutrients (nitrate, nitrite,

Table 3

Seasonal variation in the distribution of Chlorophyceae and Cyanophyceae along the salinity gradient of Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary during the present study.

Taxon	Chapora estuary						Sal estuary					
	C-1		C-2		C-3		S-1		S-2		S-3	
	M	NM	M	NM	M	NM	M	NM	M	NM	M	NM
Chlorophyceae												
<i>Actinastrum</i> sp.	+	+	+	-	+	-	-	-	+	-	+	-
<i>Chlamydomonas</i> sp.	+	-	+	+	-	-	-	-	-	-	-	-
<i>Coelastrum</i> sp.	-	-	-	-	-	-	+	-	+	-	+	-
<i>Cosmarium depressum</i>	-	-	-	-	-	-	+	-	-	-	-	-
<i>Cosmarium obsoletum</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Cosmarium</i> sp.	+	+	+	-	-	-	-	-	-	+	-	-
<i>Cosmarium subcostatum</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Golenkinia</i> sp.	+	-	+	-	-	-	-	-	-	-	-	-
<i>Oocystis</i> sp.	+	-	+	-	+	-	-	-	+	-	-	-
<i>Pediastrum</i> sp.	-	-	+	-	-	-	-	-	-	-	+	-
<i>Scenedesmus acuminatus</i>	-	-	-	+	-	-	-	-	+	-	-	-
<i>Scenedesmus quadricaudata</i>	-	+	-	+	-	-	+	-	+	-	-	-
<i>Scenedesmus</i> sp.	-	-	-	-	+	-	+	-	+	-	-	-
<i>Spirogyra</i> sp.	+	-	-	-	-	-	-	-	-	-	-	-
<i>Staurastrum anatinum</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum avicula</i>	+	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum chaetoceros</i>	-	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum clevei</i>	+	-	+	-	+	-	-	-	-	-	-	-
<i>Staurastrum elongatum</i>	-	-	-	+	-	-	-	-	-	-	-	-
<i>Staurastrum gracile</i>	+	+	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum leptocladum</i>	+	-	+	-	-	-	-	-	-	-	-	-
<i>Staurastrum longibrachiatum</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>Staurastrum sexangulare</i>	-	+	-	-	-	-	-	-	-	-	-	-
<i>Staurastrum</i> sp.	+	+	+	+	+	-	+	-	+	-	-	-
<i>Xanthidium</i> sp.	-	-	+	-	-	-	-	-	-	-	-	-
Cyanophyceae												
<i>Merismopedia</i> sp.	-	-	-	+	-	-	-	-	-	-	-	-
<i>Microcystis</i> sp.	-	-	+	-	-	-	-	-	-	-	-	-
<i>Oscillatoria</i> sp.	-	+	-	-	-	-	-	+	-	+	-	-

+ Present. - Absent.

phosphate, and silicate) was higher in monsoon as compared to non-monsoon season. Among other nutrients estimated, the concentration of nitrate along the gradient was recorded to be highest during monsoon season in both Chapora (8.28 μM) and Sal (22.39 μM) estuary. There was no significant difference in the water temperature between Chapora (26 °C–29 °C) and Sal (28 °C–30 °C) estuary during monsoon. DO concentration is an important constituent of water, and its concentration in water is an indicator of prevailing water quality (George et al., 2012). DO concentration was found to be higher in Chapora (8.90 mg L⁻¹) as compared to Sal (6.88 mg L⁻¹) estuary in monsoon season and showed remarkable seasonal variations with a range of 5.30 mg L⁻¹–8.90 mg L⁻¹ in Chapora estuary and 3.74 mg L⁻¹–6.88 mg L⁻¹ in Sal estuary. Significant changes in pH were noted in Chapora (6.59–8.04) and Sal (6.70–8.15) estuary. Published literature Locke and Sprules (2000) suggest that the pH values observed were circumneutral pH and was noted only few times during the study. Hence, it may not have high impact on the freshwater phytoplankton as they are known to have tolerance to withstand slight variation in pH.

The Suspended Particulate Matter (SPM) range (2.2–12.3 mg L⁻¹) was observed at monsoonal stations C-1 M and C-2 M and the range (3.26–7.7 mg L⁻¹) at monsoonal stations S-1 M and S-2 M respectively, which was found to be very low. However, SPM concentration was found to be < 50 mg L⁻¹ towards the monsoonal stations C-3 M and S-3 M where salinity values were found to be comparatively higher with low abundance and diversity of freshwater phytoplankton. Hence, high SPM level at this stations (C-3 M and S-3 M) will not be significant factor in the bloom formation of freshwater phytoplankton species.

An RDA analysis was carried out to understand the effect of physicochemical parameters on the distribution of freshwater

phytoplankton (Fig. 4). Based on RDA bi-plots, it was revealed that nitrate concentration, including other physicochemical parameters (salinity, temperature, DO, pH) affected the distribution of Chlorophyceae group.

Previous studies by Garnier et al. (1995) reported that Chlorophyceae develop in an N rich environment. Mishra et al. (2009), in his study on Chlorophyceae, reported nitrate as the regulating factor in the growth of phytoplankton. Reporting from the previous studies suggest nitrate as one of the prime regulating factors in the proliferation of Chlorophyceae species. Based on the observations from the present study, it can be noted that high nitrate concentration coupled with high DO concentration, and low salinity values might have led Chlorophyceae to outcompete Cyanophyceae in these estuarine environments. Patil and Anil (2011) has reported bloom conditions when nitrate level was as low as 0.4 and 8 μM in Zuari estuary along the coast of Goa. However, in the present study concentration of nitrate was more than 8 μM .

Rajagopal et al. (2010) suggest that, besides salinity and nutrients, other physicochemical parameters (DO, temperature, pH) also strongly affect Chlorophycean members in freshwater zones. Eventually, the combination of all these factors shapes the seasonal and spatial changes in the composition and distribution of species (Cloern and Dufford, 2005). It is not likely that anyone organism will have the ability to excel under all circumstances. However, a dominant group suggests its ability to maximise under the prevailing environmental conditions (Oliver and Ganf, 2000).

Published reports (Paerl et al., 2014; Carstensen et al., 2015) suggest that Chlorophyceae bloom formation occurs in the estuarine systems in low-salinity upper reaches of Scheldt estuary, responding positively to elevated freshwater input. Formation

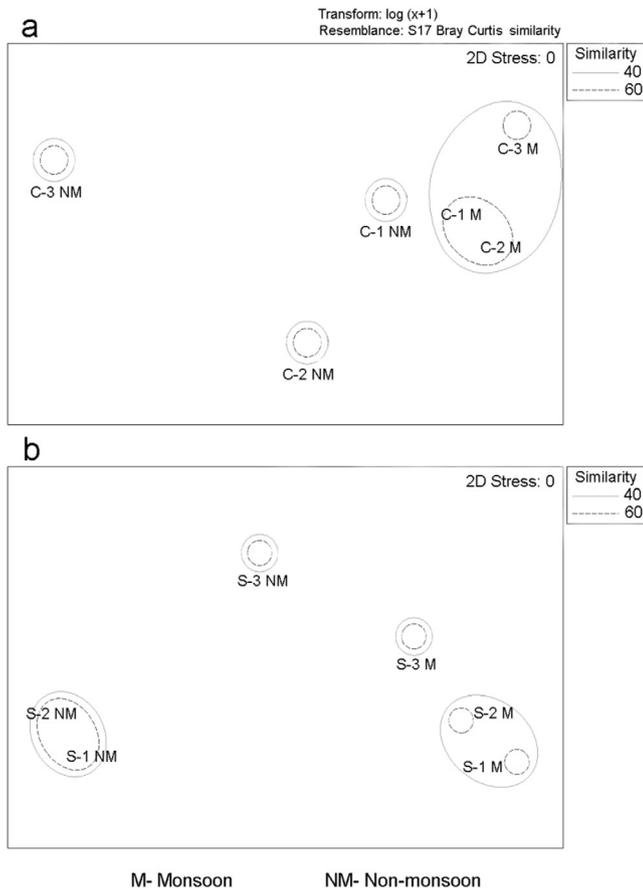


Fig. 3. Multidimensional scaling (MDS) ordination for (a) Chapora and (b) Sal estuary based on Bray–Curtis similarity of stations during the study period based on freshwater phytoplankton cell density season-wise along the salinity gradient.

of blooms in estuaries is of great concern as estuaries are high productive regions (Kannappan and Karthikeyan, 2013). Hence, it is imperative that the anthropogenic input largely influenced by the land use pattern act as one of the driving force towards the enrichment of nutrients, especially nitrogen in the tropical estuarine systems.

3.3. Presence of toxic bloom-forming cyanobacteria

The cyanobacteria (Blue-Green Algae) are the most prevalent and nuisance freshwater algal taxa in nutrient-enriched (N and P) freshwater and brackish ecosystems (Paerl, 1988; Sellner, 1997) causing serious water quality deterioration (Carmichael et al., 1997; Chorus and Bartram, 1999) through hypoxia and anoxia of underlying waters, which may lead to fish and bottom fauna mortalities (Paerl and Tucker, 1995). Historically, genera *Anabaena*, *Microcystis*, *Nodularia*, and *Oscillatoria* are the most notorious which are traditionally confined to heavily nutrient enriched impoundments (Paerl, 1988, 1995). In the present study, two toxic bloom-forming the cyanobacterial genus (*Microcystis* and *Oscillatoria*) were reported. In recent times, the distribution and frequency of occurrence of *Microcystis* and other freshwater cyanobacteria blooms are increasing and has become a worldwide concern (Fristachi et al., 2008). At higher trophic levels, *Microcystis* blooms affect fish health through impacts on the growth rate, histopathology, delayed hatching, feeding rate, and behaviour (Malbrouck and Kestemont, 2006). The reporting of these toxic bloom-forming genera in the present study is an alarming threat to the health and ecosystem function, which probably can pose a serious risk in future to water quality and public health affecting the fish diversity.

4. Conclusions

The freshwater phytoplankton community varied seasonally along the salinity gradient. It is reflected in the present study that

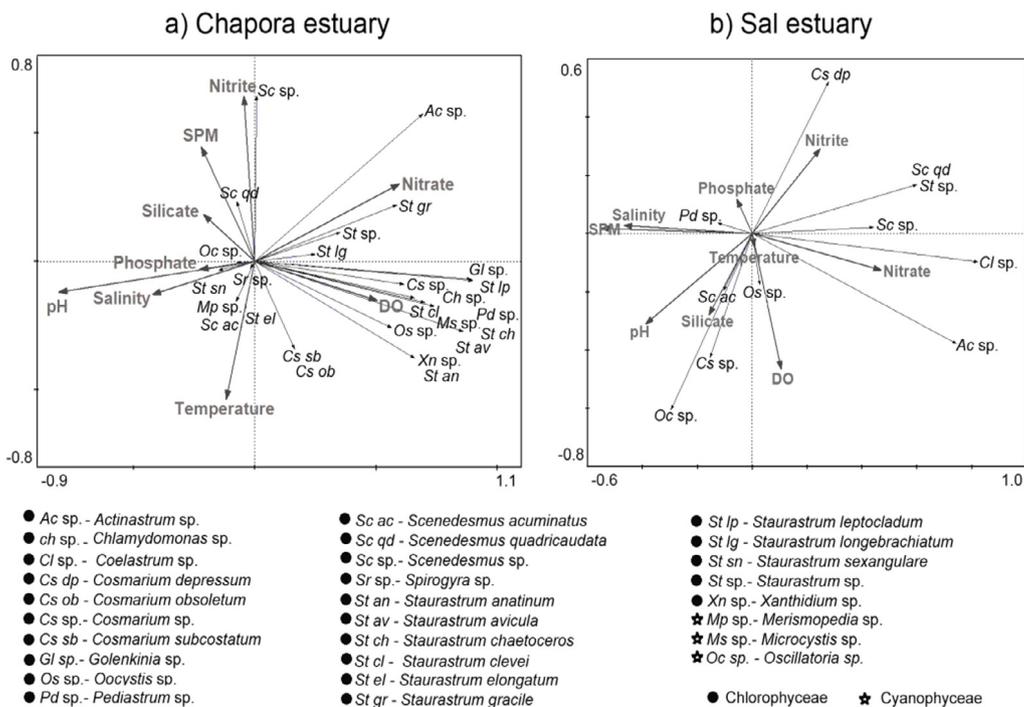


Fig. 4. Redundancy analysis (RDA) bi-plots depicting the relationship between freshwater phytoplankton (Chlorophyceae and Cyanophyceae) and environmental parameters in (a) Chapora and (b) Sal estuary.

although the growth of phytoplankton is controlled by a combination of several factors, the role of salinity on species composition and seasonal succession is found to be significant. Besides, high nitrate concentration coupled with other physicochemical parameters (DO, temperature, and pH) was found to play an important role in the dominance of Chlorophyceae over Cyanophyceae group. Further, the presence of toxic bloom-forming cyanobacterial genus (*Microcystis* and *Oscillatoria*) in these estuarine systems is of concern. As the anthropogenic nutrient input is known to be the main cause for bloom formation, there is an urgent need to identify the sources of nutrient enrichment that alter the habitat equilibrium affecting the health of the coastal ecosystems.

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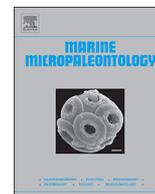
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Distribution of dinoflagellate cysts along the salinity gradient in two tropical estuaries along the West coast of India

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ABSTRACT

Dinoflagellate cysts are known to be potential seed banks which have an essential role in providing bloom history and also serve as useful indicators of eutrophication in marine environments. The present study is focused on the surface distribution of dinoflagellate cysts along the salinity gradient in two tropical estuaries, namely Chapora and Sal in Goa along the West coast of India. Surface sediments and water samples were collected monthly from February 2016–March 2017 which included all three seasons (pre-monsoon, monsoon, post-monsoon). A total of sixteen species of dinoflagellate cysts belonging to the order, Gymnodiniales, Gonyaulacales, and Peridinales were recorded in the surface sediments. Cysts of heterotrophic dinoflagellates dominated the assemblage in both estuaries. Based on Redundancy Analysis (RDA), it was observed that the distribution of the dinoflagellate cysts were influenced by various physico-chemical parameters in both estuaries with salinity and grain size being the main explanatory variable. Nitrate limitation is noted to be a triggering factor for dinoflagellate cyst formation. Also, yessotoxin producer species, such as *Gonyaulax spinifera*, encountered in both estuaries showed an inverse relationship with nitrate concentration. This suggests that, high nitrate input in these estuaries might lead to excystment of *Gonyaulax spinifera* eventually leading to harmful blooms under eutrophic conditions.

1. Introduction

Dinoflagellates are globally well-known groups due to their role in the formation of Harmful algal blooms (HABs) and their toxic effects (D'Silva et al., 2011) on aquatic ecosystems and human health (Burkholder, 1998). About 10% of the 2000 identified dinoflagellates produce cysts (Bravo and Figueroa, 2014). Dinoflagellate cysts (dinocysts) can be well preserved in sediment for several years (Lewis et al., 1999). Hence, they serve as seed banks (Anderson et al., 1995) which have important roles such as species dispersal, survival under unfavorable conditions, termination as well as initiation of blooms and provide bloom history (Heiskanen, 1993; Anderson et al., 1995; Ishikawa and Taniguchi, 1996; Dale, 2001; D'Silva et al., 2012).

In recent years, various anthropogenic activities (industrialization, sewage and agricultural runoffs) have led to nutrient enrichment leading to HABs (Gowen et al., 2012). This has adversely affected coastal regions resulting in threat to aquatic life and human health as well as economic losses to fishing industries (D'Silva et al., 2011). Estuaries are the aquatic environments which are known to be highly affected by anthropogenic activities and hence are susceptible to eutrophication (Price et al., 2016). Dinocysts have received great

attention as biological indicators of eutrophication (Matsuoka, 1999; Radi and de Vernal, 2008). Published literature (Orlova et al., 2004; Rubino et al., 2010; Baula et al., 2011; Furio et al., 2012; D'Silva et al., 2012; Narale et al., 2013) suggest the role of such dinocysts in the marine ecosystems. Recent studies (Su-Myat et al., 2012; Matsuoka et al., 2017, 2018) have been focused on coastal regions, however, few studies have been carried out in estuarine environments (Godhe et al., 2000; Wang et al., 2004; Price et al., 2016; Matsuoka et al., 2017).

Goa is developing at a rapid rate due to tourism and is largely responsible for anthropogenic output making estuaries as highly susceptible to anthropogenic influence. Most of the studies carried out along the coast of Goa on dinocysts are from the coastal waters (D'Costa et al., 2008; D'Silva et al., 2011; 2012). However, studies on the cyst assemblages along the estuaries of Goa are lacking. The present study on the distribution of dinocysts along the salinity gradient in Chapora (North Goa) and Sal estuary (South Goa) is the first of its kind. The objectives of the present study are 1) To elucidate the seasonal distribution of dinocysts along the salinity gradient 2) to reveal the influence of physico-chemical parameters on the distribution of dinocysts 3) to assess nutrient limitation as a triggering factor for dinocyst formation. Further, the information provided here may act as the baseline data on the

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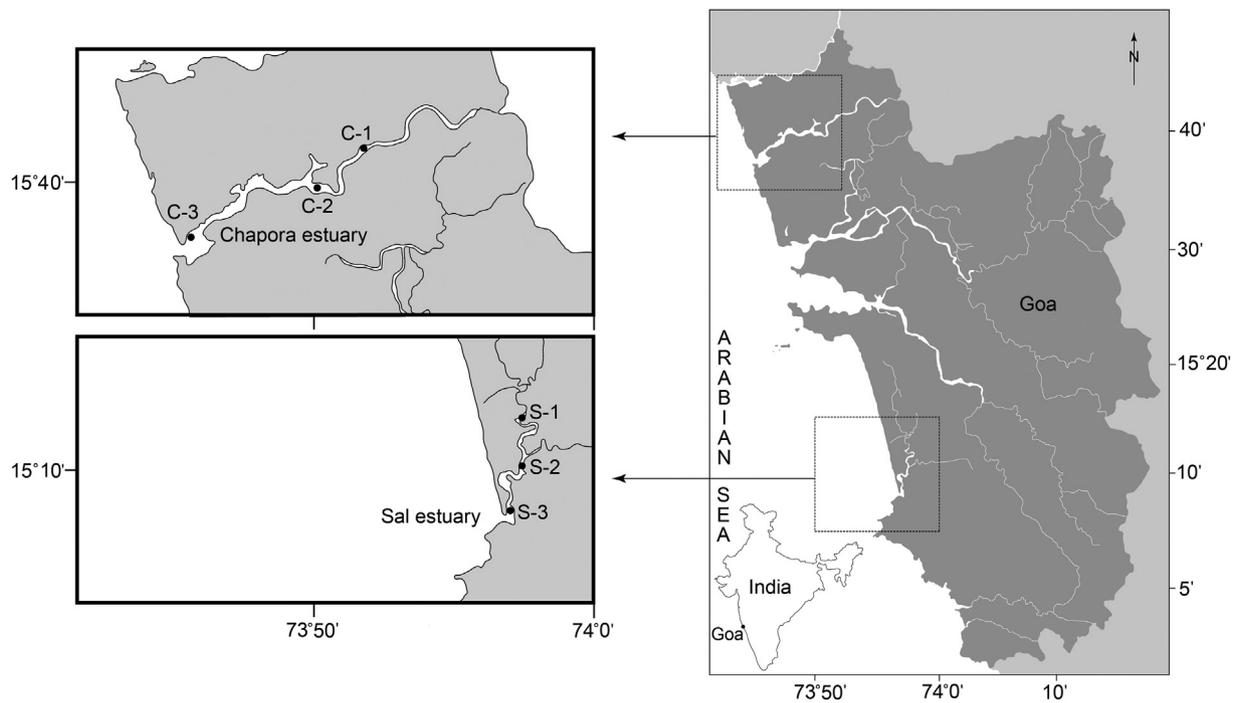


Fig. 1. Map indicating sampling locations.

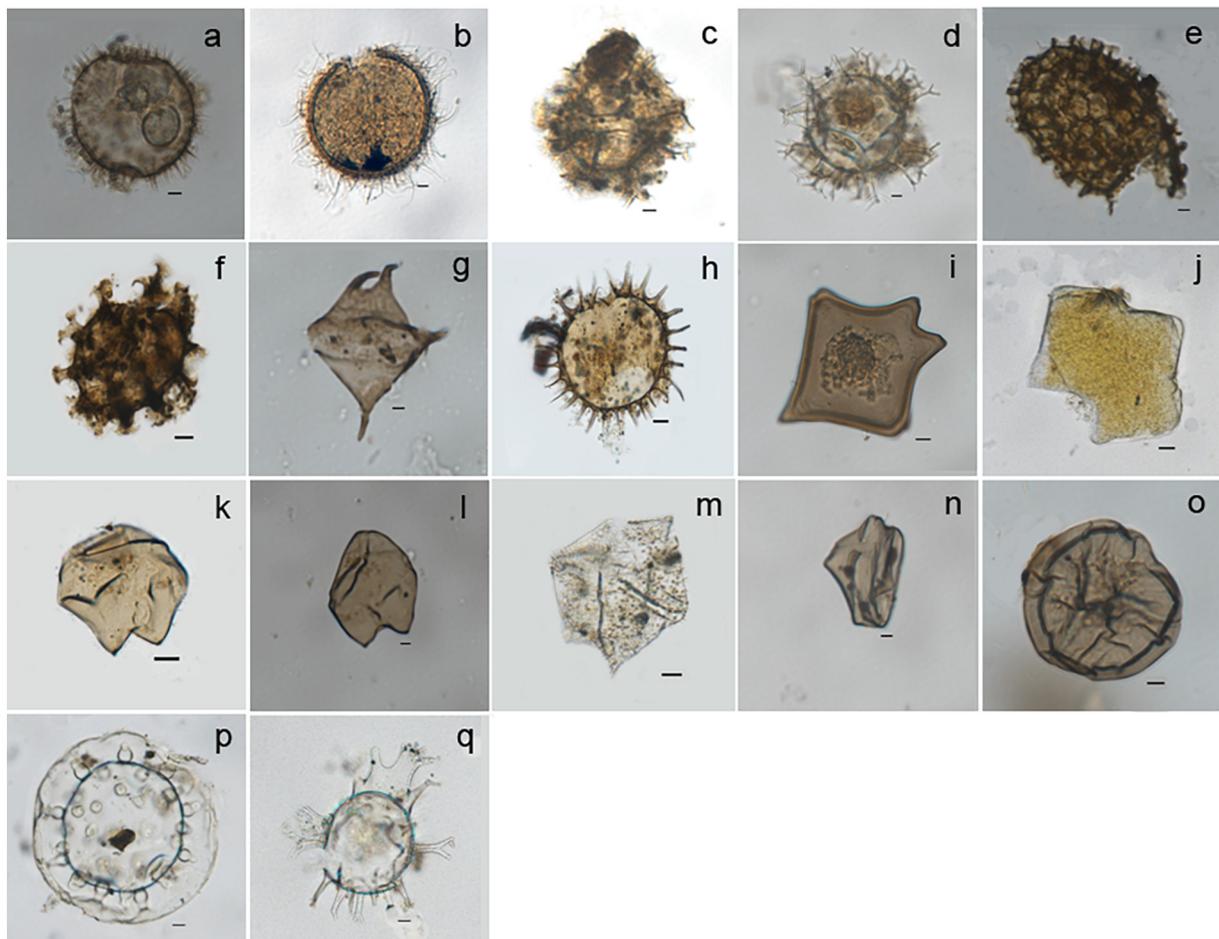


Plate 1. Dinoflagellate cysts encountered in the present study: (a) *Archaeperidinium* sp., (b) *Bitectatodinium spongium* (c) *Gonyaulax scrippsae*, (d) *Gonyaulax spinifera* complex, (e) *Polykrikos kofoidii*, (f) *Polykrikos schwartzii*, (g) *Protoperidinium compressum*, (h) *Protoperidinium conicum*, (i) *Protoperidinium latissimum*, (j) *Protoperidinium leonis*, (k) *Protoperidinium oblongum*, (l) *Protoperidinium obtusum*, (m) *Protoperidinium pentagonum*, (n) *Protoperidinium subinerme* (lateral view), (o) *Protoperidinium subinerme* (ventral view), (p) *Pyrophacus steinii*, (q) *Spiniferites mirabilis*. All scale bars 10 µm.

Table 1

Total number of dinoflagellate cysts encountered along the salinity gradient in the present study in Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3).

Estuary	CHAPORA			SAL		
	C-1 (0–8 psu) ^b	C-2 (1–16 psu) ^b	C-3 (1–26 psu) ^b	S-1 (0–27 psu) ^b	S-2 (1–31 psu) ^b	S-3 (4–36 psu) ^b
<i>Archaeoperidinium</i> sp.	3	1	8	2	0	0
<i>Bitectatodinium spongium</i>	0	2	49	6	0	0
<i>Gonyaulax scrippsae</i>	0	6	17	5	3	0
<i>Gonyaulax spinifera</i> complex ^a	4	7	22	5	3	0
<i>Polykrikos kofoidii</i>	0	1	40	2	0	0
<i>Polykrikos schwartzii</i>	0	1	6	1	1	0
<i>Protoperidinium compressum</i>	0	0	12	0	0	0
<i>Protoperidinium conicum</i>	0	0	15	1	0	0
<i>Protoperidinium latissimum</i>	0	0	11	1	0	0
<i>Protoperidinium leonis</i>	0	2	9	0	2	0
<i>Protoperidinium oblongum</i>	0	0	15	1	0	0
<i>Protoperidinium obtusum</i>	0	0	4	0	0	0
<i>Protoperidinium pentagonum</i>	0	1	2	0	0	0
<i>Protoperidinium subinerme</i>	0	3	5	0	0	0
<i>Pyrophacus steinii</i>	1	3	58	13	5	0
<i>Spiniferites mirabilis</i>	0	0	30	0	0	0

^a Toxic species.^b Values in the parenthesis indicate the salinity range at the stations.**Table 2**

List of dinoflagellate cysts encountered in the present study along with the source of identification.

Biological name	Taxon	Paleontological name	Nutritional type	Toxicity	References
<i>Archaeoperidinium</i> sp.	Ar sp.	–	Heterotrophic	–	Mertens et al., 2012
<i>Bitectatodinium spongium</i>	Bt sm	–	Autotrophic	–	Zonneveld, 1997; Zonneveld and Jurkschat, 1999
<i>Gonyaulax scrippsae</i>	Gn sc	<i>Spiniferites bulloideus</i>	Autotrophic	–	Nehring, 1997; Wall and Dale, 1968
<i>Gonyaulax spinifera</i> complex ^a	Gn sp cp	<i>Spiniferites ramosus</i>	Autotrophic	HAB	Wall and Dale, 1970; Zonneveld and Pospelova, 2015
<i>Polykrikos kofoidii</i>	Pl kf	–	Heterotrophic	–	Shin et al., 2010; Zonneveld and Pospelova, 2015
<i>Polykrikos schwartzii</i>	Pl sz	–	Heterotrophic	–	Shin et al., 2010; Zonneveld and Pospelova, 2015
<i>Protoperidinium compressum</i>	Prd cm	<i>Stelladinium stellatum</i>	Heterotrophic	–	Wall and Dale, 1968; McMinn, 1991; Nehring, 1997
<i>Protoperidinium conicum</i>	Prd cn	<i>Selenopemphix quanta</i>	Heterotrophic	–	Wall and Dale, 1968; Bolch and Hallegraef, 1990
<i>Protoperidinium latissimum</i>	Prd lm	–	Heterotrophic	–	Wall and Dale, 1968
<i>Protoperidinium leonis</i>	Prd ls	<i>Quinquecuspis concretum</i>	Heterotrophic	–	Bolch and Hallegraef, 1990; Wall and Dale, 1968
<i>Protoperidinium oblongum</i>	Prd obm	–	Heterotrophic	–	Wall and Dale, 1968; Bolch and Hallegraef, 1990
<i>Protoperidinium obtusum</i>	Prd obt	–	Heterotrophic	–	Uzar et al., 2010
<i>Protoperidinium pentagonum</i>	Prd pn	<i>Trivantedinium capitatum</i>	Heterotrophic	–	Wall and Dale, 1968; Bolch and Hallegraef, 1990; McMinn, 1991
<i>Protoperidinium subinerme</i>	Prd sb	<i>Selenopemphix nephroides</i>	Heterotrophic	–	Bolch and Hallegraef, 1990; Wall and Dale, 1968
<i>Pyrophacus steinii</i>	Py st	<i>Tuberculodinium vancompoae</i>	Autotrophic	–	Wall and Dale, 1971
<i>Spiniferites mirabilis</i>	Sp mr	<i>Spiniferites mirabilis</i>	Autotrophic	–	Bolch and Hallegraef, 1990

^a Toxic species.

distribution and composition of dinocysts that may be useful to predict, control and mitigate the formation of future bloom events.

2. Materials and methods

2.1. Study area

The study area comprises coastal waters up to 5 m depth off the central West coast of India. The continental drainage along the central West coast of India is distinguished by small rivers and streams that drain into the Arabian Sea (D'Silva et al., 2012). Goa is located on the West coast of India (14° 53' 57" to 15° 47' 59" North latitudes and 73° 40' 54" to 74° 20' 11" East longitudes). Nine dynamic estuaries traverse along the coastal zone of Goa. The present study includes Chapora and Sal estuary in North and South Goa respectively (Fig. 1). Chapora estuary is lined by patchy mangroves (upstream), with scattered distribution near the mouth of the estuary. Several anthropogenic activities (fishing, sand mining, shrimp farming ponds, discharge of domestic wastes, industrial effluents, human settlements along the river banks, agricultural runoff) are being carried out along this estuary. Along the Sal estuary, mangroves occur throughout the length of the estuary, though their density is very less downstream due to dense human habitation and agricultural fields. Fishing grounds off the mouth

of Sal estuary comprise of two different habitats (silty substratum towards the North of the mouth and submerged rock towards the South). Numerous anthropogenic activities (fishing, shellfish collection, shrimp farms, discharge of domestic and industrial wastes) are being carried out along this estuary. Dense human settlement is also being noticed along the bank of this estuary.

2.2. Sampling sites

Three stations were selected in each estuary based on salinity gradient. In Chapora estuary, station C-1, upper estuary (15° 39.072' and E 073° 47.995'), C-2, middle estuary (N 15° 38.396' and E 073° 45.848'), C-3, lower estuary (N 15° 38.137' and E 73° 45.640'), were selected and in Sal estuary, three stations namely, S-1, upper estuary (N 15° 10.095' and E 073° 56.781'), S-2, middle estuary (N 15° 10.494' and E 73° 57.502'), S-3, lower estuary (N 15° 11.842' and E 073° 58.240'), were selected (Fig. 1). Sampling was carried out during February 2016 to March 2017. Surface water samples (phytoplankton taxonomy and estimation of physico-chemical parameters) were collected during low tide at monthly intervals during pre-monsoon (February–May 2016; February–March 2017), monsoon (June–September 2016) and post-monsoon (October–December 2016; January 2017) season.

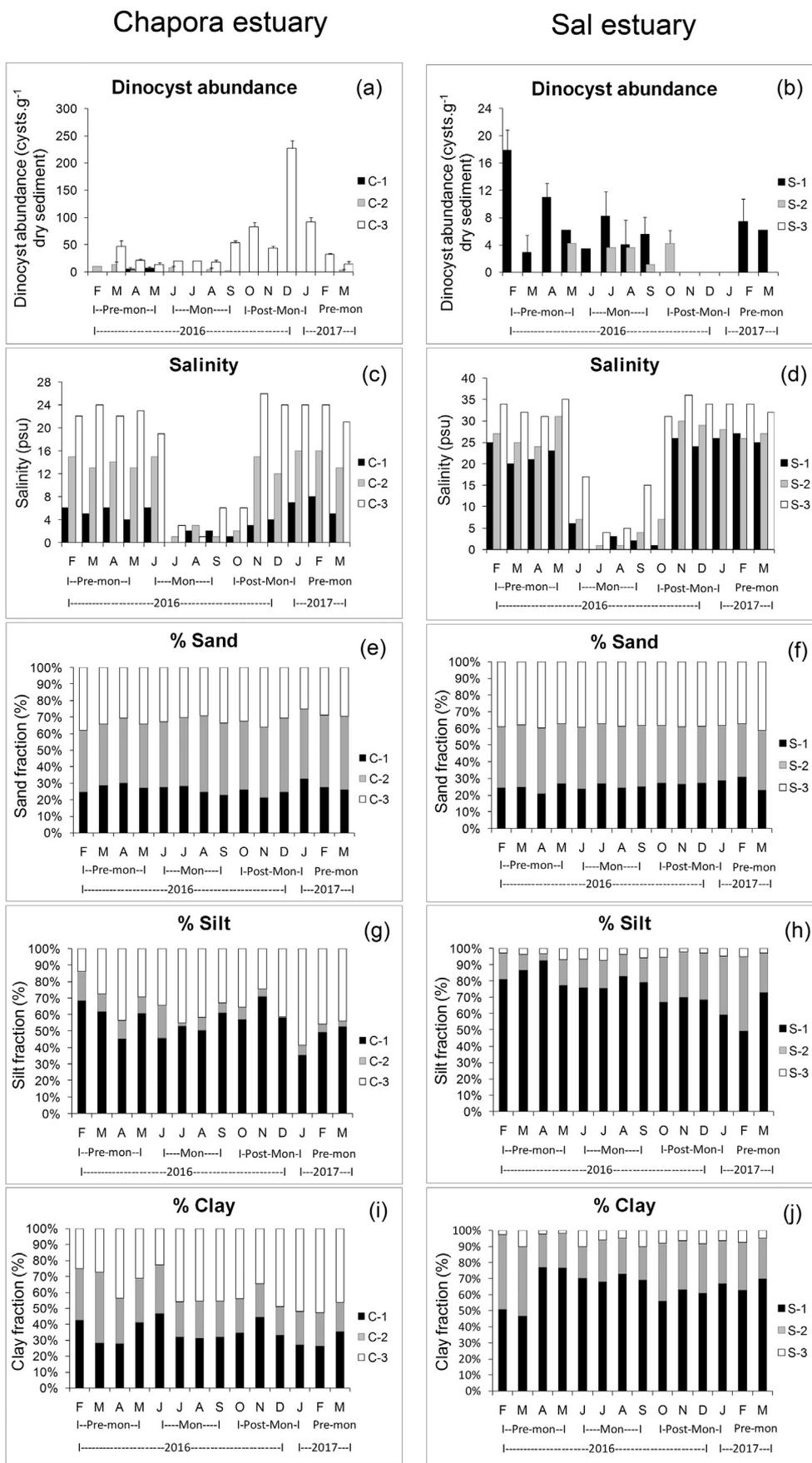


Fig. 2. Seasonal variation in (a, b) dinoflagellate cyst abundances; (c, d) salinity values and (e-j) percentage of sand, silt and clay fraction in Chapora and Sal estuary.

Table 3

Seasonal variation of dinoflagellate cysts along salinity gradient encountered in the present study along the stations in Chapora (C-1, C-2, C-3) and Sal (S-1, S-2, S-3) estuary.

Estuary	CHAPORA									SAL								
	C-1 (0–8 psu) ^b			C-2 (1–16 psu) ^b			C-3 (1–26 psu) ^b			S-1 (0–27 psu) ^b			S-2 (1–31 psu) ^b			S-3 (4–36 psu) ^b		
Season	PrM	M	PoM	PrM	M	PoM	PrM	M	PoM	PrM	M	PoM	PrM	M	PoM	PrM	M	PoM
<i>Archaeoperidinium</i> sp.	+	–	–	+	–	–	–	+	+	–	+	–	–	–	–	–	–	–
<i>Bitectatodinium spongium</i>	–	–	–	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–
<i>Gonyaulax scrippsae</i>	–	–	–	+	+	–	+	+	+	–	+	–	–	+	+	–	–	–
<i>Gonyaulax spiniferacomplex</i> ^a	+	–	–	+	+	–	+	–	+	+	+	–	–	+	+	–	–	–
<i>Polykrikos kofoidii</i>	–	–	–	+	–	–	–	+	+	+	–	–	–	–	+	–	–	–
<i>Polykrikos schwartzii</i>	–	–	–	+	–	–	–	+	+	–	+	–	–	–	–	–	–	–
<i>Protoperidinium compressum</i>	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
<i>Protoperidinium conicum</i>	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–
<i>Protoperidinium latissimum</i>	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–
<i>Protoperidinium leonis</i>	–	–	–	–	+	–	+	–	+	–	–	–	–	+	–	–	–	–
<i>Protoperidinium oblongum</i>	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
<i>Protoperidinium obtusum</i>	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
<i>Protoperidinium pentagonum</i>	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
<i>Protoperidinium subinerme</i>	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–
<i>Pyrophacus steinii</i>	+	–	–	+	–	–	+	+	+	+	+	–	+	+	–	–	–	–
<i>Spiniferites mirabilis</i>	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–

+ Present; – Absent; PrM-Pre-monsoon, M-Monsoon, PoM-Post monsoon.

^a Toxic species.

^b Values in the parenthesis indicate the salinity range at the stations.

2.5. Sediment processing and analysis

Sediment samples from the upper (0–2 cm) section of the cores were treated using the palynological method (Matsuoka and Fukuyo, 2000) with some modifications (Narale et al., 2013). Each sediment sample (3–6 g) was weighed in a beaker and washed with distilled water to remove the salt content. The samples were then sonicated and acid-digested (10% HCL and 30% HF). After rinsing the sample 3–4 times with distilled water, the acid-free sample was sieved through 100 and 10 µm mesh size to remove coarse and fine matter respectively. The residue retained on a 10 µm mesh was then transferred into a vial and a final volume was made upto 10 mL with distilled water. These vials were then stored at 4 °C in the dark for dinocyst identification.

Aliquot of 1 mL of the pre-treated sample was observed under Nikon Ti-S inverted microscope at 200×, 400× and 600× magnifications. Photographs of dinocysts were captured in Nikon imaging software (NIS elements BR) equipped with colour digital camera Ri2. Dinocysts were identified based on published descriptions (mentioned in Table 2). The dinocyst abundance was expressed as cyst.g⁻¹ dry sediment. The water content was calculated according to the formula given by Matsuoka and Fukuyo (Matsuoka and Fukuyo, 2000).

Sediment texture analysis was carried out by taking 15 g of oven dried (60 °C) sediment sample and was transferred to 1000 mL beaker containing distilled water. The sediment sample was allowed to settle for a few days, and supernatant water was decanted. This process was repeated (3–4 times). 10 mL of 10% sodium hexametaphosphate solution was then added to the sample. The following day, 5 mL of 30% hydrogen peroxide was added. Contents of the beaker were then poured over 63 µm mesh size sieve, and the filtrate was collected in a 1000 mL measuring cylinder. The contents on the mesh were washed with distilled water, till clear filtrate passed through the mesh. The volume in the cylinder was made up to 1000 mL with distilled water. Contents on the mesh were transferred to the pre-weighed beaker (sand content) and was dried in an oven (60 °C). The filtrate collected in the cylinder (for determination of clay content) was homogenized by stirring for 2 min, and was allowed to settle. 25 mL of this filtrate was pipetted out at 8 φ from a measuring cylinder at a depth of 10 cm (according to the standard table). The pipetted solution (clay content) was transferred to the pre-weighed beaker and dried (60 °C). The dried contents in the beaker were weighed and percentages of sand, silt and clay fractions

were determined.

2.6. Statistical methods

Statistical analyses were performed using PRIMER 6, CANOCO 4.5 and STATISTICA 8.0 softwares. To evaluate the relationships between the dinocyst composition and environmental variables, a constrained ordination method, Redundancy Analysis (RDA) was used. Shannon-Weiner diversity indices were computed using PRIMER 6. A Multivariate Multiple Regression (MMR) analysis was carried out with STATISTICA 8.0 statistical package at a significance level $P \leq .05$ in order to select a subset of variables having larger predictive potential.

3. Result and discussion

3.1. Seasonal distribution of dinocysts along the salinity gradient

A total of 16 dinocyst species belonging to the order, Gymnodiniales, Gonyaulacales and Peridinales were observed in both the estuaries (Plate 1). The total number of dinocysts testified along the salinity gradient in each estuaries are stated in Table 1. In Chapora estuary, among 16 dinocyst species recorded, 5 were autotrophic and 11 were heterotrophic. On the contrary, Sal estuary recorded only 11 species, wherein 4 were autotrophic and 7 were heterotrophic. Therefore, it appears that the heterotrophic dinocysts dominated the assemblage in both the estuaries (Table 2) probably in relation to monsoonal runoffs injecting nutrients and Suspended Particulate Matter (SPM) in the estuaries (Fig. 3). Our results support that reduced light intensity may explain the high abundances of heterotrophic dinocysts over autotrophic ones as observed before (Dale, 1983; Dale, 2001). Further, it is also necessary to mention that the shrimp farming activities carried out along these areas also release a significant quantity of used water with high organic load and less dissolved oxygen (Boyd, 2000; Hernandez et al., 2007) making the ecosystem more congenial for heterotrophic forms.

The observations made based on the data collected on the seasonal distribution of dinocysts suggest that there was no similarity in terms of occurrences and abundances of dinocysts at a particular season among the estuaries. However, high dinocyst abundances were noted during the post-monsoon season (Fig. 2a) in Chapora, whereas in Sal high

abundances were observed during pre-monsoon season (Fig. 2b). High dinocyst abundances in Chapora and Sal estuary during post-monsoon and pre-monsoon season were found to correlate positively with salinity, and temperature and salinity. Published studies (D'Silva et al., 2013; Satta et al., 2013; Price et al., 2016) reported salinity and temperature to be the crucial factors influencing the distribution and abundances of dinocysts. In addition, it is also probable that other factors such as tidal amplitude, turbidity and water residence time (Price et al., 2016) also might have affected the abundance of dinocysts.

The diversity and abundances of dinocysts were found to be higher in the Chapora estuary ($H' = 2.73$; 227 cysts.g⁻¹ dry sediment) than in the Sal estuary ($H' = 2.27$; 18 cysts.g⁻¹ dry sediment). High dinocyst abundances in Chapora estuary could also be attributed to the presence of fine sediments (silty-clayey), as dinocysts are known to settle out in fine silt fraction with rich organic matter in such protected areas largely due to the reduced turbulence enhancing the sedimentation rate.

Further, the size of the dinocysts is almost in similar size fraction (< 63 µm) to silts (Narale et al., 2013; Dale, 1979; Goodman, 1987). Earlier work carried out along the West coast of India (D'Silva et al., 2011) also reported positive correlation of fine silty sediment and dinocyst diversity. It is also important to note that low diversity and abundance in Sal estuary could also be due to dominance of sandy sediments as observed in the present study. The increased abundances of cysts associated with finer particles (silt/clay) observed in the present study suggest that such sediments possess high water retention capacity as compared to coarser particles (sand) and probably provide more conducive micro-niches in the surface sediments to harbor increased abundances of cyst (O'Geen, 2013).

Along the salinity gradient, stations C-3 (towards downstream) of Chapora estuary and S-1 (towards upstream) of Sal estuary where high salinity and high silt-clay loads prevail, were found to have highest dinocyst diversities (Fig. 2). Earlier studies (Shin et al., 2010; Alkawri and Ramaiah, 2010; Sildever et al., 2015; Price et al., 2016) carried out suggest that salinity is known to be a crucial parameter that affects the growth of dinoflagellates and a triggering factor in the dinocyst formation. Hence, the present study reveals that salinity and silt-clay fraction are the important parameters for the distribution of dinocysts. Based on Multivariate Multiple Regression (MMR) analysis it was noted that the silt concentration and salinity have higher predictive power on the total dinocyst abundance in Chapora estuary ($R = 0.62$, $P < .00035$). In contrast, silt concentration was found to have higher predictive power on the total dinocyst abundance in Sal estuary ($R = 0.66$, $P < .00007$) suggesting significant correlation ($P < .05$) of dinocyst abundance with silt and salinity.

3.2. Influence of physico-chemical parameters on the distribution of dinocysts

The observations made in the present study indicated that a wider salinity gradient (0–36 psu) was observed in Sal as compared to Chapora (0–26 psu). Hence both estuarine systems show different ecosystem functions with regard to effect of physico-chemical parameters and dinocyst distribution. From the Redundancy Analysis (RDA) bi-plots (Fig. 4) it appears that, apart from salinity and grain size, other physico-chemical parameters also have an impact on the distribution of dinocysts in both the estuaries. In Chapora estuary, pH, DO, SPM and phosphate concentration influenced the distribution and composition of dinocyst assemblages whereas, in Sal estuary, water temperature, pH and phosphate concentration were found to be the main influencing parameters. This is in agreement with earlier studies (Pospelova et al., 2008; D'Silva et al., 2013) reporting that dinocyst assemblages were regulated by salinity, DO, pH, nutrient concentration and water temperature coupled with sediment accumulation and encystment rates. In addition, habitat preference or competitive ability (nutrient) between the same or different set of taxa may also lead to changes in the dinocyst composition (Smayda and Reynolds, 2003).

Additionally, the diversity and abundances of dinocysts were found to be higher in the Chapora estuary as compared to the Sal estuary. The higher values can be attributed to lower levels of eutrophication, as evidenced by reduced nitrate content in the Chapora estuary (8.28 µM) as compared to the Sal estuary (22.39 µM). It is worth noting that in the Sal estuary, dinocysts were not observed during October–December 2016 and January 2017 at station S-1 (Fig. 2b). Such absence might be due to the excystment of the cysts due to favorable conditions in the overlying water column (nutrient, temperature and salinity), thus controlling the distribution of both dinoflagellates and the dinocysts (Alkawri and Ramaiah, 2010). However, two species, namely *Gonyaulax scrippsae* and *Gonyaulax spinifera*, were observed over large salinity and temperature ranges in both estuaries (Table 3.). An experimental study carried out by Hand et al. (1965) reported similar results regarding their euryhaline and eurythermal nature.

3.3. Nutrient limitation as a triggering factor for dinocyst formation

Nitrate is known as an important nutrient in the distribution of phytoplankton (Xu et al., 2010). In contrast, resting cysts result from a fate of sexuality (Anderson and Wall, 1978) formed as a response to stress or unfavorable conditions (Bravo and Figueroa, 2014). Nutrient limitation (nitrate) is one of the common limiting factors that triggers cyst formation (Rengefors and Anderson, 1998; Figueroa et al., 2006). Experimental studies similarly concluded that nitrate depletion was the most common and successful method to induce sexuality and, thereof, the formation of resting cysts in cultures (Pfiester and Anderson, 1987; McQuoid and Hobson, 2008). In the present study, high dinocyst abundances were observed in Chapora estuary congruent to low nitrate content while low dinocyst abundances in Sal estuary concurred with high nitrate content. This indicates that low nitrate concentration in Chapora estuary resulted in high-stress environmental conditions eventually leading to encystment of dinoflagellates. However, in Sal estuary, comparatively higher nitrate concentration was recorded, probably yielding a favorable habitat for dinoflagellates. Further, it is known that germination occurs when favorable environmental conditions persist (Bravo and Figueroa, 2014) possibly explaining the lower dinocyst abundances in the Sal estuary. It is imperative that encystment is enhanced during nutrient depletion (Anderson et al., 1984; Ellegaard et al., 1998), as indicated earlier that high abundance of dinocysts occurs at low nitrate concentrations (Marret and Zonneveld, 2003). Thus in the present study, nutrient dynamics especially nitrates in the overlying waters could be one of the potential reasons to enable encystment leading to seasonal bloom formation (Anderson et al., 1987; Figueroa et al., 2005).

In the present study, based on RDA bi-plots an inverse relationship between *G. spinifera* and nitrate concentration was noted (Fig. 4). *Gonyaulax spinifera* is known to be a bloom forming and a prolific yesso-toxin producer (Rhodes et al., 2006), and was here reported in both estuaries. The inverse relationship of this species with nitrate concentration indicates that high nitrate input in these estuaries might lead to encystment, eventually leading to HAB. In view of the observations made in the present study, the presence of toxic species in these estuarine areas is a matter of great concern. Indeed, once established in such tropical habitats there is a strong possibility of repeated occurrences of such blooms that are largely responsible for water quality deterioration, with its detrimental effect on the biodiversity and ecosystem function (Riccardi et al., 2009; Mudadu et al., 2017). This situation is further synergized by the anthropogenic loading in extremely vulnerable habitats largely influenced by the land use pattern (human settlement, tourism, sewage disposal, agricultural runoff, shrimp farming ponds) in the coastal habitats (personal communication). Hence, regular monitoring is necessary to assess the health status of such fragile ecosystems.

4. Conclusions

The observations made in the present study reveal that total dinocyst abundance was affected by the variations in the physico-chemical parameters with significant influence of salinity and sediment grain size. An assessment of occurrence of dinocysts within the estuaries with regard to salinity gradient suggested that the risk of forming such blooms is high with consistently in high-saline waters with silt-clay sediment texture. The dominance of heterotrophic dinocysts over autotrophic dinocysts probably resulted from high input of nutrients (nitrate) and suspended organic material from adjacent zones. Nutrient limitation (nitrate) is noted to be a triggering factor for dinocyst formation. Additionally, RDA bi-plots revealed that nitrate input might be a probable cause for the excystment of the toxic species, *Gonyaulax spinifera*. These tropical habitats support shellfish and other fishing activity along these estuaries. Hence there is a potential risk of such toxin producing species to affect the marine ecosystems as well as local population health.

Declaration of Competing Interest

The authors wish to state that there is no conflict of interest.

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