Dynamics of phytoplankton marker pigments in different aquatic ecosystems

A Thesis submitted to Goa University for the award of the Degree of

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in

Marine Sciences

By

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DECLARATION

I, Sathish K hereby declare that this thesis entitled "**Dynamics of phytoplankton marker pigments in different aquatic ecosystems**" represents work which has been carried out by me and that it has not been submitted, either in part or full, to any other University or Institution for the award of any research degree.

Place: Dona Paula
Date: Sathish K

CERTIFICATE

I hereby certify that the above Declaration of candidate, Sathish K is true and the work was carried out under my supervision.

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To the memory of Maha

SATHISH K

Dedicated to my mother

I am harvesting the seeds of hard work you planted, mom

Contents

Abbreviations

19’ BF: 19-Butanoyloxyfucoxanthin

19’ HF: 19-Hexanoyloxyfucoxanthin

AC: Approach channel

AD: Ambetkar dock

Allo: Alloxanthin

BB: Boat basin

BDE: Bharathi dock east

BDW: Bharathi dock west

BS: Berth stations

Chl *a*: Chlorophyll *a*

Chl *b*: Chlorophyll *b*

Chl c: Chlorophyll *b*

ChlB: Chlorophyll breakdown

CS: Creek stations

Diad: Diadinoxanthin

Diat: Diatoxanthin

DIN: Dissolved inorganic nutrients

DO: Dissolved oxygen

DS: Downstream

EC: Ernakulum channel

Fuco: Fucoxanthin

IP: Inner port

JD: Jawakar dock

KOPD: Kidderpore docks

LME: Lower middle estuary

Lut: Lutein

MC: Matancherry channel

MP: Middle port

NEM: North-east monsoon

Neo: Neoxanthin

NSD: Netaji subhash dock

OJS: Oil jetty stations

OP: Outer port

Peri: Peridinin

PFG: Phytoplankton functional group

Phe *a*: Pheophytin *a*

Pheid *a*: Pheophorbide *a*

PM: Post-monsoon

PMP: Phytoplankton marker pigments

Pras: Prasinoxanthin

PrM: Pre-monsoon

PS: Port sations

PSU: Practical salinity unit

RS: Riverine stations

SWM: SWM

TOC: Total organic carbon

UME: Upper middle estuary

US: Upstream

Viola: Violaxanthin

Zea: Zeaxanthin

β-car: β-carotene

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Chapter 1

General introduction

1. General introduction

Phytoplankton are unicellular microscopic algae that make up the floating pastures of the aquatic system (phyton-plant, planktos-wandering). Phytoplankton provides the food base, which supports, either directly or indirectly, to the entire animal population of the marine ecosystem, and they also contribute significantly to climatic processes. Phytoplankton are represented by considerable diversity of algal groups (diatoms, dinoflagellates, phytoflagellates, coccolithophorids, red algae, green algae, and cyanobacteria) with size ranging from 0.2 microns to several millimeters. All phytoplankton, by their photosynthetic activities, are a significant contributor to global carbon fluxes (Falkowski et al., 1998) and its size structure, species composition and temporal dynamics are influential determinants of tropical interaction in the marine ecosystem (Tester et al., 1995). Further, the variability in the phytoplankton groups reflects the rate of eutrophication, biogeochemical cycling, and potential harmful algal bloom (HAB) formations (Falkowski, 1994, Heisler et al., 2008, Cloern & Jassby 2010, Narale et al., 2013). Hence monitoring phytoplankton biomass and composition through various approaches, ranging from microscopic to remote sensing, have become high priority areas for aquatic research.

Identification and enumeration of phytoplankton community structure are usually done through the microscopic observation. Microscopic examinations, which are commonly limited to micro- and nano-plankton, are time-consuming and require a high level of taxonomic skill and expertise. There is also a possibility of missing out, a smaller group of phytoplankton, such as picoplankton during the observation (Schlüter et al., 2000, Lassen et al., 2010). Contrarily phytoplankton marker pigments (PMP) such as chlorophyll (chl) and carotenoid pigments, either singly or in combination, can effortlessly be studied to know the phytoplankton community and their physiological status. The use of high-performance liquid chromatography (HPLC) is also considered to be a more powerful tool for

classification and monitoring the phytoplankton community based on pigments (Wright and Jeffrey, 2006) as listed in Table 1.1.

Table 1.1 Chemotaxonomic relation used in the study of phytoplankton taxonomy (Gibb et al., 2001, Jeffrey et al., 1997). The relative degree of chemical stability is ranked from most (1) to least (4) stable, from Leavitt & Hodgson (2001).

Pigment	Affinity (major groups or process)	Stability
Chlorophylls		
Chl a	All photosynthetic algae, higher plants	3
Chl b	Green algae, euglenophytes, higher plants	2
Chl c family	Dinoflagellates, Diatoms, Chrysophytes	4
Carotenoids		
β-carotene	Most algae and plants	1
α-carotene	Cryptophytes, prochlorophytes, rhodophytes	2
Alloxanthin	Cryptophytes	1
Fucoxanthin	Diatoms, prymnesiophytes, chrysophytes, raphidophytes, several dinoflagellates	2
Diadinoxanthin	Diatoms, dinoflagellates, prymnesiophytes, chrysophytes, raphidophytes, euglenophytes, cryptophytes	3
19-Butanoyloxyfucoxanthin	Chrysophytes, prymnesiophytes	
Divinyl chlorophyll a	Prochlorococcus sp.	
19-Hexanoyloxyfucoxanthin	Prymnesiophytes	
Diatoxanthin	Diatoms, dinoflagellates, chrysophytes	2
Peridinin	Dinoflagellates	4
Zeaxanthin	Cyanobacteria, prochlorophytes, rhodophytes, chlorophytes	2
Canthaxanthin	colonial cyanobacteria	1
Myxoxanthophyll	colonial cyanobacteria	1
Echinenone	Cyanobacteria	1
Lutein	Green algae, euglenophytes, higher plants	1
Neoxanthin	Green algae, Euglenophytes, higher plants	4
Violaxanthin	Green algae, Euglenophytes, higher plants	4
Okenone	Purple sulphur bacteria	1
Prasinoxanthin	Prasinophytes	
Isorenieratene	Green sulphur bacteria	1
Chlorophyll degradation products		
Pheophytin a	Chl a derivative (general)	1
Pheophytin b	Chl b derivative (general)	2
Pheophorbide a	Grazing, senescent diatoms	3
Pyro-pheo(pigments)	derivatives of a and b-phorbins	1

Chlorophyll pigments, present in all phototropic organisms, are responsible for the capture and transfer of solar energy during photosynthesis and photoprotection (Porra et al., 1997, Hall et al., 1999, Graff and Rynearson., 2011). Apart from this pigment, photosynthetic organisms have accessory pigments (carotenoids) that enable them to survive under extreme light conditions. The accessory pigments which are involved in the energy transfer mechanism to reaction center during photosynthesis (e.g., fucoxanthin, 19-butanoyloxyfucoxanthin, 19-hexanoyloxyfucoxanthin,

peridinin and phycobilins) are called photosynthetic carotenoids. In contrast, the accessory pigments which helps to prevent chloroplast damage due to excess light energy (e.g., alloxanthin, diadinoxanthin, diatoxanthin, lutein, zeaxanthin, violaxanthin, and β-carotene) are called photoprotective carotenoids (Eisner et al., 2003). Presence of such accessory pigments are group-specific, and hence they can be used as biomarkers to characterize planktonic and benthic microalgal community (Brotas and Plante-Cunny 1998, Buffan-Dubau and Carman 2000, Ansotegui et al., 2001, Bianchi et al., 2002, Brotas et al., 2007, Schüller et al., 2013).

However, in an aquatic ecosystem, phytoplankton undergoes several loss processes such as grazing, program cell death, viral lysis, sinking, etc (e.g., Choi et al., 2017). In such cases, phytoplankton marker pigments (PMP), in particular, chlorophyll a (chl *a*) and its derivatives, which reflect the phytoplankton production and its pathway in the water column and sediments considered as useful indicators. Chlorophylls and pheopigments are commonly used as indicators of relatively fresh and degraded microalgal communities, respectively. (Boon et al.,1998, Wieking and Kröncke 2005, Pusceddu et al., 2009). Growth and loss processes (eg. grazing, cell sinking and senescence, photodegradation, fecal pellet sinking, physical mixing, and advective transport are known to affect chl *a* and pheopigments concentrations in the euphotic zone. However, different pathways of pigment degradation affect the integrity of chlorophyll, i.e., Mg-phorbin ring, the phytol chain, or both, and result in different degradation products such as pheophorbide (Mg-free and phytol-free chlorophyll), chlorophyllide (phytol-free chlorophyll), and pheophytin (Mg-free chlorophyll) (Fig. 1, Morata, 2011). The removal of the central magnesium atom and phytol chain from chl *a* are the early steps in chl *a* breakdown for all chlorophyll-containing organisms (Satoh and Hama 2013, Hu et al., 2015, Kuai et al., 2018 and references therein, Fig. 1.1). To the best of our knowledge, more in-depth information on chl *a* breakdown is available for leaf senescence in land plants in comparison to the

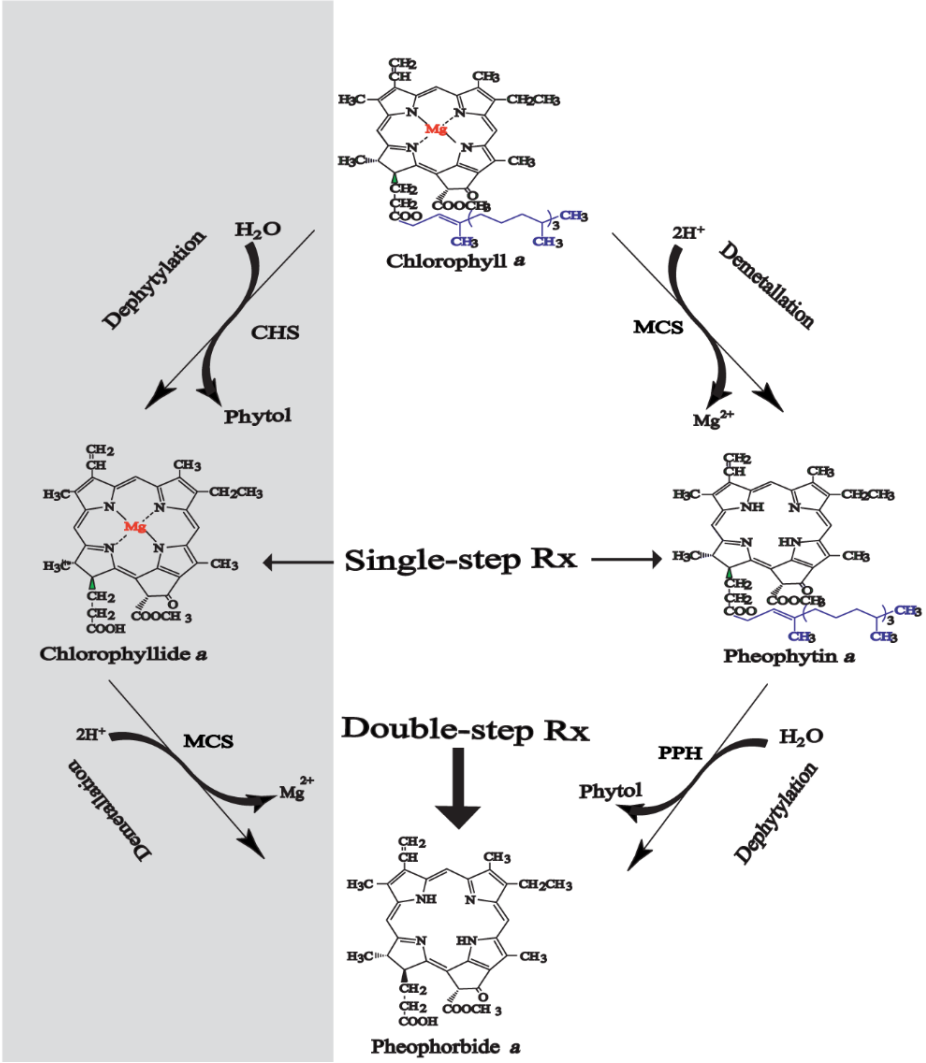

Fig 1.1 Schematic of chlorophyll breakdown pathway based on the literature (modified from Satoh and Hama, 2013).

situation for algae (Scheer 2012). Generally, pigment degradation triggered by the water column process such as grazing, cell senescence, photo-oxidation, microbial decay, and cell lysis and secondly, at the sediment-water interface (Reuss and Conley 2005 and reference therein). These processes preferentially degrade the pigments before their incorporation into sediments (Hurley and Armstrong 1991). Traditionally, the sinking of ungrazed phytoplankton or bloom to the bottom is also considered as one of the major loss factor (Calbet and Landry 2004, Choi et al., 2017). Hence

assessing marker pigments from sediments and the overlying waters in a given ecosystem will enhance the understanding of the linkages between the pelagic and benthic phytoplankton (or benthic-pelagic coupling), which is an essential process in the functioning of the ecosystem. However, research in this area is in infancy. Nevertheless, the rates of these processes are of broad general interest, and knowledge of them is fundamental to understanding the causative factors underlying the dynamics of planktonic ecosystems.

Literature suggests that the phytoplankton pigments are extensively used in the studies related to (i) photophysiology (photoprotection, photoinhibition, photoacclimation, Roy et al. 2011) (ii) dynamics of phytoplankton distribution and composition in relation to environment (Ahel et al., 1996, Ston 2002, Lohrenz et al., 2003, Dyble and Moisander 2003, Henriksen et al., 2002, Chakraborty et al., 2011, Roy et al. 2011) (iii) fate of phytoplankton under different environmental settings (Josefson and Hansen 2003, Morata et al., 2011) (iv) phytoplankton pelagic-benthic coupling (Gall and Blanchard 1995, Morata and Renaud 2008, Reuss et al., 2010, Freiberga et al., 2012) (v) palaeo-climate and palaeo-environment (Hodgson et al., 1997, Soma et al., 2001, Reuss et al., 2010) and (vi) ocean color remote sensing (Sathyendranath et al., 2004, Claustre et al., 2004, Brewin et al., 2010, Torrecilla et al., 2011, Torres-Pérez et al., 2015). In India, the work on phytoplankton marker pigments is mostly focused on the variations in the phytoplankton groups (i.e., chemotaxonomy) but restricted to few regions (Latasa and Bidigare 1998, Barlow et al., 1999, Roy et al. 2006, Parab et al. 2006, Naik et al. 2010, Aneesh Kumar and Sujatha 2012, Parab et al., 2013, Madhu et al., 2014, Roy et al., 2015). The chemotaxonomy of phytoplankton has also gained importance for remote sensing applications (Latha et al., 2014, Nagamani et al., 2017). Though some microscopic studies indicated the persistence of benthic-pelagic coupling in bloom-forming diatoms (Patil and Anil 2008) and diatom viability in sediments for several months (Anil et al., 2007) from a monsoon-influenced region but in-depth

information on phytoplankton distribution, its fate, and phytoplankton benthic-pelagic coupling are lacking. In light of above following objectives are addressed.

- Distribution of phytoplankton marker pigments in different ecosystems
- Phytoplankton marker pigment to study the fate of phytoplankton in different environmental settings
- Phytoplankton marker pigments to study benthic-pelagic linkage

India has a vast coastline with different ecosystem types (e.g., freshwater, estuaries, coastal) and so with the environmental settings in each of the ecosystems. The influence of reversible monsoons (SWM – both east and west coast and NEM – east coast), high freshwater discharges on the east-coast compared to the west-coast, different coastal morphology, and variable anthropogenic pressures are some of the events determining the environment setting in a given ecosystem along the Indian coasts. Therefore, it has been hypothesized that the phytoplankton will have a specific distribution pattern for different ecosystems, and therefore indicating fate pathways. Evaluating biomass, composition (using marker pigments), and chlorophyll degraded pigments together will provide insights into the processes involved in growth and mortality. Given this, under the Ballast Water Management Program-India, the distribution patterns of the biomass and CDP from eight-port ecosystems (categorized into freshwater, estuarine and marine port ecosystems) and adjacent water (Zuari estuary) located along the east and west coast of India are discussed from the perspective of ecosystem assessment and management.

Chapter 2

Materials & methods

2. Materials and Methods

2.1 Description of the study area

Samples were collected from 8 Port ecosystems and Zuari estuarine ecosystem, located at different locations along the east and west coast of India. The selected Ports are listed among the Major Ports of India as they handled a greater proportion of all cargo traffic. The Indian coast is influenced by monsoon reversals, a varying magnitude of freshwater influx (from major and minor rivers), and anthropogenic pressures (in particular Ports).

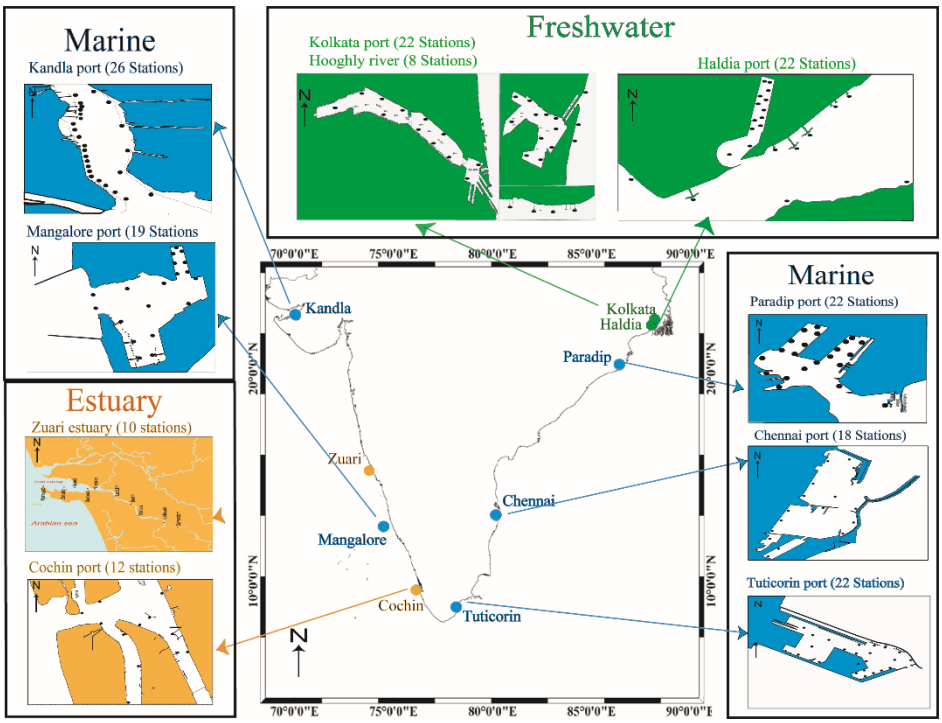

Fig. 2.1 Geographical location of the eight Ports and Zuari estuary (and the sampling stations) in different ecosystems (blue–marine, green–freshwater, and orange-estuary) along the Indian Coast. Among the eight different ecosystems, two (Kolkata and Haldia), One (Cochin), and five Ports (Kandla, Mangalore, Paradip, Chennai, and V. O. Chidhambaranar) are identified as major ports of India

freshwater, estuarine and marine Port osystems respectively. Additionally, a case study was carried out from the salinity gradient of a monsoonal estuarine system on a monthly basis, i.e., Zuari estuary (Fig 2.1).

2.1.1 Freshwater Port ecosystems

2.1.1.1 Kolkata Port

Kolkata Port is one of the major riverine Port, situated (22° 32' N, 88° 18' E) in the state of West Bengal, on the east coast of India. Kolkata Port is located on the left bank of Hooghly River and 203 km away from the mouth of the estuary. Hooghly River is a deltaic offshoot of the River Ganges (Qasim, 2003). River Ganges is one of the largest rivers in the world and the most important river system in India. This river system flows through the upland stream, warm water, swampy, and deltaic habitats during its run from the upper Himalayas to the Bay of Bengal (Bhaumik and Sharma, 2017). After traveling around 2000 km, Ganges River bisects near Rajmahal at Farakka. Hooghly is the major distributary of the Ganges River and flows towards the south and empties into the Bay of Bengal (Samanta et al., 2017). The lower reaches of the River fed by Ajay, Damodar, Rupnarayan, and Haldi (Kasai) rivers. Hooghly river channel has two major national Ports, viz., Kolkata Dock System (KDS) and Haldia Dock Complex (HDC). Hooghly River serves as a navigational channel for Kolkata and Haldia Ports. High rates of sedimentation load in this channel gives severe navigational issues for the ship traffic (Rose et al., 2015) next to the difficult bars and bends. Navigation is facilitated with the performance maintenance dredging by the Dredging Corporation of India (DCI) for every six months (Bhaskaran et al., 2014). Kolkata Port consists of two different docks and Budge Budge oil jetties, i.e., Kidderpore docks (KOPT-I and KOPT-II) and Netaji Subhas Dock (NSD). Kidderpur docks contain 18 berths and 3 dry docks with the length of the docks varying from 118 to 229 m. NSD docks contain 10 berths and two dry docks with the maximum and minimum berth length of 150 to 200 m, respectively. Budge Budge is located about 25km downstream of Kolkata and its River moorings have 6 petroleum wharves with the length between 102 to 189 m. Principle commodities

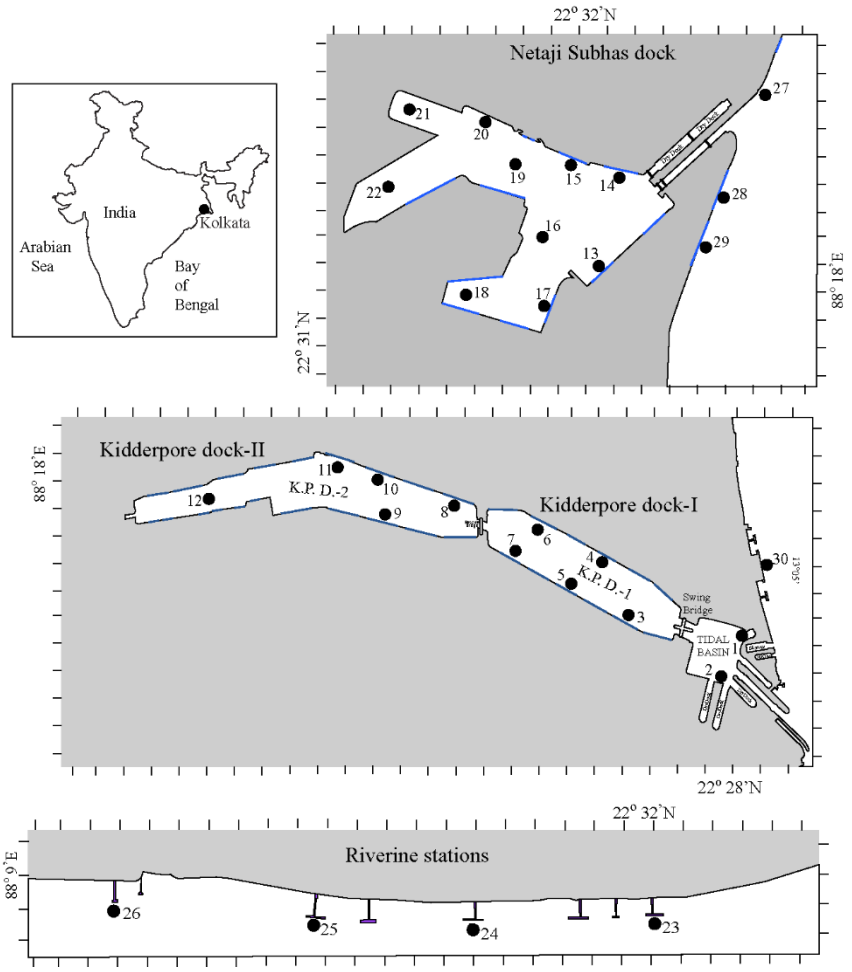

Fig. 2.2 Location of sampling stations in Kolkata Port, east coast of India.

handled by this Port are chemicals, crude oil, petroleum products, automobiles, general cargo, coal, timber, containers and pulses (http://www.kolkataPorttrust.gov.in). Sampling was carried from 22 dock stations and 8 riverine stations on 4 different occasions representing different seasons for the period from 2013-2015 [September 2013 (SWM), February 2014 (PrM 1), January 2015 (PrM 2) and December 2015 (PM)].

Hooghly River is a tidal river (Chugh, 1961) and well mixed because of its shallow depth (average ~6 m) and drains of around 6×10^4 km^2 catchment area. The tides in this region are semidiurnal, and the tidal variations at the mouth are 5.5 m during spring, and 1.8 m during neap tide (Corsolini

et al., 2012) and the saline water intrusion limited up to 70 km from the mouth (Sadhuram et al., 2005). This area experiences three different seasons, SWM (June–September), PM (October–January), and PM (February–May). The average values of freshwater discharge from the are 3000 m^3 s^{-1} and 1000 m^3 s^{-1} during SWM (June–September) and dry season (October to May) respectively (Sadhuram et al., 2005). Kolkata region experiences a tropical savannah climate with the hot and dry season with the temperature between 28 to 38°C, (March to June) and warm and wet season (mid-June to September) and a cold season with the lowest temperature ranged from 9 to 16 ºC (IMD).

2.1.1.2 Haldia Port

Haldia Port (Haldia Dock Complex) is the subsidiary Port of Kolkata situated (22° 02' N, 88° 06' E) on the west bank of Hooghly River. Haldia is a major port located in the industrial township of West Bengal, India, and located about 50 km southwest of Kolkata. Haldia Port was initially built to reduce the overflowing load of Kolkata Port. Haldia Port has 15 berths with the depths from 8.5 to 11 meters. The total length of the berths is about 3.3 km. The berths include three oil jetties, four berths for coking coal, two berths for breakbulk cargoes, two berths for liquid bulk cargoes, one berth for limestone, one berth for containers, one berth for coal, and one berth for both coal and general cargo. Major organic and inorganic materials handled by the Haldia Port are fertilizers, food grain, sugar, paper and newsprint, coal, petroleum and metallurgical coke, soda ash, iron and steel, limestone, machinery, scrap, vegetables, food grains, thermal coal, calcined petroleum coke, jute products, iron, steel, tea, metal products, machinery, mica, and other general cargoes (Source: http://www.worldPortsource.com/). Sampling was carried out in Haldia Port from 22 stations on 4 different occasions representing different seasons for the period from 2013-2015 [October 2013

(PM), February 2014(PrM 1), September 2015 (SWM) and April 2015 (PrM 2)]. Based on the location stations were demarcated as Port stations (S1 to S13) and riverine stations (S14 to S22).

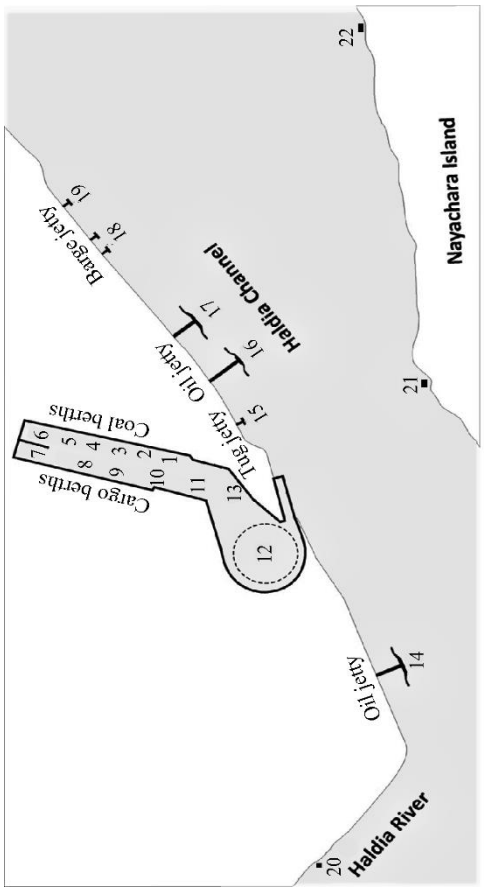

Fig. 2.3 Location of sampling stations in Haldia Port, east coast of India.

Tides in this region are semi-diurnal in nature with the occurrence of two high and two low tides every day and the inclusion of complex riverine morphology (Rose et al., 2015). Haldia climate is tropical savannah climate, with chilly winter (7 °C to 22°C) and hot summer (24 °C to 39 °C). Rainfall is heavy during SWM, and the rainy months are between May to September. The study region experiences the least amount of rainfall during December with an average of 1 mm per month. Maximum precipitation occurs in September with an average of 326 mm (Source:

http://haldiatourism.gov.in/haldia.html). During study period Haldia port experienced precipitation during the sampling period except PrM 2 (IMD).

2.1.2 Estuarine Port ecosystems

2.1.2.1 Cochin Port

Cochin Port is an all-weather Port located on the Willingdon Island, Kerala state, along the South-west coast of India (9° 58' N, 76° 14' E). Cochin Port is a natural harbor located on the bar built estuary, which is formed by six rivers (Periyar, Muvattupuzha, Pamba, Achencovil, Manimala, Meenachil). The convergence of the Cochin backwater has two permanent openings to the Arabian Sea. One is at the Cochin Port and another one is at Azhikode, where the estuary is flushed and seawater intrudes during ebb and flood tides, respectively. The Channel depth of the Cochin Port is maintained between 10 to 13 m with the help of continuous dredging whereas the depth in the estuary lies between 2 to 5 m (Qasim, 2003, Jyothibabu et al., 2006). Sampling was carried out at 12 stations located in different channels such as Approach channel (AC; S1 and S5), Mattancherry channel (MC; S2 to S4) and Ernakulum Channel (EC; S6 to S12). The Ernakulum Channel is 4.90 Km long, and the varying width between 250 to 500 m. The Mattancherry channel is 4.08 Km long, with the width varying from 180 to 250 m. Altogether, 16 berths are available in the Cochin Port with the length between 170 to 370 m (https://cochinPort.gov.in/berth-information). Cochin backwaters are potential fishing areas and used for goods transportation, industrial and domestic wastewater discharge. This port mainly deals with a lot of organic and inorganic products such as oil refineries, fertilizer plants and chemical industries. Major pollutants from these industrial products are (acids, alkalis, suspended solids, fluorides, free ammonium, insecticides, dyes, trace and heavy metals and radioactive nuclei) polluted the environment in this Port area (Menon et al., 2000; Martin et al., 2012; Anu et al., 2014).

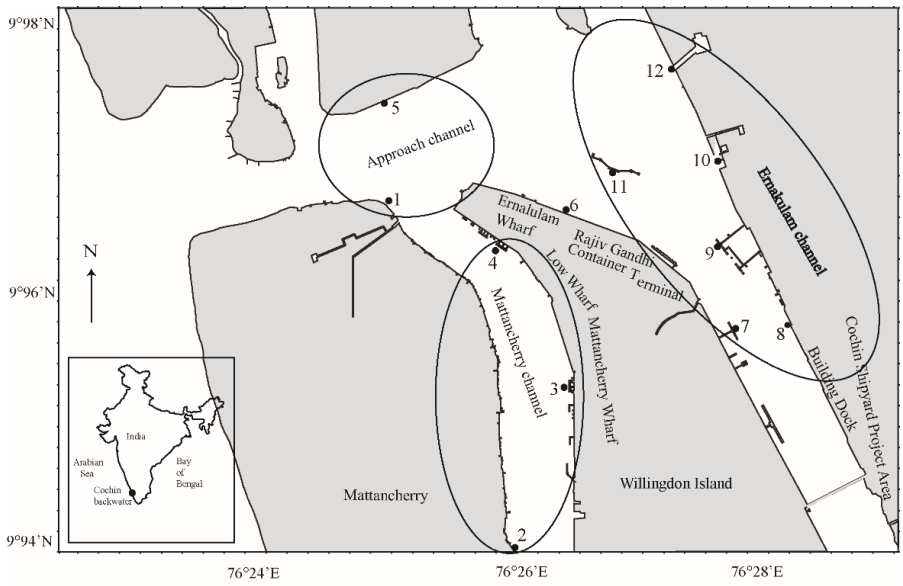

Fig. 2.4 Location of sampling stations in Cochin Port, west coast of India.

Seasonal pattern in the Cochin Port characterized as SWM (June-September), PM (October-January), and PrM (February-May). Annual air temperature varying from 20 °C to 35 °C with the maximum during PrM season. The study region receives an average annual rainfall of 3500 mm, most of about 55 to 65% contributed by the SWM season. As a result of heavy precipitation and a large amount of freshwater discharge (~3500 m^3 s^{-1}) during peak monsoon, results in a salt-wedge condition in the Cochin backwater and surface salinity over the large extent reaches near zero. During PrM (November to May), it changes to partially mixed condition whereas, during June, a large Portion of Cochin backwater is moderately stratified and partially mixed (Menon et al., 2000). Tides in this port area are mixed semidiurnal with the flood and ebb tide variation of 1m (Qasim, 2003).

2.1.2.2 Zuari estuary

The Zuari River is a major River in Goa located along the west coast of India. This river originates at Hemad-Barshen in the Western Ghats and flows for about 65 km and empties into the Arabian

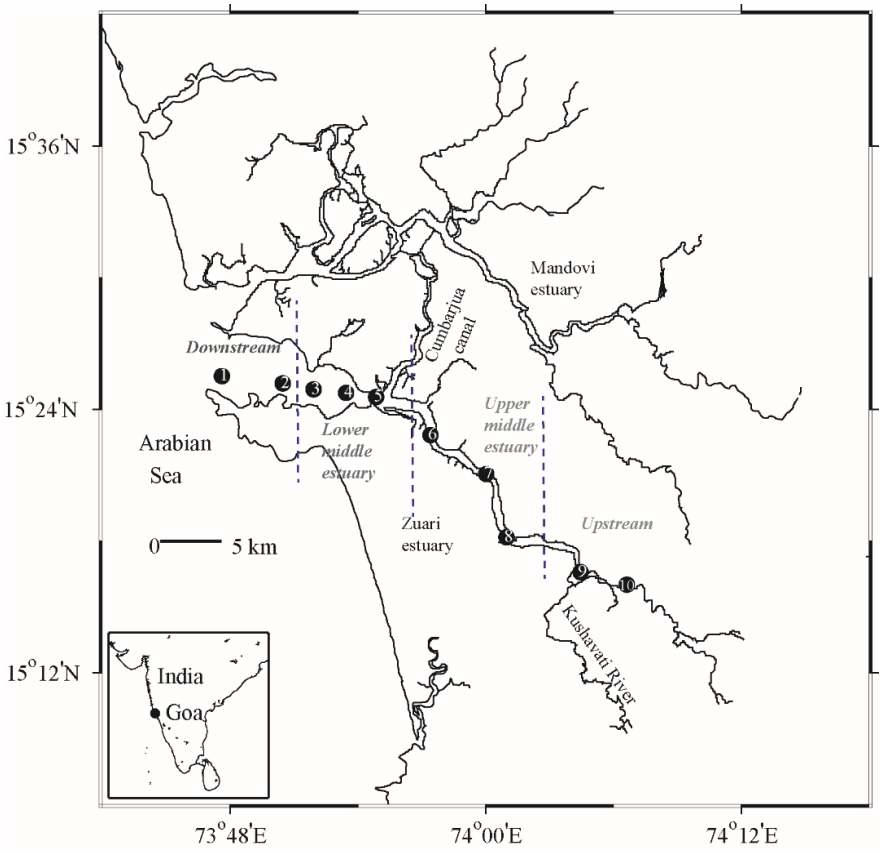

Fig. 2.5 Location of sampling stations in Zuari estuary, west coast of India

Sea. The maximum and minimum width of the estuary is varying from 5km to 0.050 km in the moth and the head, respectively. The average depth of the estuary is ~5 m, and the total catchment area was calculated (by using Globe Digital Elevation Model (DEM)) is about 1152 km^2. There are many rivers and streams drains into the estuary upstream that are the major source of freshwater influx to this estuary (Sundar and Shetye 2005). Based on the physicochemical characteristics, Zuari estuary experiences three different seasons, i.e., June to September-SWM; October to January –PM season and February to May- PrM season. Zuari estuary classified as a monsoonal estuary, which receives a large volume of freshwater influx during SWM and negligible during other seasons (Vijith et al., 2009). River discharge during SWM crossover 400 $m^3 s^{-1}$ while the other season it is around < 10 $m^3 s^{-1}$ (Shetye and Murty, 1987, Vijith et al. 2009). Zuari is a

mesotidal estuary, with the highest high and lowest low tide range between 2.3 m and 1.5 m respectively (Shetye et al., 2007; Manoj and Unnikrishnan, 2009). Tides are mixed semi-diurnal (Sundar and Shetye, 2005) and have a massive effect on the mouth compared to the upstream end of the estuary (Shetye et al., 2007). The large quantity of freshwater influx during SWM induce the drastic changes in physicochemical characteristics of the water column, whereas in the rest of the year, tidal variations dominate (Qasim and Sen Gupta, 1981)..

2.1.3 Marine Port Ecosystems (MPE)

2.1.3.1 Kandla Port

Kandla Port is situated (23° 01' N; 70° 13' E) along the western bank of Kandla creek and is approximately 90 km from the Gulf of Kutch (GoK). Kandla creek is one of the major tributaries of this creek system which is supplying water into the inner GoK and located on the North-west coast of India (Shirothkar et al., 2010). Kandla creek provides around 23 km navigable channel with the width varies from 200 to 1000m, and an average depth of 10 m (Sasikumar., 2003). Kandla Port has the least effect of strong monsoonal winds and high waves and serves as an all-weather Port. The current velocities in this area ranged between 0.08 ms^{-1} to 1.76 ms^{-1} and 0.09 ms^{-1} to 2.0 ms^{-1} during flood and ebb tide, respectively (Sinha et al., 2006). The strong ebb tidal currents translocate the Port water with the contaminants into the inner GoK (Shirothkar et al., 2010). Kandla Port currently has six oil jetties and 14 multi-purpose berths with the lengths between 182 to 300 m. Significant commodities handled by this Port are POL (petroleum, oil and lubricant products), industrial chemicals, fertilizers, edible oil, sugar, timber logs, iron and steel, food grains, metals, mineral ore, etc. (Shirodkar et al., 2010). This Port region experiences three seasons, pre-monsoon (PrM; February to May), southwest monsoon (SWM; June to September) and post-monsoon (PM; October to January). Sampling was carried out from 25 stations during four

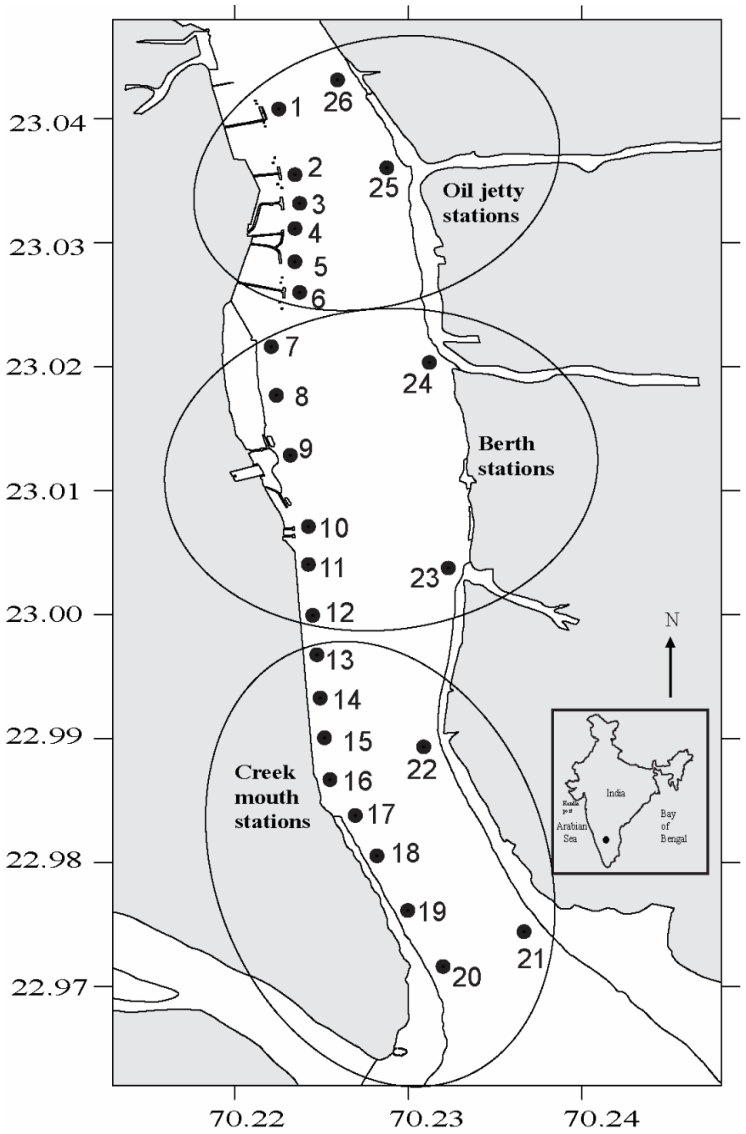

Fig. 2.6 Location of sampling stations in Kandla Port, west coast of India.

different occasions representing different seasons for the period from 2014 to 2016 [October 2014 (PM 1), July 2015 (SWM), October 2015 (PM 2), and February 2016 (PrM)]. Based on the location, stations were demarcated as Oil Jetty Stations (OJS; S1 to S6, S25, and S26), Berth stations (BS; S7 to S12, S23, and S24) and creek mouth stations (CMS; S13 to S22). Tides in this region are macro tides with the tidal variation of 0.83 to 7.2 m. Precipitation in this region is low. The annual average rainfall is about 322 m of which 90 % received during the SWM season with a maximum of 153 mm in July. Study area experienced heavy rainfall during SWM during this

sampling period (IMD). April and May are arid months with average rainfall less than 0.6 mm per month.

2.1.3.2 New Mangalore Port

New Mangalore Port is situated at Panambur, Mangalore (12° 57' N; 74° 48' E) on the west coast of India. This is a modern all-weather Port and an artificial lagoon type Port that can be reached by a 7.5 km channel and is protected by two breakwaters with a length of 770 m each. This Port contains 3.5 km of berthing space and 14 berths with a length variation of 125 m to 350 m. The boundaries of this Port area spread over 899.93 Acres. The significant commodities handled by this Port is Iron Ore Concentrates & Pellets, Iron Ore Fines, POL Products, granite stones, containerized cargo, Crude and POL products, LPG, coal, limestone, timber logs, finished fertilizers, liquid ammonia, phosphoric acid, other liquid chemicals, containerized cargo, etc. (www.newmangalore-Port.com/). Mangalore has tropical monsoon climates with the hot summer and pleasant winter. The air temperature lies between 20°C to 38°C (IMD). This Port region experiences three seasons, PrM (February to May), SWM (June to September), and PM (October to January). Sampling was carried out on four different occasions coinciding with different seasons for the period from 2011-2012, [November 2011 (PM 1), May 2012 (PrM), September 2012 (SWM), and December 2012 (PM 2)]. Samples were collected from 19 stations. Based on the geographic location stations were grouped as inner stations (IS; S1 to S9), middle stations (MS; S15 to S19), and outer stations (OS; S10 to S14). Annual rainfall in this region is 3,479 mm and maximum during SWM (~95%). PrM and PM seasons experience extremely dry conditions (IMD). Tides in this region are semidiurnal with a tidal amplitude of 0.03 m to 1.68 m and 0.26 m to 1.26 m during a spring tide (ST) and neap tide (NT) respectively.

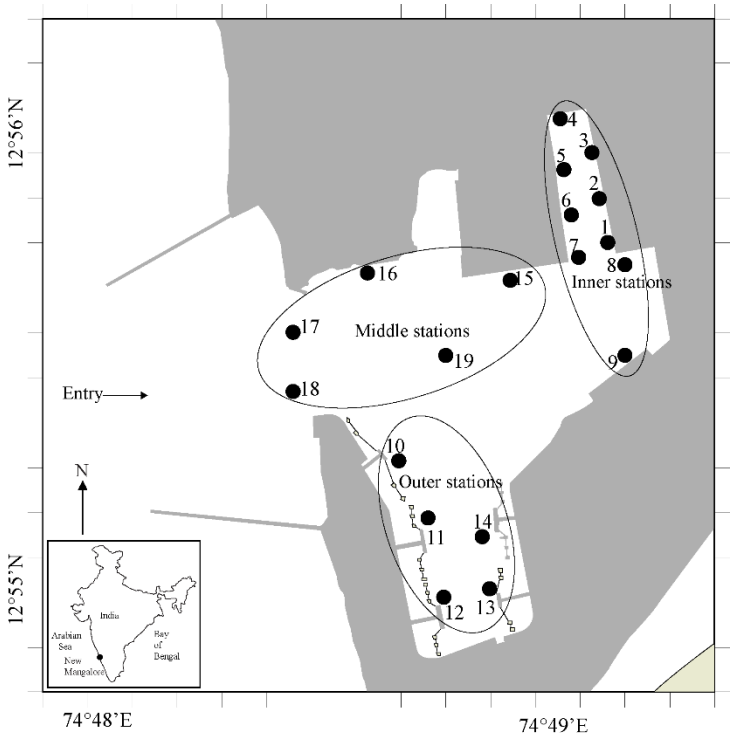

Fig. 2.7 Location of sampling stations in New Mangalore Port, west coast of India.

2.1.3.3 Paradip Port

Paradip Port is one of the major seaport situated on the east coast of India. It is an artificial lagoon type Port located at about 6.7 km south of Mahanadi River mouth in the state of Odisha. This Port has a 190 m wide approach channel, protected by the northern and southern breakwaters with a length of 0.5 km and 1 km, respectively. Paradip Port exports and imports commodities like iron ore, manganese ore, coal, chrome ore, coking coal, petroleum, sulphuric acid, limestone, etc. Paradip Port has 15 berths, three single point mooring, and 1 Ro-Ro jetty with the length from 50 m to 520 m and a well-maintained approach and entrance channel with the minimum depth of 17.1 m (https://www.paradipPort.gov.in/Port_Brochure_Static.aspx). Sampling was carried out from

12 fixed stations on four different occasions representing different seasons for the period from 2014-2015, [August 2014 (SWM), December 2014 (NEM), May 2015 (SIM) and August 2015 (FIM)]. Based on the locations stations were demarcated as inner (S1 to S5) and outer (S6 to S12) stations. Tides in this region are mixed semidiurnal type with an average tidal height of 1.87 m and 0.7 m during spring and neap tide, respectively. Paradip Port weather is tropical weather with maximum temperature ranged between 35 °C to 41.4 °C during summer and 8.9 °C to 14 °C during the winter season. The average rainfall is 150 cm, with the maximum during SWM (July to September). This area also experiences little rainfall during FIM (retreating monsoon) from October to November. January and February are the dry months. The data collected from water column and sediment in Paradip port used for objective 3.

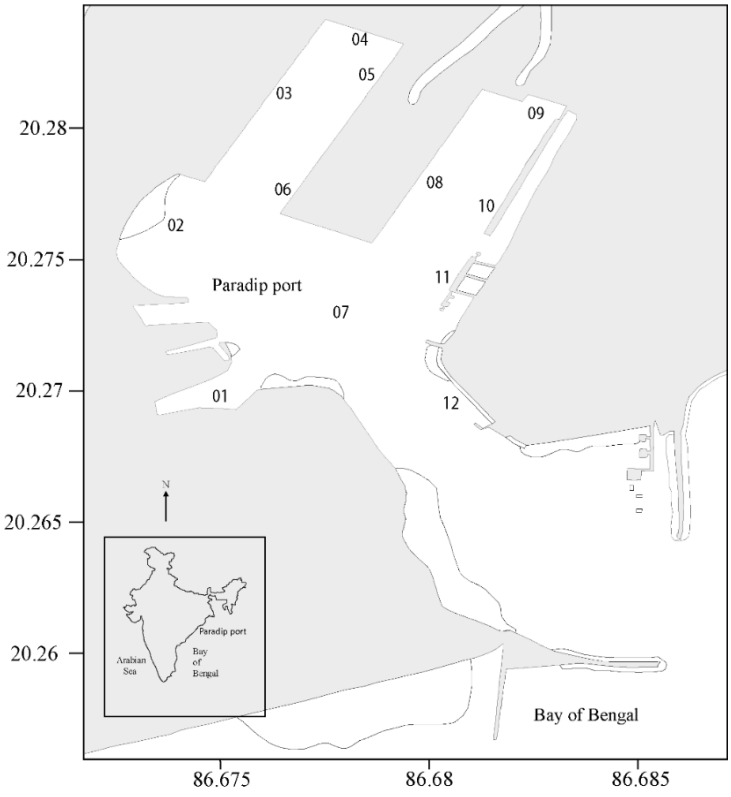

Fig. 2.8 Location of sampling stations in Paradip Port, west coast of India.

2.1.3.4 Chennai Port

Chennai Port is a major marine port located in the state of Tamil Nadu, along the east coast of India (13° 06' N, 80° 18' E). It is an artificial seaport, and the second-largest Port stands next to the Mumbai Port among the Major Ports of India. This Port is consisting of three docks, and a timber pond containing 26 berths and the entire Port area spreads over 407.51 hectares. The width of the main entrance is vast, and it varies between 244m and 410 m. The dock entrance width of Ambedkar and Bharathi docks are 125m and 350m respectively, but the entrance width of the timber pond and Jawahar dock (<50m) is very narrow (Source: http://www.chennaiPort.gov.in). Sampling was carried out from 25 stations on four different occasions representing different seasons, July 2012 (south-west monsoon-SWM), September 2012 (fall inter-monsoon-FIM), January 2013 (north-east monsoon-NEM) and March 2013 (spring inter-monsoon-SIM) at Chennai Port. 18 stations were selected including fertilizer jetty, oil berths, container berths, and iron ore berths. The sampling area was classified based on the dock position and environmental characteristics into five groups; BB-boat basin, AD- Ambethkar dock, JD-Jawahar dock, BDW-Bharathi dock west, BDE- Bharathi dock east. Boat basin (BB) stations are located in an area with low circulations. (i.e., Potential habitat for settlement of planktons and pathogens). Ambedkar dock stations are located in the middle of the Port with the maximum possibility of water circulations. Jawahar dock stations also situated in an enclosed part of the inner port with low water circulations. Bharathi dock stations are located in the outer port with relatively high water circulations and potential ballasting and deballasting site including oil berth and iron-ore berth. The climate condition of the study area is a tropical maritime climate with the air temperature ranging between 24°C and 39°C during January and May, respectively. During the study period heavy rainfall was experienced in the study area during FIM (11.2 mm to 53.1 mm; IMD). The

nature of tide in this area characterized by semi-diurnal with maximum height up to 1.2 m. The mean tidal range varies from 0.91 m to 1.22 m and 0.61 m to 0.80 m spring and neap tide, respectively. This region also experiences seasonal wind and rainfall patterns. The annual rainfall in this area is about 1250 mm with maximum and minimum during NEM (60%) and SWM (30%), respectively (Shanthi and Ramanibhai., 2011, Mishra et al., 2015).

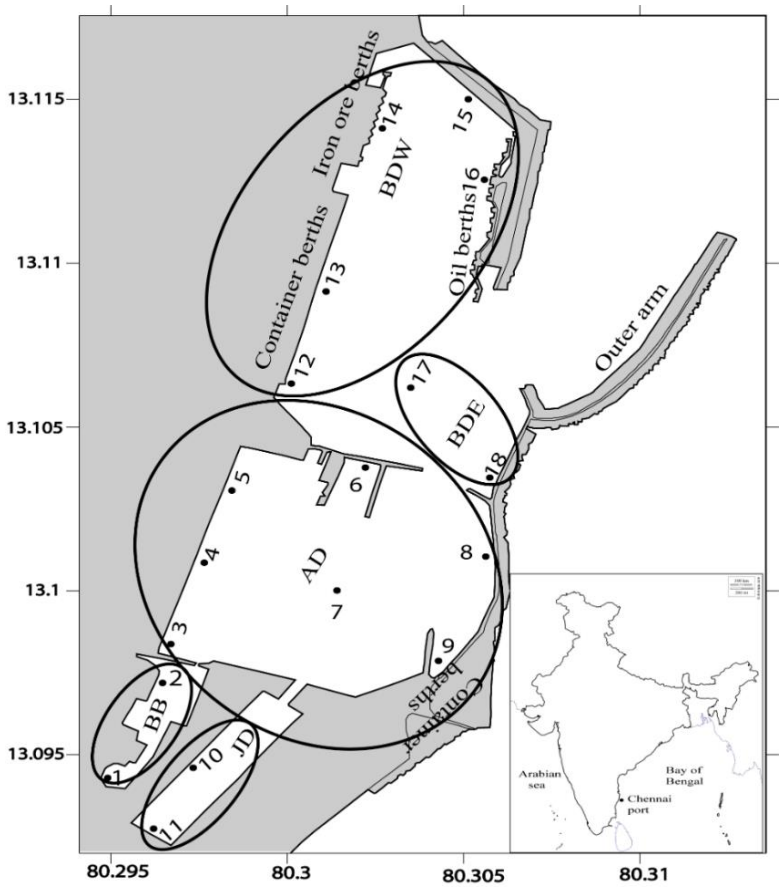

Fig. 2.9 Location of sampling stations in Chennai Port, east coast of India.

2.1.3.5 V.O. Chidambaranar Port (Tuticorin)

V.O.C. Port is an artificial deep-sea harbor located (08° 47' N, 78° 12' E) in Tuticorin, Tamil Nadu, situated along the east coast of India. V.O.C. Port is one of the major Port in India. Port area is formed with rubble mound-type parallel breakwaters projecting into the sea for about 4 km. (The

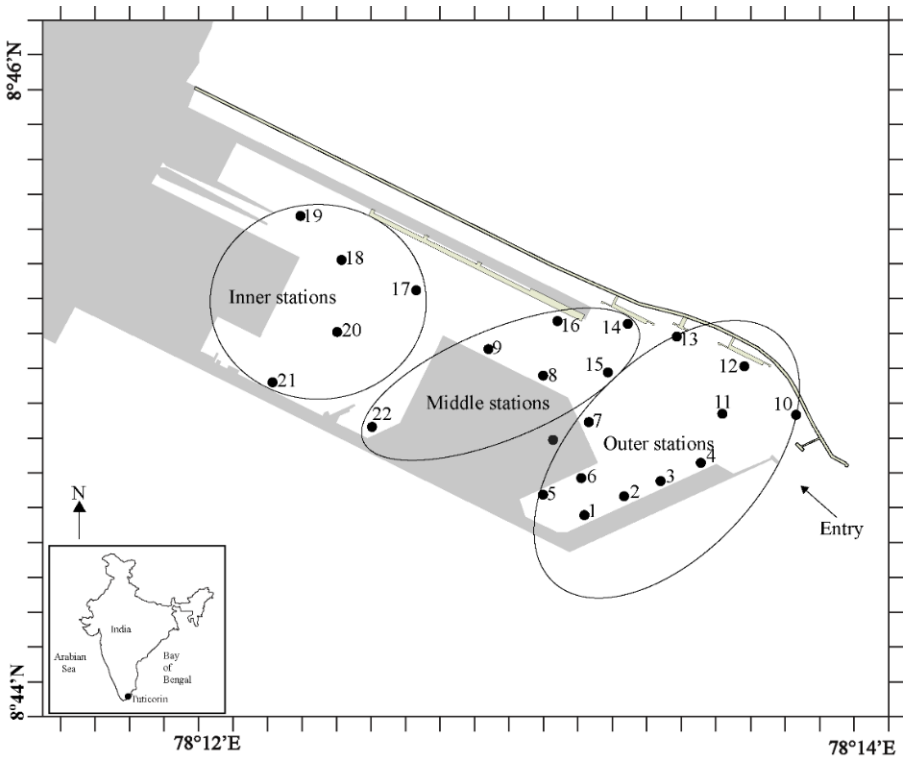

Fig. 2.10 Location of sampling stations in V.O.Chidambaranar Port, Tuticorin east coast of India.

north and south breakwater is 4098.66 m and 3873.37 m long respectively, and the distance between the breakwaters is 1275 m). The harbor basin extends to about 400 hectares of protected water area and is served by an approach channel of 2400 m length and 183 m width (Source: https://www.vocPort.gov.in/Portlayout.aspx). V.O.C. Port contains 14 berths with a total quay length of ~3000 m and a depth of about 5.85 to 10.9 m. Sampling was carried out in V.O. Chidambaranar Port (Figure 2.5) from 22 stations on four different occasions representing different seasons for the period from 2012-2013 [July 2012 (south-west monsoon), October 2012 (fall inter-monsoon), December 2012 (north-east monsoon) and March 2013 (spring inter monsoon)]. Based on the locations and the distance from the main entrance stations are grouped as inner (IP), middle (MP), and outer (OP) port stations. Tuticorin region experiences tropical climatic conditions with the minimum, and maximum air temperature is about 22 °C and 39 °C during January and May to

June, respectively. The study region receives annual rainfall varies from 762 mm to 1270 mm with the maximum during the North-east monsoon (444mm) season. There was no precipitation during the sampling period, except one-day rainfall during FIM (IMD). Tides are semidiurnal, and tidal amplitude in the area is about 0.81 m and 0.2 m during a spring tide (ST) and neap tide (NT), respectively.

2.2 sample collection

In each ecosystem, samples were collected from several stations (10 to 25) on different occasions representing different seasons. Surface and sub-surface (1m above the bottom) water samples were collected using Niskin bottles (5 L) along with the other environmental parameters such as temperature, salinity, dissolved oxygen, biological oxygen demand, phytoplankton pigments and inorganic nutrients (nitrate, nitrite, ammonia, phosphate, and silicate). The temperature was determined in situ using a multiparameter Sonde DS5X (Hydrolab). Salinity was measured with an Autosal (Guildline Autosal 8400B). Dissolved inorganic nutrients such as nitrate (NO3), phosphate (PO4), nitrite (NO2), ammonium (NH4), and silicate (SiO4) were analyzed by SKALAR SANplus ANALYSER. Dissolved oxygen (DO) and biological oxygen demand (BOD) were analyzed following standard methods (Parsons et al., 1984). For the pigment analysis, 250 to 500 ml of water sample were passed through GF/F filter (27mm; pore size 0.7 mm) and stored in at -80º C until analysis using high-performance liquid chromatography (HPLC). Additionally, (only in paradip port), sediment samples were also collected using van veen grab from 12 fixed station on four different occasions representing different seasons. Top 2cm surface sediment samples were collected using PVC corer with the diameter of 2.5 cm. The data collected from eater column and sediment were used for Objective 2.

2.3 Phytoplankton pigment analysis from the water column

The pigment samples collected from marine, freshwater and estuarine ecosystems were extracted by adding 3 ml of 90% acetone (V: V with the deionized water) to the frozen filter paper, which was sonicated using a ultrasonic probe (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) for 10 sec at 20 kHz and kept on ice to prevent excessive heat. The extracts were then stored overnight at -20 °C for analysis using HPLC (Agilent Technology; Series 1200). Before analysis, all the extracts were filtered (Nylon filter membranes, 0.2 μm pore size) immediately before injection in the HPLC to remove cells and debris from sonicated filter paper. To prevent the photolysis of the samples, extraction and injection procedures were performed under dim light. Pigment analysis was performed following the method of Van Heukelem (2002) with a slight modification. The pigment extracts were injected into the HPLC system equipped with an Agilent 1200 series pump; an Agilent diode array detector connected using a reverse-phase C8 Column maintained with 60ºC (150 X 4.6 mm, 3.5 mm particle size) with 3 phase linear solvent gradient program at a flow rate of 1.1 ml/min. The excitation and elution of the extracted pigments were detected at 450 and 665 nm. The quantitative and qualitative analysis of about 20 pigments, was carried out using the commercially available pigment standards purchased from DHI Inc (Denmark) and Sigma–Aldrich (USA).

2.4 Phytoplankton pigment analysis from the sediments

Sediment samples (0.5 to 1 g) were transferred to the 15 ml amber colored polypropylene centrifuge tubes along with 4 ml of HPLC grade acetone. The mixture was then sonicated using an ultrasonic probe (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) for 10 sec at 20 kHz and kept on ice to prevent excessive heat. After sonication, the mixture transferred to the deep freezer (-20 °C) for overnight incubation. Further, the sample mixture was centrifuged, filtered

with a syringe filter (0.2 µ), and stored in the deep freezer until analysis. Quantitative analysis of sediment pigments was carried out using the HPLC system equipped with an Agilent 1200 series pump, and an Agilent diode array detector connected using a reverse-phase C18 Column (4.6 ×250 mm, 5.5 mm particle size) with 3 phase linear solvent gradient program was used. This gradient program was a modification of Wright et al. (1991) as described by Chen et al. (2001) for enhancing the separation of pheopigments. The excitation and elution of the extracted pigments were detected at 450 and 665 nm. The quantitative and qualitative analysis of chlorophylls, carotenoids, and chlorophyll degradation products was carried out using the commercially available pigment standards purchased from DHI Inc (Denmark) and Sigma–Aldrich (USA). Chlorophyllide, was not detected in any of the surface sediment samples as well. Pigments ratios Chl a: CDP, Pheid: Phe were also calculated from analyzed sediment samples for better interpretation.

2.5 *CHEMTAX* design and analysis

CHEMTAX V1.95, a chemical taxonomy software (Mackey et al., 1996, Wright et al., 1996, 2009, 2010), was used to distinguish the relative abundance of each microalgal community to the total chl *a*. CHEMTAX is based on matrix factorization, which uses three input matrixes (marker pigments and chl *a* concentrations, input matrix for major microalgal classes and ratio limit matrix restricting the iterative adjustments) and steepest- descent algorithm to find out best fit to the current data. Totally eight phytoplankton groups (chlorophytes, chrysophytes, cyanobacteria, diatom, dinoflagellates, prasinophytes, and prymnesiophytes) were included based on the maker pigment obtained during HPLC analysis. Input pigment ratios for each microalgal groups were obtained from the previous literature (Gibb et al., 2001; Schlüter et al., 2000). Major diagnostic pigments loaded in the CHEMTAX were peridinin, 19′-butanoyloxyfucoxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthin, neoxanthin, prasinoxanthin, violaxanthin, lutein, zeaxanthin, chl *b*, and

chl *a*. To avoid the unreliable pigment:chl *a* ratios 10 initial matrixes were generated using randomizing formulae explained in Wright et al. (2009). Because CHEMTAX is sensitive to the feed values in the initial ratio matrix, it is required to be optimized. To determine each algal class concentration in terms of chl *a* the best 10% of the outputs from the 10 bins were calculated based on lower Root Mean Square (RMS) errors were used as starting matrices only when they were realistic to the study area. Final matrixes with unrealistic percentage changes were discarded after multiple trials. The input pigment ratio and output pigment ratio of the matrix derived by CHEMTAX for different ecosystems and different seasons are presented. The final data were obtained after multiple CHEMTAX trials and runs to avoid the unrealistic data.

2.6 Experiment setup to elucidate taxa specific grazing-induced phytoplankton chlorophyll breakdown pathway

To elucidate the different pathways of pigment degradation affecting the chl *a* integrity, specific experiments were performed using the mixed community of <200µm phytoplankton i.e., natural seawater (NSW) passed through a 200µm mesh to remove mesozooplankton (but not microzooplankton) and laboratory-grown monocultures. The mixed community was prepared from the seawater collected from Dona Pual Bay (Goa, India), and the filtrate obtained (herein referred to as 'NSW") was utilized for the experiments. For the experiment three bloom-forming phytoplankton species representing different classes, i.e., Bacillariophyceae (*Skeletonema costatum*), Dinophyceae (*Amphidinium* cf. *carterae*), and Chlorophyceae (*Dunaliella tertiolecta*) were selected. All three cultures were maintained using f/2 media (Guillard and Ryther 1962) and in a temperature-controlled room (25^{o}C) at 12h light: dark photocycle at a photosynthetic photon flux density of 70 µmol m^{-2} s^{-1}.

Altogether two experiments were performed and the experimental setup aimed at elucidating the influence of grazing (mainly mesozooplankton) on biomass (Chl a) buildup and pheopigment formation from the above-mentioned cultures and NSW. The experimental setup in each experiment involves two sets i.e. one set with grazers (mesozooplankton) and the other set without grazers, as control. In NSW, the dominant phytoplankton groups were diatoms and chlorophytes in experiment 1, while in experiment 2, diatoms, chlorophytes, cryptophytes, and prasinophytes dominated the NSW (Fig. 2). In each experiment, two sets of 2.5 liter polycarbonate bottles (Nalgene make) containing filtered seawater enriched with f/2 nutrients (for three culture) and NSW were prepared. In nutrient-enriched bottles of both sets, inoculums from 7-day old cultures of *Skeletonema, Amphidinium,* and *Dunaliella* were introduced separately. For each culture and NSW a total of 6 and 9 bottles in each set per culture or NSW during expt 1 and 2 respectively were used. The total volume in all the bottles was maintained the same, i.e., 2 liters. In one set of bottles, containing three cultures and NSW, an aliquot (20 ml) of zooplankton sample (obtained from well-mixed concentrate) was introduced separately to relatively maintain an equal distribution of the grazers. Zooplankton concentrate (mostly mesozooplankton) was obtained by first towing a zooplankton net (200 μm) in Dona Paula Bay, Goa, India, and then adjusting the collected sample into a known volume. The estimated duration from the zooplankton collection to the start of the experiment was approximately 5 to 6 hours. In the first experiment, zooplankton concentrate was dominated by copepods (calanoids followed by harpacticoids, cyclopoids, and others). In the second experiment, in addition to copepods, relatively high numbers of green *Noctiluca* were also present (Fig. 2). The remaining bottles of the second set with the three cultures and the NSW, which is devoid of grazers, were treated as control. All the bottles were incubated in the temperature-controlled room experiencing 12 h light: dark cycle at the same photosynthetic photon

flux density. For sampling, duplicates (expt. 1) and triplicate (expt. 2) bottles were removed daily and from each bottle, one liter of water sampling for cell counts (only in the second experiment for cultures) and pigments was performed. This procedure was repeated every day starting from day-0 (initial) to day-3. The analysis of pigments in zooplankton-free cultivations shows the metabolic processes of the algal cells. Whereas, when the zooplankton added to the alga cultures, they digest the algae during grazing by using their own enzymes and decomposition pathways. Thus the pigment extractions prepared from mixed alga-zooplankton cultures will also contain pigments extracted from the alimentary canal of zooplankton in addition to pigments extracted from the undigested algae. Given this, during the second experiment, additional samples were also collected to evaluate the likely effect of zooplankton gut contents on the results. This was done by analyzing pigments from the zooplankton and the cultures obtained from the bottles with zooplankton. First, the remaining one liter of the samples was passed through a 200-micron mesh (Fig. 1b). Then the samples for pigment analysis from the zooplankton retained on the mesh (after resuspended in known volume, i.e., 20 ml using filtered seawater) and the filtrate was collected onto the GF/F Whatman filter paper and sonicated for 30 seconds with ice (to lower the heating) for the gut pigment extraction. The cell counts were made using a haemocytometer and microscope (Olympus make), and the analysis of collected pigment samples was performed using HPLC as detailed below. The experiments were run in duplicates and triplicates in experiments one and two, respectively, and the data presented here is the average of both the experiments (i.e. n=5 for each treatment).

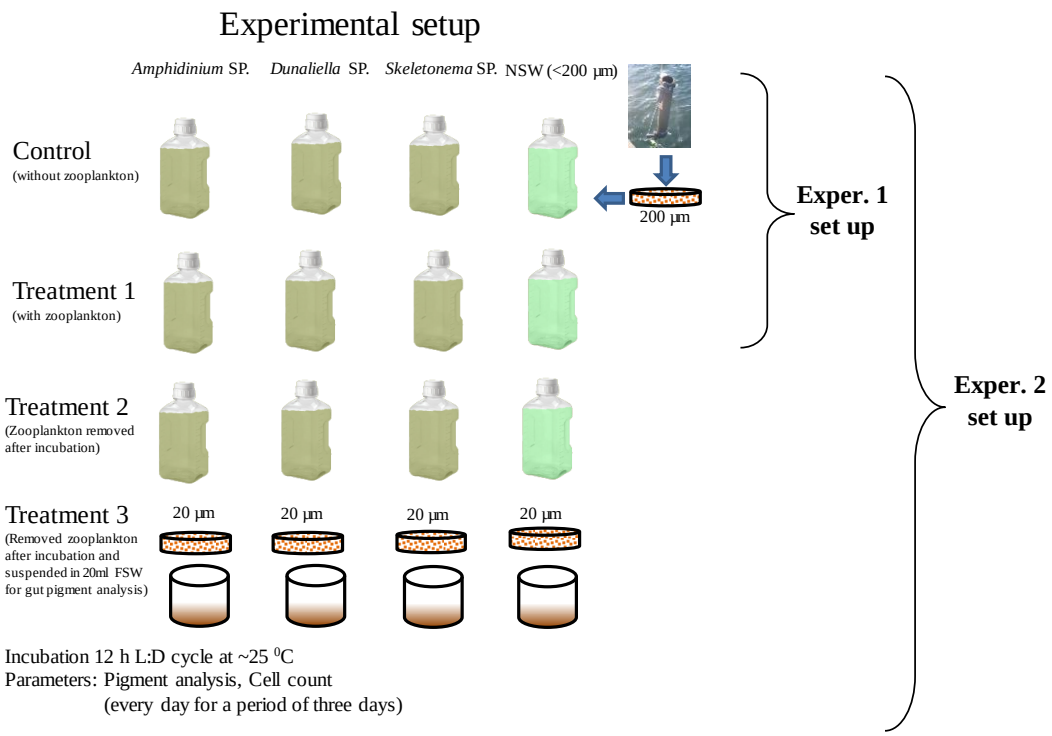

Fig. 2.11 Schematic presentation of the experimental design adapted in this study.

Chapter 3

Distribution of phytoplankton marker pigments in different ecosystems

3. Distribution of phytoplankton marker pigments in different ecosystems

3.1 Introduction

Coastal ecosystems are the productive and valuable ecosystem which act as abrading or nursery ground for the many coastal and marine species. These ecosystems are most vulnerable to anthropogenic pressure due to easy access to humans and industries. It is apparent in the most complex system like ports, wherein several anthropogenic activities can be observed (Darbra et al., 2004). Coastal ports are mainly affected by dredging, oil discharge, petroleum wastes, and different cargo handling types by ports (Bailey et al., 2004, Tripathy et al., 2005). Ports situated in the riverine and estuaries are exposed to additional anthropogenic pressure such as sewage or municipal runoff and terrestrial runoff during monsoon (Musale et al., 2015). Phytoplankton, which is the essential component of any given aquatic ecosystem, are the quickest to respond to such perturbations. Phytoplankton are important as they contribute more than 40% of the global carbon fixation (Reynolds, 1984) and are also of significant concern due to their influence on socio-economic development and human health (Wu et al., 2009). Generally, port areas are also prone to bioinvasion by introducing alien/harmful/toxic species via the ship's ballast water discharges. Therefore, monitoring phytoplankton (i.e., biomass and composition) with hydrographic parameters will better understand the ecosystem functioning and assessment.

In recent years, eutrophication and anthropogenic pressure are the major concern in port ecosystems, directly affecting the water quality and biota. In India, several studies are carried out in the ports located on the east (Vishakhapatnam and Paradip port) and west (Kandla, Cochin Murmugoa, Mumbai, and Mangalore port) coasts to understand the hydrography and biotic communities (Menon et al., 2000, Tripathy et al., 2005, D'coasta et al., 2008, 2010, Shirodkar et al., 2000, D'silva et al., 2012, 2013, Madhu et al.,2009, Mohapatra et al., 2017, Rodrigues et al.,

2019). But information on port hydrography and biotic components from Chennai, V.O.C, Kolkata, and Haldia ports are scarce. Most of these studies are focused on the water quality and distributions of phytoplankton communities (motile stage and cyst forms). These studies are mostly done by the traditional microscopic observations which have a limitation such as (i) time consuming (ii) restricted to nano- to micro-sized phytoplankton, and smaller groups are (i.e., picoplankton 0.2 to 2 µm) left out of the analysis (iii) requires a high level of taxonomic affinity and (iv) results are subjective. Considering this pigment-based HPLC analysis and CHEMoTAXonomy (CHEMTAX) is a powerful tool to understand the phytoplankton community distributions from different ecosystems. Evaluating the distribution of phytoplankton marker pigments with abiotic factors in a given ecosystem will better understand the phytoplankton composition and dynamics. The present study aims to understand the distributions of PFG's derived from the pigment-based analysis and its relationship with hydrography from different port ecosystems along the coast of India.

3.2 Methodology

Details of sampling strategies, pigments analysis, and CHEMTAX analysis from marine, estuarine, and freshwater port ecosystems are presented in chapter 2.3.

3.2.1 Data analysis

Statistical analysis was carried out for each port ecosystem and Zuari estuary separately to understand the inter-seasonal variations of PFG's variations and its relationship between hydrological parameters. ANOVA and *post hoc* Tukey tests were performed using Statistica software to evaluate the spatial and temporal variations in the environmental parameters and the distribution of PFG's. Pearson's correlation coefficients (using IBM SPSS statistics software, version 25) and redundancy analysis (RDA, using CANOCO 4.5 software, ter Braak and

Smilauer 2002) was performed to assess the relationship between phytoplankton biomass and measured environmental variables (temperature, salinity, DO, BOD, PO_4, SiO_3, NO_2, NO_3, and NH_4). For surface water, PFG's environmental parameters corresponding to surface water were used, whereas, for NBW, PFG's environmental parameters corresponding to NBW were used. Before RDA, data was initially analyzed by detrending correspondence analysis (DCA). Since the longest gradient length for all the ecosystems (marine, freshwater, and estuarine) were less than 3.0, RDA was performed.

3.3 Results

3.3.1 Environmental parameters

3.3.1.1 Freshwater port ecosystems

3.3.1.1.1 Kolkata Port

Environmental parameters (salinity, temperature, DO, BOD, and DIN) showed a distinct seasonal variation, whereas the trend between surface water and NBW was similar. Water column was warmer during SWM (31.1 ± 0.4^{o}C) and PM (25.4 ± 0.9^{o}C) whereas, it was cooler (20.6 ± 0.8^{o}C) during PrM's. Salinity variation was negligible and ranged from 0.17 to 0.35. DO values were ranged between 3.3 to 9.9 mg l^{-1} with the lowest concentration during SWM (5.8 ± 1.4 mg l^{-1}). BOD values ranged from 0.8 to 7.3 mg l^{-1} with the maximum during SWM (3.23 to 6.86 mg l^{-1}, Fig. 3.1). Among the all studied port ecosystems, the highest DIN (NO_3) was observed in Kolkata port. The highest NO_3 concentration was observed during PM, especially at KOPT stations (17.66 to 205.85 µM), followed by SWM (9.54 to 42.59 µM), PrM 2 (11.77 to 42.86 µM), and PrM 1 (0.20 to 22.93 µM). NO_2 concentrations ranged between 2.54 and 15.7 µM with the maximum concentration during PM (3.8 to 15.7 µM). PO_4 concentrations were observed higher and lower during PM (5.58 to 12.52 µM) and PrM 2 (2.3 ± 2.2 µM), respectively. SiO_4

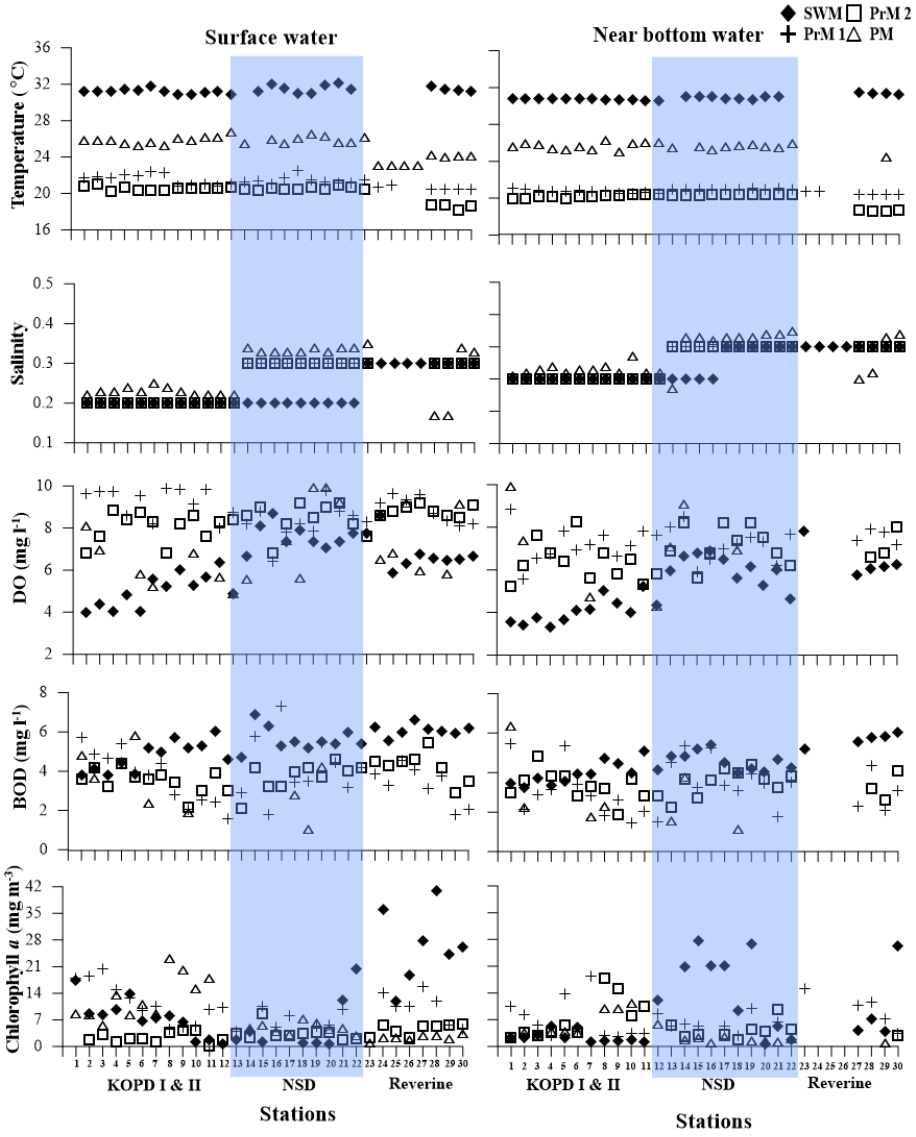

Fig. 3.1 Seasonal variations in the measured environmental variables from the surface and near bottom (NBW) waters in Kolkata port. Bars represent the values for each stations and the standard deviations

concentrations were peaked during PM across the study area (14.07 to 190.88 µM) and at riverine stations during PrM 2 (107.51 to 480.41 µM). Generally, the elevated level of PO_4 and SiO_4 concentrations were recorded at the riverine stations compared to the closed dock stations. NH_4 concentrations were observed higher (1.8 to 43.18 µM) and lower (0.5 to 8.48 µM) during PrM 1 and SWM (Fig. 3.2). ANOVA revealed significant seasonal variations (p<0.05 and an insignificant difference between the surface water and NBW.

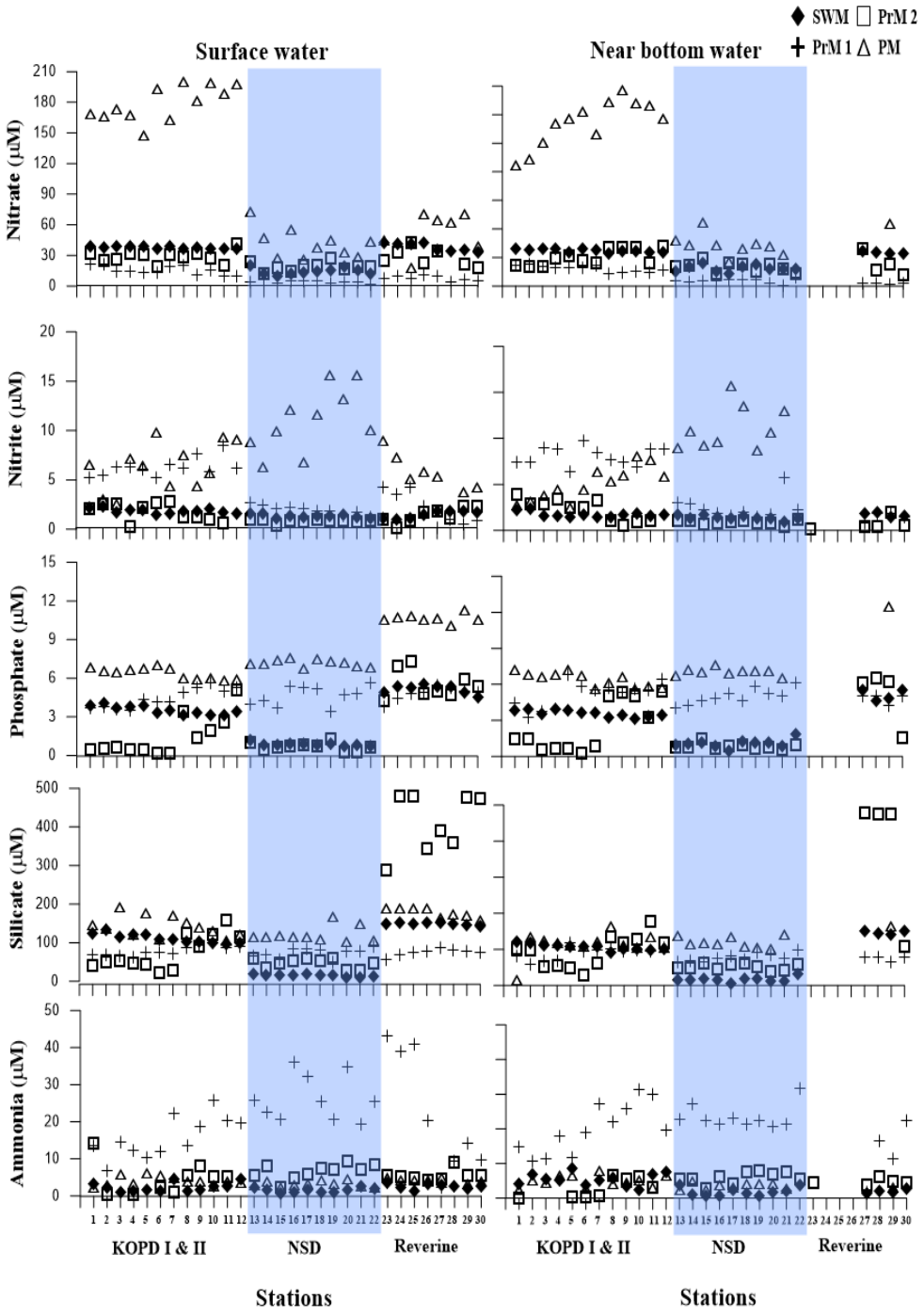

Fig. 3.2 Seasonal variations in the nutrient concentrations from the surface and near-bottom (NBW) waters in Kolkata port. Bars represent the values for each season, and the standard deviations

3.3.1.1.2 Haldia Port

Environmental parameters (salinity, temperature, DO, BOD and, DIN) showed distinct seasonal variations and a similar trend between surface and NBW. Salinity ranging between 0.6 to 8.4 was higher during PrM 1 (5.7 to 8.4) and PrM 2 (6.2 to 7.8) compared to SWM (1.0 to 3.1) and PM (0.6 to 1.8) seasons. Water temperature was higher (28.9 to 32.1ºC) during PM and SWM compared to PrM's (18.2 to 22.5ºC). Higher temperature values observed at the port stations compared that in riverine stations. DO values ranged from 3.2 to 8.6 mg l^{-1} with the higher (7.5 to 8.6 mg l^{-1}) values during PrM 1. Higher DO values were observed at the riverine stations compared to port stations, except during PrM 1. BOD values ranged between 1 to 7.3 mg l^{-1} with higher values during PM (3.8 to 5.6 mg l^{-1}, Fig. 3.3). Nutrient (except PO_4) was generally higher during SWM, and the trend was the same between surface and NBW. NO_3 concentrations were higher (9.99 to 49.52 µM) during PM and SWM, compared to PrM 2 (2.9 to 45.8 µM). The lowest NO_3 concentrations were observed during PrM 1 (1.1 to 5.9 µM). NO_2 concentrations ranged from 0.3 to 3.7 µM with the higher and lower concentration during SWM (3.0 ± 0.5 µM) and PrM 2 (0.8 ± 0.5 µM), respectively. Higher and lower PO_4 concentrations were observed during PrM's (3.8 to 8.3 µM) and SWM (3.4 ± 0.7 µM), respectively. PO_4 concentrations were higher in the port stations compared to riverine stations during all seasons. Higher and lower SiO_4 concentrations observed during SWM (152.5 ± 29 µM) and PrM 1 (79.7 ± 12.9 µM), respectively. SiO_4 concentrations observed higher in the riverine stations (156.8 ± 27.7 µM) compared to port stations (149.5 ± 28.3 µM) during SWM, whereas the reverse trend was observed during other seasons. NH_4 concentration ranged from 2.1 to 15.7 µM, with the highest concentrations observed during SWM (8.9 ± 3.8 µM) season (Fig. 3.4). ANOVA results of the

measured hydrographic parameters revealed significant seasonal variations ($p< 0.05$) and an insignificant difference between the surface water and NBW.

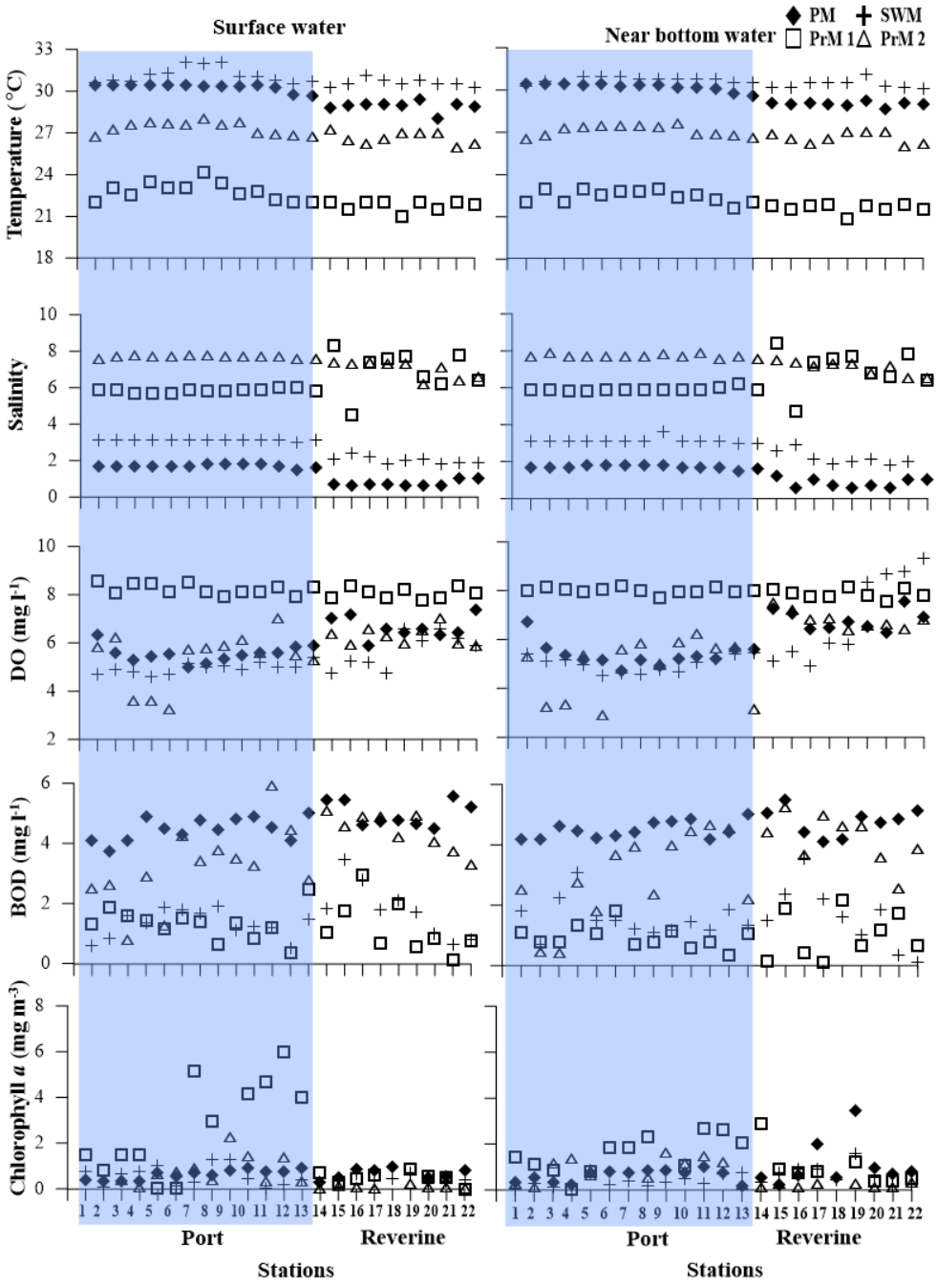

Fig. 3.3 Seasonal variations in the measured environmental variables from the surface and near-bottom (NBW) waters in Haldia port. Bars represent the values for each stations and the standard deviations

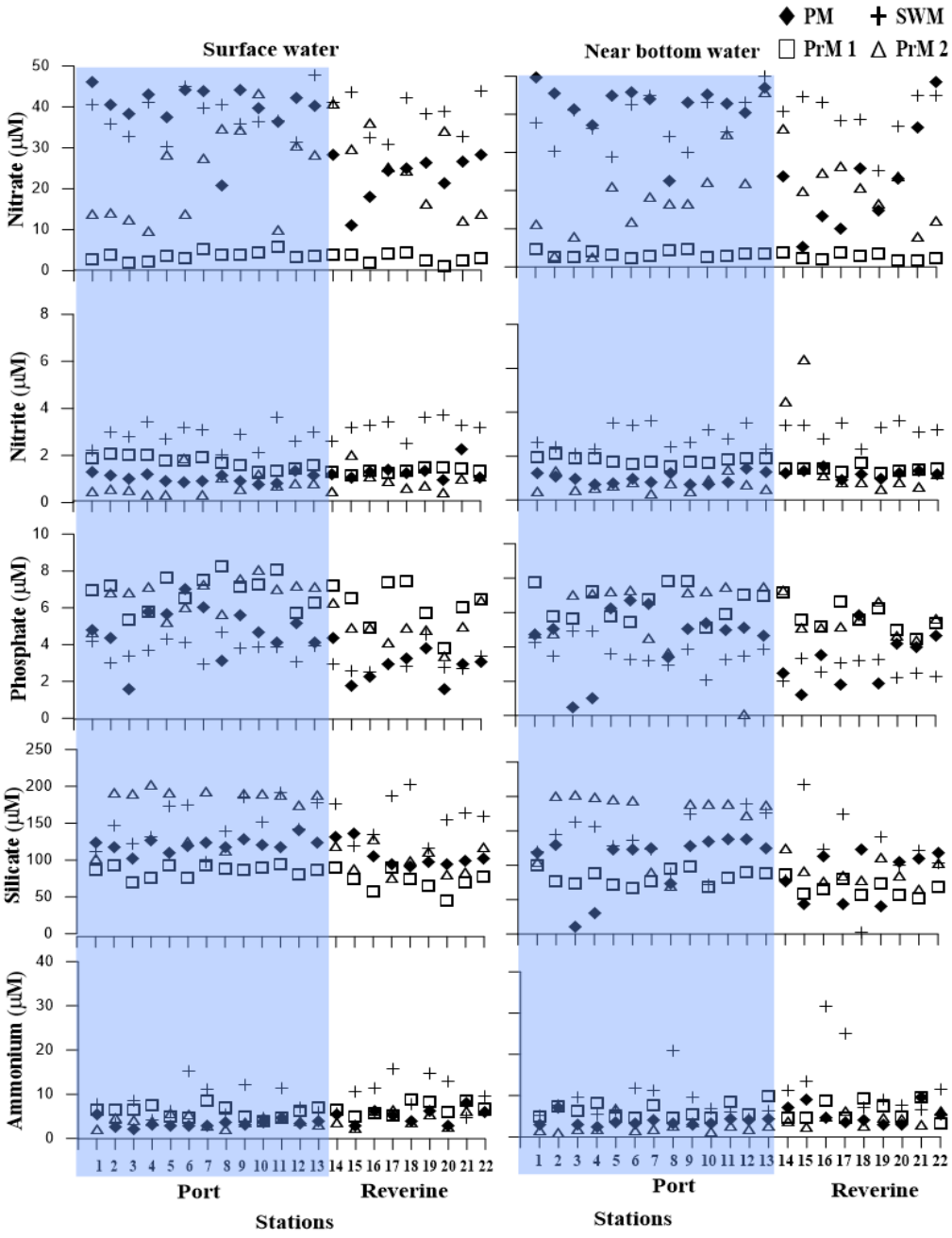

Fig. 3.4. Seasonal variations in the nutrient concentrations from the surface and near-bottom (NBW) waters in Haldia port. Bars represent the values for each season, and the standard deviations

3.3.1.2 Estuarine port ecosystems

3.3.1.2.1 Cochin Port

The hydrographic (salinity, temperature, DO, BOD, and DIN) showed a distinct variation between the seasons. The study area showed large salinity variations throughout the year. A wide range of salinity variations was observed during PrM (13.2 to 32.7) and PM's (11.2 to 32.3)

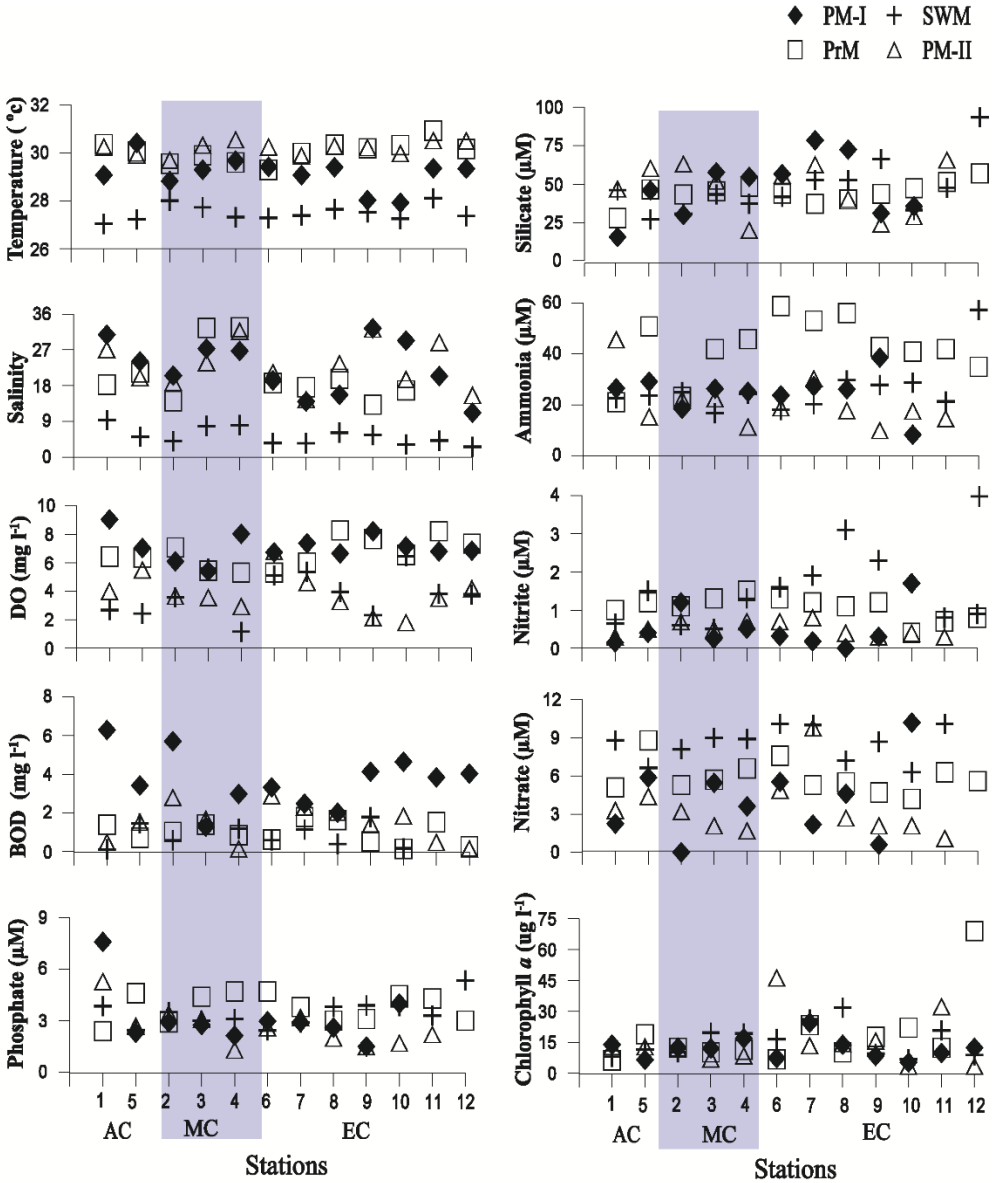

Fig. 3.5 Seasonal variations in the measured environmental variables and nutrient concentrations from the surface waters in Cochin port. Bars represent the values for each stations and the standard deviations

due to the partially mixed waters. The water column was stratified completely during SWM (2.6 to 9.4), with the freshwater discharge including extreme rainfall. The temperature ranged between 27 to 30.6 ºC with a minimum during SWM (27.5 ± 0.3 ºC) compared to other seasons. Higher DO concentrations were observed during PM 1 (5.4 to 9 mg L^{-1}) followed by PrM (6.7 ± 1 mg l^{-1}), SWM (3.9 ± 1.5 mg l^{-1}), and PM 2 (3.8 ± 1.3 mg l^{-1}). BOD values were ranged from 0.1 to 6.3 mg l^{-1} during all the seasons, with the highest concentrations during PM 1 (3.7 ± 1.4 mg l^{-1}) season (Fig. 3.5). Higher and lower PO_4 concentrations were observed during PrM (3.8 ± 0.8 µM) and PM's (1.5 to 7.6 µM), respectively. SiO_4 concentrations ranged between 15.5 to 93.5 µM with maximum concentrations during PM 1 (47.8 ± 8.9 µM) season. NO_3 concentrations were observed higher and lower during SWM (6.3 to 28.4 µM and PM's (0.6 to 10.2 µM), respectively. NO_2 concentrations ranged between 0.3 to 3.1 µM with the maximum concentrations during SWM (1.4 ± 0.8 µM). NH_4 concentrations were recorded higher during PrM (42.6 ± 11.3 µM) compared to other seasons (Fig. 3.5). ANOVA results of the measured hydrographic parameters revealed significant variations ($p< 0.05$) between the seasons.

3.3.1.2.2 Zuari Estuary

Environmental parameters such as salinity, temperature, and water transparency showed a distinct variation between the seasons. Salinity observed higher during PrM (0.5 to 34.7), followed by PM (0.04 to 32.2) and SWM (0.04 to 23.4). During SWM, freshwater influx, including heavy precipitation, lower the surface water salinity at the estuarine mouth, leading to the intense stratification from DS (21.6 ± 3.4) to the LME (15.2 ± 4.6). During PM season, the estuary slowly recovered with lower freshwater discharge and increased salinity observed at DS stations (32 ± 1.8) to LME (26.6 ± 5.1). The water column was warmer during PrM (29.1 to 30.1 ºC) compared to PM (27.6 to 29.2 ºC) and SWM (27.8 to 28.5 ºC). During PrM, the surface

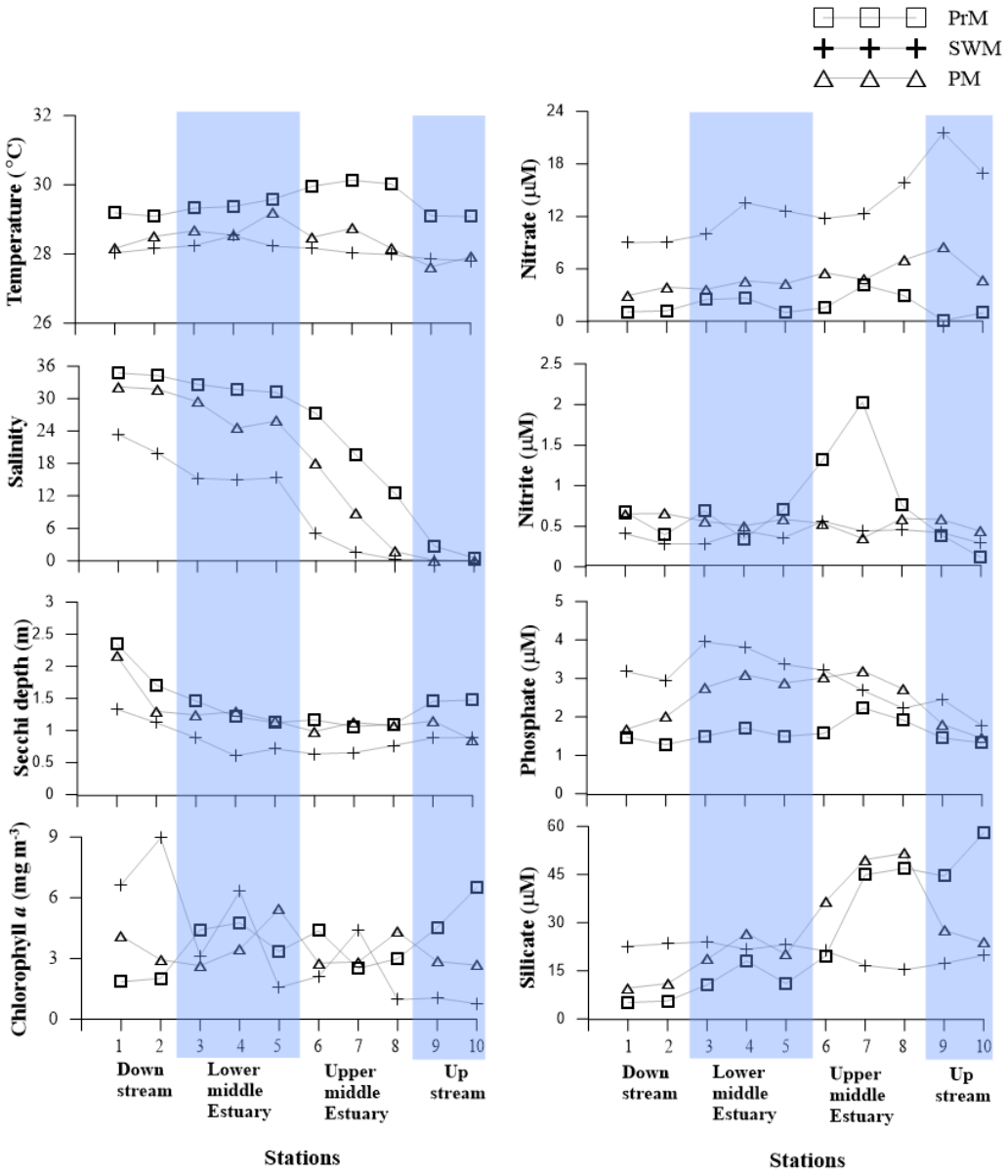

Fig. 3.6 Seasonal variations in the measured environmental variables and nutrient concentrations from the surface waters in the Zuari estuary. Bars represent the values for each station and the standard deviations

temperature was gradually rising from February to May. During the onset of SWM, the temperature dropped by an average of 1.7 °C and started increasing during PM's commencement (October) and decreased towards the end of PM (November to January). Water transparency was higher during PrM (1.06 to 2.35 m) and PM (0.85 to 2.16 m) compared to SWM (0.60 to 1.33 m)

season (Fig. 3.6). Nutrient concentrations also showed distinct seasonal and spatial variations. Generally, DIN concentrations (PO_4, NO_3) were higher during SWM than other seasons except SiO_4 and NO_2. Higher PO_4 concentrations observed from the DS (3.1 ± 2.1 µM) to LME (3.7 ± 2.4 µM) compared to the UME (2.7 ± 2.1 µM) to US (2.1 ± 1.2 µM) during SWM, whereas during non-monsoon seasons, no trend was observed. SiO_4 concentrations were higher during non-monsoon seasons, especially at UME to US, compared to DS to LME stations, whereas a reverse trend was observed during SWM. NO_3 concentrations were gradually decreasing towards the downstream region (19.3 to 9 µM) during SWM, whereas non-monsoon seasons did not show any trend along the salinity transect. Lowest NO_2 concentrations were observed during SWM (0.3 to 0.6 µM) and gradually increased during PM (0.4 to 0.7 µM) and PrM (0.1 to 2.0 µM, Fig. 3.6). ANOVA results of the measured environmental variables revealed significant seasonal variations ($p< 0.05$).

3.3.1.3 Marine port ecosystems

3.3.1.3.1 Kandla Port

Environmental parameters (temperature, salinity, DO, BOD) showed distinct seasonal variations and a similar trend between surface and NBW. Salinity data revealed that Kandla port is highly saline round the year, with the highest salinity up to 40.5 was observed during PrM season. Salinity was higher and lower during PrM (39.8 ± 0.4) and SWM (30.2 ±10.0), respectively. Freshwater influx, including precipitation, during SWM, resulted in lower salinity. Water temperature was warmer and cooler during PM 1 (30.1 ± 0.3 ºC) and PrM (20.9 ± 0.1 ºC), respectively. DO concentrations were higher (5.0 to 7.6 mg l^{-1}) during SWM and lower (3.4 to 5.4 mg l^{-1}) during PM 2. BOD was also higher and lower during SWM (0.8 to 4.4 mg l^{-1}) and PM 1 (0.3 to 2.9 mg l^{-1}), respectively (Fig. 3.7). Nutrient (NO_3, NO_2, and SiO_4) concentrations in

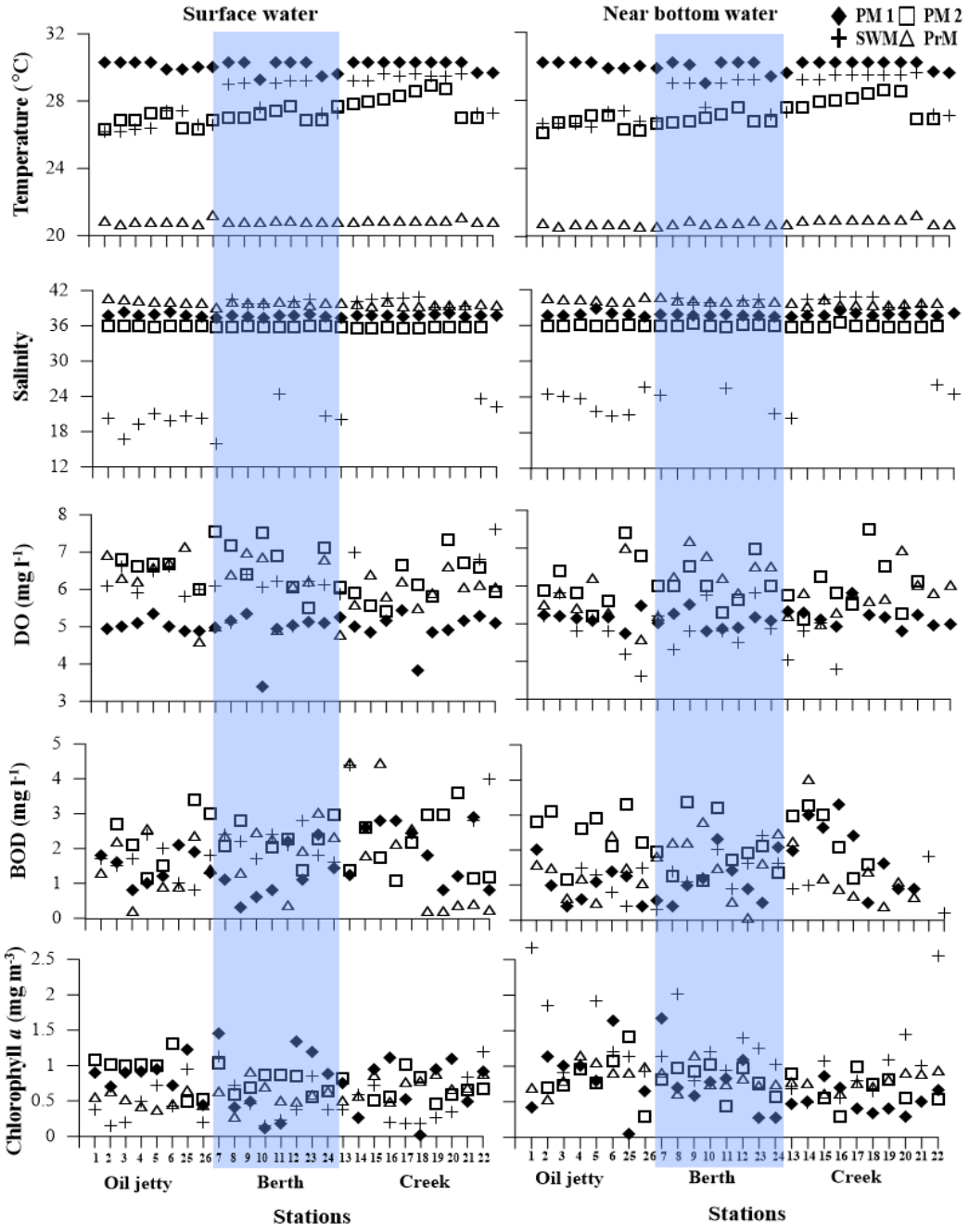

Fig. 3.7 Seasonal variations in the measured environmental variables from the surface and near-bottom (NBW) waters in Kandla port. Bars represent the values for each station and the standard deviations

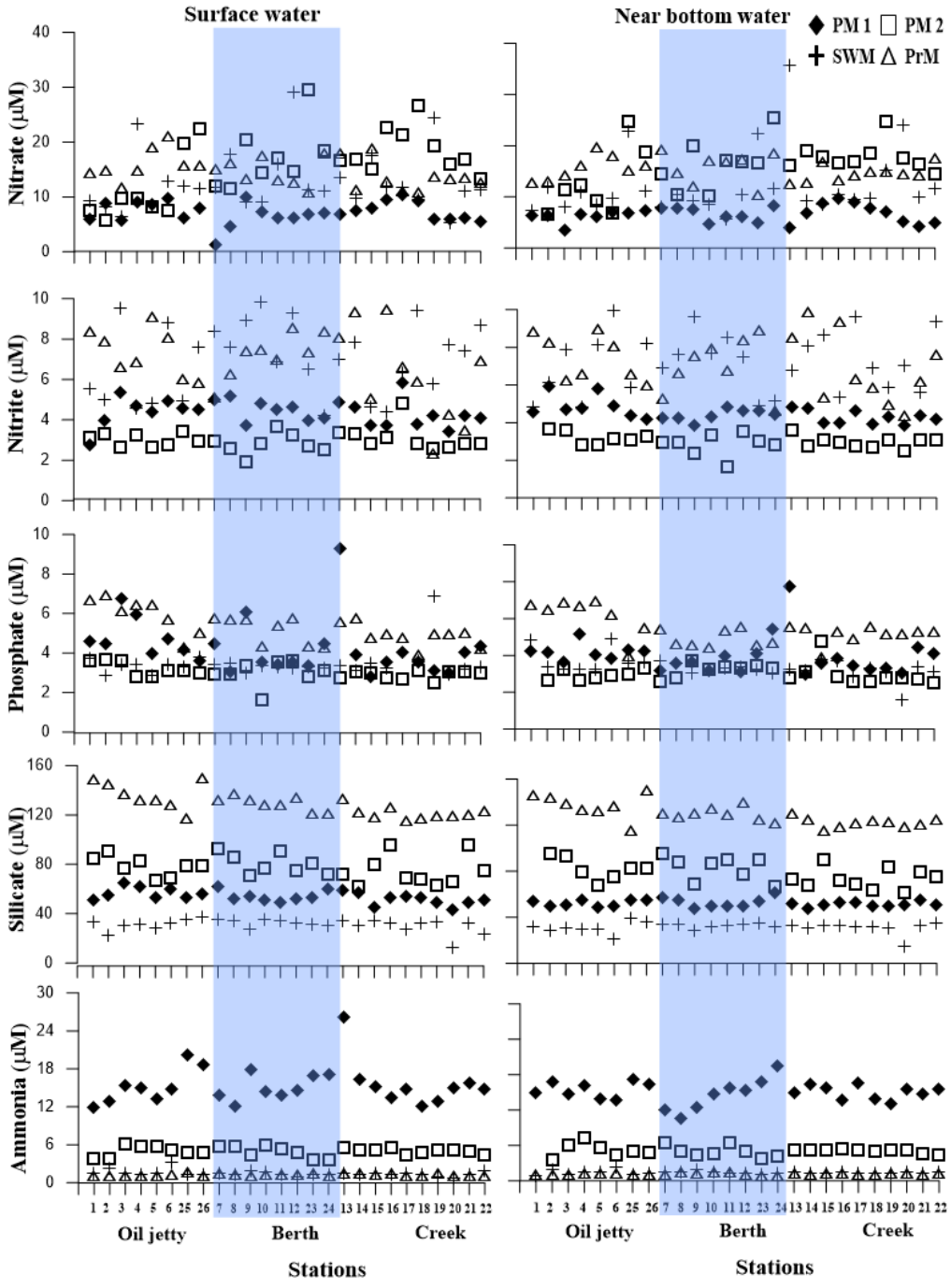

Fig. 3.8 Seasonal variations in the nutrient concentrations from the surface and near-bottom (NBW) waters in Kandla port. Bars represent the values for each season, and the standard deviations

both surface and NBW. Higher (5.3 ± 0.8 µM) and lower (3.4 ± 0.7 µM) PO_4 was observed during PrM and PM 2, respectively. SiO_4 concentrations were higher and lower during PrM (128.4 ± 9.3µM) and SWM (30.9 ± 5.2 µM), respectively. NO_3 concentrations were higher and lower during SWM (15.9 ± 6.0 µM) and PM 1 (7.2 ± 2.0 µM), respectively. NO_2 concentrations were higher (2.3 to 9.8 µM) during SWM and PrM and were lower (1.9 to 4.8 µM) during PM 2. NH_4 concentrations were higher during PM 1 and PM 2 (15.3 ± 3.0 µM and 4.9 ± 0.7 µM, Fig. 3.8). ANOVA results of measured environmental variables revealed significant seasonal variations ($p< 0.05$) and insignificant between surface and NBW.

3.3.1.3.2 Mangalore Port

The hydrographic parameters such as salinity, temperature, BOD, and dissolved inorganic nutrients showed distinct seasonal variations, and the trend was similar between the surface water and NBW. Salinity was >30.5 round the year, and the higher and lower salinity was recorded during PM 1 (35.7 to 36.1) and SWM (33.8 to 35.0), respectively. The water column temperature was lower (26.1 ± 0.5 ºC) during SWM compared to the other seasons (29.4 ± 0.5 ºC). Higher DO values were observed (up to 7.7 mg l^{-1}) during PrM, especially from the surface waters of middle port stations (6.5 ± 1.8 mg l^{-1}) and outer stations (6.2 ± 0.9 mg l^{-1}) and lower during PM 1 (3.4 ± 1.8 mg l^{-1}). In NBW, higher and lower DO values observed during PM 2 (4.2 ± 0.8 mg l^{-1}) and SWM (0.8 ± 0.4 mg l^{-1}), respectively (Fig. 3.9). NO_3 concentrations observed higher during PM 1 (1.5 to 19.4 µM) followed by PrM (0.5 to 13.0 µM) and PM 2 (1.5 to 17.5 µM). The lowest NO_3 concentrations were recorded during SWM (0.2 to 3.1 µM). NO_2 concentrations observed higher and lower during PM's (0.5 to 6.9 µM) and SWM (0.3 to 1.1 µM), respectively. NH_4 concentrations ranged from 8.6 to 48.1 µM with higher (29.1 to 48.6 µM) and lower (8.6 to 32.8 µM) concentrations during PM1 and PM2, respectively. PO_4 concentrations were observed

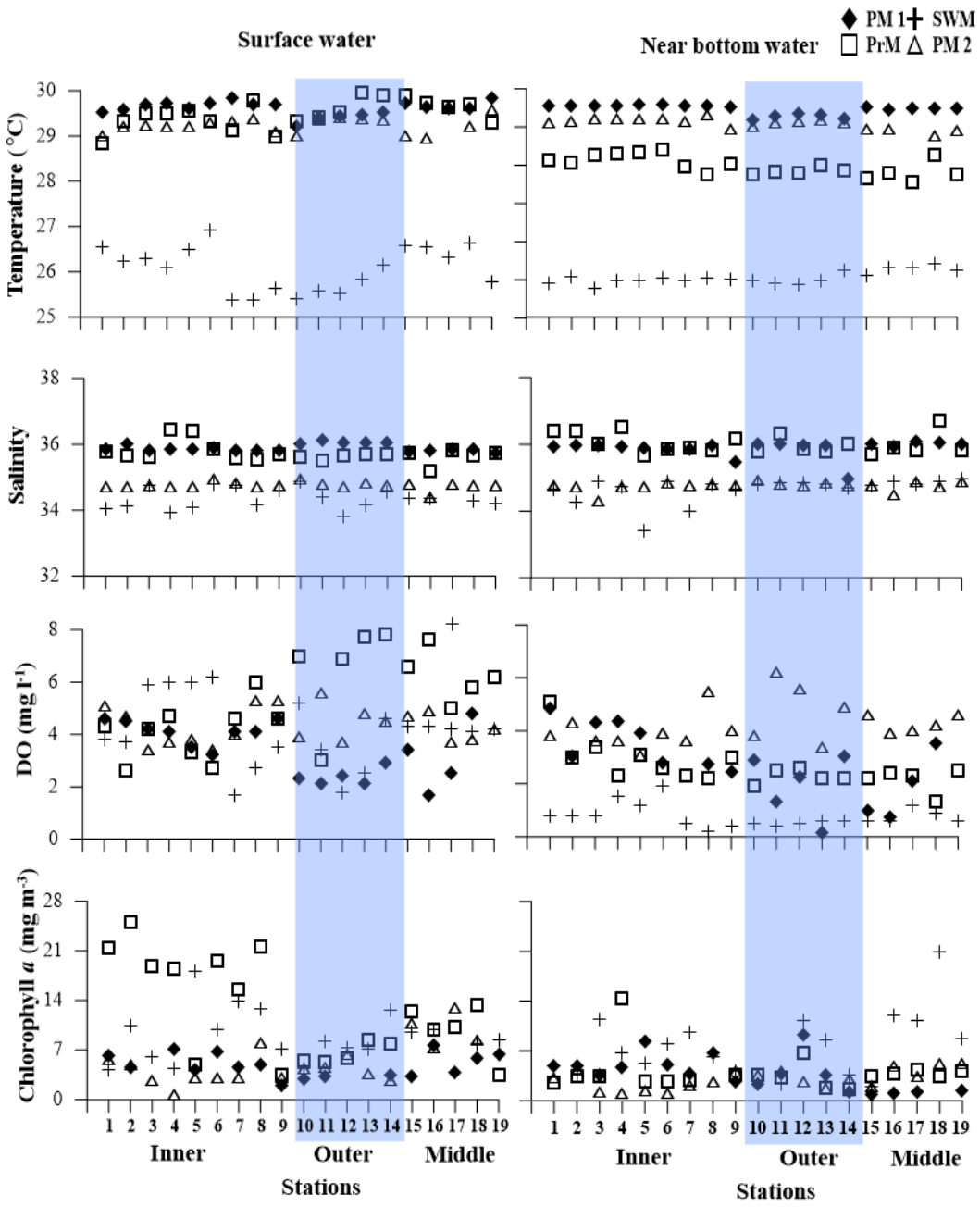

Fig. 3.9 Seasonal variations in the measured environmental variables from the surface and near-bottom (NBW) waters in Mangalore port. Bars represent the values for each station and the standard deviations

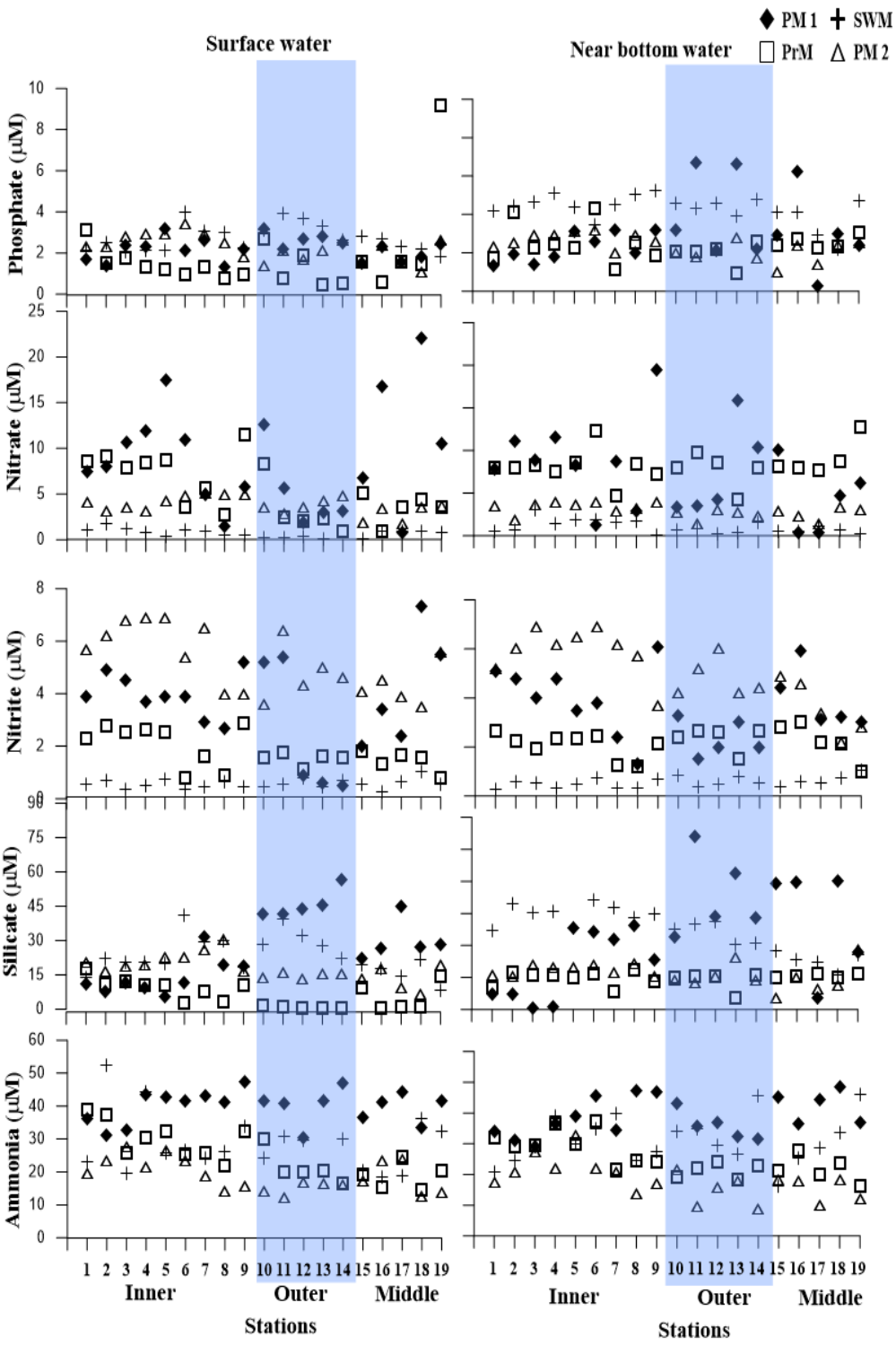

Fig. 3.10 Seasonal variations in the nutrient concentrations from the surface and near-bottom (NBW) waters in Mangalore port. Bars represent the values for each season, and the standard deviations

32.8 µM) concentrations during PM1 and PM2, respectively. PO_4 concentrations were observed higher and lower during SWM (1.8 to 5.3µM) and PrM (0.4 to 3.1 µM), respectively. Higher and lower SiO_4 concentrations were observed during PM 1 (26.6 ± 15.1 µM) and PrM (0.2 to 18.7 µM, Fig. 3.10), respectively. ANOVA of measured environmental parameters revealed significant seasonal variations ($p< 0.05$) and an insignificant difference between the surface water and NBW, except for DO.

3.3.1.3.3 Chennai Port

Environmental parameters (salinity, temperature, DO, BOD, and DIN) showed a distinct seasonal variation, and the trend was similar among the surface water and NBW. The lowest salinity (23.6 to 31.8 PSU) observed during FIM was due to the freshwater discharge, including heavy precipitation in the study area. The water column was relatively warmer (34.4 to 34.6ºC) and high saline (34.0 to 34.6) during SWM, whereas in FIM and SIM, the temperature ranged from 28.0 to 29.4ºC. Lower temperature (26.9 to 27.6ºC) and salinity (29.9 to 30.6 PSU) were observed during NEM. The high DO value was observed during FIM and NEM (4.0 to 5.4 mg l^{-1}), whereas the low DO values (up to 1.9 mg l^{-1}) were observed during SWM (Fig. 3.11).

In general, DIN levels were higher during SWM, followed by FIM, NEM, and SIM. Higher and lower NO_3 concentrations observed during the SWM (13.4 ± 3.1 µM) and SIM (6.5 ± 2.2 µM), respectively, with higher concentrations from surface waters, compared to NBW. NO_2 values were higher (2.3 ± 3.1 µM) and lower (0.9 ± 0.3 µM) during SWM and SIM, respectively. PO_4 concentrations were observed higher and lower during SWM (2.7 to 14.8 µM) and NEM (1.8 ± 0.6 µM), respectively. SiO_3 concentrations found higher and lower during SWM (23.9 ± 6.3 µM) and SIM (6.2 ± 2.1 µM), respectively. NH_4 ranged from 4.8 to 177.2 µM with higher FIM concentrations (10.8 to 177.2 µM, Fig. 3.12). ANOVA of the measured environmental variables

revealed significant seasonal variations (p<0.05) and an insignificant difference between the surface water and NBW.

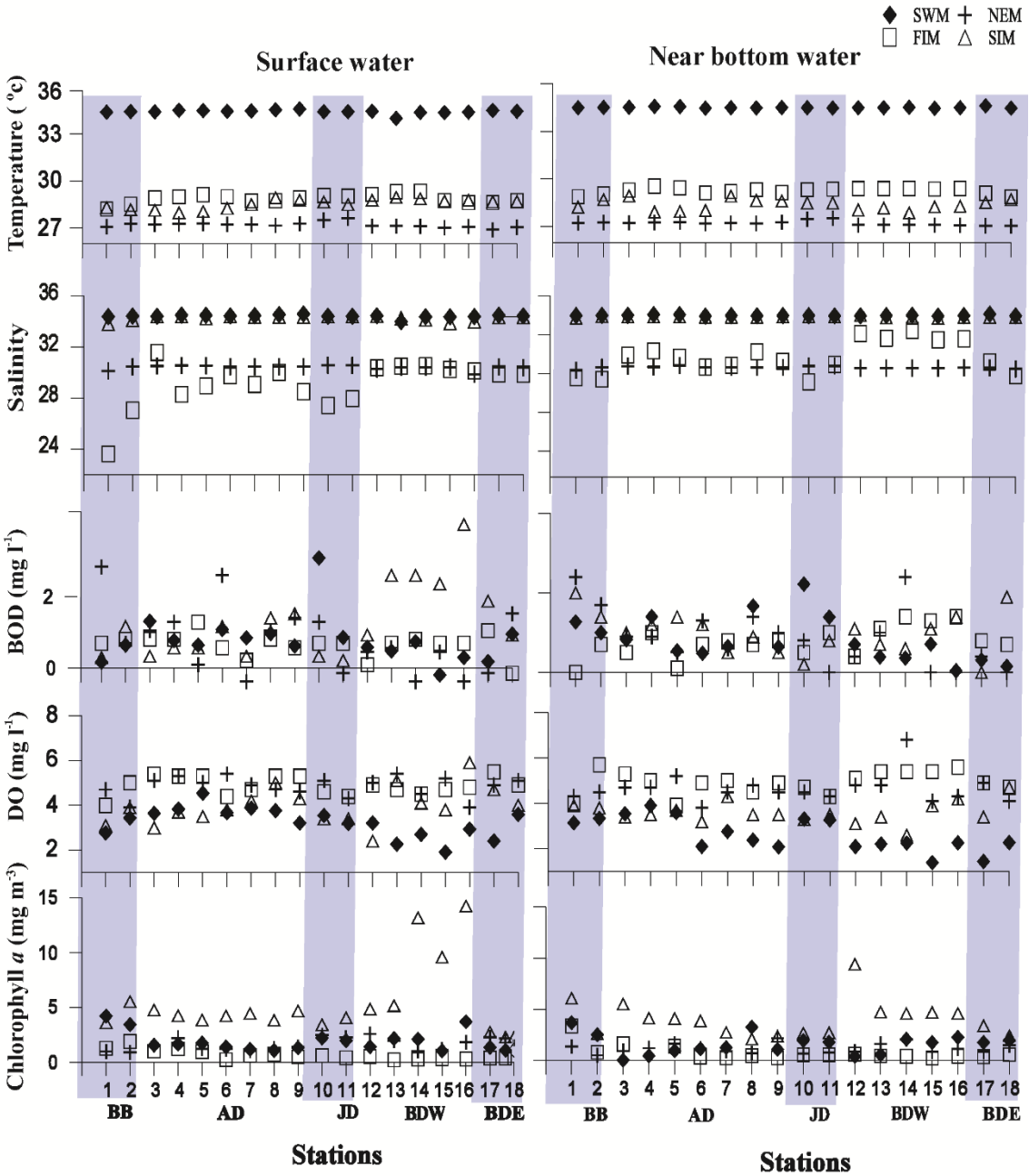

Fig. 3.11 Seasonal variations in the measured environmental variables from the surface and near-bottom (NBW) waters in Chennai port. Bars represent the values for each station and the standard deviations

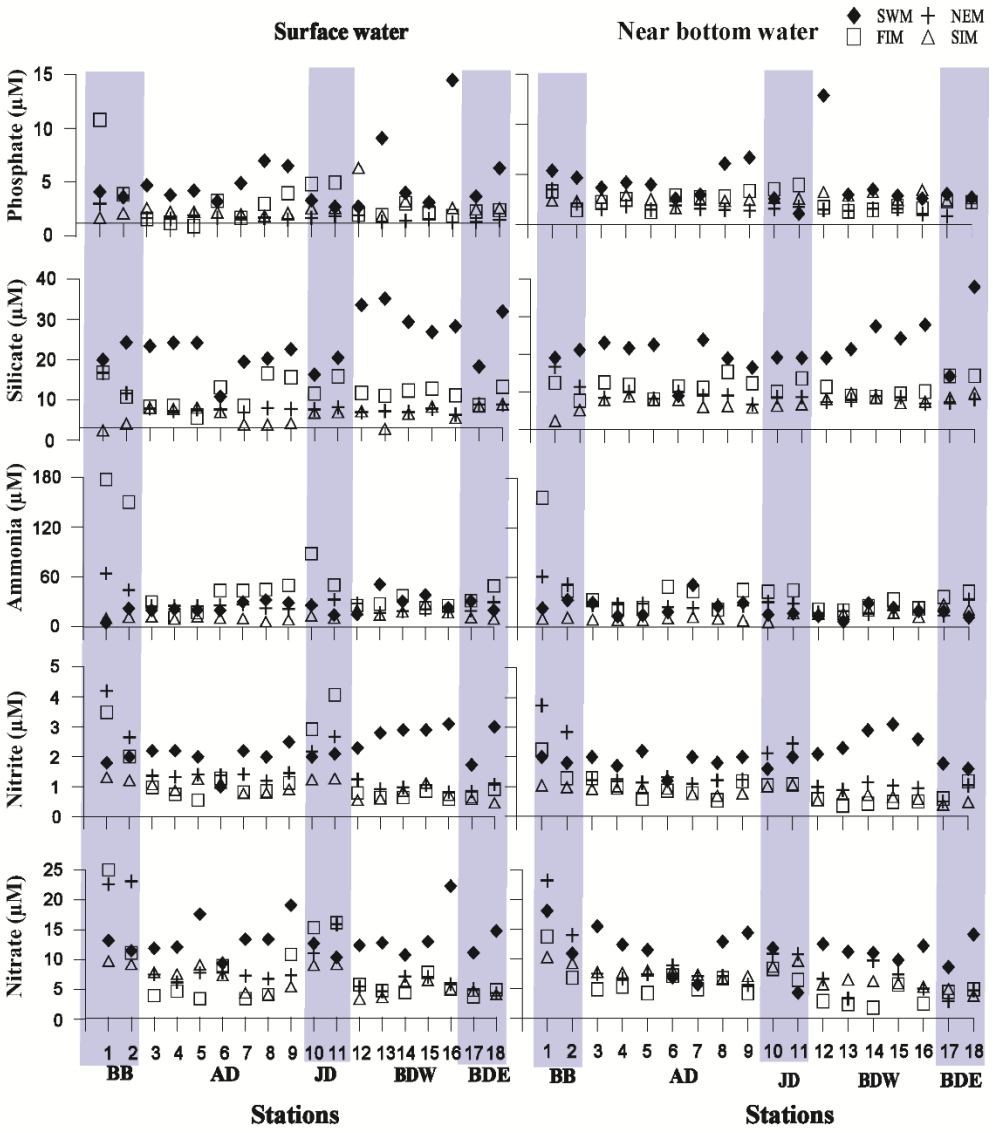

Fig. 3.12 Seasonal variations in the nutrient concentrations from the surface and near-bottom (NBW) waters in Chennai port. Bars represent the values for each season, and the standard deviations

3.3.1.3.4 V.O.C. Port

The hydrographic parameters such as salinity, temperature, BOD, and dissolved inorganic nutrients showed distinct seasonal variations, and the trend was similar between the surface water and NBW. Salinity was > 35 during SWM and FIM in the study region, whereas during NEM

and SIM, it was< 35 at many stations. Water temperature was warmer (29.46 ± 0.18ºC) and cooler (27.69 ± 0.12ºC) during SIM and NEM, respectively. DO range between 4.2 to 7.1 mg l^{-1} with maximum and minimum observed during FIM and NEM, respectively. BOD values were higher (2.7 ± 1.1 mg l^{-1}) and lower (1.3± 1.1 mg l^{-1}) during NEM and FIM, respectively (Fig. 3.13). NO_3 concentrations were higher (3.7 to 34.2 µM) and lower (0.03 to 4.6 µM) during NEM and SWM, respectively. PO_4 concentrations varied between 0.66 to 4.08 µM during the sampling period, with higher concentrations during NEM (0.98 to 3.90 µM). SiO_3 concentrations ranged from 2.51 to 23.44 µM with higher and lower values during SWM (12.3 ± 4.5 µM) and SIM (4.4 ± 0.7 µM), respectively. NH_4 concentrations were observed higher and lower during NEM (9.6 to 46.2 µM) and SWM (5.2 to 24.5 µM), respectively (Fig. 3.14). ANOVA of the measured environmental parameters revealed significant seasonal variations (p< 0.05) and an insignificant between the surface water and NBW.

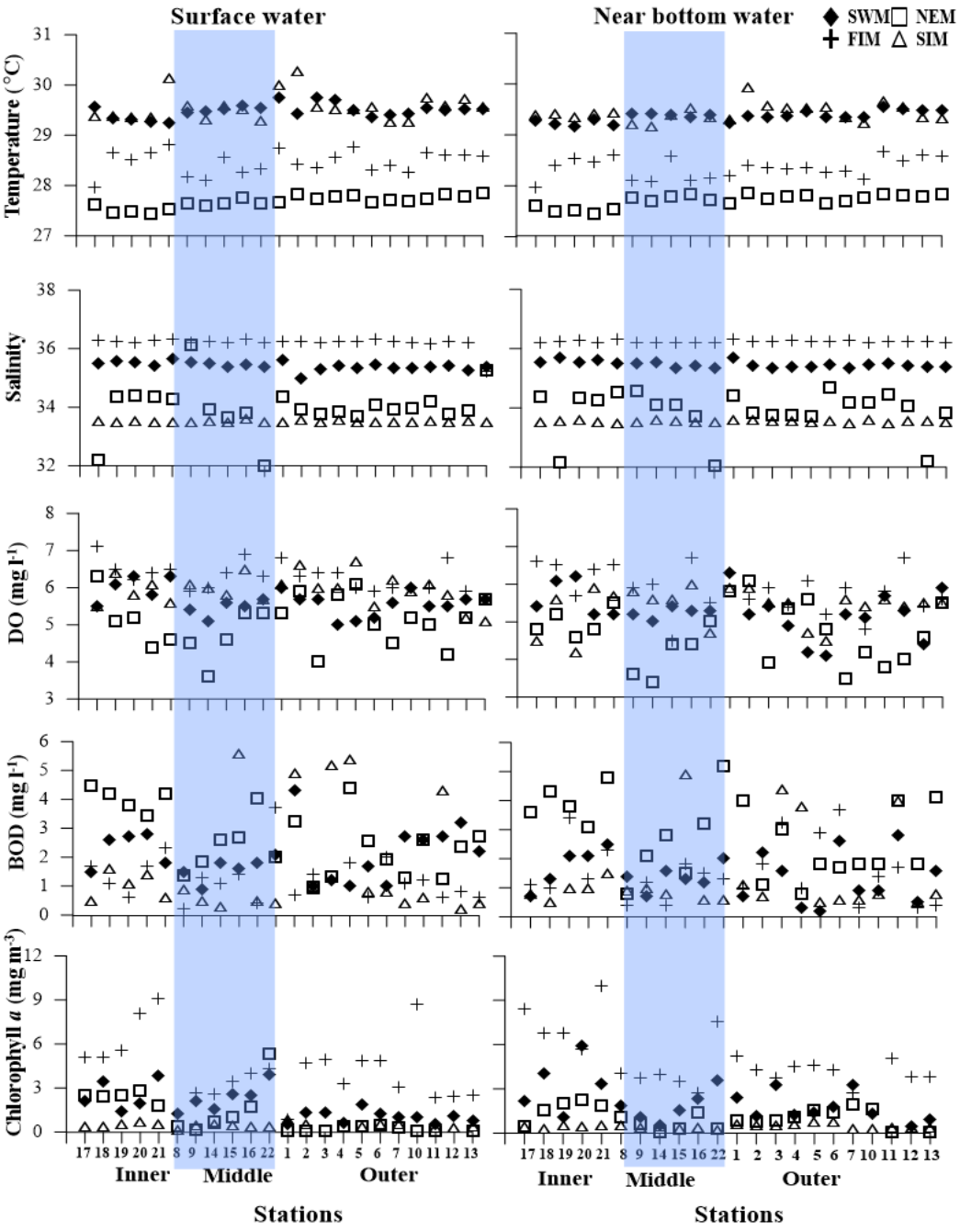

Fig. 3.13 Seasonal variations in the measured environmental variables from the surface and near-bottom (NBW) waters in V.O.C port. Bars represent the values for each station and the standard deviations

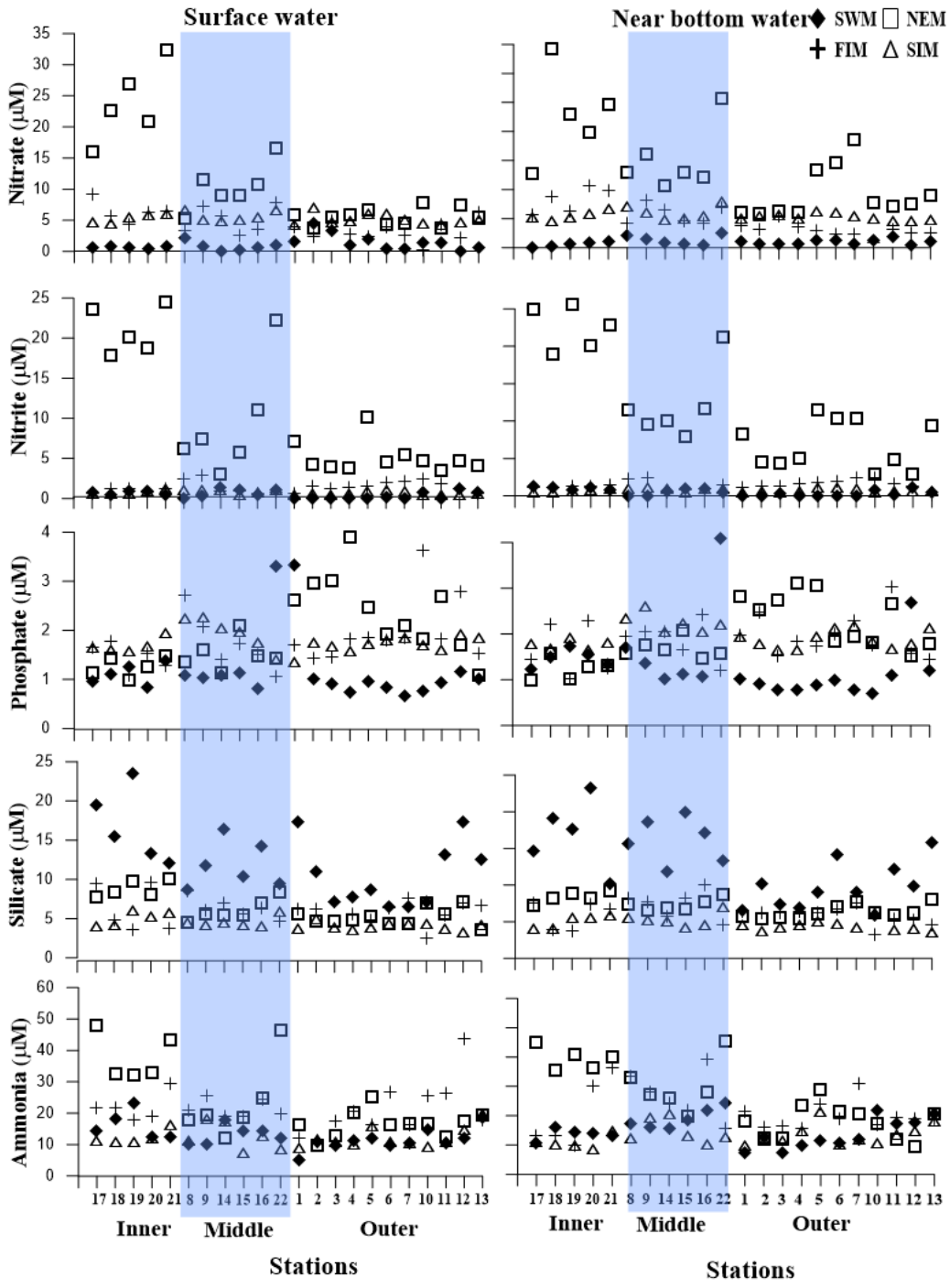

Fig. 3.14 Seasonal variations in the nutrient concentrations from the surface and near-bottom (NBW) waters in V.O.C port. Bars represent the values for each season, and the standard deviations

3.3.2 Inter-seasonal and spatial variation of phytoplankton biomass

3.3.2.1 Freshwater port ecosystems

Phytoplankton biomass exhibited distinct seasonal and spatial variations in Kolkata and Haldia ports. Among the freshwater ports, higher biomass was observed in Kolkata port compared to the Haldia port. In Kolkata port, higher chl *a* values observed during SWM (11.77 ± 11.04 µg l^{-1}) followed by PrM 1 (9.76 ± 4.69 µg l^{-1}), PM (7.18 ± 5.80 µg l^{-1}) and PrM 2 (3.41 ± 1.65 µg l^{-1}, Fig. 3.1). Higher biomass was observed in riverine stations within the port compared to port stations (except during PrM 1 and PM seasons). For instance, phytoplankton biomass during SWM and PrM 2 was higher in the riverine stations (SWM, 26.49 ± 9.11 µg l^{-1} and PrM 2, 4.51 ± 1.39 µg l^{-1}) compared to KOPD (SWM, 7.53 ± 4.73 µg l^{-1} and PrM 2, 2.27 ± 1.27 µg l^{-1}) and NSD (SWM, 5.24 ± 6.79 µg l^{-1} and PrM 2, 3.46 ± 1.85 µg l^{-1}). In Haldia port, biomass was higher during PrM 1 (1.81 ± 1.88 µg l-1), whereas during other seasons, it ranged between 0.50 to 0.74 µg l^{-1} (Fig. 3.3). Biomass was observed higher in the riverine stations compared to port stations (except during PrM's).

3.3.2.1 Estuarine port ecosystems

Among the estuarine ecosystems, phytoplankton biomass was higher in Cochin port compared to Zuari estuary. In Cochin port, higher chl *a* concentrations observed during PrM (6.21 to 69 µg l^{-1}) followed by SWM (15.77 ± 7.62 µg l^{-1}), PM 2 (15.28 ± 11.80 µg l^{-1}), and PM 1 (11.97 ± 5.02 µg l^{-1}, Fig. 3.5). Within the port, higher biomass was observed in EC and MC stations compared to AC stations. In Zuari estuary, distinct seasonal and spatial variations were observed in phytoplankton biomass along the salinity gradient. During PrM, biomass showed an increasing trend from the DS to US stations, while during SWM reverse trend was observed. During PM, biomass did not show much variation along the salinity gradient. In DS stations, higher chl *a*

concentration was observed during SWM (1.43 to 9.51 µg l^{-1}) followed by PM (1.43 to 9.51 µg l^{-1}) and PrM (0.74 to 3.04 µg l^{-1}). In LME stations, chl *a* concentrations were higher during PrM (4.17 ± 3.90 µg l^{-1}) compared to PM (3.84 ± 2.53 µg l^{-1}) and SWM (3.67 ± 3.42 µg l^{-1}). In UME stations, chl *a* concentrations were higher during PrM (3.29 ± 2.20 µg l^{-1}) and PM (3.30 ± 1.30 µg l^{-1}) compared to SWM (2.50 ± 2.69 µg l^{-1}). In US stations, chl *a* concentrations were high during PrM (5.65 ± 3.46 µg l^{-1}) followed by PM (2.76 ± 0.79 µg l^{-1}) and SWM (0.90 ± 0.63 µg l^{-1}, Fig. 3.6).

3.3.2.1 Marine port ecosystems

Among the west coast marine ports, higher and lower biomass was observed in the Mangalore and Kandla port. While a distinct seasonality was observed in east coast marine ports (Chennai and V.O.C ports). In Kandla port, chl *a* concentration in surface water ranged between 0.49 to 1.22 µg l^{-1}, whereas, in NBW, it was ranged from 0.72 to 1.22 µg l^{-1} (Fig. 3.7). Surface water chl *a* was higher during PM's (0.02 to 1.45 µg l^{-1}) followed by PrM (0.28 to 1.23 µg l^{-1}) and SWM (0.14 to 1.19 µg l^{-1}), whereas in NBW reverse trend was observed. In Mangalore port, higher biomass observed round the year with the maximum during PrM (12.15 ± 6.60 µg l^{-1}) and SWM (9.01 ± 3.53 µg l^{-1}) compared to PM 2 (5.30 ± 3.07 µg l^{-1}) and PM 1 (4.83 ± 1.62 µg l^{-1}, Fig. 3.9). East coast marine ports showed distinct seasonal and spatial variations in Phytoplankton biomass distributions. In Chennai port, biomass was higher during SIM (2.12 to 14.37 µg l^{-1}) followed by SWM (0.05 to 4.23 µg l^{-1}), NEM (0.52 to 2.60 µg l^{-1}), and FIM (0.23 to 3.37 µg l^{-1}, Fig. 3.11). While in V.O.C port, biomass was higher during FIM (0.89 to 9.98 µg l^{-1}) followed by SWM (0.47 to 5.93 µg l^{-1}), NEM (0.04 to 5.36 µg l^{-1}), and SIM (0.13 to 0.87 µg l^{-1}, Fig. 3.13). Marine ports showed an insignificant variation between the station but relatively higher biomass

observed from the inner port stations with restricted water circulations compared to the stations with high water circulations (except for Kandla port).

3.3.3 Distribution of PFG's from different port ecosystems

3.3.3.1 Freshwater port ecosystem

3.3.3.1.1 Kolkata port

Phytoplankton marker pigments and PFG's showed distinct seasonal and spatial variations. Altogether 16 marker pigments were identified, of which a high concentration of only four maker pigments (lut, fuco, zea, and allo) was detected (Table 3.1). *CHEMTAX* analysis revealed three major (chlorophytes, diatom, and cryptophytes) and five minor (cyanobacteria, prasinophytes, prymnesiophytes, chrysophytes, and dinoflagellates) groups, but the distribution pattern for the dominant PFG was not the same. Chlorophytes contributed maximum during PrM 1 (50.1% with 4.50 µg l^{-1}) and PrM 2 (61% with 2.00 µg l^{-1}) followed by PM (28.2% with 1.22 µg l^{-1}) and SWM (36% with 3.06 µg l^{-1}). Next to chlorophytes, diatom contributed maximum during SWM (41.2% with 6.45 µg l^{-1}) and PM (39.7% with 3.17 µg l^{-1}) compared to PrM 1 (30.1% with 3.18 µg l^{-1}) and PrM 2 (20.4% with 0.71 µg l^{-1}). Higher cryptophytes contributions observed during PrM 2 (14.6% with 0.47 µg l^{-1}) followed by PrM 1 (11.5% with 1.25 µg l^{-1}), SWM (10.8% with 0.84 µg l^{-1}), and PM (7.8% with 0.38 µg l^{-1}). Cyanobacteria contributed more during SWM (6.7% with 0.97 µg l^{-1}) and PM (6.1% with 0.33 µg l^{-1}) compared to PrM 1 (5.8% with 0.60 µg l^{-1}) and PrM 2 (1.3% with 0.04 µg l^{-1}). Prasinophytes evident maximum during SWM (4.8%) season (Fig. 3.15 and Fig 3.16). ANOVA results from PFG's distributions revealed significant variations (p<0.05) between the season whereas, an insignificant difference was observed between the surface water and NBW.

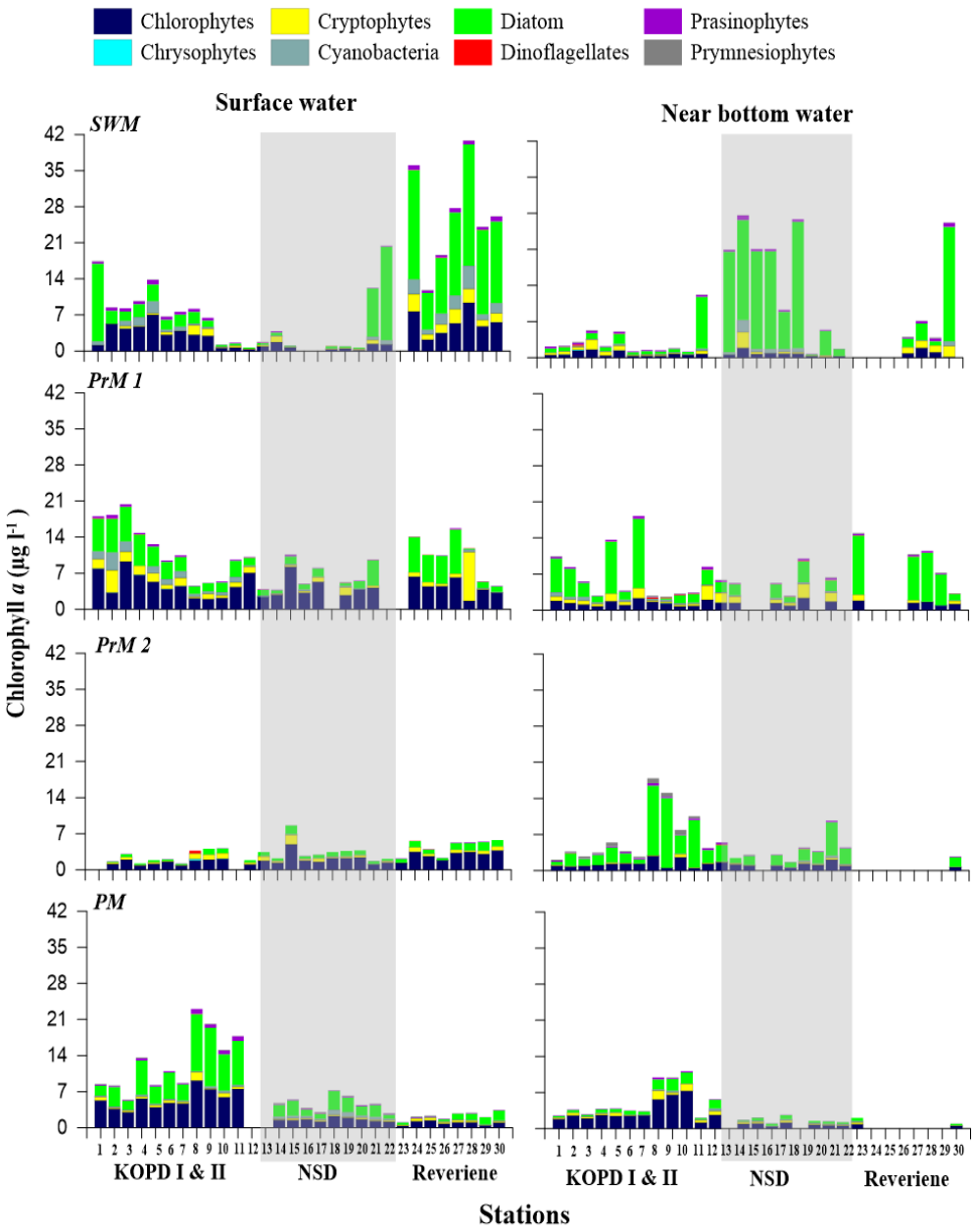

Fig. 3.15 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the overlying waters surface and near bottom in Kolkata port.

Table 3.1 Seasonal variations in the concentrations of chlorophylls, carotenoids from the surface water, near bottom water (NBW). Note SWM – southwest monsoon, PrM1 –pre monsoon 1, PrM2 – pre monsoon 2, PM – post monsoon, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids.

Pigments	Average and range of pigment concentrations from Mangalore port							
	SWM		PrM 1		PrM 2		PM	
	Surface	NBW	Surface	NBW	Surface	NBW	Surface	NBW
Chlorophylls								
Chl *a* ($\mu g\ l^{-1}$)	11.77 (0.65-40.85)	8.61 (0.72-27.62)	9.76 (3.83-20.36)	7.26 (2.62-18.24)	3.41 (1.20-8.63)	5.49 (1.68-17.89)	7.18 (1.04-23.01)	3.68 (0.93-11.14)
Chl *b* ($\mu g\ l^{-1}$)	0.70 (0.00-1.65)	0.40 (0.10-1.03)	0.60 (0.00-1.29)	0.48 (0.21-0.88)	0.20 (0.09-0.40)	0.37 (0.12-0.86)	0.53 (0.07-1.72)	0.31 (0.07-1.00)
Chl *c* ($\mu g\ l^{-1}$)	0.57 (0.00-2.65)	0.49 (0.00-2.19)	0.07 (0.01-0.21)	0.04 (0.01-0.09)	0.01 (0.00-0.08)	0.02 (0.01-0.07)	0.07 (0.01-0.24)	0.06 (0.02-0.16)
Carotenoids								
PSC								
19' bf ($ng\ l^{-1}$)	9.9 (0.0-54.9)	13.1 (0.0-231.1)	21.2 (0.0-69.8)	6.3 (0.0-42.1)	25.1 (0.0-523.3)	6.6 (0.0-38.6)	6.4 (0.0-41.4)	5.5 (0.0-21.8)
Fuco ($\mu g\ l^{-1}$)	1.78 (0.05-6.33)	1.57 (0.06-5.81)	1.21 (0.00-3.24)	1.17 (0.31-3.31)	0.51 (0.00-1.18)	1.24 (0.17-4.67)	0.60 (0.08-1.76)	0.34 (0.12-1.06)
19' hf ($ng\ l^{-1}$)	45.4 (0.0-183.0)	64.7 (0.0-265.2)	9.3 (0.0-111.6)	(0.0-179.3)	16.3 (0.0-60.8)	55.4 (0.0-227.5)	(0.0-97.8)	(0.0-54.2)
Peri ($ng\ l^{-1}$)	12.9 (0.0-71.4)	17.0 (0.0-300.5)	27.6 (0.0-90.7)	58.0 (0.0-210.6)	32.6 (0.0-680.6)	7.1 (0.0-77.4)	8.4 (0.0-53.9)	0
PPC								
Allo ($ng\ l^{-1}$)	33.4 (0.0-124.3)	28.6 (0.0-84.9)	30.7 (0.0-106.3)	55.6 (10.1-137.3)	12.8 (0.0-28.6)	36.7 (0.0-149.7)	31.8 (6.9-55.2)	21.7 (5.7-77.0)
B-car ($ng\ l^{-1}$)	202.2 (13.9-663.3)	141.1 (16.3-512.6)	177.9 (69.6-510.2)	133.6 (50.1-308.2)	41.0 (6.8-131.0)	61.3 (18.1-187.4)	129.7 (16.9-312.8)	80.8 (10.9-262.1)
Diad ($ng\ l^{-1}$)	267.7 (8.1-931.4)	308.2 (20.6-1210.8)	254.5 (0.0-649.9)	198.7 (40.8-522.9)	96.1 (17.9-242.2)	200.4 (33.7-716.0)	105.0 (10.8-269.0)	73.2 (26.1-203.9)
Diat ($ng\ l^{-1}$)	223.3 (11.2-745.2)	116.7 (21.6-394.0)	271.6 (57.3-680.7)	148.4 (59.6-288.9)	136.7 (53.4-311.2)	193.7 (44.2-643.1)	268.3 (34.8-790.0)	145.8 (47.8-356.5)
Lut ($ng\ l^{-1}$)	325.9 (30.2-984.1)	189.7 (0.0-469.7)	267.4 (0.0-516.3)	276.7 (137.6-465.8)	133.6 (47.7-297.5)	230.7 (90.3-579.2)	208.5 (26.8-558.3)	149.0 (32.0-521.9)
Viola ($ng\ l^{-1}$)	105.5 (9.1-332.8)	60.3 (15.3-209.9)	101.9 (0.0-223.1)	76.8 (0.0-182.1)	29.7 (0.0-97.7)	48.1 (0.0-118.0)	73.0 (8.5-216.0)	44.4 (17.3-127.6)
Zea ($\mu g\ l^{-1}$)	0.52 (0.01-2.22)	0.24 (0.03-1.22)	0.43 (0.09-1.90)	0.24 (0.08-0.60)	0.07 (0.01-0.22)	0.08 (0.02-0.26)	0.28 (0.05-0.54)	0.20 (0.07-0.51)
Neo ($ng\ l^{-1}$)	71.3 (0.0-333.8)	50.1 (0.0-133.8)	78.6 (0.0-163.0)	59.6 (0.0-146.8)	32.2 (0.0-104.9)	62.0 (0.0-195.0)	65.4 (0.0-154.6)	55.0 (0.0-218.3)
Pras ($ng\ l^{-1}$)	82.1 (5.5-322.4)	36.4 (0.0-142.2)	48.2 (0.0-131.8)	37.9 (0.0-153.2)	24.2 (0.0-90.6)	20.1 (0.0-100.6)	50.9 (0.0-219.4)	32.0 (0.0-123.7)

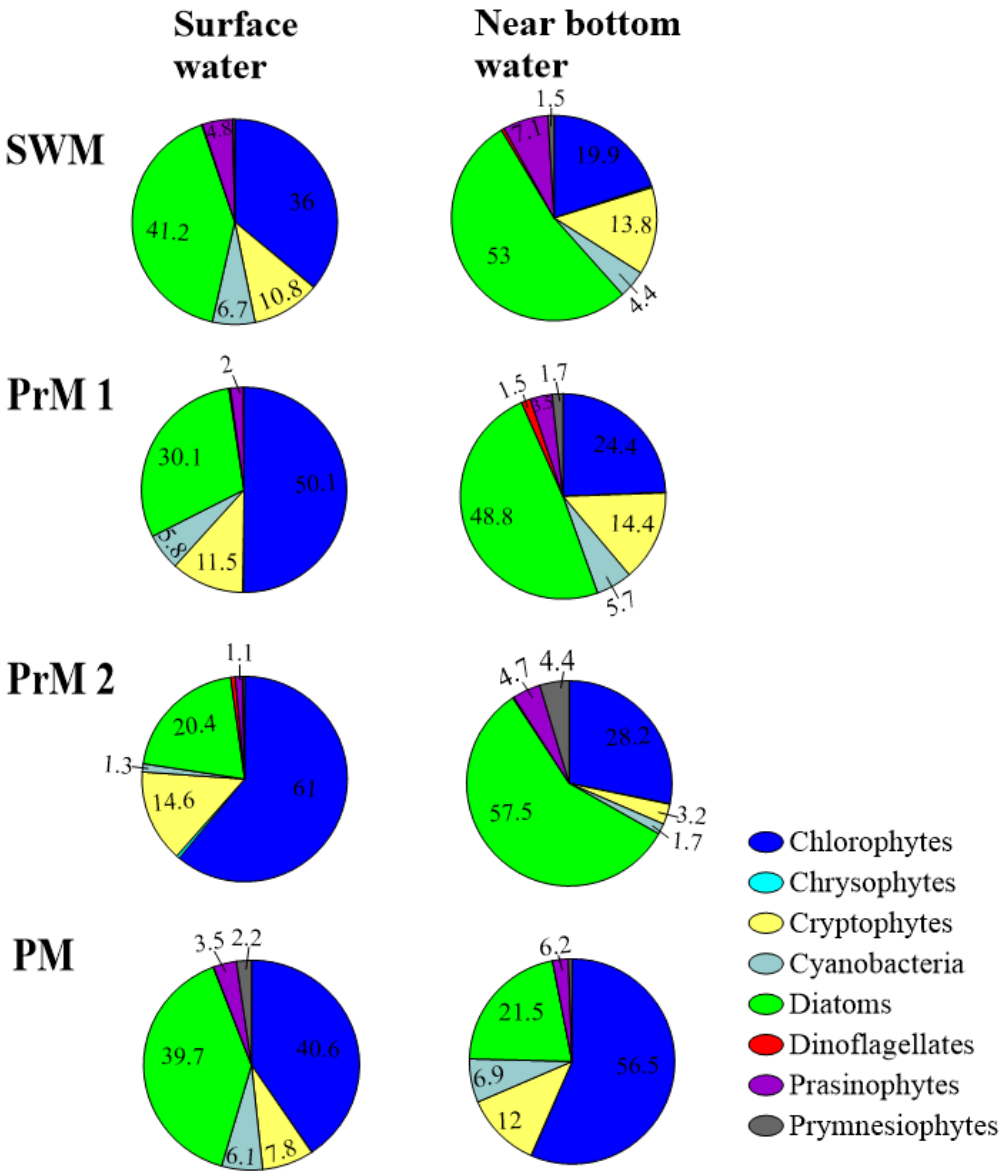

Fig. 3.16 Pie charts showing the seasonal variations in phytoplankton groups derived from CHEMTAX analysis for the surface and near bottom – NBW in Kolkata port. Average values from the 30 stations were used.

3.3.3.1.2 Haldia port (Haldia dock system)

Overall, 16 pigments were identified from the Haldia dock system but, the concentrations were lower than Kolkata port. Moreover, the presence of fuco, allo, Lut, zea, pras indicated the existence of diatom, cryptophytes, green algae, cyanobacteria, and prasinophytes (Table 3.2). *CHEMTAX* analysis revealed four major (chlorophytes, cryptophytes, cyanobacteria, and diatom)

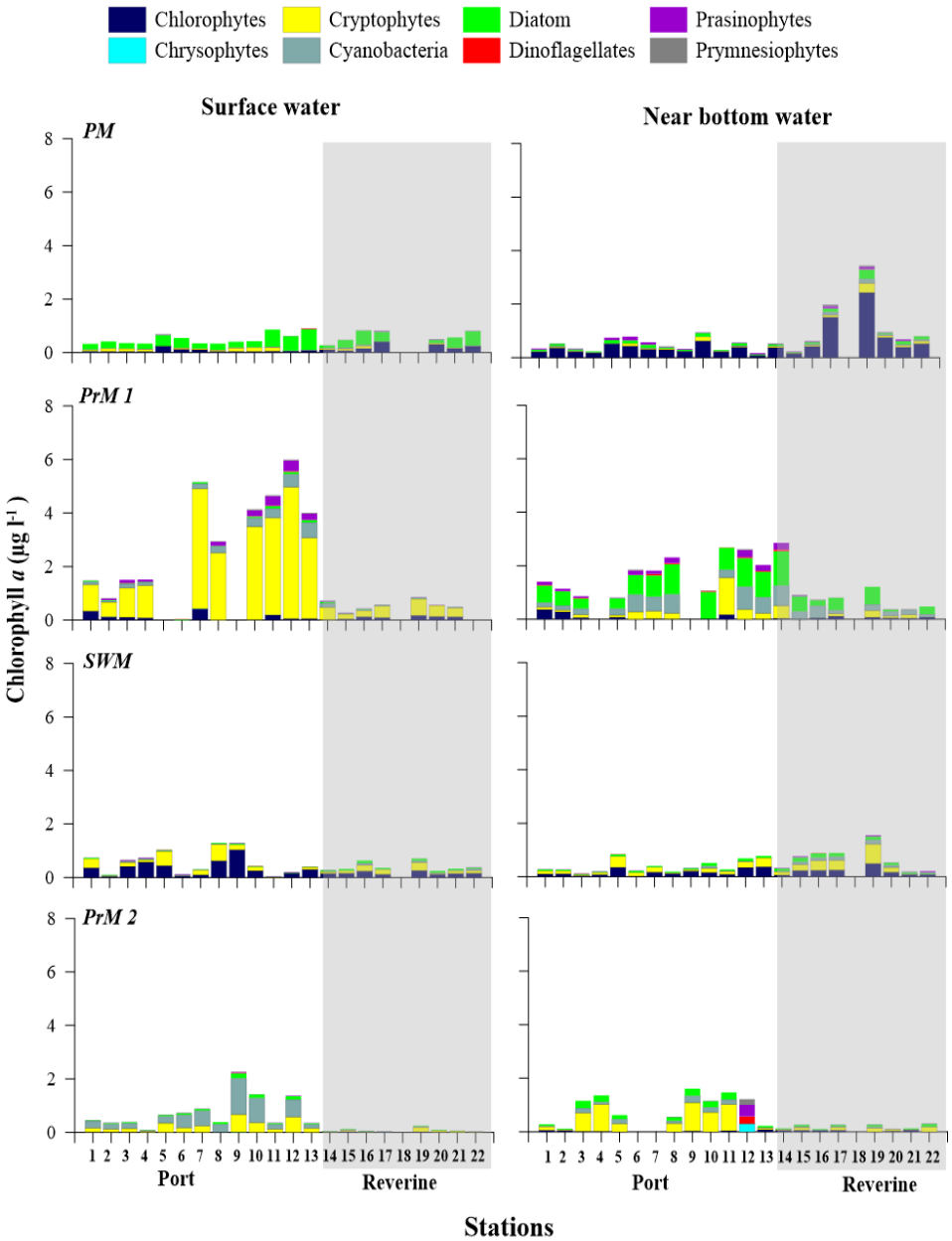

Fig. 3.17 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the overlying waters surface and near bottom in Haldia port.

Table 3.2 Seasonal variations in the concentrations of chlorophylls and carotenoids from the surface water, near bottom water (NBW) in Haldia port. Note SWM – southwest monsoon, PrM1 –pre monsoon 1, PrM2 – pre monsoon 2, PM – post monsoon, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids.

Pigments	Average and range of pigment concentrations from Mangalore port							
	PM		PrM 1		SWM		PrM 2	
	Surface	NBW	Surface	NBW	Surface	NBW	Surface	NBW
Chlorophylls								
Chl *a* (μg l^{-1})	0.54 (0.27-0.91)	0.74 (0.17-3.44)	1.81 (0.00-6.00)	1.39 (0.37-2.86)	0.51 (0.05-1.30)	0.50 (0.12-1.56)	0.50 (0.03-2.27)	0.60 (0.10-1.61)
Chl *b* (μg l^{-1})	0.07 (0.02-0.26)	0.11 (0.02-0.42)	0.08 (0.00-0.25)	0.08 (0.01-0.29)	0.04 (0.00-0.16)	0.03 (0.01-0.10)	0.02 (0.00-0.08)	0.02 (0.01-0.04)
Chl *c* (μg l^{-1})	0.01 (0.00-0.02)	0.01 (0.00-0.05)	0.02 (0.00-0.28)	0.01 (0.00-0.05)	0.003 (0.00-0.02)	0.005 (0.00-0.02)	0.004 (0.00-0.02)	0
Carotenoids								
PSC								
19'bf (ng l^{-1})	0.5 (0.0-2.2)	0	1.2 (0.0-23.0)	0	0.3 (0.0-6.7)	0	0.4 (0.0-5.8)	0
Fuco (ng l^{-1})	59.7 (20.9-151.3)	68.0 (17.2-258.7)	102.4 (5.3-272.3)	105.4 (35.2-201.3)	37.2 (0.0-98.9)	63.1 (16.6-153.3)	45.6 (5.7-185.5)	65.2 (0.0-145.0)
19'hf (ng l^{-1})	2.4 (0.0-12.2)	6.9 (0.0-50.8)	11.4 (0.0-43.2)	10.0 (0.0-34.8)	3.5 (0.0-26.8)	9.8 (0.0-32.4)	2.7 (0.0-10.3)	5.9 (0.0-23.9)
Peri (ng l^{-1})	1.4 (0.0-27.3)	0	14.7 (0.0-64.3)	18.7 (0.0-61.6)	0	0	1.5 (0.0-32.1)	0
PPC								
Allo (ng l^{-1})	3.5 (0.0-12.8)	8.1 (0.0-35.5)	3.1 (0.0-22.2)	0.9 (0.0-6.6)	7.6 (0.0-34.5)	7.9 (0.0-38.7)	1.0 (0.0-11.6)	6.1 (0.0-33.6)
B-car (ng l^{-1})	15.3 (5.8-44.0)	21.4 (2.9-97.0)	37.5 (0.0-116.5)	21.5 (0.0-49.2)	13.7 (0.0-44.7)	13.9 (0.0-28.7)	20.5 (0.0-107.6)	16.0 (0.0-35.3)
Diad (ng l^{-1})	14.1 (6.3-26.8)	14.4 (3.1-42.1)	22.3 (0.0-84.2)	22.5 (10.3-38.4)	8.1 (0.0-27.4)	11.5 (3.4-27.9)	7.6 (0.9-35.3)	11.7 (0.0-35.6)
Diat (ng l^{-1})	14.9 (4.8-23.5)	17.7 (1.8-62.5)	90.3 (0.0-287.2)	49.0 (0.0-109.3)	42.3 (7.3-168.7)	23.3 (0.0-50.7)	21.2 (0.0-114.3)	13.9 (0.0-34.4)
Lut (ng l^{-1})	21.4 (8.1-45.5)	41.7 (5.4-181.5)	18.9 (0.0-143.6)	8.5 (0.0-39.0)	26.6 (0.0-113.6)	30.8 (0.0-68.9)	0.8 (0.0-9.8)	15.7 (0.0-52.5)
Viola (ng l^{-1})	9.5 (0.0-19.7)	12.9 (0.0-32.6)	0.3 (0.0-3.2)	0.0 (0.0-0.0)	4.2 (0.0-32.2)	7.4 (0.0-33.7)	7.5 (0.0-59.4)	0.2 (0.0-2.3)
Zea (ng l^{-1})	22.3 (10.8-44.9)	38.9 (7.8-171.9)	62.0 (0.0-226.4)	50.7 (0.0-113.7)	21.6 (0.0-43.9)	32.4 (0.0-112.5)	49.1 (0.0-243.9)	54.1 (8.8-154.9)
Neo (ng l^{-1})	4.1 (0.0-14.6)	4.4 (0.0-23.3)	22.1 (0.0-125.8)	15.2 (0.0-58.7)	3.6 (0.0-23.3)	10.4 (0.0-33.5)	1.8 (0.0-13.4)	5.7 (0.0-25.1)
Pras (ng l^{-1})	1.5 (0.0-12.6)	7.6 (0.0-35.2)	39.1 (0.0-191.8)	31.4 (0.0-105.5)	4.5 (0.0-20.6)	9.0 (0.0-25.6)	3.3 (0.0-18.5)	5.0 (0.0-20.2)

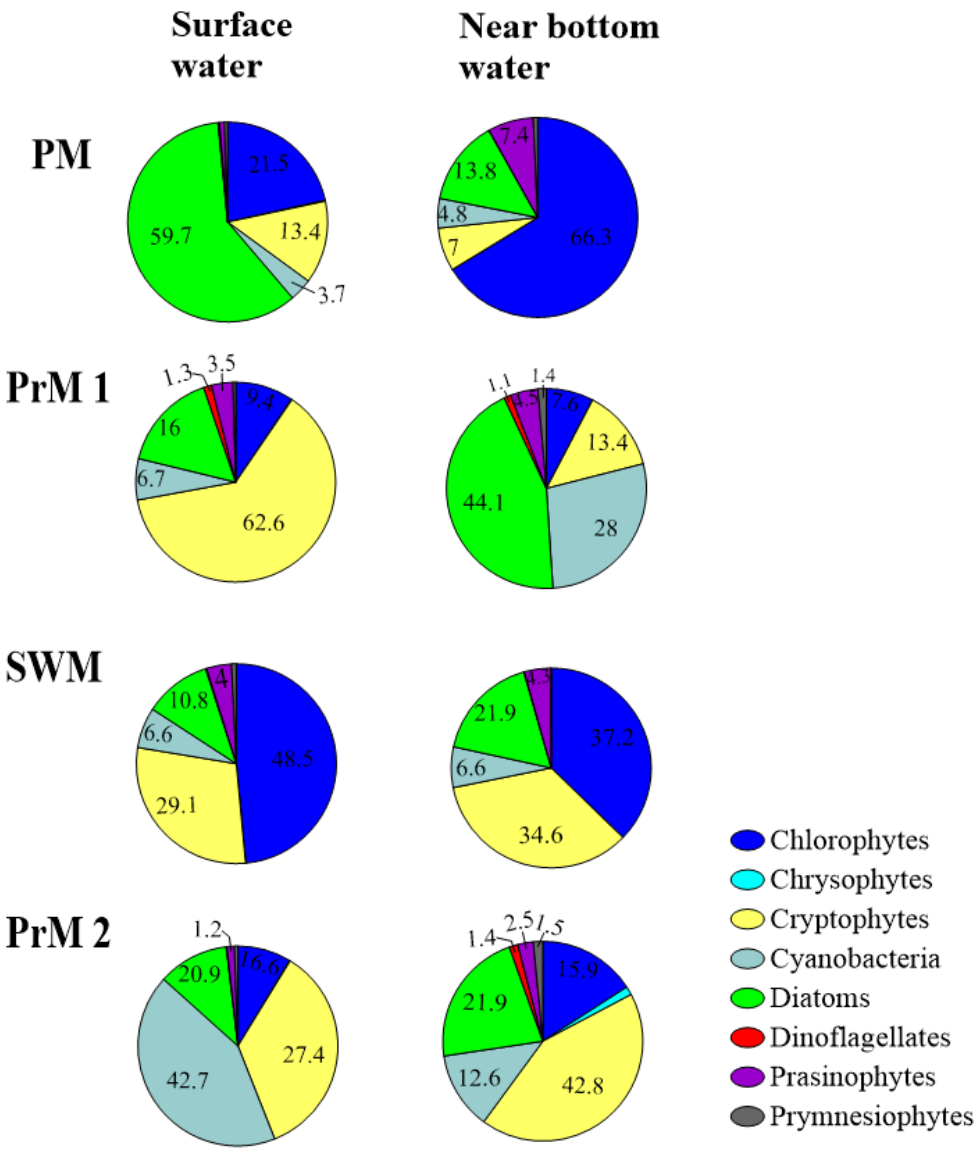

Fig. 3.18 Pie charts showing the seasonal variations in phytoplankton groups derived from CHEMTAX analysis for the surface and near bottom – NBW in Haldia port. Average values from the 22 stations were used

and four minor (chrysophytes, cyanobacteria, prasinophytes, and prymnesiophytes) groups. Chlorophytes in the surface water contributed maximum during SWM (48.5% with 0.27 µg l^{-1}) followed by PM (21.5% with 0.12 µg l^{-1}), PrM 1 (9.4% with 0.10 µg l^{-1}) and PrM 2 (8.6% with 0.07 µg l^{-1}) whereas in NBW, higher contributions observed during PM (66.3% with 0.50 µg l^{-1}) followed by SWM (37.2% with 0.18 µg l^{-1}), PrM 1 (15.9% with 0.03 µg l^{-1}) and PrM 2 (7.6% with 0.08 µg l^{-1}). Higher diatom contribution observed during PM (59.7% with 0.34 µg l^{-1}) and PrM 1 (44.1% with 0.62 µg l^{-1}) from the surface and NBW, respectively. Cryptophytes contributed more during PrM 1 (62.6% with 1.43 µg l^{-1}) and PrM 2 (33.2% with 0.16 µg l^{-1}) compared to other seasons. The highest cyanobacteria contribution observed during PrM 2 (42.7% with 0.27 µg l^{-1}) compared to other seasons. Prasinophytes were evident during PrM 1 (3.5%) and SWM (4%) seasons (Fig. 3.17 and Fig 3.18). ANOVA results of the PFG's distributions data revealed significant variations (p<0.05) between the season. In contrast, an insignificant difference was observed (except for diatom and prasinophytes) between the surface water and NBW.

3.3.3.2 Estuarine port ecosystem

3.3.3.2.1 Cochin port

Altogether, 15 marker pigments (except allo, exclusive marker pigment for cryptophytes) were detected from the Cochin port. Presence of fuco (diatom), lut (chlorophytes), zea (cyanobacteria), peri (dinoflagellates), pras (prasinoxanthin), and 19'bf (prymnesiophytes) indicating the contribution of their respective PFG's (Table 3.3). *CHEMTAX* analysis revealed three major (diatom, chlorophytes, and cyanobacteria) and four minor (chrysophytes, dinoflagellates, prasinophytes, prymnesiophytes) PFG's. Cryptophytes were absent in the Cochin Port. Among the major PFG's diatom contribution was observed higher during PM 1

(77.9% with 9.21 µg l^{-1}) followed by SWM (67.4% with 11.01 µg l^{-1}), PrM (66.9% with 13.54 µg l^{-1}), and PM 2 (64.1% with 10.64 µgl^{-1}). Diatom contribution was higher in EC and MC stations compared to AC stations (except during SWM). Chlorophytes contribution was higher during SWM (26.6% with 3.94 µg l^{-1}) followed by PrM (13.7% with 1.94 µg l^{-1}), PM 1 (13.3% with 1.80 µg l^{-1}) and PM 2 (4.5% with 0.68 µg $^{-1}$). Generally, chlorophytes contributions were higher

Table 3.3 Seasonal variations in the concentrations of chlorophylls and carotenoids from the surface water Cochin port. Note SWM – southwest monsoon, PrM –pre monsoon, PM21 – post monsoon 1, PM2 – post monsoon 2, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids

Pigments	**Average and range of pigment concentrations from Cochin port**			
	PM 1	**PrM**	**SWM**	**PM 2**
Chlorophylls				
Chl *a* (µg l^{-1})	11.97 (5.29-24.49)	18.37 (6.21-69.00)	15.77 (6.76-31.80)	15.28 (3.40-46.31)
Chl *b* (µg l^{-1})	0.22 (0.00-0.74)	0.93 (0.17-1.46)	0.70 (0.33-1.27)	0.32 (0.05-0.84)
Chl *c* (µg l^{-1})	2.05 (0.95-4.33)	4.56 (1.03-21.23)	2.36 (0.88-5.70)	2.05 (0.38-5.70)
Carotenoids				
PSC				
19' bf (ng l^{-1})	14.6 (1.0-143.1)	15.4 (1.0-62.5)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Fuco (µg l^{-1})	2.43 (1.02-5.17)	4.24 (0.93-19.74)	3.20 (1.19-6.94)	3.28 (0.46-9.53)
19' hf (ng l^{-1})	142.0 (1.0-434.0)	221.8 (1.0-2098.8)	101.8 (1.0-239.1)	220.8 (19.3-736.3)
Peri (ng l^{-1})	305.4 (96.6-765.9)	362.5 (60.8-2088.4)	220.7 (0.0-463.0)	295.4 (57.9-819.4)
PPC				
Allo (ng l^{-1})	0	0	0	0
B-car (µg l^{-1})	0.28 (0.00-0.70)	1.18 (0.33-3.84)	0.87 (0.45-1.44)	0.86 (0.26-2.95)
Diad (ng l^{-1})	263.0 (74.0-566.9)	877.3 (326.8-2915.0)	295.4 (146.7-490.1)	334.3 (52.5-1176.9)
Diat (ng l^{-1})	249.9 (71.4-826.8)	488.5 (64.1-1362.8)	678.5 (144.5-1956.1)	148.8 (43.9-254.7)
Lut (ng l^{-1})	20.1 (0.0-60.6)	34.4 (0.0-276.3)	287.2 (144.5-515.5)	95.9 (28.5-256.4)
Viola (ng l^{-1})	95.3 (0.0-450.9)	69.9 (0.0-199.5)	100.1 (33.2-186.2)	33.7 (0.0-146.8)
Zea (µg l^{-1})	0.17 (0.12-0.30)	1.08 (0.38-2.32)	0.43 (0.13-0.98)	1.10 (0.36-3.26)
Neo (ng l^{-1})	25.4 (0.0-304.6)	108.6 (0.0-200.2)	0	0
Pras (ng l^{-1})	11.4 (0.0-136.7)	87.4 (0.0-155.1)	4.9 (0.0-58.4)	12.1 (0.0-90.8)

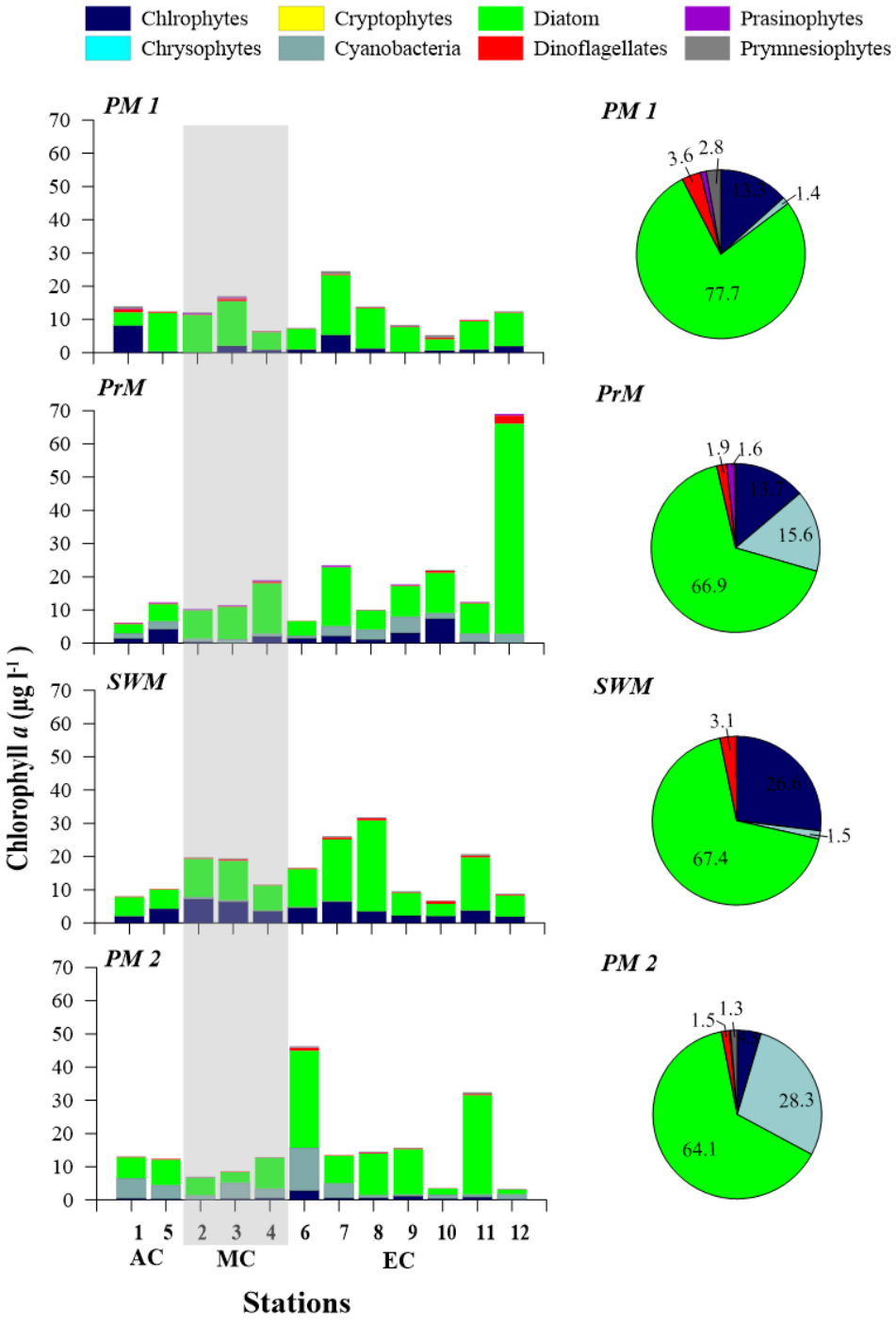

Fig. 3.19 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the surface waters in Cochin port. Pie charts showing the seasonal variations in PFG's contribution. Average values from the 12 stations were used

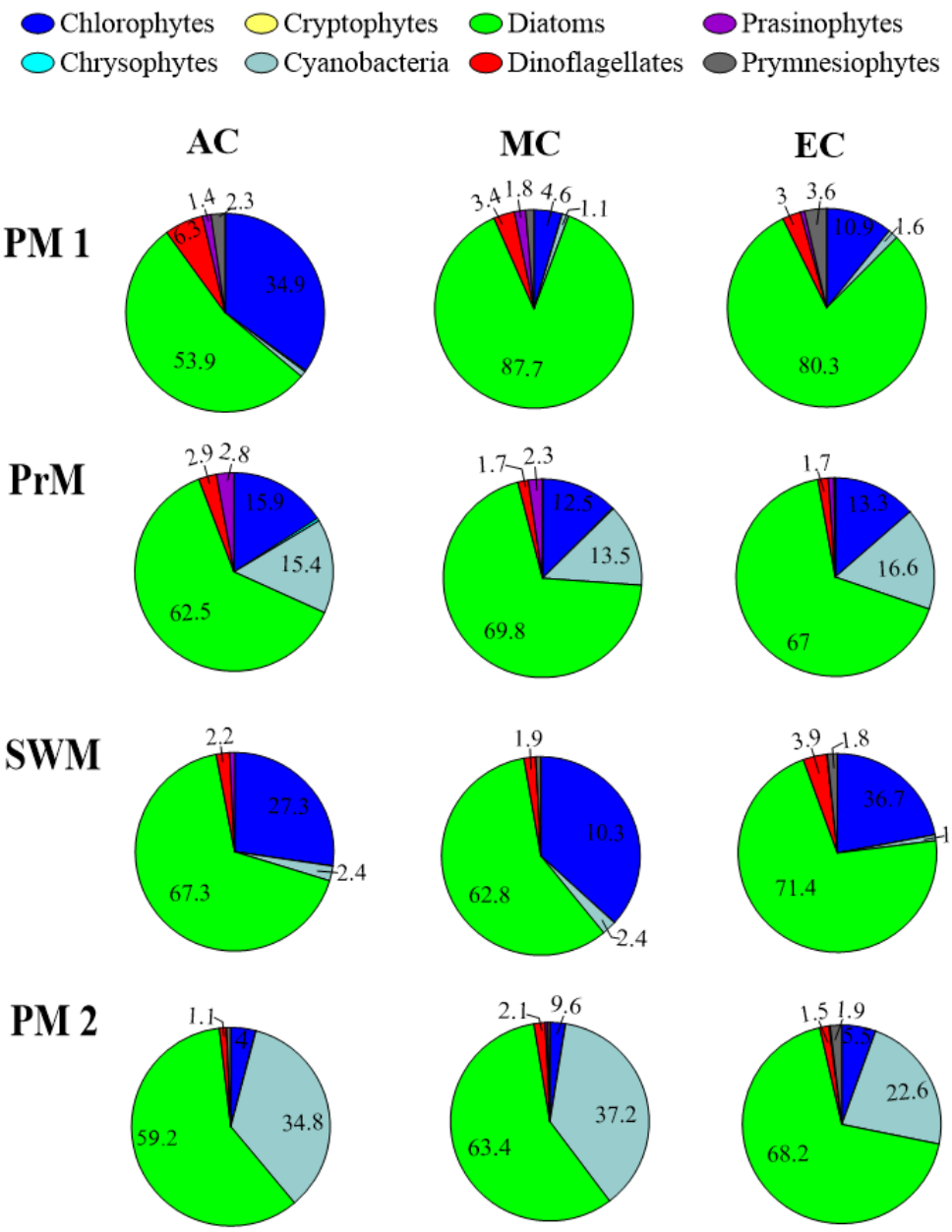

Fig. 3.20 Pie charts showing the seasonal variations in PFG's contribution from demarcated locations of the port. Note AC – approach channel, EC – ernakulam channel, MC – mattanchery channel.

from the MC than the EC and AC stations (except during PM 1). Cyanobacteria contributions were higher during PM 2 (28.3% with 3.54 µg l^{-1}) followed by PrM (15.6% with 2.19 µg l^{-1}), SWM (1.5% with 0.24 µg l^{-1}) and PM (1.4% with 0.13 µg l^{-1}). Among the minor groups,

dinoflagellate contributions was evident during PM1 (3.6%) and PrM (3.1%) while prasinophytes and prymnesiophytes evident only during PrM (1.6%) and PM (2.8%), respectively. Averaged values from the demarcated stations did not show much difference in minor PFG's distribution (Fig. 3.19 and Fig 3.20). ANOVA PFG's revealed the significant (p<0.05)and insignificant variations between the season (p<0.05) and stations, respectively.

3.3.3.2.2 Zuari estuary

Altogether 15 pigments were identified, and the pigment concentrations exhibited distinct seasonal and spatial variations along the salinity gradient of the Zuari estuary. However, the distribution pattern varied between the seasons. Allo concentrations were not calculated due to the non-availability of pigment standards. Higher chl *b* and lut were observed during PM, whereas chl *c*, fuco, 19 Hexa, zea, and pras concentrations were maximum during PrM and SWM season. Peri concentrations are evident only during SWM season. Moreover, the dominance of marker pigments such as fuco (diatom), chl *b*, viola, neo and lut (green algae), peri (dinoflagellates), 19 hexa (prymnesiophytes), zea (cyanobacteria), and pras (prasinophytes) from the Zuari estuary confirms the dominance of their respective groups (Table 3.4). *CHEMTAX* analysis revealed three major (diatom, chlorophytes, and cyanobacteria) and four minor groups (chrysophytes, dinoflagellates, prasinophytes, and prymnesiophytes). During PrM, diatom (81.3% with 3.14 µg l^{-1}) was the most dominant PFG followed by chlorophytes (7.4% with 0.18 µg l^{-1}) and cryptophytes (5.2% with 0.14 µg l^{-1}) were the dominant along the salinity gradient. The presence of prymnesiophytes (4.1% with 0.12 µg l^{-1}) and dinoflagellates (1.6% with 0.04 µg l^{-1}) were also evident. Diatom contributions were higher in LME (87.7%) and UME stations (81.7%) prymnesiophytes (4.1% with 0.12 µg l^{-1}) and dinoflagellates (1.6% with 0.04 µg l^{-1}) were also evident. Diatom contributions were higher in LME (87.7%) and UME stations (81.7%)

Table 3.4 Seasonal variations in the concentrations of chlorophylls and carotenoids from the surface water in Zuari estuary. Note DS – down-stream stations, UME – upper middle estuary, LME – lower middle estuary, US – up-stream stations SWM – southwest monsoon, PrM –pre monsoon, PM21 – post monsoon 1, PM2 – post monsoon 2, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids

Pigments	Average and range of pigment concentrations from Zuari Estuary											
	Downstream			Lower middle Estuary			Upper middle Estuary			Upstream		
	PrM	SWM	PM	PrM	SWM	PM	PrM	SWM	PM	PrM	SWM	PM
Chlorophylls												
Chl *a* (µg l^{-1})	2.07 (0.74-3.04)	7.80 (0.67-17.49)	3.52 (1.43-9.51)	4.17 (1.00-12.58)	3.67 (0.38-11.73)	3.84 (1.15-9.46)	3.29 (1.02-8.14)	2.50 (0.20-9.70)	3.30 (1.00-5.43)	5.65 (1.22-10.06)	0.90 (0.12-2.08)	2.76 (1.54-4.04)
Chl *b* (µg l^{-1})	0.14 (0.04-0.35)	0.19 (0.04-0.37)	0.33 (0.00-0.79)	0.15 (0.05-0.45)	0.18 (0.05-0.53)	0.56 (0.21-1.26)	0.20 (0.05-0.40)	0.12 (0.01-0.24)	0.58 (0.18-1.15)	0.28 (0.17-0.36)	0.10 (0.04-0.36)	0.60 (0.17-0.92)
Chl *c* (µg l^{-1})	1.41 (0.24-3.50)	2.31 (0.04-7.01)	0.41 (0.00-1.41)	1.63 (0.05-6.69)	0.34 (0.06-0.85)	0.39 (0.00-2.24)	0.70 (0.04-2.51)	0.19 (0.00-0.54)	0.20 (0.00-1.09)	0.95 (0.00-1.57)	0.05 (0.00-0.14)	0.06 (0.00-0.28)
Carotenoids												
PSC												
19BUT (ng l^{-1})	1.4 (0.0-6.4)	148.1 (0.0-879.9)	1.0 (0.0-8.3)	4.5 (0.0-29.0)	2.5 (0.0-6.9)	1.4 (0.0-17.1)	2.3 (0.0-14.5)	2.9 (0.0-12.0)	0	1.3 (0.0-9.0)	0.8 (0.0-4.6)	0
Fuco (µg l^{-1})	0.81 (0.24-1.26)	2.52 (0.14-7.18)	0.67 (0.00-2.38)	1.71 (0.10-5.98)	0.59 (0.06-1.10)	0.47 (0.00-1.55)	0.78 (0.01-0.28)	0.27 (0.04-0.52)	0.43 (0.05-1.05)	1.37 (0.06-2.69)	0.10 (0.01-0.17)	0.29 (0.08-0.60)
19Hexa (ng l^{-1})	12.6 (3.4-26.1)	54.9 (0.0-132.1)	34.2 (0.0-175.3)	13.8 (2.7-44.4)	32.0 (0.0-139.4)	12.9 (0.0-50.2)	7.5 (0.0-23.3)	21.6 (0.0-124.9)	10.4 (0.0-24.7)	18.2 (4.9-52.3)	3.2 (0.0-17.5)	12.1 (0.0-38.4)
Peri (ng l^{-1})	81.0 (32.4-155.2)	9.1 (0.0-35.3)	68.2 (0.0-226.7)	23.3 (0.0-83.5)	12.4 (0.0-57.6)	53.0 (0.0-279.5)	43.8 (0.0-334.4)	7.6 (0.0-36.3)	5.4 (0.0-23.5)	7.1 (0.0-33.2)	8.6 (0.0-51.5)	4.1 (0.0-19.7)
PPC												
Allo (ng l^{-1})	0	0	0	0	0	0	0	0	0	0	0	0
B-car (µg l^{-1})	102.3 (44.1-144.0)	329.9 (42.4-610.2)	89.1 (36.0-172.0)	189.1 (30.8-610.1)	215.0 (35.7-591.1)	146.0 (14.0-640.9)	182.6 (17.3-449.5)	151.0 (25.0-448.1)	148.5 (42.3-323.2)	215.6 (13.0-424.9)	47.2 (13.3-99.5)	60.7 (19.6-118.3)
Diad (ng l^{-1})	91.2 (36.7-154.6)	247.5 (19.0-508.4)	64.6 (0.0-177.0)	184.6 (16.9-621.4)	85.7 (11.4-204.0)	73.1 (0.0-252.0)	120.3 (9.7-343.5)	42.4 (5.1-84.0)	43.4 (0.0-135.0)	280.1 (7.7-721.0)	11.2 (0.0-21.5)	30.6 (0.0-68.6)
Diat (ng l^{-1})	45.2 (22.4-97.9)	494.2 (0.0-1575.9)	57.5 (0.0-116.0)	76.3 (13.6-141.6)	419.1 (42.3-1978.6)	286.6 (0.0-2032.6)	142.1 (22.5-232.2)	401.1 (17.0-2023.4)	243.5 (0.0-896.6)	299.3 (41.1-672.5)	67.4 (2.8-200.4)	82.5 (0.0-208.6)
Lut (ng l^{-1})	0	13.5 (0.0-45.7)	22.5 (0.0-71.5)	0	22.0 (0.0-80.5)	13.1 (0.0-64.5)	0	22.3 (0.0-76.9)	15.0 (0.0-100.4)	13.9 (0.0-52.8)	27.1 (0.0-162.7)	31.5 (0.0-99.2)
Viola (ng l^{-1})	5.2 (2.6-8.6)	15.7 (0.0-34.6)	6.5 (0.0-18.6)	8.3 (2.1-21.2)	17.6 (1.7-51.5)	4.2 (0.0-20.5)	12.0 (0.0-26.0)	14.6 (0.0-35.0)	5.9 (0.0-21.2)	24.2 (0.0-106.5)	11.1 (0.0-58.6)	3.8 (0.0-8.9)
Zea (ng l^{-1})	71.5 (32.9-115.9)	165.5 (0.0-415.0)	82.6 (0.0-149.5)	76.2 (37.3-151.3)	170.0 (24.2-318.9)	95.5 (55.8-138.6)	171.9 (33.9-414.5)	93.7 (19.0-256.9)	153.9 (0.0-364.8)	199.2 (20.0-392.0)	52.2 (0.0-82.2)	93.1 (41.2-164.3)
Neo (ng l^{-1})	6.2 (1.1-11.4)	17.3 (1.2-44.2)	5.7 (0.0-15.1)	9.9 (1.0-40.9)	21.7 (1.0-78.4)	5.7 (0.0-26.5)	16.1 (0.0-50.3)	10.5 (0.0-35.0)	7.4 (0.0-31.4)	22.7 (7.4-33.0)	13.4 (0.0-66.1)	6.8 (0.0-15.9)
Pras (ng l^{-1})	11.3 (2.1-23.8)	13.6 (1.5-28.7)	7.5 (0.0-18.4)	8.2 (3.0-15.3)	7.8 (0.0-21.7)	10.1 (0.0-37.4)	11.8 (0.0-23.6)	3.1 (0.0-13.0)	8.0 (0.0-28.6)	12.2 (0.0-37.3)	2.3 (0.0-9.0)	2.6 (0.0-10.6)

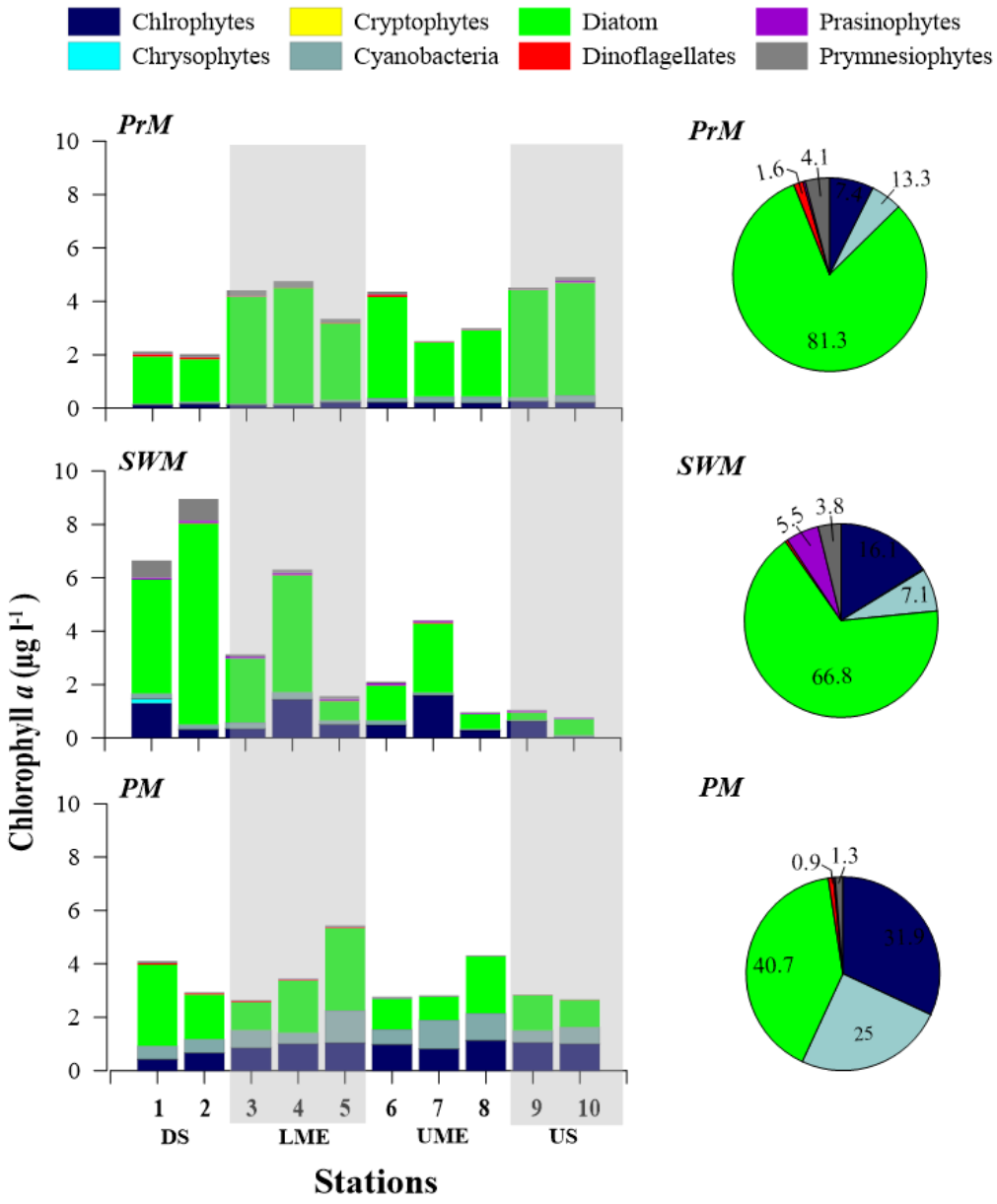

Fig. 3.21 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the surface waters in Zuari estuary. Pie charts showing the seasonal variations in PFG's contribution. Average values from the 10 stations were used

compared to DS stations (77.7%) and US stations (74.8%). Higher chlorophytes (10.8%) and prymnesiophytes (7.1%) were observed in UP stations compared to other estuary parts. Dinoflagellates (4.1%) were evident only from the US stations.

Diatoms (66.8% with 2.46 µg l^{-1}) followed by chlorophytes (16.1% with 0.69 µg l^{-1}), cyanobacteria (7.1% with 0.18 µg l^{-1}) and prasinophytes (5.5% with 0.07 µg l^{-1}) were the major contributors to the chl *a* during SWM. Averaged values indicate the higher diatom (77.8%) contribution from the DS stations than other estuary parts. Chlorophytes was higher in UME (23.6%) and US (17%) stations compared to LME (12.9%) and DS (8.6%) stations. Cyanobacteria were higher in US (6.4%) and UME (6.1%) stations than other stations. Prasinophytes (10.4%) contribution was evident from the US stations than other stations.

During PM, diatom (40.7% with 1.72 µg l^{-1}) followed by chlorophytes (31.9% with 0.90 µg l^{-1}) and cyanobacteria (25% with 0.71 µg l^{-1}) were the major contributing groups to the phytoplankton biomass. Other groups were contributing less than 4% to the phytoplankton pool. Averaged values from the demarcated stations shows that the diatom contribution was higher in DS (48.6%) and LME (42.1%) stations compared to US (38.4%) and UME (35.4%). Higher chlorophytes concentrations were observed from the US (38.7%) stations, followed by UME (32.7%) and LME (32.8%) stations. The lowest (22.7%) chlorophytes contribution was observed from the DS stations. Presence of dinoflagellate (2.6%) evident only from the US stations. Chrysophtes (0.2%) were observed only during SWM with lower (0.02 µg l^{-1}) concentration (Fig. 3.21 and Fig. 3.22). ANOVA revealed the significant variations (p<0.05) between the season, whereas the insignificant difference was observed between the stations throughout the sampling period.

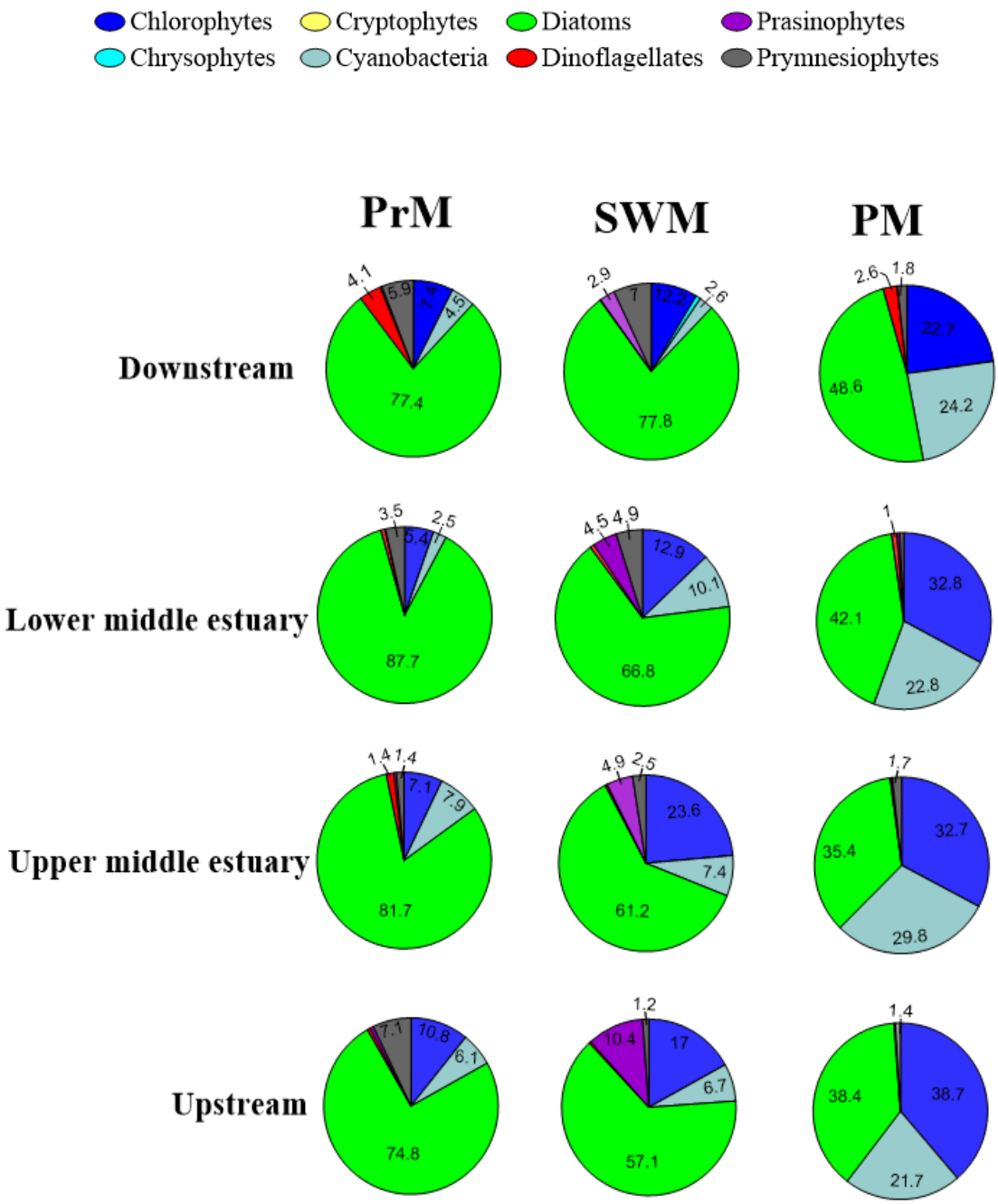

Fig. 3.22 Pie charts showing the seasonal variations in PFG's contribution from demarcated locations of the Zuari estuary.

3.3.3.3 Marine port ecosystem

3.3.3.3.1 Kandla port

Totally 16 pigments were identified from the surface and NBW. Fuco (diatom), lut (chlorophytes), allo (cryptophytes), zea (cyanobacteria) were the major pigments identified from the Kandla port. The presence of pras, peri, 19'bf indicated the existence of prasinophytes, prymnesiophytes, and dinoflagellates (Table 3.5). CHEMTAX analysis revealed four major (diatom, cryptophytes, chlorophytes, and cyanobacteria) and four minor groups (chrysophytes, dinoflagellates, prasinophytes, and prymnesiophytes). PFG's contributions revealed distinct variations between the seasons as well as among the surface water and NBW. In surface water relative diatom composition ranged between 22.2 ± 8.4 to 80.1 ± 4.1% with maximum during PrM (80.1% with 0.48 µg l^{-1} contribution to chl *a*) followed by SWM (41.2% with 0.21 µg l^{-1}), PM 2 (39.4% with 0.30 µg l^{-1}). The lowest diatom contribution was observed during PM 1 (25.4% with 0.21 µg l^{-1}) season. In NBW, diatom contribution was maximum and minimum during PrM (69.2% with 0.56 µg l-1) and PM1 (33.8% with 0.19 µg l-1), respectively. Higher cryptophytes contributions observed from the surface water during PM 1 (38.4% with 0.33 µg l^{-1}) and PM 2 (38.7% with 0.33 µg l^{-1}) followed by SWM (20.6% with 0.21 µg l^{-1}). In NBW, higher and lower cryptophytes contributed during PM 1 (41.1% with 0.21 µg l^{-1}) and PrM (20.7% with 0.17 µg l^{-1}), respectively. Maximum contributions of cyanobacteria were observed from surface water only during PM 1 (25.4% with 0.21 µg l^{-1}). Chlorophytes contribution was observed higher during SWM from surface water (17.3% with 0.07 µg l^{-1}) and NBW (18.8% with 0.24 µg l^{-1}). The higher contribution of dinoflagellates observed during SWM from the surface water (8.1% with 0.03 µg l^{-1}) and NBW (7.4% with 0.04 µg l^{-1}). Prasinophytes were evident only during PM 2 (3.1%). Prymnesiophytes contributed maximum during SWM (5%) and PrM (5.8),

Table 3.5 Seasonal variations in the concentrations of chlorophylls, carotenoids from the surface water, near bottom water (NBW) in Kandla port. Note SWM – southwest monsoon, PrM – pre-monsoon 1, PM 1 – post-monsoon 1 , PM 2 – post-monsoon 2, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids.

Pigments	Average and range of pigment concentrations from Kandla port							
	PM 1		SWM		PM 2		PrM	
	Surface	NBW	Surface	NBW	Surface	NBW	Surface	NBW
Chlorophylls								
Chl *a* (µg l^{-1})	0.76 (0.02-1.45)	0.70 (0.06-1.67)	0.49 (0.14-1.19)	1.22 (0.49-2.66)	0.78 (0.46-1.30)	0.78 (0.29-1.41)	0.62 (0.28-1.23)	0.82 (0.53-1.15)
Chl *b* (µg l^{-1})	0.02 (0.00-0.04)	0.01 (0.00-0.03)	0.01 (0.00-0.04)	0.06 (0.00-0.16)	0.03 (0.01-0.04)	0.02 (0.00-0.04)	0.02 (0.01-0.04)	0.03 (0.01-0.06)
Chl *c* (µg l^{-1})	0.06 (0.00-0.61)	0.09 (0.03-0.37)	0.09 (0.03-0.25)	0.31 (0.09-0.65)	0.12 (0.00-0.98)	0.14 (0.03-0.58)	0.02 (0.00-0.04)	0.02 (0.01-0.13)
Carotenoids								
PSC								
19' bf (ng l^{-1})	1.1 (0.0-8.8)	3.5 (0.0-9.2)	1.5 (0.0-11.2)	10.7 (0.0-40.3)	2.0 (0.0-10.7)	2.4 (0.0-8.8)	2.2 (0.0-5.5)	3.8 (0.0-7.9)
Fuco (µg l^{-1})	0.40 (0.07-0.63)	0.52 (0.13-0.71)	0.22 (0.06-0.81)	0.39 (0.10-0.72)	0.39 (0.18-0.52)	0.37 (0.14-0.57)	0.12 (0.05-0.21)	0.18 (0.00-0.28)
19' hf (ng l^{-1})	8.8 (0.0-40.5)	8.1 (0.0-37.0)	27.9 (0.0-170.7)	14.2 (0.0-99.2)	16.5 (0.0-62.7)	0	11.1 (2.0-30.8)	9.1 (0.0-25.0)
Peri (ng l^{-1})	24.4 (0.0-139.0)	31.6 (10.2-45.8)	35.0 (18.7-59.5)	81.3 (0.0-324.3)	51.8 (0.0-92.4)	45.7 (15.5-73.8)	6.1 (0.0-13.3)	9.3 (4.0-18.8)
PPC								
Allo (ng l^{-1})	7.8 (0.0-14.5)	7.7 (0.0-14.3)	5.5 (0.0-12.1)	12.7 (0.0-112.9)	16.4 (9.8-23.3)	14.2 (0.0-23.1)	7.4 (0.0-14.1)	9.3 (1.2-14.9)
B-car (ng l^{-1})	16.0 (0.0-37.7)	14.8 (5.9-36.0)	14.7 (0.0-37.5)	38.5 (11.8-133.4)	35.4 (16.7-57.9)	26.2 (10.0-63.4)	3.5 (1.4-6.8)	7.0 (2.6-16.0)
Diad (ng l^{-1})	44.0 (9.8-71.9)	53.8 (11.4-77.0)	18.9 (3.1-45.9)	54.8 (22.9-115.6)	47.5 (26.0-70.9)	46.0 (16.5-73.6)	14.9 (6.3-27.4)	19.5 (0.0-35.0)
Diat (ng l^{-1})	9.3 (0.0-32.6)	12.1 (4.1-19.2)	19.2 (0.0-38.6)	51.2 (8.4-153.5)	14.5 (6.1-24.2)	15.5 (5.9-25.3)	4.4 (0.0-9.6)	6.0 (0.0-12.2)
Lut (ng l^{-1})	1.0 (0.0-10.2)	3.1 (0.0-12.4)	9.3 (0.0-25.9)	25.4 (0.0-95.6)	1.7 (0.0-12.9)	0.4 (0.0-6.3)	0.6 (0.0-3.3)	2.5 (0.0-6.8)
Viola (ng l^{-1})	3.7 (0.0-48.5)	9.1 (0.0-25.4)	4.7 (0.0-22.0)	8.7 (0.0-59.4)	9.5 (0.0-22.7)	14.5 (0.0-31.1)	3.0 (0.0-12.6)	5.8 (0.0-14.0)
Zea (ng l^{-1})	18.3 (3.4-31.6)	23.6 (12.3-34.0)	19.2 (0.0-49.7)	52.9 (11.4-132.4)	37.4 (13.7-53.9)	42.2 (14.0-73.2)	5.0 (2.1-10.8)	9.0 (5.9-17.1)
Neo (ng l^{-1})	9.4 (0.0-33.0)	15.4 (0.0-31.3)	7.8 (0.0-33.4)	18.5 (0.0-87.5)	7.0 (0.0-25.3)	19.2 (0.0-31.0)	6.5 (0.0-14.1)	9.1 (0.0-17.0)
Pras (ng l^{-1})	3.3 (0.0-10.4)	6.6 (0.0-16.3)	1.3 (0.0-10.1)	14.6 (0.0-74.6)	14.3 (0.0-26.2)	9.9 (0.0-17.9)	1.1 (0.0-4.0)	2.6 (0.0-5.3)

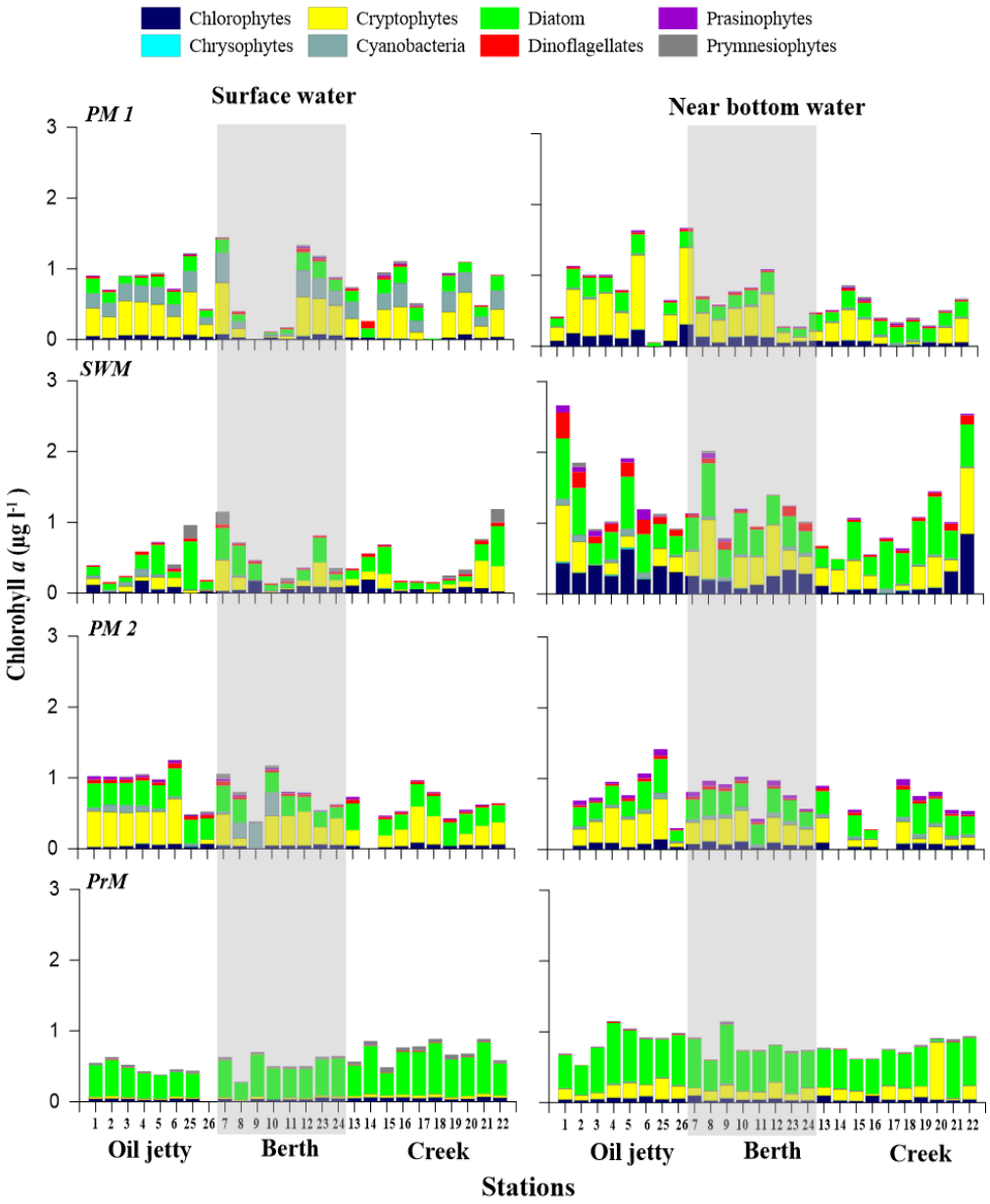

Fig. 3.23 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the overlying waters surface and near bottom in Kandla port.

whereas it was negligible during other seasons (Fig. 3.23 and Fig 3.24). ANOVA revealed significant variations ($p< 0.05$) between the seasons and the surface water and NBW.

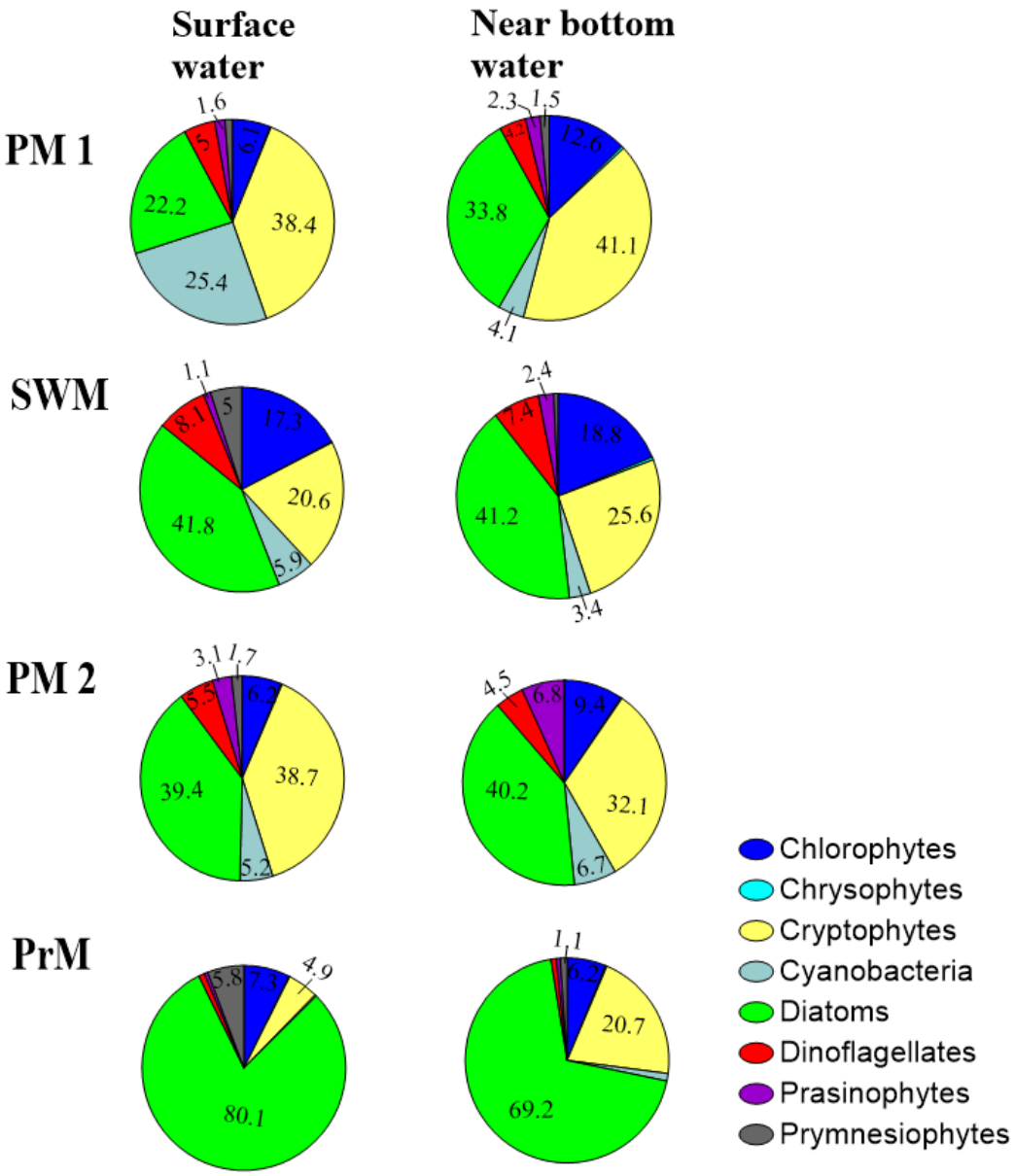

Fig. 3.24 Pie charts showing the seasonal variations in phytoplankton groups derived from CHEMTAX analysis for the surface and near bottom – NBW in Kandla port. Average values from the 22 stations were used

3.3.3.3.2 Mangalore port

Altogether 16 pigments were identified with higher concentrations during PrM and SWM compared to the PM's. Fuco (diatom), chl *b* (chlorophytes), zea (cyanobacteria), and allo (cryptophytes) were the major pigments. Moreover, the presence of pras, peri, 19'bf, indicated the existence of prasinophytes, prymnesiophytes, and dinoflagellates (Table 3.6). CHEMTAX analysis revealed three major groups (diatom, cryptophytes, and chlorophytes) and five minor groups (chrysophytes, cyanobacteria, dinoflagellates, prasinophytes, and prymnesiophytes). PFG's contributions revealed the distinct variations between the seasons and the surface water and NBW. Percentage contribution of diatom from surface water was maximum during PM 1 (80% with 3.89 µg l^{-1} contribution to chl *a*) followed by PM 2 (69.8% with 3.85 µg l^{-1}), SWM (67.8% with 6.15 µg l^{-1}) and PrM (60.2% with 7.47 µg l^{-1}). In NBW, diatom contributed maximum during PrM (86.6% with 3.48 µg l^{-1}) followed by PM 2 (63.4% with 1.98 µg l^{-1}), SWM (62.8% with 4.40 µg l^{-1}) and PM 1 (58.7% with 2.25 µg l^{-1}). Cryptophytes from the surface water contributed maximum during PrM (20% with 2.58 µg l^{-1}), SWM (12.4% with 1.24 µg l^{-1}), PM 2 (9.3% with 0.38 µg l^{-1}) and PM 1 (6.2% with 0.30 µg l^{-1}). In NBW, higher cryptophytes contributions observed during PM 2 (15.4% with 0.53 µg l^{-1}), SWM (12% with 1.01 µg l^{-1}), PM1 (7.1% with 0.31 µg l^{-1}) and PrM (3.7% with 0.15 µg l^{-1}). Chlorophytes contribution from the surface water was higher only during PrM (12.4% with 1.38 µg l^{-1}), whereas in NBW, it was maximum during PM 1 (18.2% with 0.75 µg l^{-1}) and SWM (10.3% with 0.94 µg l^{-1}). Dinoflagellates contributed maximum during SWM (10.3% with 0.94 µg l^{-1}) in surface water and NBW (5% with 0.38 µg l^{-1}). Prymnesiophytes contributions were evident around the year, with maximum contribution during PM 1 (8.7%) and PM 2 (7.8%) seasons (Fig. 3.25 and Fig 3.26). ANOVA revealed that the contribution of all PFG's (except chrysophytes and

Table 3.6 Seasonal variations in the concentrations of chlorophylls, carotenoids from the surface water, near bottom water (NBW) in Mangalore port. Note SWM – southwest monsoon, PrM – pre-monsoon 1, PM 1 – post-monsoon 1 , PM 2 – post-monsoon 2, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids.

Pigments	Average and range of pigment concentrations from Mangalore port							
	PM 1		PrM		SWM		PM 2	
	Surface	NBW	Surface	NBW	Surface	NBW	Surface	NBW
Chlorophylls								
Chl *a* (µg l^{-1})	4.83 (2.04-7.66)	3.87 (0.92-9.26)	12.15 (3.48-24.97)	4.00 (1.56-14.33)	9.01 (4.30-18.07)	7.51 (2.01-20.91)	5.30 (0.79-13.01)	3.04 (1.06-5.18)
Chl *b* (µg l^{-1})	0.07 (0.02-0.26)	0.12 (0.02-0.60)	0.31 (0.08-0.62)	0.06 (0.00-0.22)	1.02 (0.09-2.79)	0.23 (0.00-1.50)	0.18 (0.04-0.41)	0.08 (0.00-0.15)
Chl *c* (µg l^{-1})	0.08 (0.00-0.57)	0.54 (0.00-2.03)	1.19 (0.00-2.57)	1.35 (0.19-7.07)	3.91 (1.66-8.82)	1.98 (0.35-6.86)	0.03 (0.00-0.35)	0.34 (0.00-1.75)
Carotenoids								
PSC								
19' bf (ng l^{-1})	1.0 (0.0-16.1)	29.5 (0.0-247.3)	7.2 (0.0-48.4)	3.3 (0.0-59.2)	0	128.2 (0.0-1581.7)	10.2 (0.0-52.1)	45.4 (0.2-774.8)
Fuco (µg l^{-1})	1.24 (0.54-1.96)	0.98 (0.30-2.50)	3.45 (0.98-7.11)	1.25 (0.46-4.18)	2.01 (0.90-3.56)	1.95 (0.51-5.53)	1.31 (0.16-3.40)	0.66 (0.00-1.19)
19' hf (ng l^{-1})	203.0 (72.9-329.6)	162.1 (31.5-739.0)	452.8 (42.8-779.7)	107.4 (25.7-429.5)	283.9 (100.2-572.1)	636.8 (96.0-2013.0)	290.0 (34.3-773.0)	198.9 (0.1-438.1)
Peri (ng l^{-1})	61.3 (0.0-144.2)	73.8 (0.0-243.3)	174.8 (46.9-399.5)	42.7 (0.0-238.2)	565.0 (0.0-1401.4)	299.7 (0.0-1372.3)	116.3 (0.0-349.7)	52.8 (0.1-286.9)
PPC								
Allo (ng l^{-1})	18.0 (0.0-39.8)	62.8 (0.0-310.9)	104.5 (0.0-219.3)	30.0 (0.0-71.2)	48.6 (0.0-165.1)	80.2 (0.0-519.9)	47.1 (0.0-113.4)	15.6 (0.0-56.4)
B-car (ng l^{-1})	114.5 (71.5-159.1)	201.4 (28.8-672.0)	314.8 (92.0-740.6)	119.3 (40.1-322.7)	219.4 (105.5-353.4)	334.0 (68.9-1443.5)	123.1 (51.7-247.4)	98.0 (0.0-206.0)
Diad (ng l^{-1})	163.8 (57.1-244.2)	81.8 (14.9-198.1)	427.7 (157.8-750.6)	150.6 (37.5-563.3)	252.6 (71.4-466.6)	133.3 (0.0-389.8)	165.4 (15.3-330.9)	70.8 (0.0-134.7)
Diat (ng l^{-1})	19.8 (0.0-43.7)	71.1 (4.8-398.3)	203.0 (62.5-364.2)	54.2 (8.8-199.4)	61.3 (18.2-135.2)	122.7 (0.0-953.0)	29.7 (0.0-83.3)	26.2 (0.0-62.7)
Lut (ng l^{-1})	6.2 (0.0-39.0)	65.6 (0.0-356.8)	43.7 (0.0-116.8)	14.3 (0.0-109.1)	10.3 (0.0-42.0)	112.8 (0.0-1246.2)	18.2 (0.0-41.4)	23.1 (0.0-68.6)
Viola (ng l^{-1})	10.1 (0.0-54.4)	105.1 (0.0-594.5)	38.9 (12.8-72.8)	37.5 (10.1-187.2)	44.9 (0.0-116.2)	149.3 (0.0-1467.6)	30.1 (5.4-77.8)	33.2 (0.0-100.0)
Zea (ng l^{-1})	23.4 (11.7-111.5)	136.4 (15.9-600.4)	136.9 (56.9-214.8)	77.3 (29.9-199.0)	56.8 (27.5-143.2)	203.3 (31.8-1553.9)	89.9 (52.1-159.5)	74.9 (0.0-159.5)
Neo (ng l^{-1})	3.8 (0.0-60.9)	102.4 (0.0-767.1)	40.1 (0.0-86.7)	12.4 (0.0-124.0)	42.4 (0.0-182.7)	237.3 (0.0-2494.8)	20.5 (0.0-43.8)	27.2 (0.0-152.4)
Pras (ng l^{-1})	1.4 (0.0-22.8)	26.4 (0.0-213.3)	37.7 (0.0-91.2)	9.5 (0.0-54.9)	43.7 (16.1-79.4)	85.0 (0.0-578.8)	33.1 (0.0-69.2)	7.5 (0.0-39.7)

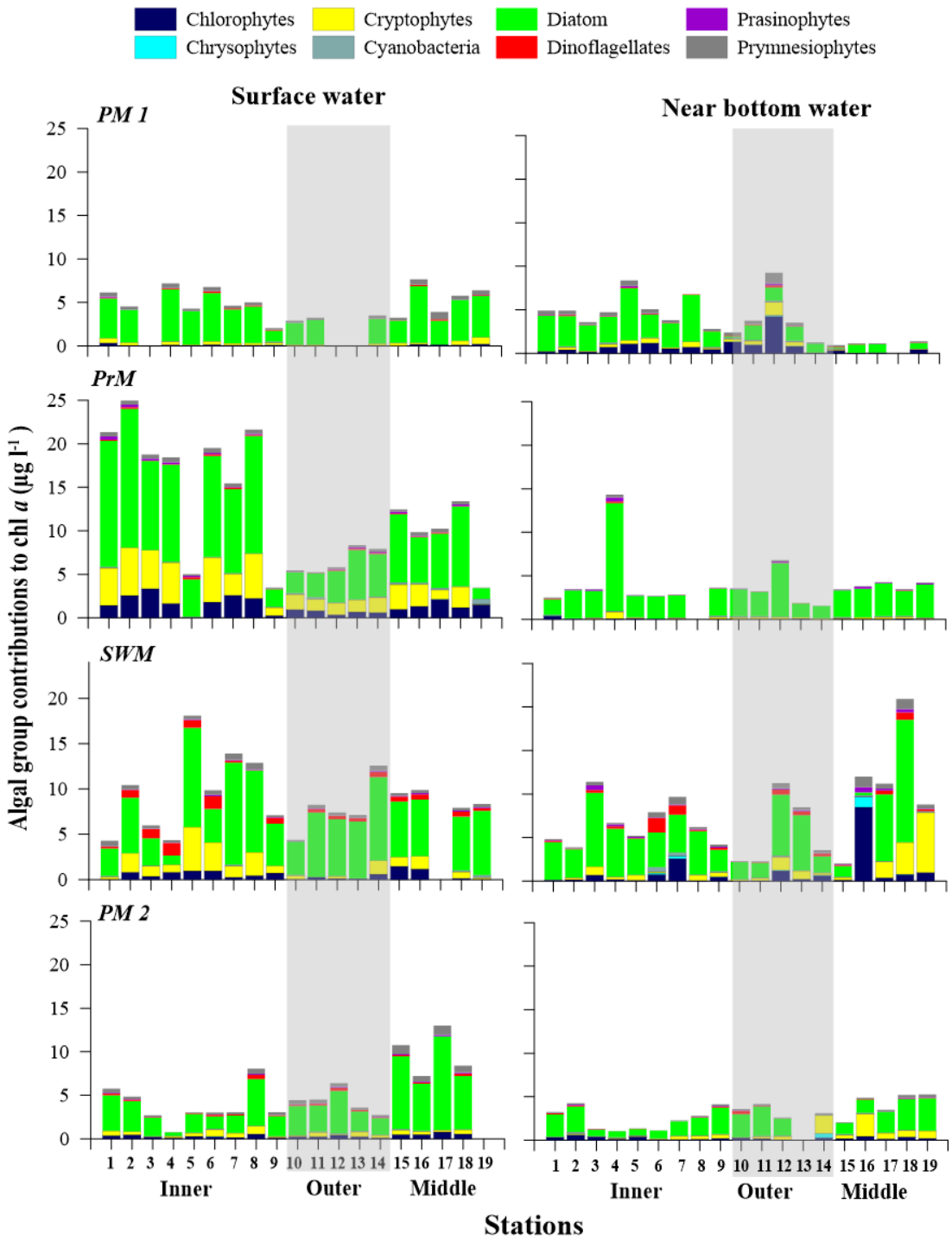

Fig. 3.25 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the overlying waters surface and near bottom in Mangalore port

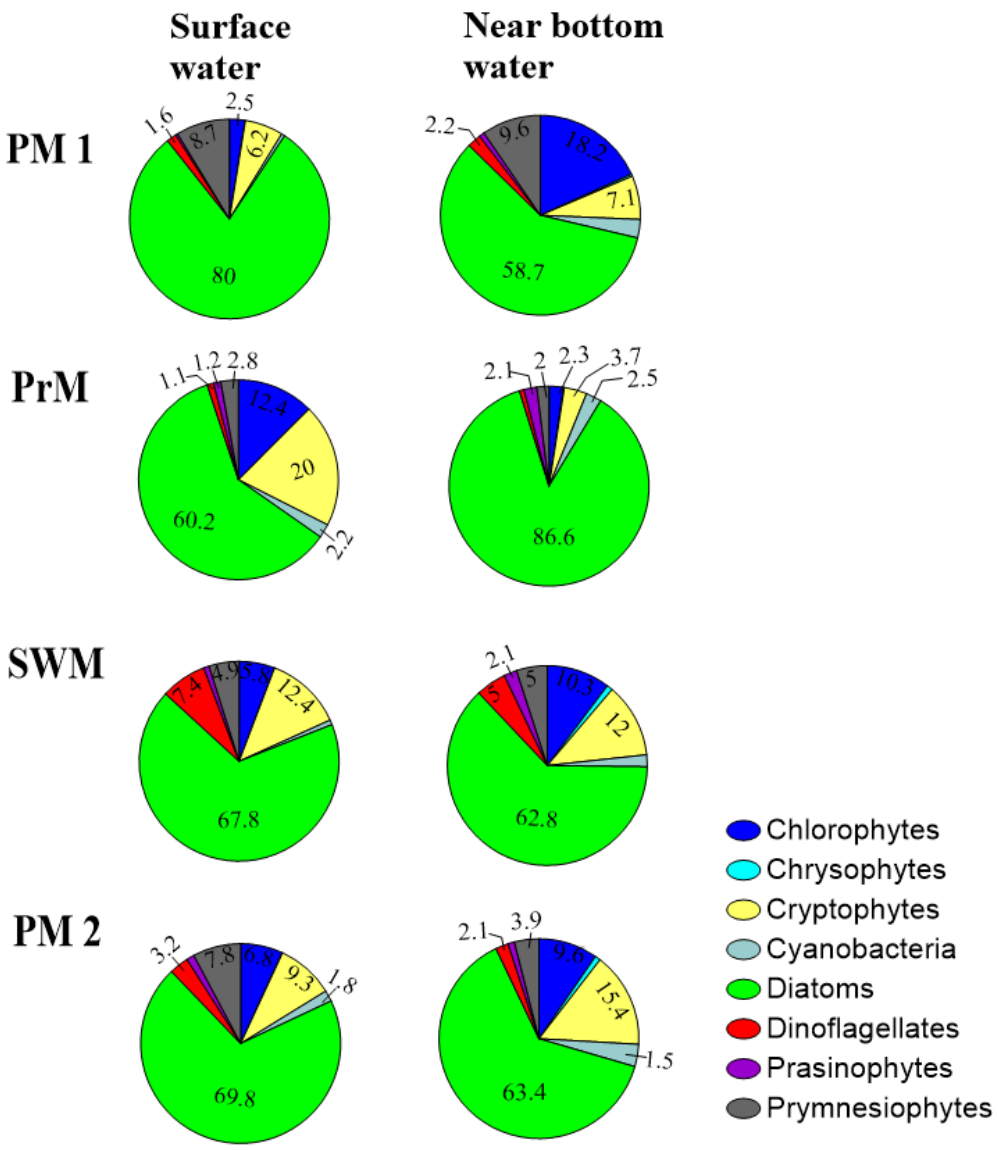

Fig. 3.26 Pie charts showing the seasonal variations in phytoplankton groups derived from CHEMTAX analysis for the surface and near bottom – NBW in Mangalore port. Average values from the 19 stations were used

prymnesiophytes) showed significant seasonal variations (p<0.05). In contrast, only diatom, dinoflagellates, prasinophytes, and prymnesiophytes showed significant seasonal variations (p<0.05) in NBW. ANOVA also revealed a significant variation (p<0.05) in some PFG's

(cryptophytes, diatom, dinoflagellates, and prymnesiophytes) between surface water and NBW but not in other PFG's.

3.3.3.3.3 Chennai port

Overall 16 pigments were identified and of which fuco (diatom), allo (cryptophytes), chl *b* (chlorophytes), and peri (dinoflagellates) were the major pigments. However, other pigments such as pras, zea, 19'bf, indicated the existence of prasinophytes, cyanobacteria, and prymnesiophytes (Table 3.7). *CHEMTAX* revealed four major (diatom, cryptophytes, chlorophytes, and dinoflagellates) and four minor (cyanobacteria, prasinophytes, prymnesiophytes, and chrysophytes) PFG's. The distribution of PFG's revealed the distinct variations between the season, and the trend was different among the surface water and NBW. The higher contribution of diatom in the surface water observed during SWM (75.2% with 1.38 µg l^{-1}) followed by SIM (73.1% with 4.06 µg l^{-1}), NEM (71.6% with 1.21 µg l^{-1}), and FIM (60.8% with 0.41 µg l^{-1}). Contrarily in NBW, diatom contributed maximum during SIM (73.4% with 2.95 µg l^{-1}) followed by FIM (45.4% with 0.33 µg l^{-1}), NEM (42.2% with 0.49 µg l^{-1}) and SWM (21.7% with 0.30 µg l^{-1}). Cryptophytes from the surface water were high during SIM (19.7% with 1.09 µg l^{-1}) and SWM (12.9% with 0.30 µg l^{-1}) whereas in NBW, the maximum contribution was observed during SWM (46.7% with 0.90 µg l^{-1}) followed by NEM (34.9% with 0.43 µg l^{-1}) and FIM (30.1% with 0.20 µg l^{-1}). Chlorophytes from the surface water contributed maximum during FIM (10.8% with 0.09 µg l^{-1}), whereas in NBW, it was observed during SWM (22% with 0.35 µg l^{-1}). Cyanobacteria contributed maximum during NEM (12.2%) and FIM (9.1% from the surface and NBW, respectively. Dinoflagellates contributions were observed higher only during FIM (20.1), especially from the surface water. Prasinophytes were evident from the surface water during SWM (7.6%), whereas in NBW, it was observed from

Table 3.7 Seasonal variations in the concentrations of chlorophylls, carotenoids and pheopigments from the surface water and near bottom water (NBW) in Chennai port. Note SWM – southwest monsoon, NEM –northeast monsoon, FIM – fall inter-monsoon, SIM – inter-monsoon, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids.

Pigments	Average and range of pigment concentrations from Chennai port							
	SWM		FIM		NEM		SIM	
	Surface	NBW	Surface	NBW	Surface	NBW	Surface	NBW
Chlorophylls								
Chl *a* (µg l^{-1})	1.95 (1.07-4.23)	1.62 (0.05-3.70)	0.68 (0.25-1.90)	0.80 (0.23-3.37)	1.66 (0.93-2.60)	1.16 (0.52-2.18)	5.62 (2.33-14.27)	3.94 (2.12-9.42)
Chl *b* (µg l^{-1})	0.18 (0.00-0.68)	0.11 (0.04-0.36)	0.09 (0.00-0.66)	0.13 (0.01-1.72)	0.12 (0.04-0.25)	0.05 (0.00-0.12)	0.18 (0.06-0.33)	0.23 (0.09-0.68)
Chl *c* (µg l^{-1})	0.52 (0.00-1.23)	1.09 (0.00-2.44)	0.32 (0.00-1.10)	0.15 (0.02-1.02)	0.42 (0.20-0.66)	0.31 (0.00-0.57)	2.43 (0.66-5.77)	1.33 (0.56-3.31)
Carotenoids								
PSC								
19' bf (ng l^{-1})	0	0	4.6 (1.0-40.0)	0	0	5.9 (0.0-48.6)	2.7 (0.0-46.0)	24.3 (1.0-149)
Fuco (µg l^{-1})	0.50 (0.11-1.15)	0.59 (0.36-1.08)	0.09 (0.03-0.22)	0.15 (0.04-0.43)	0.45 (0.21-0.73)	0.32 (0.13-0.66)	1.94 (0.64-5.04)	1.27 (0.66-3.29)
19' hf (ng l^{-1})	0	28.1 (4.7-76.0)	1.0 (1.0-1.0)	2.4 (0.0-43.8)	13.9 (0.0-36.7)	22.4 (0.0-99.7)	118.9 (25.2-416.0)	117.9 (53.9-610)
Peri (ng l^{-1})	46.6 (1.0-96.2)	24.0 (7.7-101.6)	31.1 (9.9-105.6)	41.2 (10.5-110.4)	29.3 (8.8-53.9)	29.2 (0.0-103.9)	86.9 (30.5-191.6)	58.7 (19.0-262)
PPC								
Allo (ng l^{-1})	24.8 (0.0-80.6)	13.2 (8.1-32.1)	6.0 (2.1-12.6)	14.4 (0.5-49.7)	19.8 (13.3-28.9)	19.5 (6.7-35.5)	32.4 (12.2-52.9)	24.1 (7.2-69)
B-car (ng l^{-1})	84.7 (31.3-395.2)	83.6 (33.2-298.6)	5.3 (0.0-29.8)	56.1 (9.1-427.5)	62.8 (28.5-139.1)	54.4 (0.0-174.9)	157.9 (0.0-410.5)	174.0 (75.2-1002)
Diad (ng l^{-1})	40.9 (0.0-91.5)	33.8 (5.2-86.4)	11.9 (0.0-30.8)	14.9 (2.3-75.1)	38.8 (17.5-85.2)	28.7 (0.0-61.9)	183.9 (57.4-502.3)	114.8 (57.3-265)
Diat (ng l^{-1})	41.3 (0.0-75.6)	56.2 (17.5-322.3)	11.1 (0.0-30.5)	31.7 (4.2-97.1)	32.7 (8.5-55.6)	24.4 (0.0-55.0)	67.4 (24.4-143.8)	57.6 (29.0-235)
Lut (ng l^{-1})	3.5 (0.0-49.2)	0	3.2 (0.0-58.5)	6.3 (0.0-106.7)	9.1 (0.0-29.7)	7.4 (0.0-24.1)	0	0
Viola (ng l^{-1})	2.3 (0.0-14.8)	4.3 (0.0-48.4)	0	6.5 (0.0-72.8)	6.2 (0.0-17.7)	25.9 (0.0-115.8)	5.0 (0.0-48.4)	52.3 (11.2-251)
Zea (ng l^{-1})	26.7 (0.0-53.3)	30.0 (10.6-118.5)	19.8 (8.8-36.3)	75.3 (12.2-743.6)	33.8 (7.9-79.4)	31.7 (5.7-69.2)	55.0 (30.3-136.6)	60.9 (24.5-236)
Neo (ng l^{-1})	0	0	0	17.2 (0.0-290.2)	3.4 (0.0-31.4)	15.4 (0.0-126.0)	7.5 (0.0-52.3)	142.0 (41.8-1078)
Pras (ng l^{-1})	62.9 (0.0-352.3)	46.1 (0.0-358.2)	0	5.0 (0.0-43.3)	8.1 (0.0-35.2)	37.2 (0.0-181.2)	14.9 (0.0-162.3)	26.7 (9.1-91)

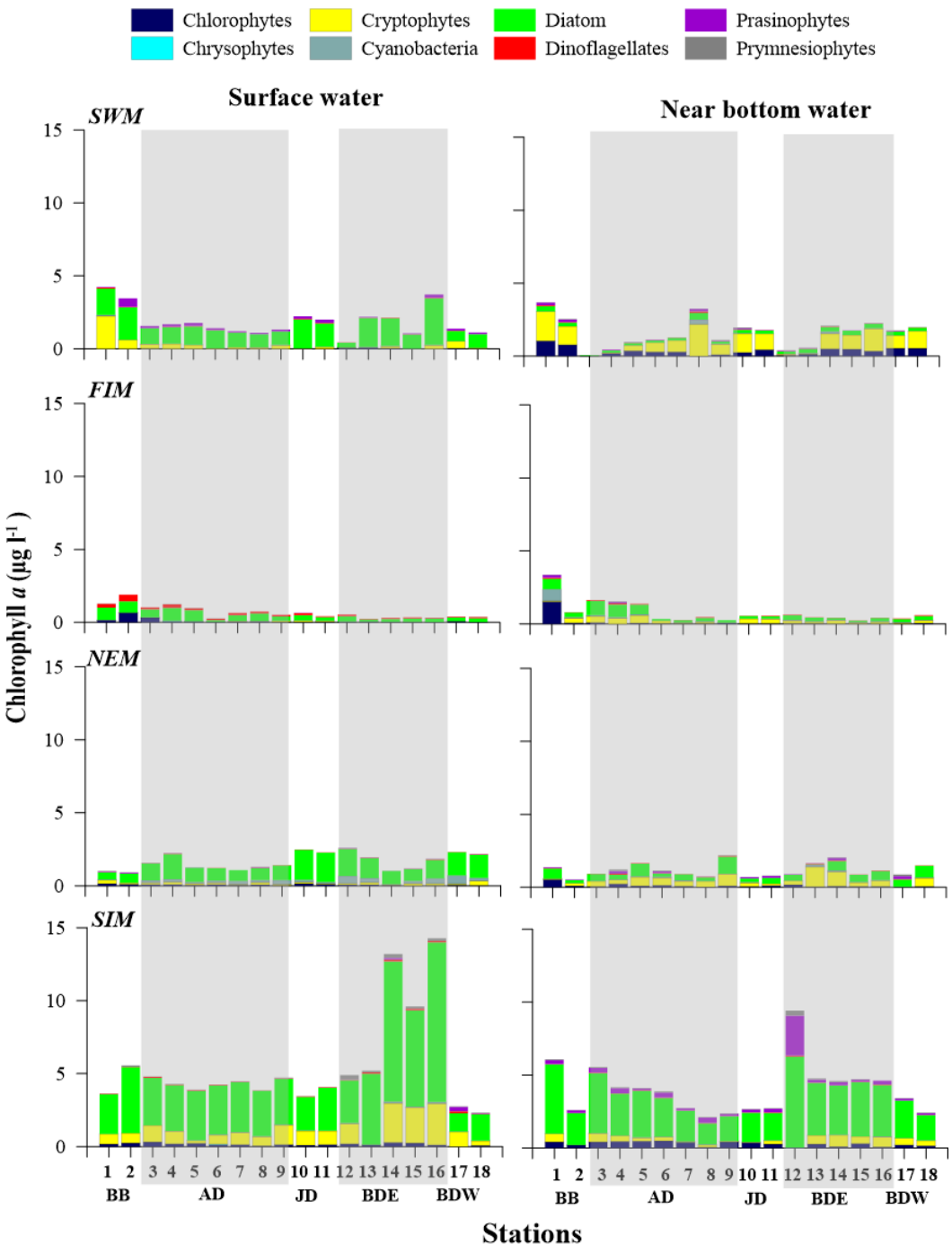

Fig. 3.27 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the overlying waters surface and near bottom in Chennai port

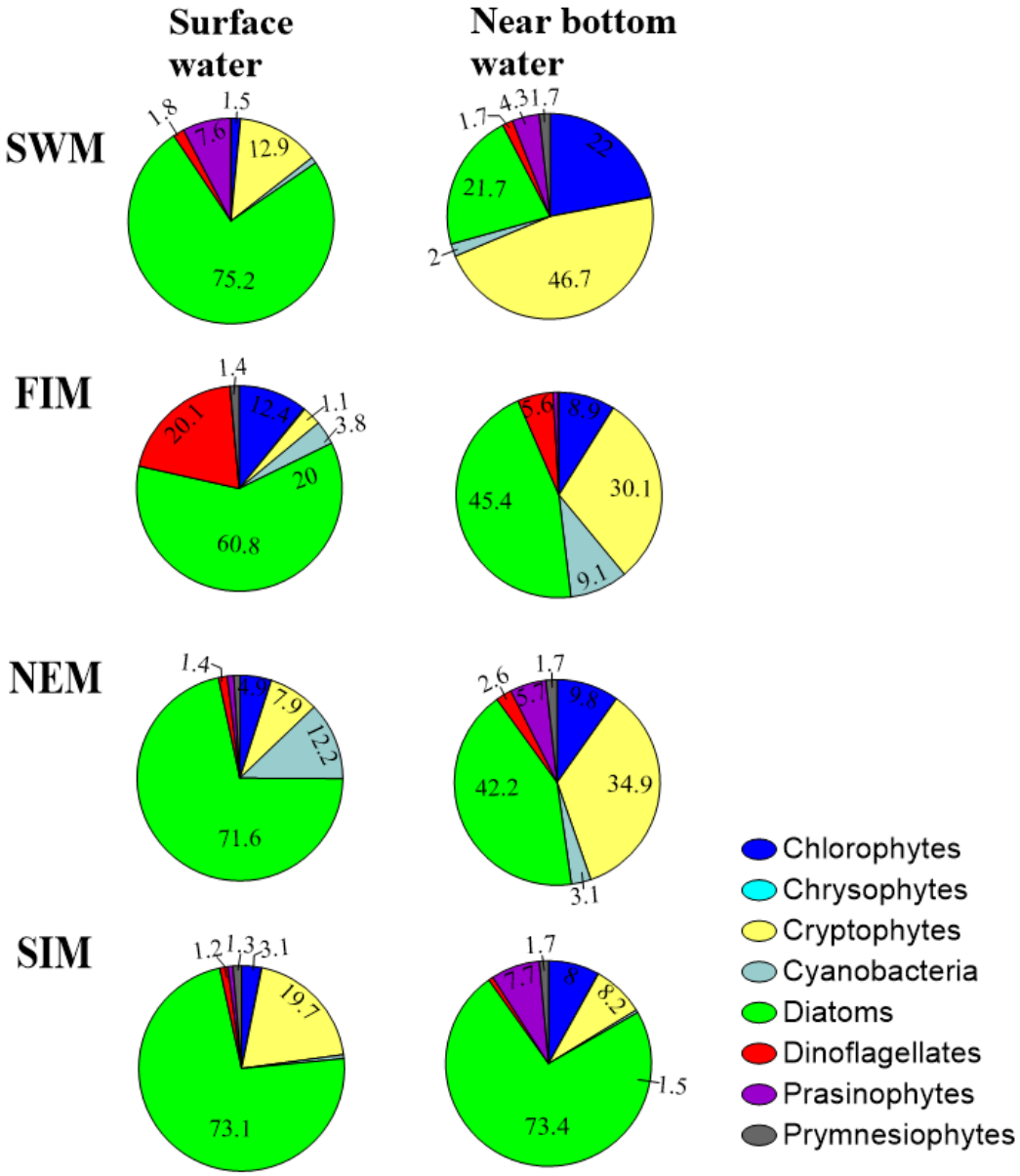

Fig. 3.28 Pie charts showing the seasonal variations in phytoplankton groups derived from CHEMTAX analysis for the surface and near bottom – NBW in Chennai port. Average values from the 18 stations were used.

SIM (7.7%), NEM (5.7%), and SWM (4.3%) seasons (Fig. 3.27 and Fig 3.28). ANOVA revealed significant variations ($p< 0.05$) between the seasons and the surface water and NBW.

3.3.3.3.4 V.O.C. port, Tuticorin

Altogether 16 pigments were identified, and of which chl *b*, fuco, zea, and allo were the major pigments, and the rest (pras, 19'bf and per) formed the minor PFG's (Table 3.8). *CHEMTAX* analysis revealed four major (diatom, cryptophytes, chlorophytes, and cyanobacteria) and four minor (dinoflagellates, prasinophytes, prymnesiophytes, and chrysophytes) groups. PFG's distributions revealed distinct variations between the season whereas, a similar trend was observed between the surface water and NBW. Diatom contributed maximum during SWM (72.7% with 1.27 µg l^{-1}) and FIM (80% with 3.65 µg l^{-1}). Contrarily, it was lower during NEM (18.5% with 0.77 µg l^{-1}) and SIM (20.9% with 0.09 µg l^{-1}). Cryptophytes observed higher during NEM (49.6% with 0.77 µg l^{-1}) and SIM (27.4% with 0.14 µg l^{-1}) compared to SWM (10.3% with 0.20 µg l^{-1}) and FIM (7% with 0.30 µg l^{-1}). Cyanobacteria contribution was maximum during SIM (from surface (32.5% with 0.42 µg l^{-1}) and NBW (41.6% with 0.18 µg l^{-1}) and NBW of SWM (23.2% with 0.42 µg l^{-1}). C chlorophytes contributed maximum during SIM (16.6% with 0.09 µg l^{-1}) and NEM (11.2% with 0.08 µg l^{-1}) compared to other seasons. Among the minor PFG's, dinoflagellates contributed maximum only during NEM (6.2% with 0.05 µg l^{-1}), whereas prasinophytes and prymnesiophytes were evident during FIM, and NEM seasons (Fig. 3.29 and Fig 3.30). ANOVA revealed significant variations ($p< 0.05$) between the seasons and the surface water and NBW.

Table 3.8 Seasonal variations in the concentrations of chlorophylls, carotenoids and pheopigments from the surface water and near bottom water (NBW) in V.O.C port. Note SWM – southwest monsoon, NEM –northeast monsoon, FIM – fall inter-monsoon, SIM – inter-monsoon, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids.

Pigments	Average and range of pigment concentrations from V.O.C. port							
	SWM		FIM		NEM		SIM	
	Surface	NBW	Surface	NBW	Surface	NBW	Surface	NBW
Chlorophylls								
Chl *a* (µg l^{-1})	1.73 (0.54-3.94)	2.13 (0.47-5.93)	4.40 (0.89-9.09)	5.02 (2.73-9.98)	1.10 (0.04-5.36)	0.47 (0.23-0.85)	0.46 (0.13-0.87)	0.46 (0.23-0.76)
Chl *b* (µg l^{-1})	0.12 (0.03-0.21)	0.21 (0.07-1.12)	0.11 (0.06-0.21)	0.24 (0.07-0.55)	0.20 (0.02-1.24)	0.10 (0.02-0.22)	0.05 (0.01-0.11)	0.10 (0.02-0.22)
Chl *c* (µg l^{-1})	0.29 (0.00-0.79)	0.35 (0.00-2.05)	1.43 (0.52-4.60)	0.27 (0.00-1.59)	0.07 (0.00-0.56)	0.004 (0.00-0.09)	0	0.004 (0.00-0.09)
Carotenoids								
PSC								
19' bf (ng l^{-1})	0	0.3 (0.0-6.1)	0	8.3 (0.0-25.1)	9.9 (0.0-40.0)	0	0	0
Fuco (µg l^{-1})	0.34 (0.08-0.76)	0.44 (0.00-2.29)	1.15 (0.00-2.35)	1.26 (0.00-3.06)	0.31 (0.00-1.16)	0.03 (0.01-0.17)	0.03 (0.00-0.05)	0.02 (0.01-0.07)
19' hf (ng l^{-1})	20.1 (0.0-93.8)	18.0 (0.0-87.5)	122.4 (39.1-323.0)	139.3 (27.0-299.8)	30.9 (0.0-78.7)	3.6 (0.0-35.1)	5.7 (0.0-42.3)	2.6 (0.0-35.1)
Peri (ng l^{-1})	35.1 (0.0-115.8)	18.1 (0.0-87.4)	95.7 (0.0-452.6)	121.6 (30.5-347.6)	37.7 (0.0-123.5)	1.0 (0.0-23.1)	0.9 (0.0-8.9)	0
PPC								
Allo (ng l^{-1})	4.0 (0.0-19.9)	0.7 (0.0-14.8)	9.4 (0.0-20.9)	26.2 (0.0-72.8)	19.2 (0.0-126.3)	4.0 (0.0-16.9)	6.4 (0.0-27.7)	3.4 (0.0-11.1)
B-car (ng l^{-1})	48.6 (16.0-150.7)	71.1 (16.0-309.3)	109.1 (44.8-219.2)	141.0 (34.6-315.7)	47.4 (0.0-150.4)	10.5 (4.3-33.6)	12.1 (0.0-25.1)	9.3 (4.3-16.5)
Diad (ng l^{-1})	38.0 (11.2-78.4)	73.4 (15.3-361.8)	83.7 (40.1-178.2)	101.3 (24.4-212.7)	32.4 (2.6-154.6)	6.4 (2.0-21.0)	3.0 (0.0-19.7)	5.7 (2.0-14.8)
Diat (ng l^{-1})	37.3 (3.3-115.2)	67.5 (7.9-449.9)	40.3 (10.6-85.7)	56.0 (0.0-111.7)	61.3 (4.2-199.7)	7.8 (4.5-28.7)	11.3 (0.0-33.6)	7.4 (5.3-18.8)
Lut (ng l^{-1})	8.6 (0.0-64.1)	3.2 (0.0-28.3)	4.1 (0.0-17.0)	18.6 (0.0-59.2)	2.3 (0.0-21.3)	9.4 (0.0-17.8)	7.1 (0.0-25.3)	9.8 (0.0-17.8)
Viola (ng l^{-1})	14.2 (0.0-26.8)	10.1 (0.0-49.1)	10.1 (0.0-20.6)	18.8 (0.0-45.8)	23.1 (0.0-86.6)	6.7 (0.0-23.4)	3.3 (0.0-14.9)	5.9 (0.0-14.6)
Zea (ng l^{-1})	70.7 (15.6-240.8)	87.4 (34.3-298.5)	39.6 (14.6-133.7)	79.2 (31.5-195.5)	52.3 (0.0-126.1)	40.5 (23.7-53.7)	40.8 (0.0-73.4)	39.8 (23.7-54.7)
Neo (ng l^{-1})	9.9 (0.0-38.6)	9.3 (0.0-61.2)	7.3 (0.0-19.3)	23.0 (0.0-66.6)	16.8 (0.0-56.3)	0	4.8 (0.0-33.5)	0
Pras (ng l^{-1})	14.8 (0.0-27.6)	12.2 (0.0-42.9)	19.4 (0.0-56.9)	60.4 (15.7-164.7)	19.0 (0.0-39.8)	7.6 (5.0-18.8)	3.4 (0.0-13.7)	7.3 (5.0-14.0)

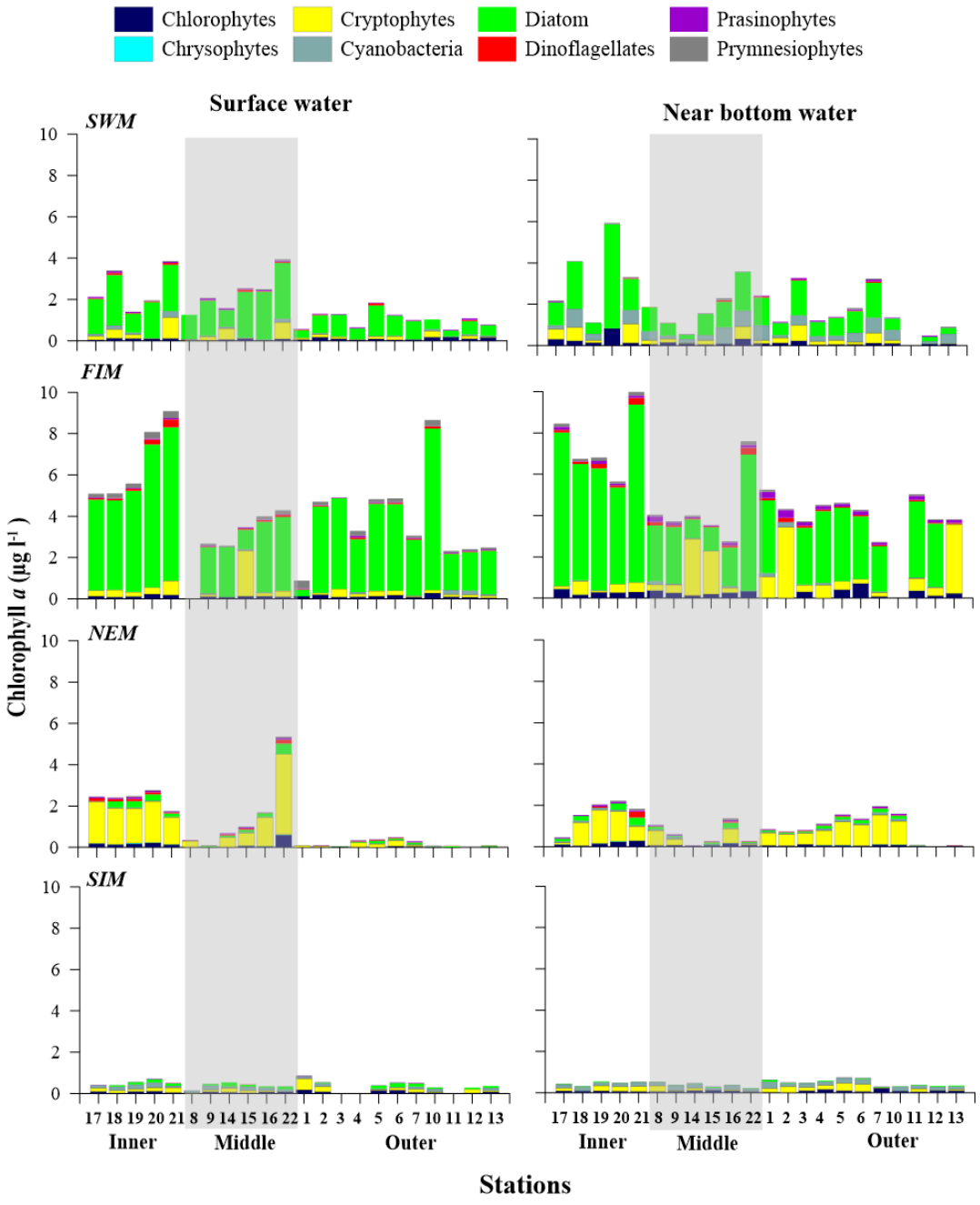

Fig. 3.29 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the overlying waters surface and near bottom in V.O.C port, Tuticorin.

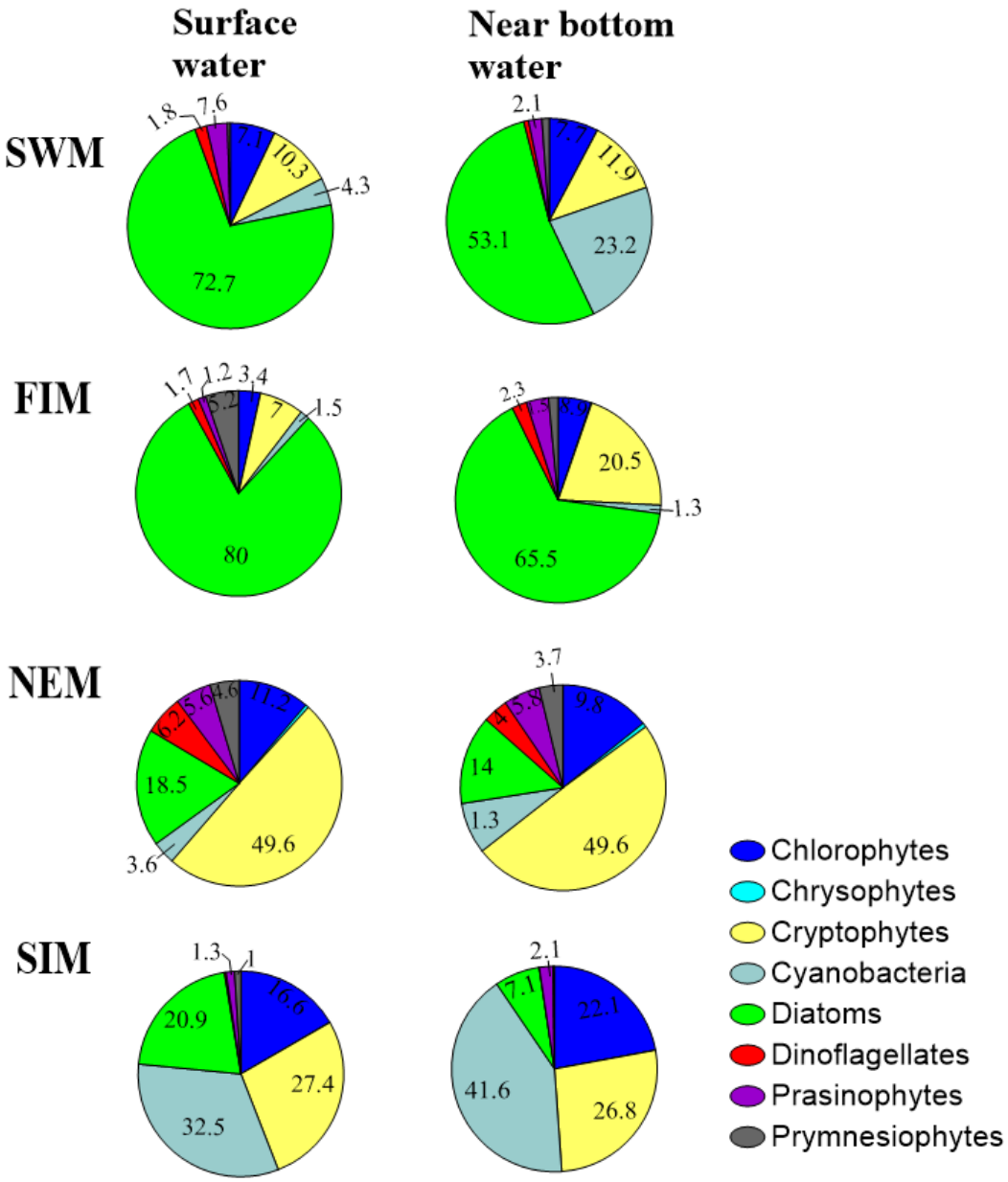

Fig. 3.30 Pie charts showing the seasonal variations in phytoplankton groups derived from CHEMTAX analysis for the surface and near bottom – NBW in V.O.C port, Tuticorin. Average values from the 22 stations were used.

3.3.4 Relationship between phytoplankton biomass, PFG's and environmental variables from different ecosystems

3.3.4.1 Freshwater port ecosystem

3.3.4.1.1 Kolkata port

Pearson's correlation of phytoplankton biomass and environmental did not showed any significant relationship (except NO_3) in the surface water. In NBW, none of the environmental variables showed a significant relationship. The RDA analysis revealed that in the surface water biplot, axes 1 and 2 (eigenvalues 0.35 and 0.09, respectively) explained 97.8 % of environmental variables and their relationship with PFG's contributions. . Chlorophytes positioned with the higher NO_3 concentration. Diatom positioned with high salinity. In the NBW biplot, axes 1 and 2 (eigenvalues 0.20 and 0.01, respectively) explained 98.3% of environmental variables and their relationship with PFG's contributions. Chlorophytes positioned with higher NO_2 concentration. Cyanobacteria positioned with the high temperature. Other PFG's did not showed any significant relationship with the environmental varibles in NBW (Fig. 3.31).

3.3.4.1.2 Haldia port

Pearson's correlations revealed that the surface phytoplankton biomass showed significant correlation only with PO_4. Whereas in NBW, phytoplankton biomass showed a significant correlation with temperature, DO, PO_4 and NO_2. The RDA revealed that in surface water biplot, axes 1 and 2 (eigenvalues 0.24 and 0.02, respectively) explained 99.3% of environmental variables and their relationship with PFG's contributions. Only DO values showed a significant relationship with the PFG's distribution. Diatom was positioned with the high NO_2 and low salinity and PO_4 concentrations. Chlorophytes and prasinophytes were placed with high DO

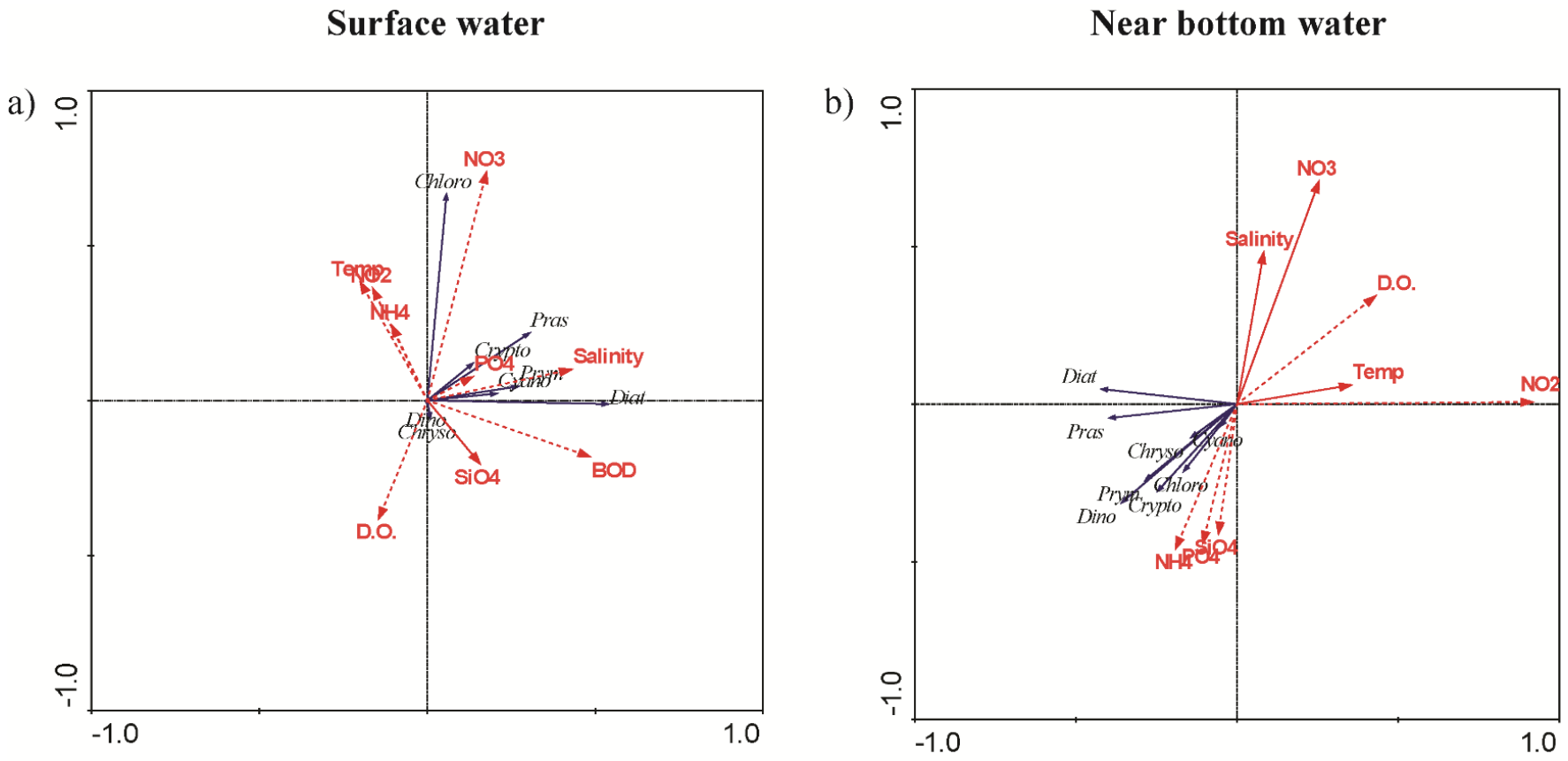

Fig. 3.31 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Kolkata port.

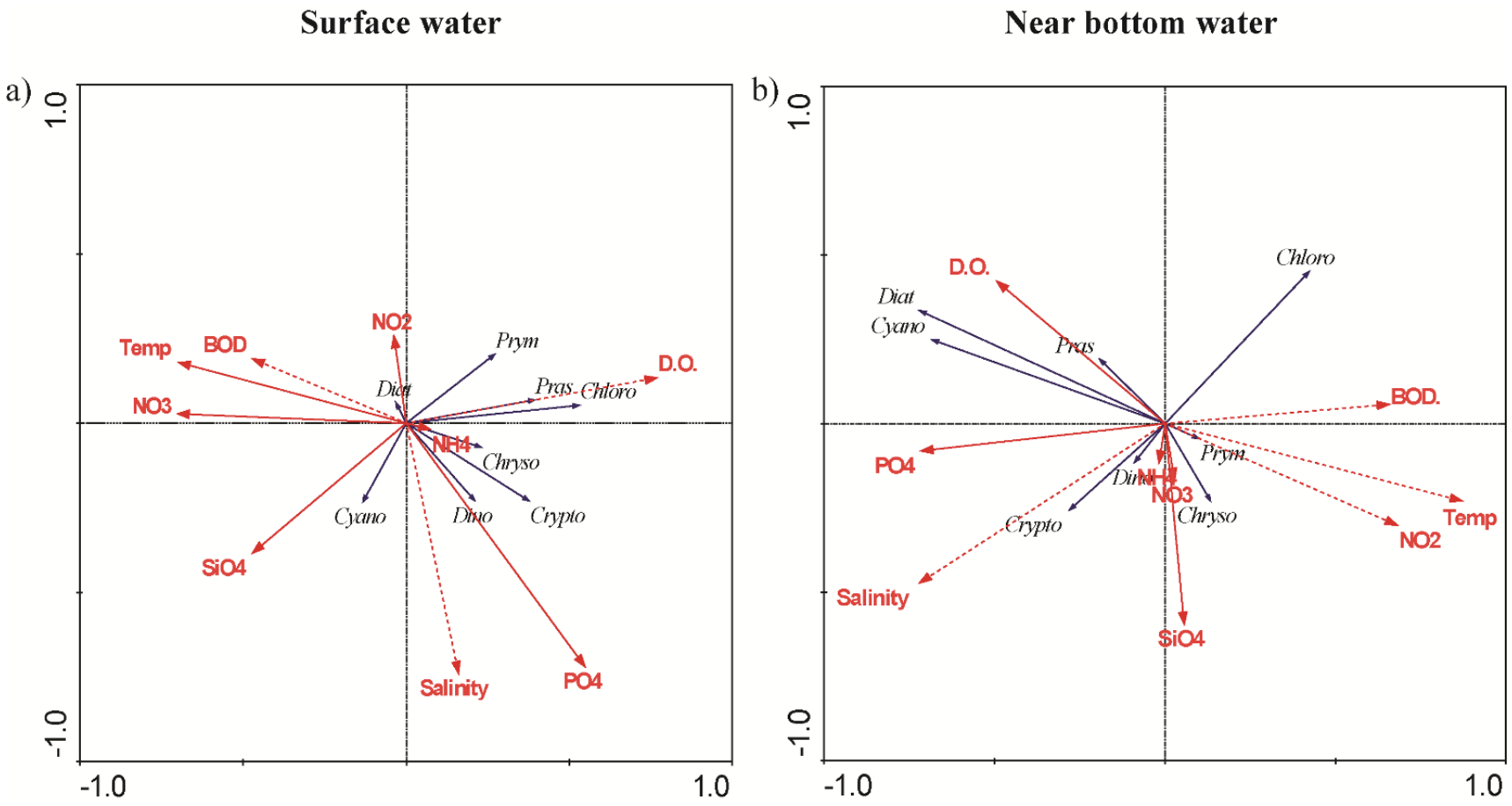

Fig. 3.32 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Haldia port.

values and low SiO_4 concentrations. Cryptophytes and dinoflagellates were positioned with high PO_4 and salinity values and low NO_2 concentration. In the NBW biplot, axes 1 and 2 (eigenvalues 0.28 and 0.13, respectively) explained 87.3% of environmental variables and their relationship with PFG's contributions. Temperature, salinity, NO_2, and PO_4 values showed a significant relationship with PFG's distribution. Chlorophytes were positioned with high BOD values and lower salinity. Cryptophytes and dinoflagellates were positioned with high salinity and PO_4 concentrations. Diatom, cyanobacteria, and prasinophytes were placed with high DO values, low temperature, and NO_2 concentration (Fig. 3.32).

3.3.4.2 Estuarine port ecosystem

3.3.4.2.1 Cochin port

Pearson's correlation revealed an insignificant correlation between phytoplankton biomass and environmental variables. The RDA analysis was of environmental parameters, and PFG's revealed that in the surface water biplot, axes 1 and 2 (eigenvalues 0.13 and 0.02, respectively) explained 95.1% of environmental variables and their relationship to PFG's. Though environmental parameters were not showing any significant ($p > 0.05$) effect on PFG's. Diatom was positioned towards high SiO_4, NH_4, and DO values. While cyanobacteria and prasinophytes were positioned with only high temperature. Dinoflagellates were positioned towards the high NO_2 concentrations while chlorophytes with high NO_3 and PO_4 concentrations (Fig. 3.33).

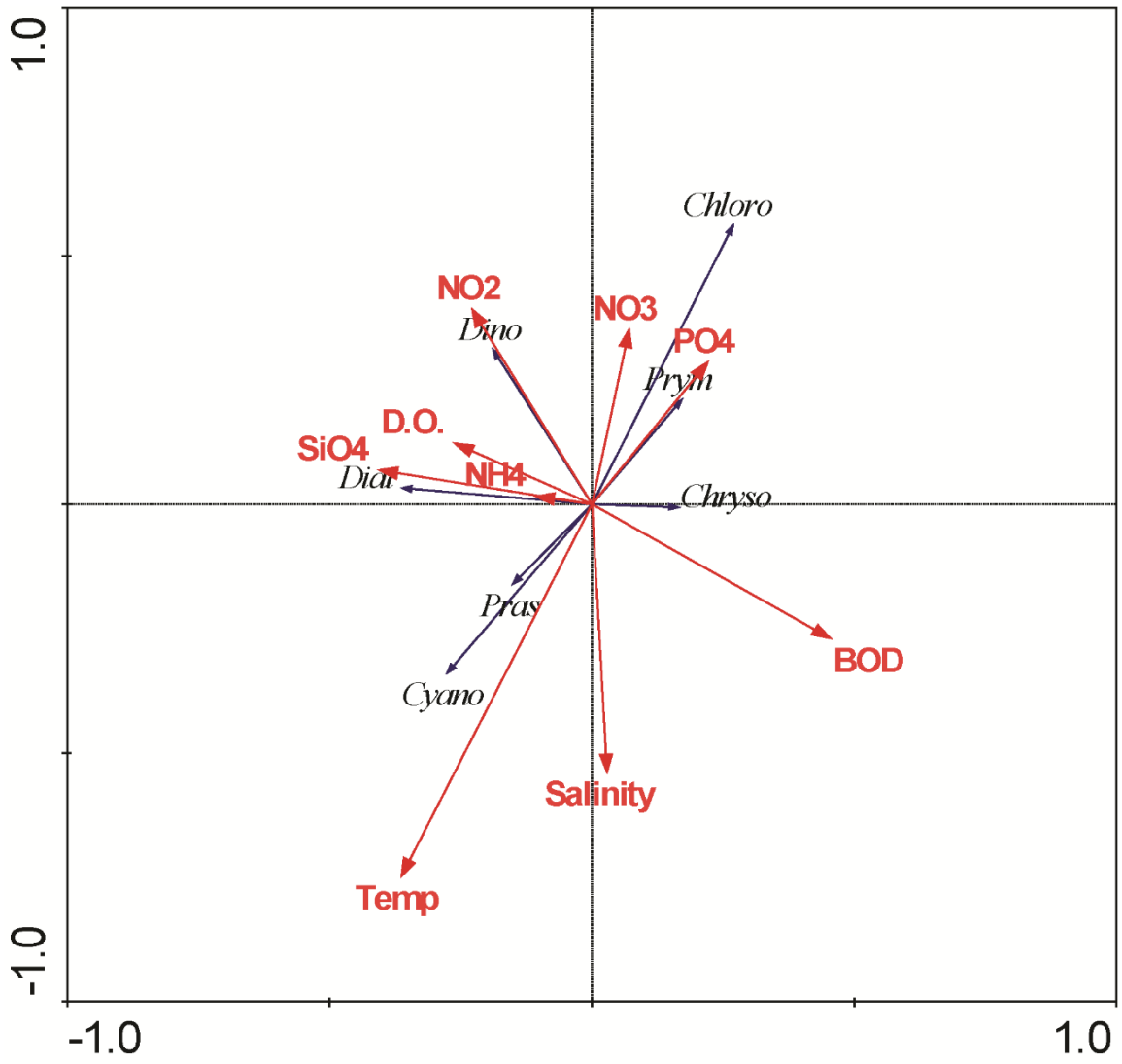

Fig. 3.33 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Cochin port.

3.3.4.2.2 Zuari estuary

Pearson's correlation analysis revealed the insignificant correlation between phytoplankton biomass and environmental variables. RDA biplot, axes 1 and 2 (eigenvalues 0.18 and 0.04, respectively) explained 97.5% of environmental variables and their relationship to PFG's. Though environmental parameters showed an insignificant effect on PFG's. Diatom was positioned towards high salinity and low NO_3 concentrations. Dinoflagellates were positioned towards high temperature and Secchi depth. Cyanobacteria were positioned with high SiO_4 and low NO_2 concentrations, while other PFG's (Chlorophytes and prasinophytes) were positioned with high PO_4 and low temperature (Fig. 3.34).

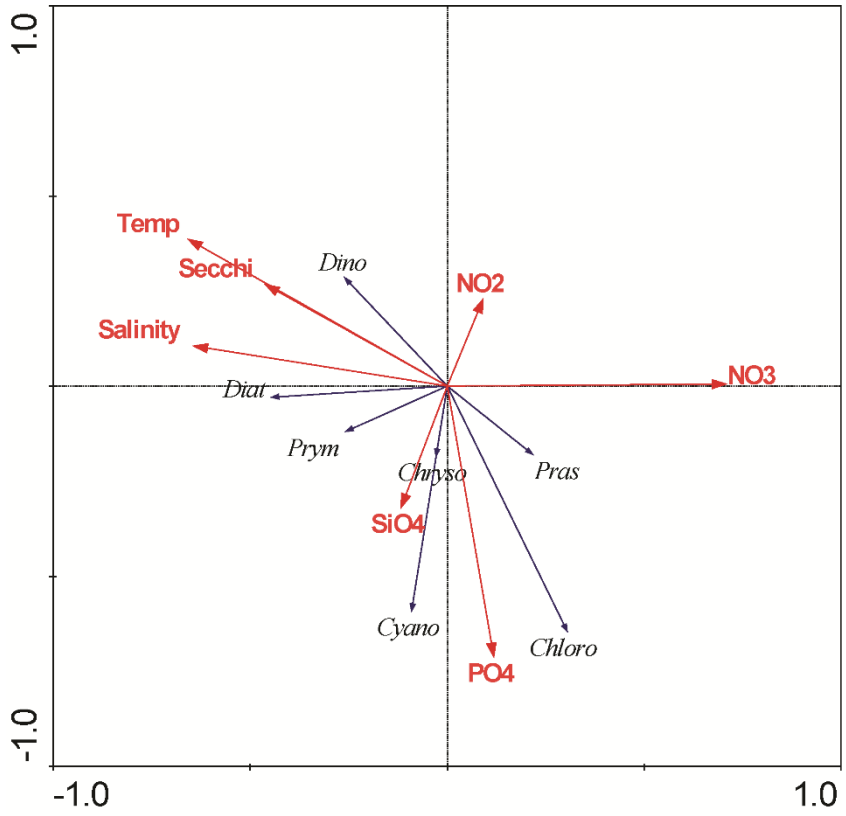

Fig. 3.34 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Zuari estuary.

3.3.4.3 Marine port ecosystem

3.3.4.3.1 Kandla port

Pearson's correlation analysis for surface water and NBW revealed a strong significant correlation between phytoplankton biomass and DIN (NH_3, NO_2, and NO_3). In the RDA biplot for surface water biplot, axes 1 and 2 (eigenvalues 0.35 and 0.09, respectively) explained 76.2 and 95.4% of the environmental variables. Temperature, NH_3, NO_3, SiO_4 are the important environmental variables influencing the PFG's in the surface waters. Diatom positioned with the high SiO_4 and low temperature. Cyanobacteria and cryptophytes positioned with higher NH_3 and lower NO_2 and NO_3 values. Dinoflagellates positioned with the high temperature. Other PFG's did not showed any significant relationship with the environment variables. In NBW biplot, axes 1 and 2 explained (eigenvalues 0.22 and 0.11, respectively) 61.8 and 91.9% variance associated with the PFG's. Diatom was positioned with high NO_2 and low NH_3. Chlorophytes dinoflagellates and chrysophytes were positioned with the low twmperature and salinity. Cryptophytes, prasinophytes and cyanobacteria were positioned with the higher inorganic nutrients (PO_4, NO_3, SiO_4; Fig. 3.35).

3.3.4.3.2 Mangalore port

Pearson's correlation revealed a significant correlation among the phytoplankton biomass and nutrients such as SiO_4 and NO_2. Whereas in NBW, temperature, NO_2, and NO_3 showed a strong significant correlation with phytoplankton biomass. The RDA biplot for surface water revealed that axes 1 and 2 (eigenvalues 0.18 and 0.07, respectively) explained 98.1% of the environmental variables. DIN (NO_2, NO_3, SiO_4), salinity and DO are the important parameter influencing PFG's in the surface water. RDA axes showed that the diatom (dominant group from Mangalore waters) was positioned with low NO_2 concentrations. Major PFG's like chlorophytes and

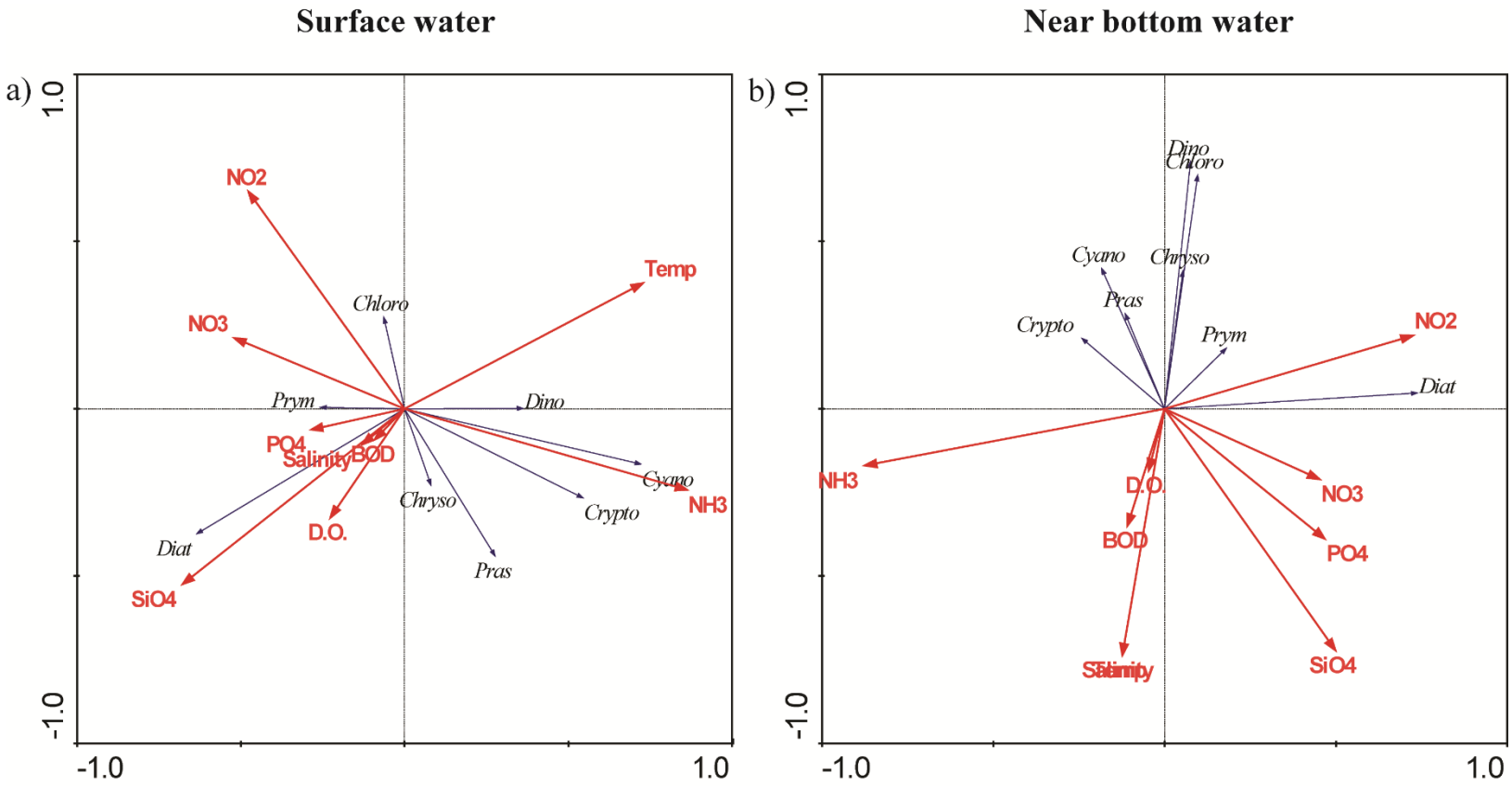

Fig. 3.35 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Kandla port.

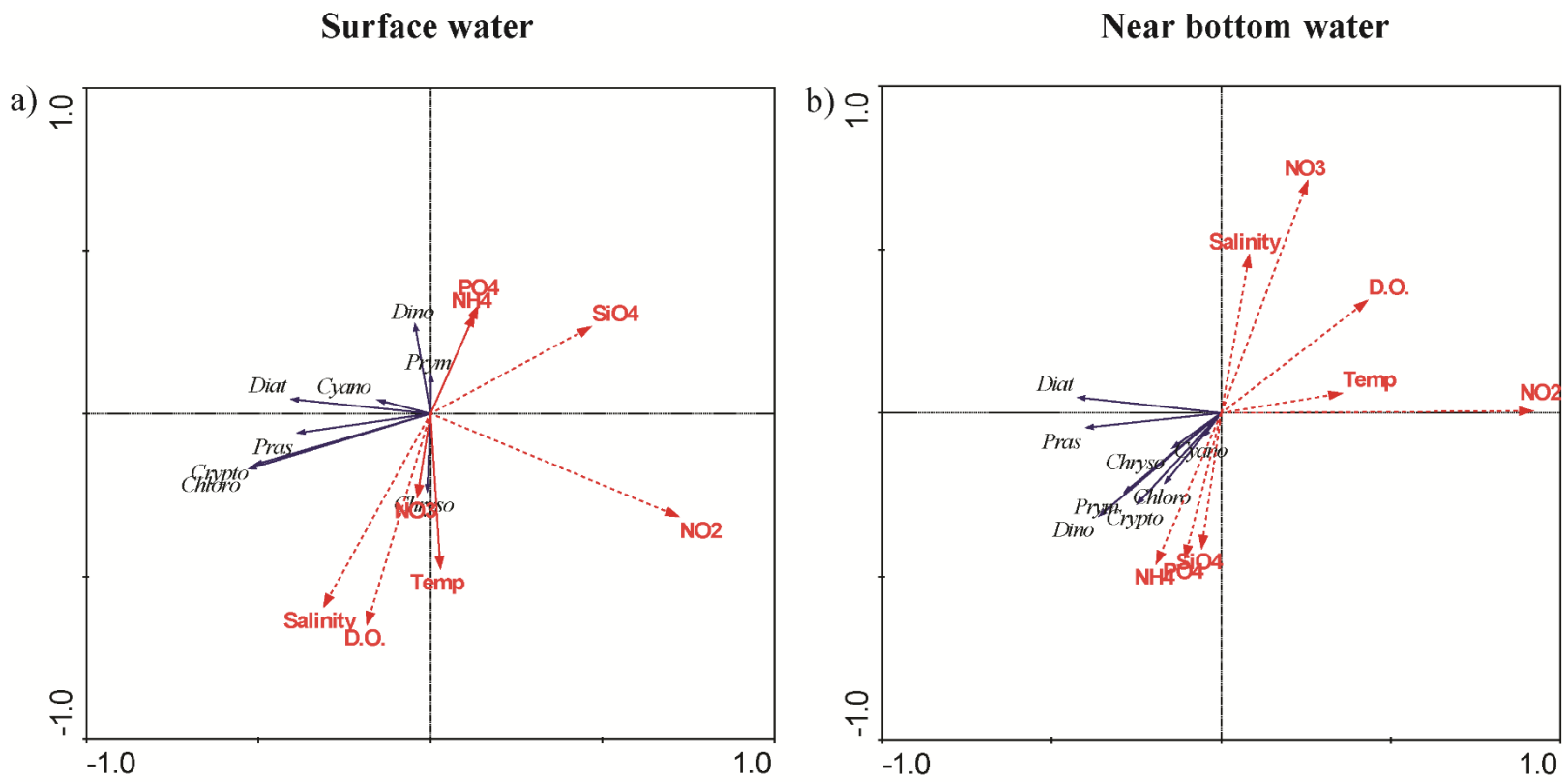

Fig. 3.36 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Mangalore port.

cryptophytes correlated positively with high salinity and DO but negatively with SiO_4. Dinoflagellates and prymnesiophytes were positively associated with PO_4 and NH_3. In NBW biplot, RDA axes 1 and 2 (eigenvalues 0.14 and 0.02, respectively) explained 96.6% variance between environmental variables and PFG's. Diatom was positioned with a low NO_2 concentration. The other major (cryptophytes and chlorophytes) and minor (chrysophytes, cyanobacteria, dinoflagellates, prasinophytes, and prymnesiophytes) groups were positioned with high nutrients (PO_4, SiO_4, and NH_4) and low values of salinity, temperature, and NO_3 (Fig. 3.36).

3.3.4.3.3 Chennai port

Pearson correlation analysis for surface water revealed a significant correlation between the phytoplankton biomass and environmental variables such as salinity, DO, SiO_4, and NH_4. In NBW, phytoplankton biomass showed a significant correlation only with salinity and DO values. In RDA biplot for surface water biplot, axes 1 and 2 (eigenvalues 0.58 and 0.003, respectively) explained 99.4% of the PFG's and environmental variance relationship. Salinity, Temperature, DO, and PO_4 are the major parameters influencing the PFG's. Diatom and chlorophytes were positioned with higher BOD values and lower SiO_4 and NO_3 concentrations. Dinoflagellates and cyanobacteria were positioned with the high NO_2 and NH_4 concentrations and lower salinity. Cryptophytes were positioned with high salinity and reduce NO_2 and NH_4 concentrations. In the NBW biplot, axes 1 and 2 (eigenvalues 0.58 and 0.003, respectively) explained 97.3% of environmental variables and their relationship with PFG's. SiO_4, salinity, temperature, NH_4, DO were the major parameters influencing the PFG's distributions. Diatom and chlorophytes were positioned with the higher salinity and BOD values and low DO and NH_4 values. Dinoflagellates

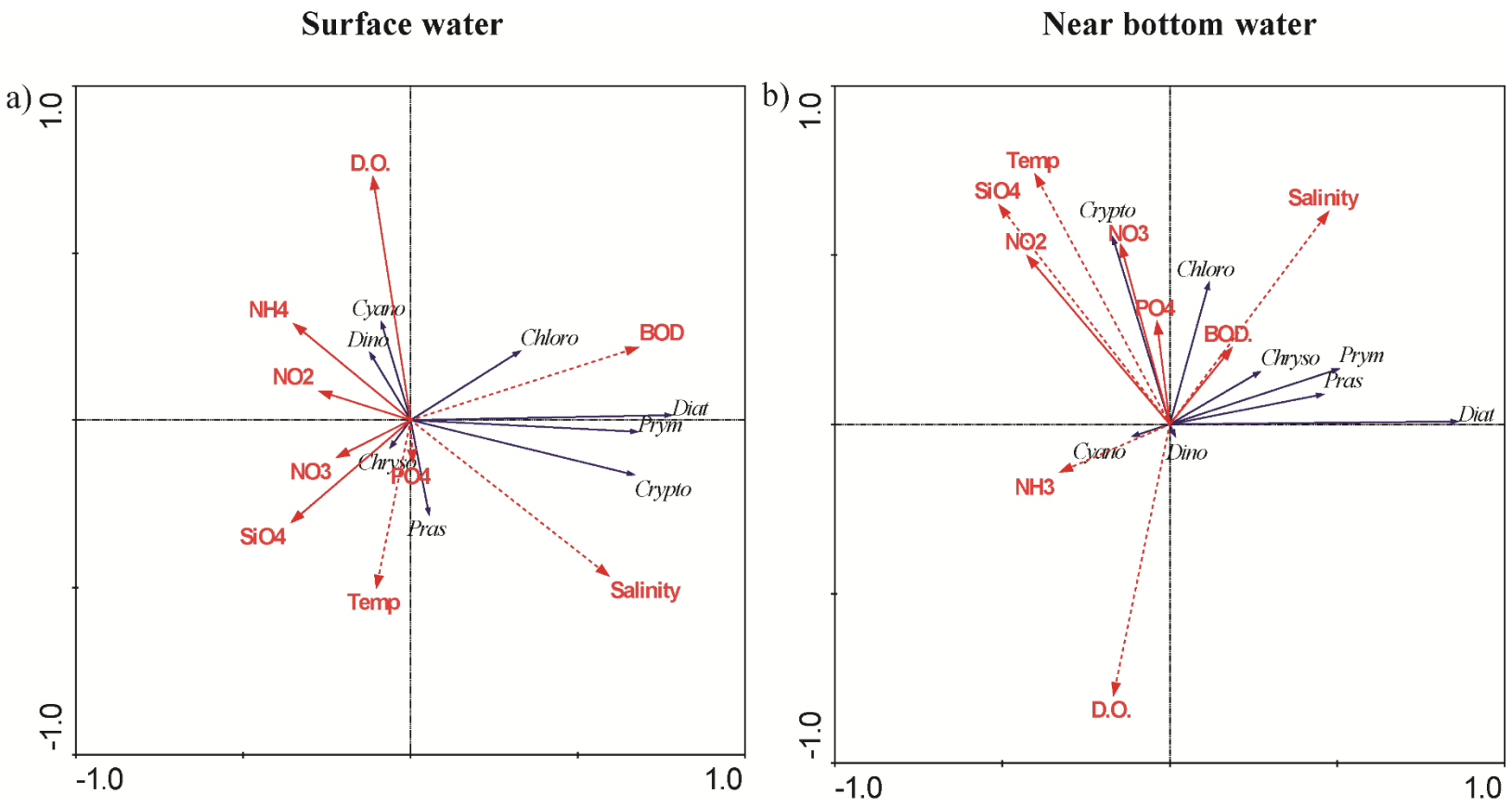

Fig. 3.37 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Chennai port.

positioned with low values of SiO_4, NO_2, and temperature. Cryptophytes were placed with high DIN concentrations (SiO_4, NO_3, NO_2, and PO_4) and high temperatures (Fig. 3.37).

3.3.4.3.4 V.O.C port, Tuticorin

Pearson's correlation analysis from surface water revealed a significant positive correlation between phytoplankton biomass and environmental parameters such as salinity, DO and, DIN (PO_4, SiO_4, and NH_4) values. Whereas in NBW, phytoplankton biomass is significantly influenced by the salinity, temperature, DO and, DIN (SiO_4, NH_4, and NO_3). In RDA biplot for surface water biplot, axes 1 and 2 (eigenvalues 0.54 and 0.07, respectively) explained 99.9% environmental variables. Salinity, NO_2, DO, SiO_4, and NH_4 are the major influencing parameters that affect phytoplankton distribution. Diatom was positioned with high salinity and DO values. Cryptophytes were positioned with high NO_2 and NO_3 concentrations. Dinoflagellates, chlorophytes, and prasinophytes were positively associated with the high concentrations of NH_4 and SiO_4. Cyanobacteria were positioned with high temperature and PO_4 concentration. In the NBW biplot, RDA axes 1 and 2 (eigenvalues 0.56 and 0.02, respectively) explained 99.5% of environmental variables and their relationship with the PFG's. Diatom was positioned with a high SiO_4 concentration. Chlorophytes were positioned with high DO values and low concentrations of DIN (NO_2, NO_3, and PO_4). Cyanobacteria were positioned with high temperature and low NH_4 concentrations (Fig. 3.38).

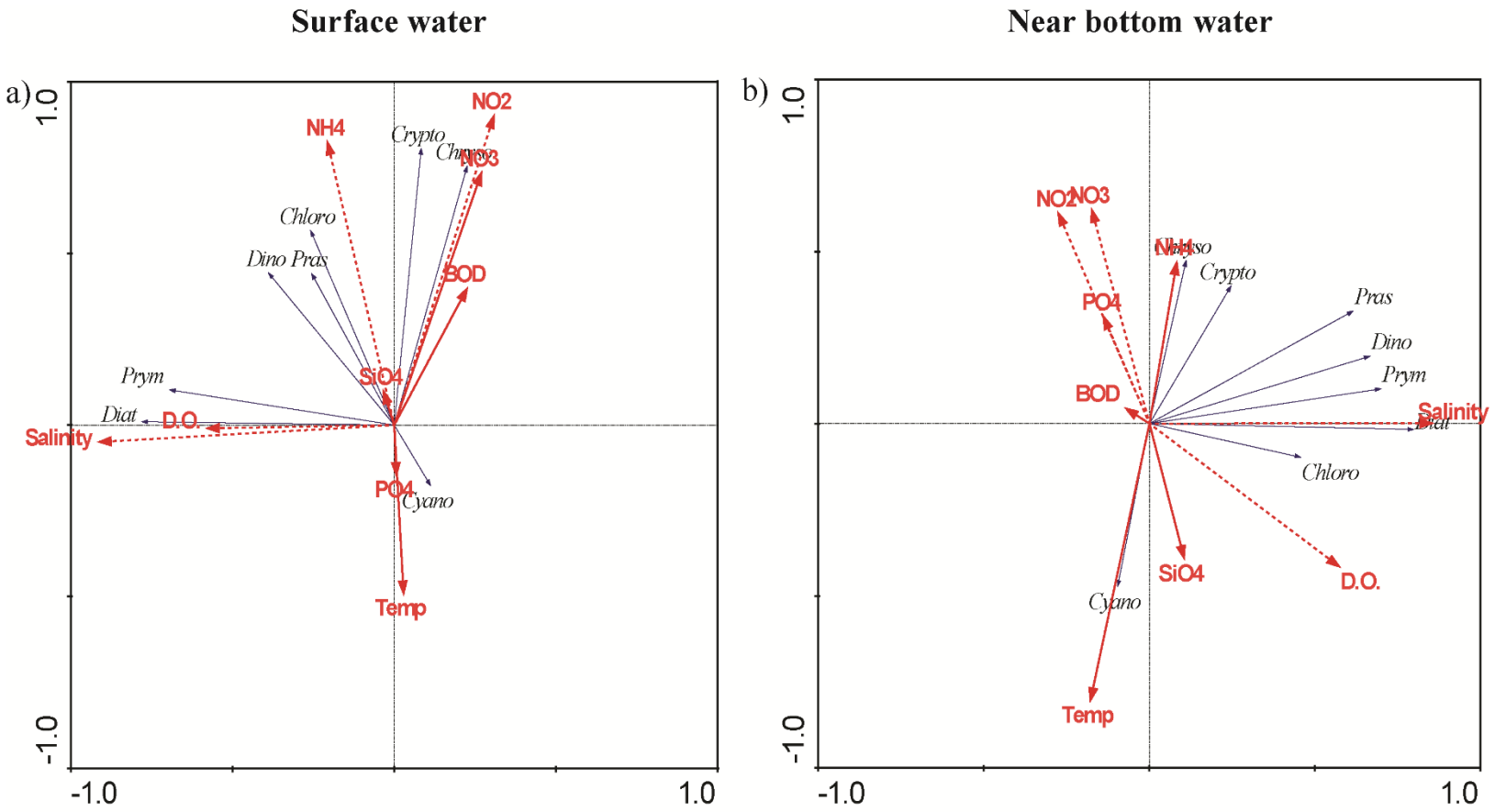

Fig. 3.38 Ordination diagram based on the RDA analysis of PFG's and environmental variables from V.O.C port.

3.4 Discussion

PMP dynamics and distributions of PFG's are strongly coupled with the hydrological parameters of all studied port ecosystems. Results have shown distinct inter-seasonal and spatial variations in measured environmental parameters, phytoplankton biomass, and groups from the marine, estuarine and freshwater port ecosystems located along the Indian coasts. However, the seasonal patterns varied according to the geographical location. Many authors have studied phytoplankton dynamics from the marine (Harnstorm et al., 2009, Rath et al., 2018, D'costa et al., 2008 and 2010, D'silva et al., 2012, Patil et al., 2017, Subramanian and Mahadevan 1999, Eshwari and Ramani Bai, 2002, Satpathy et al., 2009) freshwater (De et al., 1994, Roshith et al., 2018, Panigrahi et al., 2009, Suresh et al., 2013) and estuarine ecosystems (Patil and Anil, 2008, 2011, 2015 and 2019, Balachandran et al., 2005, Madhu et al., 2007, Martin et al., 2011, Rodrigues et al., 2019) along the Indian coast concerning seasonal changes, the effect of monsoons (SWM and NEM), food web dynamics, algal blooms, dinoflagellate cyst distributions. These studies highlight that the PFG's are sensitive to seasonal variations and changes in the environmental variables. Most of these studies are based on microscopic observations that are limited to nano and microplankton. Some studies have shown pico-phytoplankton distributions and dynamics from the monsoonal estuaries (Mitbavkar et al., 2015, Rajaneesh et al., 2013, and 2018). Still, information's on smaller groups such as chlorophytes, cryptophytes, prasinophytes, and prymnesiophytes are limited (Parab et al., 2013, Madhu et al., 2014, Bandyopadhyay e al., 2017). This study emphasizes the distributions of PFG's (comprising all size groups) from different port ecosystems (freshwater, estuarine and marine) along the coast of India with the help of pigment-based chemotaxonomy and is presented subsequently.

3.4.1 Freshwater port ecosystems

Among the nutrient-rich freshwater port ecosystems, Kolkata port is more productive compared to the Haldia port. Low biomass in Haldia port could be due to rapid salinity changes during different seasons and tides (ebb and flood) (Sadhuram et al., 2005, Deb and Chakraborty, 2012). In Kolkata, higher biomass was observed during SWM and PrM 1 compared to the PM and PrM2. Interestingly, PFG's contribution to the chl *a* and percentage composition of each algal group showed a similar trend in Haldia but different in Kolkata. However, differences in biomass distribution between riverine and port stations were observed, but the trend varied seasonally. For instance, in Kolkata port, biomass was higher in riverine stations than port stations (except during PrM1 and PM) despite higher DIN, and the same was observed in Haldia port (except PrM's wherein differences were insignificant). Increased water residence time due to enclosed systems, the fresh phytoplankton input from the upstream in Kolkata port, and salinity changes due to seawater intrusion in Haldia port could be the reasons for differences. Additionally, in both the ports, biomass was higher in the surface water compared to NBW (except during PrM 2 season). Spearman correlation indicated that temperature, PO_4, and NO_3 could be the factors influencing phytoplankton biomass. Further, in Haldia port, salinity changes due to tidal influence were also significant (Fig. 3.32).

All 8 phytoplankton groups were identified from both Kolkata and Haldia freshwater ecosystems. Diatoms (fuco), chlorophytes (lut), cryptophytes (allo), and cyanobacteria (zea) were the major PFG's and is similar to studies from freshwater systems (Wehr and Sheath, 2003, Lionard et al., 2005). At the same time, dinoflagellates (Peri), prymneisiophytes (19 Hexa), prasinophytes (Pras) and chrysophytes (19 But) formed minor PFG's. However, the seasonal patterns varied between Kolkata and Haldia port. For instance, seasonal changes in phytoplankton contributions were not

distinct in Kolkata than Haldia port, and rapid salinity variations could be the reason later. The influence of salinity changes in the shift of PFG's contributions is also reported in freshwater ecosystems elsewhere (Quilan and Phlips, 2007, Flöder et al., 2010).

Among the major groups, chlorophytes and diatom dominated round the year in Kolkata port with the maximum contribution during PrM1 and SWM, respectively. While in Haldia port, it was observed during SWM and PM when the salinity was low. In Kolkata and Haldia ports, diatom and chlorophytes showed a strong DIN relationship (NO_2, NO_3, and PO_4, Fig. 3.31 and 3.32). Previous studies from the Hooghly estuary also reported the dominance of chlorophytes, diatom, and cyanobacteria (Roy, 1955, Roshith et al., 2018, Choudhary and Pal., 2011, Mitra et al., 2011). The salinity variations in Haldia port during PrM1 and 2 favored cyanobacteria and cryptophytes at the cost of PFG's, which are low tolerance to the increasing salinity (Sellner et al., 1988, Deshpande et al., 2010 and reference therein). Changes in salinity result in osmotic stress on cells, which leads to the gain or loss of ions and affects the ionic ratio of cells (Guillard, 1962). Though the salinity tolerance is higher in brackish and marine phytoplankton, osmoregulation abilities in response to salinity changes differ prominently between different phytoplankton taxonomic groups (Redden and Rukminasari, 2008 and reference therein). RDA also indicated the prevalence of cryptophytes and chlorophytes with high and low salinity, respectively (Fig. 3.31 and 3.32). Interestingly, in Kolkata port, surface water and NBW (except during PM) were dominated by chlorophytes and diatom, respectively. It could be due to the chlorophytes affinity towards higher light conditions for the growth, while diatom adapts well to the lower light conditions (Richardson et al., 1983). Moreover, in Haldia port, distinct seasonal variations in PFG's contributions between surface water and NBW may be regulated by salinity changes influenced by tides (ebb tide and flood tide).

Diatom could proliferate in widely changing environmental conditions compared to other groups. Egge and Aksnes (1992) described the dominance of diatom could have evident irrespective of the seasons when SiO_4 concentrations surpassed the threshold of approximately 2 μM. Despite the higher SiO_4 concentrations in both ports, diatom contributions were relatively higher in the Kolkata port compared to Haldia port. In Kolkata port, diatom contributions between riverine (high during SWM) and port stations (high during PM) varied seasonally (except PrM) but not in Haldia port. Like diatoms, chlorophytes percentage contributions and absolute contributions to the chl were higher in the Kolkata port than in Haldia port. The absence and presence of seawater intrusion in Kolkata port and Haldia port, respectively, could be the possible reasons for the differences. In Haldia port, percentage contributions and absolute chlorophytes were observed only during SWM and PrM season. Unlike diatoms, chlorophytes' contributions were higher in the riverine stations than port stations in both Haldia (except during SWM) and Kolkata ports. Cryptophytes and cyanobacteria, the other major PFG, are widespread algae which could thrive in freshwater, estuarine and marine environment (Wall et al., 2014, Cunningham et al., 2019). It was found that the cryptophytes and cyanobacteria contributions were higher in Haldia (during all the seasons) and Kolkata ports (except during PrM1 for cryptophytes), respectively. Interestingly, both the PFG showed differences between port and riverine stations, but the distribution pattern was different for Kolkata and Haldia ports. For instance, higher cryptophytes were observed in riverine and port stations in Kolkata and Haldia port, respectively, while higher cyanobacteria were observed in port stations of Kolkata (except SWM) and Haldia (during PrM's but insignificant in SWM and PM) ports. RDA analysis indicated that the salinity (in Haldia port) and PO_4 (only cryptophytes) could influence their prevalence (Fig. 3.31 and 3.32). Laboratory experiments to understand the salinity effect on phytoplankton communities in a

tropical freshwater system revealed the 1 PSU could eradicate and introduce the green algae and cyanobacteria dominance, respectively. Moreover, the results also indicate that the cyanobacteria could tolerate higher osmatic pressure until the salinity reached up to 16 PSU, which is possible due to the increased production of zea as protective xanthophyll against osmatic stress (Chakraborty et al., 2011). Among the minor groups, prasinophytes, dinoflagellate, and prymnesiophytes contributions were evident in Kolkata and Haldia ports. Further, dinoflagellate and prymnesiophytes showed relatively high contributions during PrM's seasons in both ports. Chrysophytes, the least dominant PFG, were negligible in both Haldia and Kolkata ports. However, these groups didn't vary between the port and riverine stations and between the surface water and NBW in Kolkata and Haldia ports.

3.4.2 Estuarine port ecosystems

Estuaries are the most productive habitat, which acts as an essential nutrient source to the coastal ecosystem, breeding ground or food source for the marine species, the nursery for the juveniles, and potential fishery zone. Estuaries undergo the momentary physical forcing from marine source (tides, waves, and saltwater influx) as well as from the riverine source (fresh water and sediment discharges, Cloern and Nichols, 1985). The sharp gradient in these physico-chemical properties causes drastic changes in the non-conservative elements such as oxygen, carbon, nutrients, and trace metals, resulting in a massive impact on estuary biology, especially on PFG's composition (Ahel et al., 1996) which is also reported from Cochin and Zuari estuary (Anand et al., 2014, Vijith et al., 2009, Sundar et al., 2015, Madhu et al., 2009). Phytoplankton pigments (chlorophyll and other marker pigments) and PFG's distributions showed distinct seasonal and spatial variation to the rapidly changing estuarine environment conditions of both systems.

Among the estuarine ecosystems, Cochin port harbored higher biomass compared to the Zuari estuary. Many studies also reported the highly productive nature of the Cochin backwater irrespective of the seasons (Madhu et al., 2007, Martin et al., 2008, Madhu et al., 2009, Rajaneesh et al., 2015, Rodrigues et al., 2019), but the evaluation using phytoplankton pigment is scarce. Low temperature, low salinity, and high nutrient concentrations observed during SWM were attributed to the freshwater influx (precipitation and riverine discharge). The water column was partially mixed during other seasons due to the reduced freshwater inflow, as observed from other monsoon-influenced estuaries (Joseph and Kurup, 1989, Shetye, 1999). Distinct variations in environmental variables and PFG's between PM 1 (October) and PM 2 (November) was due to the samples collected during early and late PM, respectively. Phytoplankton biomass in the Cochin port showed an insignificant relationship with the environmental variables. Relatively higher biomass observed in the EC followed by MC and AC stations suggesting lower water circulations could have favored the phytoplankton biomass in inner port stations of Cochin port. Diatom (Fuco), chlorophytes (Lut), cyanobacteria (Zea), and dinoflagellates (Peri) are the major PFG's. Interestingly, cryptophytes (Allo), a major PFG's present in the freshwater and marine ports, were not observed in this study.

Among the major PFG's, diatoms were predominant similar to that reported in previous studies from the region (Madhu et al., 2007, Madhu et al., 2010). In this study, diatom dominated (64.1 to 77.7%) chl and PFG's, and the maximum percentage contribution was observed during PM 1, compared to other seasons. Still, the reverse was true in terms of absolute contribution to the chl *a* and predominance of centric diatom *Skeletonema costatum* during all the season could be the reason (BAMPI monograph series 5). Inside the port, diatom contributions were relatively higher in enclosed channels i.e. EC (SWM, PrM, and PM 2) and MC (PM 1) with lower water

circulation than AC. Diatom contribution was generally higher at salinity >11.2 (Fig. 3.33), indicating the competition between salinity ingress and egress as influencing factors. The predominance of other major PFG's chlorophytes and cyanobacteria was observed during SWM and PM 2, respectively, while both contributed equally during PrM. Among the port stations, relatively higher chlorophytes were observed in AC (PM1 and PrM) and EC stations (SWM), located adjacent to the port entrance experiencing freshwater influence from the US and the tidal action. Whereas during PM2, it was evenly distributed. During SWM, chlorophytes prevalence coincided with the relatively low temperature (27 to 28.1 °C) and low salinity (2.63 to 9.26), suggesting that freshwater influx is a conducive environment for its dominance. Such dominance was also evident in the freshwater ecosystem (above section 3.3.1). PO_4 and NO_3 were the major factors influencing chlorophytes distributions (Fig. 3.33). Cyanobacteria prevalence was maximum during warmer seasons PM2 and PrM. Flowcytometric data on the picophytoplankton community also confirmed the higher population of *Synacococcus*-PC during PM 2 (Rajaneesh et al., 2015). Generally, cyanobacteria have an affinity towards the high temperature (Sellner, 1997, Barlow et al., 2008) and was also evident in the RDA biplot (Fig. 3.33). Inside the port, relatively higher percentage contributions of cyanobacteria was observed in the EC (PM1 and PrM) and AC (SWM and PM 2) stations.

Among the minor PFG's, only dinoflagellates were observed round the year with maximum contribution during PM1 and SWM and the rest during PM2 and PrM. Parallel microscopic studies also confirmed a higher abundance of dinoflagellates during PM 1 and SWM (Rodrigues et al., 2019). Interestingly, higher dinoflagellates coincided with lower salinity (2.6 to 9.4) during SWM, and this was mainly due to the dominance of autotrophic dinoflagellate, *Pyrophacus steinii* (Rodrigues et al. 2019). Previous studies also reported the bloom of *Karenia*

mikimotai (a toxic dinoflagellate) near our study region during PM season (Madhu et al., 2011). Relatively higher dinoflagellates contributions were observed in the EC stations (except during PM 1), which are the inner port stations with low water circulations. Prasinophytes and chrysophytes were the other minor PFG's with the former showing relatively maximum contribution (biomass and composition) only during PrM, and the later contribution was negligible. RDA indicated that temperature was the major factor influencing the prasinophytes distributions as observed during PrM season (Fig. 3.33). Higher prymnesiophytes contribution to the biomass and composition was observed only during PM's seasons. Prasinophytes and prymnesiophytes did not show any distinct spatial variations.

Zuari estuary is one of the well-studied systems, and its water column is generally well-mixed along the transect and stratified up to the middle estuary during a spring tide (ST) and neap tide (NT), respectively (Shynu et al., 2013, Sundar et al., 2015). Saltwater intrude up to upstream and upper-middle estuary during ST and NT, respectively. Since the samples were collected during ST, changes in the phytoplankton community concerning saltwater intrusion is well-reflected even in the upstream region (Rajaneesh and Mitbavkar 2013). A previous study based on the microscopy reported that the phytoplankton abundance and composition are closely coupled with the various physical (advection, light, temperature, salinity, etc.), chemical (nutrients), and biological (grazing, cell senescence) factors and interaction among them (Patil and Anil 2011). Most of the studies have reported the dynamics of diatom (microscopy), dinoflagellates (microscopy), and cyanobacteria (flow cytometer) based on cell counts but to our knowledge information on the phytoplankton marker pigments and contribution of PFG's presented here will be the first report for the well-studied Zuari estuary.

Phytoplankton pigments (Chlorophylls and marker pigments) and PFG's composition and chl *a* contributions showed distinct seasonal and spatial variations and strongly coupled with the salinity transect's environmental changes. Similar to Cochin port, (i) phytoplankton biomass showed insignificant correlation with the environmental variables in Zuari estuary, and (ii) diatoms (Fuco) followed by chlorophytes (Lut), and cyanobacteria (Zea) formed the major groups while the minor groups represented by dinoflagellates (Peri), prymnesiophytes (19 Hexa), and prasinophytes (Pras). The previous study from the mouth of Mondovi estuary, an adjacent water body of Zuari estuary, also indicated the presence of major and minor PFG's (Parab et al., 2013). Among the major PFG's, diatoms, and chlorophytes wherein the former showed an increasing trend from the US to DS stations and the latter a reverse trend. Maximum diatom contribution along the salinity gradient (especially from LME to the US) during PrM could be attributed to the well-mixed water column and increased water residence time due to reduced freshwater discharge (Shetye and Murty, 1987, Shetye et al., 2007, Manoj, 2012). Lu and Gan, 2015, also observed that the low water discharge induced longer residence time, water column stability, and transparency, which favored the diatom bloom in the upper Pearl estuary during the dry season. Resident time plays a crucial role in defining the nutrient availability and utilization by PFG's (Paerl et al., 2006). Higher diatom contribution at DS during SWM (5.9 µg l^{-1}) and PM (2.4 µg l^{-1}) can be attributed to the changes driven by monsoonal discharges such as stratification, nutrient influx, resuspension of diatom benthic propagules and occurrence of diatom blooms (Patil and Anil, 2008, 2011 and 2015). The diatom dominance to monsoonal discharges is also reported from the adjacent Mandovi estuary (Parab et al., 2013) and the significant relationship between diatom and salinity (Fig. 3.34).

A further increasing trend in chlorophytes contribution from DS to US was observed only during PM followed by SWM but not during PrM. This could be attributable to high and low freshwater discharges (Paerl et al., 2006). For instance, high freshwater discharge favors fast-growing phytoplankton (such as chlorophytes and various flagellates), and low discharges favor slow-growing dinoflagellates cyanobacteria by reducing salinity and resident time. For instance, higher cyanobacteria contribution was observed during PM compared to SWM. RDA also highlighted that the high PO_4 and SiO_4 were the major factors influencing the cyanobacteria and chlorophytes distributions, respectively (Fig. 3.34). Previous studies on the size-fractionated chl *a* from the study area also indicated the maximum pico (<3µ) fraction chl *a* during PM season, particularly from the middle estuary and US stations (Rajaneesh et al., 2018). A flow cytometry study by Mitbavkar et al. (2015) from the DS stations also suggested higher pico-phytoplankton counts during PM and SWM as observed in the present study. Contrary study in the Mandovi estuary's DS stations indicating the higher contribution of cyanobacteria during PrM compared to other seasons (Parab et al., 2013). During SWM, the estuary is completely flushed with river runoff and freshwater influx (Qasim and Sen Gupta,1981, Vijith et al., 2009), whereas PM is considered as the recovery period as the changes brought about by the SWM reverted to normal seawater dominated conditions (Anand et al., 2014). These conditions could be a conducive environment for the chlorophytes and cyanobacteria to proliferate. A study from the Neuse River estuary pointed out that the well-mixed, turbid, and high-nitrate conditions increase the primary productivity by favoring the growth of diatoms, chlorophytes, and cyanobacteria (Pinckney et al., 1999) as observed in the present study.

Among the minor groups, prasinophytes evident only during SWM with the increasing contribution from the DS to the US, whereas in DS stations of the Mandovi estuary, higher

prasinophytes were observed during PrM season (Parab et al., 2013). RDA analysis also indicated the prasinophytes are positioned with high PO_4 and low salinity, temperature, and Secchi depth (Fig. 3.34). Prymnesiophytes were evident during all the seasons, with the maximum contribution to the biomass and composition during PrM and SWM seasons. Higher dinoflagellates contributions was observed during PrM and PM seasons, particularly DS station, and the same is also reported from the Mandovi estuary (Parab et al., 2013). Dinoflagellates contribution showed a decreasing trend from DS to LME while contribution was negligible at the upstream stations highlighting reduced affinity to lower salinity/freshwater regions. RDA indicated high temperature and water transparency are the major factors influencing the dinoflagellate distributions observed during PrM (Fig. 3.34). Other minor PFG's showed an insignificant relationship with the environmental variables because of their negligible contributions.

3.3.3 Marine port ecosystems

Phytoplankton biomass and distribution from the coastal water are generally driven by environmental variables (Downing, 1997, Lara-lara et al., 1990, Mallin et al., 1999) and were also reflected in the studied port ecosystems. However, the PFG's distribution pattern between the port ecosystems was different though distinct seasonality was observed in each ecosystem. This was true for environmental variables, phytoplankton biomass (chl a), and marker pigments). Interestingly, irrespective of biomass, marker pigments for all eight phytoplankton groups were observed from the marine ports located along the west and east coasts. However, the marker pigment concentrations and the contributions were different. For instance, higher and lower biomass was observed among the west coast marine ports from the Mangalore and Kandla port, respectively. In contrast, distinct seasonality was observed in east coast marine ports. To

understand such variability, RDA was performed to assess the factors influencing the variability in each of the marine ports and are discussed below.

Among the marine ports, irrespective of the season, the lowest and highest biomass was in Kandla and Mangalore port, respectively, while in east coast marine ports showed distinct seasonality in phytoplankton biomass. Despite the high DIN in Kandla port waters, turbidity has an adverse effect on phytoplankton dynamics due to low water transparency and light penetration (Shirodkar et al., 2010). Interestingly, no distinct spatial variations in biomass were observed in Kandla port, whereas higher biomass was observed in the inner port areas with low water circulations in other ports. Among the measured environmental variables, dissolved inorganic nutrients were found to be the most influential factors in determining the nature of phytoplankton biomass distribution in Kandla (NO_3, NO_2 SiO_4, and NH_3, Fig. 3.35), Mangalore (NO_2, NO_3, and SiO_4, Fig. 3.36), Chennai (SiO4 and NH_4, Fig. 3.37) and V.O.C. (PO_4, SiO_4, and NH_4, Fig. 3.38) ports. Salinity (only east coast ports) and temperature (Mangalore and VOC) were the other parameters correlated with phytoplankton biomass. Probably nutrient influx through monsoonal precipitation might have triggered phytoplankton biomass. In all the ports, phytoplankton biomass is mainly represented by diatom (Fuco), cryptophytes (Allo), and chlorophytes (Lut), while the dinoflagellates (Peri), cyanobacteria (Zea), prymnesiophytes (19 Hexa), and prasinophytes (Pras) formed the minor PFG. However, the distribution of phytoplankton biomass and PFG's inside the ports showed distinct spatial variations in Mangalore, Chennai and V.O.C ports but not in Kandla port. The prevalence of low biomass and strong tidal currents could be the possible reason for insignificant spatial variation in phytoplankton biomass and major and minor PFG's in Kandla Port. In other marine ports, relatively higher biomass is observed in the inner port stations experiencing less water circulation. Even the contribution of all the major PFGs viz,

diatoms, chlorophytes, and cryptophytes (except Chennai) and some minor PFG's (dinoflagellates) were relatively higher in inner port stations than the open stations. The other minor PFG's (except cyanobacteria in Mangalore and Chennai ports) showed no spatial variations. In cyanobacteria, spatial variations were observed in Mangalore (i.e., higher in inner and outer port stations from surface and NBW, respectively) and Chennai ports (in outer ports such as BDE and BDW), but the distribution pattern was different, unlike major PFG's. Generally, low water movement and current effects from the inner port area of Ceuta port (North Africa) and Tolo Harbour (Hong Kong) explain the port structure negatively influence the benthic community (Guerra-Garcia and García-Góme, 2003) and induce algal blooms (Wong and Wong 2004), respectively. Marine ports (Mangalore, Chennai, and V.O.C ports), which is considered a semi-enclosed body, also support this assumption of relatively higher nutrient concentrations, higher PFG contributions, and restricted circulations.

Among the major PFG's, diatoms dominated in Kandla, New Mangalore, Chennai, and V.O.C (only during SWM and SIM) ports and were in accordance with other previous studies (based on microscopy data) from the west (Rehnstam-Holm et al., 2010, Harnstrom et al., 2009) and east coast (Eshwari and Ramanibhai, 2001, Satpathy et al., 2009, Anand et al., 2015, Anand and Kala, 2015, Bharathi et al., 2018). RDA analysis indicated a strong positive relationship with diatom and salinity in all marine ports (except in Mangalore port, Fig. 3.35 to 3.38). In Kandla port, diatom dominated despite low light penetration and low biomass condition as they are capable of adapting to the low light conditions (Lionard et al., 2005) and its predominance in PrM, could be related to the high salinity (39.1 to 40.6) and relatively low-temperature (20.6 to 21.3 °C) conditions. Barlow et al., 2008 also mentioned the diatom affinity towards the lower temperature. Parallel microscopic observations in Mangalore port also confirmed the high

abundance of centric diatoms (*Leptocylindrus danicus*, *Paralia sulcata*, and *Rhizosolenia imbricata*) during PrM (mainly from the inner port) and succession of *Skeletonema costatum* during SWM season (Rath et al., 2018). Among the east coast marine ports, diatoms dominated round the year in Chennai port, while in V.O.C. port, it was observed only during SWM and FIM. Balakrishnan et al., 2017 also noticed the low DIN during summer (SWM) due to increased nutrient uptake by the Tuticorin waters' phytoplankton. The dominance of diatom during FIM in V.O.C. port coincided with a moderate temperature and low DIN. The lowest diatom contribution during NEM and SIM seasons could result from low salinity, and high DIN diminished diatom contributions and favored the prevalence of other PFG's (cyanobacteria and cryptophytes), respectively. The previous study also indicated the decrease in the biomass and diatom contribution during NEM despite the high DIN due to the low salinity and light availability (Bharathi et al., 2018).

Cryptophytes formed the next major PFG in all the marine ports. The cryptophytes, showing higher contributions during PM's and PrM in Kandla and Mangalore port, respectively, coincided with the higher temperature and can be attributable to its photophysiological plasticity to tolerate high irradiances (Mendes et al., 2018). Among the east coast marine ports, absolute and percentage contributions were higher in both Chennai and V.O.C (mainly during NEM and SIM) ports. Interestingly, in Chennai port, higher contributions were observed from the NBW compared to surface water. Some studies demonstrated a clear preference for dissolved inorganic or organic N compounds such as urea, river dissolved organic nitrogen (Altman and Pearl, 2012, Cira et al., 2016) and was also evident in all the ports. Statistical analysis indicated that the DIN could be one of the major factors influencing cryptophytes contribution in Kandla (NO_2 and NO_3, Fig. 3.35), Mangalore (NO_2, Fig. 3.36), Chennai (NO_2, Fig. 3.37), and Tuticorin (NO_2 and NO_3,

Fig. 3.38) ports. The relationship between temperature (Kandla and Tuticorin, Fig. 3.35 and 3.38) and salinity (Chennai, Fig. 3.37) was also evident in some ports.

Chlorophytes, the last major PFG, the prevalence was observed in both west and east coast marine ports. However, the occurrence pattern was different. In Kandla port, maximum percentage contributions were during SWM (from NBW) wherein Mangalore port it was observed during PrM and PM's. Absolute chlorophytes contributions were higher in Mangalore port compared to Kandla port. In contrast, chlorophytes are present round the year in east coast marine ports with relatively higher contributions in the NBW compared to surface water. Similar to cryptophytes, chlorophytes showed a strong positive relationship with NO_2 and NO_3 in all marine ports (Fig. 3.35 to 3.38). Striking shifts in PFG's occurred in Chennai and V.O.C. port during low productive seasons, when diatom was replaced by other PFG's such as chlorophytes, cryptophytes, and cyanobacteria.

Cyanobacteria, dinoflagellates, prasinophytes, and prymnesiophytes formed the minor PFG's in marine ports. Among these, cyanobacteria and dinoflagellate showed relatively higher contribution compared to prymnesiophytes and prasinophytes. In west coast marine ports, higher absolute and percentage contributions of cyanobacteria were observed in the Mangalore and Kandla port. Higher cyanobacteria contribution coincided with the relatively warmer temperature in west coast marine ports due to its high temperature affinity (Sellner, 1997, Barlow et al., 2008). RDA analysis also pointed out the significant relationship between cyanobacteria and warmer temperature (Fig. 3.35 and 3.36). Among the east coast marine ports, higher cyanobacteria contributions were observed in Chennai and V.O.C port during NEM and SIM, respectively, coinciding with the relatively low biomass. A previous study pointed out that the increasing zooplankton and cyanobacteria abundance coincided with the decreasing diatom

contributions indicating significant influence of selective zooplankton grazing on the phytoplankton composition (Bharathi et al., 2018). Dinoflagellate contribution was evident in all the marine ports, and its higher percentage and absolute contributions were observed when the marine ports experiences relatively lower salinity. For instance higher contributions of dinoflagellates detected in Chennai and V.O.C port was during FIM (salinity, 23.6 to 31.6) and SIM (33.5 to 33.6), respectively, while in Mangalore (30.5 to 34.9) and Kandla port (15.9 to 40.9) it was observed during SWM. Some studies also reported dinoflagellates sensitivity to salinity changes (Naik et al., 2011 B, Sahu et al., 2014). A study from Galveston, Texas, have reported the dinoflagellate bloom associated with the low salinity resulting in the mortalities of demersal fish and benthic invertebrates (Harper Jr and Guillen, 1989). Microscopic observations from Chennai port also confirmed the prevalence of dinoflagellates, *Tripos furca* (a potential harmful algal bloom species, unpublished data), during FIM. Stimulation of *Tripos fusus* growth due to low salinity and elevated nutrient concentration caused by the heavy rainfall-induced freshwater discharge is also reported from Sagami Bay, Japan, Baek et al. (2009). Other minor PFG's such as prasinophytes and prymnesiophytes were observed in both west and east coast marine ports. Among the west and east coast marine ports, relatively high contributions of prasinophytes and prymnesiophytes were observed in Mangalore and Chennai ports, respectively, due to the prevalence of high biomass compared to other marine ports in the respective coasts. However, due to negligible contribution, it was impossible to ascertain the specific causative factors influencing their Spatio-temporal variations.

3.3 Conclusion

This study concludes that a total of four major (diatom, cryptophytes, chlorophytes, and cyanobacteria) and minor (dinoflagellates, prasinophytes, prymnesiophytes, and chrysophytes) PFG's were identified from all the studied ecosystems irrespective of the biomass distribution (chl *a* concentrations). However, the seasonal distribution pattern of the total biomass and the PFG's varied between the ecosystems and the influencing factors. Interestingly, in each port (except Kandla) the phytoplankton biomass was relatively higher in the enclosed stations than open stations highlighting the influence of flow patterns. For the first time, this study provides an overview of the PFG distribution (including those not reported earlier) from the ecosystems that are least studied with significant human activities. Therefore, this study will be valuable in the studies related to ecosystem assessment and management as demonstrated in the subsequent chapters.

Chapter 4

Phytoplankton marker pigments to study the fate of phytoplankton

4. Phytoplankton marker pigments to study the fate of phytoplankton

4.1 Introduction

In an aquatic ecosystem, phytoplankton, which are diverse and major primary producers, undergoes several loss processes such as grazing, program cell death, viral lysis, sinking, etc. (e.g., Choi et al., 2017). Phytoplankton marker pigments, in particular, chlorophyll *a* (chl *a*) and its derivatives resulting from degradation, which reflect phytoplankton production and the pathway of degradation in the water column and sediment, are considered useful indicators of relatively fresh and degraded microalgal communities, respectively (Boon and Duineveld.1998, Wieking and Kröncke, 2005, Pusceddu et al., 2009). Processes such as growth, grazing, cell sinking and senescence, photodegradation, fecal pellet sinking, physical mixing, and advective transport are known to affect chl *a* and pheopigments concentrations in the euphotic zone. The role of biotic factors (e.g., the catabolic activity of phytoplankton, chewing/grazing and bacterial degradation) in the breakdown of chl *a*, which is a multistep pathway (Fig. 4.1), are frequently reported (Satoh and Hama, 2013, Schelbert et al., 2009, Eckardt, 2009). However, different pathways of pigment degradation affect the integrity of chlorophyll I.e., demetalation and dephytylation. To the best of our knowledge, more in-depth information on chl *a* breakdown is available for leaf senescence in land plants in comparison to the situation for algae (Scheer 2012). Demetalation (i.e., removal of the central magnesium atom from chl *a*) and dephytylation (i.e., removal of the phytol chain from chl *a*) are the early steps in chl *a* breakdown for all chlorophyll-containing organisms (Satoh and Hama, 2013, Hu et al., 2015, Kuai et al., 2018 and references therein). The cleavage of the phytol chain and magnesium atom from chl *a* is catalyzed by hydrolase enzymes (chlorophyllase and pheophytinase) and metal chelating compounds and/or low pH, respectively. Dephytylation of chl *a* by chlorophyllase activity during cell senescence was widely accepted as the only pathway until

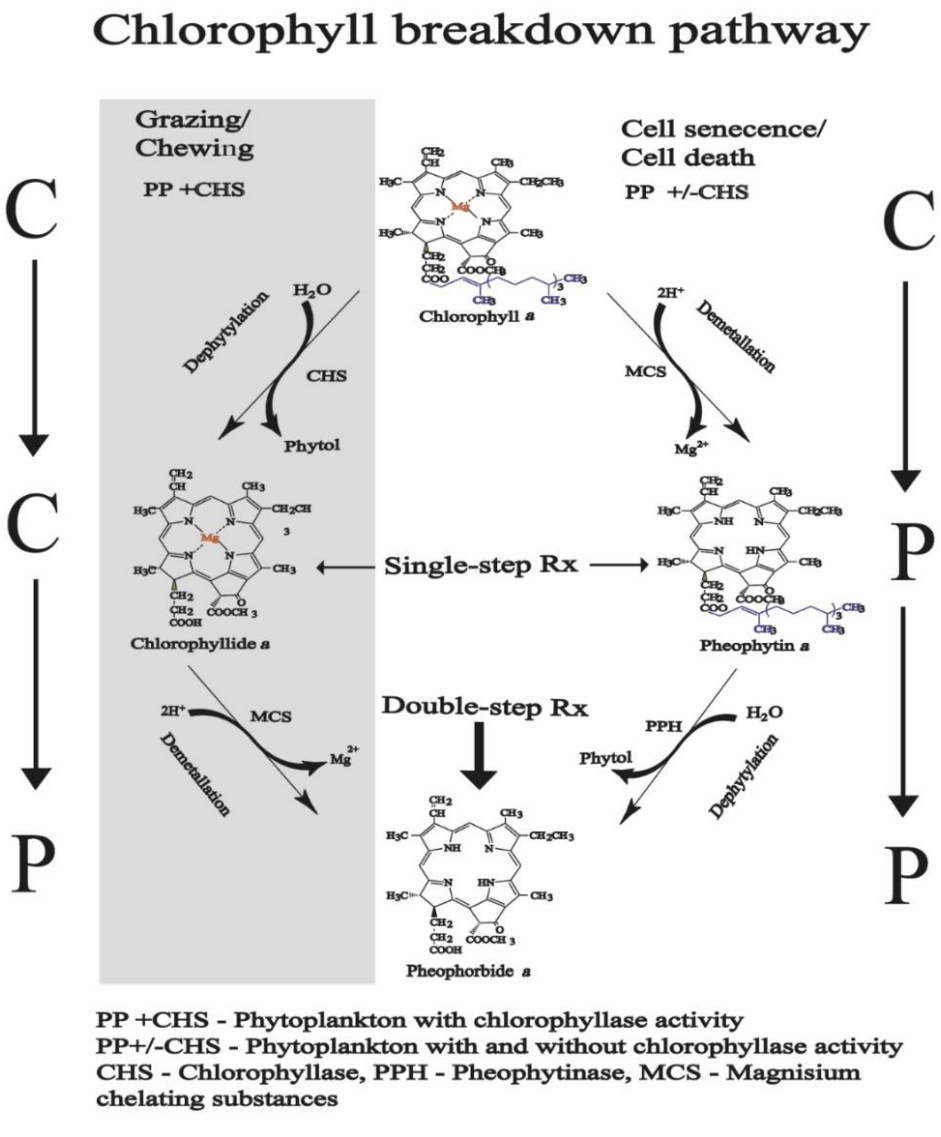

Fig. 4.1. Schematic of chlorophyll breakdown pathway based on the literature (modified from Satoh and Hama, 2013).

the discovery of (i) the role of pheophytinase, a chloroplast-located enzyme (Schelbert et al., 2009, Eckardt 2009, Guyer et al., 2014) and (ii) the involvement of chlorophyllase activity in the conversion of chlorophyll into chlorophyllide when leaf cells are disrupted or when chlorophyllase is genetically mislocalized to chloroplasts (Hu et al., 2013). The primary derivatives obtained from

demetalation and dephytalation are referred to as pheophytin (magnesium-free chl *a*) and chlorophyllide (phytol-free chl *a*), respectively. The subsequent breakdown of these derivatives, i.e., dephytylation of pheophytin and demetalation of chlorophyllide results in pheophorbide (magnesium-free and phytol-free chl *a*), which is a genuine breakdown product of chl *a* (Langmeier et al.,1993). During senescence, demetalation is the first step, followed by dephytylation, and it was previously understood that it happened the opposite way around for pheophorbide formation (Schelbert et al.,2009, Eckardt 2009). Additionally, laboratory experiments on relationship between grazing pressure and pheopigment production (Klein et al., 1986, Penry and Frost, 1991, Gieskes et al., 1991, Head and Harris 1996, Penry et al., 1991, Pandolfini et al., 2000, Goericke et al., 2000) have shown that the presence of colourless products such as 132, 173–cyclopheophorbide *a* enol (CPP516), while pheopigments productions with grazers were recorded little to none. Unfortunately, to our knowledge, this new information (i.e., on the role of hydrolase enzymes and the sequence in the early breakdown steps) has not been used for any studies on aquatic ecosystems even though there exists considerable literature. However, research in this area is in infancy. Nevertheless, the rates of these processes are of broad general interest, and knowledge of them is fundamental to understanding the causative factors underlying the dynamics of planktonic ecosystems.

Literature on aquatic systems suggests the following (i) pheophorbide and pheophytin are the primary degradation products of chl *a* from grazing in the water column (Carpenter et al., 1986, Spooner et al., 1994, Strom 1993, Cartaxana et al.,2003), sediment (Abele-Oeschger and Theede, 1991, Bianchi et al., 1988, Buffan-Dubau et al., 1996, Cartaxana et al., 2003, Coelho et al., 2011), intertidal region (Brotas and Plante-Cuny 1998, Lucas and Holligan 1999, Cartaxana et al., 2003) and Ice algae (Morata et al., 2011) and (ii) chlorophyllide produced by chlorophyllase activity is

the main product during cell senescence (Jeffrey and Hallegraeff, 1987, Louda et al.,1998, Louda et al.,2002). At the same time, few reports also raised doubts over the use of pheopigments as a relevant indicator of herbivorous activities (Head and Harris 1994, Villanueva and Hastings 2000, Ford and Honeywill 2002). Ford and Honeywill (2002) suggested that any use of pheophorbide as a marker pigment for grazing in surface intertidal sediments in the future should be tested (especially for biological reworking and deep oxygen diffusion) before use. This knowledge, which has been applied in environmental research to monitor the degradation process, needs to be updated based on new discoveries. Some studies reported that pheophorbide formation is mainly due to zooplankton grazing, whereas pheophytin is formed during grazing, cell senescence, and microbial degradation (Leavitt 1993, Morata 2007). Several studies even suggested that pheophorbide can be used as a proxy to trace grazing activity (Jeffrey 1974, Carpenter et al.,1986, Spooner et al., 1994, Cartaxana et al.,2003, Brotas and Plante-Cuny, 1998, Riaux-Gobin et al., 2000) while others have raised doubts (Ford and Honeywill, 2002). Some studies indicated that pheophorbide is also produced during bacterial degradation (Symczak-Zyla et al., 2008) and cell senescence (Head and Harris 1994). Satoh and Hama (2013) suggested the following sequence chlorophyll, chlorophyllide, pheophorbide, and pyropheophorbide as the main pathway. Since the chlorophyllase activity of unicellular algae shows distinct variation between species of the same or a different class (Jeffrey and Hallegraeff, 1987), it is hypothesized that there is taxon-specific chlorophyll breakdown (ChlB) pathways. In this study, an attempt also made to identify grazing-induced ChlB pathways in bloom-forming phytoplankton species representing different taxonomic groups. To our knowledge, much of the information on CDP has come from sediments, but from the water column, information is limited.

Nevertheless, the presence of CDP in the water indicates the presence of zooplankton activity or cellular senescence (Bidigare et al., 1986, Coupel et al., 2015). Since there exists distinct spatial and temporal variability in biological production within or between ecosystems, it is possible that the ChlB pathway will also exhibit distinct variability. For example, ChlB into pheophorbide can be either via chlorophyllide or pheophytin. Laboratory experiments confirmed that under grazing pressure, ChlB pathway (i.e., via chlorophyllide or pheophytin) is taxa-specific and grazers feeding habitat (unpublished data). India has a vast coastline with different ecosystem types (e.g., freshwater, estuaries, coastal) and so with the environmental settings in each of the ecosystems. The influence of reversible monsoons (SWM – both east and west coast and NEM – east coast), high freshwater discharges on the east-coast compared to the west-coast, different coastal morphology, and variable anthropogenic pressures are some of the events determining the environment setting in a given ecosystem along the Indian coasts. Therefore, it has been hypothesized that the phytoplankton will have a specific distribution pattern for different ecosystems, and therefore indicating fate pathways. Evaluating biomass, composition, and CDP together will provide insights into the processes involved in growth and mortality. Given this, under the Ballast Water Management Program-India, the distribution patterns of the biomass and CDP from eight-port ecosystems (categorized into freshwater (FPE), estuarine (EPE) and marine (MPE) port ecosystems) located along east and west coast of India are discussed from the perspective of loss processes. Further, the field data generated will have implications for ecosystem assessment and management. For instance, in ballast water management (BWM), it is assuming that the pheophorbide: pheophytin ratio will be useful for assessment of the (i) nature of the environment in the ballast water discharge point (e.g. ports), and (ii) the ballast water sampling. Generally, ports are at the receiving end for ships ballast water, and therefore the port environment

determines the fate of the introduced organisms. For instance, the introduced organisms survive/proliferate and do not under a conducive and non-conducive environment respectively. Irrespective of the climatic zones, the geographic location (riverine, estuarine, and marine) and the seasons (wet and dry seasons) determined the port environment and was evident in this study. Therefore, the biological production (e.g. plankton biomass and production) or occurrences of blooms vary between the ports and the seasons and so with its fate. Given, this the chlorophyll and its breakdown products (e.g. pheophytin, pheophorbide) and ratios can be useful ecological indicators for determining the fate of phytoplankton, including introduced viable phytoplankton and blooms,) during productive and non-productive conditions (Morata et al., 2011, Grippo et al., 2009, Allison et al., 2013).

4.2 Methodology

Details of sampling strategies, pigments analysis (Chl *a* and pheopigments), from marine, estuary, and freshwater port ecosystems are presented in chapter 2.

4.2.1 Data analysis

However, for this study, only the total chlorophylls and two degradation pigments (pheophytin and pheophorbide) were considered. Chlorophyllide, another degradation pigment, was not detected in any of the samples analyzed. Additionally, following pigment ratios Chl *a*: Pheophytin, Chl *a*: Pheophorbide, pheophorbide: pheophytin for better interpretation. Further, the data on chlorophyll, pheopigments, and pheophorbide: pheophytin ratios were subjected to clustering (using Bray Curtis similarity and average group method) and non-metric multidimensional scaling (NMDS) to understand the ecosystem wise distribution. Before analysis, data were not transformed. Clustering and NMDS were performed using Primer version 6 software.

4.3 Results

4.3.1 Chlorophyll and pheopigments formation in the presence and absence of zooplankton

The experiments showed that the increase in chl *a* and pheopigments (pheophytin and pheophorbide) concentration with incubation occurred in all three cultures and natural seawater (Fig. 4.2). An increment in phytoplankton cell abundance in cultures and NSW with incubation also followed a trend similar to that of chl *a* concentration (data not presented) It is noted that the magnitude of the difference in pheopigment concentrations varied between the presence and absence of zooplankton. For example, pheopigments concentration was significantly higher ($p<0.02$) in the presence of mesozooplankton than without mesozooplankton (Fig. 4.2). Results also showed that pheophorbide and respectively (Fig. 4.3). These results indicate that the herbivory also results in pheophytin formation, but again species-dependent. The distinct differences, as observed in some mesozooplankton species eg. naupli, copepodites) which were not separated initially. Despite that distinct increase in pheophorbide and pheophytin was observed in the presence of mesozooplankton (Fig. 4.2 and 4.3). These findings suggested that the increase was due to the added mesozooplankton and the contribution coming from the already present microzooplankton was insignificant in this study. In NSW, the growth of diatoms resulted in an increment in cell abundance with incubation. The contribution of diatoms, from day 0 to 3, increased from 42% to 72% and 37% to 78% in the bottles with zooplankton and without zooplankton, respectively (Fig. 4.4). In contrast, the contribution of other prominent groups declined with incubation, and this was observed in NSW (Fig. 4.4). mesozooplankton and not from microzooplankton which was present initially in NSW (which was used in the experiments). A separate experiment revealed that the contribution of chl *a* and pheopigments was significantly higher in the samples after removing without (-zoo) natural zooplankton (zoo) assemblage.

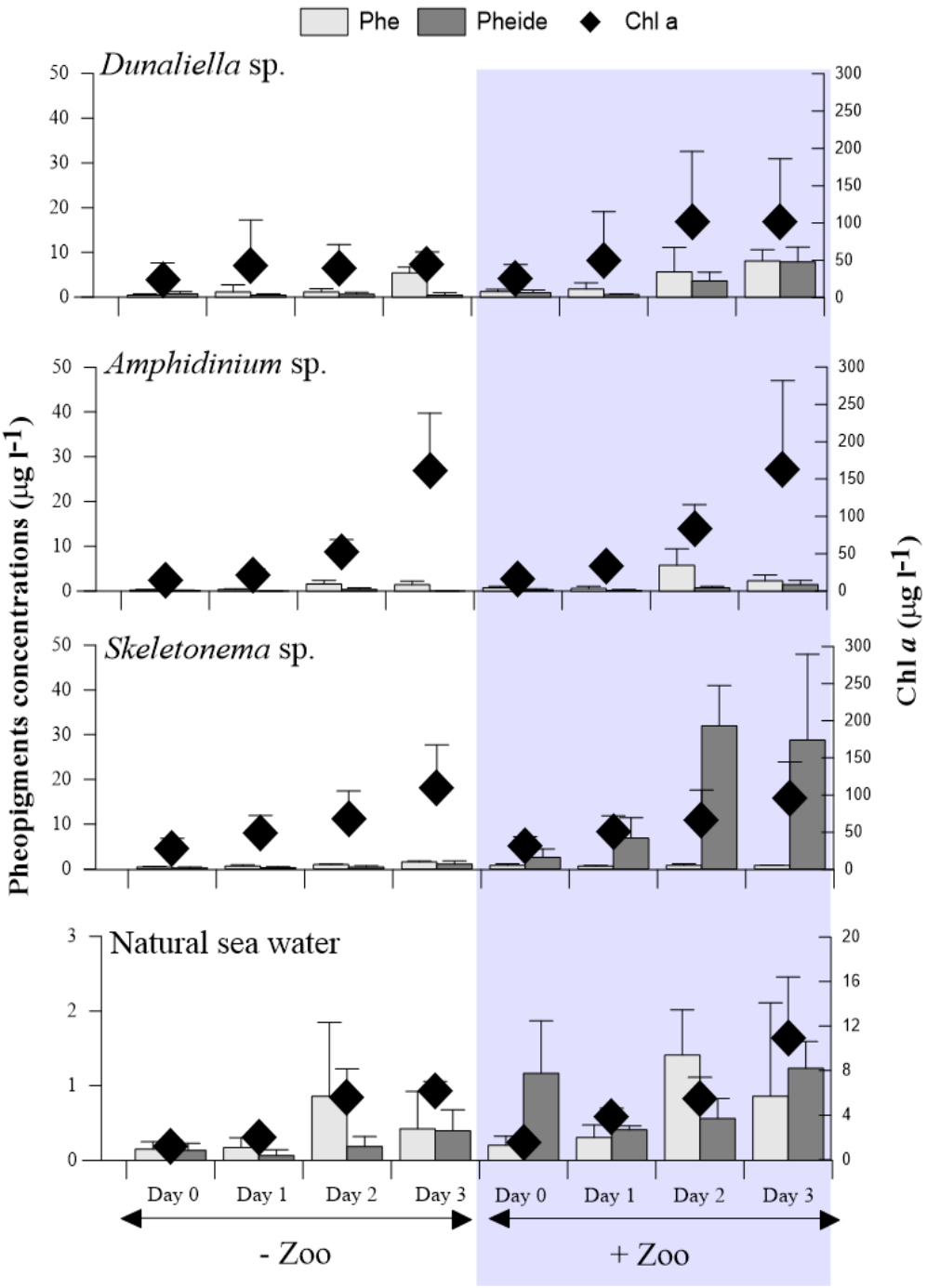

Fig.4.2 The daily variations in the concentrations of chlorophyll (chhl a), pheophytin (Phe), and pheophorbide (Pheide) from different phytoplankton cultures (*Skeletonema* (diatoms), *Dunaliella* (green algae), and*Amphidinium* (dinoflagellate)) and natural seawater incubated for three days with (+zoo) and without (-Zoo) grazers.

The data presented is the average (including erro bars) from two parallels (n=5). mesozooplankton and almost negligible **(**<1μgl^{-1}**)** in removed mesozooplankton (Fig. 4.5). This was observed for all the cultures and NSW set up, revealing higher pheopigments (both pheophytin and pheophorbide) in the cell suspension than the added mesozooplankton. Despite such community changes, the pheophorbide and pheophytin formation observed in the NSW incubation was mainly due to the addition of addition of mesozooplankton and not from microzooplankton which was present initially in NSW (which was used in the experiments). A separate experiment revealed that the contribution of chl *a* and pheopigments was significantly higher in the samples after removing mesozooplankton and almost negligible **(**<1μgl^{-1}**)** in removed mesozooplankton (Fig. 4.5). This as observed for all the cultures and NSW set up, revealing higher pheopigments (both pheophytin and pheophorbide) in the cell suspension than the added mesozooplankton.

4.3.2 Pigments ratios in the presence and absence of zooplankton

Generally, Chl *a*: pheopigments ratios were calculated to understand the phytoplankton physiological state, such as actively growing microalgae or freshness of organic matter. In this study, despite variations between the zooplankton presence and absence in the bottles, the ratio was always >1 in all treatments (Fig. 4.6), indicating that the culture/NSW were physiologically active. However, chl *a*: total pheopigments was relatively lower in bottles with zooplankton but not in the bottles without zooplankton indicating reduced microalgal activity (due to grazing-induced mortality) in the former than the latter bottles (Fig. 4.6). However, the magnitude varied with cultures and NSW. For instance, in the presence of zooplankton, chl *a*: total pheopigments ratio was distinctly lower for *Skeletonema* and *Dunaliella* compared to that of *Amphidinium* and NSW, suggesting more grazing pressure in the former than latter. Among the pheopigments, the pheophorbide formation was evident only in the bottles inoculated with zooplankton, while pheo-

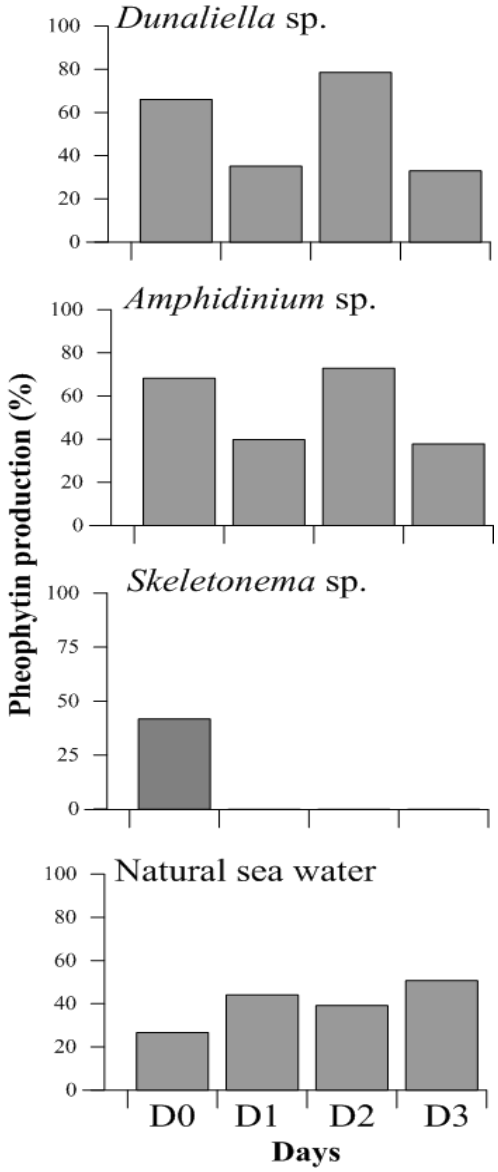

Fig. 4.3 The daily variations in the grazing-induced pheophytin (difference in pheophytin production with and without zooplankton) production for different phytoplankton cultures (*Skeletonema* (diatoms), *Dunaliella* (green algae), and *Amphidinium* (dinoflagellate)) and natural seawater.

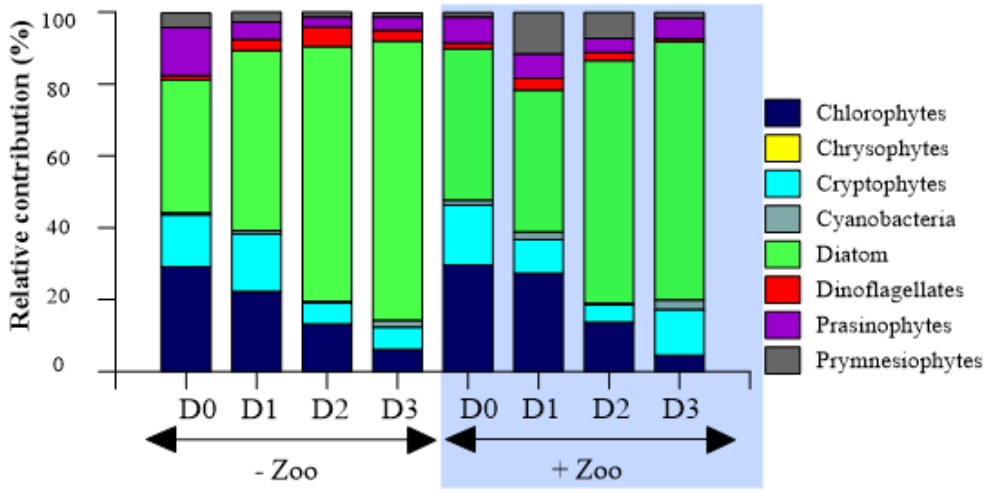

Fig. 4.4 The daily variations in the relative contribution of phytoplankton groups of natural seawater incubated for three days with (+zoo) and without (-zoo) natural zooplankton (zoo) assemblage.

-phytin formation was observed in all the bottles inoculated with and without zooplankton and this was reflected in the chl *a*: pheophytin and chl *a*: pheophorbide ratios (Fig. 4.6). Pheophorbide formation was negligible in the bottles without zooplankton, whereas in bottles with zooplankton pheophorbide formation was observed with chl *a*: pheophorbide ratios ranged between 1 to ~500 (Fig. 7). The highest and lowest chl *a*: pheophorbide ratio were observed with *Dunaliella* and *Skeletonema*, respectively. Unlike chl *a* : pheophorbide ratios, the differences in chl *a*: pheophytin ratios between the microalgae with and without zooplankton were not distinct. However, there exist variations between the species (Fig.4.4). For example, the highest and lowest chl *a*: pheophytin ratios were observed with *Skeletonema* and *Dunaliella*, respectively (Fig. 4.6).

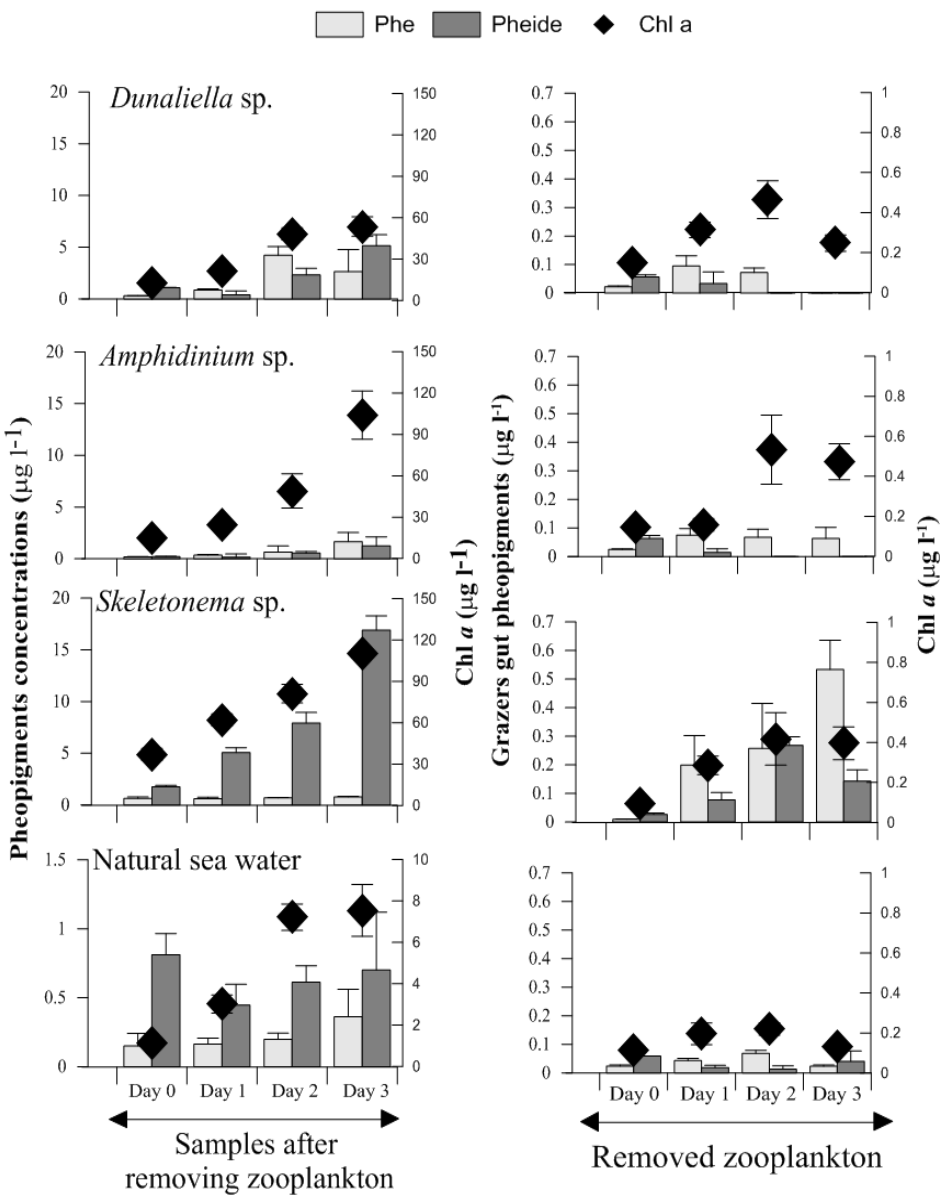

Fig. 4.5 The daily variations in the concentrations of chlorophyll (chl *a*), pheophytin (Phe), and pheophorbide (Pheide) from the samples (*Skeletonema*, *Dunaliella*, *Amphidinium* and natural seawater) after removing mesozooplankton and removed mesozooplankton. The data presented is the average (including erro bars) from two parallels (n=5).

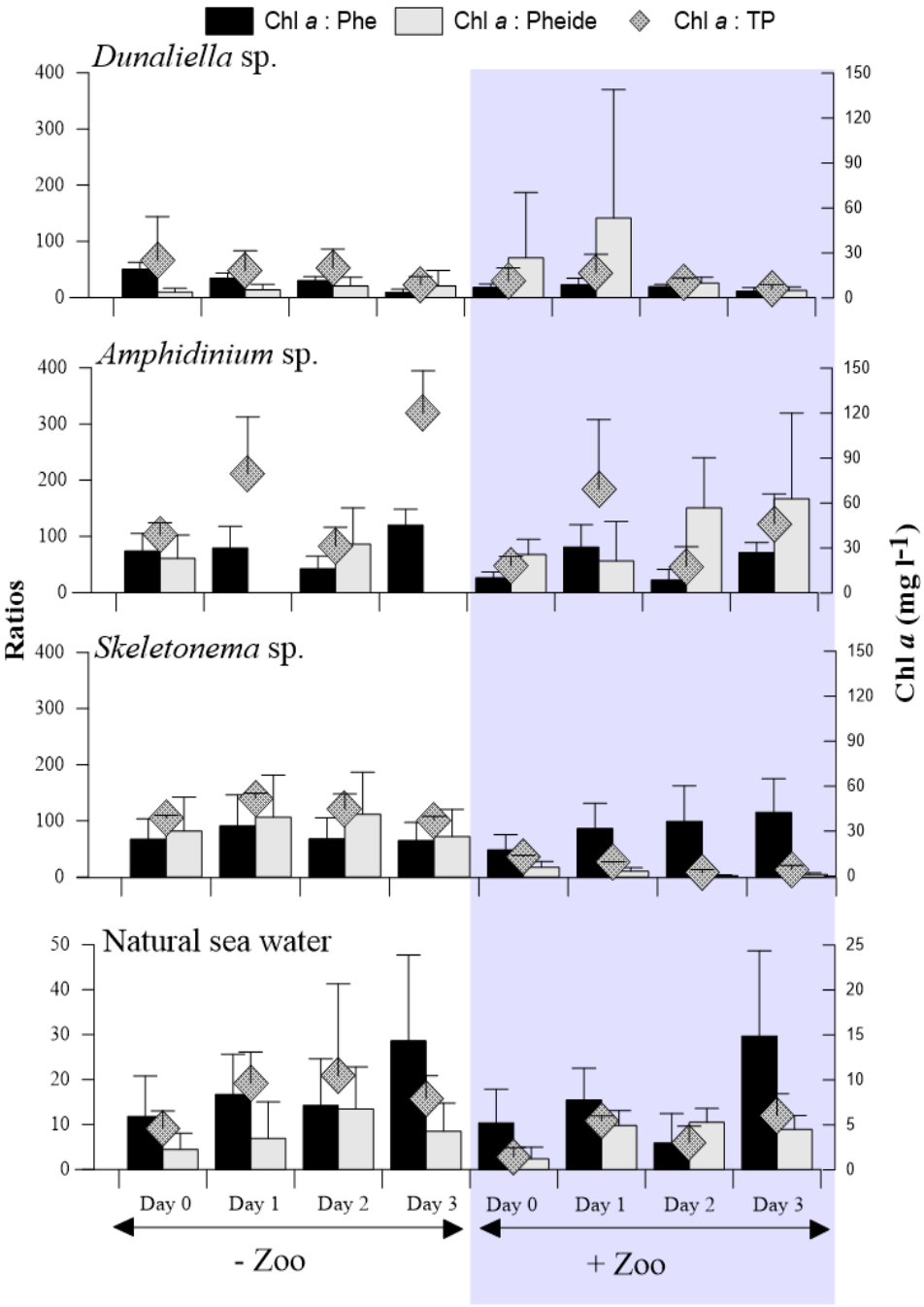

Fig. 4.6 The daily variations in pigment ratios (Chl *a*:pheophorbide (Pheide), Chl *a*:pheophytin (Phe) and Chl *a*) and from different phytoplankton cultures (*Skeletonema* (diatoms), *Dunaliella* (green algae), and *Amphidinium* (dinoflagellate)) and natural seawater incubated for three days with (+zoo) and without (-zoo) natural zooplankton (zoo) assemblage. . The data presented is the average (including erro bars) from two parallels (n=5).

Pheophorbide: pheophytin ratio was also calculated to determine the nature of the pathway, i.e., chl *a*-chlorophyllide-pheophorbide (or herbivory) is dominant (>1) or not dominant (<1). The ratios were <1 in the cultures and NSW without zooplankton, whereas in the presence of zooplankton, the ratios recorded varied up to 40. The highest was observed for *Skeletonema* (4 to 40) followed by NSW (0.6 to 9) and the lowest (<1) for *Amphidinium* and *Dunaliella* (Fig. 4.7).

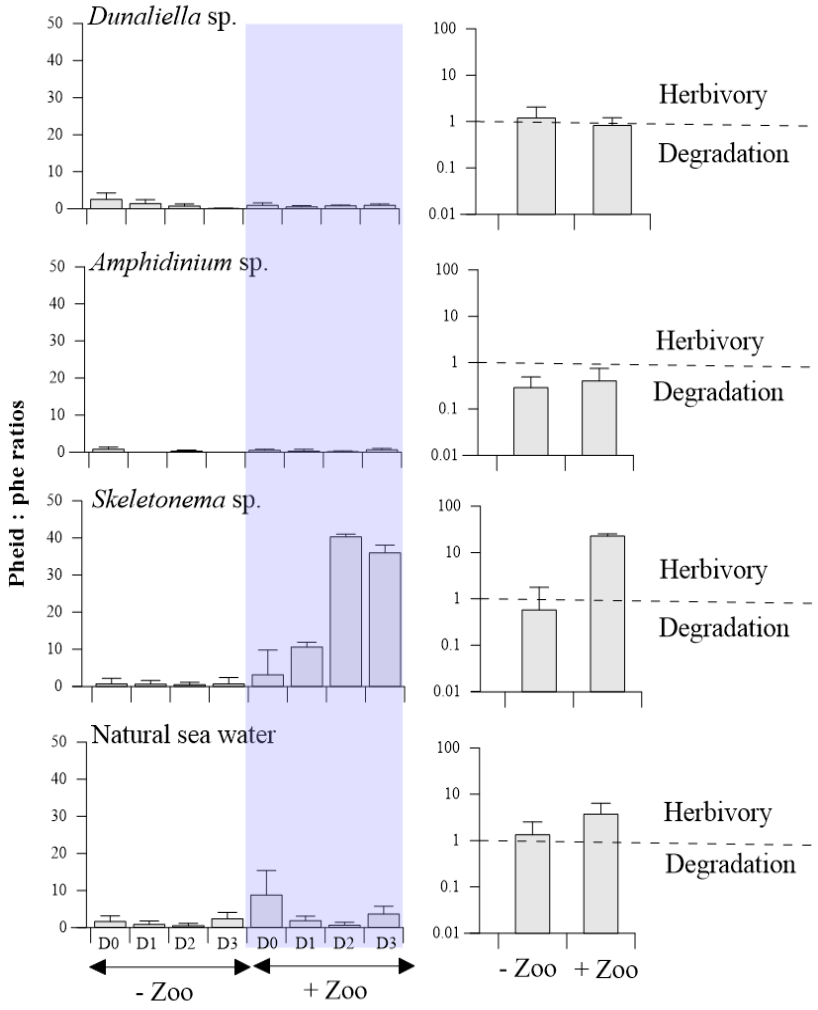

Fig. 4.7 The daily variations in pigment ratios (pheophorbide (Pheide): pheophytin (Phe), Chl *a*: CDP) and from different phytoplankton cultures (*Skeletonema* (diatoms), *Dunaliella* (green algae), and *Amphidinium* (dinoflagellate)) and natural seawater incubated for three days with (+zoo) and without (-zoo) natural zooplankton (zoo) assemblage.

4.3.3. Environmental parameters

The measured environmental parameters from the different ecosystems showed distinct seasonal variations in all the studied ports. However, the seasonal patterns varied according to the geographical location of the ports. Water temperatures in the ports are relatively warmer and cooler during southwest monsoon (SWM) and northeast (east coast) or post (west coast) monsoon, respectively. The averaged salinity variations were negligible (Kolkata: < 0.4 round the year and Haldia: 0.6 to 7.8), marginal (25 to 35) and large (5.1 to 26) in freshwater, marine and estuarine ports (Cochin) respectively. The low salinity observed in marine and estuarine ports were due to freshwater influx during monsoons. Interestingly, the freshwater and estuarine ports systems are nutrient (NO_3, PO_4, and SiO_4) rich compared to marine ports. However, the magnitude varies between the ports. For instance, among the nutrient-rich freshwater influenced ports the magnitude of nutrient concentrations was higher for Kolkata and Haldia ports compared to Cochin port (Table 2). Among the marine ports nutrients showed a distinct seasonal variability, but the seasonal patterns varied between the ports located on east (Tuticorin, Chennai, and Paradip) and west coast (Kandla and Mangalore) of India.

4.3.4. Phytoplankton biomass (chl a) and pheopigments (pheophytin and pheophorbide)

Results indicate that the chl *a* and degraded pigments showed significant seasonal variations in each of the ecosystems (except Haldia and Kandla due to the prevalence of low chl *a* in all the seasons). However, the seasonality pattern was different between the ecosystem, and this was true for chl *a* and pheopigments (Figs. 4.8 and 4.9). The detailed results for each of the ecosystems are provided below.

Table 4.1 Environmental parameters from river influenced ports systems (RIPS) and no river influenced port systems (NRIPS). SWM – southwest monsoon, PM- post-monsoon, PrM – pre-monsoon, NEM – northeast monsoon, SIM – spring inter-monsoon, FIM – fall inter-monsoon, DO – dissolved oxygen (mg l^{-1}). The units for nutrients (ammonia, nitrate, nitrite, phosphate and silicate) are in μM.

Environmental parameters from RIPS

Stations	Season	Salinity	DO	Phosphate	Silicate	Nitrate	Nitrite	Ammonia
Kochi	PM 1	22.6 ± 6.7	7.1 ± 1.0	3.2 ± 1.7	47.8 ± 9.9	4.0 ± 3.0	0.5 ± 0.5	24.9 ± 7.7
	PrM	20.5 ± 6.8	6.7 ± 1.1	3.8 ± 0.8	44.3 ± 9.3	5.9 ± 1.3	1.1 ± 0.3	42.6 ± 11.8
	SWM	5.3 ± 2.2	3.9 ± 1.6	3.5 ± 0.8	47.6 ± 18.1	10.2 ± 5.9	1.4 ± 0.8	26.2 ± 10.5
	PM2	23.1 ± 2.2	3.8 ± 1.4	2.6 ± 1.1	47.2 ± 16.7	3.4 ± 2.4	0.5 ± 0.2	20.5 ± 10.1
Kolkata	SWM	0.20 ± 0.05	6.2 ± 1.5	2.3 ± 1.4	66.9 ± 48.8	27 ± 12.2	1.6 ± 0.3	2.0 ± 1.0
	PrM 1	0.25 ± 0.05	8.8 ± 0.9	4.5 ± 0.7	76.4 ± 12.4	10.0 ± 6.6	4.3 ± 2.3	20.6 ± 7.8
	PrM 2	0.25 ± 0.05	8.2 ± 0.7	1.1 ± 1.2	61.6 ± 31.3	24.3 ± 7.0	1.3 ± 0.8	5.7 ± 3.5
	PM	0.28 ± 0.06	7.0 ± 1.8	6.7 ± 0.5	132. 3 ± 26.1	116.5 ± 71.5	8.4 ± 3.6	3.7 ± 1.1
Hooghly	PM	0.3 ± 0	6.7 ± 0.8	5.1 ± 0.3	148.3 ± 3.1	38.2 ± 4.1	1.5 ± 0.4	2.5 ± 0.7
	PrM 1	0.3 ± 0	8.9 ± 0.6	4.8 ± 0.5	74.7 ± 1.8	7.6 ± 2.6	2.1 ± 1.7	22.3 ± 16.3
	PrM 2	0.3 ± 0	8.8 ± 0.3	5.5 ± 1.1	411.5 ± 76.1	28.1 ± 8.7	1.4 ± 0.8	5.6 ± 1.5
	SWM	0.25 ± 0.10	7.0 ± 1.5	10.7 ± 0.3	177.7 ± 13.0	51.5 ± 18.7	5.8 ± 1.8	4.2 ± 0.6
Haldia	PM	1.3 ± 0.5	6.0 ± 0.7	4.0 ± 1.5	114.7 ± 14.4	33.0 ± 10.2	1.1 ± 0.3	4.1 ± 1.6
	PrM 1	6.3 ± 0.9	8.2 ± 0.2	6.6 ± 1.1	79.7 ± 12.9	3.4 ± 1.1	1.6 ± 0.3	6.3 ± 1.4
	PrM 2	2.7 ± 0.6	5.3 ± 0.6	3.4 ± 0.7	152.5 ± 29.0	38.1 ± 5.0	3.0 ± 0.5	8.9 ± 3.8
	SWM	7.4 ± 0.5	5.7 ± 1.0	6.0 ± 1.3	142.9 ± 46.0	24.3 ± 10.7	0.8 ± 0.5	4.3 ± 1.4

Environmental parameter from NRIPS

Stations	Season	Salinity	DO	Phosphate	Silicate	Nitrate	Nitrite	Ammonia
Kandla	PM 1	37.7 ± 0.3	5.0 ± 0.4	4.3 ± 1.4	54 ± 5.2	7.2 ± 2.0	4.4 ± 0.7	15.3 ± 3.0
	SWM	30.2 ± 10.2	6.2 ± 0.6	3.4 ± 0.7	30.9 ± 5.2	12.8 ± 5.6	7.0 ± 1.8	1.4 ± 0.5
	PM 2	35.8 ± 0.1	6.5 ± 0.6	3.0 ± 0.4	77.6 ± 9.8	15.9 ± 6.0	3.0 ± 0.5	4.9 ± 0.7
	PrM	39.8 ± 0.4	6.1 ± 0.7	5.3 ± 0.8	128.4 ± 9.7	14.6 ± 2.8	6.8 ± 1.8	1.2 ± 0.2
Mangalore	PM 1	35.9 ± 0.1	3.8 ± 1.2	2.2 ± 0.6	26.6 ± 15.5	8.5 ± 5.9	4.3 ± 3.2	39.9 ± 5.0
	PrM	35.7 ± 0.3	5.7 ± 1.9	1.8 ± 1.9	6.1 ± 5.7	5.2 ± 3.2	1.8 ± 0.7	25.3 ± 8.3
	SWM	34.1 ± 0.9	4.7 ± 1.6	2.7 ± 0.6	23.5 ± 8.4	0.7 ± 0.5	0.6 ± 0.2	29.4 ± 9.2
	PM2	34.8 ± 0.1	4.7 ± 0.9	2.3 ± 0.8	17.6 ± 5.6	3.9 ± 1.2	5.2 ± 1.2	18.8 ± 4.8
Paradip	SWM	26.3 ± 0.5	5.2 ± 0.4	1.9 ± 0.3	20.4 ± 5.0	4.9 ± 1.1	0.9 ± 0.2	25.0 ± 21.2
	NEM	31.0 ± 1.3	4.7 ± 1.0	1.8 ± 0.2	42.1 ± 15.0	4.9 ± 2.0	1.1 ± 0.2	12.5 ± 4.8
	SIM	33.8 ± 0.3	4.7 ± 0.4	2.5 ± 0.5	78.2 ± 13.7	8.7 ± 5.9	1.8 ± 0.2	5.0 ± 2.9
	FIM	29.6 ± 1.3	4.6 ± 0.6	0.2 ± 0.1	63.0 ± 38.9	4.5 ± 0.9	1.6 ± 0.7	3.7 ± 1.2
Chennai	SWM	34.4 ± 0.1	3.2 ± 0.7	5.1 ± 2.9	23.9 ± 6.3	13.4 ± 3.2	2.3 ± 0.5	24.8 ± 10.2
	FIM	29.1 ± 1.8	4.9 ± 0.4	3.1 ± 2.3	11.8 ± 3.1	8.0 ± 5.8	1.3 ± 1.1	51.3 ± 44.3
	NEM	30.4 ± 0.2	4.8 ± 0.5	1.8 ± 0.6	8.4 ± 2.4	9.1 ± 5.6	1.6 ± 0.9	27.2 ± 11.2
	SIM	34.3 ± 0.2	4.0 ± 0.8	2.6 ± 1.0	6.2 ± 2.1	6.5 ± 2.2	0.9 ± 0.3	12.9 ± 5.1
Tuticorin	SWM	35.4 ± 0.1	5.6 ± 0.4	1.2 ± 0.7	12.3 ± 4.6	1.1 ± 1.1	0.5 ± 0.5	12.9 ± 3.9
	FIM	36.2 ± 0.2	6.3 ± 0.4	1.8 ± 0.6	6.2 ± 1.8	4.4 ± 2.2	1.4 ± 0.7	21.5 ± 6.8
	NEM	34.0 ± 0.8	5.0 ± 0.7	1.9 ± 0.8	6.2 ± 1.8	11.0 ± 8.1	9.8 ± 7.5	23.2 ± 11.3
	SIM	33.5 ± 0.03	5.9 ± 0.4	1.8 ± 0.2	4.4 ± 0.7	5.3 ± 0.8	0.8 ± 0.2	12.3 ± 3.3

4.3.4.1. Biomass

Results revealed higher biomass in freshwater (except Haldia) and estuarine ports compared to marine ports. In each port (except Haldia and Kandla due to the prevalence of low chl *a* in all the seasons), biomass showed significant seasonal variations. However, the seasonality pattern for chl *a* was different between the port ecosystems (Figs. 4.8 and 4.9). Among freshwater port ecosystems, the highest (up to 40.8 µg l^{-1}) and lowest (0.5 to 1.8 µg l^{-1}) concentration of chl *a* are observed in Kolkata–port (including Hooghly riverine stations) and Haldia-port respectively. In Kolkata, high chl *a* concentration was observed in the stations located in Hooghly River (up to 40.8 µg l^{-1}) compared to enclosed port stations (up to 20.3 µg l^{-1}). In the estuarine port (Cochin) chl *a* remained high (11.97 to 18.37 µg l^{-1}) round the year, and the high and low chl *a* was observed during SWM and PRM season respectively. Among marine ports, and Mangalore ports respectively, whereas, in the rest of the ports, chl *a* was lowest (<1 µg l^{-1}) and highest (up to 25 µg l^{-1}) chl *a* were observed in Kandla variable, i.e., high in only one or two seasons. Clustering and non-metric MDS at 60% similarity also revealed two groups and three ungrouped individual ports. The ports characterized by low (Haldia and Kandla) and high biomass (Mangalore, Kolkata/Hooghly, and Cochin) for most of the year formed two groups (Fig. 4.12). In the remaining individual ports, high biomass was recorded once or twice during the observation (Fig. 4.12).

4.3.4.2. Pheophytin

Similar to chl *a*, higher pheophytin is observed in freshwater (except Haldia) and estuarine ports compared to marine ports. Among freshwater ports, high (up to 3.4 µg l^{-1}) and low (< 0.1 µg l^{-1}) concentrations were recorded in Kolkata (including riverine stations) and Haldia ports respectively during all the occasions. Interestingly, high pheophytin concentration (but lesser than freshwater)

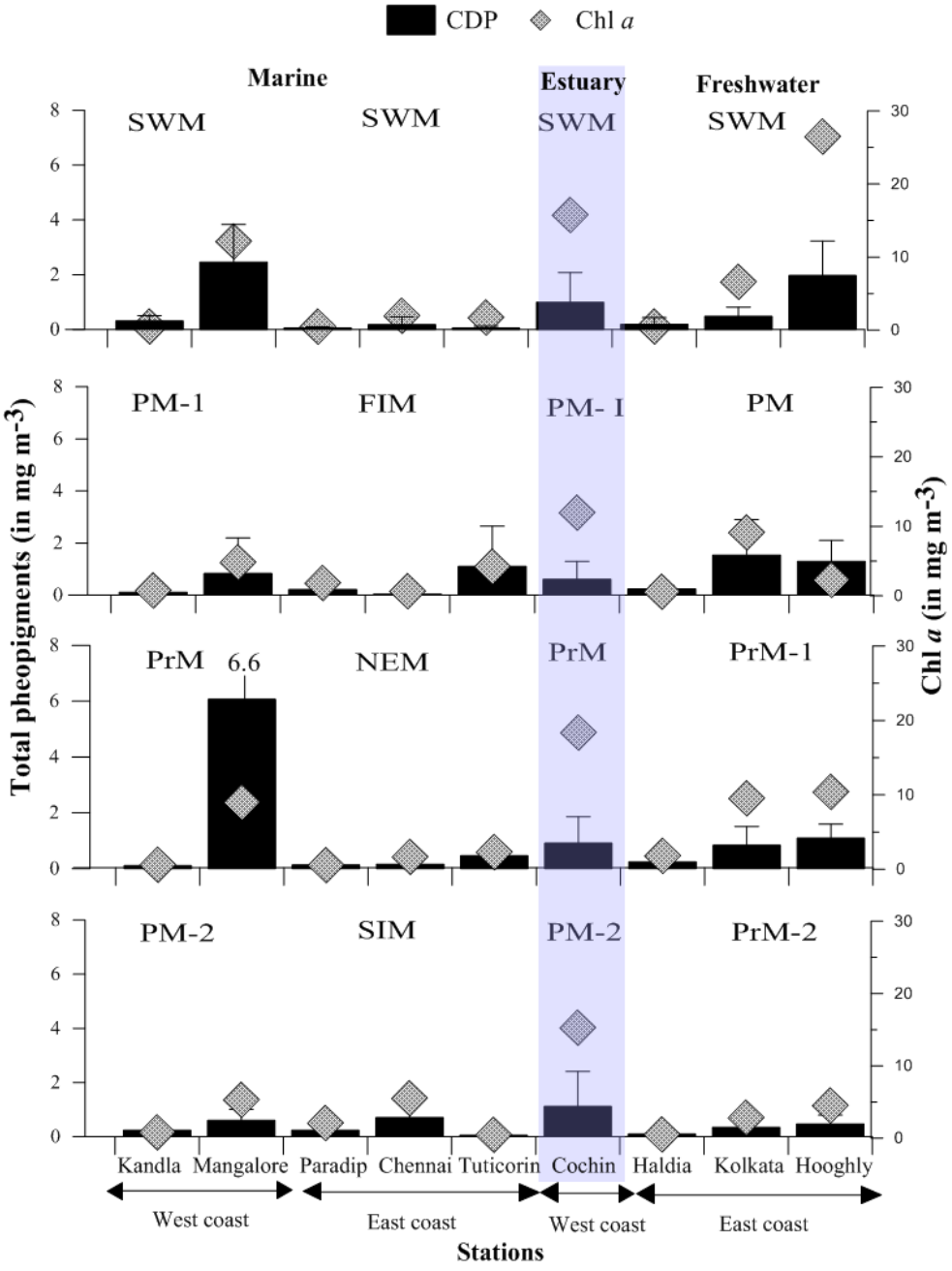

Fig. 4.8. The seasonal variations in the concentrations of chlorophyll and chlorophyll degraded pigments (CDP) from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon, FIM – Fall inter-monsoon, NEM – northeast monsoon, SIM – spring intermonsoon, PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

were observed most of the year in estuarine port (Cochin). Among the marine ports, pheophytin showed a distinct seasonal trend. The highest and lowest pheophytin was recorded in Mangalore (0.1 to 1.2 µg l^{-1}) and Kandla (0.03 to 0.13 µg l^{-1}) ports, respectively. In the remaining three ports pheophytin distribution was variable (Fig. 4.9). Clustering and non-metric MDS at 60% similarity also revealed two groups and four ungrouped individual ports. The ports characterized by low (Haldia, Paradip, and Kandla) and relatively higher pheophytin (Mangalore, Kolkata, and Cochin) for most of the year formed two groups (Fig. 4.12). However, some stations located in Hooghly river (part of Kolkata sampling) recorded the highest pheophytin, whereas the remaining two individual ports recorded high pheophytin were recorded once or twice during the observation (Fig. 4.12).

4.3.4.3. Pheophorbide

Unlike chl *a* and phoephytin, the pheophorbide distribution was not same between the ports. On the contrary to high biomass Kolkata port, pheophorbide concentrations were low except during PM (0.1 to 2.0 µg l^{-1}), whereas in Haldia-port low pheophorbide concentrations (0.09 to 0.19 µg l^{-1}) were recorded throughout the year. Unlike freshwater systems, the estuarine-port (Cochin) recorded higher pheophorbide concentrations, and the maximum was during post-monsoon followed by southwest monsoon and premonsoon (Fig. 4.9).

Among the marine ports, pheophorbide distribution showed a distinct seasonal trend. Unlike in freshwater and estuarine ecosystems, pheophorbide in marine ports exhibited a distinct relationship with chl *a*. Among ports, highest (up to 20.1 µg l^{-1}) and lowest (<0.2 µg l^{-1}) pheophorbide concentrations were recorded in Mangalore and Kandla port, respectively. In the remaining marine ports, pheophorbide distribution showed distinct seasonality, but the seasonal patterns were different due to the geographic location of the ports. Clustering and non-metric MDS at 60% simi-

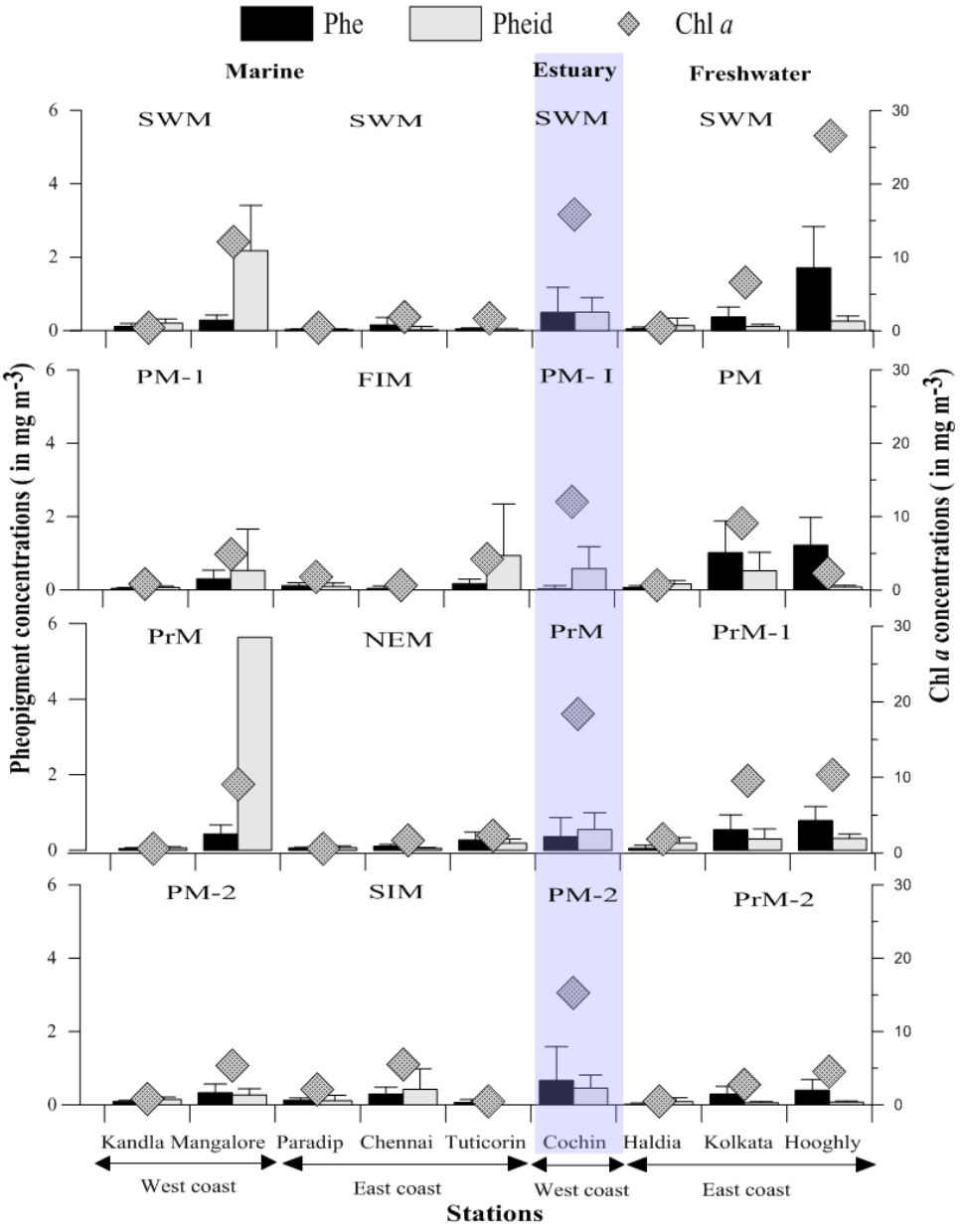

Fig. 4.9. The seasonal variations in the concentrations of pheophytin (Phe), pheophorbide (Pheid) and chlorophyll (Chl) from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon, FIM – Fall inter-monsoon, NEM – northeast monsoon, SIM – spring intermonsoon, PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

-larity also revealed two groups and three ungrouped individual ports. The ports characterized by low (Kolkata and Tuticorin) and relatively higher pheophorbide (Hooghly, Haldia, Kandla, and Paradip) for most of the year formed two groups (Fig. 4.12). However, Mangalore port followed by Cochin recorded the highest pheophorbide for most of the year, whereas the remaining Chennai port high pheophorbide concentration was recorded once during the observation (Fig. 4.12).

4.3.5. Pigment ratios

4.3.5.1. Chl a to pheopigments ratio

Generally, Chl *a*: pheopigments ratios were calculated to understand the freshness of organic matters from the overlying water column from different ecosystems. In this study, pigment ratio (Chl *a*: CDP, Chl *a*: pheophytin and Chl *a*: pheophorbide) showed a distinct spatial (between ecosystem types such as freshwater, estuary and marine ports) and seasonal variations (Fig. 4.10). High Chl *a*: CDP ratios and high- Chl *a:* pheophorbide ratios were observed in freshwater (except Haldia due to low biomass) and estuarine ports compare to marine ports. On the contrary, high Chl *a*: pheophytin ratios were mostly observed in marine ports (except Paradip due to low biomass) than freshwater ports (except Cochin and Haldia). Among the freshwater ports, high and low ratios were encountered in Kolkata and Haldia, respectively, whereas among the marine ports, high and low ratios were encountered in Mangalore and Paradip/Kandla, respectively (Figs. 4.10 and 4.12). The low ratios in freshwater and marine ports were mainly due to the prevalence of low chlorophyll.

4.3.5.2. Pheophorbide vs. Pheophytin

The ratios of pheophorbide to pheophytin were calculated to understand the ecological process of the different ecosystems, i.e., CCP/herbivory is dominant (>1) or not dominant (<1). Like Chl to CDP ratios, pheophorbide: pheophytin ratio also showed a distinct spatial and seasonal variation (Fig. 4.11). The average pheophorbide: pheophytin ratio ranged from a low of 0.2 in Kolkata to in

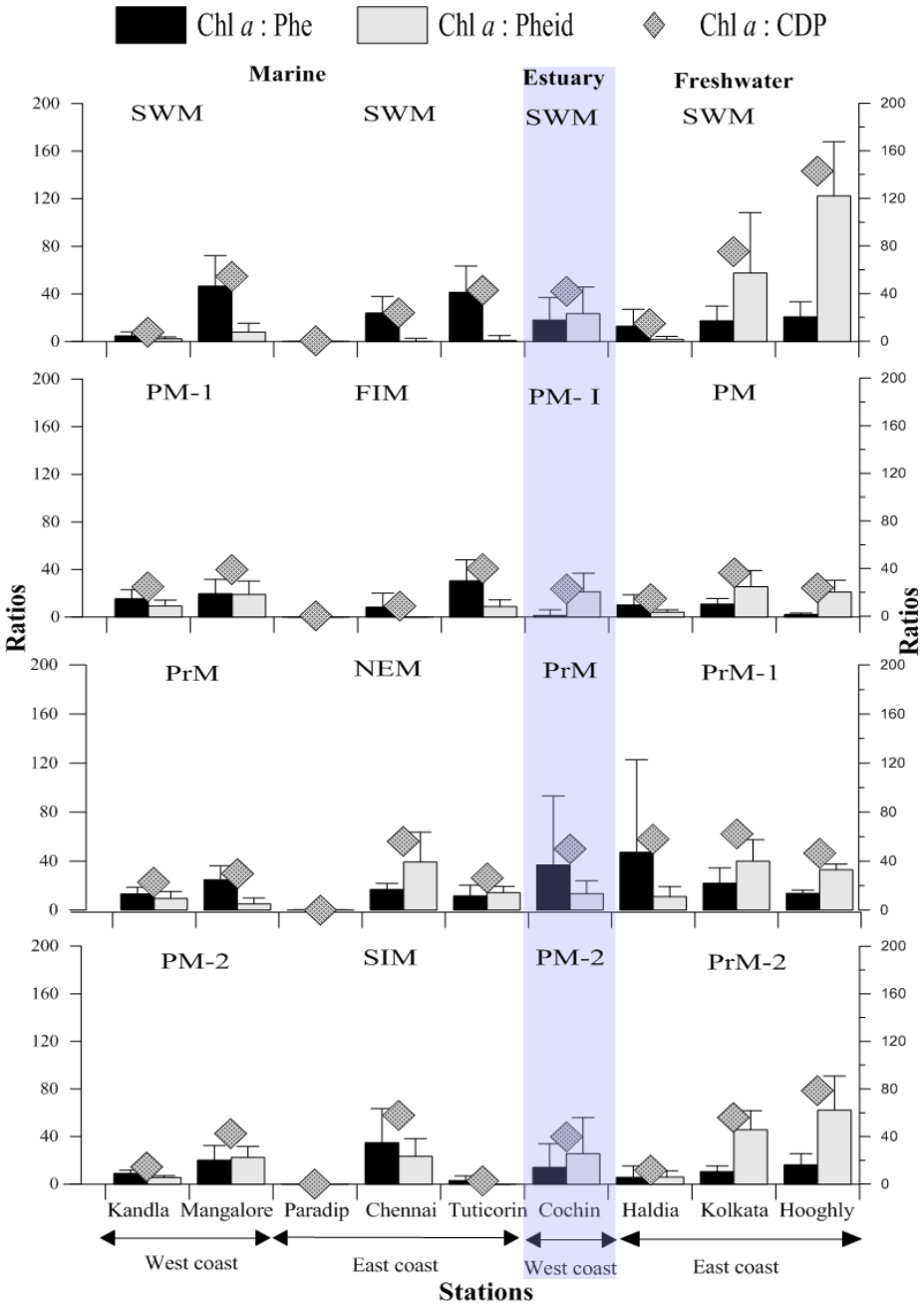

Figure 4.10. The seasonal variations in the pigment ratios (Chl:pheophorbide, Chl:pheophytin and Chl:CDP) from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon, FIM – Fall inter-monsoon, NEM – north east monsoon, SIM – spring inter monsoon, PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

freshwater (except Haldia) and estuarine ports were <1, (i.e., ranges between 0.2 to 0.9) whereas in marine ports the average ratio was mostly >1, i.e., ranges between 0.4 to 11.5 (Figs. 6 and 7). The low ratios in marine ports (especially in Chennai and Tuticorin) are observed only on a couple of occasions when the biomass is very low (<1 µg/l). Clustering and non-metric MDS at 60% similarity the highest ratio (up to 11.2) for the most of the year, whereas in the remaining Chennai and Tuticorin-port, high ratios (>1) were recorded only once during the observation (Fig. 4.12).

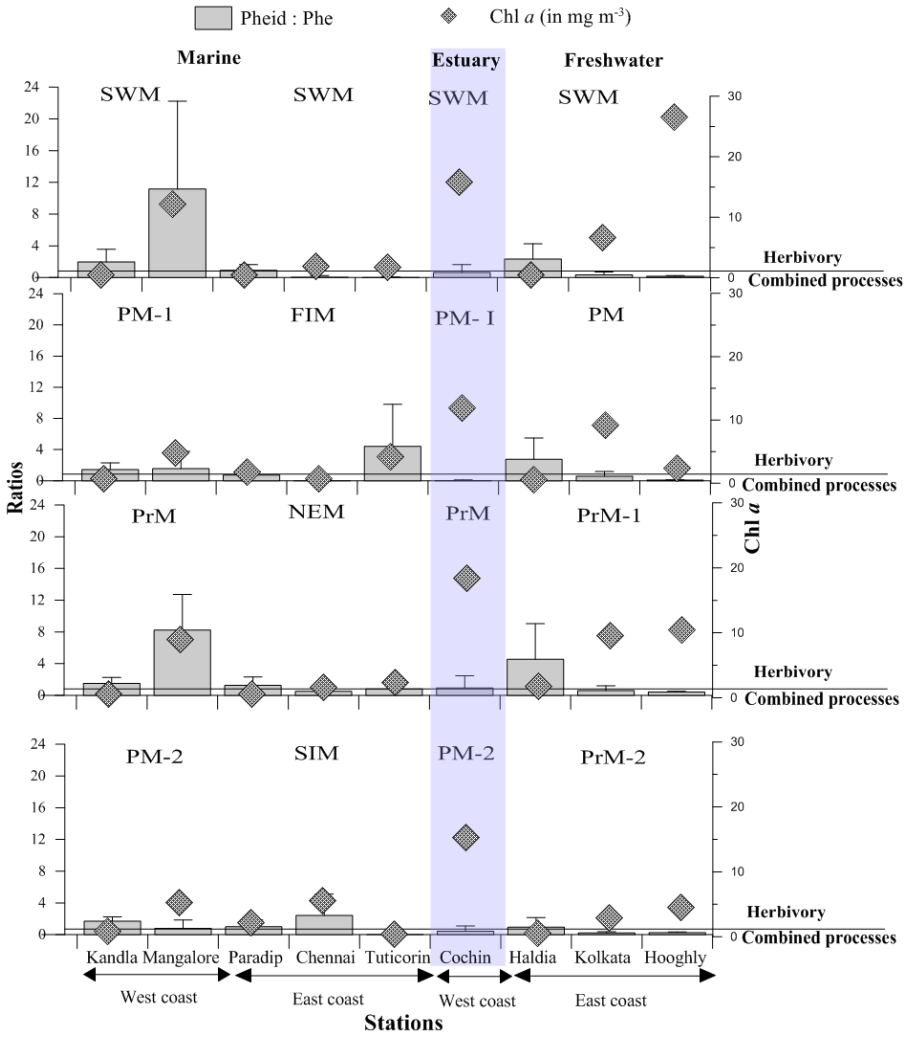

Figure 4.11. The seasonal variations in the pheophorbide : pheophytin ratios from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon, FIM – Fall inter-monsoon, NEM – northeast monsoon, SIM – spring intermonsoon, PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

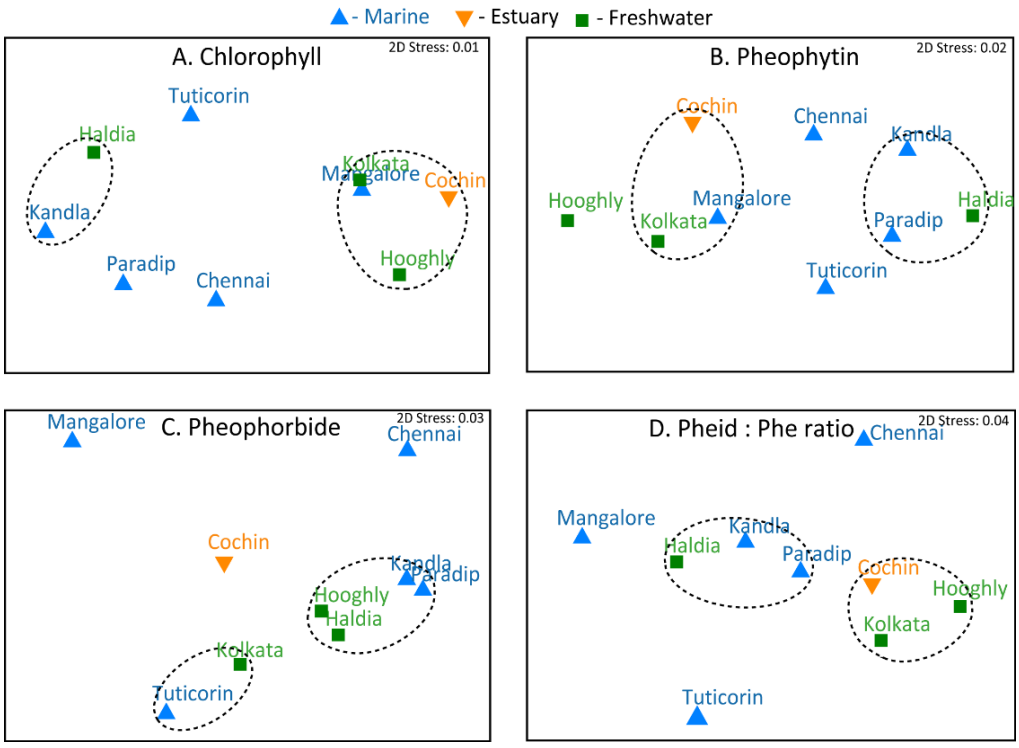

Fig. 4.12. Non-metric multidimensional scaling (NMDS) ordination based on the Bray–Curtis similarity coefficient of (a) chlorophyll, (b) pheophytin, (c) pheophorbide and (d) pheophorbide:pheophytin ratio for the ports located in different ecosystems (marine, freshwater and estuarine). The grouping is based on the 60% similarity level of the cluster dendrograms constructed using the Bray–Curtis similarity coefficient and group average method. The text and symbols in blue, green and orange colour represents marine, freshwater and estuarine ecosystems respectively.

4.4 Discussion

4.4.1 Effect of grazing on chlorophyll breakdown pathway

The three chl *a* derivatives or reaction fractions (RF) are derived from three break-down reactions starting from chl *a* to pyropheophorbide (triple RF) via chlorophyllide/pheophytin (single RF) and pheophorbide (double RF) (Satoh and Hama, 2013). This study focuses on single and double RF as the triple RF was not detected in this study. The grazing-induced mortality resulted in high pheopigments formation compared to non-grazed cultures/NSW (Fig. 4.2). This is because the analysis of pigments in zooplankton-free cultivations shows metabolic processes of the algal cells

while the pigments extracted from the mixed alga-zooplankton cultures was mostly contributed by the undigested algae i.e. culture/NSW suspension suggesting that the contribution from the alimentary canal of zooplankton showed the least effect on the results (Fig. 4.5). Even the low chl a: total pheopigments [an indicator of the freshness of organic matter deposited in the benthos (Morata et al. 2011) or actively growing community (Grippo et al. 2009, Allison et al. 2013) ratios in bottles inoculated with grazers compared to the bottles without grazers also indicated that higher pheopigment production (due to grazing-induced mortality) in the former than the latter bottles (Figs. 4.2 and 4.6). In this study, the formation of only pheophorbide (bottle with zooplankton) and pheophytin (bottles with and without zooplankton) are observed, but not chlorophyllide, indicating that the chl *a* has undergone single and double break-down reactions, respectively. Hence one would expect the occurrence of the chl*a*–pheophytin–pheophorbide pathway, but this may not be the case as the magnitude of increase was not the same among the phytoplankton cultures and NSW incubations. The chl *a*: pheophorbide ratio was lower for the diatom, *Skeletonema* (Fig. 4.6), and the reverse was observed with the chl *a*: pheophytin ratio. An increase in pheophytin was also evident (except *Skeletonema*) in the bottles with zooplankton compared with no zooplankton (Fig. 4.3). These results suggest that grazing can also contribute to pheophytin pool, and the contribution can go up to 80% depending on the phytoplankton species (Fig. 4.3). Interestingly, the pheophytin formation from *Skeletonema*, a preferred zooplankton diet, was negligible. Traditionally, diatoms (including *Skeletonema*) have been considered as preferential food for zooplankton grazers (herbivores) such as copepods. However, in the last two decades, this beneficial role is debated after the discovery of toxic metabolite (oxylipins) production from *Skeletonema marinoi*) that induces reproductive failure in zooplankton grazers (Lauritano *et al.*, 2015). However, the preference (as feed) and non-preference (inhibition) by copepods are also

dependent on the species or strains (Md Amin et al., 2011) as well as the growth phase of *Skeletonema* (Barofsky et al., 2010). For example, copepods showed a higher preference for *S. marinoi* cultures in stationary phase than those in the exponential phase (Barofsky et al., 2010). In this study, the reduced growth of *Skeletonema* in the presence of grazers (than the absence of grazers) implies its mortality due to grazing. Does this mean that the pheophytin formation is negligible in preferred and higher in non-preferred feed?. There are divergent reports of either pheophytin increase (e.g., Fundel et al.,1998) or decrease (e.g., Downs, 1989, Gieskes et al.,1991, Strom, 1993) during grazing experiments, and the difference was attributed to different digestive equipment and feeding modes of the herbivores tested (Fundel et al.,1998).The chl *a*: pheophytin ratio data also indicated such differences in this study (Fig. 4.6), but neither the feeding mode nor the digestive equipment is the reasons, as the grazer composition was the same in all the treatments. These findings highlight that such differences occur in the plankton community, and the food type can be one of the factors. Further, the relative amounts of pheophorbide and pheophytin accumulation in sediments also seem to be dependent on both the composition of the grazed and the grazer communities (Cartaxana et al., 2003). The experimental findings raise the question of why there these differences occur even when all the selected phytoplankton has the potential to produce both pheopigments.

Jeffrey and Hallegraeff (1987) reported high chlorophyllase activity in *Skeletonema* (80-100%) and *Dunaliella* (74%) but not in *Amphidinium*. Hence it is hypothesized that taxon-specific breakdown pathways exist, and this is discussed schematically (Fig. 4.14A and 4.14B). Generally, the location of chlorophyllase enzyme (in the cytoplasm) and chlorophyll (in chloroplasts) are compartmentalized. Hence the catalysis of chlorophyll dephytylation takes place only when they come in contact, and this happens whenever there is cell destruction like chewing or grazing (Hu

et al., 2015). Under such circumstances, the phytoplankton with high chlorophyllase activity will have the chl *a*–chlorophyllide–pheophorbidepathway under grazing pressure. However, this, in

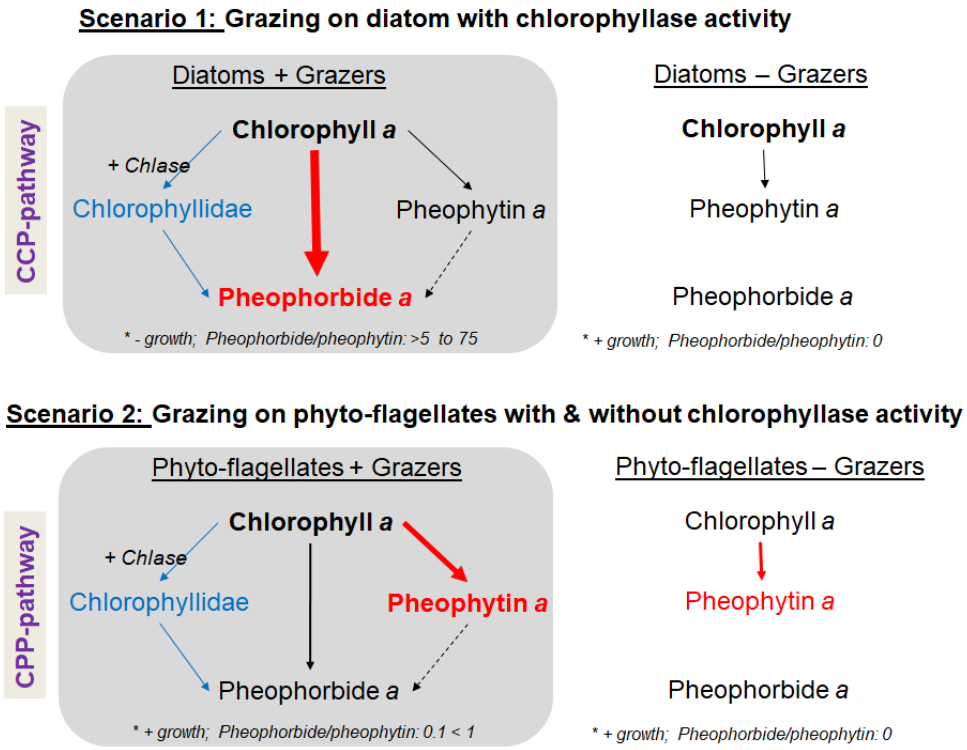

Fig. 4.13A Schematic showing the chlorophyll breakdown pathway in different phytoplankton species with (*Skeletonema* a diatom and *Amphidinium* a dinoflagellate) and without (*Dunaliella* a green algae) chlorophyllase (Chlase). The arrow thickness (black and red) corresponds to relative concentration. Red indicates an active pathway based on the experimental findings. Blue colored text and arrows are an assumption based on literature.

turn depends on grazer feeding habits. In the case of *Skeletonema*, high pheophorbide (also low pheophytin) concentration and decreased growth were observed in bottles with zooplankton compared to control, and the reverse trend was noticed in *Dunaliella* and *Amphidinium* (Figs. 4.14A and 4.14B) because *Skeletonema* is a preferred food for most of the zooplankters relative to the other two phytoplankters used in this study. Further, high grazing results in more production of

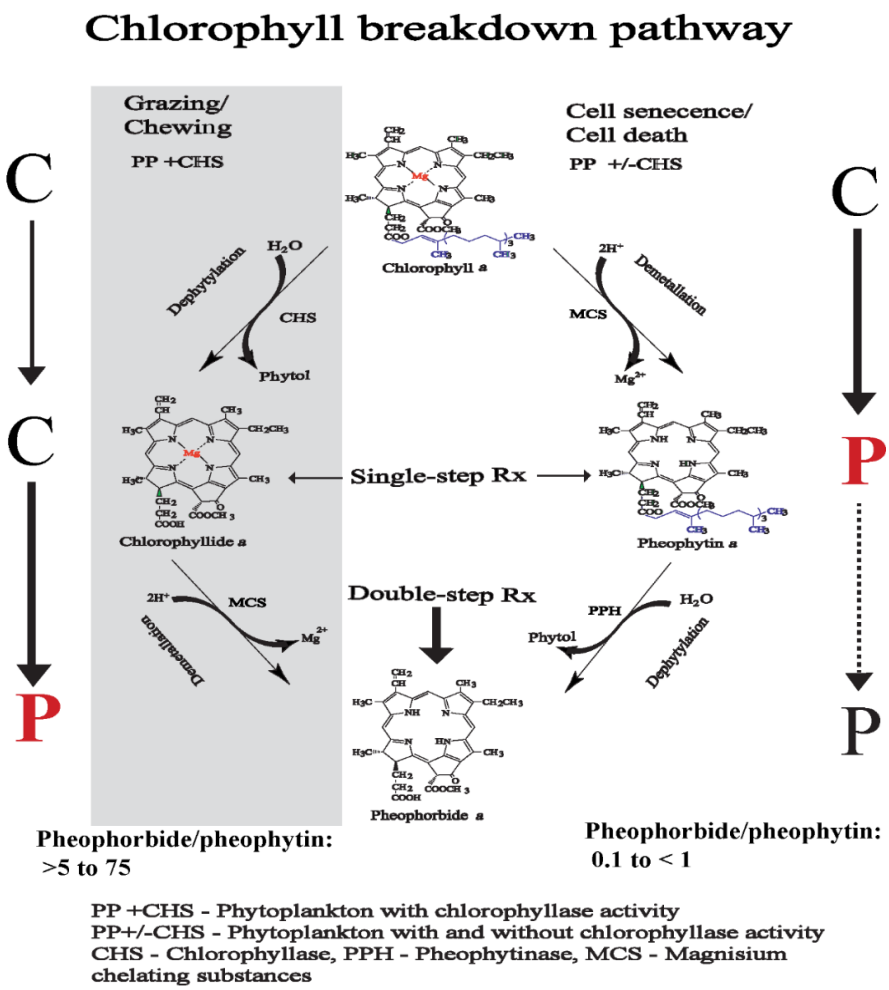

Fig. 4.13B. Schematic of chlorophyll breakdown pathway based on the exprimental results. The arrow thickness (black and red) corresponds to relative concentration. Red indicates an active pathway based on the experimental findings.

fecal material in which pheophorbide is the principal pheopigment (Schuman and Lorenzen, 1975). In some cases (e.g., *Hydrobia ulvae*), pheophorbide can be used as a measure of microalgal ingestion (Coelho et al., 2011). Therefore, *Skeletonema* encountered active grazing and chl *a*-chlorophyllide-pheophorbide pathway but not *Dunaliella* and *Amphidinium* (Fig. 4.14A and 4.14B). Pheophorbide:pheophytin ratio also indicated insignificant variations between with and

without grazers for *Dunaliella* and *Amphidinium* but was siginificant for *Skeletonema* ($p<0.01$). Further, compared to *Skeletonema*, significantly higher pheophytin and lower pheophorbide formation (very low pheophorbide:pheophytin ratio i.e. <1) were observed in *Dunaliella* and *Amphidinium*, suggesting the chl *a*–pheophytin–pheophorbide pathway through ademetalation followed by dephytylation catalyzed by the pheophytinase enzyme (a chloroplast-located and senescence-induced hydrolase enzyme).

Schelbert et al., (2009) reported that the pheophytinase orthologs are widely distributed in eukaryotic photosynthetic organisms (plants and algae), and therefore the involvement of the chl *a*–pheophytin–pheophorbide pathway cannot be ruled out. To our knowledge, no direct studies are available, and there is a need for in-depth studies on pheophytinase enzymes from microalgae. In this study, the chl *a*–pheophytin–pheophorbideand chl *a*–chlorophyllide–pheophorbide pathways resulted in lower and higher pheophorbide production, respectively. Even the chl *a* breakdown pathway is specific to the algal taxon as well as the grazer's feeding habits. Since chlorophyllide was not detected, the pheophorbide: pheophytin ratio can be a good indicator to determine the dominant mortality process, i.e., herbivory is dominant (>1) or not dominant (<1). This ratio is several times higher (up to 40) for *Skeletonema*, whereas for the rest, it was <1 (Figs. 4.7 and 4.14). However, the ratio was found to increase with incubation in all the cultures (Fig. 4.7), suggesting that the mortality due to herbivory (directly or indirectly) was gaining momentum. Nevertheless, higher (>1) and lower (<1) pheophorbide: pheophytin ratios in a bloom-forming *Skeletonema* and flagellates (*Amphidinium* and *Dunaliella*) respectively indicated grazing induced mortality or growth control in the former but not in the latter. Some studies have also shown that toxin-producing phytoplankton deters grazing for certain member of zooplankton (e.g., Turner, 2010), however, such deterrent properties may also be highly specific to species and/or condition (e.g.,

Davis and Gobler, 2010, Stauffer et al., 2017) and could be the case with *Amphidinium* (a potential harmful species, Patil et al. 2015). Given this, the incorporation of the ChlB pathway indicator to determine bloom fate will be a step ahead in the microalgal bloom research (including mitigation of blooms). Technically, several methods such as chemical, physical (including clay dispersal) and biological can be used to control HAB as long as they are capable of killing the HAB organisms and inhibiting bloom formation (Yu et al., 2017 and the references therein). Therefore, ChlB pathway will be useful in determining the factors (e.g. herbivory or senescence) responsible for the decline of the phytoplankton population or as a potential proxy for evaluating the efficacy of the bloom control measures such as grazing and algicidal microbes (Secher, 2009, Choi et al., 2017, Paerl et al., 2018).

Further, the pheopigments distribution is also known to exhibit distinct spatial and seasonal variability similar to that seen in biological variables, particularly plankton. A separate seasonal study from eight major ports (located along the east and west coasts of India) encompassing different ecosystems showed that the pheophytin is more in freshwater and estuarine ecosystems, but its distribution was not linked to chlorophyll variations while the pheophorbide concentrations were more in the marine ecosystem followed by estuarine systems and its distribution linked with seasonal chlorophyll variations. Even the pheophorbide: pheophytin ratios exhibited apparent differences between marine (ratios >1), and freshwater influenced port ecosystems (ratios <1 for estuarine and freshwater systems), respectively. Therefore, documenting the pheopigment distribution and their ratios along with the phytoplankton (biomass and composition) and key environmental variables will aid in ecosystem assessment.

4.4.2 Chlorophyll breakdown pathway in different ecosystems

In aquatic ecosystems, loss (mortality) processes (e.g., grazing, program cell death, viral lysis, sinking) determine the fate of phytoplankton and under such circumstances PMP can be used as a proxy to understand the potential chlorophyll breakdown (ChlB) pathways. In the ChlB pathway, pheophorbide formation is an important step, formed either via chlorophyllide or pheophytin. Laboratory experiments reported that the initial ChlB-pathway induced by grazing and senescence are not the same, i.e., chlorophyll-chlorophyllide-pheophorbide (CCP) and chlorophyll-pheophytin-pheophorbide (CPP) in the former and latter case, respectively. Therefore, documenting these different breakdown factions will aid in determining the dominant ChlB pathway. Like plankton and environmental variables, the ChlB-pathway will also differ in different ecosystems (i.e., FPE, EPE, and MPE) and was documented in this study. The highest concentration of chl *a* and CDP were encountered in FPE (except Haldia with least values) and EPE compared to MPE (Figs. 4.8, 4.9, and 4.12), suggesting the existence of system-specific growth and loss process occurring at different time scales. Chl *a*: total pheopigments, an indicator for degradation of pigments (Boon and Duineveld 1996) or freshness of organic matter (Morata et al., 2011), did not reveal a clear pattern between port ecosystems as observed with the concentrations (Fig. 4.10). However, irrespective of the river influence, the systems with very low biomass (<1 μgm^{-1}) showed very low Chl *a*: CDP ratio suggesting the higher degradation activity (Fig. 4.10). Low and high ratios were observed in Cochin and Kolkata/Hooghly respectively. In the case of MPE, low and high ratios in Paradip and Mangalore respectively suggested the predominance of less or more actively growing biomass, respectively.

In this study, the pool of ChlB fractions is mainly contributed by pheophytin and pheophorbide, suggesting the prevalence of CCP and CPP pathways even when the chlorophyllide fraction was

not detected. However, there exists considerable spatial and temporal variability in the ChlB pathways, as indicated by pheophytin and pheophorbide distribution. The presence of pheophytin (demetalated chlorophyll) and pheophorbide (demetalated and dephytalated chlorophyll) indicates chlorophyll has undergone a single-step and double-step breakdown reactions. Generally, pheophytin concentration is always higher in FPE and EPE compared to MPE (Figs. 4.9 and 4.12) due to the prevalence of high biomass as well as the contribution from the terrigenous influx. On the contrary, the distribution of pheophorbide concentrations was variable even with the high biomass in FPE and EPE, whereas in MPE, distribution showed high, low, and variable under high, low, and variable biomass, respectively (Figs. 4.9 and 4.12). In Cochin (EPE) and Mangalore ports (MPE) high pheophorbide concentrations were recorded,suggesting that these ecosystems are conducive for the chlorophyll to undergo double-step breakdown reactions. Overall pheophorbide concentrations showed independent and dependent on biomass in FPE/EPE and MPE, respectively (Fig. 4.10). Since chlorophyllide is not detected, the identification of ChlB pathway is not straightforward.

The laboratory experiments suggested that the ChlB to pheophorbide via CCP pathway is relatively faster compared to CPP pathway (unpublished data). In such circumstances, pheophorbide: pheophytin ratio will be a potential proxy to understand the dominant pathway or nature of mortality (an important ecological process) in different ecosystems. For instance, if the ratio is >1, then the CCP pathway is more active than the CPP pathway and <1 then the CCP pathway is less active than CPP pathway. Both CCP and CPP pathways may be considered in equilibrium if the ratio is equal to 1. The laboratory grazing experiments using monocultures of phytoplankton belonging to different groups (diatoms, dinoflagellates, and green algae) and natural communities revealed that pheophorbide: pheophytin ratio can be proxy to determine herbivory is dominant (>1)

or senescence is dominant (<1), Satish et al. submitted). These findings suggest that the additional information on the taxonomic composition of both phytoplankton and zooplankton along with the environmental settings (e.g., FPE, EPE, and MPE) will be useful to identify the cause for ChlB pathway. Given this, a schematic indicating the higher ratios for no-river influenced coastal systems (>1) followed by estuarine (0.5), and freshwater (0.2 to 0.4) systems is proposed (Fig. 4.13), which can aid in better ecological assessment and the details on the possible usefulness of the above insights is provided subsequently. For example, the systems with low (<1) and high (>1) pheophorbide: pheophytin ratios suggested the dominance of cell senescence (suggesting CPP pathway due to high pheophytin concentrations) and cell destruction due to herbivory or grazing (suggesting CCP pathway due to high pheophorbide concentrations), respectively. Here the following scenarios: ballast water management (treatment and post-voyage discharge), the fate of algal bloom (the decline of bloom is due to herbivory or senescence or exhaustion of resource) and the ecological perspective are envisaged to elucidate the usefulness of pheophorbide: pheophytin ratio in the ecosystem assessment.

Generally, ports are at the receiving end for ship's ballast water discharge and therefore the information on the phytoplankton growth and loss according to the environmental conditions at discharge point will be valuable for ballast water management and is summarized in Table 4.2.

The present study indicated that the pheophorbide: pheophytin ratio exhibited a distinct spatial (between/within ecosystems) and seasonal variability (Figs. 6 and 7). In FPE (except Haldia) and EPE, the ratio was <1 during all the seasons suggested that the CPP pathway is more active compared to CCP pathway (Fig. 6). In Kolkata and Hooghly systems, the pheophorbide concentration was minimal even when high biomass was present whereas, in the case of Cochin systems, the ratio was relatively higher as there was evidence of high pheophorbide production

compared to freshwater ports during all the sampling occasion. Unfortunately, in Haldia, high ratios (0.9 to 4.6) were observed despite the prevalence of low biomass on all the occasions (Fig. 4.11). One possible reason for such a different scenario is due to the prevalence of different phytoplankton composition. For instance, in Cochin and Kolkata/Hooghly, diatoms (63% - 78%) and non-diatoms (65% - 75%) dominated the phytoplankton community respectively. Generally, diatoms, which have high chlorophyllase activity (Jeffrey and Hallegreff 1987), are the preferred feed for grazers. Therefore, under such circumstances, the CCP pathway will be active and was the case in Cochin ecosystems along with CPP pathway. Even the laboratory experiments also indicated higher pheophorbide production for *Skeletonema* (diatom) than species belonging to other groups, including species (*Dunaliella*) with high chlorophyllase activity (unpublished data). It should also be noted that the chlorophyllase activity varied between the species of the same group (Jeffery and Hallaegreff, 1987). Therefore, the presence of low herbivore population/biomass or the non-preferred diet of the prevailing grazer in Kolkata/Hooghly cannot be ruled out. On the contrary, irrespective of the phytoplankton biomass, pheophorbide: pheophytin ratio in MPE ranged up to 11.2 (Figs. 4.11 and 4.12). This suggests that chlorophyll has undergone double-step ChlB reactions, unlike FPE, with one-step reactions. In MPE, the phytoplankton composition is dominated by diatoms round the year, and therefore, the observed high pheophorbide production via CCP pathway cannot be ruled out. In low (Kandla) and high (Mangalore) biomass systems, CCP pathway is active round the year. In the case of Paradip, Chennai and Tuticorin systems, biomass was variable and so with the ratios. A distinct seasonality in the pheophorbide production in diatom-dominated marine ports was observed, and this is because of the ChlB reactions of seasonal high biomass via CCP pathway, i.e., through herbivory.

Nevertheless, this study suggests that the ChlB pathways exhibit apparent differences between the freshwater (enclosed, Kolkata port), riverine (Hooghly), estuarine (Cochin) and marine ecosystems, respectively. This generally means that the dominant loss factor for phytoplankton (including introduced species) will be cell senescence and herbivory in FPE (throughout the year) and MPE (due to seasonal diatom peaks) respectively, whereas both cell senescence and herbivory are responsible in EPE (due to prevalence of high biomass and freshwater influx). Given the above it is also envisaged that the time window of the conducive environmental condition for the growth of ballast introduced viable phytoplankton is lower for MPE (except Mangalore) and FPE (except diatoms and dinoflagellates) compared to that for EPE (Table 4.2). These findings suggest that the introduction of ballast water with viable phytoplankton during the growing season involves higher risk, and the risk level varied between the ports and season. Nevertheless, the study revealed grazing (in MPE and EPE) and senescence (in FPE and EPE) as the dominant loss factor for phytoplankton. From the ballast water management perspective, the risk level involved in the studied ports is summarized for the reference (Table 4.2). Further, most of the studied port ecosystems are prone to blooms and the knowledge on taxa, chlorophyll, and pheophorbide: pheophytin ratio will be useful to determine whether the decline of bloom is due to herbivory or other reasons (senescence, microbial degradation, resting stage formation). Here, the former scenario (mostly related to seasonal diatom blooms as noticed in MPE and EPE) signifies a good ecological state, and the latter (unusual or harmful algal blooms-HAB) is a matter of ecological and environmental concern. Some studies have also shown that the members of micro- and mesozooplankton don't prefer toxin-producing HAB species (e.g., Turner, 2010), however, such deterrent properties may also be highly specific to species and/or condition (e.g., Davis and Gobler, 2010, Stauffer et al., 2017) and was evident in the laboratory experiment.

Table 4.2. Summary on the distribution of phytoplankton pigments, level of invasion risk and potential loss factor for different major port ecosystems along Indian coast.

Port location	Ecosystems	Biomass (Chlorophyll)	Dominant groups	Pheophytin (Phe)	Pheophorbide (Pheid)	Pheid:Phe	Seasonal	Phytoplankton loss processes	Invasion risk
Cochin	Estuarine	High	Diatoms, Chlorophytes	High	Variable	<1	Yes	Degradation & herbivory (biomass independent)	High
Kolkata	Freshwater	High	Chlorophytes					Degradation (biomass independent)	High*
Haldia	Freshwater	Low	Chlorophytes	Low	Low	>1	No	Herbivory	Low
Kandla	Marine	Low	Diatoms	Low	Low	>1	Yes	Herbivory (biomass dependent)	Low
Mangalore	Marine	High		High	High	Variable			High
Tuticorin	Marine	Variable	Diatoms	Variable	Variable	Variable	Variable		Variable
Chennai	Marine								
Paradip	Marine								

The grazing experiment with a bloom-forming diatom (*Skeletonema*) and dinoflagellate (*Amphidinium*) revealed higher (>1) and lower (<1) pheophorbide: pheophytin ratios respectively suggesting grazing induced mortality or growth control in the former but not in the latter. The global increase in the occurrence of HABs continue to underscore the many remaining gaps in knowledge such as technological advances for early detection (Stauffer et al., 2019). Given this, the incorporation of the ChlB pathway indicator to determine bloom fate will be a step ahead in the ecological/environmental assessment and HAB research per se. For bloom events to occur, the growth rate must exceed losses, through biological and physical processes (Caron et al., 1989, Mitra and Flynn 2006, Tillmann 2004). Therefore, biological control measures such as grazing and algicidal microbes are considered as promising techniques to control algal blooms (e.g., Secher 2009, Choi et al., 2017, Paerl et al., 2018). Given this, it is assumed that ChlB pathway will be a potential proxy for evaluating the efficacy of the bloom control techniques. Traditionally, in addition to grazing, the sinking of ungrazed phytoplankton or bloom to the bottom is considered as one of the major loss factor (Calbet and Landry, 2004, Choi et al., 2017). Thereby assessing the dynamics of PMP from the sediments and the overlying waters in a given ecosystem will improve the understanding of the linkages between the pelagic and benthic phytoplankton, which is an essential process in the functioning of the ecosystem. In view of this, it is assumed that chlorophyll: pheopigment and pheophorbide: pheophytin ratios together can be used as potential proxies to determine the fate of phytoplankton (in particular bloom) or pelagic primary production as the former is an indicator for actively growing microalgal community in the water column and freshness of organic input into the surface sediment (Morata et al., 2011, Grippo et al., 2009, Allison et al., 2013) whereas the latter for determining the factors (eg. herbivory or senescence)

responsible for decline of phytoplankton population or bloom. However, specific studies in these directions will be a step ahead.

The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 (BWM Convention), entered into force globally on 8 September 2017. From this date of the BWM Convention, all ships must conform to the two ballast water management standards (D-1 and D-2). The D-1 standard requires ships to exchange their ballast water in open seas, away from coastal areas (i.e., ~200 nautical miles from land and in water at least 200 meters deep) whereas the D-2 standard specifies the maximum amount of viable organisms allowed to be discharged, including specified indicator microbes harmful to human health. According to the convention, BWM systems (e.g., use of filters and ultraviolet light or electrochlorination) have to be tested in a land-based facility and on-board ships to prove that they meet the performance standard set out in the treaty. Given this Ballast water sampling from the ships has started receiving the importance of proving that ships carrying ballast water comply with BWM standards (Gollasch and David 2017). Even port authorities may also sample ballast water for subsequent analyses to prove compliance with the ballast water performance standards. In view of this, analysing the degraded pigments and their ratios along with the modern tools/methodologies for viable phytoplankton cells will be added advantage in the evaluation of the impact of (i) ballast tank conditions (eg. dark) during voyages, and (ii) treatment of the ships ballast water using approved BWM systems before discharge. During voyages, in ballast tanks, prolong darkness is the prominent stress factors, and this condition can have varying effects on the organisms. The laboratory experiments have shown that phytoplankton population decreased with increase in the length of the dark incubation (Carney et al., 2011, Desai et al., 2017, Patil and Anil 2018), but the duration of dark survival or tolerance varies and is species-specific (Peters 1996, Paters and

Thomas 1996, Unpublished data). The decline in the population could be due to senescence or microbial degradation and grazing or both. The sampling from the ballast tanks during voyages revealed different findings. In one study, decrease and increase in plankton (both phyto and zoo) abundance and bacteria was observed respectively till the end of the voyages (Desai et al., 2018) and in such condition senescence or microbial degradation will be dominant. Whereas in another study, sampling from ballast tanks observed a large fish and increase in copepod (a dominant zooplankton and a grazer) populations (Gollasch et al., 2000, Gollasch and David 2019) suggesting herbivory dominance cannot be ruled out. Moreover, most of the ballast water treatments involve killing or destruction of the cells, and in such cases, phytoplankton degradation process will be similar to that observed during herbivory. The involvement of chlorophyllase activity in the conversion of chlorophyll into chlorophyllide is the reason, and this happens whenever there is cell destruction like chewing or grazing (Hu et al., 2013). Nevertheless, in each of the scenarios, the proposed pheophorbide: pheophytin ratio can be useful to determine the reason for phytoplankton population decline. Since the chl *a* breakdown pathway under grazing-pressure is taxon-specific and the grazer's feeding habits (Sathish et al. submitted), additional studies in this direction will be valuable in ecological surveillance and assessment.

4.5 Conclusion

This study confirms that the chl *a* breakdown under grazing pressure is specific to the taxon as well as the grazers feeding habits. The grazed taxa with high chlorophyllase activity produced higher pheophorbide suggesting chl *a* breakdown through chl *a* -chlorophyllide-pheophorbide pathway. While, the non-grazed taxa (with and without chlorophyllase activity) produced higher pheophytin, and lower pheophorbide suggesting chl *a* – pheophytin –pheophorbide pathway. Pheophytin and pheophorbide are single-step (of chl a – pheophytin –pheophorbide pathway) and two-step

breakdown reactions. Since chlorophyllide (single-step reaction of chl a -chlorophyllide-pheophorbide pathway) was not detected, pheophorbide: pheophytin ratio can be a potential proxy for determining dominant breakdown pathway, i.e., chl a-chlorophyllide-pheophorbide (or herbivory) is dominant (>1) or not dominant (<1).

Moreover, this study also concludes that the distribution of single (pheophytin) and double (pheophorbide) step chlorophyll-breakdown reaction fractions exhibit distinct spatial and seasonal variations. Pheophytin is higher in the FPE

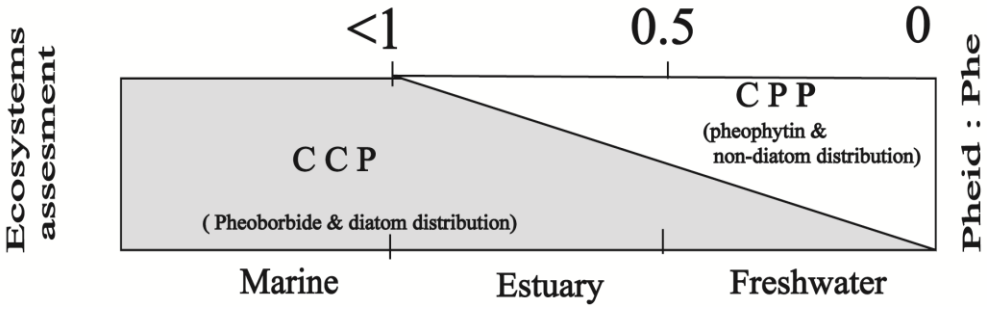

Figure. 4.14 Schematic illustrating the chlorophyll-breakdown pathway in different ecosystems. CCP and CPP indicates chlorophyll-chlorophyllide-pheophorbide and chlorophyll-pheophytin-pheophorbide pathways respectively. Non-diatoms indicates that the phytoplankton is dominated by other groups such as chlorophytes, cyanobacteria, dinoflagellates. Pheid:Phe indicated phoephorbide:pheophytin ratio.

and EPE, but its distribution is not linked to biomass variations. Whereas pheophorbide is more in MPE followed by estuarine systems and its distribution could be linked with biomass distribution. Since chlorophyllide (single-step reaction of CCP pathway) was not detected, pheophorbide: pheophytin ratio can be a potential proxy for determining the nature of the breakdown pathway, i.e., CCP or herbivory dominant (>1) or not dominant (<1). The ratios exhibited clear differences between different ecosystems, i.e., the higher ratios in marine (up to 11.2) followed by estuarine (up to 0.94) and freshwater (0.2 to 0.4) port systems. This generally means that the dominant

degradation pathway for phytoplankton (including introduced species) will be cell senescence and herbivory in FPE (throughout the year) and MPE (due to seasonal diatom peaks) respectively, whereas both cell senescence and herbivory are responsible in EPE (due to prevalence of high biomass and freshwater influx). Since laboratory experiments revealed that the chl *a* breakdown pathway under grazing-pressure is taxon-specific and the grazer's feeding habits (Sathish et al. submitted) additional studies in this direction will be valuable in ballast water management (treatment and post-voyage discharge), algal bloom research (eg. understanding fate and in control measures), and in the ecological surveillance. Also confirming the role of hydrolase enzymes and the sequence in the early as well as post-pheophorbide breakdown steps in different aquatic ecosystems will enhance the understanding of the ChlB pathways, which further alleviate the ecosystem assessment.

Chapter 5

Phytoplankton marker pigments to study benthic-pelagic linkages

5. Phytoplankton marker pigments to study the benthic-pelagic linkage

5.1 Introduction

Phytoplankton are accountable for about half of the global primary production and establish a base to the food web, which supports either directly or indirectly to the entire marine ecosystems (Jeffrey and Vesk, 1997, Falkowski et al., 2004). Phytoplankton are represented by considerable diversity of algal groups (diatoms, dinoflagellates, phytoflagellates, coccolithophorids, red algae, green algae, and cyanobacteria) with size ranging from 0.2 microns to several millimeters. All phytoplankton, by their photosynthetic activities, are a significant contributor to global carbon fluxes (Falkowski 1998). Variations in the taxonomic and phytoplankton functional groups (PFG's) influence the pelagic environment all through its biogeochemical cycle, tropical interaction, nutrients, and food chain (Agirbas and Karadeniz 2020). In the coastal area, benthic faunal changes are determined by the fluctuation of phytoplankton, which is major organic input (Zhang et al., 2015). Since phytoplankton are the principle element of biogeochemical cycles and food web dynamics, accessing phytoplankton biomass and its functional groups from pelagic and benthic environment plays a crucial role in environmental assessments.

Benthic-pelagic coupling is demonstrated as the exchange of energy, biomass, or inorganic nutrients between benthic and pelagic habitats. It plays a crucial part in the nutrient cycling and food web dynamics of aquatic ecosystems. Coastal and estuarine systems are strongly affected by the anthropogenic activities, but the understating of organic matter and inorganic nutrient exchange between the benthic environment and water column are minimal (Griffiths et al., 2017). Therefore, knowledge of the phytoplankton composition and biomass from the pelagic ecosystem is essential to understand the linkage and dynamics (Mendes et al., 2011, Agirbas et al., 2014). The water column phytoplankton changes also fuel the benthic production (Graff and Rynearson, 2011,

Freiberg et al. 2011). Sedimentary pigments reflect phytoplankton production and its pathway in the water column and sediment. Hence, it is hypothesized that assessing the dynamics of phytoplankton marker pigments from sediments and water column will improve the understanding of the linkages between the pelagic and benthic phytoplankton, which is an essential process in the functioning of the ecosystem (Frieberg et al. 2011).

Apart from the group-specific phytoplankton marker pigments, chlorophyll *a* and its degradation products often used as a diagnostic indicator for the fresh and degraded microalgal community, respectively (Lorenzen, 1967, Welschmeyer et al., 1984, Boon and Duineveld, 1998, Wieking and Kröncke, 2005, Pusceddu et al., 2009). The major chlorophyll *a* degradation products observed in aquatic ecosystems are chlorophyllide *a* (Chlide *a*), pheophytin *a* (Pheo *a*), pheophorbide *a* (Pheid *a*), and pyropheophytin *a* (pPheo *a*). Processes such as growth, grazing, cell sinking and senescence, photodegradation, fecal pellet sinking, physical mixing, and transport affect chl *a* and pheopigments concentrations in the euphotic zone. Hence assessing these pheopigments and its ratios will strengthen the understating of phytoplankton from the given ecosystem.

Literature suggests that the phytoplankton pigments are extensively used in the studies related to photophysiology (Roy et al. 2011, Patil et al., 2017), dynamics of phytoplankton distribution and composition in relation to environment (Ahel et al. 1996, Ston 2002, Lohrenz et al. 2003, Dyble and Moisander 2003, Henriksen et al. 2002, Chakraborty et al. 2011, Roy et al. 2011, Agirbas et al. 2017, Nunes et al. 2018, Naik et al. 2018), sedimentary pigments as a biomarker (Gall and Blanchard 1995, Morata and Renaud. 2008, Reuss et al. 2010, Freiberg et al. 2011, Aneeshkumar and Sujatha 2012, Rasiq et al. 2016, Symczak-Zyla et al. 2008, Sañé et al. 2019) and palaeo-climate and palaeo-environment (Hodgson 1997, Soma et al. 2001, Reuss et al. 2010). These studies are either focused on the water column pigment distributions or sedimentary pigment biomarkers,

including the Indian coasts. Though some microscopic studies indicated the persistence of benthic-pelagic coupling in bloom-forming diatoms (Patil and Anil 2008), diatom viability in sediments for several months (Anil et al. 2007), recent dinoflagellate cyst distributions (D'Silva et al., 2012, Narale et al., 2013, D'Silva et al., 2013, Rodrigues et al., 2019, Prabhudessai et al., 2020) and phytoplankton pigment linkage between benthic and pelagic from large shallow the lake (Frieberg et al. 2011). Though some microscopic studies indicated the persistence of benthic-pelagic coupling in bloom-forming diatoms (Patil and Anil 2008), diatom viability in sediments for several months (Anil et al. 2007) and phytoplankton pigment linkage between benthic and pelagic from large shallow lake (Frieberg et al. 2011). The studies related to phytoplankton biomass and composition from the coastal sediment and its coupling between the water columns are limited to the best of our knowledge. This study expands the existing knowledge for the area in terms of distribution and dynamics of PFG's, phytoplankton benthic-pelagic linkage, and the fate of ungrazed phytoplankton using marker pigments and the ratios indicating phytoplankton physiological status and the nature of loss process.

5.3 Methodology

Details of sampling strategies, pigments analysis (water column and sediment) from paradip port ecosystems are presented in chapter 2.

5.3.1 Data analysis

ANOVA and followed by *post hoc* Tukey tests were performed using Statistica software to evaluate the spatial and temporal variations in the contributions of PFG's and pheopigments ratios (Pheid:Phe and Chl *a*:CDP's). To evaluate the relationship between PFG's and measured environmental parameters (temperature, salinity, DO, BOD, PO_4, SiO_3, NO_2, NO_3 and NH_4) redundancy analysis (RDA) was performed by using the CANOCO 4.5 software (ter Braak and

Smilauer 2002). For surface water PFG's environmental parameters corresponding to surface water were used, whereas for NBW and sediment PFG's environmental parameters corresponding to NBW were used. Before RDA, data was initially analyzed by detrending correspondence analysis, and since the longest gradient length for the water column (Surface and NBW) and sediment was less than 3.0, RDA was performed.

5.3. Results

5.3.1 Environmental parameters

The results revealed that the measured environmental parameter showed a distinct seasonality. Water temperature ranged from 25.2 to 30.4 ºC with the highest temperature during FIM (29.7 ± 0.4 ºC). The water column remained cooler during NEM (25.2 to 26.9 ºC) and warmer during SWM (19.1 to 28.6) and SIM (27.4 to 28.8 ºC). Salinity variations were wide (25.6 to 33.9), with the highest (33.8 to 33.9) salinity observed during SIM followed by NEM (28.9 to 32.6) and FIM (28.1 to 31.5). The lowest salinity was found during SWM (25.6 to 28.7). Higher salinity was observed in the NBW compared to surface water during all seasons, suggesting the freshwater influence in the port area. ANOVA revealed an insignificant difference (p >0.05) in salinity and temperature between surface and NBW. DO concentration ranged from 2.6 to 5.6 µg l^{-1} with the maximum concentration (5.2 ± 0.4 µg l-1) in the surface water during SWM.

There was no considerable difference in the averaged DO concentrations during NEM, FIM, and SIM and ranged between 4.0 to 4.7 µg l^{-1}. ANOVA revealed a significant difference (p <0.001) in DO concentrations for surface and NBW, except during NEM. BOD values ranged from negligible to 2.7 µg l-1 with the maximum and minimum demand during FIM (0.6 to 2.7 µg l-1) and SWM (0 to 0.8 µg l-1), respectively (Fig. 5.1).

NO_3^- concentrations were high during FIM (1.2 to 14.7 μM) compared to other seasons and during the rest of the seasons (SWM, NEM, and FIM) magnitude of the averaged values (4.0 to 4.8 μM) was same. NO_2^- concentration ranged from 0.4 to 3.3 with the maximum and minimum during

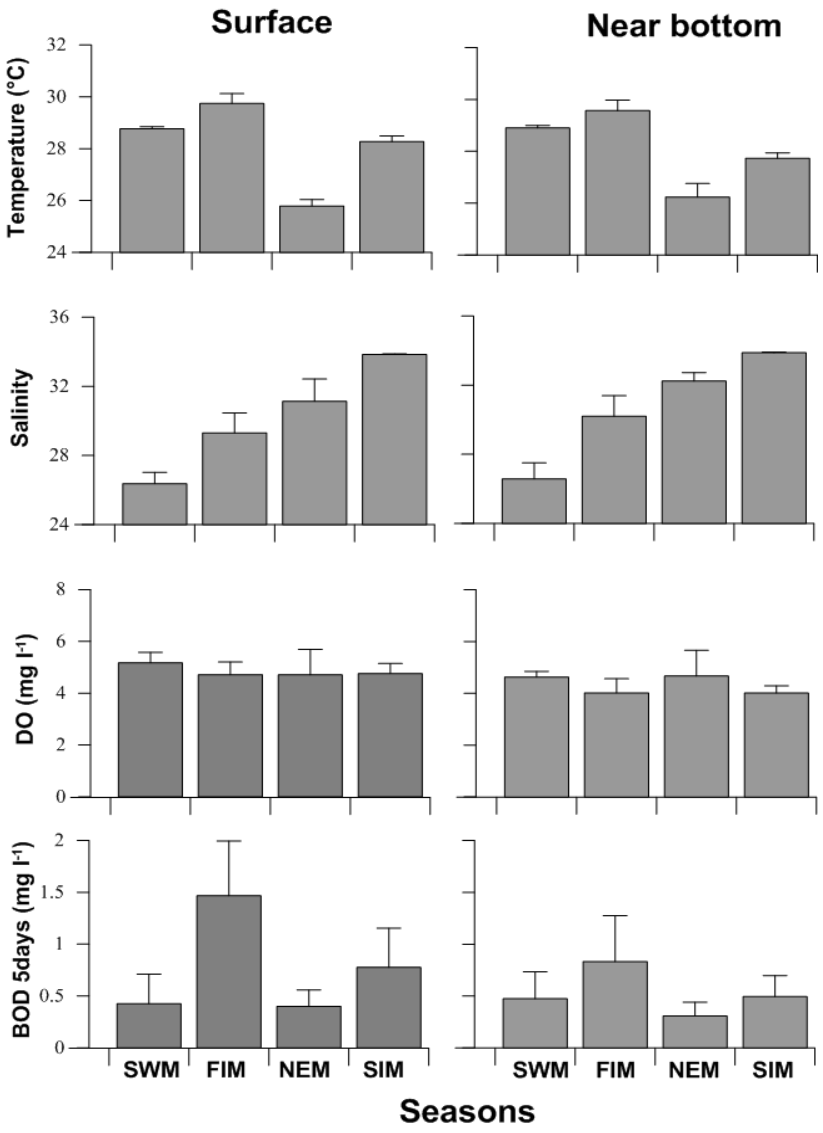

Fig. 5.1 Seasonal variations in the measured environmental variables from the surface and near bottom (NBW) waters. Bars represent the average values and the standard deviations

SIM (1.8 ± 0.2 μM) and SWM (0.9 ± 0.2 μM) respectively. PO_4^{3-} concentrations ranged up to 2.8 μM with the higher and lowest values during SIM (2.2 to 3.0 μM) and FIM (0 to 0.5 μM),

respectively. Highest SiO_4^{4-} levels were observed during FIM (8.1 to 134.9 µM) and SIM (52.3 to 100.0 µM) compared to NEM (31.8 to 106.4 µM) and SWM (13.7 to 27.0 µM). Interestingly, SiO_4^{4-} concentrations (71.1 ± 41.9 µM) were relatively higher in the NBW compared to surface water (60.3 ± 37.6 µM) during FIM. There was no significant difference (p >0.05) in PO_4^{3-}, NO_3^-, NO_2^- and SiO_4^{4-} values between surface and NBW (Fig. 5.2).

5.3.2 Phytoplankton pigment concentrations

Altogether 16 pigments were observed in the water column, and 11 pigments were observed in the surface sediment samples. Chl *a*, Chl *b*, Fuco, Zea, Allo, Peri, Phe *a*, and Pherd *a* were the primary pigments observed in the water column and sediment. The results indicated a distinct seasonality in the distribution of biomass (chl *a*) and marker pigments (for different groups and degradation) from the sediment and overlying water column. However, the seasonal trend in the surface and NBW was the same but not with the sediment. In the surface and NBW, chl *a* ranged between 0.1 to 8.8 µg l^{-1}. The higher values were observed during spring (1.1 to 8.8 µg l^{-1}) and fall (0.7 to 3.1 µg l^{-1}) inter-monsoons compared to the southwest (0.2 to 0.7 µg l^{-1}) and northeast (0.1 to 1.6 µg l^{-1}) monsoons. The chl *a* in sediments ranged between 0.01 to 0.2 µg $g^{-1,}$ and the higher values were observed during southwest (0.03 to 0.14 µg g^{-1}) and north-east (0.02 to 0.24 µg g^{-1}) monsoons compared to fall (0.01 to 0.05 µg g^{-1}) and between 0.03 to 3.19 µg g^{-1} in the water column and ranging from 0.01 to 0.13 µg g^{-1} in the sediment. High Fuco concentrations were observed in the water column during inter-monsoons (fall and spring, 0.08 to 3.19 µg l^{-1}), and low values during monsoons (southwest and north-east, 0.05 to 0.58 µg l^{-1}) and the reverse trend was observed in the sediments. Moreover, significantly higher concentrations of marker pigments such as Chl *b*, Zea, Allo, Peri, were observed during inter-monsoons (fall and spring) compared to monsoons (southwest and north-east, Table 5.1).

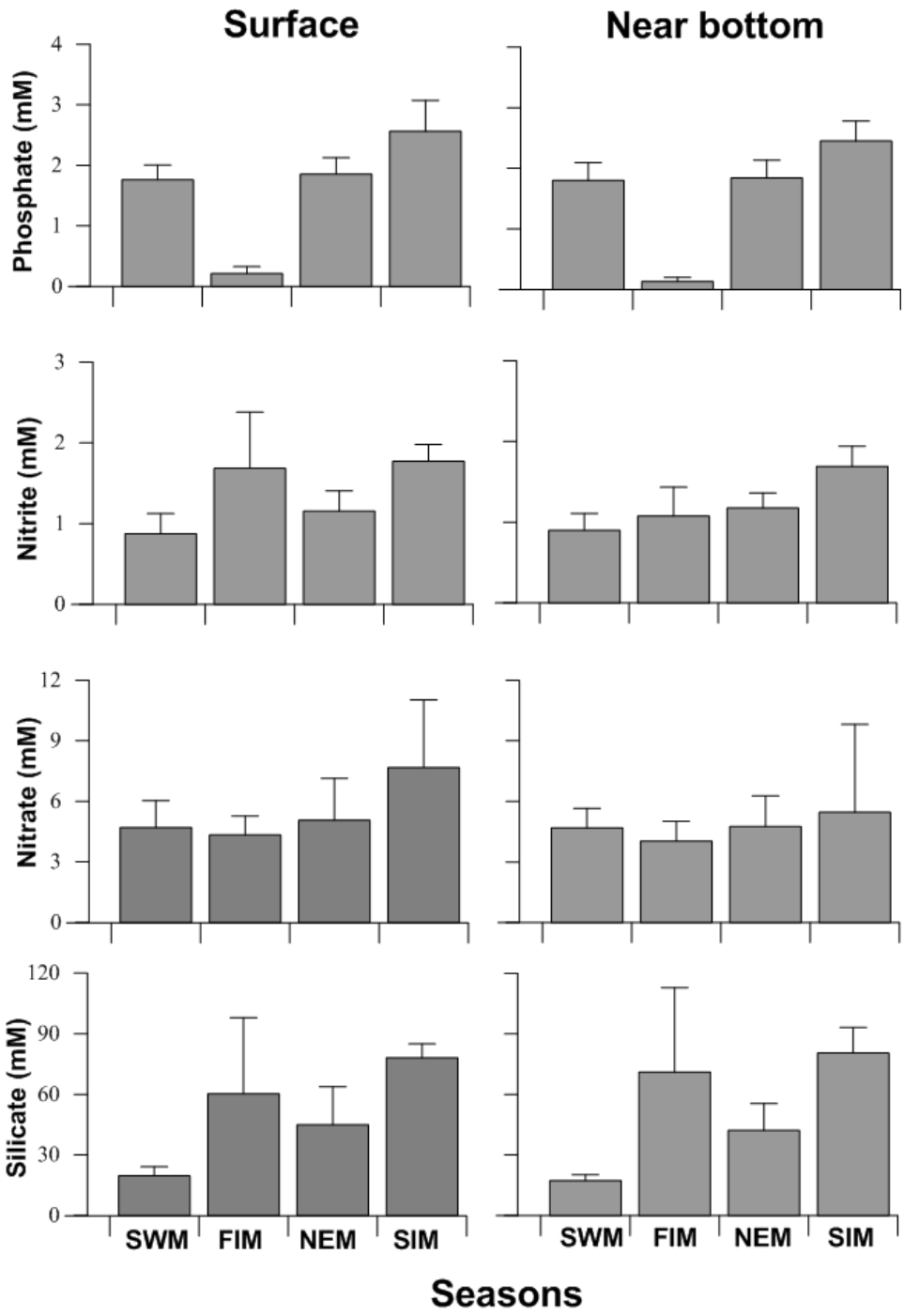

Fig. 5.2 Seasonal variations in the nutrient concentrations from the surface and near bottom (NBW) waters. Bars represent the average values and the standard deviations

5.3.3 Distribution of phytoplankton functional group's (PFG's)

CHEMTAX derived relative contribution (%) of each PFG's revealed the presence of 4 major and four minor groups in the water column and sediment. Phytoplankton groups contributing to the total chl *a* were different in the water column and sediment. For instance, diatoms, cryptophytes, chlorophytes, and cyanobacteria were the dominant groups in the water column. However, in sediment, chlorophytes followed by diatoms, dinoflagellates, and cryptophytes were the dominant groups. PFG's were showing distinct variability between the seasons. During SWM surface water was dominated by diatoms (43.7 %) with higher contribution to chl *a* (0.16 µg l^{-1}) and cryptophytes (37.1 % with 0.15 µg l^{-1} contribution to chl *a*) followed by chlorophytes (8.9 %), prasinophytes (7.6%) and cyanobacteria (3.4%). Other groups such as dinoflagellates, chrysophytes, and prymnesiophytes were contributing less than 1% during SWM. In NBW, diatoms (48.8 % with 0.21) and chlorophytes (32.9 % with 0.14 µg l^{-1}) were the dominant groups followed by cryptophytes (6.8%) and prasinophytes (4.7%). In contrast, other groups were contributing less than 3% to the total chl *a*. Contrarily, in surface sediment, during SWM, chlorophytes (34% with 0.03 µg g^{-1}) were dominating over the diatom (23% with 0.02 µg g^{-1}) followed by cyanobacteria (14.6%), cryptophytes (14.1%) and dinoflagellates (13.9%). Phytoplankton biomass in the surface water during FIM was mainly contributed by diatom (35.4% with 0.46 µg g^{-1}) and cryptophytes (35.5% with 0.52 µg g^{-1}) followed by chlorophytes (6.9%) and prasinophytes (5.6%). Other groups such as dinoflagellates, chrysophytes, and prymnesiophytes were contributing less than 3% to the total chl *a*. In NBW, phytoplankton biomass was mainly contributed by diatom (53.2% with 0.62 µg l^{-1}) and cryptophytes (27.2% with 0.32 µg l^{-1}). In contrast, phytoplankton biomass from the surface sediments was mostly contributed by chlorophytes (65.1% with 0.02 µg g^{-1}) followed by diatom (17.9% with 0.01 µg g^{-1}) and dinoflagellates (11.2%). Other groups were contributing less

Table 5.1 Seasonal variations in the concentrations of chlorophylls, carotenoids and pheopigments from the surface water, near bottom water (NBW) and surface sediments. Pigment values from water (surface and NBW) and sediments are in L^{-1} and g^{-1} dry weight, respectively. Note SWM – southwest monsoon, NEM –northeast monsoon, FIM – fall inter-monsoon, SIM – inter-monsoon, PSC – photsynthetic carotenoids and PPC – photoprotective carotenoids.

Pigments	Average and range of pigment concentrations											
	SWM			FIM			NEM			SIM		
	Surface	NBW	Sediment	Surface	NBW	Sediment	Surface	NBW	Sediment	Surface	NBW	Sediment
chlorophyll												
Chl *a* (µg)	0.38 (0.20-0.69)	0.42 (0.25-0.65)	0.08 (0.03-0.15)	1.42 (0.72-2.57)	1.13 (0.67-3.08)	0.03 (0.01-0.05)	0.56 (0.32-1.04)	0.56 (0.12-1.63)	0.15 (0.02-0.24)	2.54 (1.08-8.8)	1.10 (0.77-1.86)	0.07 (0.03-0.11)
Chl *b* (µg)	0.03 (0.01-0.07)	0.05 (0.02-0.12)	0.07 (0.05-0.11)	0.18 (0.04-0.34)	0.05 (0.01-0.16)	0.04 (0.03-0.06)	0.08 (0.03-0.20)	0.08 (0.01-0.15)	0.15 (0.03-0.23)	0.26 (0.09-0.91)	0.10 (0.05-0.14)	0.10 (0.03-0.19)
Chl *c* (ng)	3.1 (0.7-8.4)	7.6 (2.7-14.3)	0	22.4 (11.2-42.9)	48.2 (10.6-263)	0	16.5 (2.1-39.3)	20.9 (8.5-33.9)	0	79.4 (0.0-740.8)	25.7 (11.9-55.7)	0
carotenoids												
PSC												
19BUT (ng)	2.6 (0-5.2)	10.9 (0-23.1)	0	27.0 (0-94.2)	7.2 (0-18.9)	0	0.6 (0-7.2)	1.8 (0-6.0)	0	18.2 (0.0-77.1)	7.0 (0.0-17.7)	0
Fuc (µg)	0.07 (0.05-0.12)	0.13 (0.07-0.19)	0.07 (0.02-0.13)	0.29 (0.17-0.46)	0.37 (0.08-1.25)	0.02 (0.01-0.02)	0.07 (0.03-0.12)	0.13 (0.05-0.54)	0.08 (0.01-0.13)	0.60 (0.19-3.19)	0.34 (0.24-0.58)	0.05 (0.01-0.09)
19Hexa (ng)	1.6 (0-5.8)	12.3 (0-40.5)	0	18.2 (0-46.8)	15.4 (0-38.4)	0	14.9 (0-44.7)	15.3 (0-78.1)	0	38.6 (0.0-208)	15.6 (0.0-38.3)	0
Per (ng)	4.7 (0-13.9)	6.2 (0-13.1)	17.8 (7.7-52.6)	49.6 (25.5-91.8)	20.4 (0-39.5)	6.2 (3.6-8.3)	2.3 (0-8.9)	8.7 (0-28.0)	44.0 (4.1-74.8)	68.9 (4.9-174)	14.5 (0.0-30.2)	19.4 (4.2-31.4)
PPC												
Allo (µg)	0.01 (0.00-0.02)	0.01 (0-0.02)	0.03 (0.01-0.20)	0.04 (0.01-0.1)	0.01 (0.00-0.02)	0.02 (0.01-0.03)	0.01 (0.00-0.02)	0.01 (0-0.02)	0.03 (0-0.08)	0.07 (0.03-0.19)	0.03 (0.02-0.07)	0.03 (0.01-0.11)
B-car (ng)	5.7 (1.4-18.3)	5.3 (2.0-9.9)	0	23.5 (4-49.4)	11.6 (2.0-32.5)	0	16.5 (10.4-29.3)	12.2 (0-25.3)	0	61 (20-185)	19.1 (8.8-58.2)	0
Diad (ng)	7.7 (4.5-17.2)	17.0 (5.6-28.3)	19.9 (5.3-63.1)	42.4 (0-108.1)	45.1 (13.3-159)	5.8 (2.3-9.2)	13.6 (3.9-24.3)	12.1 (4.5-33.0)	20.0 (2.9-37.1)	85.3 (32.1-362)	30.9 (19.8-48.5)	12.6 (0.0-20.9)
Diat (ng)	1.7 (0-2.9)	8.9 (0-20.4)	50.6 (16.2-143)	15.2 (0-28.5)	5.5 (2.0-14.2)	23.8 (15.6-35.6)	6.5 (1.7-14.9)	5.0 (0-18.4)	105 (14.1-208)	28.9 (8.5-114)	10.0 (5.7-16.2)	54.5 (14.3-130.3)
Lut (ng)	4.0 (2.3-8.3)	11.3 (4.9-27.3)	96.5 (0-402.6)	39.4 (10.6-91.5)	8.1 (2.5-15.5)	47.5 (23.1-65.6)	17.8 (2.4-49.7)	19.4 (5.2-37.5)	151 (26.2-244)	29.4 (13.0-72.3)	12.6 (0.0-18.7)	151.3 (34.1-442.1)
Viola (ng)	3.0 (0.0-5.1)	19.6 (3.7-41.3)	4.7 (0-12.8)	28.2 (9.1-51.8)	14.9 (0-25.6)	3.4 (0-5.7)	10.7 (0-20.1)	14.5 (0-37.6)	7.9 (0.0-13.7)	43.8 (15.1-185)	28.3 (18.9-47.4)	9.3 (0.0-55.9)
Zea (µg)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.06 (0.03-0.12)	0.18 (0.03-0.43)	0.04 (0.01-0.08)	0.03 (0.01-0.04)	0.04 (0.02-0.10)	0.05 (0.02-0.10)	0.14 (0.03-0.28)	0.12 (0.04-0.28)	0.05 (0.02-0.10)	0.08 (0.02-0.20)
Neo (ng)	3.7 (0-8.7)	19.7 (6.8-40.2)	10.1 (2.6-24.1)	37.5 (8.3-86.3)	5.9 (0-13.9)	5.7 (2.1-11.5)	9.7 (0-26.6)	9.1 (0-28.6)	17.2 (4.1-26.8)	67.5 (12.8-341)	25.3 (8.9-52.7)	15.2 (4.9-42.9)
Pras (ng)	4.1 (2.0-8.7)	10.3 (0-23.2)	0	42.7 (20.3-99.7)	15.6 (0-30)	0	12.9 (0-30.2)	12.4 (0-23.7)	0	62.2 (17.1-310)	16.7 (10.0-24.6)	0
Deg product												
Pheo *a* (µg)	0.02 (0.01-0.04)	0.049 (0-0.07)	0.51 (0.13-0.99)	0.11 (0.04-0.18)	0.11 (0.09-0.19)	0.15 (0.06-0.21)	0.06 (0.03-0.1)	0.05 (0-0.09)	1.26 (0.30-4)	0.14 (0.06-0.22)	0.11 (0.06-0.21)	0.57 (0.15-1.92)
Pheide *a* (µg)	0.02 (0.01-0.09)	0.078 (0-0.15)	0.32 (0.05-0.8)	0.07 (0.000-0.12)	0.11 (0.03-0.26)	0.06 (0.04-0.07)	0.08 (0-0.20)	0.04 (0-0.11)	0.27 (0.05-0.56)	0.14 (0.05-0.67)	0.13 (0.06-0.24)	0.15 (0.04-0.25)

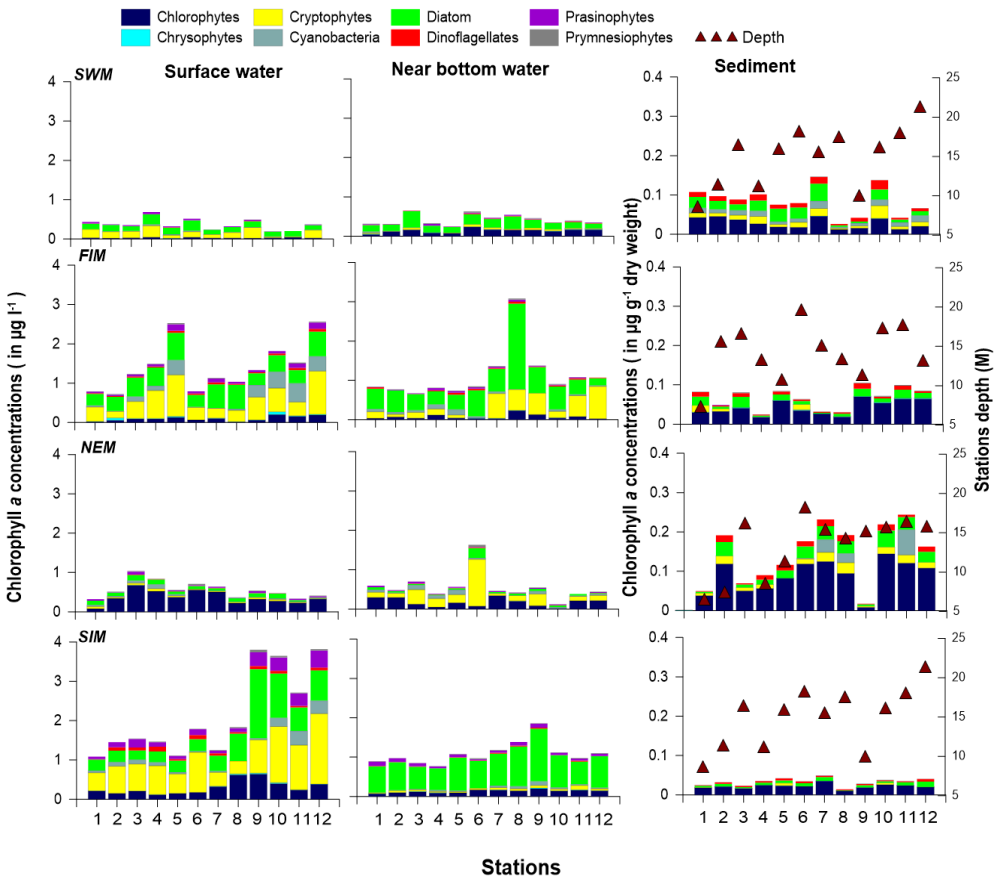

Fig. 5.3 Station wise variations in the phytoplankton groups derived from CHEMTAX analysis for the overlying waters (surface and near bottom - NBW) and sediments.

-than 3% to the total phytoplankton biomass. During NEM, chlorophytes (63.8% with 0.37 µg l^{-1}) and diatom (16% with 0.08 µg l^{-1}) were the dominant contributors to the total chl *a* in surface water. Other groups, such as cryptophytes, cyanobacteria, and prasinophytes, were contributing less than 10% to the total phytoplankton biomass. Whereas in NBW, the predominance of chlorophytes (34.9% with 0.25 µg l^{-1} contribution to chl *a*) and cryptophytes (34.7% with 0.17 µg l^{-1} contribution to chl *a*) followed by cyanobacteria (11.5%) and diatom (10.1%) were observed. During NEM, chlorophytes (62.2% with 0.09 µg g^{-1} contribution to chl *a*) make the most significant contribution to the total chl *a* in the surface sediment followed by diatom (15.5%) and cryptophytes (10.1%).

Other groups such as dinoflagellate and cryptophytes were contributing up to 8% to the total chl *a*. Phytoplankton biomass in surface waters during SIM, mostly contributed by cryptophytes (40.1% with 0.83 µg l^{-1}) and diatom (25.8% with 0.58 µg l^{-1}) followed by chlorophytes (15%). Other groups were contributing less than 10% to total chl *a*. In NBW, Chl *a* mainly contributed by diatom and chlorophytes with relative contribution of 71% and 12%, respectively. In contrast, reverse trend was observed in the sediment. The predominance of chlorophytes (64.2%) followed by the diatom (17.4%) and dinoflagellates (9.2%) was observed in the surface sediment (Fig. 5.4).

5.3.4 Chlorophyll a and Pheopigment ratios

In this study, chl *a*:total phoepigments (TPhe) and Pheophorbide:pheophytin ratios were considered as the former and latter indicates the micro-algal status and nature of the degradation pathway respectively. Chl *a*: TPhe ratio was high in the water column (3.95 to 9.17) compared to sediment (0.11 to 0.17). In surface water, highest Chl *a*: TPhe ratio was observed during SIM (9.17 ± 3.57) followed by SWM (9 ± 3.59) and FIM (8.46 ± 3.76). The lowest value was observed during NEM (5.59 ± 5.02). Whereas in NBW, Chl *a*:TPhe ratio ranged between 3.95 to 6.28 with the maximum during NEM. Chl *a*: TPhe ratio from surface sediment (0.40 to 0.76) (Fig. 5.6). In surface water, Pheid: Phe ratio was high during NEM (1.39 ± 1.33) followed by SIM (1 ± 1.02), SWM (0.99 ± 0.78) and FIM (0.71 ± 0.45), while in NBW highest Pheid: Phe ratios were observed during SIM (1.24 ± 0.5) and SWM (1.19 ± 0.66) compared to FIM (0.88 ± 0.35) and NEM (0.67 ± 0.46). Pheid: Phe ratio in the surface sediment ranged between 0.40 to 0.76 with the maximum during SWM (Fig. 5.6). ANOVA results suggested that there was no significant (p >0.05) variations in Pheid: Phe ratio between the stations or seasons.

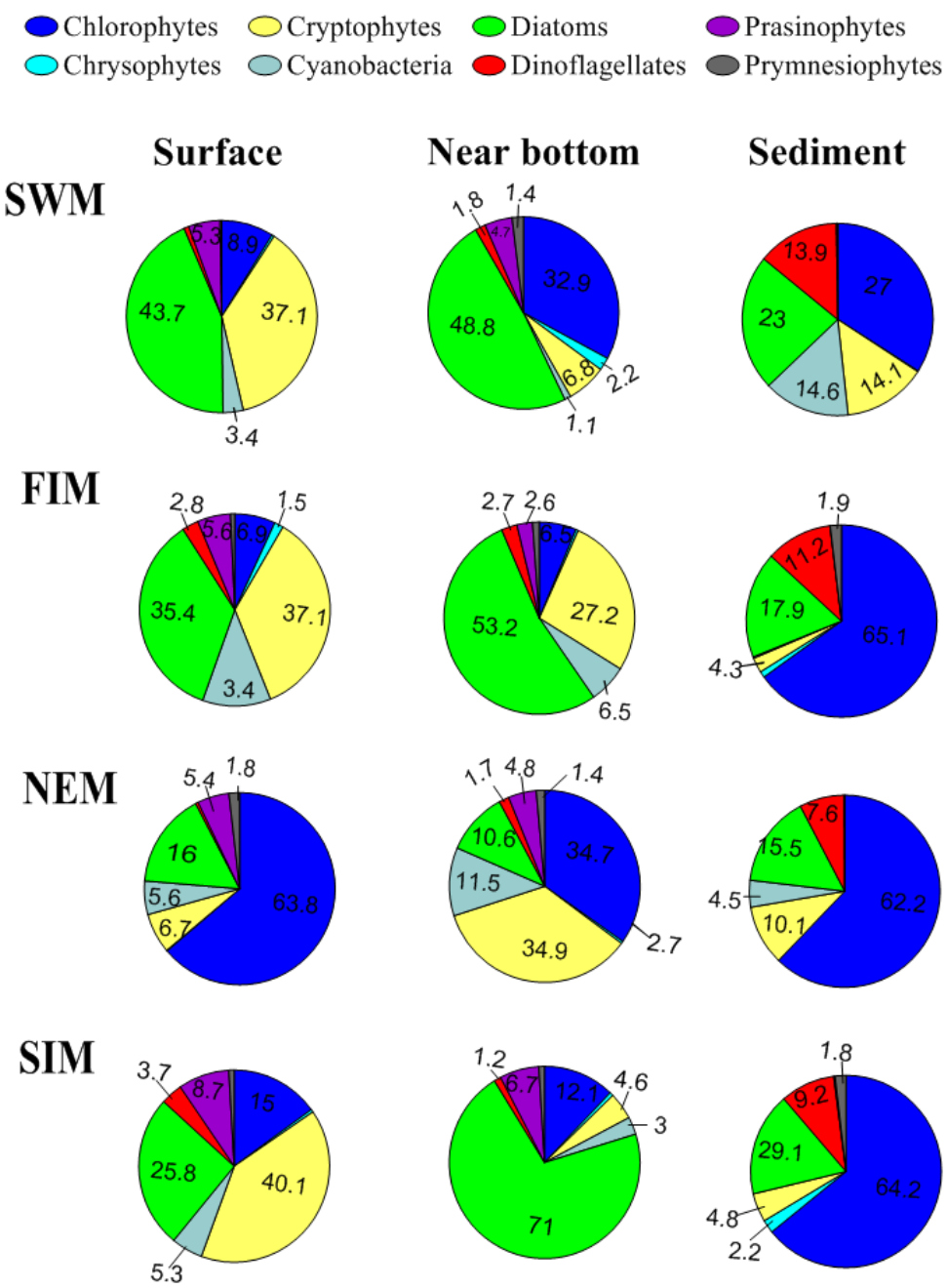

Fig. 5.4 Pie charts showing the seasonal variations in phytoplankton groups derived from CHEMTAX analysis for the overlying waters (surface and near bottom - NBW) and sediments. Average values from the 12 stations were used.

5.3.4 Relationship between environmental parameters and PMP contribution

Redundancy analysis was performed to understand the relationship between environmental variables and group specific PMP's contributions (Fig. 8). Temperature, salinity, PO_4, NO_2 are the major variables significantly influencing the PMP's from the water column and sediment. In surface water biplot, axis 1 and 2 explained 69.1 and 91.8 % variance associated with the marker pigments and environmental variables, respectively. Fuco positioning with the temperature and low salinity, SiO4 and NO_3 suggested that the warmer and low salinity conditions favoured diatoms during inter-monsoons (fall and spring) and SWM by utilizing SiO_4 and NO_3. Chl *b* and 19' Hexa positioned with the high salinity and low temperature conditions suggesting the cooler temperature, and high salinity during NEM favoured chlorophytes and prymnesiophytes. Peri and Allo positioned with the high temperature, Chl *a*, NO_2 suggesting that these conditions during inter-monsoons coincided with the higher dinoflagellates and cryptophytes. Zea and Pras was positioned with the high NO_3, SiO_4 and low PO_4 and NH_3 values and is mainly due to the increased contributions of cyanobacteria and prasinophytes during FIM and NEM.

In NBW biplot, axis 1 and 2 explained 94.4 and 97.4 % variance associates with the marker pigments and environmental variables, respectively. Relationship with the PMP contributions and environmental variables in NBW is different compared to the surface water (except for Fuco). Like surface water, Fuco positioned with the high temperature and Chl *a* concentrations suggesting that the warmer conditions during inter-monsoons favoured phytoplankton biomass, particularly diatoms.. On the other hand high PO_4, NO_2 and NO_3 and low SiO_4 concentrations favoured the next dominant pigment Chl *b* during NEM and SWM. While the third dominant pigment Zea and the least contributed pigment Pras dominance during NEM coincided with high PO_4, NH_3 and DO values and lower temperature and Chl. Positioning of Peri with high SiO_4 and low PO_4, NO_2 and

NO_3 concentrations suggesting their role in favouring dinoflagellates during FIM and NEM while the 19 hexa and Allo showed the insignificant relationship with the environmental variables in NBW.

In sediment biplot, axis 1 and 2 explained 57.3 and 84.7 % variance associates with the marker pigments and environmental variables, respectively. Relationship trend between sediment PMP contributions and environmental variables is different compared to surface water and NBW. The contribution of the most dominant PMP Chl *b* was positioned with the high salinity and SiO_4 and lower NH_3 and DO values indicated their influence on the chlorophytes dominance during inter-monsoons (fall and spring). The increase in Fuco contribution during SWM and NEM seasons could be due the prevalence of high DO, Chl *a*, NH_3 values and lower salinity, Chl *a*, SiO_4, and NO_2 concentrations.. The notable contribution of Allo was coincided with the high temperature and low PO_4, DO and Chl *a* suggesting their role in favouring cryptophytes during SWM and FIM. The Zea and Peri contributions positioned with the environmental settings (high salinity, Chl *a*, NO_2 and PO_4 values and low temperature) coincided with higher cyanobacteria and dinoflagellates during NEM and SIM.

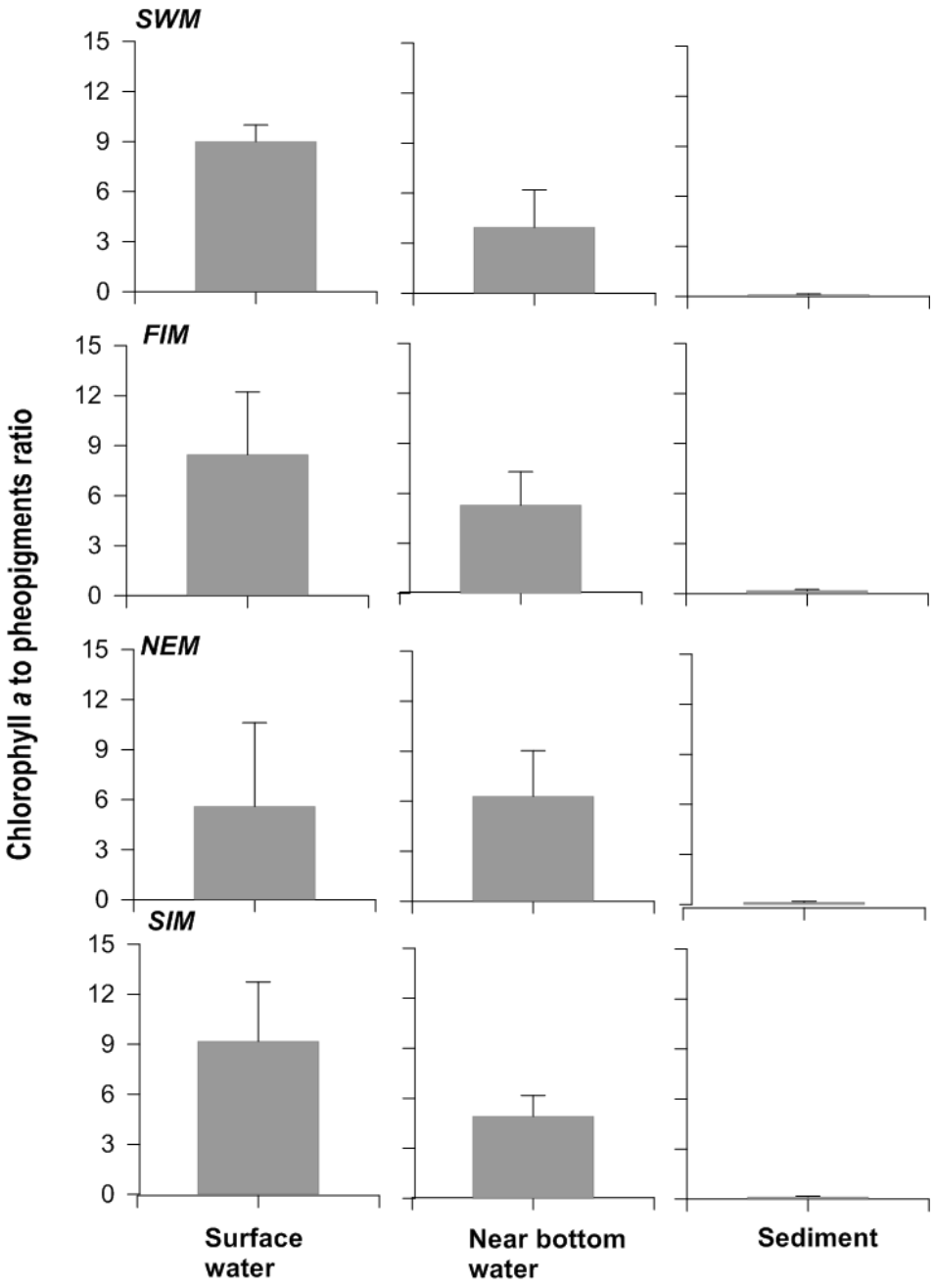

Fig. 5.5 Seasonal variations in the chlorophyll *a* to total pheopigment ratios from the surface and near bottom waters. Bars represent the average values and the standard deviations

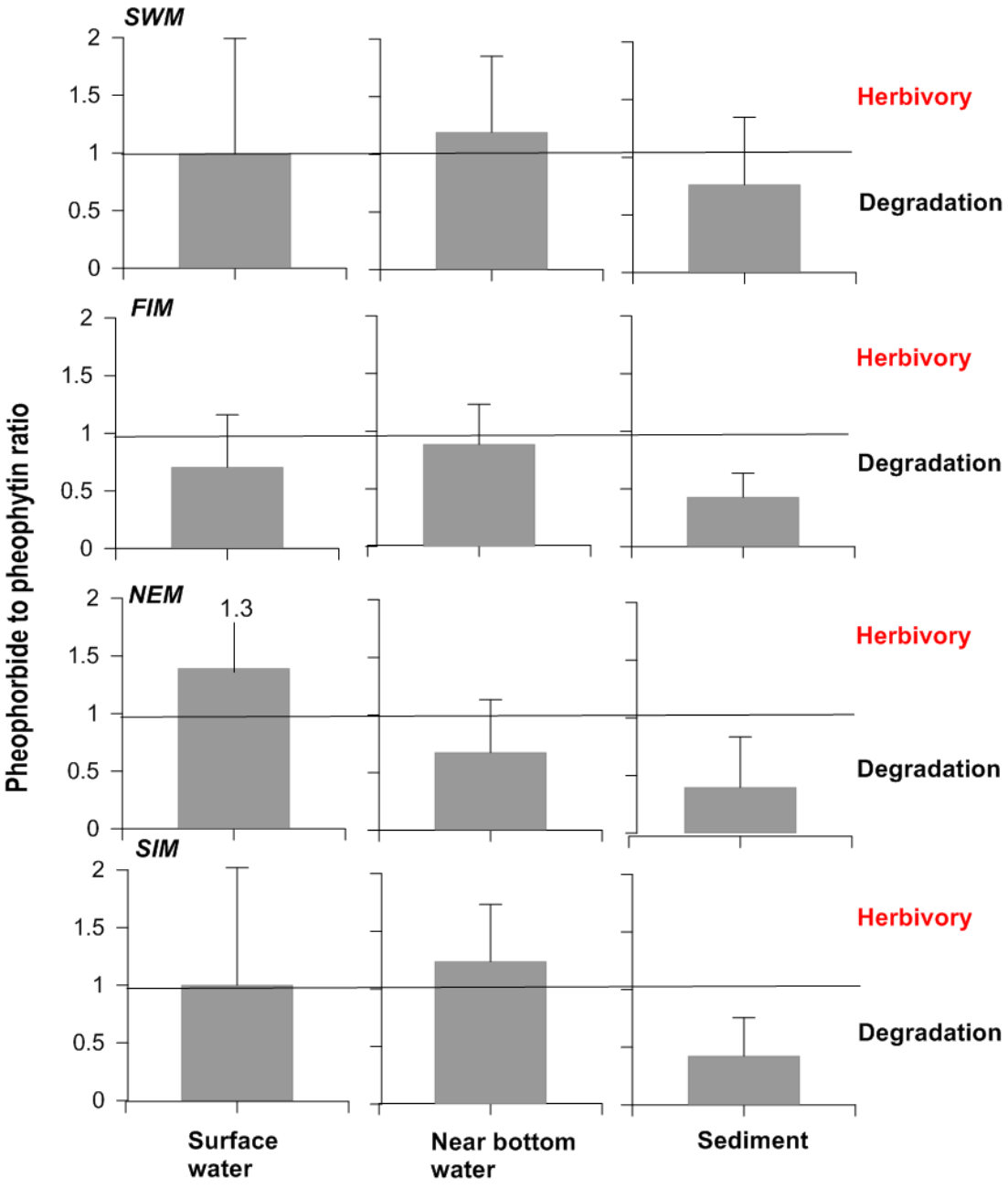

Fig. 5.6 Seasonal variations in the pheophorbide to pheophytin ratios from the surface and near bottom waters. Bars represent the average values and the standard deviations

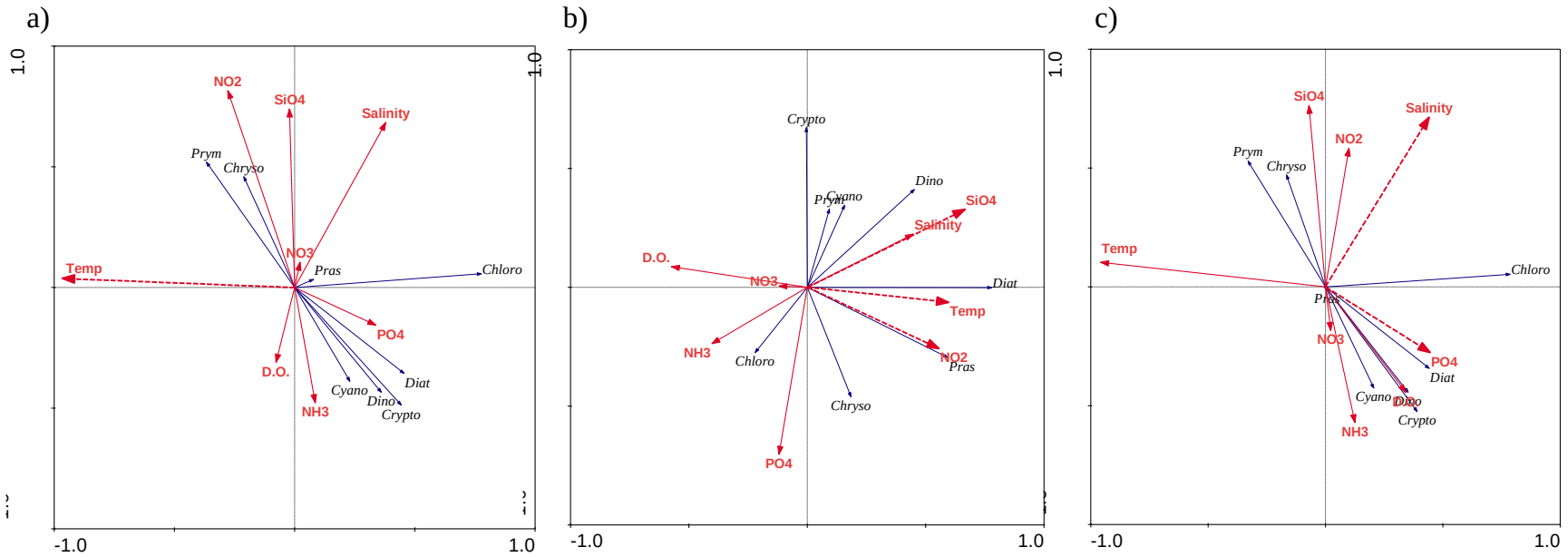

Fig. 5.7 Ordination diagram based on the RDA analysis of PFG's and environmental variables from Paradip port.

5.5 Discussion

The present study elucidates the usefulness of phytoplankton marker pigments (such as PFG's, chlorophyll *a* and pheopigments ratios) to understand the pelagic and benthic phytoplankton linkages from the Paradip port ecosystem. The measured environmental variables showed a distinct seasonal variation and this was also reflected in the distribution of phytoplankton biomass and functional groups (Fig. 5.1 and 5.7). For instance, (i) water temperature followed the classic radiation pattern i.e. warmer and cooler during FIM (August) and NEM (December), respectively (Sarma et al., 2013), (ii) seasonal freshening of surface water (during SWM and SIM), (iii) seasonal influx of high inorganic nutrients during SIM due to the freshwater influx from Mahanadi estuary (Sundaray et al., 2006). The nutrient concentrations were not limited in the study area throughout the year (except phosphate during FIM, Fig. 5.2). Generally, low water movement and current effects from the inner port area of Ceuta port (North Africa) and Tolo Harbour (Hong Kong) explain the port structure negatively influenced by the benthic community (Guerra-Garcia and García-Góme, 2003) and induced algal blooms (Wong and wong., 2004), respectively. Paradip port, which is considered as a semi-enclosed body, also supports this assumption of relatively higher nutrient concentrations and restricted circulations. Previous microscopic studies from Paradip port (Mohapatra and Panda, 2017) as well as from the east coast of India (Gouda and Panigrahy 1989, Gouda and Panigrahy 1996, Madhupratap et al. 2003, Panigrahy et al. 2006, Paul et al. 2008, Naik et al. 2008, Chakraborty et al. 2011, Baliarsingh et al. 2012) suggest that diatom and dinoflagellates are the dominant phytoplankton groups from the water column. However, the information on the smaller or other taxonomic groups of phytoplankton is lacking. In our study, the distribution and dynamics of sedimentary pigments did not match the dynamics of phytoplankton pigments in surface water and NBW. PFG's observed from the sediment could be

the recent settlement of phytoplankton due to the high sedimentation and resuspension (Anand and Sundar., 1990). The chlorophyll *a* distribution indicates that the Paradip port waters are more productive during inter-monsoons (fall and spring) compared to the monsoons (southwest and northeast), but pigment markers from the surface sediment exhibit the reverse trend i.e. high and low during monsoons and inter-monsoons respectively (Fig. 5.2). This could be due to the termination and sedimentation of water column phytoplankton chlorophyll *a* to the sediment towards the end of inter-monsoons. Recent studies from the Paradip port also support this assumption as low and high values of total suspended solids (TSS) observed during SIM (April) and NEM (December), further substantiated by low phytoplankton growth during NEM (Mohapatra and Pandra., 2017).

Further, a distinct reverse trend was observed in the seasonal distribution of chl *a* between water and sediment, suggesting that high sediment chlorophyll observed during monsoons could be due to the deposition of water column chlorophyll occurred during inter-monsoons. However, the contribution of the major phytoplankton groups was different in water-column and sediments. The high concentrations of Fuco, Chl c_2, Diad, Chl *a* with β-car and dominance of Allo concentrations indicates the dominance of diatoms and cryptophytes in the water column, respectively. Additionally, significant concentration of Chl *b* associated with lutein, a typical marker pigments of chlorophytes and prasinophytes (Jeffrey and Vesk., 1997) were also observed, especially high during NEM. The optimum concentrations of detected pigments in the water column such as, Pras, Per and Zea indicate the presence of prasinophytes, dinoflagellates and cyanobacteria (Table 5.1). Marker pigments from the water column agree with the relative contributions of PFG's like diatom, cryptophytes, chlorophytes, cyanobacteria, prasinophytes, and dinoflagellates. However, the concentrations of marker pigments and relative contributions of PFG's varied vertically in the

surface water, NBW, and surface sediments (Table 5.1 and Figs. 5.3 & 5.4). In surface sediment, Fuco, Allo, and Pras concentrations are decreased compared to water column, whereas the leutin, peridinin concentrations increased markedly. Higher Zea and Allo concentrations during monsoons (SWM and NEM) coincided with the higher sediment chlorophyll *a* concentration. The variations in pigment concentrations corresponded to the increase in the relative contributions of chlorophytes and dinoflagellates as well as a decrease in the diatom, cryptophytes, and prasinophytes (Table 1, Figs. 5.3 & 5.4). CHEMTAX derived relative phytoplankton contribution data indicates that the organic input to the sediment was majorly from the chlorophytes followed by diatom, dinoflagellates and cyanobacteria (Fig. 5.4).

The diatom and cryptophytes dominance in the water column was not reflected in the sediment and the possible reasons are delineated in this study. Some recent studies suggested that the nano and picophytoplankton might play a crucial role to fuel organic matters to the benthic community (Chen et al., 2016). The dominance of Chlorophytes in the sediment can be explained by the following (i) selective grazing by zooplankton on diatom and other groups decreases the percentage contribution (ii) much of the settled diatom and cryptophytes could be grazed by benthic invertebrates (iii) marker pigments of diatom and cryptophytes (Fuco, decay rate constant (k)= 15 to 26 year^{-1} and Allo, k = 0.0001 ±0.0011) are least stable than marker pigments of chlorophytes (Chl *b*, k = -0.001 ± -0.005 and leutin, k = -0.0003 ± -0.0064; Leavitt and Hodgson 2001, Bianchi et al., 2000, Schüller et al., 2015). Additionally, several studies also reported the influence of microphytobenthos on the phytoplankton group contributions from the shallow coastal benthic ecosystems (Macintyre et al., 1996, Hardison et al., 2013, Semcheski et al., 2016). In this study, even though Paradip port is a shallow coastal water (depth 6.3 to 21.1m, Fig. 5.3), the influence of microphytobenthos may not influence the outcome of this study significantly as the biomass distribution showed distinct

variations with respect to season as well as between water and also the contribution of active microalgae in sediment is negligible compared to overlying waters as evidence from chl *a* to total pheopigment ratios (Fig. 5.5). Aquatic ecosystems undergoe several losses (mortality) processes (e.g., grazing, sedimentation, cell senescence, and viral lysis), and therefore, it is important to determine the fate of phytoplankton from the sediment and overlying water column. In such cases, phytoplankton marker pigments (PMP) and CDP's can be used to understand the chlorophyll breakdown (ChlB) pathway, which will lead to a better understanding of the phytoplankton benthic-pelagic linkage from the given ecosystem. In the ChlB pathway, pheophorbide formation is an important step, formed either via chlorophyllide or pheophytin. Laboratory experiments and some previous studies reported that the initial ChlB-pathway induced by grazing and senescence are not the same, i.e., chlorophyll-chlorophyllide-pheophorbide (CCP) and chlorophyll-pheophytin-pheophorbide (CPP) in the former and latter case, respectively (Satoh and Hama, 2013, Hu et al., 2013, Sathish et al., 2020). In such cases, Pheophorbide to pheophytin (Pheid: Phe) ratios can be used to determine the dominant chlorophyll breakdown pathway and the fate of phytoplankton in the given ecosystem, i.e., herbivory dominant (>1, CCP pathway) or degradation dominant (<1, CPP pathway) as explained in Chapter 4. In this study, CDP's pool is mainly derived by the pheophytin and pheophorbide, which can be attributed to the presence of CCP or CPP pathway, even with the absence of chlorophyllide.

Degradation rate vary abundantly among the marker pigments (Leavitt and Hodgson 2001, Schüller et al., 2015) and between the different habitats (Hurley & Armstrong, 1990). Pigment degradation in the water column is rapid compared to the sediment (Leavitt and Hodgson 2001, and references therein). In different habitats (i.e. fresh and marine water) pigments degradation mainly depend on the photo oxidation, selective grazing, senescence, sinking rate (different species of living and dead

cells) and microbial decay, cell lysis and enzymatic metabolism during senescence and secondly at the sediment-water interface (Louda et al., 1998, Reuss and Conley. 2005, Fietz et al., 2005 and reference within). Subsequently, phytoplankton chlorophyll (cells and detritus matters) settled on the surface sediment, degrade more rapid (Bianchi et al., 2000), and less rapid (Hurley & Armstrong, 1990, Yacobi et al., 1991) during oxic and anoxic conditions, respectively. Further, this is depending on the life strategies of phytoplankton, burrowing invertebrates, and physical condition of water (Leavitt and Carpenter, 1989, Yacobi and Ostrovsky, 2012). Higher NBW, oxic conditions (4.0 to 4.9 µg l^{-1}) and increasing Chlorophyll degradation products from the surface sediment obtained in the present study support this statement. Conversely, degradation of carotenoids highly depends on the chemical structure, the number of oxygen atoms, and the presence of 5,6-epoxy groups (Schüller et al., 2015, and references within). Previous literatures suggesting that the Chlorophyllide *a* shows the lowest decay constant (K^a) of −0.007 ± -0.007 $year^{-1}$ followed by chlorophyll *a* (-0.004 ± -0.003 $year^{-1}$) pheophorbide *a* (-0.003 ± -0.001 $year^{-1}$) and pheophytin *a* (-0. 003 ± -0.003 $year^{-1}$, Schüller et al., 2015). Hence chlorophyll and pheopigments ratios can be a good indicator to understand the fate of phytoplankton.

The present study indicates that the Pheid: Phe ratio did not exhibit significant variations between the seasons, but the variations between the water column and sediments were apparent. Low and high Pheid : Phe ratios in the sediment (0.40 to 0.76) and water column (0.67 to 1.39) suggested the dominance of cell senescence (suggesting CPP pathway due to high pheophytin concentrations) and cell destruction due to herbivory or grazing (suggesting CCP pathway due to high pheophorbide concentrations), respectively (Fig. 5.6). The ratios indicate active grazing in the water column compared to sediment, which could be the reason for the lower contribution of diatom and cryptophytes from the sediment (Fig. 5.6). Chlorophyll : TPhe (total pheopigments),

an indicator of the freshness of organic matter deposited in the benthos (Moratta et al. 2011) or actively growing community (Grippo et al., 2009, Allison et al., 2013), did not reveal any significant difference between the season. Higher Chlorophyll: TPhe values in the surface water and NBW (3.95 to 9.17) compared to surface sediment (0.11 to 0.17), indicating the presence of actively growing microalgal community in the water column and the organic input to the sediment was not fresh (Fig. 5.5). Taking these findings into consideration, it is assumed that (i) much of the phytoplankton (mostly diatoms) is lost due to herbivory before reaching bottom sediments, and (ii) selective benthic grazing and PMP decay constants determine pigment contribution. These findings suggest that the knowledge on phytoplankton marker pigments in combination with the chlorophyll degradation products from the benthic and pelagic environment will give a better understanding of the linkages between pelagic and benthic phytoplankton, which will be useful in ecosystem assessment and algal bloom research.

5.5 Conclusions

This study concludes that the distribution of chlorophyll and PMP (for different groups and degradation) from Paradip port revealed a distinct seasonality, but the seasonal trends between water and sediment are not the same. Pheophorbide to pheophytin ratio indicated the dominance of herbivory in the water column compared to sediment. The contribution of actively growing microalgae was high in the water-column but negligible in sediment, suggesting organic input was not fresh. It is assumed that (i) much of phytoplankton is lost due to herbivory in the water column before reaching bottom sediments, and (ii) selective benthic grazing and PMP decay constants determine pigment contribution. Documenting the phytoplankton (biomass and composition), chlorophyll breakdown products and key environmental variables will be usefulin ecosystem assessment and algal bloom research.

Chapter 6

Summary

6. Summary

Phytoplankton are the key primary producers, which play a crucial role in food web dynamics and nutrient cycling. Hence, monitoring phytoplankton and its functional groups has become a high priority area of aquatic research. Port ecosystems are highly vulnerable to anthropogenic activities, bioinvasions, and harmful algal blooms (HAB). The effect of these ecological stressors needs the maximum attention as it can affect the port ecosystem and nearby water bodies. Phytoplankton pigments can easily be studied to understand the phytoplankton groups through the accessory pigments. Given this phytoplankton marker pigments were evaluated from the samples collected from several stations (10 to 30) during 4 different occasions representing different seasons from the seven ports and Zuari estuary representing diverse ecosystems such as freshwater (Kolkata and Haldia), estuary (Cochin and Zuary), marine (Kandla, Mangalore, Chennai, and V.O.C) port ecosystems. This study revealed distinct spatial (between and within ports) and seasonal variations in environment and phytoplankton marker pigments. Pigment-based chemotaxonomy analysis revealed that among the river-influenced ports, eight phytoplankton groups (chlorophytes, chrysophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, prasinophytes, and prymnesiophytes) were present in Kolkata, Haldia, Zuari estuary, and Kochi (except cryptophytes and chrysophytes). In Estuarine ports, environmental parameters showing an insignificant relationship with PFG's distribution. Diatoms and chlorophytes were the dominant groups in river-influenced port ecosystems. Among the freshwater ports in Kolkata, all the environmental variables (except salinity and SiO_4) are showed a distinct relationship with environmental parameters, whereas, in Haldia port, environmental variables such as DO, temperature, salinity, and NO_2 showed a significant relationship with PFG's distributions. Among marine ports, Kandla and New Mangalore ports

(both located along the west coast of India), all the groups (except chrysophytes) were present during all the seasons. Salinity, PO_4, and NO_3 are the major environmental variables influencing PFG's distributions in west coast marine ports. However, in the marine ports (V.O.Chidambaranar, Chennai, and Paradip) located along the east coast of India, dominant groups were diatoms, chlorophytes, and cryptophytes. Salinity, temperature, DO, PO_4, SiO_4 and NO_3 are the major factors influencing PFG's distributions in east coast marine ports. The prevalence of high abundance of harmful dinoflagellates (*Tripos furca* and Dinophysis caudate; in Chennai), prasinophytes (in Paradip), and cyanobacteria (in Tuticorin) in specific ports were also evident. Interestingly, higher dinoflagellate contributions coincided with the lower salinity in all the ecosystems. It is proposed that the baseline data generated will have implications in the studies relevant to ecosystem assessment and management.

The roles of biotic factors (grazing/senescence) in chlorophyll *a* (chl *a*) breakdown (ChlB) to colorless derivatives in a multistep-pathway are frequently reported. In aquatic microalgae, the information on CHLB-pathway is scarce compared to land plants. The enzymatic activities (e.g., chlorophyllase–dephytalation; pheophytinase–demetalation) involved in CHLB vary significantly among phytoplankton species, but how this influences the CHLB-pathway is unknown and delineating the same will aid in determining the fate of high microalgal biomass, particularly blooms. The grazing experiments using three species of bloom-forming marine phytoplankton (*Skeletonema costatum* (Bacillariophyceae), *Dunaliella tertiolecta* (Chlorophyceae), and *Amphidinium carterae* (Dinophyceae)) and mixed natural assemblage confirmed that the CHLB-pathway under grazing-pressure is species-specific and the grazer's feeding habits. In *Dunaliella* and *Amphidinium*, with and without chlorophyllase-activity, respectively, higher pheophytin and lower pheophorbide formation suggested the chl *a*–

pheophytin–pheophorbide pathway. *Skeletonema* with high chlorophyllase activity produced higher pheophorbide suggesting CHLB through the chl *a*-chlorophyllide–pheophorbide pathway. In the presence of grazers, the pheophorbide: pheophytin ratio was several times higher in *Skeletonema* (3-40) than others (0.1-<1), suggesting a potential proxy for determining chl *a* -chlorophyllide–pheophorbide (>1) or chl *a*–pheophytin–pheophorbide pathway (<1) dominance. This study highlights that incorporating such CHLB- pathway indicator to determine bloom fate will be a step ahead in the microalgal bloom research and ecosystem assessment.

So far, much of the information on chlorophyll-breakdown is available from sediments, but data from the water column is limited. Chapter 3 addressed CHLB pathway on a seasonal basis from eight major ports (18-30 stations/port) representing freshwater, estuarine, and marine ecosystems. The distribution of chlorophyll and its breakdown fractions (pheophytin, pheophorbide) exhibited distinct spatial and seasonal variations. Fresh-water (except Haldia-port) and estuarine ports are characterized by high-biomass, high-pheophytin, and low-pheophorbide, whereas marine-ports by low-biomass (except Mangalore-port), low-pheophytin, and high-pheophorbide. Pheophytin and pheophorbide distribution were biomass independent and dependent, respectively. The pheophorbide: pheophytin ratio indicated a potential proxy for determining the dominant breakdown pathway, i.e., herbivory dominant (>1) or not dominant (<1). However, CHl-BP is taxa-specific and grazer's feeding habits. The ratios exhibited apparent differences between different ecosystems, i.e., the higher ratios in marine (up to 11.2) followed by estuarine (up to 0.9) and freshwater (up to 0.4; except Haldia) systems. The diatoms (preferred grazer diet) contribution to total phytoplankton was more in marine followed by estuarine and freshwater systems. The low and high ratios suggested the prevalence of

chlorophyll-breakdown via senescence and grazing mode, respectively. In view of these studies, it is proposed that such scaling will have implications in the management of ships ballast water (ballast tank conditions (eg. dark) during voyages, post-voyage discharge – including treated water using approved BWM systems, and the nature of ports, potential discharge point), which is considered as major vector for translocation of the non-native species and marine bioinvasions as well as in the understanding of bloom fate and in bloom control measures.

Assessing the dynamics of phytoplankton marker pigments (PMP; for biomass and functional groups) and the ratios (indicating freshness and fate) from the sediments and overlying waters in an aquatic ecosystem will improve the understanding of phytoplankton pelagic-benthic linkages. Given this, the seasonal distribution of PMP from water and surface sediments was evaluated from the coastal port ecosystem (Paradip port, Odisha, east coast of India) as a case study. The results indicated a distinct seasonality in biomass (chlorophyll) and PMP (for different groups and degradation) distribution, but the seasonal trend was different for water and sediments. In the water-column, high and low values were observed during inter-monsoons (fall and spring) and monsoons (south-west and north-east), respectively, whereas a reverse trend was recorded in sediments. High sediment chlorophyll observed during monsoons could be due to the deposition of water-column chlorophyll during inter-monsoons. However, the major phytoplankton groups' contribution was different: in the water-column diatoms followed by flagellates, cyanobacteria and dinoflagellate were the dominant groups whereas, in sediment, flagellates followed by cyanobacteria, diatoms, dinoflagellates were dominant. Selective grazing (in water column and sediments) and stability of benthic PMP (marker pigments for diatoms and cryptophytes are least stable than other major groups) could be the possible reasons for such differences. High (>1) and low (<1) ratios of chlorophyll:pheopigment and

pheophorbide: pheophytin in the water column and sediments, respectively, indicated the dominance of actively growing microalgae and herbivory in the former but not in the latter. Given these findings, it is assumed that (i) much of the phytoplankton (mostly diatoms) is lost due to herbivory before reaching bottom sediments, and (ii) selective benthic grazing and PMP decay constants determine pigment contribution. This study of PMP from different ecosystems revealed that documenting the phytoplankton (biomass and composition), chlorophyll breakdown products and key environmental variables will be useful in ecosystem assessment, ballast water management, benthic-pelagic linkage, and algal bloom research (fate and bloom control measures).

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Publications

List of publications:

- **Sathish, K**., Patil, J.S., Anil, A.C., 2020. Phytoplankton chlorophyll breakdown pathway: implication in ecosystem assessment. Journal of Environmental Management, 258 (2020) 109989

- **Sathish K**., Patil, J.S. and Anil, A.C., 2022. Benthic-pelagic coupling assessed using phytoplankton marker pigments: a case study from the Paradip port, East Coast of India. Environmental Science and Pollution Research, pp.1-18.

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- Received ICBAB Best Student Paper Award for the year 2021. ICBAB award was constitute by the organizers of the International Conference on Biofouling and Ballast Water Management (ICBAB), which was held in 2008 to the student community of CSIR-NIO, Goa.

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Research article

Phytoplankton chlorophyll-breakdown pathway: Implication in ecosystem assessment

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ABSTRACT

The chlorophyll-breakdown to pheophorbide is determined by biotic factors such as grazing (via chlorophyllide) or senescence (via pheophytin). So far, much of the information on chlorophyll-breakdown is available from sediments, but information from the water column is limited. This study addressed chlorophyll-breakdown-pathways (Chl-BP) on a seasonal basis from eight major ports (18–30 stations/port) representing freshwater, estuarine, and marine ecosystems. The distribution of chlorophyll and its breakdown fractions (pheophytin, pheophorbide) exhibited distinct spatial and seasonal variations. Fresh-water (except Haldia-port) and estuarine ports are characterized by high-biomass, high-pheophytin, and low-pheophorbide, whereas marine-ports by low-biomass (except Mangalore-port), low-pheophytin, and high-pheophorbide. Pheophytin and pheophorbide distribution were biomass independent and dependent, respectively. The pheophorbide: pheophytin ratio indicated a potential proxy for determining the dominant breakdown pathway, i.e., herbivory dominant (>1) or not dominant (<1). However, CHl-BP is taxa-specific and grazer's feeding habits. The ratios exhibited apparent differences between different ecosystems, i.e., the higher ratios in marine (up to 11.2) followed by estuarine (up to 0.9) and freshwater (up to 0.4; except Haldia) systems. The diatoms (preferred grazer diet) contribution to total phytoplankton was more in marine followed by estuarine and freshwater systems. The low and high ratios suggested the prevalence of chlorophyll-breakdown via senescence and grazing mode, respectively. We proposed that such scaling will have implications in the ballast water management – BWM (ballast tank conditions (eg. dark) during voyages, post-voyage discharge – including treated water using approved BWM systems, and the nature of ports, potential discharge point) and algal bloom research (e.g. understanding fate and in control measures).

1. Introduction

Phytoplankton, which are unicellular/filamentous and autotrophic micro-organisms, comprised of diverse algal groups (diatoms, dinoflagellates, phytoflagellates, coccolithophorids, red algae, green algae, and cyanobacteria) with sizes ranging from 0.2 μm to several millimeters. Phytoplankton are significant contributor to global carbon fluxes (Falkowski et al., 1998) and also influential determinants of tropical interaction in the marine ecosystem (Tester et al., 1995). However, in an aquatic ecosystem, phytoplankton undergoes several loss processes such as grazing, program cell death, viral lysis, sinking, etc.(e.g., Choi et al., 2017). In such cases, phytoplankton marker pigments (PMP), in particular, chlorophyll *a* (chl *a*) and its derivatives, which reflect the phytoplankton production and its pathway in the water column and sediments are considered as useful indicators. Chl *a* and pheopigments are commonly used as indicators of relatively fresh and degraded microalgal communities, respectively (Boon and Duineveld, 1998; Wieking and Kröncke, 2005; Pusceddu et al., 2009). Processes such as growth, grazing, cell sinking and senescence, photodegradation, fecal pellet sinking, physical mixing, and advective transport are known to affect chl *a* and pheopigments concentrations in the euphotic zone. However, different pathways of pigment degradation affect the integrity of chlorophyll, i.e., Mg-phorbin ring, the phytol chain, or both, and result in different degradation products such as pheophorbide (Mg-free and phytol-free chlorophyll), chlorophyllide (phytol-free chlorophyll), and pheophytin (Mg-free chlorophyll) (Fig. 1; Morata et al., 2011). The removal of the central magnesium atom and phytol chain from chl *a* are the early steps in chl *a* breakdown for all chlorophyll-containing organisms (Satoh and Hama, 2013; Hu et al., 2013; Kuai et al., 2018 and references therein). To the best of our knowledge, more in-depth

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information on chl *a* breakdown is available for leaf senescence in land plants in comparison to the situation for algae (Scheer, 2012). Additionally, laboratory experiments on relationship between grazing pressure and pheopigment production (Klein et al., 1986; Penry and Frost, 1991; Gieskes et al., 1991; Head and Harris, 1996; Penry and Frost, 1991; Pandolfini et al., 2000; Goericke et al., 2000) have shown that the presence of colourless products such as 132, 173–cyclopheophorbide *a* enol (CPP516), while pheopigments productions with grazers were recorded little to none. However, research in this area is in infancy. Nevertheless, the rates of these processes are of broad general interest, and knowledge of them is fundamental to understanding the causative factors underlying the dynamics of planktonic ecosystems.

Several studies have shown that pheophorbide and pheophytin as the primary chl *a* degradation products (CDP) from grazing in the water column (Carpenter et al., 1986; Spooner et al., 1994; Strom, 1993), sediment (Abele-Oeschger and Theede, 1991; Bianchi et al., 1988; Buffan-Dubau et al., 1996; Cartaxana et al., 2003; Coelho et al., 2011), intertidal region (Brotas and Plante-Cuny, 1998; Lucas and Holligan, 1999; Cartaxana et al., 2003) and Ice algae (Morata et al., 2011). At the same time, few reports also raised doubts over the use of pheopigments as a relevant indicator of herbivorous activities (Head and Harris, 1994; Villanueva and Hastings, 2000; Ford and Honeywill, 2002). Ford and Honeywill (2002) suggested that any use of pheophorbide as a marker pigment for grazing in surface intertidal sediments in the future should be tested (especially for biological reworking and deep oxygen diffusion) before use. To our knowledge, much of the information on CDP has come from sediments, but from the water column, information is limited.

Nevertheless, the presence of CDP in the water indicates the presence of zooplankton activity or cellular senescence (Bidigare et al., 1986; Coupel et al., 2015). Since there exists distinct spatial and temporal variability in biological production within or between ecosystems, it is possible that the chlorophyll breakdown pathway will also exhibit distinct variability. For example, chlorophyll breakdown into pheophorbide can be either via chlorophyllide or pheophytin. Laboratory experiments confirmed that under grazing pressure, chlorophyll breakdown pathway (i.e., via chlorophyllide or pheophytin) is taxa-specific and grazers feeding habitat (unpublished data). India has a vast coastline with different ecosystem types (e.g., freshwater, estuaries, coastal) and so with the environmental settings in each of the ecosystems. The influence of reversible monsoons (SWM – both east and west coast and NEM – east coast), high freshwater discharges on the east-coast compared to the west-coast, different coastal morphology, and variable anthropogenic pressures are some of the events determining the environment setting in a given ecosystem along the Indian coasts. Therefore, it has been hypothesized that the phytoplankton will have a specific distribution pattern for different ecosystems, and therefore indicating fate pathways. Evaluating biomass, composition, and CDP

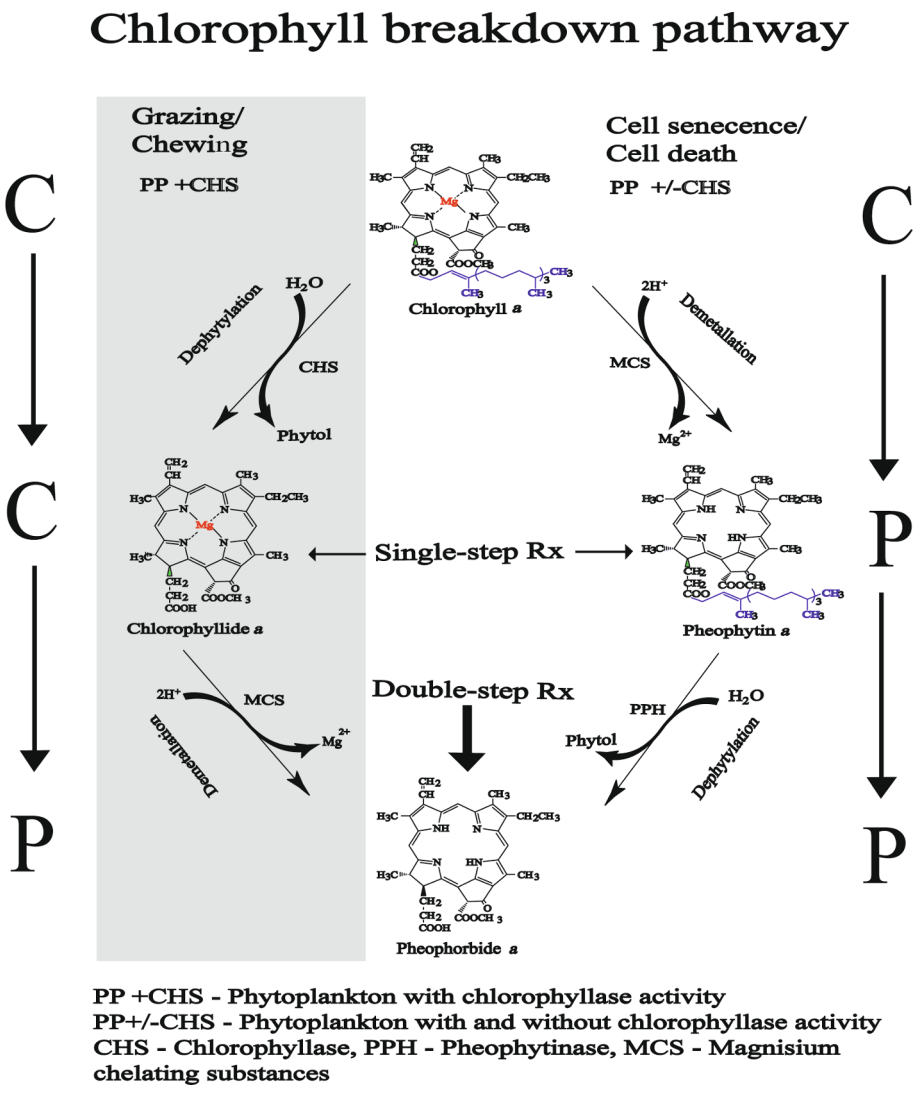

Fig. 1. Schematic illustrating the chlorophyll-breakdown pathway.

together will provide insights into the processes involved in growth and mortality. Given this, under the Ballast Water Management Program-India, the distribution patterns of the biomass and CDP from eight-port ecosystems (categorized into freshwater (FPE), estuarine (EPE) and marine (MPE) port ecosystems) located along east and west coast of India are discussed from the perspective of loss processes. Further, the field data generated will have implications for ecosystem assessment and management.

The discharge of water from ships' ballast water tanks is widely considered as the most critical vector for "bioinvasion" i.e. unintentional translocation of nonindigenous organisms from diverse taxonomic groups (such as microbes, flora, and fauna) across their bioregions. In ballast water management (BWM), we assume that the pheophorbide: pheophytin ratio will be useful for assessment of the (i) nature of the environment in the ballast water discharge point (e.g. ports), and (ii) the ballast water sampling. Generally, ports are at the receiving end for ships ballast water, and therefore the port environment determines the fate of the introduced organisms. For instance, the introduced organisms survive/proliferate and do not under a conducive and non-conducive environment respectively. Irrespective of the climatic zones, the geographic location (riverine, estuarine, and marine) and the seasons (wet and dry seasons) determined the port environment and was evident in this study. Therefore, the biological production (e.g. plankton biomass and production) or occurrences of blooms vary between the ports and the seasons and so with its fate. Given this, the chlorophyll and its breakdown products (e.g. pheophytin, pheophorbide) and ratios can be useful ecological indicators for determining the fate of phytoplankton, including introduced viable phytoplankton and blooms) during productive and non-productive conditions (Morata et al., 2011; Grippo et al., 2009; Allison et al., 2013; Satish et al. submitted).

2. Materials and methods

2.1. Study area and sample collection

Water samples (surface and sub-surface) were collected from 8 ports located at different locations along the east and west coast of India. The selected ports are listed among the major ports of India as they handled greater proportion of all cargo traffic. The Indian coast is influenced by monsoon reversals, a varying magnitude of freshwater influx (from major and minor rivers), and anthropogenic pressures (in particular ports). Therefore, the ports along the Indian coast represent different ecosystems. Among the eight ports, two (Kolkata and Haldia), one (Cochin), and five ports (Kandla, Mangalore, Paradip, Chennai, and Tuticorin) are identified as freshwater, estuarine and marine port ecosystems respectively. In each port, samples were collected from 12 to 25 stations on different occasions representing different seasons (Fig. 2, Table 1). Surface and sub-surface (1 m above the bottom) water samples were collected using Niskin bottles (5 l) along with the other environmental parameters such as temperature, salinity, dissolved oxygen, biological oxygen demand, phytoplankton pigments and inorganic nutrients (nitrate, nitrite, ammonia, phosphate, and silicate). The temperature was determined *in situ* using a multiparameter Sonde DS5X (Hydrolab). Salinity was measured with an Autosal (Guildline Autosal 8400B). Dissolved inorganic nutrients such as nitrate (NO_3), phosphate (PO_4), nitrite (NO_2), ammonium (NH_4), and silicate (SiO_4) were analyzed by SKALAR SANplus ANALYSER. Dissolved oxygen (DO) and biological oxygen demand (BOD) were analyzed following standard

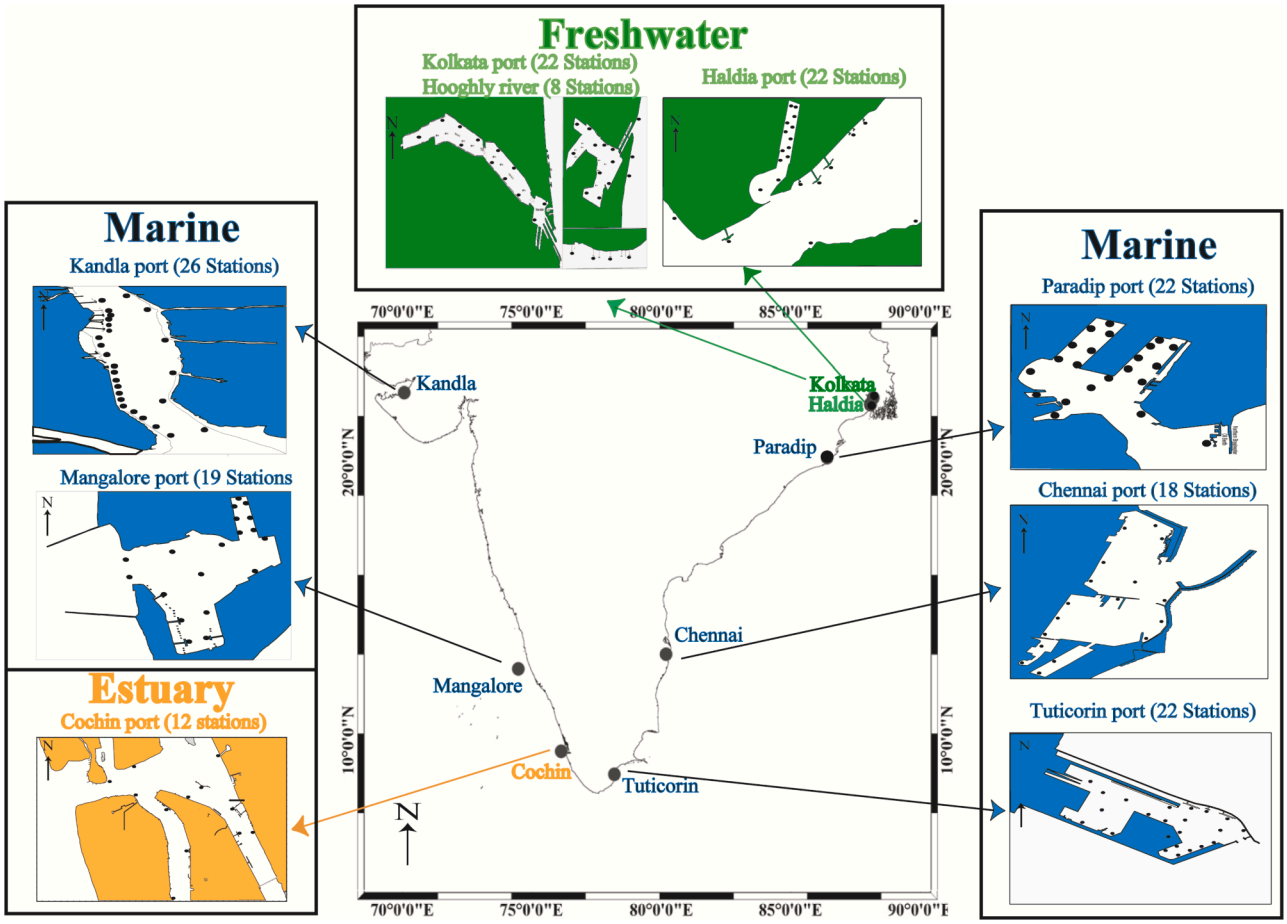

Fig. 2. Geographical location of the eight ports (and the sampling stations within the harbor) in different ecosystems (marine, freshwater and estuary) along the Indian Coast.

Table 1
Details of sampling stations (numbers and location), sampling period, water temperature and salinity.

Stations	No.Stations	Latitude	Longitude	Sampling period	Temperature	Salinity
Cochin	12	9.58	76.14	October 2011, May, August & November 2012	29.2 ± 1.2	17.9 ± 8.5
Kolkata	22	22.32	88.18	September 2013, Febraury 2014 January & December 2015	25.5 ±5.0	0.3± 0.02
Hooghly	8	22.33	88.17	September 2013, Febraury 2014 January & December 2015	23.5 ±5.7	0.3 ± 0.05
Haldia	22	22.04	88.08	October 2013, Febraury & September 2014, April 2015	27.5 ± 0.7	4.4 ± 2.9
Mangalore	22	12.57	74.48	November 2011, May, September & December 2012	28.1 ± 1.8	35.2 ±0.6
Kandla	26	23.01	70.13	October 2014,July & October 2015, Febraury 2016	26.5 ± 0.5	35.9 ±2.7
Paradip	25	20.15	86.4	August & December 2014, May & August 2015	28.1 ± 0.3	30.1 ±3.2
Chennai	18	13.08	80.18	June & October 2012, January & April 2013	29.8 ± 3.2	32.1 ±2.7
Tuticorin	25	8.47	78.12	July, October, & December 2012, March 2013	28.8 ± 0.9	34.8 ± 1.2

methods (Parsons et al., 1984). For the pigment analysis, 250–500 ml of water sample were passed through GF/F filter (27 mm; pore size 0.7 mm) and stored in at −80 °C until analysis using high-performance liquid chromatography (HPLC).

2.2. Phytoplankton pigment analysis using HPLC

The collected pigment samples were extracted by adding 3 ml of 90% acetone (V: V with the deionized water) to the frozen filter paper, which was sonicated using a ultrasonic probe (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) for 10 s at 20 kHz and kept on ice to prevent excessive heating. The extracts were then stored overnight at −20 °C for analysis using HPLC (Agilent Technology; Series 1200). Before analysis, all the extracts were filtered (Nylon filter membranes, 0.2 μm pore size) immediately before injection in the HPLC to remove cells and debris from sonicated filter paper. To prevent photolysis of the samples, extraction and injection procedures were performed under dim light. Pigment analysis was performed following the method of Van Heukelem (2002) with a slight modification. The pigment extracts were injected into the HPLC system equipped with an Agilent 1200 series pump; an Agilent diode array detector connected using a reverse-phase C8 Column maintained at 60 °C (150 × 4.6 mm, 3.5 mm particle size) with 3 phase linear solvent gradient program at a flow rate of 1.1 ml/min. The absorption of the extracted pigments were detected at 450 and 665 nm. The quantitative and qualitative analysis of about 20 pigments, including the chlorophyll degradation products was carried out using the commercially available pigment standards purchased from DHI Inc (Denmark) and Sigma–Aldrich (USA). However, for this study, only the total chlorophylls and two degradation pigments (pheophytin and pheophorbide) were considered. Chlorophyllide, another degradation pigment, was not detected in any of the samples analyzed. Additionally, following pigment ratios Chl *a*: Pheophytin, Chl *a*: Pheophorbide, pheophorbide: pheophytin were also considered for better interpretation. Further, the data on chlorophyll, pheopigments, and pheophorbide: pheophytin ratios were subjected to clustering (using Bray Curtis similarity and average group method) and non-metric multidimensional scaling (NMDS) to understand the ecosystem wise distribution. Before analysis, data were not transformed. Clustering and NMDS were performed using Primer version 6 software.

3. Results

3.1. Environmental parameters

The measured environmental parameters showed distinct seasonal variations in all the studied ports. However, the seasonal patterns varied according to the geographical location of the ports. Water temperatures in the ports are relatively warmer and cooler during southwest monsoon (SWM) and northeast (east coast) or post (west coast) monsoon, respectively. The averaged salinity variations were negligible (Kolkata: < 0.4 round the year and Haldia: 0.6 to 7.8), marginal (25–35) and large (5.1–26) in freshwater, marine and estuarine ports (Cochin) respectively. The low salinity observed in marine and estuarine ports were due to freshwater influx during monsoons. Interestingly, the freshwater and estuarine ports systems are nutrient (NO_3, PO_4, and SiO_4) rich compared to marine ports. However, the magnitude varies between the ports. For instance, among the nutrient-rich freshwater influenced ports the magnitude of nutrient concentrations was higher for Kolkata and Haldia ports compared to Cochin port (Table 2). Among the marine ports nutrients showed a distinct seasonal variability, but the seasonal patterns varied between the ports located on east (Tuticorin, Chennai, and Paradip) and west coast (Kandla and Mangalore) of India.

3.2. Phytoplankton biomass (chl a) and pheopigments (pheophytin and pheophorbide)

Results indicated that the chl *a* and degraded pigments showed significant seasonal variations in each of the ecosystems (except Haldia and Kandla due to the prevalence of low chl *a* in all the seasons). However, the seasonality pattern was different between the ecosystem, and this was true for chl *a* and pheopigments (Figs. 3 and 4). The detailed results for each of the ecosystems are provided below.

3.2.1. Biomass

Results revealed higher biomass in freshwater (except Haldia) and estuarine ports compared to marine ports. In each port (except Haldia and Kandla due to the prevalence of low chl *a* in all the seasons), biomass showed significant seasonal variations. However, the seasonality pattern for chl *a* was different between the port ecosystems (Figs. 3 and 4). Among freshwater port ecosystems, the highest (up to 40.8 μg l^{-1}) and lowest (0.5–1.8 μg l^{-1}) concentration of chl *a* are observed in Kolkata–port (including Hooghly riverine stations) and Haldia-port respectively. In Kolkata, high chl *a* concentration was observed in the stations located in Hooghly River (up to 40.8 μg l^{-1}) compared to enclosed port stations (up to 20.3 μg l^{-1}). In the estuarine port (Cochin) chl *a* remained high (11.97–18.37 μg l^{-1}) round the year, and the high and low chl *a* was observed during SWM and PRM season respectively. Among marine ports, lowest (<1 μg l^{-1}) and highest (up to 25 μg l^{-1}) chl *a* were observed in Kandla and Mangalore ports respectively, whereas, in the rest of the ports, chl *a* was variable, i.e., high in only one or two seasons. Clustering and non-metric MDS at 60% similarity also revealed two groups and three ungrouped individual ports. The ports characterized by low (Haldia and Kandla) and high biomass (Mangalore, Kolkata/Hooghly, and Cochin) for most of the year formed two groups (Fig. 7). In the remaining individual ports, high biomass was recorded once or twice during the observation (Fig. 7).

3.2.2. Pheophytin

Similar to chl *a*, higher pheophytin is observed in freshwater (except Haldia) and estuarine ports compared to marine ports. Among freshwater ports, high (up to 3.4 μg l^{-1}) and low (<0.1 μg l^{-1}) concentrations were recorded in Kolkata (including riverine stations) and Haldia ports respectively during all the occasions. Interestingly, high pheophytin concentration (but lesser than freshwater) were observed most of the year in estuarine port (Cochin). Among the marine ports, pheophytin showed a distinct seasonal trend. The highest and lowest pheophytin

Table 2
Environmental parameters from river influenced ports systems (RIPS) and marine port systems (MPS). SWM – southwest monsoon, PM-post-monsoon, PrM – pre-monsoon, NEM – northeast monsoon, SIM – spring inter-monsoon, FIM – fall inter-monsoon, DO – dissolved oxygen (mg l^{-1}). The units for nutrients (ammonia, nitrate, nitrite, phosphate and silicate) are in μM.

Environmental parameters from RIPS								
Stations	Season	Salinity	DO	Phosphate	Silicate	Nitrate	Nitrite	Ammonia
Cochin	PM1	22.6 ±6.1	7.1 ±1.0	3.2 ±1.7	47.8 ±9.9	4.0 ±3.0	0.5 ± 0.5	24.9 ±7.7
	PrM	20.5 ±6.8	6.7 ±1.1	3.8 ±0.8	44.3 ±9.3	5.9 ±1.3	1.1 ±0.3	42.6 ±11.8
	SWM	5.3 ±2.2	3.9 ±1.6	3.5 ±0.8	47.6 ±18.1	10.2 ±5.9	1.4 ±0.8	26.2 ±10.5
	PM2	23.1 ±2.2	3.8 ±1.4	2.6 ±1.1	47.2 ±16.7	3.4 ±2.4	0.5 ±02	20.5 ±10.1
Kolkata	SWM	0.20 ±0.05	6.2 ±1.5	2.3 ± 1.4	66.9 ±48.8	27 ± 122	1.6 ±0.3	2.0 ± 1.0
	PrM 1	0.25 ±0.05	8.8 ±0.9	4.5 ±0.7	76.4 ±12.4	10.0 ±6.6	4.3 ±2.3	20.6 ±7.8
	PrM 2	0.25 ±0.05	8.2 ±0.7	1.1 ±1.2	61.6 ± 31.3	24.3 ± 7.0	1.3 ±0.8	5.7 ±3.5
	PM	0.28 ±0.06	7.0 ±1.8	6.7 ± 0.5	132. 3 ±26.1	116.5 ±71.5	8.4 ±3.6	3.7 ± 1.1
Hooghly	SWM	0.3 ±0	6.7 ±0.8	5.1 ±0.3	148.3 ±3.1	38.2 ±4.1	1.5 ±0.4	2.5 ±0.7
	PrM 1	0.3 ±0	8.9 ±0.6	4.8 ±0.5	74.7 ±1.8	7.6 ±2.6	2.1 ± 1.7	22.3 ±16.3
	PrM 2	0.3 ±0	8.8 ±0.3	5.5 ±1.1	411.5 ±76.1	28.1 ±8.7	1.4 ±0.8	5.6 ±1.5
	PM	0.25 ± 0.10	7.0 ±1.5	10.7 ±0.3	177.7 ±13.0	51.5 ± 18.7	5.8 ±1.8	4.2 ±0.6
Haldia	PM	1.3 ± 0.5	6.0 ±0.7	4.0 ±1.5	114.7 ±14.4	33.0 ±10.2	1.1 ±0.3	4.1 ±1.6
	PrM 1	6.3 ± 0.9	8.2 ±0.2	6.6 ±1.1	79.7 ±12.9	3.4 ±1.1	1.6 ±0.3	6.3 ±1.4
	SWM	2.7 ± 0.6	5.3 ±0.6	3.4 ± 0.7	152.5 ±29.0	38.1 ±5.0	3.0 ±0.5	8.9 ±3.8
	PrM 2	7.4 ±0.5	5.7 ±1.0	6.0 ±1.3	142.9 ±46.0	24.3 ± 10.7	0.8 ± 0.5	4.3 ±1.4
Environmental parameter from MPS								
Stations	Season	Salinity	DO	Phosphate	Silicate	Nitrate	Nitrite	Ammonia
Kandla	PM 1	37.7 ± 0.3	5.0 ± 0.4	4.3 ± 1.4	54 ±5.2	7.2 ±2.0	4.4 ±0.7	15.3 ±3.0
	SWM	30.2 ± 10.2	6.2 ± 0.6	3.4 ± 0.7	30.9 ±5.2	12.8 ±5.6	7.0 ± 1.8	1.4 ±0.5
	PM 2	35.8 ± 0.1	6.5 ± 0.6	3.0 ± 0.4	77.6 ±9.8	15.9 ±6.0	3.0 ±0.5	4.9 ±0.7
	PrM	39.8 ± 0.4	6.1 ± 0.7	5.3 ± 0.8	128.4 ±9.7	14.6 ±2.8	6.8 ± 1.8	1.2±0 2
Mangalore	PM 1	35.9 ± 0.1	3.8± 1.2	2.2 ± 0.6	26.6 ± 15.5	8.5 ± 5.9	4.3 ±3.2	39.9 ±5.0
	PrM	35.7 ± 03	5.7 ± 1.9	1.8±1.9	6.1 ±5.7	52 ±3.2	1.8 ±0.7	25.3 ±8.3
	SWM	34.1 ± 0.9	4.7 ± 1.6	2.7 ±0.6	23.5 ± 8.4	0.7 ±0.5	0.6 ±0.2	29.4 ±9.2
	PM2	34.8 ± 0.1	4.7 ± 0.9	2.3 ± 0.8	17.6 ±5.6	3.9 ± 1.2	5.2 ± 1.2	18.8 ±4.8
Paradip	SWM	26.3 ± 0.5	5.2 ± 0.4	1.9 ±03	20.4 ±5.0	4.9 ±1.1	0.9 ±0.2	25.0 ±212
	NEM	31.0± 13	4.7 ± 1.0	1.8 ± 02	42.1 ± 15.0	4.9 ±2.0	1.1 ±0.2	12.5 ±4.8
	SIM	33.8 ± 03	4.7 ± 0.4	2.5 ±0.5	782 ± 13.7	8.7 ±5.9	1.8 ±0.2	5.0 ±2.9
	FIM	29.6± 13	4.6±0.6	0.2 ±0.1	63.0 ±38.9	4.5 ±0.9	1.6 ±0.7	3.7± 12
Chennai	SWM	34.4 ± 0.1	3.2 ±0.7	5.1 ±2.9	23.9 ±6.3	13.4 ±32	2.3 ±0.5	24.8 ± 102
	FIM	29.1 ± 1.8	4.9±0.4	3.1 ±23	11.8 ±3.1	8.0 ± 5.8	1.3 ± 1.1	51.3 ±443
	NEM	30.4 ± 02	4.8 ± 0.5	1.8 ±0.6	8.4 ±2.4	9.1 ± 5.6	1.6 ±0.9	27.2 ± 112
	SIM	34.3 ± 02	4.0 ± 0.8	2.6 ±1.0	6.2 ±2.1	6.5 ±2.2	0.9 ±0.3	12.9 ±5.1
Tuticorin	SWM	35.4 ± 0.1	5.6±0.4	1.2 ±0.7	123 ±4.6	1.1 ±1.1	0.5 ± 0.5	12.9 ±3.9
	FIM	36.2 ± 02	6.3 ± 0.4	1.8 ±0.6	6.2 ±1.8	4.4 ±2.2	1.4 ±0.7	21.5 ±6.8
	NEM	34.0 ± 0.8	5.0±0.7	1.9 ±0.8	6.2 ±1.8	11.0 ±8.1	9.8 ±7.5	23.2 ± 113
	SIM	33.5 ± 0.03	5.9 ± 0.4	1.8 ±02	4.4 ±0.7	53 ±0.8	0.8 ±0.2	12.3 ±3.3

was recorded in Mangalore (0.1–1.2 μg l^{-1}) and Kandla (0.03–0.13 μg l^{-1}) ports, respectively. In the remaining three ports pheophytin distribution was variable. Clustering and non-metric MDS at 60% similarity also revealed two groups and four ungrouped individual ports. The ports characterized by low (Haldia, Paradip, and Kandla) and relatively higher pheophytin (Mangalore, Kolkata, and Cochin) for most of the year formed two groups (Fig. 7). However, some stations located in Hooghly river (part of Kolkata sampling) recorded the highest pheophytin, whereas the remaining two individual ports recorded high pheophytin were recorded once or twice during the observation (Fig. 7).

3.2.3. Pheophorbide

Unlike chl *a* and phoephytin, the pheophorbide distribution was not same between the ports. On the contrary to high biomass Kolkata port, pheophorbide concentrations were low except during PM (0.1–2.0 μg l^{-1}), whereas in Haldia-port low pheophorbide concentrations (0.09–0.19 μg l^{-1}) were recorded throughout the year. Unlike freshwater systems, the estuarine-port (Cochin) recorded higher pheophorbide concentrations, and the maximum was during post-monsoon followed by southwest monsoon and premonsoon (Fig. 4). Among the marine ports, pheophorbide distribution showed a distinct seasonal trend. Unlike in freshwater and estuarine ecosystems, pheophorbide in marine ports exhibited a distinct relationship with chl *a*. Among ports, highest (up to 20.1 μg l^{-1}) and lowest (<0.2 μg l^{-1}) pheophorbide concentrations were recorded in Mangalore and Kandla port, respectively. In the remaining marine ports, pheophorbide distribution showed distinct seasonality, but the seasonal patterns were different due to the geographic location of the ports. Clustering and non-metric MDS at 60% similarity also revealed two groups and three ungrouped individual ports. The ports characterized by low (Kolkata and Tuticorin) and relatively higher pheophorbide (Hooghly, Haldia, Kandla, and Paradip) for most of the year formed two groups (Fig. 7). However, Mangalore port followed by Cochin recorded the highest pheophorbide for most of the year, whereas in the remaining Chennai port high pheophorbide concentration was recorded once during the observation (Fig. 7).

3.3. Pigment ratios

3.3.1. Chl a to pheopigments ratio

Generally, Chl *a*: pheopigments ratios were calculated to understand the freshness of organic matters from the overlying water column from different ecosystems. In this study, pigment ratio (Chl *a*: CDP, Chl *a*: pheophytin and Chl *a*: pheophorbide) showed a distinct spatial (between ecosystem types such as freshwater, estuary and marine ports) and seasonal variations (Fig. 5). High Chl *a*: CDP ratios and high- Chl *a:* pheophorbide ratios were observed in freshwater (except Haldia due to low biomass) and estuarine ports compare to marine ports. On the contrary, high Chl *a*: pheophytin ratios were mostly observed in marine ports (except Paradip due to low biomass) than freshwater ports (except Cochin and Haldia). Among the freshwater ports, high and low ratios

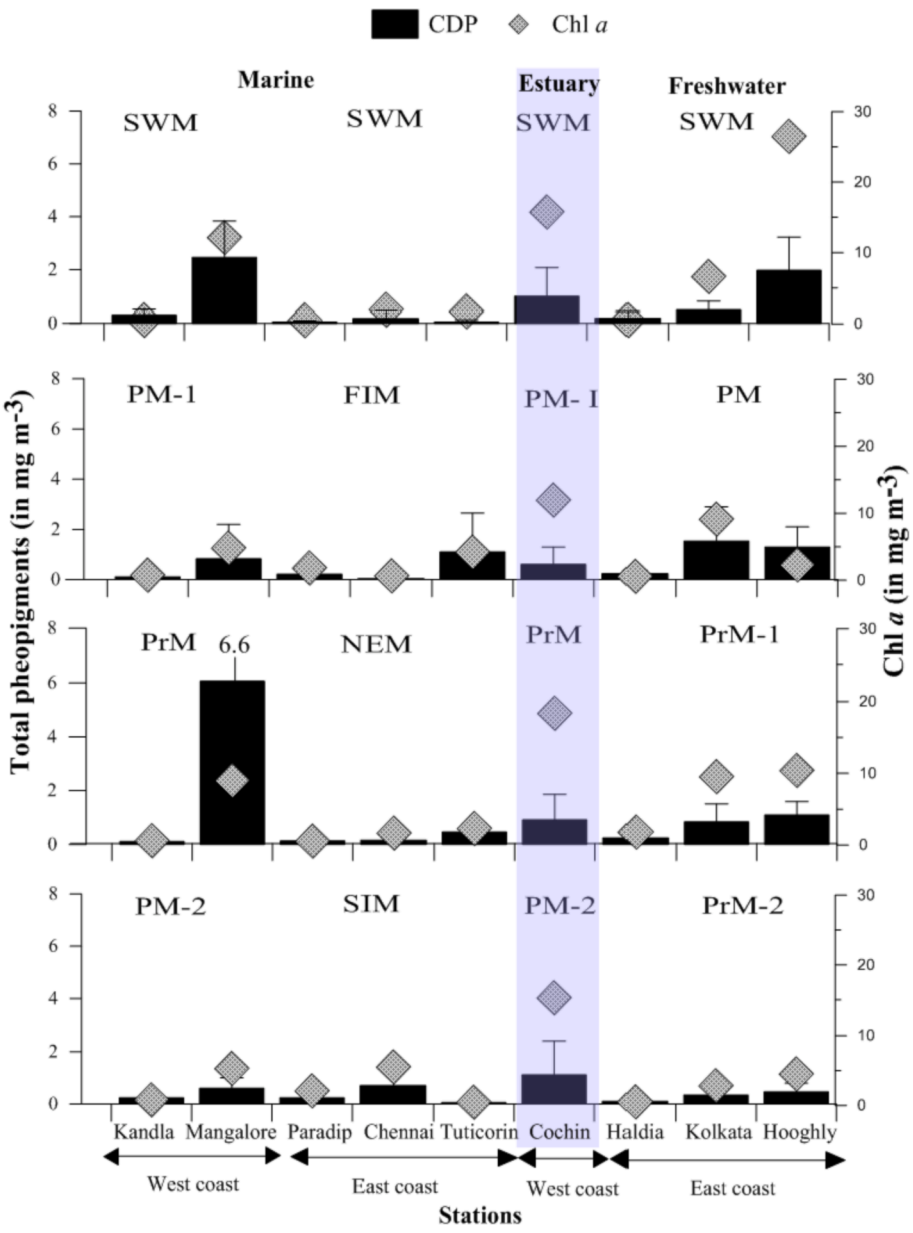

Fig. 3. The seasonal variations in the concentrations of chlorophyll and chlorophyll degraded pigments (CDP) from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon; FIM – Fall inter-monsoon; NEM – northeast monsoon; SIM – spring intermonsoon; PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

were encountered in Kolkata and Haldia, respectively, whereas among the marine ports, high and low ratios were encountered in Mangalore and Paradip/Kandla, respectively (Figs. 5 and 7). The low ratios in freshwater and marine ports were mainly due to the prevalence of low chlorophyll.

3.3.2. *Pheophorbide vs. pheophytin*

The ratios of pheophorbide to pheophytin were calculated to understand the ecological process of the different ecosystems, i.e., CCP/herbivory is dominant (>1) or not dominant (<1). Like Chl to CDP ratios, pheophorbide: pheophytin ratio also showed a distinct spatial and seasonal variation (Fig. 6). The average pheophorbide: pheophytin ratio ranged from a low of 0.2 in Kolkata to a maximum of 11.5 in Mangalore (Fig. 6). Pheophorbide: pheophytin ratios in freshwater (except Haldia) and estuarine ports were <1, (i.e., ranges between 0.2 and 0.9) whereas in marine ports the average ratio was mostly >1, i.e., ranges between 0.4 and 11.5 (Figs. 6 and 7). The low ratios in marine ports (especially in Chennai and Tuticorin) are observed only on a couple of occasions when the biomass is very low (<1 μg/l). Clustering and non-metric MDS at 60% similarity also revealed two groups and three ungrouped individual ports. The ports characterized by low, i.e., <1 (Kolkata, Hooghly, and Cochin) and relatively higher ratio, i.e.,>1 (Haldia, Kandla, and Paradip) for the most of the year formed the two groups (Fig. 7). However, Mangalore port recorded with the highest ratio (up to 11.2) for the most of the year, whereas in the remaining Chennai and Tuticorin-port, high ratios (>1) were recorded only once during the observation (Fig. 7).

4. Discussion

In aquatic ecosystems, loss (mortality) processes (e.g., grazing, program cell death, viral lysis, sinking) determine the fate of phytoplankton and under such circumstances PMP can be used as a proxy to understand the potential chlorophyll breakdown (ChlB) pathways. In the ChlB pathway, pheophorbide formation is an important step, formed either

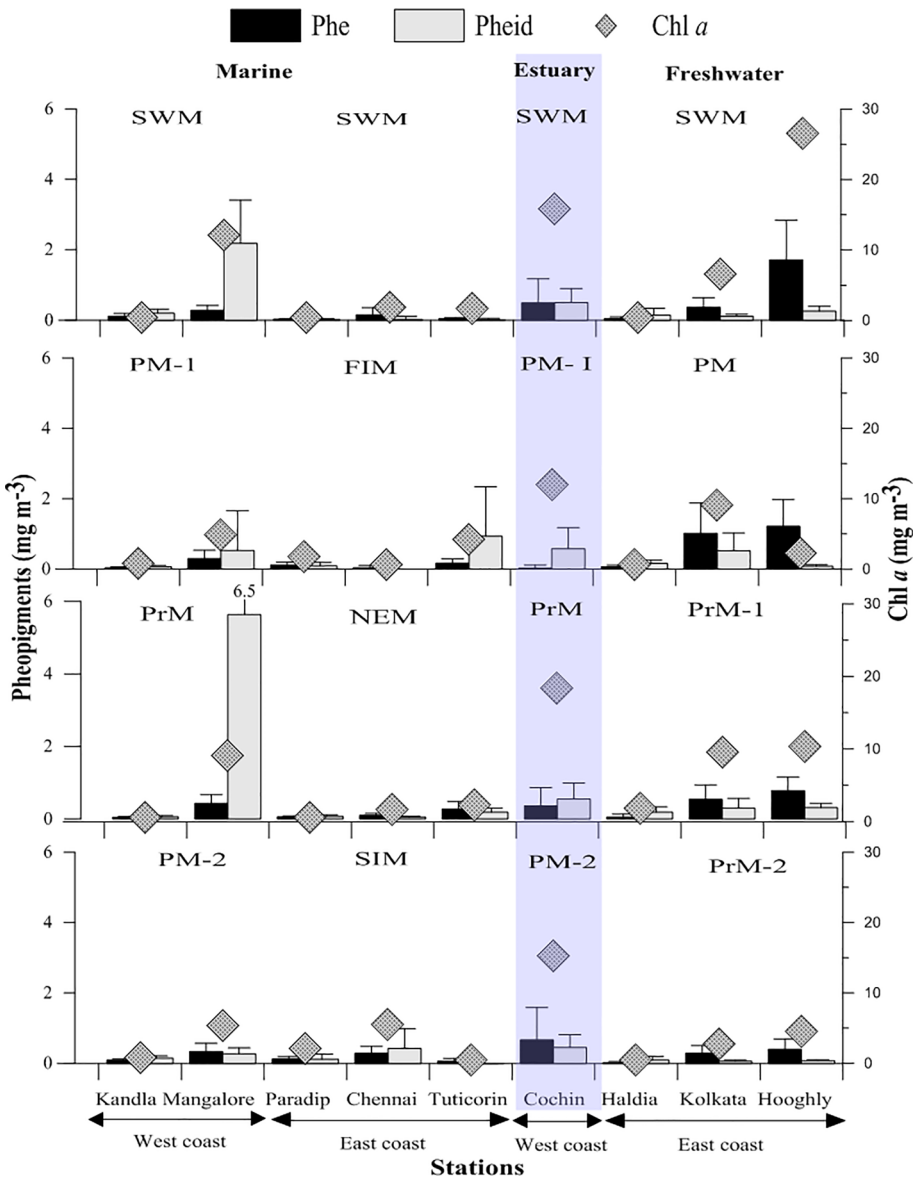

Fig. 4. The seasonal variations in the concentrations of pheophytin (Phe), pheophorbide (Pheid) and chlorophyll (Chl) from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon; FIM – Fall inter-monsoon; NEM – northeast monsoon; SIM – spring intermonsoon; PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

via chlorophyllide or pheophytin. Laboratory experiments reported that the initial ChlB-pathway induced by grazing and senescence are not the same, i.e., chlorophyll-chlorophyllide-pheophorbide (CCP) and chlorophyll-pheophytin-pheophorbide (CPP) in the former and latter case, respectively. Therefore documenting these different breakdown factions will aid in determining the dominant ChlB pathway. Like plankton and environmental variables, the ChlB-pathway will also differ in different ecosystems (i.e., FPE, EPE, and MPE) and was documented in this study. The highest concentration of chl *a* and CDP were encountered in FPE (except Haldia with least values) and EPE compared to MPE (Figs. 3, 4 and 7), suggesting the existence of system-specific growth and loss process occurring at different time scales. Chl *a*: total pheopigments, an indicator for degradation of pigments (Boon and Duineveld, 1996) or freshness of organic matter (Morata et al., 2011), did not reveal a clear pattern between port ecosystems as observed with the concentrations (Fig. 5). However, irrespective of the river influence, the systems with very low biomass (<1 $\mu g m^{-1}$) showed very low Chl *a*: CDP ratio suggesting the higher degradation activity (Fig. 5). Low and high ratios were observed in Cochin and Kolkata/Hooghly respectively. In the case of MPE, low and high ratios in Paradip and Mangalore respectively suggested the predominance of less or more actively growing biomass, respectively.

In this study, the pool of chlorophyll breakdown fractions is mainly contributed by pheophytin and pheophorbide, suggesting the prevalence of CCP and CPP pathways even when the chlorophyllide fraction was not detected. However, there exists considerable spatial and temporal variability in the chlorophyll breakdown pathways, as indicated by pheophytin and pheophorbide distribution. The presence of pheophytin (demetalated chlorophyll) and pheophorbide (demetalated and dephytalated chlorophyll) indicates chlorophyll has undergone a single-step and double-step breakdown reactions. Generally, pheophytin concentration is always higher in FPE and EPE compared to MPE (Figs. 4 and 7) due to the prevalence of high biomass as well as the contribution from the terrigenous influx. On the contrary, the distribution of pheophorbide concentrations was variable even with the high biomass in FPE and EPE, whereas in MPE, distribution showed high, low, and variable under high, low, and variable biomass, respectively (Figs. 4 and 7). In Cochin (EPE) and Mangalore ports (MPE) high pheophorbide

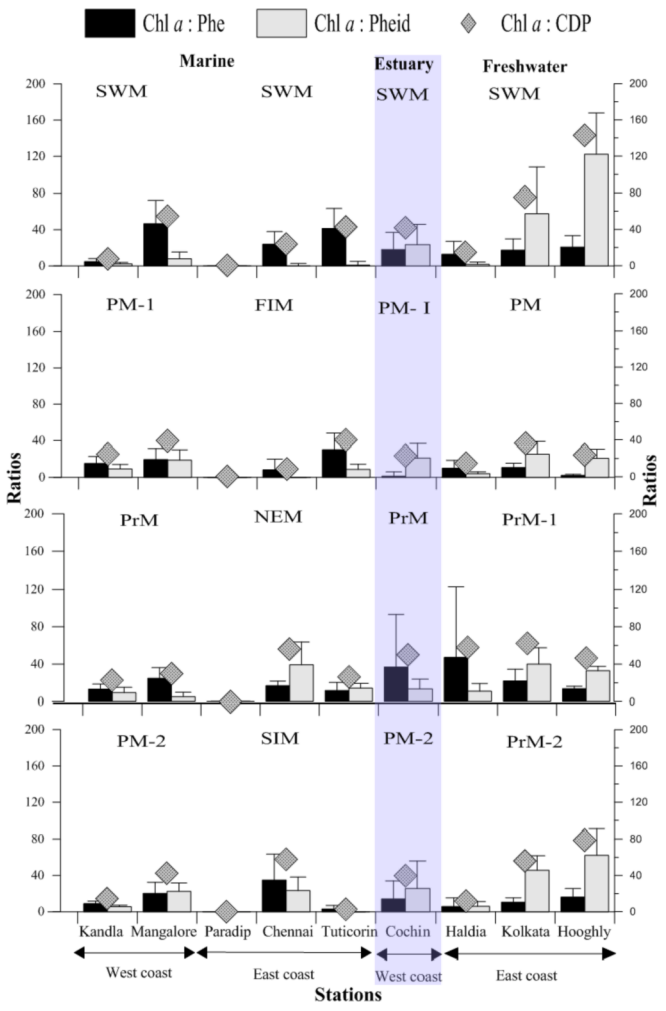

Fig. 5. The seasonal variations in the pigment ratios (Chl:pheophorbide; Chl: pheophytin and Chl:CDP) from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon; FIM – Fall inter-monsoon; NEM – north east monsoon; SIM – spring inter monsoon; PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

concentrations were recorded, suggesting that these ecosystems are conducive for the chlorophyll to undergo double-step breakdown reactions. Overall pheophorbide concentrations showed independent and dependent on biomass in FPE/EPE and MPE, respectively (Fig. 5). Since chlorophyllide is not detected, the identification of chlorophyll breakdown pathway is not straightforward. The laboratory experiments suggested that the chlorophyll breakdown to pheophorbide via CCP pathway is relatively faster compared to CPP pathway (unpublished data). In such circumstances, pheophorbide: pheophytin ratio will be a potential proxy to understand the dominant pathway or nature of mortality (an important ecological process) in different ecosystems. For instance, if the ratio is >1, then the CCP pathway is more active then the CPP pathway and <1 then the CCP pathway is less active than CPP pathway. Both CCP and CPP pathways may be considered in equilibrium if the ratio is equal to 1. The laboratory grazing experiments using monocultures of phytoplankton belonging to different groups (diatoms, dinoflagellates, and green algae) and natural communities revealed that pheophorbide: pheophytin ratio can be proxy to determine herbivory is dominant (>1) or senescence is dominant (<1); Satish et al. submitted). These findings suggest that the additional information on the taxonomic composition of both phytoplankton and zooplankton along with the environmental settings (e.g., FPE, EPE, and MPE) will be useful to identify the cause for chlorophyll breakdown pathway. Given this, a schematic indicating the higher ratios for no-river influenced coastal systems (>1) followed by estuarine (0.5), and freshwater (0.2–0.4) systems is proposed (Fig. 8), which can aid in better ecological assessment and the details on the possible usefulness of the above insights is provided subsequently. For example the systems with low (<1) and high (>1) pheophorbide: pheophytin ratios suggested the dominance of cell senescence (suggesting CPP pathway due to high pheophytin concentrations) and cell destruction due to herbivory or grazing (suggesting CCP pathway due to high pheophorbide concentrations), respectively. Here the following scenarios: ballast water management (treatment and post-voyage discharge), the fate of algal bloom (the decline of bloom is due to herbivory or senescence or exhaustion of resource) and the ecological perspective are envisaged to elucidate the usefulness of pheophorbide: pheophytin ratio in the ecosystem assessment.

Generally, ports are at the receiving end for ship's ballast water discharge and therefore the information on the phytoplankton growth and loss according to the environmental conditions at discharge point will be valuable for ballast water management and is summarized in Table 3. The present study indicated that the pheophorbide: pheophytin ratio exhibited a distinct spatial (between/within ecosystems) and seasonal variability (Figs. 6 and 7). In FPE (except Haldia) and EPE, the ratio was <1 during all the seasons suggested that the CPP pathway is more active compared to CCP pathway (Fig. 6). In Kolkata and Hooghly systems, the pheophorbide concentration was minimal even when high biomass was present whereas, in the case of Cochin systems, the ratio was relatively higher as there was evidence of high pheophorbide production compared to freshwater ports during all the sampling occasion. Unfortunately, in Haldia, high ratios (0.9–4.6) were observed despite the prevalence of low biomass on all the occasions (Fig. 6). One possible reason for such a different scenario is due to the prevalence of different phytoplankton composition. For instance, in Cochin and Kolkata/Hooghly, diatoms (63%–78%) and non-diatoms (65%–75%) dominated the phytoplankton community respectively. Generally, diatoms, which have high chlorophyllase activity (Jeffery and Halletraeff, 1987), are the preferred feed for grazers. Therefore, under such circumstances, the CCP pathway will be active and was the case in Cochin ecosystems along with CPP pathway. Even the laboratory experiments also indicated higher pheophorbide production for *Skeletonema* (diatom) than species belonging to other groups, including species (*Dunaliella*) with high chlorophyllase activity (unpublished data). It should also be noted that the chlorophyllase activity varied between the species of the same group (Jeffery and Halletraeff, 1987). Therefore, the presence of low herbivore population/biomass or the non-preferred diet of the prevailing grazer in Kolkata/Hooghly cannot be ruled out. On the contrary, irrespective of the phytoplankton biomass, pheophorbide: pheophytin ratio in MPE ranged up to 11.2 (Figs. 6 and 7). This suggests that chlorophyll has undergone double-step chlorophyll breakdown reactions, unlike FPE, with one-step reactions. In MPE, the phytoplankton composition is dominated by diatoms round the year, and therefore, the observed high pheophorbide production via CCP pathway cannot be ruled out. In low (Kandla) and high (Mangalore) biomass systems, CCP pathway is active round the year. In the case of Paradip, Chennai and Tuticorin systems, biomass was variable and so with the ratios. A distinct seasonality in the pheophorbide production in diatom-dominated marine ports was observed, and this is because of the chlorophyll breakdown reactions of seasonal high biomass via CCP pathway, i.e., through herbivory. Nevertheless, this study suggests that the chlorophyll breakdown pathways exhibit apparent differences between the freshwater (enclosed; Kolkata port), riverine (Hooghly), estuarine (Cochin) and marine ecosystems, respectively. This generally means that the dominant loss factor for phytoplankton (including introduced species) will be cell senescence and herbivory in FPE (throughout the year) and MPE (due to seasonal diatom peaks) respectively, whereas both cell senescence and herbivory are responsible in EPE (due to prevalence of high biomass and freshwater influx). Given the above it is also envisaged that the time window

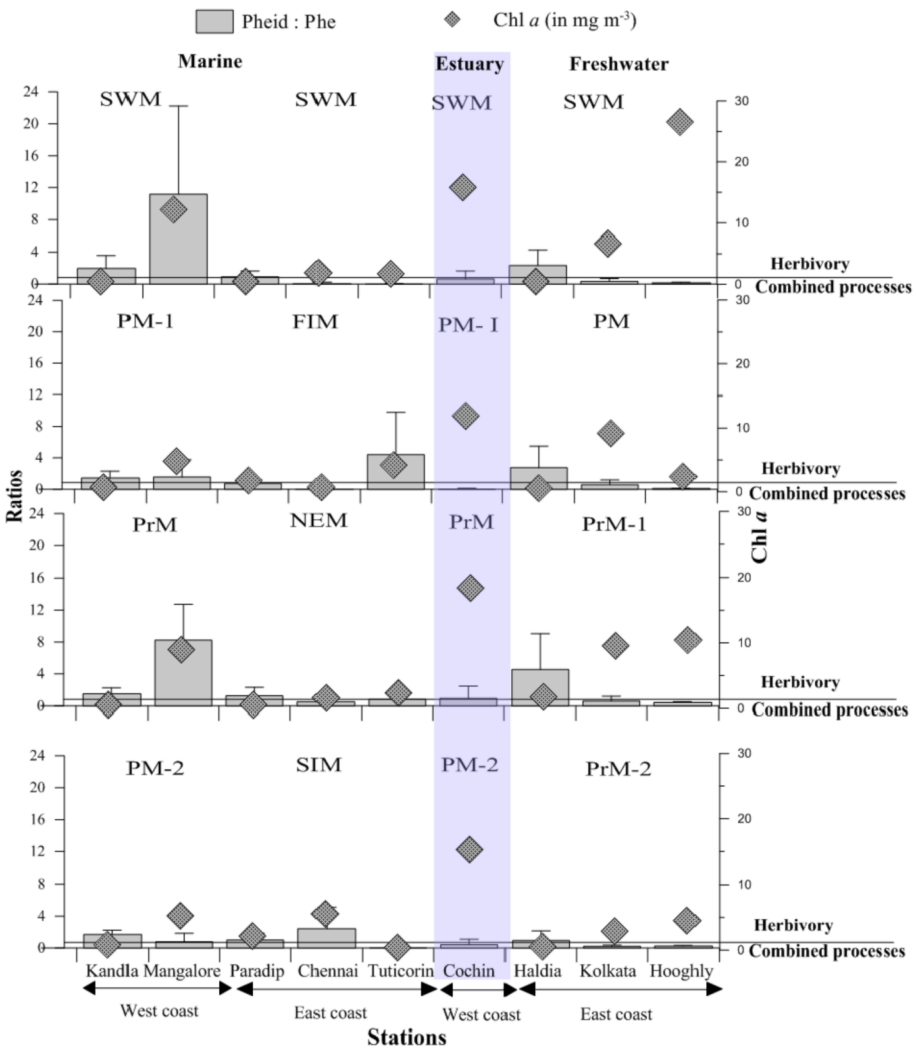

Fig. 6. The seasonal variations in the pheophorbide: pheophytin ratios from different port ecosystems (freshwater, estuarine and marine) located along the east and west coast of India. SWM – southwest monsoon; FIM – Fall inter-monsoon; NEM – northeast monsoon; SIM – spring intermonsoon; PrM 1 and PrM 2 – pre-monsoon of first and second sampling season, PM 1 and 2 – post-monsoon first and second sampling season.

of the conducive environmental condition for the growth of ballast introduced viable phytoplankton is lower for MPE (except Mangalore) and FPE (except diatoms and dinoflagellates) compared to that for EPE (Table 3). These findings suggest that the introduction of ballast water with viable phytoplankton during the growing season involves higher risk, and the risk level varied between the ports and season. Nevertheless, the study revealed grazing (in MPE and EPE) and senescence (in FPE and EPE) as the dominant loss factor for phytoplankton. From the ballast water management perspective, the risk level involved in the studied ports is summarized for the reference (Table 3).

Further, most of the studied port ecosystems are prone to blooms and the knowledge on taxa, chlorophyll, and pheophorbide: pheophytin ratio will be useful to determine whether the decline of bloom is due to herbivory or other reasons (senescence, microbial degradation, resting stage formation). Here, the former scenario (mostly related to seasonal diatom blooms as noticed in MPE and EPE) signifies a good ecological state, and the latter (unusual or harmful algal blooms-HAB) is a matter of ecological and environmental concern. Some studies have also shown that the members of micro- and mesozooplankton don't prefer toxin-producing HAB species (e.g., Turner, 2010); however, such deterrent properties may also be highly specific to species and/or condition (e.g., Davis and Gobler, 2010; Stauffer et al., 2017) and was evident in the laboratory experiment. The grazing experiment with a bloom-forming diatom (*Skeletonema*) and dinoflagellate (*Amphidinium*) revealed higher (>1) and lower (<1) pheophorbide: pheophytin ratios respectively suggesting grazing induced mortality or growth control in the former but not in the latter (Sathish et al. unpublished data). The global increase in the occurrence of HABs continue to underscore the many remaining gaps in knowledge such as technological advances for early detection (Stauffer et al., 2019). Given this, the incorporation of the chlorophyll breakdown pathway indicator to determine bloom fate will be a step ahead in the ecological/environmental assessment and HAB research per se. For bloom events to occur, the growth rate must exceed losses, through biological and physical processes (Caron et al., 1989; Mitra and Flynn, 2006; Tillmann, 2004). Therefore biological control measures such as grazing and algicidal microbes are considered as promising techniques to control algal blooms (e.g., Secher, 2009; Choi et al., 2017; Paerl et al., 2018). Given this, we assume that chlorophyll breakdown pathway will be a potential proxy for evaluating the efficacy of the bloom control techniques. Traditionally, in addition to grazing, the sinking of ungrazed phytoplankton or bloom to the bottom is considered as one of the major loss factor (Calbet and Landry, 2004; Choi et al., 2017). Thereby assessing the dynamics of PMP from the sediments and the overlying waters in a given ecosystem will improve the understanding of the linkages between the pelagic and benthic phytoplankton, which is an essential process in the functioning of the ecosystem. In view of this, it is assumed that chlorophyll: pheopigment and pheophorbide: pheophytin ratios together can be used as potential

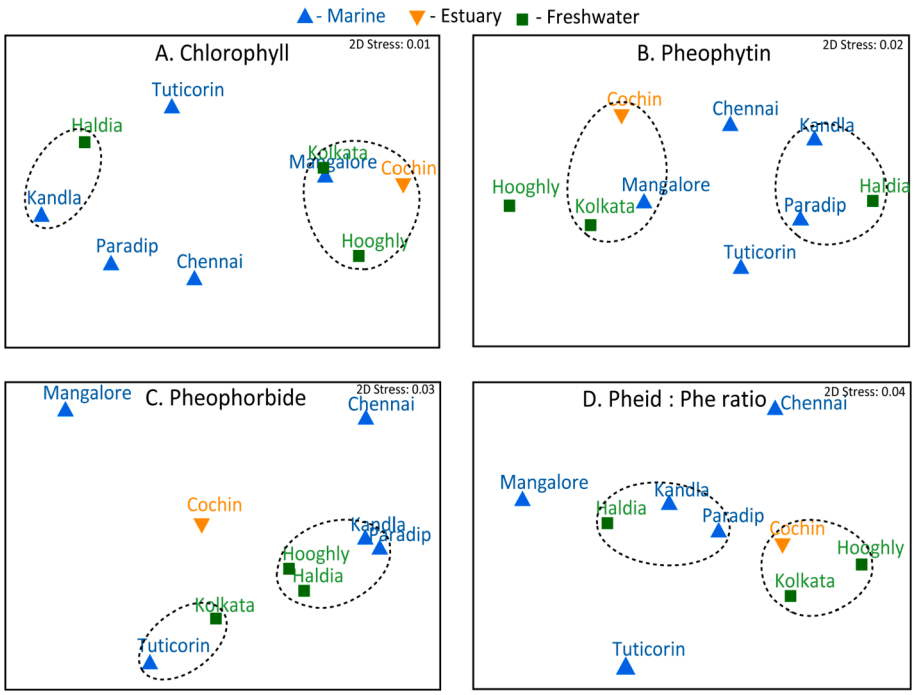

Fig. 7. Non-metric multidimensional scaling (NMDS) ordination based on the Bray–Curtis similarity coefficient of (a) chlorophyll, (b) pheophytin, (c) pheophorbide and (d) pheophorbide:pheophytin ratio for the ports located in different ecosystems (marine, freshwater and estuarine). The grouping is based on the 60% similarity level of the cluster dendrograms constructed using the Bray–Curtis similarity coefficient and group average method. The filled symbols triangle, square and inverted triangle represents marine, freshwater and estuarine ecosystems respectively.

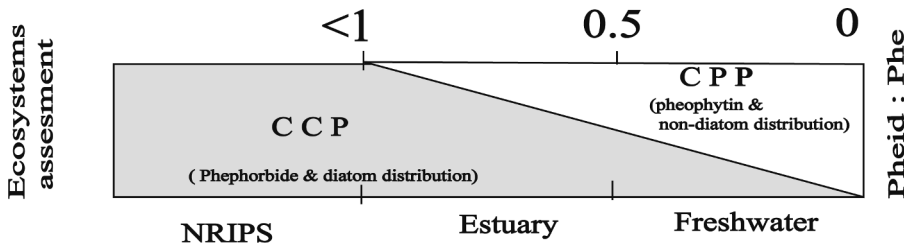

Fig. 8. Schematic illustrating the chlorophyll-breakdown pathway in different ecosystems. CCP and CPP indicates chlorophyll-chlorophyllide-pheophorbide and chlorophyll-pheophytin-pheophorbide pathways respectively. Non-diatoms indicates that the phytoplankton is dominated by other groups such as chlorophytes, cyanobacteria, dinoflagellates. Pheid:Phe indicated phoephorbide:pheophytin ratio.

Table 3
Summary on the distribution of phytoplankton pigments, level of invasion risk and potential loss factor for different major port ecosystems along Indian coast.

Port location	Ecosystems	Biomass (Chlorophyll)	Dominant groups	Pheophytin (Phe)	Pheophorbide (Pheid)	Pheid: Phe	Seasonal	Phytoplankton loss processes	Invasion risk
Cochin	Estuarine	High	Diatoms, Chlorophytes	High	Variable	<1	Yes	Degradation & herbivory (biomass independent)	High
Kolkata	Freshwater	High	Chlorophytes					Degradation (biomass independent)	High[a]
Haldia	Freshwater	Low	Chlorophytes	Low	Low	>1	No	Herbivory	Low
Kandla	Marine	Low	Diatoms	Low	Low	>1	Yes	Herbivory (biomass	Low
Mangalore	Marine	High		High	High	Variable		dependent)	High
Tuticorin	Marine	Variable	Diatoms	Variable	Variable	Variable	Variable		Variable
Chennai	Marine								
Paradip	Marine								

[a] Excluding diatoms and dinoflagellates.

proxies to determine the fate of phytoplankton (in particular bloom) or pelagic primary production as the former is an indicator for actively growing microalgal community in the water column and freshness of organic input into the surface sediment (Morata et al., 2011; Grippo et al. (2009); Allison et al. (2013)] whereas the latter for determining the factors (eg. herbivory or senescence) responsible for decline of phytoplankton population or bloom (Satish et al. submitted). However, specific studies in these directions will be a step ahead.

The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 (BWM Convention), entered into force globally on 8 September 2017. From this date of the BWM Convention, all ships must conform to the two ballast water management standards (D-1 and D-2). The D-1 standard requires ships to exchange their ballast water in open seas, away from coastal areas (i.e., ~200 nautical miles from land and in water at least 200 m deep) whereas the D-2 standard specifies the maximum amount of viable organisms allowed to be discharged, including specified indicator microbes harmful to human health. According to the convention, BWM

systems (e.g., use of filters and ultraviolet light or electrochlorination) have to be tested in a land-based facility and onboard ships to prove that they meet the performance standard set out in the treaty. Given this Ballast water sampling from the ships has started receiving the importance of proving that ships carrying ballast water comply with BWM standards (Gollasch and David, 2017). Even port authorities may also sample ballast water for subsequent analyses to prove compliance with the ballast water performance standards. In view of this, analyzing the degraded pigments and their ratios along with the modern tools/methodologies for viable phytoplankton cells will be added advantage in the evaluation of the impact of (i) ballast tank conditions (eg. dark) during voyages, and (ii) treatment of the ships ballast water using approved BWM systems before discharge. During voyages, in ballast tanks, prolong darkness is the prominent stress factors, and this condition can have varying effects on the organisms. The laboratory experiments have shown that phytoplankton population decreased with increase in the length of the dark incubation (Carney et al., 2011; Desai et al., 2018; Patil and Anil, 2018), but the duration of dark survival or tolerance varies and is species-specific (Peters, 1996; Peters and Thomas, 1996; Unpublished data). The decline in the population could be due to senescence or microbial degradation and grazing or both. The sampling from the ballast tanks during voyages revealed different findings. In one study, decrease and increase in plankton (both phyto and zoo) abundance and bacteria was observed respectively till the end of the voyages (Desai et al., 2018) and in such condition senescence or microbial degradation will be dominant. Whereas in another study, sampling from ballast tanks observed a large fish and increase in copepod (a dominant zooplankton and a grazer) populations (Gollasch et al., 2000; Gollasch and David, 2019) suggesting herbivory dominance cannot be ruled out. Moreover, most of the ballast water treatments involve killing or destruction of the cells, and in such cases, phytoplankton degradation process will be similar to that observed during herbivory. The involvement of chlorophyllase activity in the conversion of chlorophyll into chlorophyllide is the reason, and this happens whenever there is cell destruction like chewing or grazing (Hu et al., 2013). Nevertheless, in each of the scenarios, the proposed pheophorbide: pheophytin ratio can be useful to determine the reason for phytoplankton population decline. Since the chl *a* breakdown pathway under grazing-pressure is taxon-specific and the grazer's feeding habits (Sathish et al. submitted), additional studies in this direction will be valuable in ecological surveillance and assessment.

5. Conclusion

This study concludes that the distribution of single (pheophytin) and double (pheophorbide) step chlorophyll-breakdown reaction fractions exhibit distinct spatial and seasonal variations. Pheophytin is higher in the FPE and EPE, but its distribution is not linked to biomass variations. Whereas pheophorbide is more in MPE followed by estuarine systems and its distribution could be linked with biomass distribution. Since chlorophyllide (single-step reaction of CCP pathway) was not detected, pheophorbide: pheophytin ratio can be a potential proxy for determining the nature of the breakdown pathway, i.e., CCP or herbivory dominant (>1) or not dominant (<1). The ratios exhibited clear differences between different ecosystems, i.e., the higher ratios in marine (up to 11.2) followed by estuarine (up to 0.94) and freshwater (0.2–0.4) port systems. This generally means that the dominant degradation pathway for phytoplankton (including introduced species) will be cell senescence and herbivory in FPE (throughout the year) and MPE (due to seasonal diatom peaks) respectively, whereas both cell senescence and herbivory are responsible in EPE (due to prevalence of high biomass and freshwater influx). Since laboratory experiments revealed that the chl *a* breakdown pathway under grazing-pressure is taxon-specific and the grazer's feeding habits (Sathish et al. submitted) additional studies in this direction will be valuable in ballast water management (treatment and post-voyage discharge), algal bloom research (eg. understanding fate and in control measures), and in the ecological surveillance. Also confirming the role of hydrolase enzymes and the sequence in the early as well as post-pheophorbide breakdown steps in different aquatic ecosystems will enhance the understanding of the chlorophyll breakdown pathways, which further alleviate the ecosystem assessment.

Author contributions

Sathish K: Port sampling, sample analysis, data interpretation and statistical analysis; JS Patil: Original concept, Port sampling, data interpretation, manuscript elaboration and supervision; AC Anil: Port sampling, Manuscript elaboration and also co-supervised all works.

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RESEARCH ARTICLE

Benthic-pelagic coupling assessed using phytoplankton marker pigments: a case study from the Paradip port, East Coast of India

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Abstract

This study addresses the seasonal distribution of phytoplankton marker pigments (PMP) and the ratios (indicating freshness and fate) from water and surface sediments from the coastal port ecosystem (Paradip port, Odisha, east coast of India) and its utility in understanding phytoplankton pelagic-benthic linkages. Chlorophyll and PMP (for different groups and degradation) distribution revealed distinct seasonality, but the seasonal trend was different for water and sediments. High and low values were observed in the water column during inter-monsoons (fall/spring) and monsoons (southwest/northeast), respectively, whereas, in sediments, the reverse was recorded. However, the contribution of group-specific PMP was different: fucoxanthin > chlorophyll b > zeaxanthin > alloxanthin > peridinin dominated in water and chlorophyll b > zeaxanthin > fucoxanthin > alloxanthin > peridinin in sediment. Selective grazing and stability of sedimentary PMP (fucoxanthin, diatoms PMP, is least stable than other groups) could contribute to such differences. Relatively high chlorophyll:pheopigment ratios in the water and low pheophorbide: pheophytin in sediments indicated the dominance of actively growing microalgae and chlorophyll degradation via chlorophyllidae pathway in the water but not in sediments. These findings suggest that (i) much of the phytoplankton (primarily diatoms) is lost due to herbivory before reaching bottom sediments, and (ii) pigment contribution is determined by selective grazing in water and PMP decay constants in sediments. Documenting such information will give new insights into ecosystem assessment and algal bloom research.

Keywords Phytoplankton · Pelagic-benthic linkages · Chlorophyll · Marker pigments · Pheopigments

Introduction

Phytoplankton are accountable for about half of the global primary production and establish a base to the food web, which supports either directly or indirectly to the entire marine ecosystems (Jeffrey and Vesk 1997; Falkowski et al. 2004). Phytoplankton are represented by considerable diversity of algal groups with size ranging from 0.2 µm to several millimeters. All phytoplankton, by their photosynthetic activities, are a significant contributor to global carbon fluxes (Falkowski 1998). In the coastal area, benthic faunal changes are determined by the fluctuation of phytoplankton, which is major organic input (Zhang et al. 2015). Since phytoplankton are the principle element of biogeochemical cycles and food web dynamics, accessing phytoplankton biomass and its functional groups from pelagic and benthic environments plays a crucial role in environmental assessments.

Benthic-pelagic coupling is demonstrated as the exchange of energy, biomass, or inorganic nutrients between benthic and pelagic habitats. It plays a crucial part in the nutrient cycling and food web dynamics of aquatic ecosystems. Coastal and estuarine systems are strongly affected by anthropogenic activities, but the understating of organic matter and inorganic nutrient exchange between the benthic environment and water column is minimal (Griffiths et al., 2017). Therefore, knowledge of the phytoplankton composition and biomass from the pelagic ecosystem is essential to understand the linkage and dynamics (Mendes et al. 2011; Agirbas et al. 2015). The water column phytoplankton changes also fuel the benthic production (Graff and

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Rynearson 2011, Freiberg et al. 2011). Sedimentary pigments reflect phytoplankton production and its pathway in the water column and sediment. Hence, it is hypothesized that assessing the dynamics of phytoplankton marker pigments from sediments and water columns will improve the understanding of benthic-pelagic coupling, which is an essential process in the functioning of the ecosystem (Freiberg et al. 2011). Apart from the group-specific phytoplankton marker pigments, chlorophyll *a* and its degradation products (chlorophyllide *a*, Chlide *a*; pheophytin *a*, Pheo *a*; pheophorbide *a*, Pheid *a*; and pyropheophytin *a*, pPheo *a*) often used as a diagnostic indicator for the fresh and degraded microalgal community, respectively (e.g., Wieking and Kröncke 2005; Pusceddu et al. 2009). Processes such as growth, grazing, cell sinking and senescence, photodegradation, fecal pellet sinking, physical mixing, and transport affect chl *a* and pheopigments concentrations in the euphotic zone. Hence, assessing these pheopigments and their ratios will strengthen the understating of phytoplankton from the given ecosystem. So far, several studies are either focused on the water column pigment distributions or sedimentary pigment biomarkers, including the Indian coasts (Ahel et al. 1996; Ston 2002; Lohrenz et al. 2003; Dyble and Moisander 2003; Henriksen et al. 2002; e.g., Chakraborty et al. 2011; Roy et al. 2011; Agirbas et al. 2017; Nunes et al. 2018; Naik et al. 2018, Morata and Renaud 2008; Reuss et al. 2010; Freiberg et al. 2011; Aneeshkumar and Sujatha 2012; Rasiq et al. 2016; Symczak-Zyla et al. 2008; Sañé et al. 2019). However, some microscopic studies indicated the persistence of benthic-pelagic coupling in bloom-forming diatoms (Patil and Anil 2008), diatom viability in sediments for several months (Anil et al. 2007), and phytoplankton pigment linkage between benthic and pelagic from the large shallow lake (Freiberg et al. 2011). The studies related to phytoplankton biomass and composition from the coastal sediment and its coupling between the water columns are limited to the best of our knowledge. This study expands the existing knowledge for the area in terms of distribution and dynamics of phytoplankton marker pigments, benthic-pelagic couling, and the fate of ungrazed phytoplankton using marker pigments and the ratios indicating phytoplankton physiological status and the nature of the loss process.

Materials and methods

The present study was conducted in Paradip port, which is one of the major seaport situated on the east coast of India (Fig. 1). It is an artificial lagoon type port located at about 6.7 km south of Mahanadi River mouth in Odisha. This port has a 190-m wide approach channel, protected by the northern and southern breakwaters with a length of 0.5 km and 1 km. Paradip port exports and imports commodities like iron ore, manganese ore, coal, chrome ore, coking coal, petroleum, sulfuric acid, and limestone. Paradip port has 15 berths, three single point mooring, and 1 Ro-Ro jetty with the length from 50 to 520 m and a well-maintained approach and entrance channel with the minimum depth of 17.1 m (https://www.paradipPort.gov.in/Port_Brochure_Static.aspx). Tides in this region are mixed semidiurnal type with an average tidal height of 1.87 m and 0.7 m during spring and neap tide, respectively. The average rainfall is 150 cm, with the maximum during active southwest monsoon SWM (June to August) and the little rainfall during FIM (retreating monsoon) from October to November. In general, the sediment texture is dominated by silt (59.0% ± 26.8%), followed by sand (37.3% ± 26.3%) and clay (2.8% ± 9.5%) during observation (Noyel and Desai, 2020).

Sampling strategy

Water and sediment sampling was carried out from 12 fixed stations in the Paradip port (Fig. 1) on four different occasions representing different seasons for the period from 2014 to 2015 [8–15 August 2014 (SWM), 13–22 December 2014 (NEM), 11–20 May 2015 (SIM), and 29 August to 8 September2015 (FIM)]. Since September represents the transition between SWM and NEM seasons (Mukherjee et al. 2014), it is referred to as FIM in this study. During 2015 SWM, peak rainfall was observed during June to August 2015 and a decrease after that (Suppl. Figure 1). All the sampling was conducted between low tide (1–1.5 m) and high tide (2–3 m) in the morning. Additionally, during each sampling season, a 24-h time series sampling (2-h interval) at the center of the port (station no. 7, Fig. 1) was also carried out to assess the chl *a* and pheopigments variations due to tides (Suppl. Figures 2–4). Water samples were collected using Niskin bottles (5 l) to measure environmental parameters such as salinity, dissolved oxygen, biological oxygen demand, phytoplankton pigments, and inorganic nutrients (nitrate, nitrite, ammonia, phosphate, and silicate). The water temperature was determined in situ using a multiparameter Sonde DS5X (Hydrolab). Salinity was measured with an Autosal (Guildline Autosal 8400B). Dissolved inorganic nutrients such as nitrate (NO_3), phosphate (PO_4), nitrite (NO_2), ammonium (NH_4), and silicate (SiO_4) were analyzed by SKALAR SANplus Analyzer. Dissolved oxygen (DO) and biological oxygen demand (BOD) were analyzed following standard methods (Parsons et al. 1984). For the pigment analysis, 250 to 500 ml of water samples were passed through GF/F filter (27 mm; pore size 0.7 mm) and stored in at – 80 °C until analysis using high-performance liquid chromatography (HPLC). For the sediment pigment analysis, sediment samples were collected using Van Veen grab. The top 2-cm surface sediment samples were collected using PVC corer

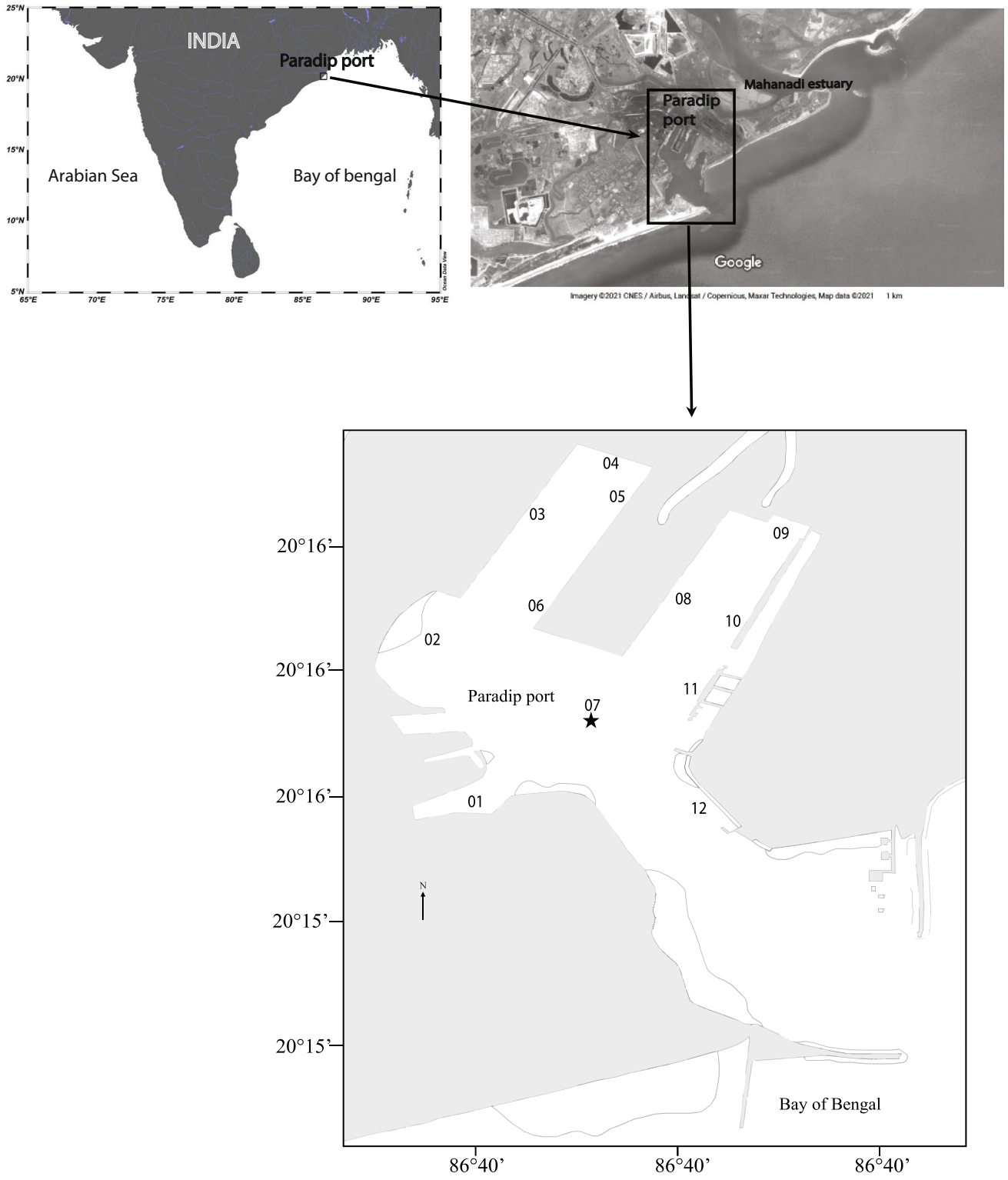

Fig. 1 Location of the study region and the sampling stations. 1 Boat basin, 2 area adjacent to fertilizer berth, 3 fertilizer berth II, 4 North Quay II, 5 Central Quay III, 6 Central Quay I, 7 turning circle, 8 East Quay II, 9 North Quay I, 10 coal berth I, 11 coal berth II, and 12 stone pitching side. Star symbol indicates location of time series station

with a diameter of 2.5 cm and stored in – 80 °C until further analysis.

Phytoplankton pigment analysis from the water column

The collected pigment samples were extracted by adding 3 to 4 ml of 90% acetone (V: V with the deionized water) to the frozen filter paper, which was sonicated using an ultrasonic probe (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) for 10 s at 20 kHz and kept on ice to prevent excessive heat. The extracts were then stored overnight at – 20 °C for analysis using HPLC (Agilent Technology; Series 1200). Before analysis, all the extracts were filtered (Nylon filter membranes, 0.2-µm pore size) immediately before injection in the HPLC to remove cells and debris from sonicated filter paper. To prevent the photolysis of the samples, extraction and injection procedures were performed under dim light. Pigment analysis was performed following the method of Van Heukelem (2002) with a slight modification as detailed subsequently. The pigment extracts were injected into the HPLC system equipped with an Agilent 1200 series pump, an Agilent diode array detector connected using a reverse-phase C8 Column maintained with 60 °C (150 × 4.6 mm, 3.5 mm particle size) with 3 phase linear solvent gradient program at a flow rate of 1.1 ml/min. The excitation and elution of the extracted pigments were detected at 450 and 665 nm. The quantitative and qualitative analysis of about 20 pigments, including pheopigments, was carried out using the commercially available pigment standards purchased from DHI Inc. (Denmark) and Sigma–Aldrich (USA). Chlorophyll degradation products such as pheophytin and pheophorbide were also calculated along with the other accessory pigments to understand the different season's ecosystem processes. Chlorophylls, carotenoids, and other chlorophyll breakdown products were quantified using the commercially available pigment standards purchased from DHI Inc (Denmark). Chlorophyllide, another degradation pigment, was not detected in any of the samples analyzed. Additionally, the following pigment ratios Chl a: total phoepigments (TPhe) and pheophorbide: pheophytin (Pheid: Phe) were also considered for better interpretation. Among these, only the average ratio of Pheid: Phe ratio presented in this manuscript was taken from Sathish et al. (2020), and it is used only to compare with the data from the near bottom and surface sediment to delineate the factors influencing benthic-pelagic coupling.

Phytoplankton pigment analysis from the sediments

Sediment samples (0.5 to 1 g) were transferred to the 15-ml amber colored polypropylene centrifuge tubes along with 4 ml of HPLC grade acetone. The mixture was then sonicated using an ultrasonic probe (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) for 10 s at 20 kHz and kept on ice to prevent excessive heat. After sonication, the mixture transferred to the deep freezer (– 20 °C) for overnight incubation. Further, the sample mixture was centrifuged, filtered with a syringe filter (0.2 µ), and stored in the deep freezer until analysis. Quantitative analysis of sediment pigments was carried out using the HPLC system equipped with an Agilent 1200 series pump, and an Agilent diode array detector connected using a reverse-phase C18 Column (4.6 × 250 mm, 5.5 mm particle size) with 3 phase linear solvent gradient program was used. This gradient program was a modification of Wright et al. (1991) described by Chen et al. (2016)to enhance the separation of pheopigments. The excitation and elution of the extracted pigments were detected at 450 and 665 nm. The quantitative and qualitative analysis of chlorophylls, carotenoids, and chlorophyll degradation products was carried out using the commercially available pigment standards purchased from DHI Inc (Denmark) and Sigma–Aldrich (USA). Chlorophyllide was not detected in any of the surface sediment samples as well.Pigment ratios Chl a: TPhe and Pheid: Phe were also calculated from analyzed sediment samples for better interpretation.

Statistical analysis

ANOVA and followed by post hoc Tukey tests were performed using Statistica software to evaluate the spatial and temporal variations in the PMPs (both concentrations and contributions) and pheopigment ratios (Pheid:Phe and Chl *a*: TPhe). To evaluate the relationship between relative contribution (%) of PMPs and measured environmental parameters (temperature, salinity, DO, BOD, PO_4, SiO_3, NO_2, NO_3, and NH_4), redundancy analysis (RDA) was performed by using the CANOCO 4.5 software (ter Braak and Smilauer 2002). For RDA, arcsine transformed data of relative contribution of PMPs were used. For surface water, PMPs and environmental parameters corresponding to surface water were used, whereas for NBW and sediment PMPs, environmental parameters corresponding to NBW were used. Before RDA, data was initially analyzed by detrended correspondence analysis. Since the most extended gradient length for the water column (Surface and NBW) and sediment was less than 3.0, RDA was performed.

Results

Environmental parameters

The results revealed that the measured environmental parameter showed a distinct seasonality. Water temperature ranged

from 25.2 to 30.4 °C with the highest temperature during FIM (29.7 ± 0.4 °C). The water column remained cooler during NEM (25.2 to 26.9 °C) and warmer during SWM (19.1 to 28.6) and SIM (27.4 to 28.8 °C). Salinity variations were wide (25.6 to 33.9), with the highest (33.8 to 33.9) salinity observed during SIM followed by NEM (28.9 to 32.6) and FIM (28.1 to 31.5). The lowest salinity was found during SWM (25.6 to 28.7). Higher salinity was observed in the NBW compared to surface water during all seasons, suggesting the freshwater influence in the port area. ANOVA revealed an insignificant difference ($p > 0.05$) in salinity and temperature between surface and NBW. DO concentration ranged from 2.6 to 5.6 μg l^{-1} with the maximum concentration (5.2 ± 0.4 μg l^{-1}) in the surface water during SWM. There was no considerable difference in the averaged DO concentrations during NEM, FIM, and SIM and ranged between 4.0 and 4.7 μg l^{-1}. ANOVA revealed a significant difference ($p < 0.001$) in DO concentrations for surface and NBW, except during NEM. BOD values ranged from negligible to 2.7 μg l^{-1} with the maximum and minimum demand during FIM (0.6 to 2.7 μg l^{-1}) and SWM (0 to 0.8 μg l^{-1}), respectively (Fig. 2).

NO_3 concentrations were high during FIM (1.2 to 14.7 μM) compared to other seasons, and during the rest of the seasons (SWM, NEM, and FIM), magnitude of the averaged values (4.0 to 4.8 μM) was the same. NO_2 concentration ranged from 0.4 to 3.3 with the maximum and minimum during SIM (1.8 ± 0.2 μM) and SWM (0.9 ± 0.2 μM), respectively. PO_4 concentrations ranged up to 2.8 μM with the higher and lowest values during SIM (2.2 to 3.0 μM) and FIM (0 to 0.5 μM), respectively. Highest SiO_4 levels were observed during FIM (8.1 to 134.9 μM) and SIM (52.3 to 100.0 μM) compared to NEM (31.8 to 106.4 μM) and SWM (13.7 to 27.0 μM). Interestingly, SiO_4 concentrations (71.1 ± 41.9 μM) were relatively higher in the NBW compared to surface water (60.3 ± 37.6 μM) during FIM. There was no significant difference ($p > 0.05$) in PO_4, NO_3, NO_2, and SiO_4 values between surface and NBW (Fig. 3).

Phytoplankton pigment concentrations

Altogether 16 pigments were observed in the water column, and 11 pigments were observed in the surface sediment samples. Chl *a*, Chl *b*, Fuco, Zea, Allo, Peri, Phe *a*, and Pherd *a* were the primary pigments observed in the water column and sediment. The results indicated a distinct seasonality in the distribution of biomass (chl *a*) and marker pigments (for different groups and degradation) from the sediment and overlying water column. However, the seasonal trend in the surface and NBW was the same but not with the sediment. In the surface and NBW, chl *a* ranged between 0.1 and 8.8 μg l^{-1}. The higher values were observed during spring (1.1 to 3.8 μg $^{-1}$) and fall (0.7 to 3.1 μg l^{-1}) inter-monsoons compared to the southwest (0.2 to 0.7 μg l^{-1}) and northeast (0.1 to 1.6 μg l^{-1}) monsoons. The chl *a* in sediments ranged between 0.01 and 0.2 μg g^{-1}, and the higher values were observed during southwest (0.03 to 0.14 μg g^{-1}) and northeast (0.02 to 0.24 μg g^{-1}) monsoons compared to fall (0.01 to 0.05 μg g^{-1}) and spring (0.03 to 0.10 μg g^{-1}) inter-monsoons (Fig. 4 and Table 1). Next to chl *a*, Fuco, a marker pigment of diatom was the dominant accessory pigment with average of all stations ranged between 0.07 and 0.60 μg g^{-1} in the water column and ranging from 0.01 to 0.13 μg g^{-1} in the sediment. High Fuco concentrations were observed in the water column during fall and spring inter-monsoons (average for all stations, 0.29 to 0.60 ug l^{-1}) and low values during southwest and northeast monsoons (average 0.07 to 0.13 μg l^{-1}), and the reverse trend was observed in the sediments. Moreover, significantly higher concentrations of Chl *b*, followed Zea, Allo, and Peri, which are considered as marker pigments for chlorophyte, cyanobacteria, cryptophytes, and dinoflagellates, respectively, were observed during inter-monsoons (fall and spring) compared to monsoons (southwest and northeast; Table 1). Interestingly, 19' hexafucoxanthin and prasinoxanthin, which are marker pigments for prymesiophytes and prasinophytes, were present on most of the occasions, but the contribution was negligible in the water column and was not detected in the sediments. Phe *a*, and Pherd *a* are the primary and secondary breakdown product of the Chl *a*. The average concentration of Phe *a* and Pherd *a* in the water column ranged between 0.02 and 0.14 μg l^{-1}, whereas in the sediments, the ranges for former and latter were 0.15–1.26 and 0.06 to 0.32 μg g^{-1}, respectively. Two-way ANOVA of all the detected pigments revealed significant variations ($p < 0.001$) between seasons, but not between the stations and two hourly sampling (i.e., no tidal effect).

Phytoplankton marker pigment contributions

Group-specific phytoplankton marker pigments such as Chl *b* (chlorophytes), Fuco (diatom), Zea (cyanobacteria), Allo (cryptophytes), Peri (dinoflagellates), Pras (prasinoxanthin), and 19' Hexa (prymesiophytes) showed distinct seasonal and depth variations (among the surface, NBW, and sediment) in the contributions. However, the percentage contribution varied between the water column depth and sediment (Fig. 5). Among the PMP's, Fuco, Chl *b*, and Zea were dominant marker pigments in both water column and sediment. In water column, Fuco (Avg. 31.9 to 69.2%) was the dominant marker pigment followed by the Chl *b* (Avg. 10.4 to 34.4%) and Zea (Avg. 4.3 to 19.9%), whereas in sediment, Chl b (Avg. 30 to 38.1%) contribution was higher followed by Zea (Avg. 24.2 to 30.2%) and Fuco (Avg. 14 to 27%). Fuco contributions in the surface water ranged between the

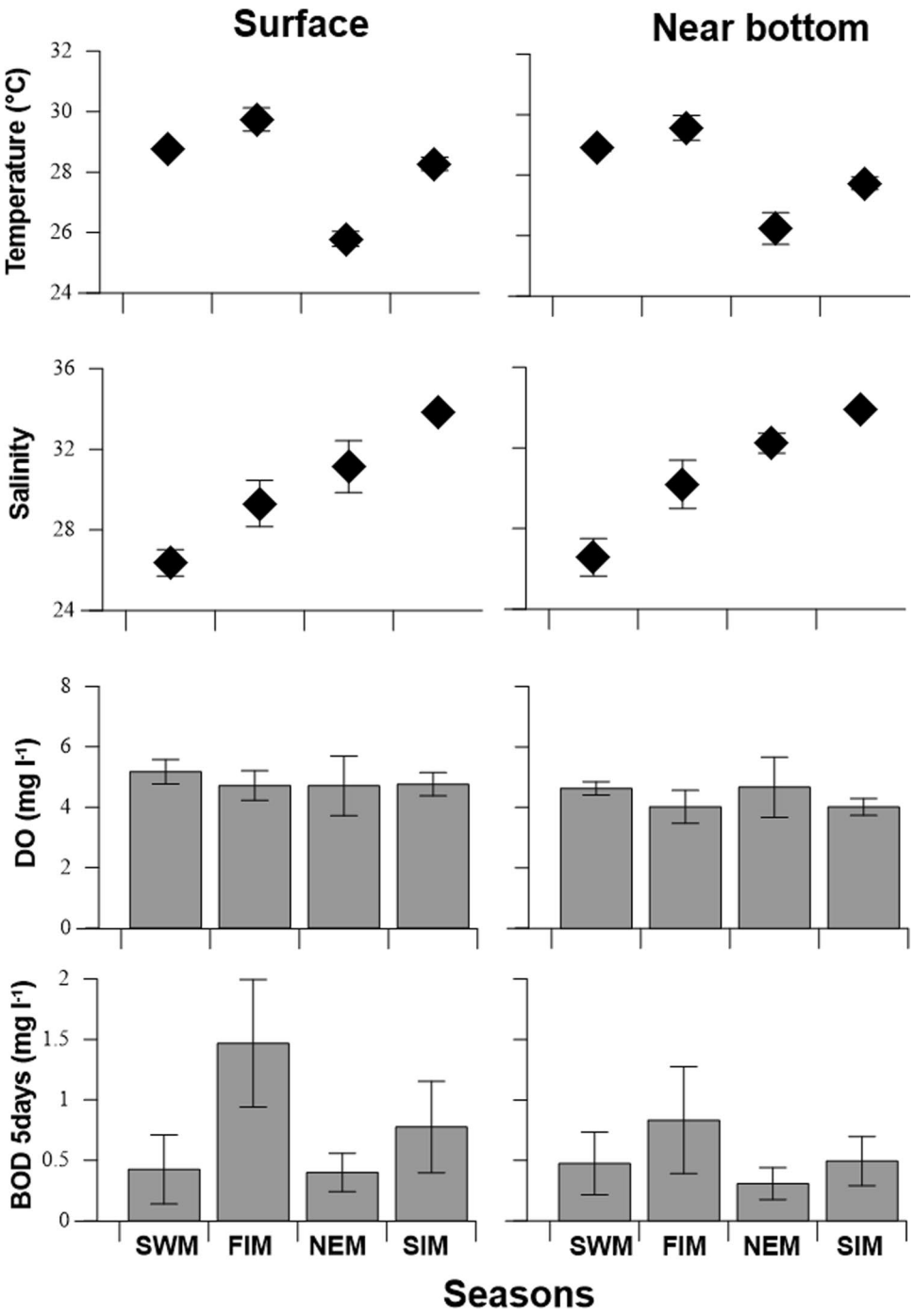

Fig. 2 Seasonal variations in the measured environmental variables from the surface and near bottom (NBW) waters. SWM, southwest monsoon; NEM, northeast monsoon; FIM, fall inter-monsoon; SIM, spring inter-monsoon. Bars represent mean value for the 12 stations, and the error bars indicate standard deviations from 12 stations

18.3 to 70.7% with the maximum contributions during SWM (52.3 ± 7.9%) and SIM (43.8 ± 10.2%) compared to FIM (40.3 ± 13.5%) and NEM (31.9 ± 9.7%), whereas in NBW, Fuco contributions ranged between 21.9 to 80.1% with the higher contribution during spring (61.7 ± 4.1%) and fall (69.2 ± 10.2%) inter-monsoons compared to SWM (55.3 ± 7.8%) and NEM (37.6 ± 15.2%). In sediment, Fuco contributions ranged between 6.9 and 46.1% with maximum contribution during SWM (27 ± 9.4%) and NEM (17.7 ± 4.5%) compared to SIM (16.2 ± 6.1%) and FIM (14 ± 4.3%). Next to Fuco, Chl *b* was the most dominant pigment in the water column, whereas in sediment, Chl *b* was the dominant PMP. In water column, Chl *b* ranged between 2.9 and 49.3% with the maximum contribution during NEM (2.9 to 49.3%) and SWM (12.8 to 35.4%) compared to SIM (10.6 to 32.6%) and FIM (5 to 27.2%), whereas in sediment, Chl *b* ranged between 13.6 and 62.9% with the higher contributions during FIM (27.1 to 47.4%) and SIM (19.3 to 62.9%) compared to NEM (13.6 to 58.7%) and SWM (20.8 to 41.4%). The

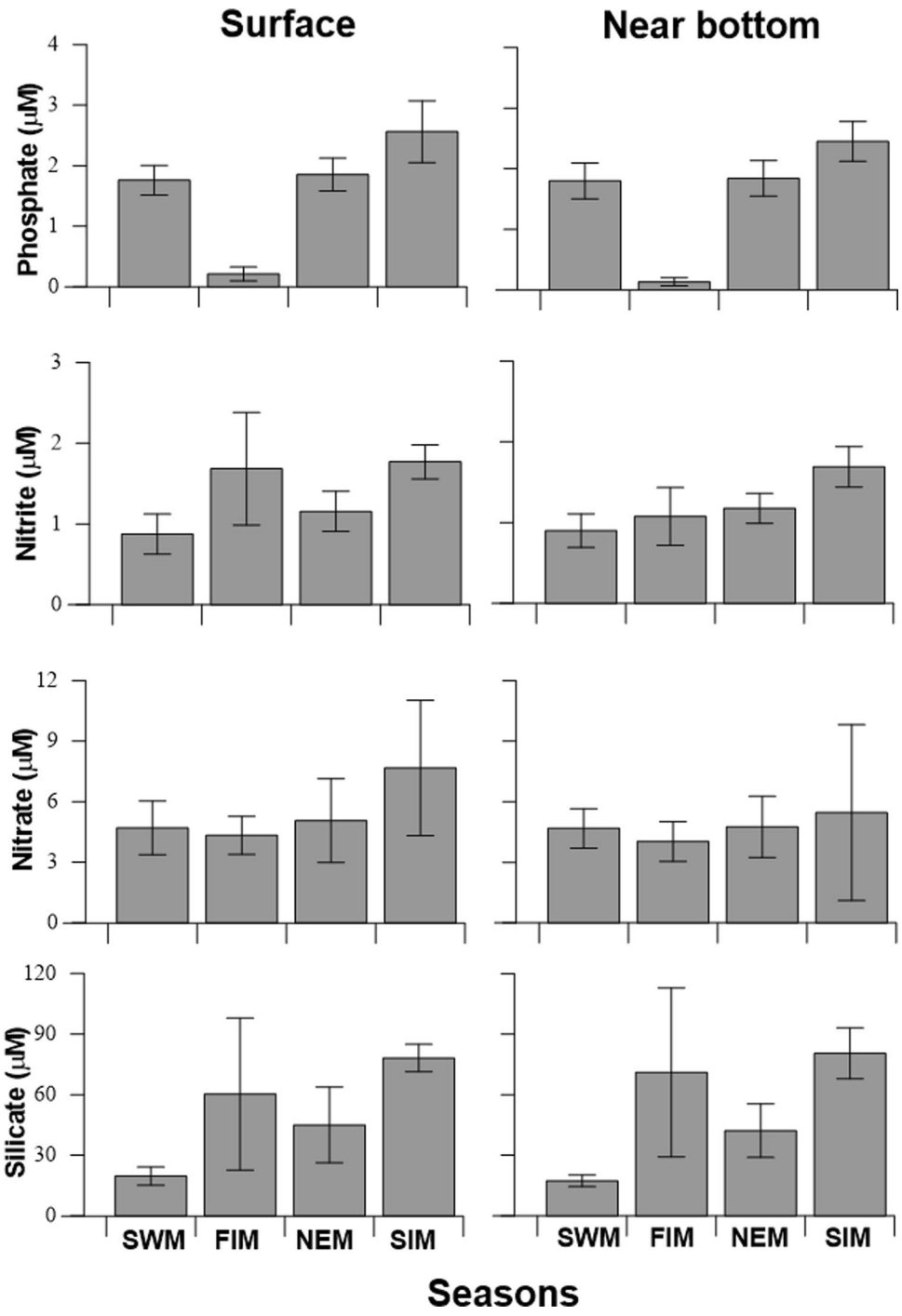

Fig. 3 Seasonal variations in the nutrient concentrations from the surface and near bottom (NBW) waters. SWM, southwest monsoon; NEM, northeast monsoon; FIM, fall inter-monsoon; SIM, spring inter-monsoon. Bars represent mean value for the 12 stations, and the error bars indicate standard deviations reflecting the differences among stations

contribution of Zea, the third highest contributor, in the water column ranged between 0 and 38.9% with the maximum contributions during NEM (2.8 to 38.9%) and FIM (0 to 38.4%) compared to SWM (4.3 to 15.7%) and SIM (3.5 to 18.9%), whereas in sediment, it ranged between 14.1 and 41.3% with higher contribution during NEM (18.3 to 40.5%) and SIM (17.6 to 41.3%) compared to SWM (15.8 to 35.9%) and FIM (14.1 to 35.7%). The contribution of other PMP was minimal and notable but not similar between water column and sediment. For instance, Peri and Allo contributions were higher in the sediment (Avg. Peri; 5.7 to 9.5% and Allo; 7.8 to 16.6%) compared to water column (Avg. Peri; 0.8 to 7.7% and Allo; 2.4 to 6.2%). In the water column, Allo contribution was maximum during SIM (2.8 to 11.6%) and SWM (2.2 to 12.8%) and minimum during NEM (0 to 9.2%) and FIM (1.1 to 8.2%), whereas in sediment, it was maximum during SWM (7.1 to 24.0%) and FIM (8.2

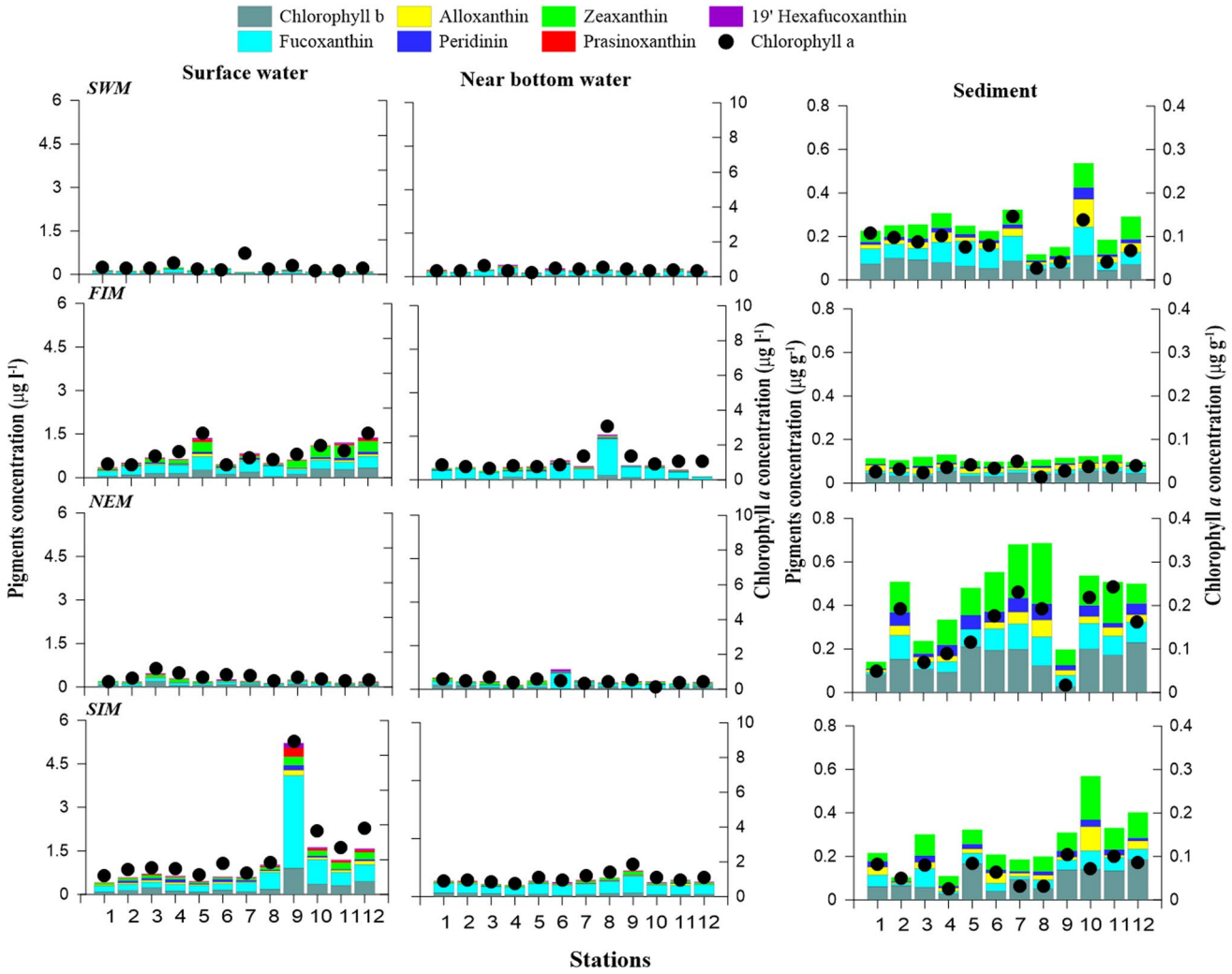

Fig. 4 Spatial and seasonal variations in the concentrations of phytoplankton marker pigments from the overlying waters (surface and near bottom, NBW) and sediments. SWM, southwest monsoon; NEM, northeast monsoon; FIM, fall inter-monsoon; SIM, spring inter-monsoon

to 22.5%) compared to SIM (3.3 to 23%) and NEM (0 to 12.6%). In the case of Peri, contribution was higher during SIM (0 to 17.1%) and FIM (0 to 12%) compared to SWM (0 to 8.3%) and NEM (0 to 10.3%) in the water column, whereas in sediment, it ranged between 2.9 and 15% with maximum contribution during NEM (2.9 to 15%) and SIM (3.5 to 12.4%). While 19 hexa (Avg. 1.2 to 6.4%) and Pras (Avg. 2.8 to 5.5%) were the least contributor and were detected only in water column. Their maximum contributions were observed during NEM and FIM (only Pras). Two-way ANOVA of PMP revealed significant ($p < 0.01$) variations between the sampling seasons (except for Peri and Pras) and verticals (i.e., among the surface water, NBW, and sediment), but the variation was insignificant ($p > 0.05$) between the sampling stations and two hourly sampling (i.e., no tidal effect).

Chlorophyll a and pheopigment ratios

In this study, chl *a*:total phoepigments (TPhe) and Pheophorbide:pheophytin ratios were considered as the former, and the latter indicates the microalgal status and nature of the degradation pathway, respectively. Chl *a*: TPhe ratio was high in the water column (average 3.95 to 9.17) compared to sediment (0.11 to 0.17) (Fig. 6). In surface water, the maximum of the average was observed during SIM (9.17 ± 3.57) followed by SWM (9.00 ± 3.59) and FIM (8.46 ± 3.76). The lowest value was observed during NEM (5.59 ± 5.02). Whereas in NBW, the maximum Chl *a*:TPhe ratio (6.28 ± 2.75) was observed during NEM, while the minimum (3.95 ± 2.26) was observed during SWM (Fig. 6). Chl *a*: TPhe ratio from the surface sediments was observed < 0.17 round the year. ANOVA results indicate there was no significant ($p > 0.05$) seasonal or spatial

Table 1 Seasonal variations in the concentrations of chlorophylls, carotenoids, and pheopigments from the surface water, near bottom water (NBW) and surface sediments

Average and range of pigment concentrations												
Pigments	**SWM**			**FIM**					**NEM**	**SIM**		
	Surface	**NBW**	**Sediment**	**Surface**	**NBW**	**Sediment**	**Surface**	**NBW**	**Sediment**	**Surface**	**NBW**	**Sediment**
chlorophyll												
Chl *a* (µg)	0.38 (0.20–0.69)	0.42 (0.25–0.65)	0.08 (0.03–0.15)	1.42 (0.72–2.57)	1.13 (0.67–3.08)	0.03 (0.01–0.05)	0.56 (0.32–1.04)	0.56 (0.12–1.63)	0.15 (0.02–0.24)	2.54 (1.08–8.8)	1.10 (0.77–1.86)	0.07 (0.03–0.11)
Chl *b* (µg)	0.03 (0.01–0.07)	0.05 (0.02–0.12)	0.07 (0.05–0.11)	0.07 (0.05–0.11)	0.05 (0.01–0.16)	0.04 (0.03–0.06)	0.08 (0.03–0.20)	0.08 (0.01–0.15)	0.15 (0.03–0.23)	0.26 (0.09–0.91)	0.10 (0.05–0.14)	0.10 (0.03–0.19)
Chl *c* (ng)	3.1 (0.7–8.4)	7.6 (2.7–14.3)	0	22.4 (11.2–42.9)	48.2 (10.6–263)	0	16.5 (2.1–39.3)	20.9 (8.5–33.9)	0	79.4 (0.0–740.8)	25.7 (11.9–55.7)	0
carotenoids												
PSC												
19BUT (ng)	2.6 (0–5.2)	10.9 (0–23.1)	0	27.0 (0–94.2)	7.2 (0–18.9)	0	0.6 (0–7.2)	1.8 (0–6.0)	0	18.2 (0.0–77.1)s	7.0 (0.0–17.7)	0
Fuc (µg)	0.07 (0.05–0.12)	0.13 (0.07–0.19)	0.07 (0.02–0.13)	0.29 (0.17–0.46)	0.37 (0.08–1.25)	0.02 (0.01–0.02)	0.07 (0.03–0.12)	0.13 (0.05–0.54)	0.08 (0.01–0.13)	0.60 (0.19–3.19)	0.34 (0.24–0.58)	0.05 (0.01–0.09)
19Hexa (ng)	1.6 (0–5.8)	12.3 (0–40.5)	0	18.2 (0–46.8)	15.4 (0–38.4)	0	14.9 (0–44.7)	15.3 (0–78.1)	0	38.6 (0.0–208)	15.6 (0.0–38.3)	0
Per (ng)	4.7 (0–13.9)	6.2 (0–13.1)17.8 (7.7–52.6)	17.8 (7.7–52.6)	49.6 (25.5–91.8)	20.4 (0–39.5)	6.2 (3.6–8.3)	2.3 (0–8.9)	8.7 (0–28.0)	44.0 (4.1–74.8)	68.9 (4.9–174)	68.9 (4.9–174)	19.4 (4.2–31.4)
PPC												
Allo (µg)	0.01 (0.00–0.02)	0.01 (0–0.02)	0.03 (0.01–0.20)	0.04 (0.01–0.1)	0.01 (0.00–0.02)	0.02 (0.01–0.03)	0.01 (0.00–0.02)	0.01 (0–0.02)	0.03 (0–0.08)	0.07 (0.03–0.19)	0.03 (0.02–0.07)	0.03 (0.01–0.11)
B-car (ng)	5.7 (1.4–18.3)	5.3 (2.0–9.9)	0	23.5 (4–49.4)	11.6 (2.0–32.5)	0	16.5 (10.4–29.3)	12.2 (0–25.3)	0	61 (20–185)	19.1 (8.8–58.2)	0
Diad (ng)	7.7 (4.5–17.2)	17.0 (5.6–28.3)	19.9 (5.3–63.1)	42.4 (0–108.1)	45.1 (13.3–159)	5.8 (2.3–9.2)	13.6 (3.9–24.3)	12.1 (4.5–33.0)	20.0 (2.9–37.1)	85.3 (32.1–362)	30.9 (19.8–48.5)	12.6 (0.0–20.9)
Diat (ng)	1.7 (0–2.9)	8.9 (0–20.4)	50.6 (16.2–143)	15.2 (0–28.5)	5.5 (2.0–14.2)	23.8 (15.6–35.6)	6.5 (1.7–14.9)	5.0 (0–18.4)	105 (14.1–208)	28.9 (8.5–114)	10.0 (5.7–16.2)	54.5 (14.3–130.3)
Lut (ng)	4.0 (2.3–8.3)	11.3 (4.9–27.3)	96.5 (0–402.6)	39.4 (10.6–91.5)	8.1 (2.5–15.5)	47.5 (23.1–65.6)	17.8 (2.4–49.7)	19.4 (5.2–37.5)	151 (26.2–244)	29.4 (13.0–72.3)	12.6 (0.0–18.7)	151.3 (34.1–442.1)
Viola (ng)	3.0 (0.0–5.1)	19.6 (3.7–41.3)	4.7 (0–12.8)	28.2 (9.1–51.8)	14.9 (0–25.6)	3.4 (0–5.7)	10.7 (0–20.1)	14.5 (0–37.6)	7.9 (0.0–13.7)	43.8 (15.1–185)	28.3 (18.9–47.4)	9.3 (0.0–55.9)
Zea (µg)	0.02 (0.01–0.04)	0.02 (0.01–0.04)	0.06 (0.03–0.12)	0.18 (0.03–0.43)	0.04 (0.01–0.08)	0.03 (0.01–0.04)	0.04 (0.02–0.10)	0.05 (0.02–0.10)	0.14 (0.03–0.28)	0.12 (0.04–0.28)	0.05 (0.02–0.10)	0.08 (0.02–0.20)
Neo (ng)	3.7 (0–8.7)	19.7 (6.8–40.2)	10.1 (2.6–24.1)	37.5 (8.3–86.3)	5.9 (0–13.9)	5.7 (2.1–11.5)	9.7 (0–26.6)	9.1 (0–28.6)	17.2 (4.1–26.8)	67.5 (12.8–341)	25.3 (8.9–52.7)	15.2 (4.9–42.9)

Table 1 (continued)

Average and range of pigment concentrations

Pigments	SWM			FIM		NEM		SIM	
	Surface	NBW	Sediment	Surface	NBW	Sediment	Surface	NBW	Sediment	Surface	NBW	Sediment
Pras (ng)	4.1 (2.0–8.7)	10.3 (0–23.2)	0	42.7 (20.3–99.7) 15.60.11 (0.04–0.18)	15.6 (0–30)	0	12.9 (0–30.2)	12.4 (0–23.7)	0	62.2 (17.1–310)	16.7 (10.0–24.6)	0
Deg product												
Pheo *a* (µg)	0.02 (0.01–0.04)	0.049 (0–0.07)	0.51 (0.13–0.99)	0.11 (0.04–0.18)	0.11 (0.09–0.19)	0.15 (0.06–0.21)	0.06 (0.03–0.1)	0.05 (0–0.09)	1.26 (0.30–4)	0.14 (0.06–0.22)	0.11 (0.06–0.21)	0.57 (0.15–1.92)
Pheide *a* (µg)	0.02 (0.01–0.09)	0.078 (0–0.15)	0.32 (0.05–0.8)	0.07 (0.000–0.12)	0.11 (0.03–0.26)	0.06 (0.04–0.07)	0.08 (0–0.20)	0.04 (0–0.11)	0.27 (0.05–0.56)	0.14 (0.05–0.67)	0.13 (0.06–0.24)	0.15 (0.04–0.25)

Chl values from water (surface and NBW) and sediments are in l^{-1} and g^{-1} dry weight, respectively. *SWM* southwest monsoon; *NEM* northeast monsoon; *FIM* fall inter-monsoon; *SIM* inter-monsoon; *PSC* photsynthetic carotenoids; and *PPC* photoprotective carotenoids

variation in the Chl *a*: TPheo ratio from the water column as well as from the sediment. Pheid: Phe ratios were also high in the water column (average 0.67 to 1.39) compared to surface sediment (0.40 to 0.76) (Fig. 7). Box and whiskers plot (Fig. 7) indicating the higher Pheid:phe median values observed in the NBW (0.89 to 1.17) compared to surface water (0.55 to 0.72) except during NEM season (surface water, 1.04; NBW, 0.62). Whereas in sediment, median values are much lower (0.24 to 0.39) compared to water column depths. Variation between the quartiles was higher in the NBW compared to surface water during all the seasons, while in sediment, large variations observed only during SWM. Statistical outliers observed in the surface water (during SWM, NEM, and SIM) and sediment (FIM, NEM, and SIM) due to some stations with higher Pheid: Phe ratios. In surface water, Pheid: Phe ratio was high during NEM (1.39 ± 1.33) followed by SIM (1 ± 1.02), SWM (0.99 ± 0.78), and FIM (0.71 ± 0.45), while in NBW, highest Pheid: Phe ratios were observed during SIM (1.24 ± 0.5) and SWM (1.19 ± 0.66) compared to FIM (0.88 ± 0.35) and NEM (0.67 ± 0.46). Pheid: Phe ratio in the surface sediment showed the maximum (0.76 ± 0.59) during SWM and the minimum (0.40 ± 0.44) during NEM (Fig. 7). ANOVA results suggested that there was no significant ($p > 0.05$) spatial and temporal (seasonal and two hourly) variations in Pheid: Phe ratio between the stations or seasons.

Relationship between environmental parameters and PMP contribution

Redundancy analysis was performed to understand the relationship between environmental variables and group-specific PMP's contributions (Fig. 8). Temperature, salinity, PO_4, and NO_2 are the major variables significantly influencing the PMP's from the water column and sediment. In surface water biplot, axes 1 and 2 explained 69.1 and 91.8% variance associated with the marker pigments and environmental variables, respectively. Fuco positioning with the temperature and low salinity, SiO4, and NO_3 suggested that the warmer and low salinity conditions favored diatoms during inter-monsoons (fall and spring) and SWM by utilizing SiO_4 and NO_3. Chl *b* and 19' Hexa positioned with the high salinity and low temperature conditions suggesting the cooler temperature and high salinity during NEM favored chlorophytes and prymnesiophytes. Peri and Allo positioned with the high temperature, Chl *a*, and NO_2, suggesting that these conditions during inter-monsoons coincided with the higher dinoflagellates and cryptophytes. Zea and Pras were positioned with the high NO_3, SiO_4, and low PO_4 and NH_3 values and are mainly due to the increased contributions of cyanobacteria and prasinophytes during FIM and NEM.

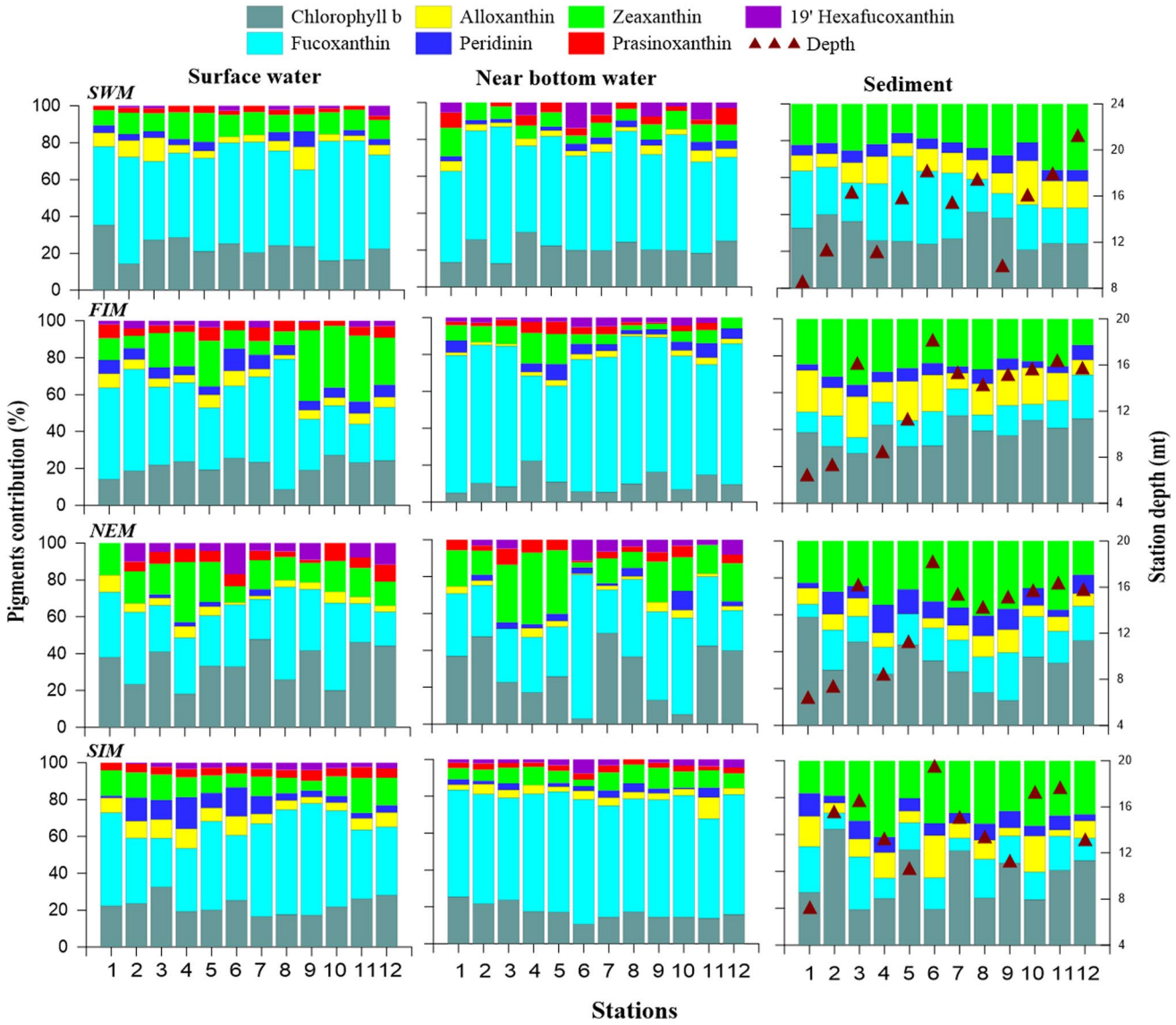

Fig. 5 Seasonal variations in the chlorophyll *a* to total pheopigment ratios from the surface and near bottom waters. SWM, southwest monsoon; NEM, northeast monsoon; FIM, fall inter-monsoon; SIM, spring inter-monsoon. Bars represent mean value for the 12 stations, and the error bars indicate standard deviations reflecting the differences among stations

In NBW biplot, axes 1 and 2 explained 94.4 and 97.4% variance associates with the marker pigments and environmental variables, respectively. Relationship with the PMP contributions and environmental variables in NBW is different compared to the surface water (except for Fuco). Like surface water, Fuco positioned with the high temperature and Chl *a* concentrations suggesting that the warmer conditions during inter-monsoons favored phytoplankton biomass, particularly diatoms. On the other hand, high PO_4, NO_2, and NO_3 and low SiO_4 concentrations favored the next dominant pigment Chl *b* during NEM and SWM. While the third dominant pigment Zea and the least contributed pigment Pras dominance during NEM coincided with high PO_4, NH_3, and DO values and lower temperature and Chl. Positioning of Peri with high SiO_4 and low PO_4, NO_2, and NO_3 concentrations suggesting their role in favoring dinoflagellates during FIM and NEM while the 19 hexa and Allo showed the insignificant relationship with the environmental variables in NBW.

In sediment biplot, axes 1 and 2 explained 57.3 and 84.7% variance associates with the marker pigments and environmental variables, respectively. Relationship trend between sediment PMP contributions and environmental variables is different compared to surface water and NBW. The contribution of the most dominant PMP Chl

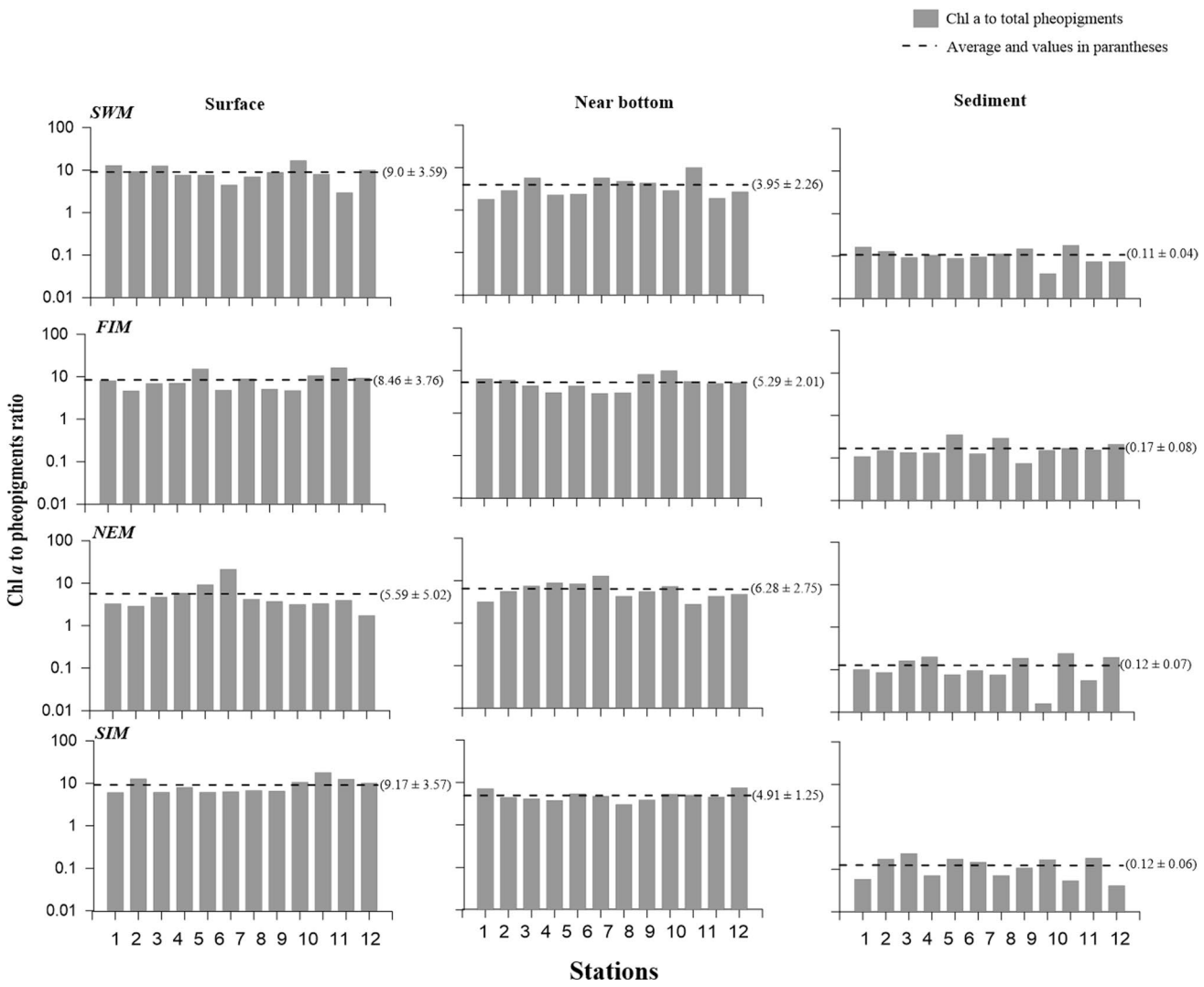

Fig. 6 Seasonal variations in the pheophorbide to pheophytin ratios from the surface and near bottom waters. Bar plot represents (a) station wise data and (b) mean value for the 12 stations, and the error bars indicate standard deviations reflecting the differences among stations for each season. SWM, southwest monsoon; NEM, northeast monsoon; FIM, fall inter-monsoon; SIM, spring inter-monsoon. Bars represent the mean value for the 12 stations, and the error bars indicate standard deviations reflecting the differences among stations. *Numbers in the parenthesis represent values for outliers

b was positioned with the high salinity and SiO_4, and lower NH_3 and DO values indicated their influence on the chlorophytes dominance during inter-monsoons (fall and spring). The increase in Fuco contribution during SWM and NEM seasons could be due the prevalence of high DO, Chl *a*, and NH_3 values and lower salinity, Chl *a*, SiO_4, and NO_2 concentrations. The notable contribution of Allo was coincided with the high temperature and low PO_4, DO, and Chl *a* suggesting their role in favoring cryptophytes during SWM and FIM. The Zea and Peri contributions positioned with the environmental settings (high salinity, Chl *a*, NO_2, and PO_4 values and low temperature) coincided with higher cyanobacteria and dinoflagellates during NEM and SIM.

Discussion

The present study elucidates the usefulness of phytoplankton marker pigments (such as chlorophylls, carotenoids, and pheopigments) to understand the benthic-pelagic coupling from the Paradip port ecosystem. The measured environmental variables showed a distinct seasonal variation, and this was also reflected in the distribution of phytoplankton biomass and group-specific marker pigments (Fig. 2, 3 and 8). For instance, (i) water temperature followed the classic radiation pattern and so with the phytoplankton community (Fig. 8; Sarma et al. 2013), i.e., flagellates dominated in cooler and diatoms in warmer during NEM and FIM, respectively; (ii) seasonal freshening of surface water (during

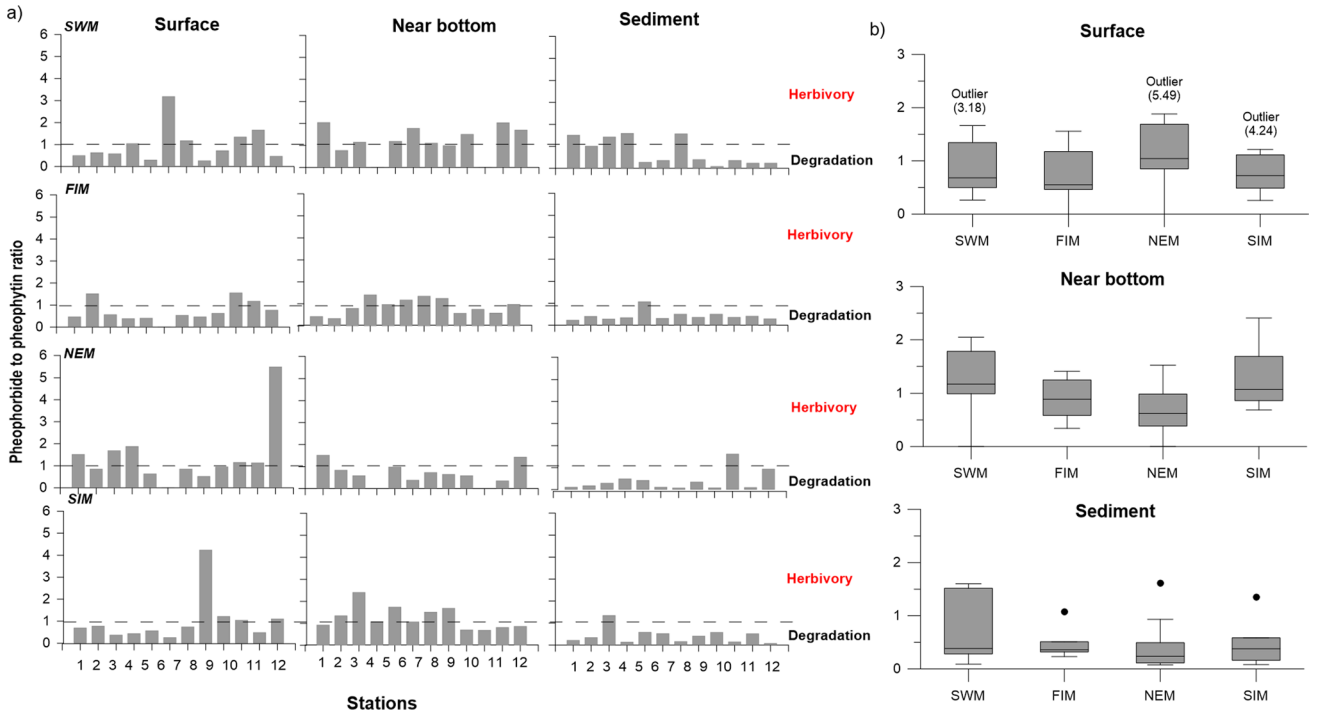

Fig. 7 Redundancy analysis biplot to understand the relationship between the measured environmental variables and relative contribution of phytoplankton marker pigments (–Fuco, Fucoxanthin; chl b, chlorophyll b; zea, zeaxanthin; allo, alloxanthin; peri, peridinin; pras, prasinoxanthin; 19 hex, 19' hexafucoxanthin) for (a) surface water, (b) near bottom water, and (c) surface sediments. In case of sediments, environmental variables corresponding to near bottom waters were used

SWM) coincided with low biomass; and (iii) seasonal influx of high inorganic nutrients (Sundaray et al. 2006) coincided with high phytoplankton biomass. The nutrient concentrations were not depleted in the study area throughout the year (except phosphate during FIM; Figs. 3 and 8). Generally, low water movement and current effects from the inner port area of Ceuta port (North Africa) and Tolo Harbour (Hong Kong) explain that the port structure negatively influences the benthic community (Guerra-García and García-Gómez 2005) and induced algal blooms (Wong and Wong 2004), respectively. Paradip port, which is considered as a semi-enclosed body, also supports this assumption of relatively higher nutrient concentrations and restricted circulations. Though Paradip port handles large volume of cargo, the influence

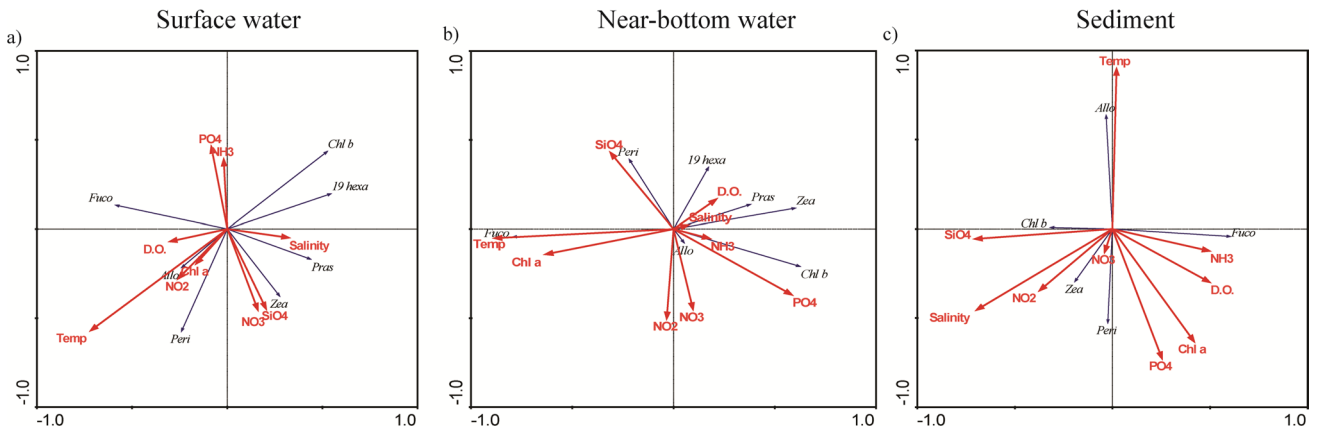

Fig. 8 Bar graphs showing the spatial and seasonal variations in the contribution (%) of phytoplankton marker pigments for the overlying waters (surface and near bottom, NBW) and sediments. SWM, southwest monsoon; NEM, northeast monsoon; FIM, fall inter-monsoon; SIM, spring inter-monsoon

of anthropogenic activities on the ecosystem (particularly on nutrient concentrations and phytoplankton biomass) is much less compared to some of the major ports along the Indian coasts. For instance, the nutrient concentrations and phytoplankton (biomass and composition) are much less than the other major ports along the Indian coasts (Sathish et al. 2020). Further, the statistically no significance between the stations also signifies nearly homogenous (except at few instances during inter monsoons) phytoplankton pigment distribution during the study period (Fig. 4). Nevertheless, these findings on spatio-temporal variations agree (only distribution trends) with previous studies conducted during 2011–2012 (Mohapatra and Panda, 2017).

Previous microscopic studies from Paradip port (Mohapatra and Panda 2017) as well as from the east coast of India (Gouda and Panigrahy 1989; Gouda and Panigrahy 1996; Madhupratap et al. 2003; Panigrahy et al. 2006; Paul et al. 2008; Naik et al. 2008; Chakraborty et al. 2011; Baliarsingh et al. 2012) suggest that diatom and dinoflagellates are the dominant phytoplankton groups from the water column. However, the information on the smaller or other taxonomic groups of phytoplankton is lacking. In our study, the distribution and dynamics of sedimentary pigments did not match the dynamics of phytoplankton pigments in surface water and NBW. PMP observed from the sediment could be the recent settlement of phytoplankton due to the high sedimentation and resuspension (Ananth and Sundar, 1990). The chlorophyll *a* distribution indicates that the Paradip port waters are more productive during inter-monsoons (fall and spring) compared to the monsoons (southwest and northeast), but pigment markers from the surface sediment exhibit the reverse trend, i.e., high and low during monsoons and inter-monsoons, respectively (Fig. 3). This could be due to the termination followed by sedimentation of water column phytoplankton chlorophyll *a* to the sediment towards the end of inter-monsoons.

However, the contribution of the major phytoplankton marker pigments was different between the surface and near bottom waters as well as between those and sediments (Table 1 and Fig. 8). The high concentrations of Fuco, chl b, and Zea in the water column indicate the dominance of diatom, chlorophytes, and cyanobacteria (Jeffrey and Vesk 1997). Diatom was the dominant group during warmer seasons, except during NEM (i.e., winter) season. Higher diatom contribution observed in the NBW compare to the surface water (Fig. 8) was likely attributable to their ability to adapt in low light conditions (Lionard et al., 2005). Additionally, relatively higher Pheid:phe ratio also suggests the prevalence of herbivory over the degradation due to senescence in the NBW compared to surface water (except during NEM). Apart from the diatom, chlorophytes followed by cyanobacteria are other dominant group with the higher contribution during NEM. These groups, which proliferated under relatively saline and high concentrations of macronutrients (PO4, NO3 and SiO4), showed a significant depth wise (between surface and NBW stations) and seasonal variations. PMPs such as Pras, Per, and 19 Hexa, which indicates the presence of prasinophytes, dinoflagellates, and prymnesiophytes respectively (Jeffrey and Vesk 1997), were the least contributors (Table 1 and Fig. 8) and also showed a significant difference between surface and NBW. Since, in all seasons, the water column is well-mixed as evidenced from the same water temperature, salinity, and DO between the surface and bottom waters, the light availability and the biological activity (as evidenced from BOD data and pheophorbide: pheophytin ratio) could influence the variability in PMP relative contribution, chl *a*, and its degradation between surface and bottom water. In surface sediment, higher contributions of Chl *b*, Zea, Fuco, and Allo indicated the dominance of chlorophytes, cyanobacteria, diatom, and cryptophytes, respectively. Chl *b* and Zea were major contributors followed by Fuco and Allo. Fuco, Pras, and 19 Hexa contributions decreased compared to the water column, whereas the Zea, Allo, Chl b, and peridinin concentrations increased markedly. Higher Zea and Allo concentrations during monsoons (SWM and NEM) coincided with the higher sediment chlorophyll *a* concentration. The variations in pigment concentrations indicate increase in the relative contributions of chlorophytes, cyanobacteria, cryptophytes, and dinoflagellates as well as a decrease in the diatom. Marker pigments representing prymnesiophytes, and prasinophytes are negligible, and it could be existed in the below the detection limits (Table 1; Figs. 4 and 8). The relative contribution PMP for phytoplankton groups indicates that the organic matter in the sediment was majorly from the chlorophytes followed by cyanobacteria, diatoms, cryptophytes, and dinoflagellates (Fig. 8).

The diatom dominance in the water column was not reflected in the sediment, and the possible reasons are delineated in this study. Some recent studies suggested that the nano and picophytoplankton might be crucial in fueling organic matters to the benthic community (Chen et al. 2016). The dominance of chlorophytes (chl b) in the sediment can be explained by the following (i) selective grazing by zooplankton on diatom and other groups decreases the percentage contribution (ii) much of the settled diatom could be grazed by benthic invertebrates (iii) marker pigments of diatom are least stable than marker pigments of chlorophytes and cyanobacteria. Bianchi et al. (2000) observed comparable decay constant (0.04–0.07 day 1) for both Chl a and Fuco in laboratory sediment microcosms. Meanwhile, Schüller et al. (2015) reported the decay rate constant k of phytoplankton pigments in sediment cores from New Zealand fjords, which suggested that Allo, Zea, and Chl b were less degradable than Chl a: $k\ (y-1) = -0.004$ for Chl a, -0.001 for Chl b, -0.0002 for Zea, $+0.0001$ for

Allo, where negative and positive value means pigments decay and accumulate within the core, respectively. Previous studies have pointed out that chl b, leutin, Zea, and allo are more chemically stable than Fuco, which attributed to the presence of 5,6 epoxides in Fuco (Repeta and Gagosian 1987). This would also be consistent with the result that the contribution by Fuco was lower and the contribution by Zea was higher in the sediments, compared to those in the water column. In spite of the smaller Allo contributions in the water column, elevated levels in the sediment could be due to the higher stability of Allo than others as suggested by Schüller et al. (2015). Additionally, several studies also reported the influence of microphytobenthos on the phytoplankton group contributions from the shallow coastal benthic ecosystems (Macintyre et al. 1996; Hardison et al. 2013; Semcheski et al. 2016). In this study, even though Paradip port is a shallow coastal water (depth 6.3 to 21.1 m; Fig. 4), microphytobenthos may not influence the outcome of this study significantly as the biomass distribution showed distinct variations with respect to the season as well as between water, and also the contribution of active microalgae in sediment is negligible compared to overlying waters as evidenced by substantially low chl *a* to total pheopigment ratios (Fig. 6). Phytoplankton in aquatic ecosystems undergoes several losses (mortality) processes (e.g., grazing, sedimentation, cell senescence, and viral lysis), and therefore, it is important to determine the fate of phytoplankton from the sediment and overlying water column. In such cases, PMP and chlorophyll degraded pigments (such as pheopigments) can be used to understand the chlorophyll breakdown (ChlB) pathway, which will lead to a better understanding of the benthic-pelagic coupling from the given ecosystem. To the best of our knowledge, more in-depth information on chl *a* breakdown is available for leaf senescence in land plants compared to algae (Scheer, 2012). In the ChlB pathway, pheophorbide formation is an important step, formed either via chlorophyllide or pheophytin. Some studies reported that the initial ChlB pathway induced by grazing and senescence is not the same, i.e., chlorophyll-chlorophyllide-pheophorbide (CCP) and chlorophyll-pheophytin-pheophorbide (CPP) in the former and latter case, respectively (Satoh and Hama 2013; Hu et al. 2013; Sathish et al. 2020). Dephytylation of chl *a* by chlorophyllase activity during cell senescence was widely accepted as the only pathway until the discovery of (i) the role of pheophytinase, a chloroplast-located enzyme, and (ii) the involvement of chlorophyllase activity in the conversion of chlorophyll into chlorophyllide when leaf cells are disrupted (e.g., during grazing/chewing) or when chlorophyllase is genetically mislocalized to chloroplasts. In phytoplankton, the enzymatic activities involved in chlorophyll breakdown varied significantly among the species and were confirmed through grazing studies. For instance, (i) ChlB pathway under grazing pressure is species-specific and the grazer's feeding habits (i.e., selective grazing), and (ii)under grazing pressure, the pheophorbide concentration was higher, and also the maximum pheophorbide:pheophytin ratio was observed in diatoms or diatom dominated mixed assemblage (> 1 to 40) than other tested autotrophic flagellates (0.1 to < 1) suggesting a potential proxy for determining nature of breakdown pathway. In such cases, pheophorbide to pheophytin (Pheid: Phe) ratios can be used to determine the dominant chlorophyll breakdown pathway and the fate of phytoplankton in the given ecosystem. In this study, pool of chlorophyll degraded pigments is mainly derived from the pheophytin and pheophorbide, which can be attributed to the presence of CCP or CPP pathway, even with the absence of chlorophyllide.

Degradation rates vary abundantly among the marker pigments (Leavitt and Hodgson 2001; Schüller et al. 2015) and between the different habitats (Hurley and Armstrong 1990). Pigment degradation in the water column is rapid compared to the sediment (Leavitt and Hodgson 2001, and references therein). In different habitats (i.e., fresh and marine water), pigment degradation mainly depends on the photooxidation, selective grazing, senescence, sinking rate (different species of living and dead cells) and microbial decay, cell lysis, and enzymatic metabolism during senescence (Louda et al. 1998; Reuss and Conley 2005; Fietz et al. 2005 and reference within). Subsequently, phytoplankton chlorophyll (cells and detritus matters) settled on the surface sediment, degrade more rapidly (Bianchi et al. 2000), and less rapidly (Hurley and Armstrong 1990; Yacobi et al. 1991) during oxic and anoxic conditions, respectively depending on the life strategies of phytoplankton, burrowing invertebrates, and the physical condition of water (Leavitt and Carpenter 1989; Yacobi and Ostrovsky 2012). Oxic conditions (4.0 to 4.9 μg l^{-1}) in NBW and increasing chlorophyll degradation products from the surface sediment obtained in the present study support this statement. Also, the degradation of carotenoids highly depends on the chemical structure, the number of oxygen atoms, and the presence of 5,6-epoxy groups (Schüller et al. 2015, and references within). Perhaps, the decay constant (K^a) of chlorophyllide *a* (-0.007 ± -0.007 $year^{-1}$) is lower than that of chlorophyll *a* (-0.004 ± -0.003 $year^{-1}$), pheophorbide *a* (-0.003 ± -0.001 $year^{-1}$), and pheophytin *a* (-0.003 ± -0.003 $year^{-1}$; Schüller et al. 2015); i.e., it would be transformed faster than other degradation products, so only chlorophyllide *a* was not detected in our samples. Hence, chlorophyll and pheopigment ratios can be a good indicator to understand the fate of phytoplankton.

The present study indicates that the Pheid: Phe ratio did not exhibit significant variations between the seasons and stations, but the variations between the water column and sediments were apparent. Additionally, the station data were averaged and presented in this study considering

enclosed port systems and well-mixed water columns. Low and high Pheid: Phe ratios (average) in the sediment (0.40 to 0.76) and water column (0.67 to 1.39) suggested that the contribution by CCP is smaller (or CPP is larger) to Pheid in sediments than that in water columns. Meanwhile, the ratios indicate active cell destruction via grazing in the water column compared to sediment, which could be the reason for the lower contribution of diatom (fuco) and cryptophytes (allo) from the sediment (Fig. 7) when assuming that zooplankton feed selectively on them. Chlorophyll: TPhe (total pheopigments), an indicator of the freshness of organic matter deposited in the benthos (Morata et al. 2011) or actively growing community (Grippo et al. 2009; Allison et al. 2013), did not reveal any significant difference between the season. Higher Chlorophyll: TPhe values in the surface water and NBW (3.95 to 9.17) compared to surface sediment (0.11 to 0.17), indicating the presence of actively growing microalgal community in the water column and the organic matters in the sediment was not fresh (Fig. 6). In a parallel investigation, multivariate index of trophic state (TRIX) indicated good water quality in near bottom water and high organic carbon load in the sediment might resulted in the stressed environment (Noyel and Desai 2020). Taking these into consideration, it is assumed that (i) much of the phytoplankton (mostly diatoms) is lost due to herbivory before reaching bottom sediments, and (ii) given low Pheid: Phe ratios in sediment samples, the effect of benthic grazing on PMPs will not be significant, and PMP decay constants will determine pigment contribution. Generally, in shallow systems, the autochthonous and allochthonous supply do contribute to the pool of degraded pigments. Therefore, the additional knowledge on the degree of suspended organic matter degradation and terrestrial material contribution would have strengthened the findings. Since Paradip port is a sea port, the magnitude of salinity variation in the port (even during peak monsoon) is very less compared to other estuarine ports, e.g., Cochin port (5.3 to 22.6; Sathish et al. 2020) or Marmugao port (3.8 to 36.2) where salinity variations are large. This is because the Mahanadi estuary is located north of the port, and therefore, the influence of river water entering the port will be minimal. Considering the minimal riverine flux (as evidenced from salinity data), and the presence of the (i) lowest pheopigment concentration compared to estuarine and other seaports (up to 6.07 mg m^{-3}; Sathish et al. 2020), (ii) active growing phytoplankton as evidence from high chlorophyll: pheopigment ratios, and (iii) the distinct seasonality in chlorophyll distribution (with lowest (< 1 mg m^{-3}) and highest (1.5–2.5 mg m^{-3}) during monsoon and inter-monsoon, respectively), it is assumed that the phytoplankton marker pigments in this study are mostly of autochthonous origin. These findings suggest that the knowledge on phytoplankton marker pigments combined with the chlorophyll degradation products from the benthic and pelagic environment will better understand the benthic-pelagic coupling, which will be helpful in ecosystem assessment and algal bloom research.

Conclusion

This study concludes that the distribution of chlorophyll and PMP (for different groups and degradation) from Paradip port revealed a distinct seasonality. Still, the seasonal trends between water and sediment are not the same. Pheophorbide to pheophytin ratio indicated that the contribution by CCP is smaller (or CPP is larger) to Pheid in sediments than that in water columns when a lower ratio is found in sediments than water samples. The contribution of actively growing microalgae was high in the water column but negligible in sediment, suggesting organic input was not fresh. It is assumed that (i) much of phytoplankton is lost due to herbivory in the water column before reaching bottom sediments, and (ii) PMP decay constants determine pigment contribution. Documenting the phytoplankton (biomass and composition), chlorophyll breakdown products, and key environmental variables will be helpful in ecosystem assessment and algal bloom research.

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Author contribution Sathish K: Port sampling, sample analysis, data interpretation, and statistical analysis; JS Patil: Original concept, port sampling, data interpretation, manuscript elaboration, and supervision; AC Anil: Manuscript elaboration and also co-supervised all works including acquisition of funds and project administration.

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Data availability All data concerned to the study are included in this manuscript and some are also provided as supplementary. However, it is not applicable for material availability.

Declarations

Ethics approval No animal testing was performed during this study.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests The authors declare no competing interests.

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