Effect of environmental factors on protozooplankton and metazooplankton communities from estuarine to open water system off Goa, Arabian Sea

A thesis submitted to the

Goa University

For the award of the Degree of

DOCTOR OF PHILOSOPHY
IN
MARINE SCIENCE

By

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Under the Guidance of
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February 2018

Dedicated to Lotus Feet of
my Beloved Supreme Lord
Sri Krishna

CERTIFICATE

This is to certify that the Doctoral thesis entitled “Effect of environmental factors on protozooplankton and metazooplankton communities from estuarine to open water system off Goa, Arabian Sea” is an original research investigation carried out by Mr. Priya Brata Das under my supervision for the degree of Doctor of Philosophy in the department of Marine Sciences, Goa University. This thesis has not been submitted for any other degree or diploma in any Universities or Institutions.

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STATEMENT

As required under the University ordinance OB-9.9 (v-vi), I state that the present thesis entitled “Effect of environmental factors on protozooplankton and metazooplankton communities from estuarine to open water system off Goa, Arabian Sea” is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

The literature associated with the current research has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

Place: Dona Paula- Goa
Date: --/--/--

Priya Brata Das

Acknowledgements

The work presented in this thesis would not have been possible without my close association with many people who were always there when I needed them the most. I take this opportunity to acknowledge them and extend my sincere gratitude for helping me make this Ph.D. thesis a possibility.

Foremost, I would like to express my sincere gratitude to my guide **Dr. Mangesh Uttam Gauns**, Principal Scientist, CSIR-National Institute of Oceanography, Goa, for his continuous support, encouragement, motivation throughout my Ph.D. work. His guidance helped me in all the time of research and writing of this thesis. I am immensely grateful to him for providing me a conducive working environment.

I convey my heartfelt gratitude to **Dr. S.W.A. Naqvi**, Former Director, CSIR-National Institute of Oceanography, Goa, not only for his valuable suggestions, tremendous academic support but also for giving me a wonderful opportunity to participate in a one-month training programme at Kaiserslautern University, Germany.

My sincere appreciation and thanks go to **Dr. Z.A. Ansari**, Retired Scientist, CSIR-National Institute of Oceanography, Goa, for his constructive comments and suggestions throughout my work.

I am deeply indebted to the Director, CSIR-NIO for providing logistics and laboratory facilities for execution of my Ph.D. work.

I am thankful to **Dr. Hema Naik,** Senior Scientist, CSIR-NIO for providing nutrient and dissolved oxygen data. I acknowledge the support extended by **Dr**. **Siby Kurian,** Senior Scientist and **Dr. Damodar Shenoy,** Principal Scientist, CSIR-NIO for execution of this work.

I am very grateful to **Dr. Anil Pratihary**, Senior Scientist, CSIR-NIO for the valuable suggestions, discussion, and correction of the articles.

Also, I am grateful to Professor **Dr. Thorsten Stoeck**, HOD, Division of Ecology, Kaiserslautern University, Germany for allowing me to do the molecular analysis in their laboratory.

A very special thanks go out to all down at Council of Scientific and Industrial Research **(**CSIR) and Ministry of Earth Sciences (MoES) for helping and providing the funding for the work.

I would like to acknowledge with thanks the scientific officials of CSIR-NIO for giving me an opportunity to work and also for utilizing the facilities of the research vessel R.V. Sindhu Sankalp. My sincere thanks go to the Captains, Officers, Crew, the scientific and technical team of various cruises for their skilled co-operation and assistance during the cruises.

With regards and love, I am deeply grateful to **my mother**, **father** and all the **family** members who have provided me with moral and emotional support in my life. Their prayer for me was what sustained me this far. Especially my hearty thanks goes to my beloved wife **Mrs. Madhusmita Mishra** for her continuous support to finish this thesis; without her help, it would not have been achievable.

I would like to thank all of my friends who supported me in thesis writing and incented me to strive towards my goal. My special thanks to Dr. Sai Elangovan for his extended support in this thesis preparation. I would like to thank Mr. Wassim, Mr. Sumant, Ms. Analiza, Ms. Prachi, Ms. Lakshmi priya, Ms. Bhagyashri, Ms. Sujata, Ms. Prajoti, Mr. Nandadeep and all the laboratory colleagues for their help throughout this work.

Special thanks go to Mr. Mithun, Dr. Dusmant, Mr. Chandrasekhar, Mr. Aseem, Dr. Lalita, Dr. Rajaneesh and Dr. Dheeraj, for their encouragement and kind support.

I would like to extend my thanks to every one working in CSIR-NIO, it was a great experience of working with all of you.

Contents

List of Figures

List of Tables

Chapter 1 Introduction

1.1 General introduction and review of literature

To our understanding, the marine ecosystems are characterised by different regions such as open ocean, coastal (outer shelf and inner shelf) and estuarine systems. These regions vary in physico-chemical and biological properties and processes, which influence the biological diversity, and species composition in the ecosystem (Harrison et al., 2014; Chase, 2003). Thus, the spatial variability among ecological sites experiencing different environmental conditions can shape the community structure (Akaska and Takamura, 2012). The community living in habitats separated by a small distance tend to have a low beta diversity due to higher degree of exchange of organisms between taxonomic groups through natural processes and anthropogenic activities. Moreover, the similar environmental conditions are also responsible for decreasing beta diversity (Gonzalez, 2009; Thomaz et al., 2007). Seasonal changes can varied impact on biogeochemistry on different ecosystems such as estuaries, shelves and open ocean and consequently, spatial variability in species composition and diversity is observed. Hence, the characterization of different forms of living organisms (protozooplankton to metazooplankton) and their association with ecological regimes are important to understand the ecosystems functioning.

Plankton are defined as the free floating and drifting organisms with limited movement in aquatic ecosystems (Dybas, 2006). The plant forms are known as phytoplankton and the animals are called zooplankton, which floats with the water current and play an intermediate role in functioning of biogeochemical cycles in the marine environment. Among these, the protozooplankton (kingdom Protista) comprises various groups of organism with diverse nutritional modes such as microbial and classic planktonic food

webs (Wittaker, 1969; Margulis, 1974). Bacterioplankton are the smallest among the plankton community which play a significant role as decomposers, food web regulator and exploiters of dissolved organic matter in the marine ecosystems. In higher category, metazooplankton are generally defined as multicellular zooplankton of a range of size classes with functional differences (Sieburth et al., 1978; Dupuy et al., 2016). This is an advanced term for zooplankton community which is basically refered as metazoan planktonic forms. For the nutritional necessity, the protozooplankton (ciliates and flagellates) feed on bacteria and metazooplankton (copepods; holoplanktonic larvae; meroplanktonic larvae and gelatinous plankton) feed on protozooplankton. All these processes are facilitated by inter linking of food chains which is known as the microbial loop (Azam et al., 1983). Hence, the spatio-temporal distribution of protozooplankton, metazooplankton and other of planktonic larvae are important to understand the ecosystem functioning in marine pelagic ecosystems.

1.2 Plankton categorisation

In general, plankton are classified with regard to their size, trophic level, nutrition mode and the characteristics of life cycle. The plankton are classified in different size groups by following logarithmic scale of equatorial spherical diameter (ESD). Thus, femtoplankton (0.02-0.2 µm): virus; picoplankton (0.2-2 µm): cyanobacteria; nano plankton (2-20 µm): heterotrophic and autotrophic flagellates; microplankton (20-200 µm): diatoms, dinoflagellates, ciliates and small metazoans, and mesoplankton (200-2000 µm) comprising of metazooplankton e.g. copepods (Sieburth et al., 1978) are the various planktonic forms in ocean. In addition, some protozooplankton and phytoplankton are also found in these categories (Sieburth et al., 1978). However, these logarithmic size classes are not ideal for protozooplankton which are larger in size (15 µm; Boenigk and Arndt, 2002). The trophic mode of energy is obtained either through

autotrophy (photosynthesis) or through heterotrophy wherein energy is obtained by ingestion of organic matter. Most of the protozoan are strictly heterotrophic and some other forms are mixotrophic (both autotrophic and heterotrophic). Moreover, the phagotrophs and the osmotrophs are distinct from their nutritional mode. The phagotrophs feed on available food particles (particulate organic matter through engulfment) whereas the osmotroph synthesize their food from the dissolved organic and inorganic nutrients (Weisse et al., 2016). In this context, herbivorous and bacteriovorous ciliates, dinoflagellates and nanoflagellates represent the phagotrophic forms. Whereas, the heterotrophic nanoflagellates engulfing the dissolved organic carbon are denoted as osmotrophs (Jugens and Massana, 2008). Based on life cycle, zooplankton are classified into three broad catogories, such as holoplankton, meroplankton and tycoplankton (Raymont, 1983; Omori and Ikeda, 1992). The species which spend their entire lifecycle in the water column are kown as holoplankton (e,g: calanoid copepods, eupahausids, ostracods and appendicularians). Whereas, the species which spend their life cycle partially in pelagic and benthic are known as meroplankton (e.g: eggs and larval stages; Lenz, 2000). The tycoplankton are known to be planktonic forms (e.g: mysids and other crustaceans) in shallow waters which depends on diurnal migration as their feeding strategy and some other forms e.g. harpacticoid copepods, gammarid amphipods, cumaceans, isopods etc. are planktonic in the early phase of their life cycle but moves to the bottom at later phase (Raymont, 1983).

1.3 Impact of environmental parameters in estuarine and marine ecosystems

The population dynamics of biology in the estuarine and marine environments are influenced by the physical, chemical and biological parameters. These common environmental parameters are described as:

Tides: The high and low tides basically occur by the gravitational forces of moon and sun. It has significant impact on coastal and estuarine systems by sea level rise and fall in about every 6 hours in a day. As a result, erosion, sediment deposition, and transportation of different water mass through water currents play a significant role in these ecosystems (Devassy and Goes, 1988; Vijith et al., 2009).

Light: It is one of the most important factor which influence the pelagic zone of aquatic ecosystems. The light is an energy source for phytoplankton productivity (photosynthesis), and furthermore this energy is transferred to primary, secondary and tertiary consumers and it establishes the food web in the estuarine and marine ecoystems (Patil and Anil, 2011).

Temperature: It is an important abiotic factor which influences the life cycle, metabolism, migratory behaviour and adaption of planktonic organisms (phytoplankton and zooplankton) and impacts the ecological process of estuarine and marine ecosystems (Brierley, 2009).

Dissolved Oxygen: It is a primary requirement which support the aquatic organisms for a healthy ecosystems. Continental shelves and estuaries get nutrient input mainly through riverine discharge, land run-off, coastal upwelling and atmospheric inputs (Hickey and Banas, 2003). whereas open ocean can get nutrients thourgh upwelling, eddies and transport of upwelled waters from shelf. The excess nutrients cause blooms and their detritus maximize the organic load to subsurface depth, which can deplete the oxygen leading to hypoxic, suboxic and at times anoxic condition in the water column. The low dissolved oxygen affects the distribution pattern of biological community and thus can be used as an indicator to understand the state of ecosystem (Omar, 2010).

Nutrients: Nutrients (nitrogen, phosphorus and silicate) are the key factors for primary production, and the limited and excess nutrient causes the oligotrophic and eutrophic

condition respectively in the estuarine and marine ecosystems (Howarth, 1988; Andersen et al., 1991). Thus, the eutrophication leads to changes in the community structure, ecology and diversity mainly through two processes such as oxygen depletion (indirect mode) and nutrient enrichment (Yang et al., 2008).

Salinity: It has a significant effect on species abundance in the aquatic environments. The estuarine and coastal waters experience the variation in salinity due to the tidal cycles and due to mixing of intruding seawater and outflowing river water. Moreover, the amount of precipitation and the rate of evaporation plays a major role in determining salinity. The associated biological community structures vary from upstream (freshwater) to mouth (saline) areas (www.userpages.umbc.edu). In this context, some species are adaptable to euryhaline and stenohaline conditions in aquatic ecosystems (Collins and Williams, 1982; Padmavati and Goswami, 1996).

Turbidity: It is a measurement to determine the optical characterstics (light transmission) in the water column. Thus, dissolved organic matter from the industrial effluent, land runoff and shoreline erosion which increases the suspended particulate matter in the water column (Hoover and Mackenzie, 2009). Comparatively, the maximum effect of high turbidity is found in intertidal area of estuaries and coastal waters than open ocean (Uncles, 2002). It has a considerable effect on living organisms, which directly dependent on light such as aquatic plants which require light energy for photosynthesis.

1.4 Importance and role of proto- and metazooplankton in aquatic food web

In a brief note, the protozooplankton and metazooplankton are the vital intermediaries in aquatic food webs (Montagnes et al., 2010). Protozooplankton are considered to be a vital component of nano and microplankton (Porter et al., 1985; Carlough and Meyer, 1989). These planktonic forms include heterotrophic nanoflagellates, heterotrophic

dinoflagellates, mixotrophic dinoflagellates, ciliates (heterotrophic and mixotrophic), planktonic foraminifera, acantheria and radiolarian. These protozooplankton are well known for the potential prey for zooplankton (Stoecker et al., 2017).

Microplankton are the important food source for zooplankton which prey on nanoplankton (2-5 μm) to a lesser extent (Berggreen et al., 1988). Whereas in case of larger metazooplankton, only pelagic tunicates, cladocerans and pteropods feed on smaller nanoplankton (Sanders and Wickham, 1993). However, heterotrophic flagellates and phytoplankton are the important food source for zooplankton communities (Sanders and Porter, 1990; Work, 2003). Metazooplankton play a significant role in aquatic food webs being grazer of phyto and protozoolplankton, and prey for small pelagic fishes, shrimps and mysids (Harris et al., 2000; Pont, 1995; Calbet et al., 2008; Pollack et al., 2008; Spinelli et al., 2012). Numerous studies suggested that the changes in metazooplankton abundance and their composition may affect higher trophic levels and fisheries (Beaugrand, 2003). In the marine environment, copepods represent as a major group of metazooplankton. Based on their feeding habit, they are classified into two groups such as herbivores and carnivores (Weisse et al., 2016). In this context, the food web is more complex with the interlinking of different trophic levels between copepods, microzooplankton and nanoplankton (Rassoulzadegan et al., 1988). Zooplankton support the growth of bacteria through thier excretes and also they graze on bacterial population. Therefore, the zooplankton is well known as an essential component of food chain in marine environment (Longhurst et al., 1989) (Fig.1).

The physico-chemical and biological circulation of carbon from surface to bottom water in the marine environment is known as a solubility pump and the conversion of

inorganic carbon to organic matter through photosynthesis in the food web known as biological pump (Longhurst, 1991).

The zooplankton grazing on phytoplankton has a significant effect on marine biogeochemical cycles. In ecological perspective, the biological pump is characterized by the photosynthetic production of organic matter by phytoplankton and their sinking, decomposition of organic debris, zooplankton grazing and their migratory activity (Longhurst, 1991; Fortier et al., 1994). The main process which transfers carbon into the deep water is the sinking of dead phytoplankton and the feeding behaviour of zooplankton and microbial mineralization (Longhurst and Harrison, 1989; Longhurst, 1991). The zooplankton migrate vertically to consume the organic matters in the surface water at night and process the ingested food during the day time at depth. Their faecal pellets which are carried to surface water by diel vertical migration are vital role in the carbon fluxes (Angel, 1985; Fowler and Knauer, 1986). Thus, the zooplankton are important component in the marine food web for exporting the organic material from the euphotic zone (Dam et al., 1995; Le Borgne and Rodier, 1997). The spatial assemblages of zooplankton species varies with respect to the available nutrient and different physical parameters such as temperature, salinity, turbidity and currents (Kimmel et al., 2006). In the Arabian Sea, spatial variability of zooplankton is mainly influenced by temperature, salinity and food supply (Fernandes and Ramaiah, 2009).

A comprehensive research has been done on the zooplankton community distribution in Mandovi- Zuari estuarine system. Goswami and Singbal (1974) showed the ecological role of zooplankton communities with respect to hydrographic variations in Mandovi Zuari estuaries during monsoon. Among zooplankton community, the abundance and ingression of the penaeid larvae has been observed in these estuarine systems (Selvakumar et al., 1977; Achuthankutty et al., 1977). Studies on diel variations of

penaeid larvae in estuaries and coastal waters of Goa were investigated by Goswami and George (1978). Nair and Selvakumar (1979) carried out studies on the ecological distribution of chaetognaths in the estuarine systems of Goa. The contrasting patterns of biogeochemical and ecological features of the Mandovi-Zuari estuary in Goa, associated with eastern coastal Arabian Sea, were reported by Qasim and Sengupta (1981). Valubale information on secondary production and zooplankton composition in the zuari estuary were given by Nair et al. (1983). Copepod distribution, diversity, coexistance and succession of copepod species were investigated in the Mandovi-Zuari estuary (Goswami, 1982; Goswami, 1983). Goswami and Devassy (1991) reported the seasonal occurrence of *cladocera* in Mandovi-Zuari estuarine ecosystems. Goswamy (1992) reported the zooplankton community associated with mangroves areas of the upper reaches of Mandovi and Zuari estuatries. A comprehensive research has been carried out on ecological diversity of zooplankton communities in estuarine networks of Goa by Padmavati et al. (1997). Also, temporal and epehermal variations of copepod communities were investigated in both these estuaries by (Dalal and Goswami, 2001). But in the context of protozooplankton, there is a lack of studies on their ecological diversity in the Mandovi estuary. Very little information is available on microzooplankton community structure in Zuari estuary. Gauns et al. (2015) reported the seasonal variation in abundance and grazing rates of microzooplankton only in the Zuari estuary.

Among protozooplankton category, ciliates are the important constituent of microplankton in the different aquatic habitats such as seas, estuaries and freshwater systems (Rassoulzadegan and Gostan, 1976; Pace and Orcutt, 1981; Smetacek, 1981; Banse, 1982; Sherr et al., 1986). They are the vital component of a planktonic food web (Porter et al., 1985) and abundant in neritic waters (Burkill, 1982; Variety, 1987; Sherr

and Sherr, 1988; Pierce and Turner, 1992). The community response of microzooplankton to coastal upwelling and summer stratification in the southeastern Arabian Sea were summarised by Gauns et al. (1996) and Jyothibabu et al. (2008). Heterotrophic dinoflagellates are known to be ubiquitous protist in the marine environment (Lessard, 1991; Hansen, 1991; Verity et al., 1993). They are important constituent of protozooplankton (microzooplankton) community which play a significant role in the plankton ecology. Additionally, some of the studies in the Arabian Sea suggest that protozooplankton play a singnificant role in microbial food web (Gauns et al., 1996; Madhupratap et al., 1996; Madhupratap et al., 2001; Nair et al., 1999). Worldwide, study on ecological and biogeochemical role of the protozooplankton are stated that their role in carbon flow is significant in the marine environment (Gast, 1985; Pierce and Turner, 1992).

Mandovi and Zuari are the estuarine system which is located in the coast of Goa (west coast of India). They are geographically important due to monsoonal regime, tidal variations and the riverine water influx. In the marine food web, both proto- and metazooplankton have the biological importance to characterize the function of a pelagic ecosystem. Despite their ecological importance and ubiquity, the catalogue of protozoan community have not been studied enough and the data about their distribution are very limited (Leveque et al., 2005).

In the view of the earlier studies and special emphasis on oxygen minimum zone, this present study was carried out to understand the zooplankton (proto and metazooplankton) community structure, seasonal distribution and their composition across the estuarine, coastal and open ocean of the Arabian Sea .

1.5 Objective and scope of the study

To understand the role of proto- and metazooplankton in the central and eastern Arabian Sea, the observation of proto- and metazooplankton in a stretch from the estuarine to open ocean is necessary. The aim of proposed work was to document the distribution pattern of protists and metazooplankton which are present in the epipelagic and mesopelagic waters of the estuarine to open ocean systems of the Arabian Sea. The multicellular zooplankton (metazooplankton: copepods, holoplankton, meroplankton and gelatinous plankton), fungi, and bacteria have vastly different community structures in the pelagic environments (Jebaraj et al., 2010; Fuchs et al., 2005; Wishner et al., 2008; Morrison et al., 1999), and it is important to analyse their seasonal variation, community structure and their community shift from estuarine to open water system.

As mentioned earlier, the diversity patterns of heterotrophic flagellates and ciliates are poorly studied in the Arabian Sea. Previously enumeration of ciliates, heterotrophic flagellates and thraustochytrids in the central and north-eastern Arabian Sea was based on microscopic observations (Gauns et al., 1996; Garrison et al., 2000; Raghukumar et al., 2001). These studies were instrumental to demonstrate the general occurrence of specific taxon groups related to oxygen gradients in the water column. However, no attempts were made to relate these taxon groups to specific environmental conditions. Moreover, microscopic studies are usually only successful in the identification of the most abundant and conspicuous protists, while smaller and low-abundant species often spurt from microscopic observation (MacManus and Katz, 2009). In contrast, even though high-throughput sequencing strategies have their pitfalls, its approach is much more sensitive than microscopy and paints a complete picture of protistan community structures (Stoeck et al., 2014). Accordingly, this approach has been widely used for

studying protistan diversity in a variety of ecosystems, including oxygen-depleted environments (Stoeck et al., 2010; Jing et al., 2015; Parris et al., 2014; Duret et al., 2015).

In this connection, this ecological investigation was summarized with the following objectives.

- To study spatial and temporal distributions of zooplankton community (metazooplankton) structure in estuarine (Mandovi and Zuari), shelf and open ocean waters of the Arabian Sea.
- To study ecology and distribution of protists in the Arabian Sea with special reference to the oxygen minimum zone.
- To study the changing phylogenetic composition of the HNF community in relation to oxygen concentration.

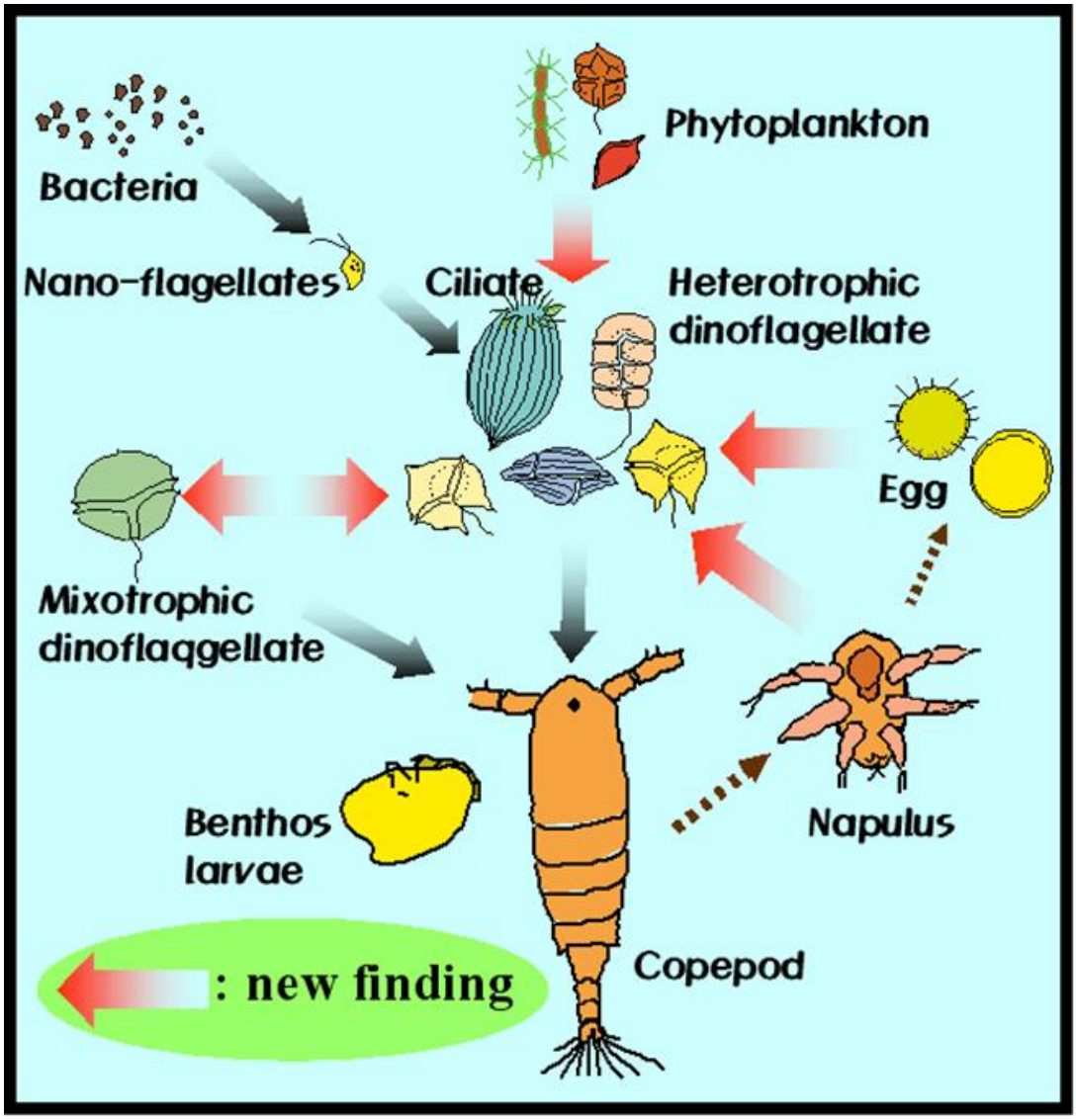


Figure 1. A schematic representation of proto and metazooplankton in aquatic food web (Source: http://hosting03.snu.ac.kr/~hjjeong/Food%20webs%20index.htm).

Chapter 2 Study area

The contrasting ecosystems as the estuarine systems (Mandovi and Zuari), coastal and open ocean waters of the Arabian Sea were considered for this ecological investigation. The significantly varying environmental characteristics between these ecosystems can exert reasonable effect on their distribution patterns of associated organisms ranging from unicellular to multicellular level. A wide range of study on the planktonic community in these particular environments have been carried out previously and the biological, chemical and physical characteristics of these different biogeochemical regimes have been reported in reputed scientific journals (Qasim et al., 1972; Devassy and Goes, 1988; Goswami and Singbal, 1974; Goswami and Devassy, 1991; Padmavati and Goswami, 1996, Vijith et al., 2009; Shetye et al., 2007; Qasim and Sengupta, 1981; Gauns et al., 2015; Gauns et al., 1996). The above studies on protozooplankton and metazooplankton have not addressed their community composition in a spatial stretch from estuarine waters to open ocean region of the Arabian Sea in a single timescale. This is the first ever study to report the molecular diversity of protist communities with special attention to ciliates and heterotrophic flagellate communities in the different biogeochemical regions of the Arabian Sea through next generation sequencing. The studies on microzooplankton (ciliates) carried out in the coastal, central Arabian Sea and in the estuarine regions (Mandovi and Zuari) are very limited. After JOGFS (Joint Global Ocean Flux studies) there are no such studies reported the ciliates and heterotrophic flagellate communities varying in estuarine, coastal and open waters of the Arabian Sea along with the oxygen gradients. So, my current research investigation has focused on the diverse protist communities with special attention to ciliates and heterotrophic flagellate communities present in the spatially varying biogeochemical regimes of the Arabian Sea. The metazooplankton communities

(copepods, gelatinous zooplankton and planktonic larval forms) were also investigated in spatially varying ecosystems such as estuary, shelf and open waters of the Arabian Sea.

2.1 Geographical description of estuarine systems (the Mandovi and Zuari estuaries)

There are nine major rivers originate in the Western Ghats and falls into the Arabian Sea. Among these, rivers Mandovi and Zuari are the key estuarine systems, which has major role in the economic significance of the state as they are used for the mining industries, tourism and fishery. Both the estuarine systems cover 69% of the total geographical area of Goa and meet the Arabian Sea near Mormugao harbour. Study area of Mandovi and Zuari estuarine systems ranges along the Latitude 15° 16 'N to 15° 32 'N and Longitude 73° 47 'E to 74° 06 'E (Fig. 2). The sampling sites in these estuaries were selected in near mouth (M1 and Z1), mid estuarine (M3 and Z4) and upstream (M6 and Z7) region. The Mandovi-Zuari estuarine system experience strong seasonality over the course of fall intermonsoon (October-November), northeast monsoon (December-March), spring intermonsoon (April-May) and southwest monsoon (Jun-Sep). These two estuaries are well known for its networking waterways. Moreover, these waters provide the fishery resources throughout the year and particularly these are most dependable fishery sources during the seasonal monsoon period when the fishing activities are banned in the shelf waters due to rough weather.

2.1.1 Estuarine geomorphology and their hydrology

Mandovi and Zuari river originate from the Sahyadri hills situated in the Western Ghats and cover a distance of ~70 km towards the Arabian Sea. The width of Mandovi estuary is 3.2 km at its mouth while it narrows down up to 0.25 km at upstream region. The major source of water to the estuarine system is the monsoon precipitation (~3000

mm / annum) and run-off from the catchment area of 1150 km^2. The 42% area of Goa is covered by its basin of 1530 Km^2.

During the non-monsoon period, systematic replacement of water occurs in estuaries by semi-diurnal tides with a highest range of 2.3 m (Murty and Das, 1972). This river has a large tributary system demarcated with numbers of islands, narrow channels and shallow depths. It also receives discharges from forest lands covering an area of 435 km^2. A numbers of small and medium scale industries found in its basin and 16×10^6 m^3 of effluent is discharged annually to this river through the associated tributaries. The estuarine system is actively used for the transport of most (2/3) of mining of Goa. Moreover, 20 large mining industries produce 7000-8000 tonnes of waste materials annually which are dumped into this estuarine region.

Apart, the total area of Goa, 36% is used for agriculture, 25% is under forest cover and the remaining area is used for mining and residential areas. Agricultural activities by means of synthetic and organic fertilisers are washed into the riverine systems, which determine the river water quality.

On the other hand, river Zuari originates from the Sahyadri hills of the Dighi ghat and falls into the Arabian Sea close to Mormugao harbour. The mouth area is of 5.5 km wide whereas it tappers to only 0.5 km at the upstream region. Like Mandovi river, Zuari river is also fed by the monsoonal precipitation and the total freshwater discharge from the catchment area is about 550 km^2. Comparatively, its catchment covers less area i.e. 975 km^2 (27%) of the total Goa than Mandovi river. Its flow also regulated by semi diurnal tides up to a highest range of 2.3 m. The estuary receives discharges from the forest land of 309 km^2 and 8.6×10^6 m^3 of effluents from the industries situated along its basin. Thus a huge quantity (1500 – 5000 tonnes) of mining waste is added to this riverine system per day.

A man-made canal known as Cumbarjua canal, connects these two rivers thereby allowing a dynamic interaction between two estuarine ecosystems. The canal is 17 km long and 0.2 to 0.5 km wide and it flows 14 km and 11 km from the mouth area of the Mandovi and Zuari respectively. The canal becomes very useful particularly during monsoon season when a sandbar is developed near mouth region of the Mandovi estuary and thus the traffic roots of all boats and barges to Mormugao harbour are diverted via this canal to the Zuari river. Therefore, the Mandovi-Zuari estuarine system includes Cumbarjua canal which is also influenced by the seawater intrusion during non-monsoon season and by freshwater domination during south west monsoon. During the period of monsoon and non-monsoon season, these estuarine systems show a marked variation in hydrological characteristics. Mandovi and Zuari estuary are termed as coastal plain estuaries and a type of drowned river valley. They display semidiurnal tide and are affected by the seawater inflow particularly during dry season.

2.2 Environmental settings of the Arabian Sea

2.2.1 Physical and biological observations from the central and eastern Arabian Sea (coastal and open ocean)

Coastal belt of Goa covers a distance about 100 km and the geographically it is located between 14°54' N and 15°48' N latitude and between 72°41' E and 74°20' E longitude. The Arabian Sea is well-known for its dynamic nature, which is geographically placed in the north-western part of the Indian Ocean. It is partly land locked by the African and Asian continents which is one of the important reasons for its varying hydrographical and biogeochemical characteristics. The major rivers such as Indus, Tapti and Narmada with lower freshwater discharge rates fall into the Arabian Sea. In contrast, the Bay of Bengal situated on the other side of Indian peninsula, receives a large amount of riverine runoffs from the major rivers such as Irrawady, Brahmaputra,

Ganga, Mahanadi, Godavari, Krishna, and Cauvery. One of the important characteristics of the Arabian Sea is the higher evaporation than the precipitation and lower run-off except from the west coast of India during southwest monsoon (Venkateswaran, 1956). The Arabian coast receives maximum evaporation (100-150 cm y^{-1}) over precipitation and its rate gradually reduced towards the southeast. Thus the Arabian Sea attains the high surface salinities due to its high evaporation rates. Moreover, the Red Sea and the Persian Gulf being situated in the arid zones experience intense evaporation and show the highest surface salinity among the world oceans. The outflow from these two seas also increases salinity of the Arabian Sea. High salinity along with the winter cooling in the northern region forms dense water masses which sink (Dietrich, 1973). The sinking high saline water masses travel at subsurface level into the Arabian Sea via the gulf of Oman and Aden. At the same time the surface waters of the Arabian Sea flows in to Red Sea and Persian Gulf in order to keep the balance between outflow and inflow of water masses (Grasshoff, 1969; Grasshoff, 1975; Hartmann et al., 1971).

The Arabian Sea is renowned for being affected by monsoon regimes which induces seasonal changes in physico-chemical and biological characteristics. Seasonally reversing wind regimes results in summer or south west monsoon (SWM) and winter or northeast monsoon (NEM). These seasonal changes drive the variation in physico-chemical-biological characteristics in different regions such as coastal (outer shelf and inner shelf) and open ocean of the Arabian Sea. Keeping these features as background, in the present study was conducted in the inner shelf (G5), Outer shelf (G12) and open Ocean (ASTS) sites (Fig. 2). In summer season strong wind blows from the south western part of the Arabian Sea mediated by the pressure difference between Ocean and land masses. But, during winter (NEM) cold dry wind blows from north to

southern part of the Indian Ocean. In this way these two seasonal periods (SWM and NWM) greatly influence the water circulation in the Arabian Sea (Shetye, 1998; Shankar et al., 2002). Also, the coastal current has significant effect in this process. During the summer monsoon, the combined influence of the West Indian Coastal Current and the monsoon current transports the high saline waters to the Bay of Bengal which reverses during the winter period (Kumar et al., 2004).

The direction of winds over the north Indian Ocean plays a major role in transferring the water flow from south to the equator along the coast of India (Shetye et al., 1990; Muraleedharan and Prasanna Kumar, 1996). In this way a large scale surface circulation happens in the Arabian Sea, is particularly a sign of Somali current system that flows northward along the coast of Somalia attaining its highest strength in July (Schott, 1983). But in the month of May, a low level flow moves towards north along the east African coast and with the acceleration it forms the little Somali Jet (Findlater, 1969) passing over the Arabian Sea from Somalia to Gujarat coast of India. Due to this reason a shallow mixed water column forms in the north direction of the Jet by the effect of the cyclonic wind stress curl. As a result of these processes the northern sector experiences biological blooms by nutrient enrichment (Bauer et al., 1991).

At the same time southern part experiences anticyclonic curl which forms the deep mixed layer (Muraleedharan and Prasanna Kumar, 1996). Moreover, the cold upwelled water is transported along the west coast of India during this period (Shetye et al., 1990). However, it is uncertain to find its extension towards the 15°N. But the earlier study shows the signatures of terupwelling along the southwest coast of India; intensity of which reduces towards the northern region (Shetye et al., 1990). Additionally, the studies reported the annual successions of wind stress mechanisms along the coast and the ability of the local wind flow for driving the surface circulation.

During northeast monsoon (December-March) the surface circulations of the Arabian Sea becomes like that in the Pacific and the Atlantic Oceans. In the region to equator, water flows from east to west by means of northeast monsoon current. This becomes more intense in the month of February and weakens by April (Wyrtki, 1973). Consequently, during this period, west coast of India receives low saline waters from the Bay of Bengal through the NE monsoon current. On the other hand, the other part of this monsoonal current shifts to the south off Somalia and combines with south equatorial current. Also, counter current and under water current are noticed and the surface circulation occurs anticlockwise during this period (Wyrtki, 1973).

During the winter the atmospheric temperature remains low (near about 22° C) in the north, from where dry continental air passing over the northern Arabian Sea develops surface cooling due to the evaporation. Also, the reduced solar insolation further enhances surface cooling. As a result, the combined effect of enhanced evaporation and reduced solar insolation during winter results in reduced SST. Subsequently, the cooling and densification of surface water column leads to its sinking by the process of convective mixing which results in a deeper mixed layer in the northern Arabian Sea. In this way the nutrient enriched water from the upper thermocline region is injected to the surface water column (Prasanna kumar and Prasad, 1996; Madhupratap et al., 1996a). Earlier studies (Madhupratap et al., 1996b) in the northern region during winter cooling reported the constant availability of nitrate (2-4 µM) in the upper water column, which greatly influences chlorophyll *a* and primary production.

Another seasonal regime is the spring inter monsoon (April-May) found in-between NE and SW monsoons during which atmospheric temperature increases up to 28-30° C. The weak (< 4 m s^{-1}) surface wind predominantly flows from the south of 17° N to the north and it gradually becomes stronger on the way to north-west direction. In this

period, a thin mixed layer is formed over the most parts of the Arabian Sea and in the later stage it leads to strong stratification (Wyrtki, 1973). During this period, low nutrient availability is the main reason for the low chlorophyll a and primary productivity.

2.2.1.1 Biological productivity and biogeochemical processes in the Arabian Sea

Among the world ocean, the Arabian Sea is one of the most productive regions due to upwelling induced nutrient enrichment during the southwest monsoon and convective mixing during northeast monsoon (Naqvi et al., 2003). The association of trophic structures and the biological productivity is greatly enhanced by the strength of physical forcing in the Arabian Sea. The biological productivity by means of primary and secondary producers and their abundance reflects seasonal changes in physico-chemical characteristics of the water column (Sawant and Madhupratap, 1996; Gauns et al., 1996; Ramaiah et al., 1996). The maximum primary production (1782 mg C M^{-2} d^{-1}) in the eastern Arabian Sea was recorded during the southwest monsoon (Prasanna kumar et al., 2001). But the winter convection in the Arabian Sea leads to the high organic matter production and dissolved organic carbon accumulation in the surface mixed layer (Madhupratap et al. 1996a; Barber et al., 2001). In this connection an important biogeochemical process happens with the build-up of organic carbon which is termed as microbial loop that yields bacteria as a food source for the secondary producer (zooplankton) during the inter monsoon periods (Madhupratap et al., 1996b). Though, the primary production decreases during intermonsoon, the bacterial and zooplankton biomass remains high in this region. In spite of lower phytoplankton production, the zooplankton maintains stable biomass by feeding on the bacterial population during the intermonsoon period when microbial loop is most active.

In the view of biogeochemistry research in the Arabian Sea, a number of projects like Joint Ocean Flux Study (JGOFS) and the land Ocean Interactions in the Coastal zone (LOICZ) have been carried out as the two major wings of the International Geosphere Biosphere Programme (IGBP). These studies clearly find the distinct observation between the eastern and western part of the Arabian Sea (Kumar, 2006). The main difference is characterised as Eastern Arabian Sea being net heterotrophic, whereas the western Arabian Sea is net autotrophic (Sarma, 2004). The transfer of organic matter from the surface layer and its biological degradation is the vital reason for the acute oxygen depletion in mesopelagic water column (150-1000 m; Banse et al., 2014; Naqvi et al., 2006b). One of the significant features of the Arabian Sea is the harbouring oxygen minimum zones (OMZ) at intermediate depths. However, the evolution mechanism and intensity of oxygen deficiency in the open ocean varies from those in the shelf regions. Particularly, the former one is perennial while the latter occurs seasonally along the west coast of India (Naqvi et al., 2006a). It creates a significant impact on socioeconomics of coastal regions. Dumping of organic debris and fertilizer runoffs from the coastal area are the main causes of coastal pollution which contributes to intensification of oxygen deficiency in the water column. Extensive hypoxia even anoxia occurs in coastal water column of the eastern Arabian Sea during the summer monsoon (Naqvi et al., 2006a). An important reason for such hypoxia is the formation of low saline surface layer due to monsoonal rain and river discharges which spreads over the upwelled low oxygen waters. This less dense low saline water acts as a barrier which does not permit the diffusion of atmospheric oxygen and contribute to the coastal hypoxia and accumulation of nitrous oxide up to ~800 nM formed by the process of nitrification and denitrification in this region, the maximum concentration found in the world ocean (Naqvi et al., 2000). Seasonal variation of hydrographical parameters in

the coastal area causes the evolution of hypoxia, which turns to anoxia leading to hydrogen sulphide build-up in the water column (Naqvi et al. 2006). JGOFS study discovered the seasonal variation of dissolved oxygen and nutrients in the water column and showed oxygen concentrations below detectable limit at 400m during winter (de Sousa et al., 1996). These regions of low oxygen have significant implications for nitrogen and carbon cycling in the Arabian Sea. This is mediated by microbial processes such as nitrification and denitrification. In a comparative account with the world ocean, only the open Ocean of eastern tropical south (Peru) and north (Mexico) Pacific Ocean experiences mesopelagic nitrate reduction (Codispoti et al., 1992). Though the Arabian Sea is a small basin, its diverse physical attributes experiences significant biological regimes, which draws the attention of oceanographers across the world to carry out research in this region. Recently, SIBER INDIA has come forward to continuously monitor the ecological, physical and biogeochemical processes in this region of the Arabian Sea by following regular time series observations.

2.2.2 Sampling sites and time periods

In the Mandovi and Zuari estuary three sites each were selected for this research work. In the Mandovi estuary three stations include M1 (near mouth), M3 (mid-estuarine) and M6 (upstream) stations selected depending on their physico-chemical features. Likewise, three stations in the Zuari estuary e.g. Z1 (near mouth), Z4 (mid-estuarine) and Z7 (upstream) stations were selected. To find out the seasonal variation of metazooplankton community, all these stations of Mandovi and Zuari estuary were sampled during the period from October 2011 to September 2012. Sampling period was divided into four seasons as (i) fall intermonsoon (FIM; October-November 2011), (ii)

north east monsoon (NEM; December 2011-March 2012), (iii) spring inter monsoon (SIM; April-May 2012) and (iv) southwest monsoon (SWM; June-September 2012).

On a contrary, a different time period was considered to compare the seasonal variation of metazooplankton communities from the estuaries and with the sample collected from coastal and open ocean sites. This was decided based on the time period of the cruise. Accordingly, estuarine (stations: M2, M3, M6, Z2, Z4 and Z7), outer shelf (G12), inner shelf (G5) and open ocean stations were considered to observe the spatio-temporal variation of metazooplankton communities from estuarine to open ocean region of the Arabian Sea. For temporal variation, data from only three seasons was considered in the present study, that is SSK 56 (October-November 2013-FIM), SSK 69 (September-October 2014-Monsoon) and SSK 79 (March 2015). Additionally, during 2013 (SSK 56) whole protist community was studied with special attention to heterotrophic protists (heterotrophic flagellates and ciliates) which were identified through the advanced next-generation sequencing (NGS) approach from estuarine to open water system of the Arabian Sea. Moreover, the ciliates were sampled for the visual microscopic taxonomy.

2.2.2.1 Geographical location of the sampling sites

Location details of the estuarine, shelf and open ocean sampling sites are presented in the Fig. 2. The geographical locations of each sampling sites and their average depth information are as follows

Estuarine stations (M1 and Z1):

Both the stations M1 (15.47°N, 73.78°E) and Z1 (15.43°N, 73.80°E) represents the mouth areas, where these two rivers meet the Arabian Sea. The distance between these two stations is about 5.2 km. Station M1 is ~30 km far from its upstream point (M6),

where Z1 is located at a distance of ~43 km from the upstream station Z7. The average depth of M1 and Z1 station is about 8m.

Estuarine Stations (M2 and Z2):

The station M2 (15.49°N, 73.81°E) is located ~4.3 km away from the mouth of the estuary and the station Z2 (15.40°N, 73.90°E) is ~12 km away from its Mouth. The average depth at M2 is around 4 m while at Z2 it was 5 to 6 m.

Estuarine stations (M3 and Z4):

M3 (15.50°N, 73.95°E) station is located at ~6km away from the station M2, whereas the Z4 (15.34°N, 74°E) sampling point is ~13 km far from Z2. The average depth at M3 is 6m and at Z4 is 8m.

Upstream estuarine stations (M6 and Z7):

The upstream station M6 (15.50°N, 73.99°E) is located at a distance of ~20 km from M3 and the upstream station Z7 (15.26°N, 74.10°E) is about ~20 km far from the station Z4. The average depth at M6 is 6 m, whereas Z7 is about 4 m.

Coastal station-inner continental shelf (G5):

This represents inner continental shelf station (15.50°N, 73.67°E) fixed at a distance of ~12.25 km from the Mandovi mouth and about ~15.7 km from the mouth of Zuari. The average depth of this station was 26 km.

Coastal station-outer continental shelf (G12):

Station G12 represented outer continental shelf station (15.24°N, 72.98°E) located about ~79.3 km away from the inner shelf station. The station depth was about 160 m.

Open ocean station (ASTS):

It is the open ocean station (17°N, 68°E) located at ~572 km from the Indian west coast. The average depth of this station is about 3600 m.

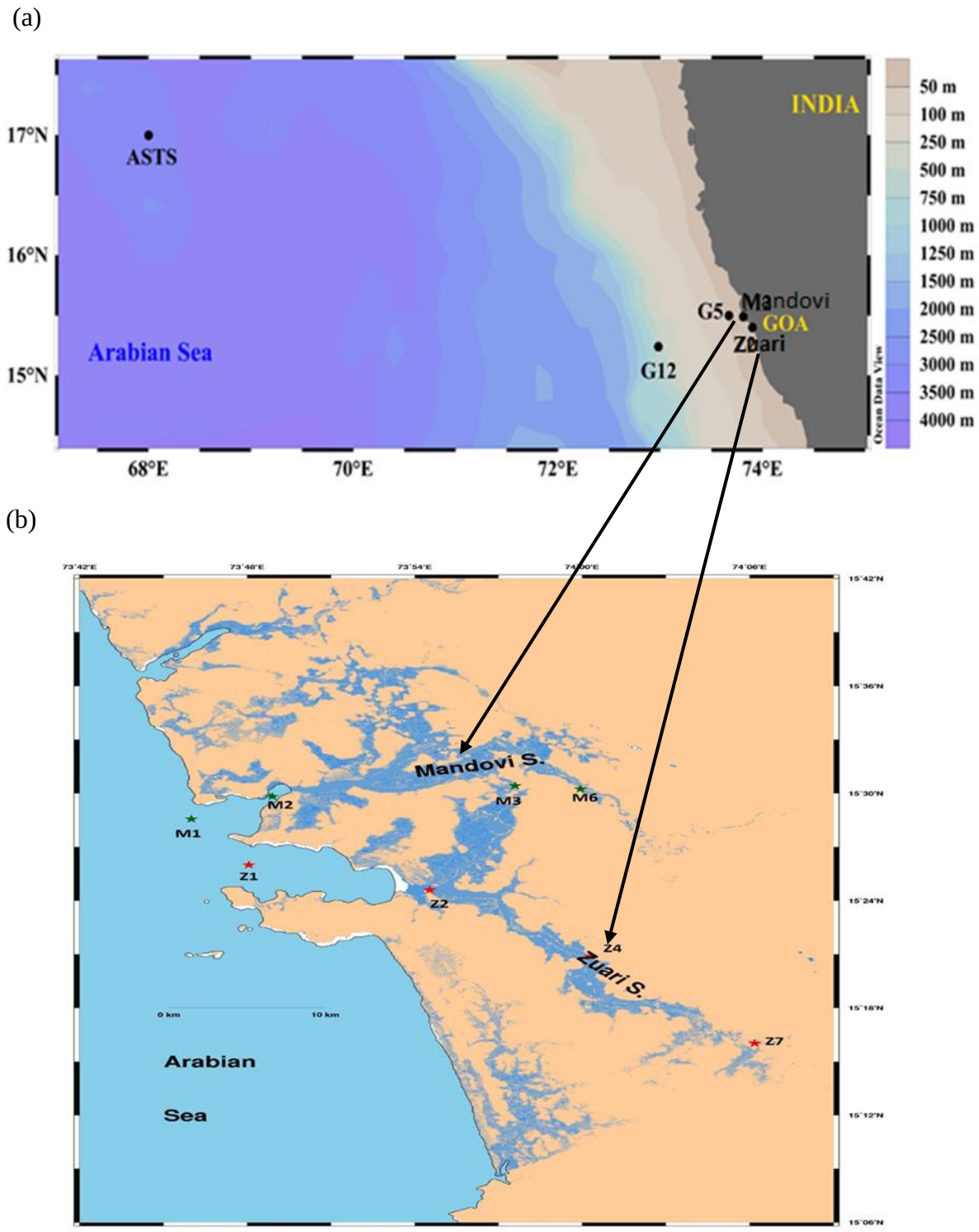


Figure 2. (a) Map showing sampling sites located in different regions of the Arabian Sea ranging from estuarine to open ocean stations (Estuary: Mandovi-M2, Zuari-Z2; Open ocean-ASTS; Outer continental shelf-G12; Inner continental shelf-G5); **(b)** spatial stretch of sampling sites in estuarine systems (Mandovi-M1,M3,M6; Zuari-Z1,Z4,Z7).

Chapter 3 A

Estuarine (Mandovi-Zuari) variability

3a.1 Introduction

Spatio-temporal distribution is the fundamental measurement to define the patterns of living entity in a specific landscape (Whittaker, 1972; Legendre et al., 2005). The aquatic ecosystems are commonly diversified into different regions such as estuary, coastal and open ocean sites and their environmental characteristics vary with respect to their surrounding physicochemical processes. As per the niche theory, the species have different ecological responses with respect to associated environmental attributes (Chase and Leibold, 2003). Thus, the community structure is shaped by ecological processes occurring in different aquatic environments (Chase et al., 2011).

Estuarine Systems (Mandovi and Zuari)

Estuarine systems are an integral part of the coastal waters and they form a buffer zone in between the saline and freshwater regimes. These represent the habitats with fluctuating salinity and temperature for the different trophic level organisms. Different estuarine systems and their ecological processes are influenced by hydrological components with fresh and marine water. These estuarine systems act as nourishing and breeding grounds for several metazooplankton communities. Two prominent estuarine systems Mandovi and Zuari found on the west coast of India. Mandovi estuary is well defined by the regular flushing and flooding by the semidiurnal tides affecting environmental conditions of the surrounding area (Vijith et al., 2009). This estuarine flow is governed by the inward tide at the Zuari estuary, which also arrives at the Mandovi estuary through the cambuja canal and the flow was reversed by the outgoing tide. Four distinct seasons of the region (FIM, SIM, NEM and SWM) determine the environmental fluctuations in the estuary. Mostly, summer and monsoon period (SWM)

are the cause to the extent of high saline and low saline environments. Likewise, the Zuari estuary also influenced by the southwest monsoon, where the maximum rainfall received by the Zuari estuary during the monsoon period (June- September) leading to defined monsoonal estuary (Shetye et al., 2007; Vijith et al., 2009). Monsoonal estuaries are very dynamic and unstable as compare to temperate estuarine systems (Anand Subha et al., 2014). Both these estuaries have a perennial connection with the coastal Arabian Sea. The changing environments of these estuarine systems influence the trophic relationship. Zooplankton has key role in marine pelagic systems in the food chain as an intermediate link between primary producers and higher trophic levels. Most of the earlier studies on zooplankton ecology have vowed the relation of salinity as an important factor driving the community distribution in this estuarine ecosystem (Padmavati et al., 1996; Goswami 1982; Goswami and Singbal, 1974).

Coastal waters (outer and inner continental shelf) and open Ocean

In a comparative note, shelf coastal waters are less variable in water temperature and salinity, makes them more stable than the dynamic estuarine environments. Coastal regimes reflect seasonal changes with respect to consecutive seasons. River discharge during monsoonal periods is a prime factor for diluting the coastal areas and the mixing process increases turbidity in the water column. The anthropogenic activities and industrial wastes are the major contributing factors in changing water quality on a wide scale. But majorly coastal upwelling and river discharge brings nutrients to the coastal area resulting in high productivity (Banse, 1968) and major changes in hydrography which influence the variation in biological processes. Moreover, its effect induces primary production and enhances the secondary productivity in the coastal zooplankton communities (Madhupratap et al., 1990). In addition, there is coastal hypoxia occurring along the west coast of India (Naqvi et al., 2006). In every year this process has been

estimated as a growing concern for coastal living resources. With the spreading of hypoxia, anoxic conditions have also reported in the eastern Arabian Sea during summer monsoon (Naqvi et al., 2000; Naqvi et al., 2006a).

Open waters are mostly characterized by poor nutrients mediating low primary productivity (Krey and Babenerd, 1976; Bhattathiri et al., 1996). Advection of upwelled water can lead to higher productivity in some seasons. Environmental conditions of open ocean system are more stable than the estuarine and coastal environments. Observations has made during JGOFS studies revealed sustenance of high zooplankton biomass both in coastal and open Ocean recorded both in coastal and open ocean waters during spring intermonsoon (Wishner et al., 1998). Also, comparable rates of primary production and the quantity of mesozooplanktonic biomass were obtained in the open ocean due to upwelling and lateral advection (Kumar et al., 2001). Overall, the Arabian Sea is a perfect choice site for carrying the field investigations of pelagic ecosystems due to its wide range of trophic interactions which happening in a land covered system, narrow continental margins, and with strong physical forcing. By keeping all these significant features as a background the present study was an attempt to understand the spatio-temporal variation of metazooplankton communities at a single time scale from the dynamic estuarine, coastal and open ocean environments with the association of surrounding environmental attributes. Further, this study also focused on the seasonal and spatial specific zooplankton communities with respect to environmental parameters.

3a.2 Material and methods

3a.2.1 Study area The detailed description of sampling sites and their geographical mapping details are given in Chapter 2. In this chapter, I have reported the seasonal variation of metazooplankton in the Mandovi and Zuari estuaries covering four seasons

fall-intermonsoon (FIM: October-November 2011); northeast monsoon (NEM: December 2011-March 2012); spring intermonsoon (SIM: April-May 2012) and southwest monsoon (June-September 2012). Besides, the second part of the chapter covers the spatio-temporal variations of zooplankton communities with respect to Open Ocean and coastal stations.

3a.2.2 Sampling strategy Two ways of sampling were carried out to access the spatio-temporal distribution of metazooplankton communities in estuarine as well as open waters of the Arabian Sea.

1. Monthly sampling (October 2011 to September 2012) were carried out in both the estuaries. Covering three distinct regions with three stations. The selected stations were located at the mouth (M1 and Z1), middle (M3 and Z4) and upstream (M3 and Z7) region. From all these stations surface water samples were collected using Niskin Sampler (for details please see chapter 2).
2. Cruise sampling in the coastal and open ocean was carried out based on the availability of cruise time covering the seasonal transitions of the Arabian Sea. The seasonal periods covered were southwest monsoon (SWM: September-2014), fall intermonsoon (FIM: October-November 2013) and northeast monsoon (NEM: March 2015). Sampling was carried out on a spatial scale from the estuarine (M1, Z1, M3, Z4, M6 and Z6), coastal (G5: inner shelf, G12: outer shelf) and open ocean (ASTS) sites of the Arabian Sea. The detail position and descriptions of the sampling sites are given in chapter 2.

Estuarine Sample collection

Water samples were generally collected from 1m below surface of all the estuarine stations using a 5 L Niskin sampler for hydrographical and biological parameters. The collected chlorophyll *a* (Chl *a*) and nutrient samples were stored in an icebox soon after the collection to avoid losing its actual concentration. Care was taken not to expose Chl *a* samples to bright light. Temperature and salinity were immediately noted using the hand held thermometer and salinometer (ATAGO). The sample collected for dissolved oxygen (DO) also fixed immediately with Winkler's solution. The metazooplankton samples were collected by using plankton net. Details of sample collection, preservation and analysis are mentioned below.

Coastal and Open Ocean sampling

Water samples were collected from the coastal (inner shelf: G5; outer shelf: G12) and open ocean (ASTS) sampling sites in the Arabian Sea by using 10-liter Niskin bottles mounted on a Sea-Bird Electronics CTD (conductivity-temperature-depth)-Rosette system which provide vertical profiles of temperature and salinity from the respective station. Water samples from the desired locations and depth were collected using CTD for the analysis of biological (chlorophyll *a*) and hydrographical parameters (salinity; temperature; nutrients: nitrate and nitrite; dissolved oxygen). Appropriate plankton net (200 µm mesh size) was used to collect zooplankton from the desired strata.

3a.2.3 Measurements of biological and hydrological parameters

3a.2.3.1 Metazooplankton

In the present study metazooplankton mostly represents copepods and other zooplankton groups such as holoplanktonic, meroplanktonic larval forms and gelatinous plankton. These zooplankton samples were collected from the estuarine sampling

stations by using the Heron-Tranter net (mesh size 200 µM) with the mouth area of 0.25 m^2. The net was towed obliquely for five minutes and the volume of water filtered was calculated using the digital flow meter (Hydrobios German, 438110). After retrieving the net on the deck, zooplankton samples collected at the cod-end bucket of the net was transferred to a plastic bottle. Further, the samples were passed through a 200 µM mesh to retain metazooplankton and immediately then soaked on an absorbent paper to remove water content. The bio-volume measurement was followed by the process of transferring the collected zooplankton to a measuring cylinder with a known volume of water. The volume displaced in the measuring cylinder was considered to determine the total wet biomass. The biomass measurement for the estuarine sampling sites was not taken to consideration for result and data analysis because of its biomass values were interrupted by dense content of resuspended estuarine debris. The metazooplankton samples were preserved with 4% buffered formalin.

In the coastal and openwaters of the Arabian Sea the metazooplankton samples were collected by using Multi-Plankton Net (Hydro-Bios, mouth area 0.25 m^2, meshsize 200 µm) during day/night hours subjected to the arrival of station. Sampling was done based on the surface mixed layer and the oxygen profile at coastal, outershelf and opensea stations.

In most of the samples, since copepod contribution was much higher to the total stock of metazooplankton. Present study focused more on the copepod species taxonomy and to some extent on other metazooplankton groups.Total metazooplankton and copepod numerical counts were calculated for the whole sample in the terms of ind. /100m^{-3}. Taxonomic composition of all zooplankton groups and copepods were identified through the stereo zoom microscope (Nikon SMZ1500) by using standard identification keys (Kasturirangan, 1963; Sewell, 1999; Conway et al., 2003).

3a.2.3.2 Chlorophyll *a*

The photosynthetic pigments are essential for fixing carbon and capable of absorbing blue-violet and red light. The particular pigments like chlorophyll *a*, fluoresce in the red wavelength after extraction in acetone excited by the blue wavelength of light. It was measured using fluorometer following before and after acidifying the sample. Predetermined acidification factor was calculated following JGOFS Protocols used to find the relative strength of chlorophyll and phaeopigments can be calculated for both chlorophyll *a* and phaeopigment concentrations (JGOFS Protocols 1994). In estuarine sites, 500 ml of water samples were collected for the chlorophyll *a* analysis whereas, 1 liter of samples was processed for the coastal and open ocean sampling sites. These samples were filtered through Whatman (GF/F) filter paper with the pore size of 0.7 µm. Then the extraction process was carried out for 24 hours with 10 ml of 90% acetone in the dark place at -20°C. After this process samples were brought down to room temperature and the fluorescence was measured in a fluorometer (Turner Designs, Model no. 10-AU) before and after acidification of samples by using 2 drops of 1.2 M HCl. Particularly the pigment chlorophyll *a* was calculated from the fluorescence reading with following the calibration factor (JGOFS Protocols 1994). Prior to analysis, fluorometer was calibrated with the standard chlorophyll *a* (Sigma Aldrich from Spinach), which was dissolved in 90% acetone for 2 hours and then measured by fluorometrically. The concentration of chlorophyll *a* and phaeopigments was calculated with the following methods:

Chl *a* (µg/l) = $[F_m/ (F_m-1)]*(F_0-F_a) * (K_x/V_0) * [Vol_{ex}/Vol_s]$

Phaeo (Chl equiv. µg/l) = $[Fm/ (Fm-1)] * \{(Fm * Fa) –F_0\} *K_x –Vol_{ex}$

Where

F_m = acidification coefficient (F_0/F_a) for pure Chl *a*

F_0 = Reading before acidification

F_a = Reading after acidification

Kx = Door factor from calibration calculations (for 10 ml standard)

Vol_{ex} = Extraction volume (ml)

Vol_s = Sample volume (ml)

V_0 = Volume used for calibration in ml (usually 10 ml)

3a.2.3.3 Temperature and Salinity

In the estuarine sampling sites, the temperature was also measured by a quality centigrade reversing thermometer attached to the 5L Niskin sampler apart from CTD Sensor. The salinity was noted from a portable CTD sensors (Conductivity, Temperature, and Depth) profiler (SBE 19plus V2 SeaCAT Profiler). Whereas in the open ocean, Seabird CTD was used to measure temperature and salinity levels.

3a.2.3.4 Dissolved Oxygen

Standard protocol for all hydrochemical analysis was followed as per Grasshoff et al. (1983). The analysis of dissolved oxygen was followed by Winkler's method where oxygen was fixed with Winkler's reagents. After the collection, water samples were immediately fixed by adding 1ml of Winkler's A (manganous chloride) and 1 ml of Winkler B (alkaline iodide) solution to 125 ml of samples collected from respective depths. Samples collected from the estuarine sites were analyzed by titration method (Grasshoff et al. 1983). Whereas the samples collected from coastal and open ocean sites were measured by colorimetric technique (Pai et al. 1993).

3a.2.3.5 Nutrients

Water samples for the nutrient were collected in 60 ml plastic bottles. After collection, the samples were frozen until the time of analysis. All the samples collected from

estuarine and Arabian Sea were analyzed by SKALAR autoanalyzer. Water samples were brought down to attain room temperature prior to analysis. The automated analyses are based on the colorimetric methods described by Grasshoff et al. (1983). All standards were carefully prepared with accuracy (Grasshoff et al., 1983) and stored at low temperature (4°C). The standards were compared and fully checked with CSK nutrient standards.

Nitrite and nitrate

Nitrite (NO^{2-}) analysis involves the diazonium coupling reaction in which an aromatic amine N-(1-napthyl) ethylenediamine dihydrochloride reacts with NO^{2-} present in the sample to form a diazonium compound. Further, it reacts with the secondary aromatic amine (sulphonamide hydrochloride) to form a pink colored azodye (Bend Schneider and Robinson, 1952). The absorbance of the color was measured at 543 nm. In case of NO^{3-} analysis, the reaction process, color formation and its measurement remains same as that for nitrite. Before diazo coupling reaction, the sample was passed through a packed column of activated copperised cadmium in a buffered medium of pH 8.2 (Grasshoff, 1970) so that NO^{3-} is almost quantitatively reduced to No^{2}-. The concentrations measured by this method are actually the sum of nitrate and nitrite.

$No^{3-} + Cu(s) + 2H^{+} \rightarrow No^{2-} + CU^{2+} + H2O$

3a.2.4 Statistical analysis

The statistical analysis of environmental parameters and metazooplankton data are analysed by using software packages PRIMER (V. 6.1). The diversity indices were measured for the total metazooplankton community and as well as copepod species diversity by using PIMER (V. 6.1). Spatio-temporal differences of environmental factors were accessed by one way Analysis of Variance (ANOVA) with Turkey's Post

Hoc test in SPSS 16. Further PERMANOVA was used to compare the statistical difference between stations, while seasonal difference was analysed following PRIMER (V 6.1). Differences between the sampling sites were visualised based on the abundance data by non- metric multi-dimensional scaling plots (nMDS) (Clarke and Gorley, 2001). This statistical analysis revealed the grouping of sampling sites based on the Bray-Curties similarity index. Additionally, SIMPER analysis was used to find the species contribution for different grouping. Seasonal and station wise correlation between environmental factors and total metazooplankton abundance as well with copepod community abundance were determined by using Spearman rank correlation analysis (Microsoft Excel, windows 2013). The overall correlation (with respect to all sampling sites and Seasons) of environmental and metazooplankton abundance were analysed by using SPSS 16. Spatio-temporal variations in the effect of environmental influence on metazooplankton community distribution were accessed by Canonical Correspondence Analysis (CCA). This analysis was performed by CANACO (ter Braak and smilauer, 2002). All the wide scale abundance data were logarithmically log(X+1) transformed to meet the normality.

3a.3 Results

3a.3.1 Seasonal cycle of hydrography in the estuarine system on the basis of monthly sampling

On a seasonal scale covering four seasons (FIM, NEM, SIM and SWM) from October 2011- September 2012, selected hydrographical parameters were recorded from three stations (M1, M3, M6) of Mandovi estuary (Fig. 3) and three stations of Zuari estuary (Z1, Z4 and Z7) (Fig. 4). Sampling period was selected for four seasons such as FIM, NEM, SIM, and SWM.

Mandovi estuary

Temperature:

Spatially higher average temperature was recorded at upstream regimes (stns. M3 and M6; ~28°C (Fig. 3). Likewise fluctuation in water temperature was much larger at upstream region (M6) than at the mouth and mid reach station (M1 and M3). However on a seasonal scale water temperature was much warmer (>28°C; Fig. 3) during FIM and SIM compared to NEM and the least was recorded in SWM. Interestingly, large fluctuation in temperature (~ 5°C) was observed during NEM and SIM (Fig. 3).

Salinity: Salinity also showed large fluctuation in the estuarine system (near zero salinity to 36 PSU) with an annual average of 18 PSU (±13 PSU) spatially. Unlike water temperature, fluctuations in salinity were much larger at the mouth and mid reach of estuary (M1 and M3). On seasonal scale also, lowest saline water prevailed during SWM followed by FIM and the most saline water prevailed during SIM where water column found to be more stable from salinity view point (Fig. 3).

Dissolved oxygen (DO): On an annual scale, surface waters of the estuarine system remained well oxygenated (avg. 179 ± 44.66 µM). Upstream region M6 found to be more oxygenated (avg. 223.3 µM) than other two stations. High saline warm water that prevailed during SIM found to hold less dissolved oxygen (avg. 174.1 µM) compared to freshwater dominating during SWM (avg. 205.4 µM). Similar to salinity trend, dissolved oxygen concentration was also much lower during SIM (FIG. 3).

Nitrate: Spatial nitrate concentration fluctuated in the estuarine system (from below detectable limit to 23 µM) with an average of 5µM (± 7 µM). The average nitrate was comparatively less (avg. 2.96; ± 6.38 µM) at the mouth than the mid reach station M3 (avg. 4.89 ± 6.26) and upstream station M6 (8.14 ± 6.47 µM). Seasonally the average

nitrate concentrations fluctuated from lowest value (avg. 1.54 ± 2.31 µM) during SIM to the highest average of 9.13 (±8.88 µM) of SWM (FIG. 3).

Nitrite: Similar to nitrate, wide fluctuations in nitrite concentration was also recorded. The range of annual nitrite varied from below detectable limit to 6 µM). Spatially varied mouth (M1) and mid reach stations (M3) revealed less nitrite concentration than the upstream (Fig. 3). On the other hand, nitrite concentrations showed seasonal fluctuations with the highest average concentration (2.26 ±1.95 µM) during NEM and the lowest values were in SWM and FIM. Somewhat similar to water temperature and salinity trend (Fig. 3).

Chlorophyll *a*:

Annual scale of Chl *a* varied from 0.1-6.44 (avg. 2.73 ±1.76 µg L^{-1}). Highest concentration was noticed at the mid reach station M3 (avg. 3.52 ±1.81 µg L^{-1}) than at the mouth M1 (avg. 2.49 ±1.11µg L^{-1}) and upstream station M6 (avg. 2.66 ±1.33 µg L^{-1}). Less productive waters of the estuarine system prevailing during SWM (avg. 1.78 ±1.2 µg L^{-1}) found to attain its peak during SIM (avg. 4.4 ±2.2 µg L^{-1}) despite being relatively low in nutrient-NO_3^- (Fig.3).

Zuari estuary

Temperature: During the annual cycle water temperature varied from 24.8-31.5°C (avg. 28.4 ±1.9°C) in the Zuari estuary. Unlike Mandovi estuary, Middle reach station (Z4) depicted comparatively higher surface temperature than the other two regions. Highest temperature (avg. 31.5 ±0.1°C) was reported during SIM and lowest temperature (avg. 27.06 ±2.7°C) was recorded in NEM. The annual fluctuation in temperature observed at the mouth region (Z1) was much lower than at the other two regions. Seasonally not much variation was observed during transition period (FIM and

SIM) when warm sea surface temperature (SST) prevailed, maximum during SIM. Lower SST is reached during winter (avg. 27.66 ±2.1°C) encountered much larger fluctuations than other periods (Fig. 4).

Salinity: Unlike SST, variation in salinity is the region was much larger varying between 0.03-35.95 PSU. This low salinity water always prevailed at the upstream region, salinity of which did not fluctuate much compared to observed wide fluctuation at station Z4 (~29 PSU). However on a seasonal scale salinity did not fluctuate much between FIM, NEM and SIM. Salinity fluctuation recorded during SWM was lower (~31.6 PSU compared to SIM (35.7 PSU), NEM (34.6 PSU) and FIM (33.1 PSU) (Fig. 4).

Nitrate: The value of nitrate concentration in the region ranged from below detectable limit to 18.25 µM with an annual average of 4.34 (± 4.88 µM). Higher concentration was recorded at upstream stations (Z4 and Z6). At times station Z4 also experienced undetectable concentration of NO^{3-} concentration. This fluctuates on an annual scale at station Z4 and Z6 were much larger (16-18 µM) than at Z1 (~8.5 µM). Higher values were recorded during SWM at all the stations (Z1, Z4, and Z7) of this estuary (Fig. 4). Average concentration remained well below 1 µM during SIM as compared to other seasons with a maximum during SWM (avg. 7 µM).

Nitrite: Similarly, the annual variation of nitrite ranged from the below detectable limit to 5.6 µM (avg. 0.83 ±1.25). Overall, the station Z4 revealed the highest concentration (avg. 4.88 ±1.83 µM). As expected seasonal fluctuation were lower during transition period (FIM and SIM) than in NEM and SWM (avg. 16-18µM). Build-up of nitrite concentration (>1 µM) was found to be associated with NEM season (avg. 1.5 ±1.9µM) and the least seasonal concentration was recorded (avg.0.3 µM ±0.3 µM) during FIM.

During this period the lowest value of 0.12 (± 0.03 µM) was recorded at all upstream strata Z7.

Dissolved oxygen: Yearly variation of dissolved oxygen (ml l^{-1}) fluctuated between 145-262 µM (avg. 198 ±28µM). Spatially, whole estuarine surface water remained well oxygenated (avg. > 179µM) throughout the study period. Nonetheless, upstream station Z6 was more oxic compared to near mouth region. Annual variation observed at these sites was well within 22.3 µM. Further average seasonal variation was also well within 22.3µM. The seasonal average concentration at Z1 during FIM, NEM, SIM, and SWM were 181 (± 2), 199 (± 9), 206(± 36) and 183 (± 32) µM respectively. At the station Z4, the highest value was noticed with an average of 187 (± 24) and 211 (± 20) µM during SIM and SWM. Comparatively the station Z7 obtained high concentrations 234-235 (± 6-27) µM at the station Z7 during FIM and SWM (Fig. 4).

Chlorophyll *a*: The annual range of chlorophyll *a* varied from 1 to 14.7 µg L^{-1} in the whole estuary. The highest amount of chlorophyll *a* was recorded at upstream station (Z7; Avg. 6.95 ±3.72 µg L^{-1}), which is roughly two fold lower than Chl *a* concentration recorded at Z1 (Avg. 3.3 ±3.6 µg L^{-1}). Both at Z1 and Z7 annual fluctuations were 2 fold more that at mid reach station (Z4). Average concentrations during NEM and SEM to be found comparable (avg. 5 µg L^{-1}) that was much lower than the recorded concentrations of SIM (avg. 6.8 ±2.8 µg L^{-1}). The highest chl *a* concentrations were recorded during SWM (14.7 µg L^{-1}), followed by FIM (9.87 µg L^{-1}; Fig.4).

3a.3.2 Correlation between the environmental parameters and metazooplankton abundance

Spearman's rank correlation analysis was applied to measure the overall correlation between the environmental parameters and total metazooplankton abundance during the

annual cycle (October 2011-September 2012). Moreover, the seasonal correlation of environmental parameters at each stations (M1, M3, and M; Z1, Z4 and Z7) were analysed through spearman's rank correlation analysis. In this context only the seasonal period of NEM and SWM revealed the statistical significant correlation ('r' value). Whereas the sampling season of FIM and SIM could not be calculated statistically due to less number of samples obtained in that period.

Mandovi

The relationship between environmental variables and metazooplankton was studied using spearman's rank correlation. Annually, the total zooplankton and copepod species showed positive correlation with salinity (r= 0.527, r= 0.526) in the Mandovi estuary (Table 1).

During NEM, the metazooplankton and copepod species showed positive correlation with salinity both at stations M1 (r=0.62; r=0.63) and M3 (r=0.75). Whereas at upstream station (M6), the total metazooplankton and copepod species showed positive correlation with salinity (r=0.93 and r= 0.92) and temperature (r= 0.51 and r= 0.55).

During SWM, the total metazooplankton and copepod species showed strong positive correlation with nitrate (r= 0.93, r= 0.94) and nitrite (r= 0.74, r= 0.72) at station M1. Wherein at station M3, the total metazooplankton and copepods species also showed positive correlation with temperature (r= 0.70, r= 0.67), apart from salinity (r= 0.98, r= 0.97) and chlorophyll *a* (r= 0.62, r= 0.63). At station M6, the total metazooplankton and copepod species showed strong positive correlation with temperature (r= 0.89, r= 0.91), salinity (r= 0.72, r= 0.78), chlorophyll *a* (r= 0.89, r= 0.92, nitrite (r= 0.83, r= 0.78) (Table 2).

Zuari

Annually, the total metazooplankton and copepod species showed significant correlation with salinity (r= 0.522, r= 0.469) and DO (r= -0.456, r= -0.437) in the Zuari estuary (Table 3).

During NEM, the total metazooplankton and copepod species showed negative correlation with temperature (r= -0.86, r= -0.81), salinity (r= -0.88, r=0.82), Nitrate (r= 0.67, r= 0.69), DO (r= 0.94, r= 0.89), chlorophyll *a* (r= 0.68, r= 0.75) at station Z1. Wherein station Z4, the total metazooplankton and copepod species showed strong positive correlation with nitrate (r= 0.98, r=0.97) and negative correlation with chlorophyll *a* (r= -0.52, -0.52). At station Z7, the total metazooplankton was positively related with temperature (r= 0.69, r= 0.79) and salinity (r= 0.58, r= 0.45).

During SWM, the total metazooplankton was positively related with nitrate (r= 0.82), DO (r= 0.69) and chlorophyll *a* (r= 0.52). And the total copepod species was positively related with DO (r= 0.93) and Chlorophyll *a* (r= 0.94) at Z1. Wherein at station Z4, the total metazooplankton and copepod species was positively related with temperature (r= 0.90, r= 0.90), salinity (r= 0.76, 0.77) and nitrite (r=0.57, 0.59) and negatively related with DO (r=-0.56, r= -0.58) and nitrate (r= -0.70, r= -0.69). At station Z7, the total metazooplankton was positively related with DO (r= 0.73) and negatively related with nitrate (r= -0.56) and nitrite (r= -0.76; Table 4).

3a.3.3 ANOVA and PERMANOVA (among environmental variables of Mandovi)

In Mandovi estuary, as per one-way ANOVA with respect to seasonal variations of measured environmental parameters, temperature ($p<0.01$), salinity ($p<0.01$), nitrite ($p<0.05$) and chlorophyll *a* ($p<0.05$) were statistically significant (Table 5.a). As per station wise variation of environmental parameters salinity ($p<0.01$), nitrite ($p<0.05$)

and DO ($p<0.01$); (Table 5.b). Based on these environmental differences further PERMANNOVA was used to find out the statistical significant difference among the stations and seasons. As per this pairwise tests significant differences were observed in between NEM and SWM, NEM and SIM, SIM and SWM ($p<0.5$) (Table 6.a). Also there was a significant difference environmental parameters in between near mouth and upstream ($p<0.01$) and mid reach and upstream ($p<0.01$) stations of the Mandovi estuary (Table 6. b).

In case of Zuari estuary, temperature and nitrate ($p<0.05$) showed their significant differences within the different seasonal periods whereas with respect to different stations, salinity, nitrite, DO and chlorophyll *a* ($p< 0.05$) were statistically different (Table 7. a and b). Based on these environmental differences PERMANOVA (pairwise test) results were agreed with statistical seasonal difference between FIM and NEM ($p< 0.05$), NEM and SWM ($p<0.05$), and SIM and SWM ($p<0.05$) (Table 8.a). Moreover the spatial differences were significantly different ($p<0.01$) between all the stations (nearmouth, midreach and upstream) (Table 8.b).

3a.3.4 Seasonal cycle of metazooplankton abundance in estuarine systems on the basis of monthly sampling

3a.3.4.1 Seasonal abundance of total metazooplankton and copepod abundance in the Mandovi estuary

At station M1 the average seasonal total metazooplankton abundance varied from 55616 (±64685) to 212560 (±166651) ind. $100m^{-3}$. Highest zooplankton abundance was recorded during FIM with 84% of copepod contribution; the lowest contribution of copepod was in NEM. At M3, highest zooplankton abundance of 633136 (±420017) ind. $100m^{-3}$ was also recorded during FIM. The highest copepod abundance contributed

as much as 83% of the highest zooplankton abundance during FIM. In case of the upstream station (M6), highest zooplankton abundance with an average of 17333 ind. $100m^{-3}$ (±13553) was obtained during NEM and the lowest abundance was found in SWM with an average of 329 ind. $100m^{-3}$ (±406). All over the copepod abundance at the station M1 accounts 84-94% of the total zooplankton community, highest during SWM. At the station M3 the percentage of copepod contribution ranged from 61-93% where the highest contribution was recorded during NEM and the lowest in SWM. Similarly at the station M6, the abundance of copepod community ranged in between 45 and 89% of the total zooplankton abundance (Fig. 5.a and b).

3a.3.4.2 Seasonal abundance of total metazooplankton and copepod abundance in the Zuari estuary

On a seasonal scale total zooplankton density varied from 27833-98770 (±17422-83190) ind. $100m^{-3}$ at the station Z1 with the highest contribution during SIM and the lowest in NEM. The copepod abundance ranged from 18181-85966 (±9441-75161) ind. $100m^{-3}$. The highest copepod abundance accounted of 87% of the bulk zooplankton community. At the station Z4, seasonal variation of zooplankton abundance ranged from 52719-222146 (±39937-266933) ind. $100m^{-3}$ where highest abundance was recorded during SWM and lowest was recorded during SIM. The seasonal copepod abundance during this period varied from 26105-211978 (±2531-258844) ind. $100m^{-3}$ with the highest contribution (95%) in NEM and lowest (49%) during SIM. Total zooplankton abundance at the station Z7 was comparatively low that ranged between 4032-30978 (± 5506-12234) ind. $100m^{-3}$. The highest abundance was recorded during SIM and the lowest was obtained in SWM. Highest copepod abundance accounted 77% of total zooplankton abundance during SIM whereas the lowest contribution (13%) was recorded during SWM. Overall, copepod contribution at Z1 over the four seasons varies

from 34-87% with the highest contribution during the period of SIM. Seasonal variation of copepod contribution at the station Z4 ranged from 49-95% whereas the variation of seasonal copepod abundance at the station Z7 ranged from 13-86% (Fig. 6.a and b).

3a.3.4.3 Metazooplankton community composition in the Mandovi estuary

Station M1

The metazooplankton community structure during FIM revealed highest abundance of calanoid copepods (161840 ind. $100m^{-3}$) followed by decapod larvae (10240 ind. $100m^{-3}$), pelecypoda larvae (7120 ind. $100m^{-3}$), appendicularia (4400 ind. $100m^{-3}$) and copepod juveniles (7440 ind. 100m-3). In case of NEM the dominant communities were represented by calanoida copepods (45700 ind. 100m-3), poecilostomatoida (1233 ind. 100m-3), barnacle naupli (1165 ind. 100m-3), decapod larvae (947 ind. 100m-3) and copepod juveniles (2212 ind. 100m-3). The SIM showed maximum availability of calanoida copepods (63694 ind. $100m^{-3}$), siphonophorae (6574 ind. $100m^{-3}$), decapod larvae (2414 ind. $100m^{-3}$), poecilostomatoida copepods (1677 ind. $100m^{-3}$), cyclopoida copepods (1619 ind. $100m^{-3}$), appendicularia (1411 ind. $100m^{-3}$), harpacticoida (1411 ind. $100m^{-3}$) and pelecypoda larvae (1328 ind. $100m^{-3}$). Interestingly, unlike other seasons, cyclopoida copepods (41502 ind. $100m^{-3}$) were dominant during SWM followed by calanoida copepods (28090 ind. $100m^{-3}$), harpacticoida copepods (3886 ind. $100m^{-3}$), gastropod larvae (1857 ind. $100m^{-3}$) and pelecypoda larvae (620 ind. $100m^{-3}$) (Fig. 7. a).

Station M3

At this station M3, metazooplankton community during FIM was represented by dominant calanoida copepods (420730 ind. $100m^{-3}$), copepod juveniles (58991 ind. $100m^{-3}$), polychaete larvae (34690 ind. $100m^{-3}$), cyclopoida copepods (23003 ind.

100m^{-3}), gastropod larvae (20035 ind. 100^{m-3}) and barnacle nauplii (15026 ind. 100m^{-3}). During NEM maximum abundance was again contributed by calanoida copepods (69017 ind. 100m^{-3}), apart from cyclopoida copepods (30241 ind. 100m^{-3}), copepod juveniles (5245 ind. 100m^{-3}) and barnacle nauplii (2473 ind. 100m^{-3}). SIM did not reveal much difference in abundant communities compared to NEM. In case of SWM, maximum abundance of metazooplankton groups were displayed by calanoida copepods (56640 ind. 100m^{-3}), gastropod larvae (42108 ind. 100m^{-3}), cyclopoida copepods (18694 ind. 100m^{-3}) and decapod larvae (3276 ind. 100m^{-3}) (Fig. 7.b).

Station M6

The community composition of metazooplankton during FIM revealed highest contribution of calanoida copepods (3531 ind. 100m^{-3}), gastropod larvae (3031ind. 100m^{-3}), cyclopida copepods (321 ind. 100m^{-3}), poecilostomatoida (271 ind. 100m^{-3}) and cypris larvae (157 ind. 100 m-3). Compared to FIM, NEM obtained maximum numbers of groups with the contribution of calanoida copepods (12136 ind. 100m^{-3}), gastropod larvae (1790 ind. 100m^{-3}), copepod juveniles (1699 ind. 100m^{-3}), decapod larvae (596 ind. 100m^{-3}), cyclopoida copepods (335 ind. 100m^{-3}), barnacle nauplii (171 ind. 100m^{-3}), harpacticoida (152 ind. 100m^{-3}) and hydroidomedusae (112 ind. 100m^{-3}). During SIM harpacticoida and decapod larvae were higher in counts compared to NEM. Compare to other seasons, the community composition in SWM revealed very low abundance of calanoida copepods (113 ind. 100m^{-3}), decapod larvae (93 ind. 100m^{-3}), gastropod larvae (43 ind. 100m^{-3}) and cladocerans (22 ind. 100m^{-3}). At this station cladocerans were only found during SWM (Fig. 7.c).

3a.3.4.4 Copepod species composition in the Mandovi estuary

Station M1

During the study period, 32 copepod species belong to 44 genera were recorded at station M1. The dominant copepods with percentage contribution to the total copepod community during FIM were *Acrocalanus gibber* (33%), *Acrocalanus gracilis* (21%), *Paracalanus parvus (21%), Temora turbinata* (3%), *Pseudodiaptomus serricaudatus* (2%), *and Pseudodiaptomus jonesii* (2%). During NEM, *Acrocalanus gibber* (47%), *Paracalanus parvus (11%), Paracalanus aculeatus (7%), Acrocalanusgracilis* (7%) and *Euterpina acutifrons* (1%) were predominant forms. In case of SIM, *Acrocalanus gibber* (30%), *Lebidocera sp.* (21%), *Acrocalanus sp.* (14%), *Acrocalanus gracilis* (11%) and *Acartia pacifica* (6%) dominated the copepod community. While during, SWM, the dominant copepods were *Oithona sp.* (33%), *Oithona brevicornis* (19%), *Paracalanus sp*. (9%) and *Acrocalanus sp.* (9%) (Table 9).

Station M3

Overall, maximum number of copepod species (27) was found at this location during entire period of study. The highest and lowest copepod diversity was SIM-M3 and NEM-M3 season, respectively. The copepod community was mostly dominated by *Paracalanus parvus* (43%), *Acrocalanus gibber* (14%) and *Copepod juveniles* (11%) during FIM whereas *Oithona* sp. (21%), *Acrocalanus gibber* (20%) and *Oithona brevicornis* (7%) dominated during NEM. Likewise, SIM period was dominated by *Acrocalanus gibber* (30%), *Lebidocera* sp. (22%) and *Acrocalanus* sp. (11%) and SWM by *Paracalanus parvus* (29%), *Oithona brevicornis* (14%), *Pseudodiaptomus sewelli* (7%) and *Acartia tropica* (4%). The detail distributions of copepod communities are during different seasons is given in Table 9.

Station M6

At this upstream station altogether (23) numbers of copepod species were recorded during the entire study period. Some of the distinct copepod species of this station living at low saline condition were *Acartia southwelli, Allodiaptomus mirabilipes, Diaptomus* sp., *Cyclops* sp. and *Acartiella* sp. During FIM, only 11 species were found at this site and the most dominant form were *Acartia southwelli* (32%), *Allodiaptomus mirabilipes* (23%), *Acrocalanus gibber* (13%), *Paracalanus parvus* (8%), *Onceae venusta* (7%), and *Diaptomus* sp. (6%). Contrary, the *Cyclops* sp. (5%) was highly abundant during FIM compared to other seasons. During NEM, highest number of copepod species (23) was found and the dominant forms were *Acrocalanus gibber* (28%), *Acartia southwelli* (13%) and *Diaptomus* sp. (12%). Compared to other seasons, *Acartiella Keralensis* (2%) revealed its major contribution during this period. Out of the 14 copepod species that prevailed during SIM, *Acartia southwelli* (36%), *Acrocalanus* sp. (19%) and *Diaptomus* sp. (7%) were the most abundant forms. While during SWM, copepod community was represented by 18 species with the dominance of *Diaptomus* sp. (34%), *Acrocalanus gracilis* (11%) and *Acartiella Keralensis* (7%) (Table 9).

3a.3.4.5 Metazooplankton community composition in the Zuari estuary

Station Z1

At the near mouth estuarine station, cladocerans were abundantly (52884 ind. $100m^{-3}$) present during FIM followed by calanoida copepods (27774 ind. $100m^{-3}$), barnacle nauplii (1721 ind. $100m^{-3}$), harpacticoida (1447 ind. $100m^{-3}$), fish eggs (1195 ind. $100m^{-3}$) and cyclopoida copepods (958 ind. $100m^{-3}$). In NEM, metazooplankton community was mainly represented by calanoida copepods (14226 ind. $100m^{-3}$), barnacle nauplii (2160 ind. $100m^{-3}$), poecilostomatoida copepods (2038 ind. $100m^{-3}$),

fish eggs (1833 ind. $100m^{-3}$), pelecypoda larvae (1346 ind. $100m^{-3}$), copepod nauplii (1261 ind. $100m^{-3}$) and decapod larvae (1221 ind. $100m^{-3}$). Highest calanoid copepods (80216 ind. $100m^{-3}$) were obtained during SIM followed by other groups as decapod larvae (6908 ind. $100m^{-3}$), harpacticoida copepods (2740 ind. $100m^{-3}$), poecilostomatoida copepods (1909 ind. $100m^{-3}$), barnacle nauplii (1713 ind. $100m^{-3}$) and fish eggs (1381 ind. $100m^{-3}$). Though hydromedusae were very less (33 ind. $100m^{-3}$) at this site, but they accounted its presence only during this period. During SWM, pelecypoda larvae (16058 ind. $100m^{-3}$), fish larvae (3445 ind. $100m^{-3}$), cyclopoida (2015 ind. $100m^{-3}$), copepod nauplii (1799 ind. $100m^{-3}$) and siphonophorae (193 ind. $100m^{-3}$) were comparatively more abundant than the other seasons (Fig. 8.a)

Station Z4

Community composition of metazooplankton was represented by calanoida copepods (75321 ind. $100m^{-3}$), cladocerans (9481 ind. $100m^{-3}$), poecilostomatoida copepods (4030 ind. $100m^{-3}$) and decapod larvae (1373 ind. $100m^{-3}$). Compared to other seasons cladocerans, poecilostomatoida copepods and harpacticoida copepods were dominant during FIM. Calanoida copepods (204272 ind. $100m^{-3}$) and chetognatha (3706 ind. $100m^{-3}$), cyclopoida (1458 ind. $100m^{-3}$) and lucifers (407 ind. $100m^{-3}$) displayed their maximum abundance during NEM. During SIM, decapod larvae (24789 ind. $100m^{-3}$), barnacle nauplii (1035 ind. $100m^{-3}$) and pelecypoda larvae (402 ind. $100m^{-3}$) displayed their prominent contribution in comparison to other seasonal periods. The abundant groups during SWM were calanoida copepods (188694 ind. $100m^{-3}$), decapod larvae (9585 ind. $100m^{-3}$), cypris larvae (4852 ind. $100m^{-3}$) and gastropod larvae (1691 ind. $100m^{-3}$). Cladoceran were present only during FIM and SWM. Moreover, cypris larvae were most dominantly found in SWM compared to other seasons (Fig. 8.b).

Station Z7

In comparison to other stations, there was very low density of metazooplankton communities were recorded at this upstream station with reference to four distinct seasons. Community compositions in FIM were dominated by calanoida copepods (6195 ind. $100m^{-3}$), decapod larvae (3684 ind. $100m^{-3}$) and cladocerans (433 ind. $100m^{-3}$). Cypris larvae (959 ind. $100m^{-3}$), polychaete larvae (747 ind. $100m^{-3}$), poecilostomatoida copepods (454 ind. $100m^{-3}$), cyclopoida copepods (451 ind. $100m^{-3}$) and gastropod larvae (325 ind. $100m^{-3}$) were found abundantly during NEM in comparison to other season. Comparatively higher abundance was noticed in SIM with the maximum abundance of calanoida copepods (15837 ind. $100m^{-3}$), copepod juveniles (7272 ind. $100m^{-3}$) and decapod larvae (3684 ind. $100m^{-3}$). Pelecypoda larvae (2890 ind. $100m^{-3}$) were only observed during SIM. During the period of SWM cladocerans were highly abundant (2728 ind. $100m^{-3}$) followed by decapods larvae (539 ind. $100m^{-3}$) and calanoida copepods (356 ind. $100m^{-3}$; Fig. 8. C).

3a.3.4.6 Copepod composition in the Zuari estuary

Station Z1

This nearmouth station was represented by 25 copepod species during entire period of study. Overall, the seasonal range of copepod species varied from 18-25. Least diversity (18) was recorded during FIM. The maximum copepod species contributed were *Acrocalanus gibber* (61%), *Acrocalanus gracilis* (9%), *Euterpina acutifrons* (5%), *Paracalanus aculeatus* (4%), and *Temora turbinata* (4%). Whereas, during NEM, 20 copepod species were observed in this region. Amongst, *Acrocalanus gibber*, *Acrocalanus* sp. (15%), *Paracalanus aculeatus* (12%) and *Paracalanus parvus* (8%) were dominant forms. Seasonal period of SIM revealed maximum number of copepod

species (25) with the dominance of *Paracalanus* sp. (32%), *Acrocalanus* sp. (20%), *Acrocalanus gibber* (17%), *Paracalanus parvus* (13%), *Acrocalanus gracilis* (4%) and *Euterpina acutifrons* (3%). Similarly, 25 species of copepods were observed during SWM. The dominant copepods were *Acrocalanus gibber* (30%), *Acrocalanus* sp. (15%), *Acartia centrura* (12%), *Paracalanus aculeatus* (9%), *Paracalanus parvus* (4%), *Acrocalanus gracilis* (3%) and *Acartia tropica* (2%; Table 10).

Station Z4

This middle reach station was represented by 16-21 number of copepod species over the whole seasonal cycle. Maximum number of copepod species were recorded during FIM and the community was mainly represented by *Acartia centrura* (14%), *Paracalanus parvus* (11%), *Acrocalanus sp.* (11%), *Acartia erythreae* (10%), *Acartiella keralensis* (8%), *Acartia pacifica* (8%), *Acartia tropica* (6%), *Acartiella sewelli* (4%) and *Onceae venusta* (4%). Comparable number of copepod diversity (20) was also observed in NEM. The dominant copepods were *Paracalanus parvus* (20%), *Paracalanus aculeatus* (18), *Acartia centrura* (12%), *Acartia tropica* (12%), *Acartiella gravelyi* (7%), *Acartiella keralensis* (6%) and *Pseudodiaptomus sewelli* (5%). Likewise, SIM was represented by 21 species and *Paracalanus parvus* (53%), *Acartia tropica* (13%), *Acrocalanus gibber* (11%), *Acartiella keralensis* (4%) and *Acartia* sp. (3%) were the most abundant forms. Least diversity of copepods (16 species) was found during SWM with dominant copepods *Paracalanus aculeatus* (35%), *Paracalanus parvus* (34%), *Acrocalanus gibber* (11%) and *Acartia* sp. (3%; Table 10).

Station Z7

In all 19 copepod species were recorded at this upstream station with respect to different seasonal periods. Overall, *Diaptomus* sp., *Cyclops* sp. and *Allodiaptomus*

mirabilipes were only forms documented at this upstream station. Very less number (9) of species were encountered during FIM. Amongst, *Diaptomus* sp. (64%), *Acrocalanus gibber* (14%), *Acartiellla keralensis* (7%), *Psudodiaptomus jonesii* (4%) *and Acrocalanus gracilis* (2%) were dominant forms. Maximum number (19) of copepods were observed during the period of NEM. Highest contribution (34%) of copepod juveniles were noticed during this period. *Acartiella keralensis*, *Acartia sewelli* (12%), *Acartia pacifica* (9%), *Acartia tropica* (8%), *Pseudodiaptomus* sp. (7%) and *Acartiella sewelli* (2%) were dominant copepods in this season. Copepods were equally diverse (15) during SIM. Contribution of copepod juveniles were sizably large 31%. *Pseudodiaptomus jonesii* (24%), *Acartiella keralensis* (8%), *Diaptomus* sp. (6%) and *Acartiella gravelyi* (5%) were predominant forms during this season. In SWM, total 17 species were encountered with the dominance of *Diaptomus* sp. (18%), *Acrocalanus* sp. (9%), *Cyclops* sp. (7%) and *Acartia tropica* (6%; Table 10).

3a.3.4.7 Diversity

Mandovi

The range of total number of species (S), metazooplankton diversity index (H´), species richness (d) and evenness (j´) based on the metazooplankton groups in the Mandovi estuary was statistically evaluated. Seasonally, the number of groups (S) in Mandovi estuary revealed the variation of 15-19 (at M1), 13-18 (at M3) and 6-17 (at M6). Comparatively, NEM showed pronounced group abundance at all 3 stations than other seasons. The seasonal diversity index (H´) ranged from 3.1-3.4 (M1), 2.7-3.48 (M3) and 2.2- 3.4 (M6). The highest diversity was recorded during SWM. Similarly, species richness (d) on a seasonal scale that varied from 2.1-2.8 (M1), 1.8-2.5 (M3) and 0.9-3 (M6) also showed higher values in SWM. Highest value of species evenness was similarly during SWM (at M6) and in SIM (at M3; Fig. 9 and Table 11).

Copepod diversity evaluated based on the number of copepod species (S) varied from 24-29 (M1), 22-27 (M3) and 11-23 (M6). The maximum number of species were recorded at the station M1 during NEM. Highest diversity index (H´) value of 4.2 was noted at the station M3 during SWM whereas the lowest copepod diversity (3.2) was obtained during FIM at the station M6. Overall the species richness ranged from 3.1-4.1 (M1), 2.7-3.7 (M3) and 1.9-4.4 (M6) with the highest abundance at M6 during SWM. Maximum evenness (0.95) was observed at the station m6 during SWM and the lowest value (0.84) was recorded at M1 in the period of SWM (Fig. 10 & Table 12).

Zuari

Seasonal variation of number of metazooplankton groups were recorded at Z1 (12-17), Z4 (10-18) and at Z7 (05-10). Seasonal range of zooplankton group diversity at the station Z1 ranged from 2.7-3.7 and the highest diversity was recorded in NEM. At station Z4, the diversity range came down between 2.3 and 2.66 whereas the station Z7 revealed wide range (2.1-2.9) of diversity than the Z4 station. Wide range of species richness (d) was also observed at the station Z4 and Z7, than at Z1.The seasonal range of species evenness were found at Z1 (0.76-0.91), Z4 (0.63-0.78) and Z7 (0.75-0.87) (Fig. 11 and Table 13).

High diversity of copepod species (25) was recorded at the station Z1 during the season SIM and SWM. The pronounced range of seasonal diversity was noticed at the station Z1 (3.6-4.2) and Z4 (3.8-4.2). The station Z1 and Z4 revealed high copepod diversity in SWM (4.23) and FIM (4.29). The species richness was highest at the station Z1 during SWM and the lowest value was recorded at the station Z7 during FIM. Species evenness followed the range 0.85-0.92 (Z1), 0.8-0.96 (Z4) and 0.9-0.96 at Z7 (Fig. 12 and Table 14).

3a.3.4.8 Spatio-temporal variation of zooplankton (major groups and copepods) Mandovi

nMDS based on metazooplankton groups and copepod species abundance clearly explains the extent to which three distinct regions (mouth, midreach and upstream) differ with respect to corresponding seasons (Fig. 13. a & b). nMDS plot based on the Bray-Curtis similarity index of zooplankton group abundance indicated 4 clusters at the level of 60% similarity (Fig. 13. a). Cluster 1 grouped SIM-M1, NEM-M1, FIM-M1 and FIM-M3. Cluster 2 grouped SWM-M1, NEM-M3 and NEM-M6. Cluster 3 grouped NEM-M3, SWM-M3 and SIM-M3, and cluster 4 grouped FIM-M6 and SWM-M6. Further **SIMPER** analysis was carried out to highlight the account of zooplankton groups which makes prominent contribution for the dissimilarity between stations and seasons. High difference of zooplankton groups were recorded between mouth (M1) and upstream stations (M6) followed by midreach (M3) and upstream station (M6). Gastropod larvae (17%), cyclopoida copepods (10%) and decapod larvae (10%) revealed the contribution of higher difference between near mouth (M1) and upstream (M6) stations whereas cyclopoida copepods (14%) accounted higher difference in between midreach (M3) and upstream stations (M6). On a temporal scale maximum average dissimilarity between SIM and SWM was due to the contribution of cyclopida copepods (15%), Gastropod larvae (13%) and Decapod larvae (9%) (Table 15. a).

Similarly, in case of copepod species nMDS plot also produced 4 clusters (i) included FIM-M6 and SWM-M6, (ii) with SIM-M3, NEM-M3 and SWM-M3, (iii) with FIM-M3, SIM-M1, FIM-M1 and NEM-M1, and (iv) with SWM-M1, NEM-M6 and SIM-M6 (Fig. 13.b). As in zooplankton groups, **SIMPER** analysis revealed highest scale of average dissimilarity between mouth and upstream station by the maximum contribution *Acartia southwelli* (11%), *Diaptomus* sp. (9%), and *Allodiaptomus*

mirabillipes (4%); (Table 15.b). On a temporal scale, maximum copepod average dissimilarity was found between the season FIM and SWM followed by SIM and SWM. For instance, the copepod species contributing to the maximum dissimilarity between FIM and SWM were *Oithona* sp. (6%), *Paracalanus parvus* (6%), *Oithona brevicornis* (6%), *Acrocalanus gibber* (5.88) and *Acartia southwelli* (5.41%) and *Diaptomus* sp. (5.22%); (Table 15.b).

Zuari

In the Zuari estuary, nMDS plot based on abundance of zooplankton groups formed only 3 clusters where the largest cluster represented eight sites (NEM-Z7, SIM-Z7, SIM-Z4, SIM-Z1, NEM Z4, SWM-Z4, FIM-Z4 and FIM-Z7). Among other two clusters one showed the grouping between FIM-Z1 and SWM-Z7 while, the other formed cluster with NEM-Z1 and SWM-Z1 (Fig. 13a). Following to this, **SIMPER** analysis was used to detect the contribution of zooplankton groups for responsible for spatial and temporal distinction. Overall, on a spatial scale prominent average dissimilarity of groups were observed between mouth (Z1) and upstream stations (Z7) followed by mouth (Z1) and midreach stations (Z4). Highest contribution of groups to the difference between mouth and upstream stations were cladocerans (17%), copepod juveniles (12%), pelecypoda larvae (9%), calanoida copepods (7%) and decapod larvae (7%) (Table 16.a). Temporally highest average groups difference were noticed in between SIM and SWM, which was mainly due to the contribution of cladocerans (23%), pelecypoda larvae (16%), decapod larvae (15%) and calanoida copepods (9%).

Copepeod abundance data were subjected to nMDS analysis which further formed three major groups at the scale of 60% similarity. One group formed with FIM-Z7, SIM-Z7, SWM-Z7, NEM Z7 and FIM-Z4. The second group included SWM-Z4, SIM-Z4, NEM-Z4, FIM-Z4 and FIM-Z1 while the third group represented NEM-Z1, SIM-Z1

and SWM-Z1 (Fig. 14b). Additionally, **SIMPER** analysis confirmed the large variation of copepod species between mouth and upstream stations. This was mainly by the contributions of *Diaptomus* sp. (8%), *Acrocalanus gibber* (7%) and *Acartiella Keralensis* (6.05%); (Table 16.b). Overall, the temporal variation of copepod species revealed the highest difference between FIM and NEM followed by FIM and SIM. The maximum average dissimilarity between FIM and NEM was due to the contribution of *Paracalanus aculeatus* (6.21%), *Acartia centrura* (6%), *Diaptomus sp*. (5.8%), *Paracalanus parvus (*5.8%*), Acartia tropica (*5.4%*), Acartiella keralensis* (4.8%). The copepod species such as *Paracalanus parvus* (8%), *Diaptomus* sp. (7%), *Paracalanus* sp., *Acrocalanus* sp. and *Acartia* sp. were attained maximum difference between the period of FIM and SIM (Table 16.b).

3a.3.4.9 Effect of environmental influence on copepod population

CCA triplot (Fig. 15a) with 2 axes explained 35% and 66.9% of association between copepod species and environmental factors of three stations in the Mandovi estuary on a seasonal scale. Eigen values of axis 1 and axis 2 are given in (Table 17.a).

CCA plot explains the influence of environmental factors on the distribution of copepod species. Analysis overall revealed the most influence of temperature on the copepod distribution at the first axis, whereas chlorophyll *a* and salinity were represented on the second axis. Among all the factors, temperature and nitrate were the favourable factors for the distribution of copepod species at the stations M1 (NEM), M1 (SIM) and M3 (SIM). In this context CCA ordination indicated copepod species such as *Oithona rigida, Paracalanus aculeatus, Lebidocera pectinata, Acrocalanus monachus, Centropages furcatus, Acartia danae, Acartia pacifica, Centropages tenuremis, Cathocalanus pauper, Oncaea conifera and Macrosetella gracilis* are influenced mostly by temperature. Moreover, salinity was the favourable factor for the association

of *Acartia spinicaudata, Pseudodiaptomus serricaudatus, Temora turbinata, Acrocalanus gracilis, Macrosetella norvegica and Labidocera acuta* at the station M1 (FIM) and M3 (FIM). The copepod species such as *Acrocalanus giber*, *Temora turbinata*, *Temora* sp., *Temora stylifera*, *Corycaeus* spp. and *Faranulla* spp. positioned at the centre of ordination plane indicated the influence of all environmental attributes for their distribution.

In case of Zuari estuary, CCA triplot (Fig. 15. b) with first two axes explained 32% and 56% of the association between the distribution of copepod species and the environmental factors on a saptio-temporal scale (Table 17 b). Temperature, dissolved oxygen and salinity seem to be the most influencing factor for the distribution of copepod species at the sampling sites of M1 (FIM), M1 (NEM), M1 (SIM) and M1 (SWM). *Oithona rigida, Temora stylifera*, *Oithona brevicornis, Temora turbinata, Centropages furcatus, Clausocalanus* sp., *Labidocera pectinata and Acrocalanus gibber* revealed the major influence with temperature and dissolved oxygen. On other axis 2, the environmental parameters such as chlorophyll *a* and Nitrate prominently influence the association of *Paracalanus aculeatus, Paracalanus parvus, Pseudodiaptomus bowmani, Acartiella gravelyi, Acartiella keralensis, Pseudodiaptomus sewelli, Acartia tropica and Acartia centrura* at the station NEM-M3, SWM-M3 and NEM-M6. Nitrite revealed a strong association between *Acartia erythraea*, *Acartiella* sewelli and *Oncaea venusta* (Fig. 15).

3a.4 Discussion

Mandovi and Zuari estuaries are very dynamic ecosystems with spatially and temporally varying physic-chemical and biological features. This underscores system to monitor its community composition in relation to environmental attributes. Though there are lot of studies on zooplankton ecology (Padmavati and Goswami, 1996;

Goswami and Singbal, 1974; Dalal and Goswami, 2001) in both the estuarine systems but these are decade old studies zooplankton community composition in these ecosystems. To trace the recent change in zooplankton community composition and abundance with respect to the current environmental status, monitoring the variation of biotic and abiotic parameters on a spatial and temporal scale will help for better understanding of spatio-temporal heterogeneity in the estuarine systems. Present study depicted the marked seasonality in the region with fluctuating salinity on an annual scale, observation similar to previously published work (Qasim and Sengupta, 1981; Pednekar et al. 2011; Shetye et al. 2007; Vijith et al. 2009). Heavy monsoonal rainfall on the Sahyadri ranges and consequent high riverine run-off causes wide fluctuation in salinity in the Mandovi and Zuari estuaries (Shetey et al., 2007). On an average Goa receives 80% of its total rain fall from June to August (Vijith et al., 2009). During the monsoon (Jun-September) most of the freshwater discharge into the two estuaries comes from its large catchment area (Shetye et al., 2007). Therefore, it is natural to expect the wide range of salinity in the Mandovi (0.04-35.34 psu) and Zuari estuary (0.04-35.53 psu). Wide variation in salinity (4- 31psu) observed in the Mandovi estuary between SWM and SIM indicates the massive freshwater discharge during SWM whereas the intrusion of seawater (tidal influence) raises the salinity to the maximum during SIM. During SIM the mid-estuarine region shows wide variation in salinity. Similarly the upstream station, represented mesohaline zone with the variation of salinity from (0.04-16 psu).

Common zooplanktonic groups such as copepoda (calanoida, cyclopoida, poecilostomatoida and harpacticoida), decapod larvae, chaetognatha, appendicularia, cladocerans, hydromedusae, pelecypoda larvae, gastropod larvae, cypris larvae, polychaete larvae, fish larvae and eggs are dominant in both the estuarine systems. As

per spearman correlation analysis, significant correlation of salinity with the annual variation of zooplankton abundance indicates that the majority groups are of marine origin. Also, seasonal abundance of zooplankton groups revealed positive correlation with salinity particularly during NEM and SWM at all the station (except M1 during SWM). Thus, the associated high abundance of zooplankton in the comparatively low saline waters prevailing at the mouth (M1) was due to the dominance of gastropod larvae and decapod larvae possibly advected from the mid-estuarine stations.

Among the zooplankton community, copepods are major component in these estuaries encompassing brackish, marine and freshwater forms. Some of the copepod species are stenohaline carried by means of tidal current coming from shelf to estuaries (Goswami, 1983). Many of the species are able to thrive in wide range of salinity in both the estuaries are known as euryhaline species. In view of this, food availability is not the limiting factor for the distribution of kind of species found in the estuary. As per Devassy and Goes (1989), the food supply by phytoplankton is available for alternative pathways in these estuarine systems. It clearly states the copepod species of Mandovi and Zuari estuary are possibly able to thrive under a wide range of salinity. Few forms of copepod species such as *Heliodiaptomus cinctus, Heliodiaptomus contortus*, *Allodiaptomus mirabillipes, Diaptomus* sp. *Acrtiella gravelyi* and *Acartia sewelli* were abundant at upstream stations (M6 and Z7) of both the estuary preferring low saline water. The results of the present study demonstrate the role of space and season in the distribution of metazooplankton groups and copepod communities. The seasonal characteristics of copepod species and metazooplankton abundance are regulated by the variation of salinity caused by high fresh water discharge due to heavy rainfall. Spearman correlation analysis revealed a significant correlation of salinity ($p < 0.05$) with the total zooplankton as well as with copepod abundance in both the estuaries. In

addition, the water temperature is also an important factor for the distribution of copepod species at the different reaches of estuary following seasonal transitions. Though temperature variation is less compared to variation of salinity, there was noticeable seasonal variation of copepod species abundance with respect to the temperature. Seasonal changes of zooplankton assemblages in the coastal and estuarine system are regulated by temperature and salinity (Smoot and Hopcroft, 2017; Miron et al., 2014; Araujo et al., 2008).

Due to influence of salinity, the seasonal variation of metazooplankton composition is clearly reflected in NMDS analysis. Three distinct major groups formed at different locations in the estuaries which could be due to change in the season. This could be also the reason for the changes in copepod species composition such as the abundance of *Acartia southwelli* and *Diaptomus* sp. and for the difference in abundance near mouth and upstream point indicating the oligohaline nature of the species.

The CCA analysis revealed that seasonal changes in temperature and salinity are more prominent in the Mandovi then the Zuari estuary. Overall, salinity gradient was a common feature in both the estuaries which was observed to have a profound effects on the spatial variation of copepod composition and their abundance. Though variation of temperature is less as compared to salinity, it has significant effect on the distribution and copepod species composition as observed elsewhere previously (Calbet et al., 2001; David et al., 2005; Lionard et al., 2005). For instance, few copepods were restricted to the upstream part of the estuary, especially during SWM experiencing high riverine flow (Qasim and Sengupta, 1981; Pradeep Ram et al., 2003). Copepods such as *Heliodiaptomus cinctus, Diptomus* sp. and *Cyclops* sp. were observed in upstream stations which indicates their preference for near-freshwater conditions. We hypothesize that these freshwater-specific species dominant in upstream region are

advected to mid-estuarine stations through the riverine flow. On the other hand, *Acrocalanus gibber*, *Paracalanus parvus*, *Acrocalanus gracilis* were revealed cosmopolitan nature as they were abundantly present at all three different regions of the estuary. In both the estuaries, *Acartia* sp. were dominantly found in middle and upstream part of the estuary showing their euryhaline nature and their successful colonization.

Marine zooplanktons tend to become more abundant at the mouth region, particularly in SIM, where the salinity is high. This study highlighted the major difference in copepod and total zooplankton abundance across the length of estuaries (near mouth to upstream). Such difference is apparently due to the variation of physico-chemical features of estuarine systems. The stability of the water mass in the mid-estuarine stations compared to mouth due to low hydrodynamics, shallow depth, frequent salinity changes and high nutrient enrichment (Marques et al., 2002) result in higher zooplankton abundance. The two estuarine systems experience different environmental conditions.

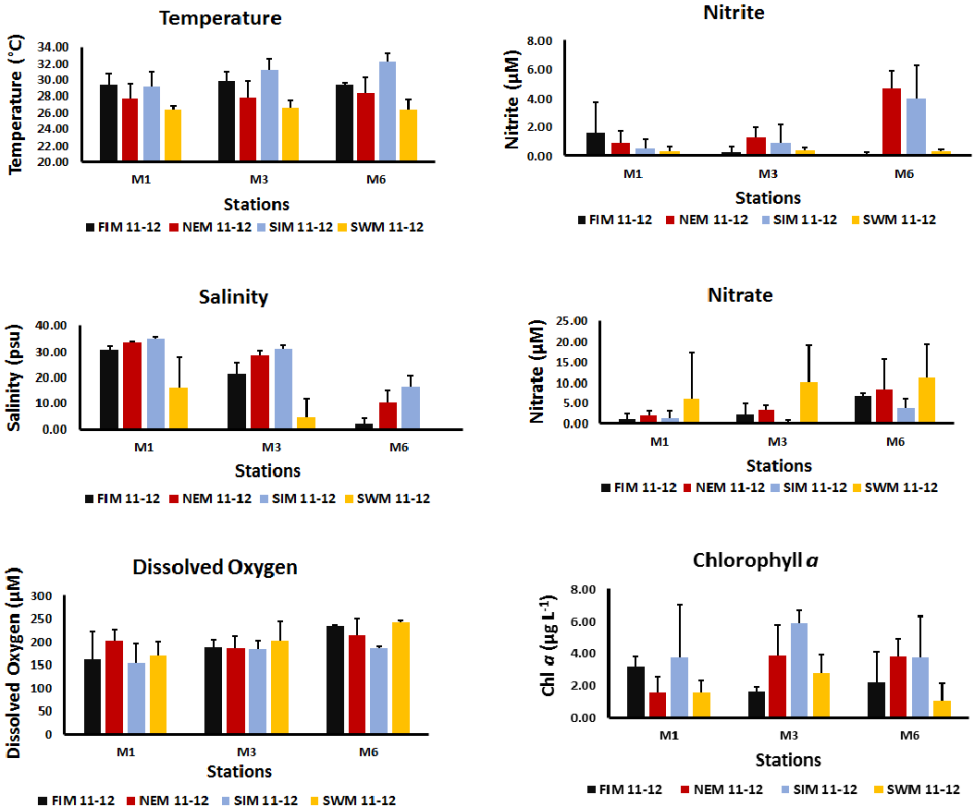


Figure 3. Seasonal variation of hydrographical parameters (temperature, salinity, Dissolved oxygen (DO), Nitrate, Nitrite and Chlorophyll *a* in the spatial stretches (M1, M2, M3 and M6) of Mandovi estuary.

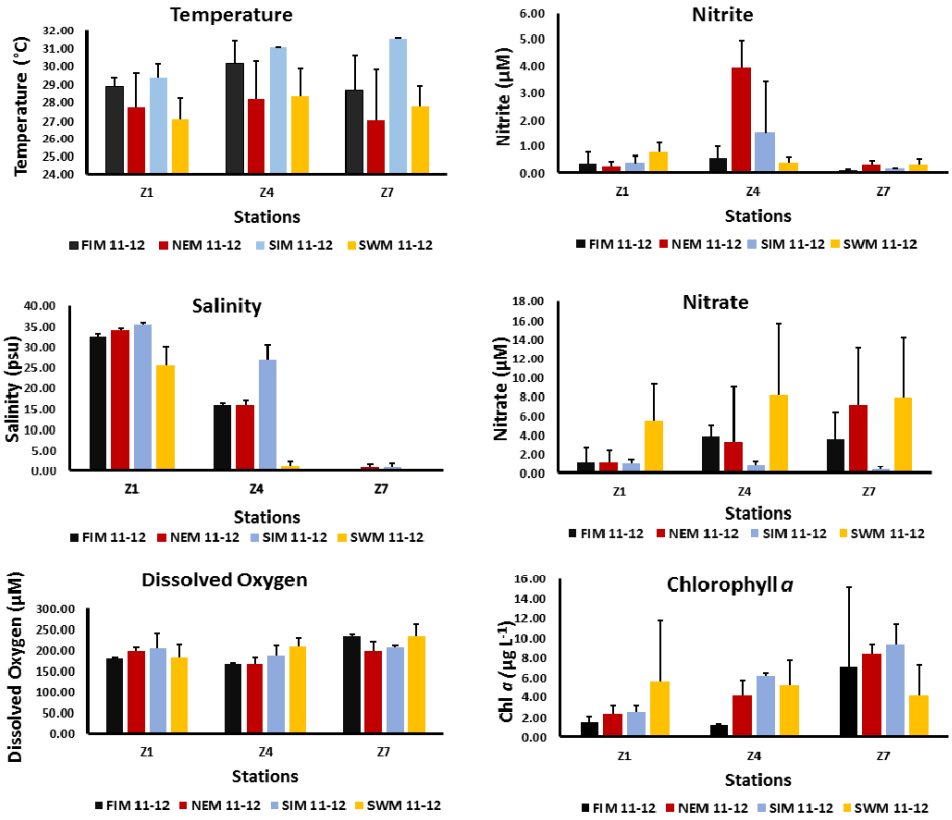
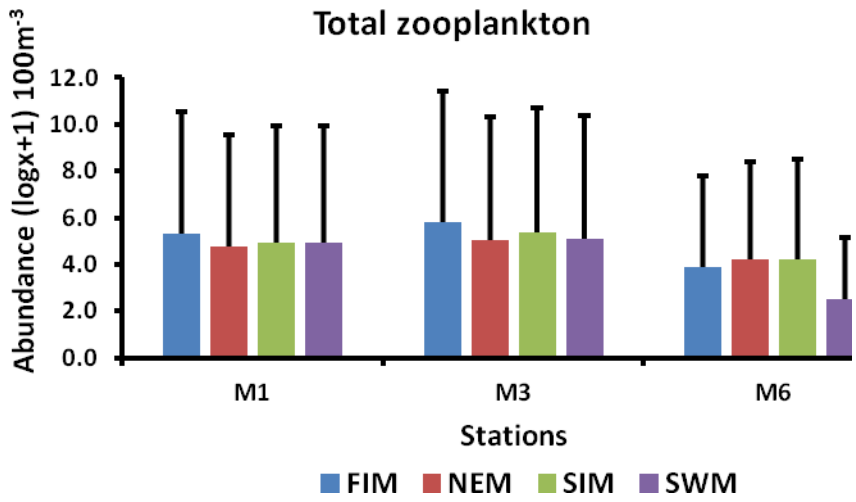


Figure 4. Seasonal variation of hydrographical parameters (temperature, salinity, Dissolved oxygen (DO), Nitrate, Nitrite and Chlorophyll *a* in the spatial stretches (Z1, Z2, Z4 and Z7) of Zuari estuary.

(a)



(b)

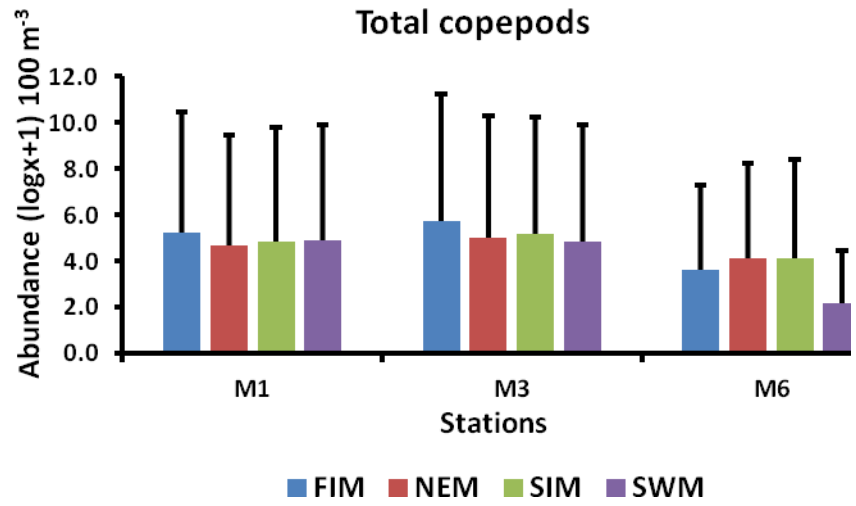
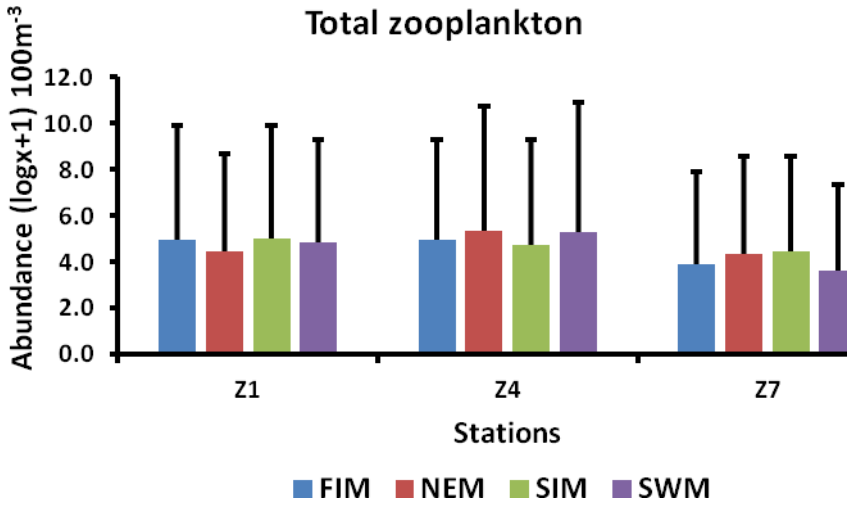


Figure 5. (a) Seasonal variation of total zooplankton and **(b)** total copepod abundance at three spatial points (M1, M3, M6) of Mandovi estuary. Abundance value were calculated in the form of log(x+1) $100m^{-3}$.

(a)



(b)

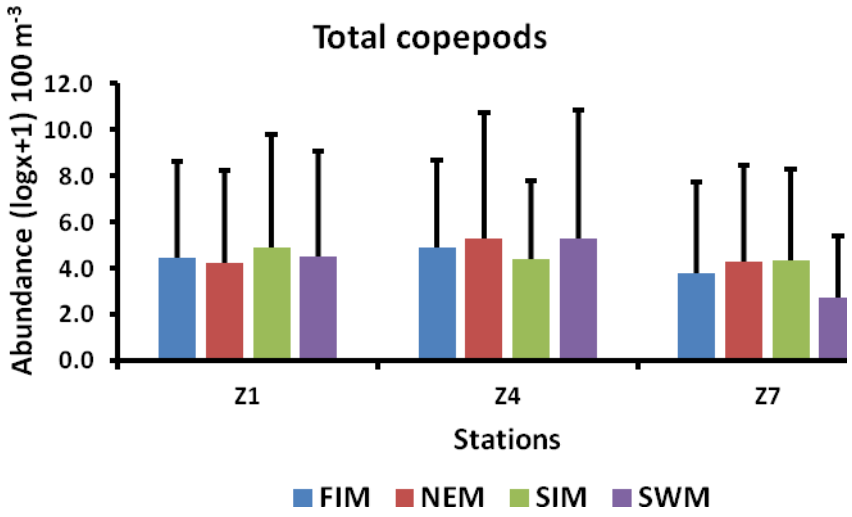
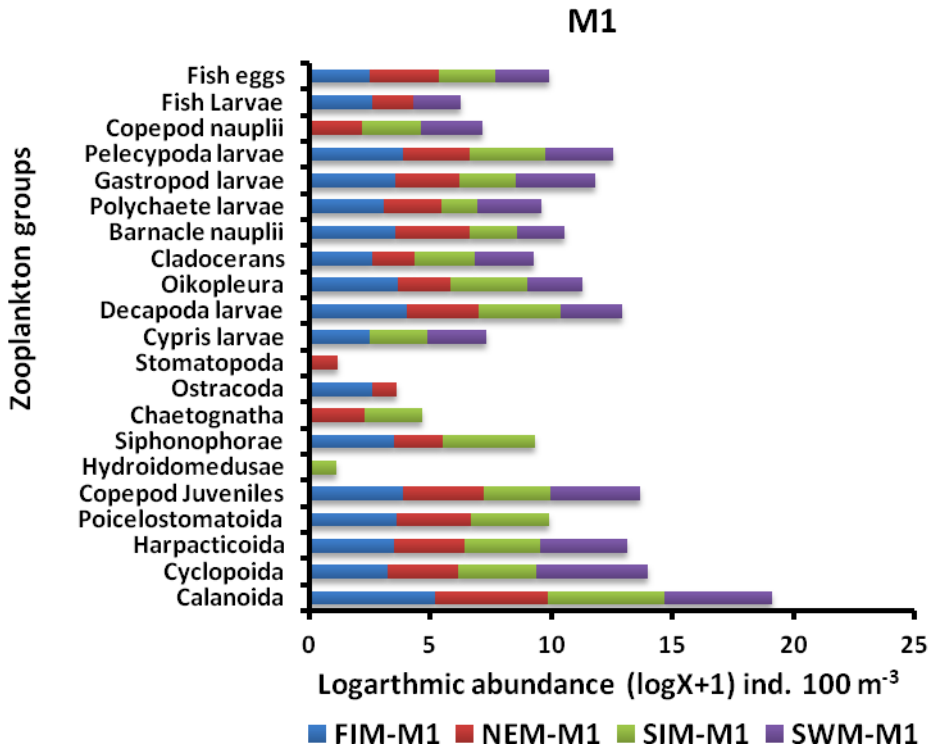


Figure 6. (a) Seasonal variation of total zooplankton and **(b)** total copepod abundance at three spatial points (Z1, Z4, Z7) of Zuari estuary. Abundance value were calculated in the form of log(x+1) $100m^{-3}$.

(a)



(b)

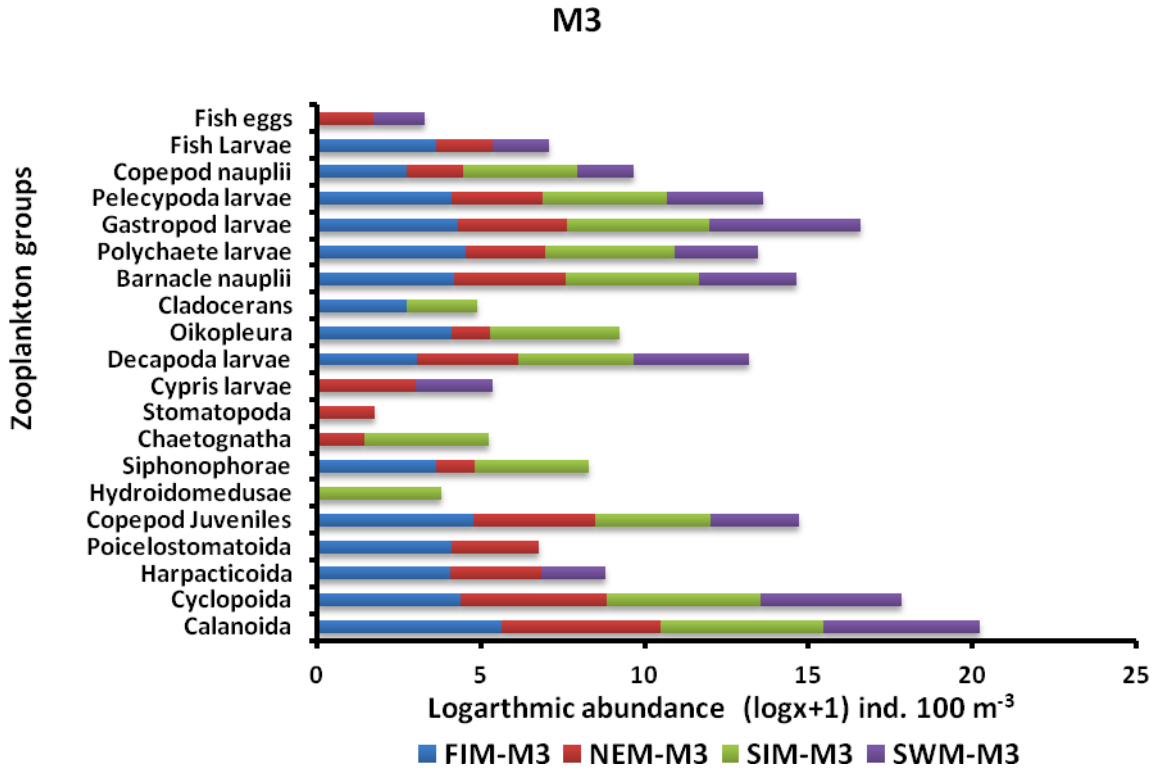


Figure 7.a-b. Seasonal variation of zooplankton community composition in spatial sites of Mandovi estuary (M1, M3). Zooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

(c)

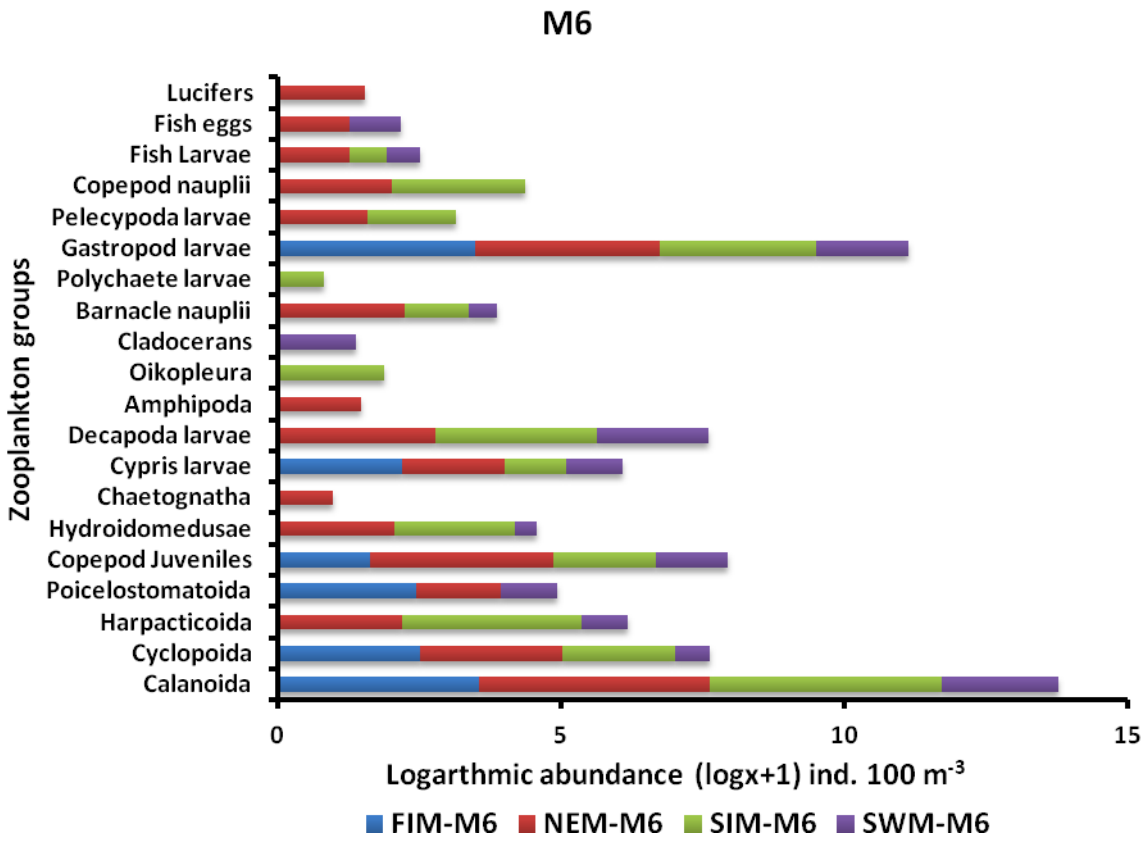
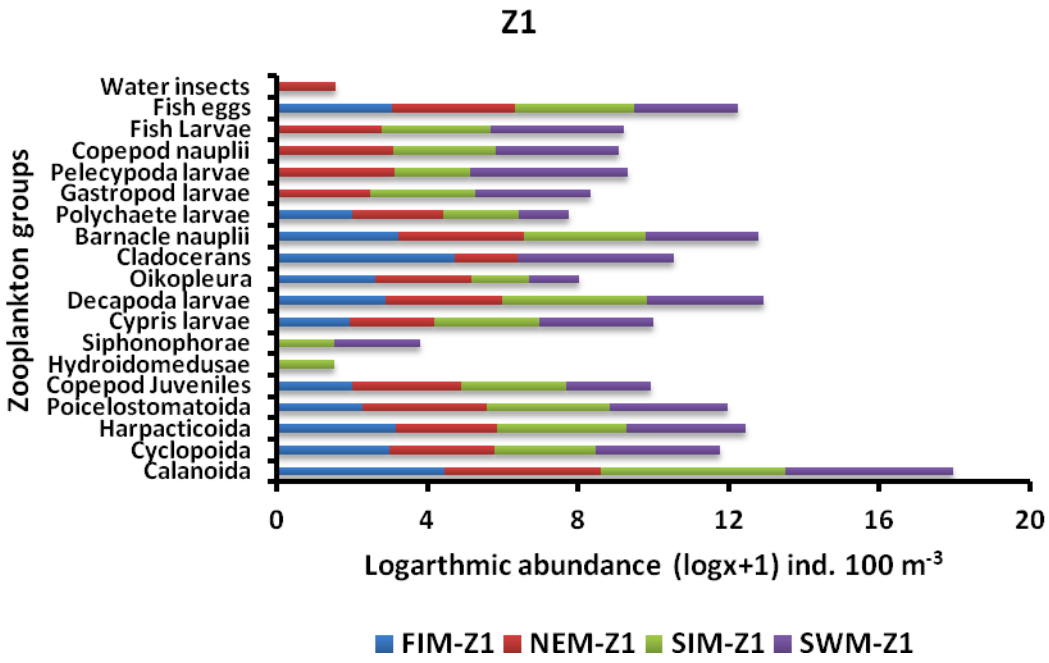


Figure 7.c. Seasonal variation of zooplankton community composition in spatial sites of Mandovi estuary (M6). Zooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

(a)



(b)

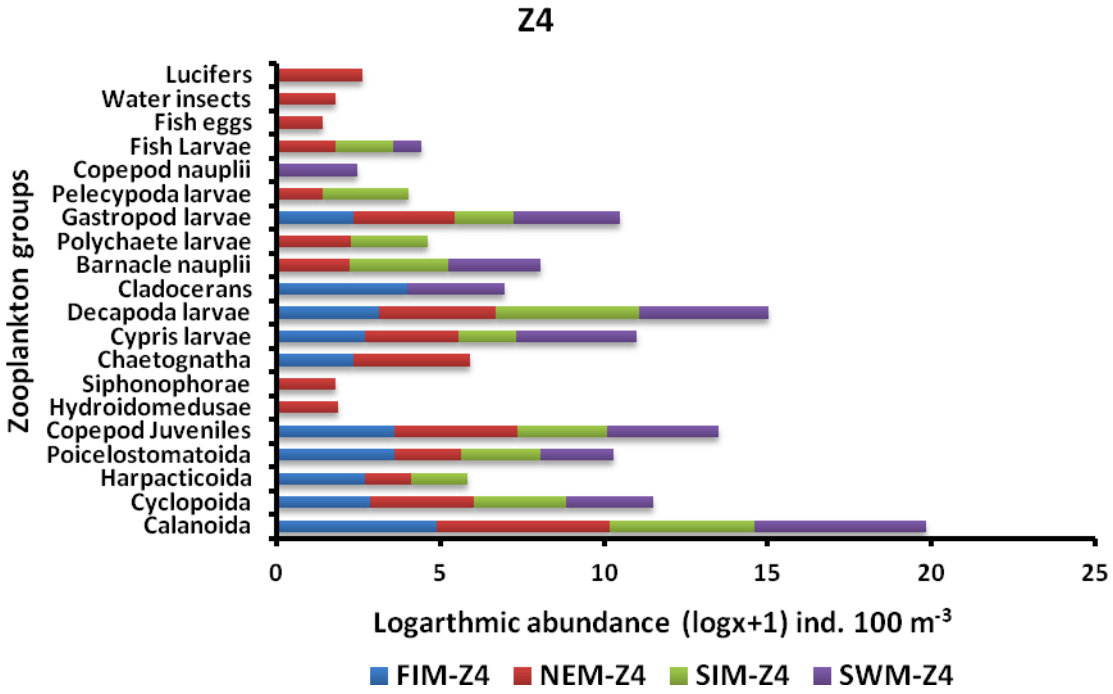


Figure 8. a-b. Seasonal variation of zooplankton community composition in three spatial sites of Zuari estuary (Z1, Z4). Zooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

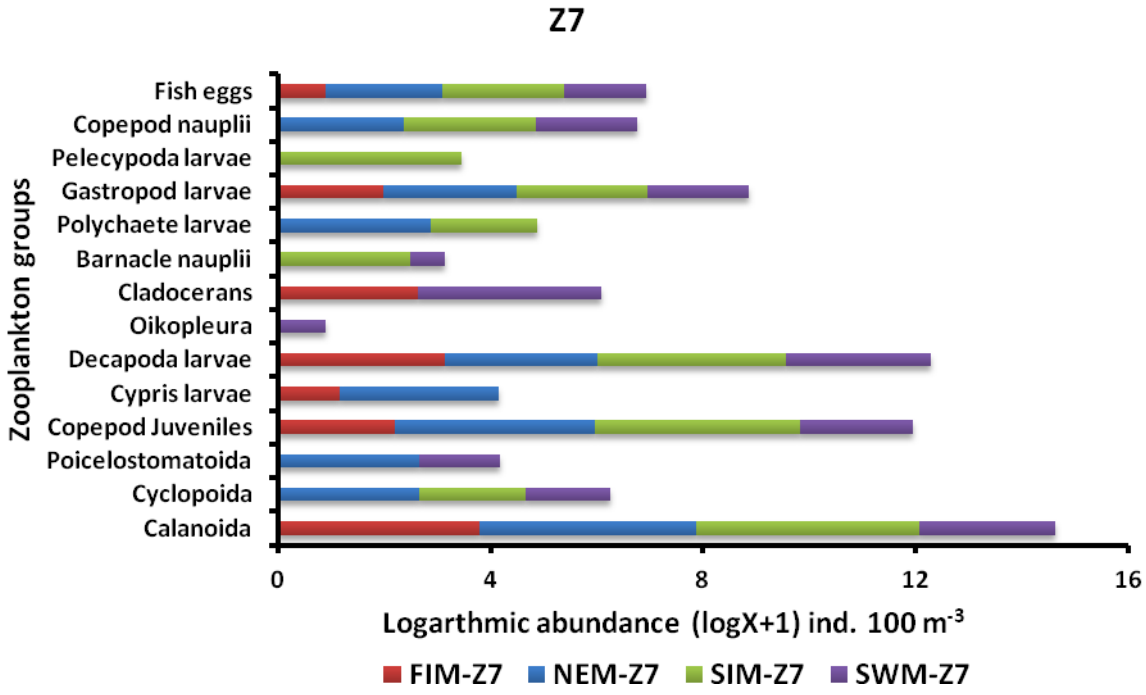


Figure 8.c. Seasonal variation of zooplankton community composition in three spatial sites of Zuari estuary (Z7). Zooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

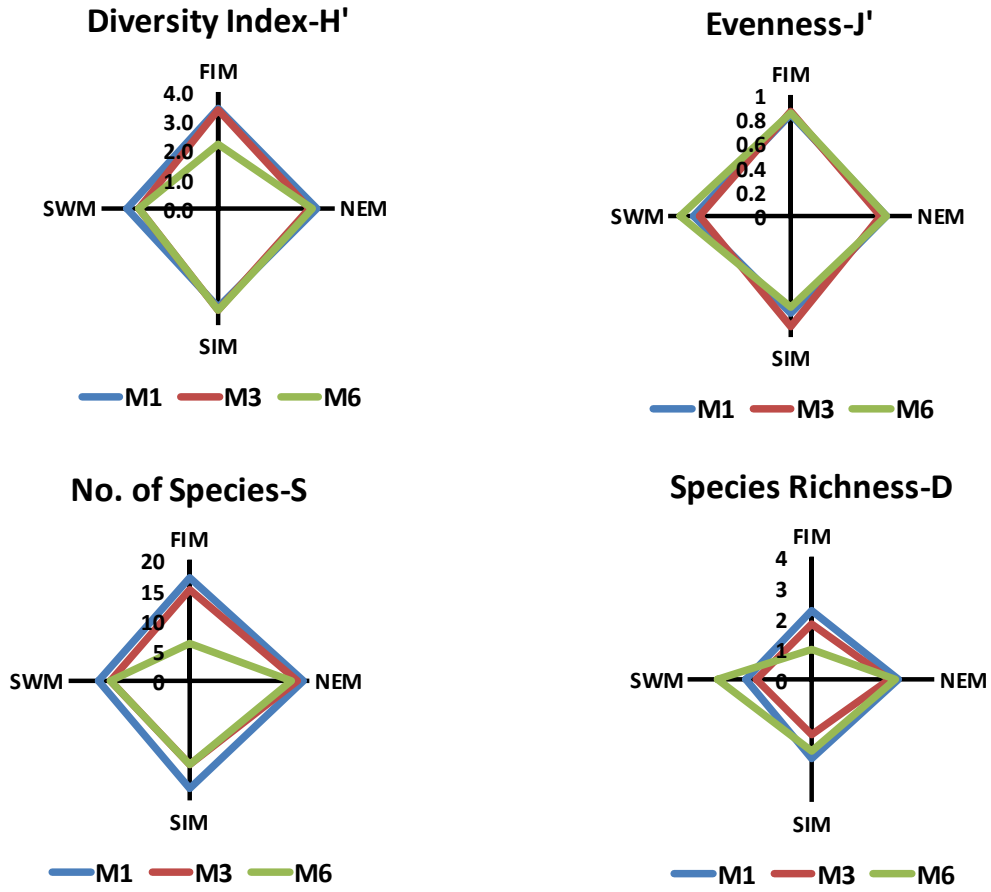


Figure 9. Seasonal and spatial variation of diversity index (H´), number of species (S), species richness (d) and evenness (J) for metazooplankton community in Mandovi estuary.

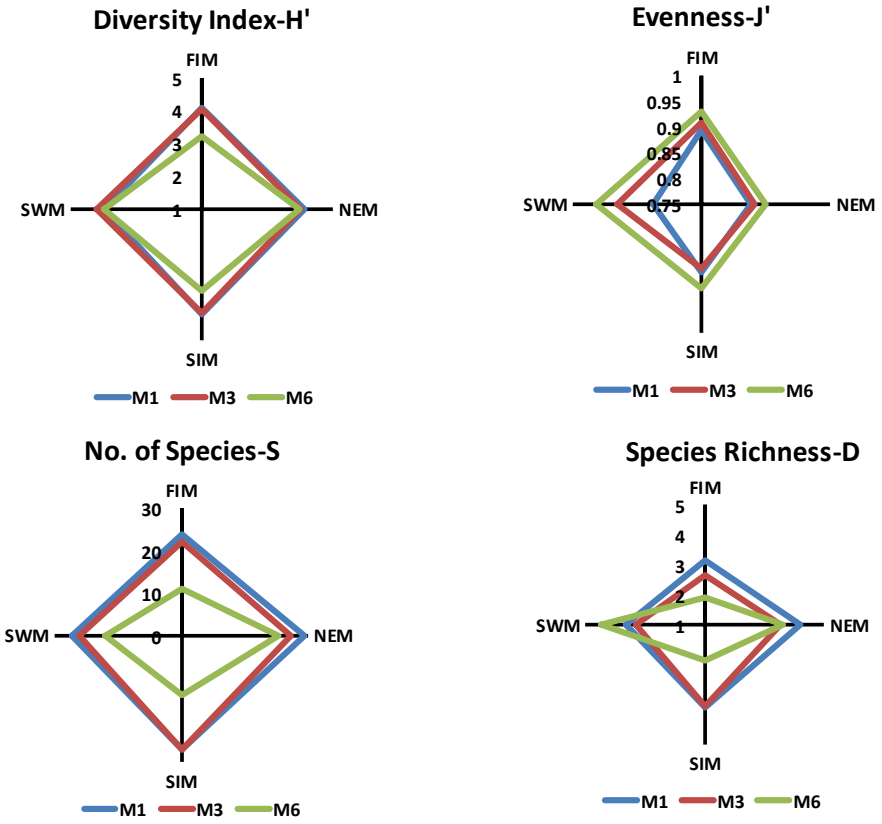


Figure 10. Seasonal and spatial variation of diversity index (H′), number of species (S), species richness (d) and evenness (J) for copepod community in Mandovi estuary.

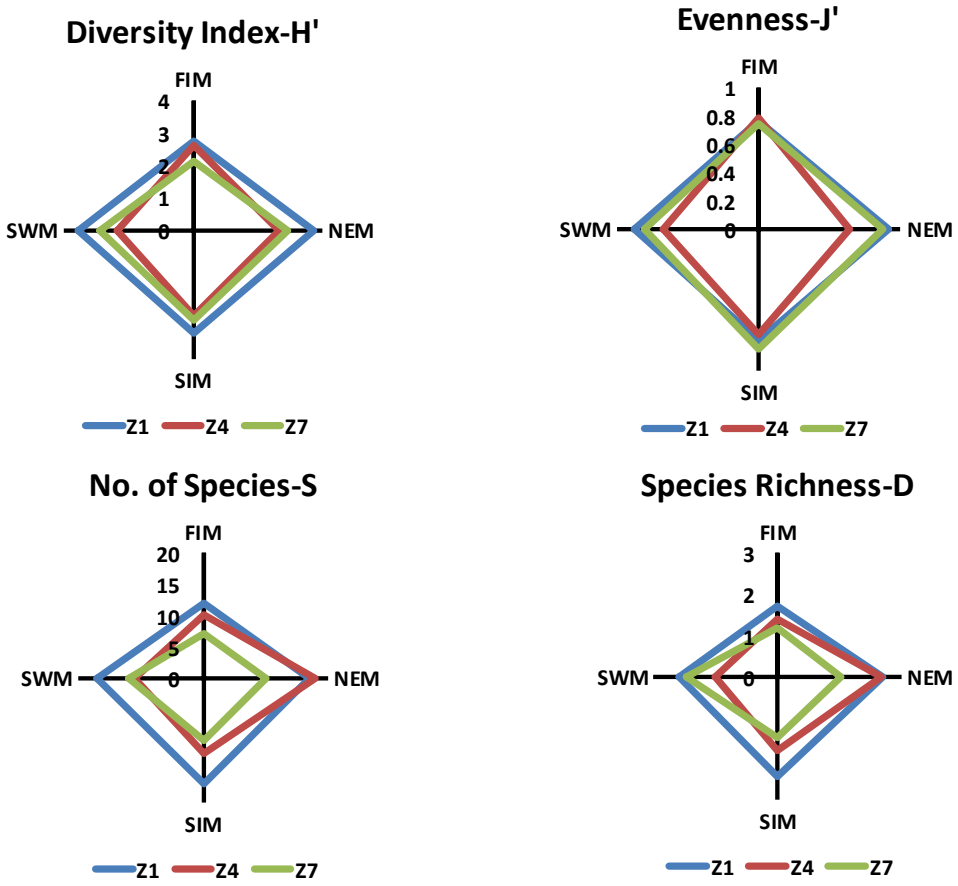


Figure 11. Seasonal and spatial variation of diversity index (H´), number of species (S), species richness (d) and evenness (J) for metazooplankton community in Zuari estuary.

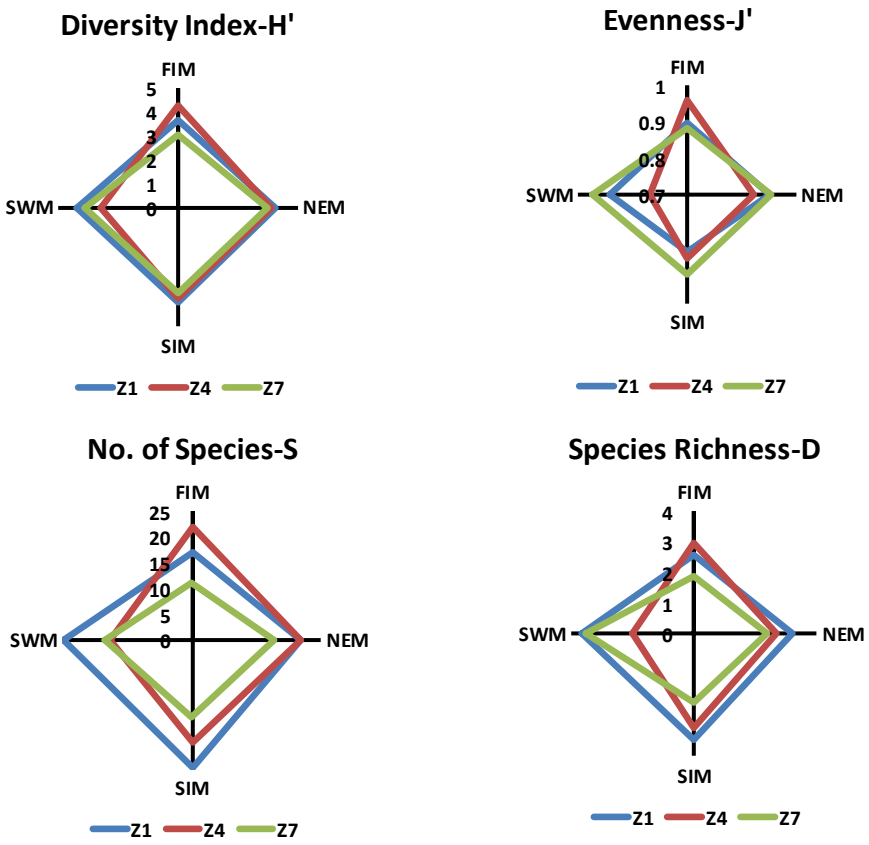
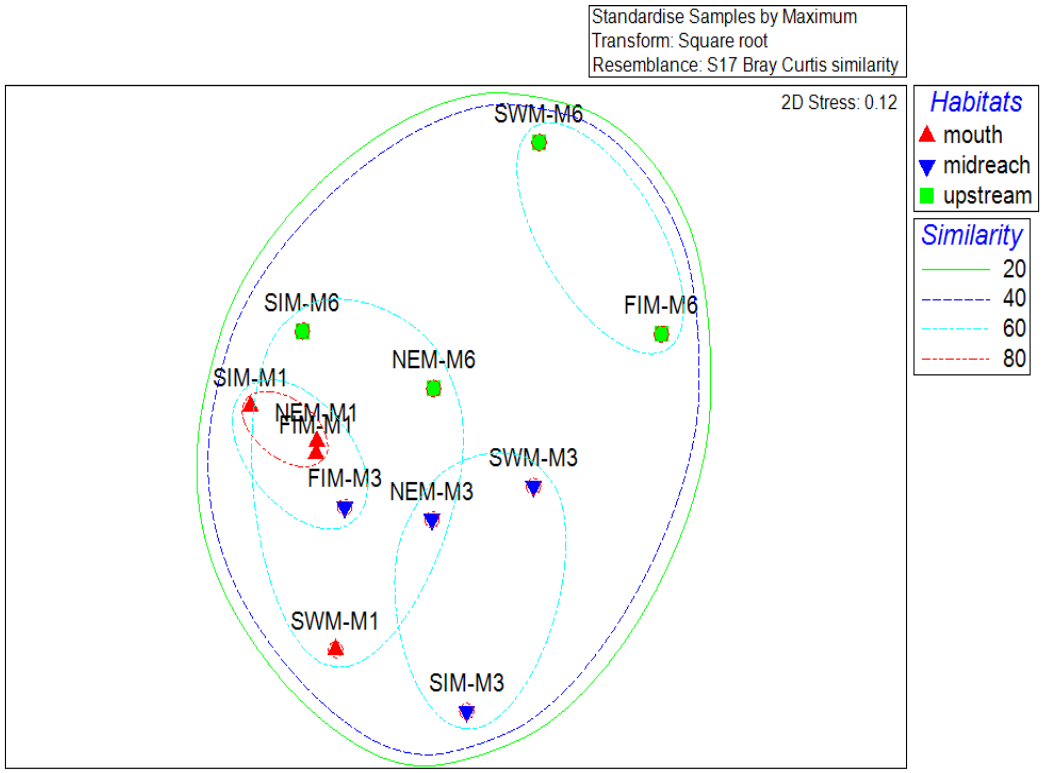


Figure 12. Seasonal and spatial variation of diversity index (H´), number of species (S), species richness (d) and evenness (J) for copepod community in Zuari estuary.

(a)



(b)

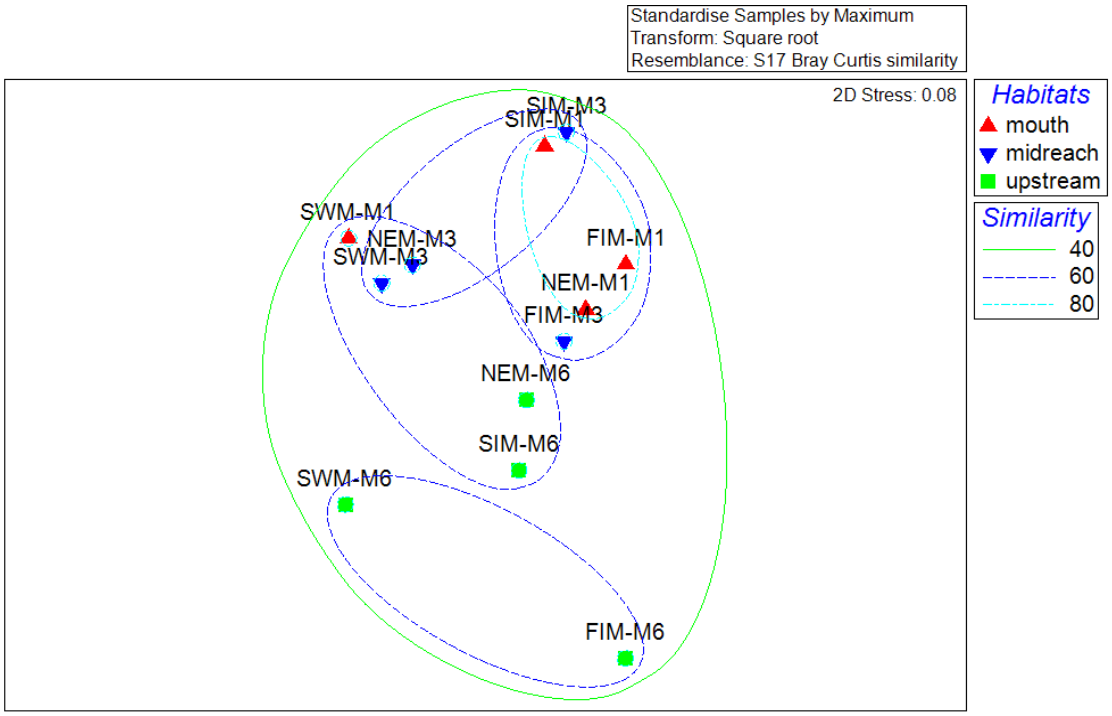
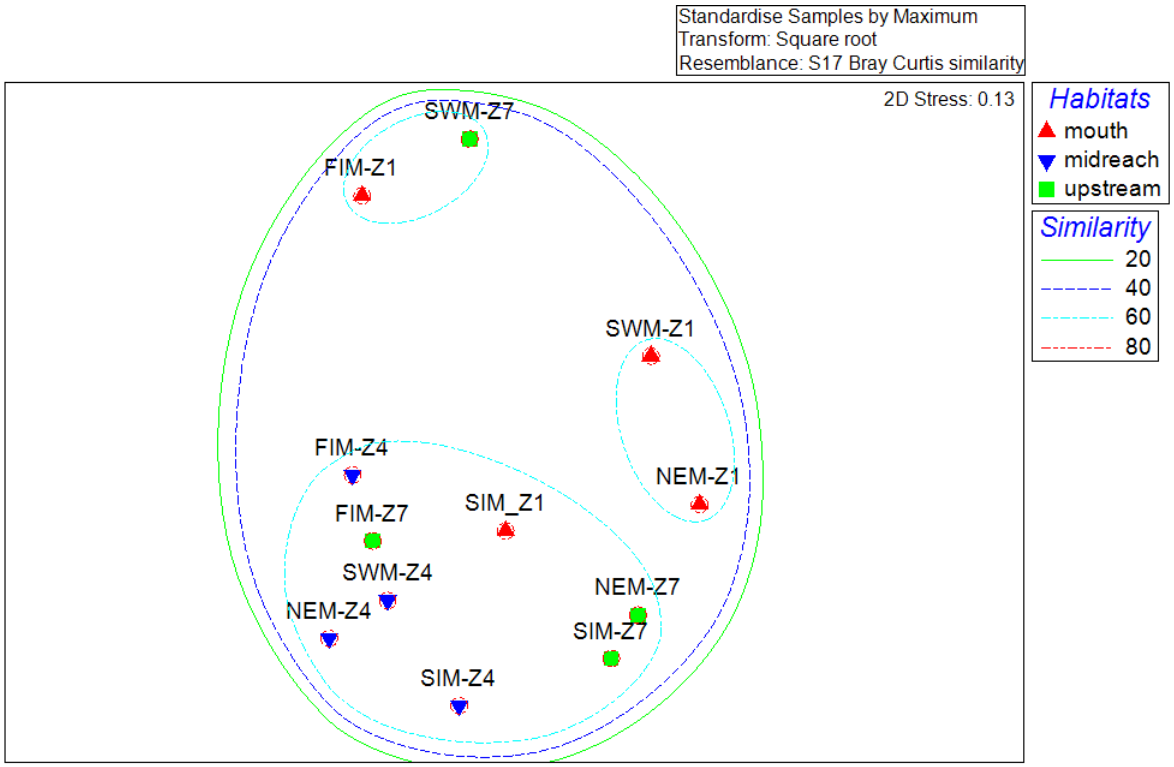


Figure 13. (a) nMDS plots based on seasonal variation of zooplankton group abundance **(b)** copepod species abundance in different sampling sites of Mandovi estuary.

(a)



(b)

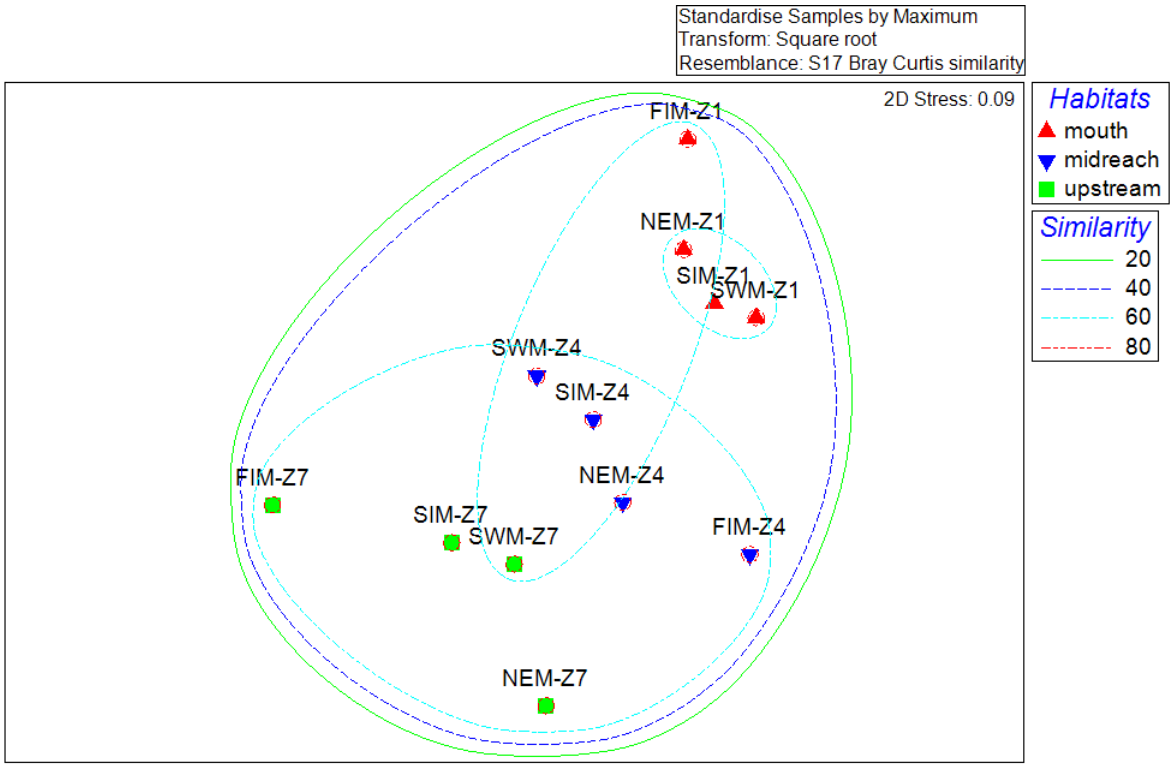
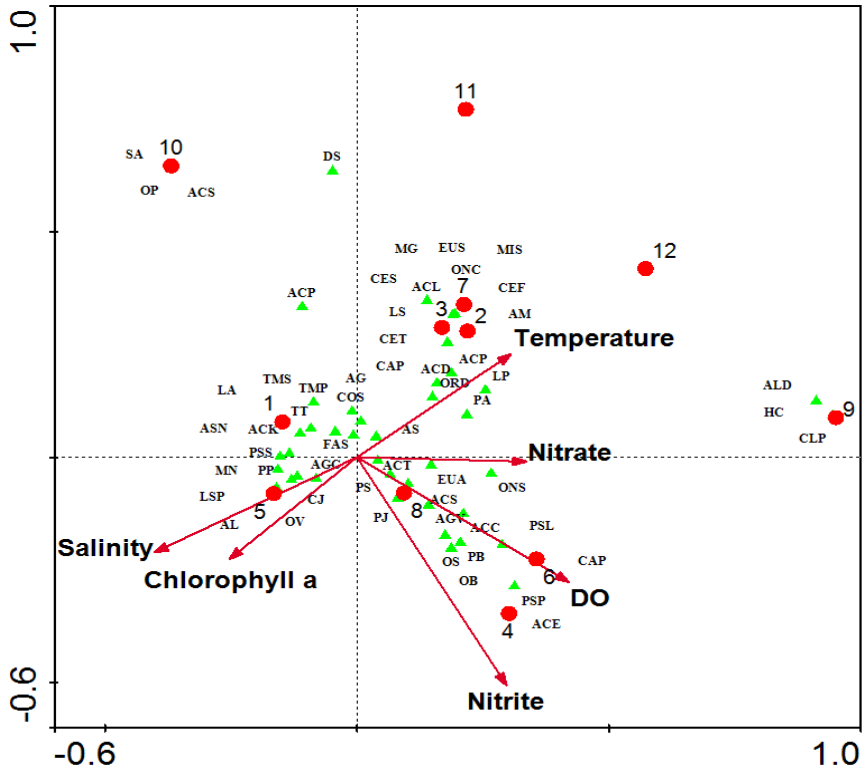


Figure 14. (a) nMDS plots based on seasonal variation of zooplankton group abundance **(b)** copepod species abundance in different sampling sites of zuari estuary.

(a)



(b)

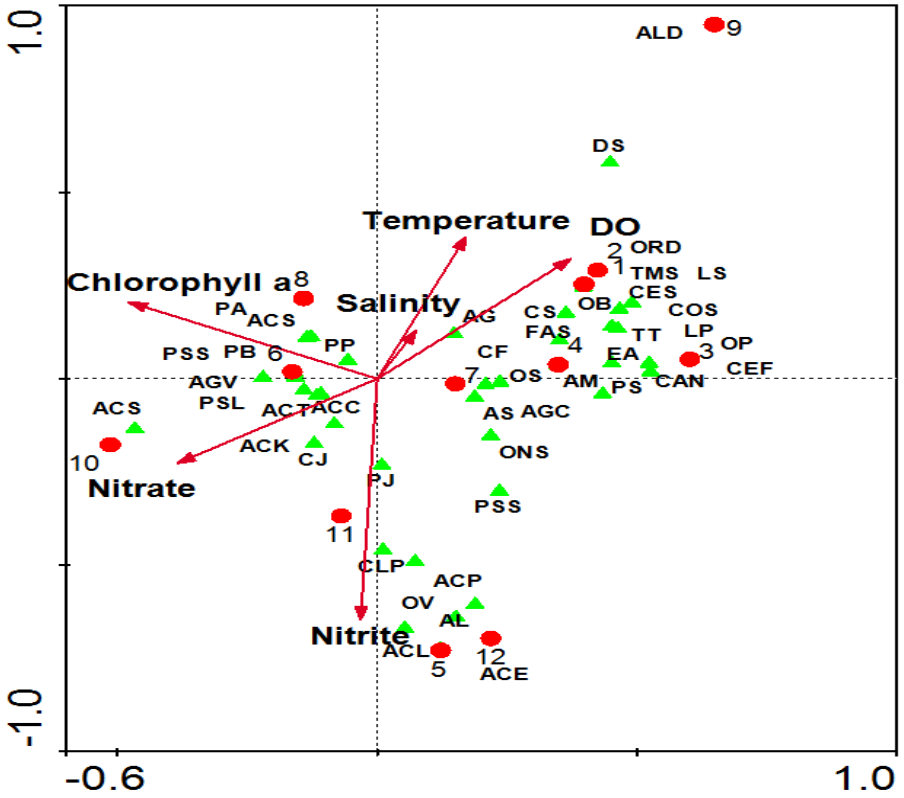


Figure 15. CCA triplot depicting **(a)** Environmental association of copepod community in Mandovi estuary and **(b)** copepod community in Zuari estuary.

Table 1. Spearman's correlation analysis results overall correlation between environmental factors and biological communities (metazooplankton and copepods) throughout the annual cycle in Mandovi estuary.

			Temperature	Salinity	Nitrate	Nitrite	DO	Chlorophyll	Total Zooplankton	Total Copepods
Spearman's rho	**Temperature**	**Correlation Coefficient**	1.000	.324	-423*	0.162	-0.360*	0.314	.245	.250
		Sig. (2-tailed)		.054	.010	.345	.031	.063	.151	.142
		N	36	36	36	36	36	36	36	36
	Salinity	**Correlation Coefficient**	.324	1.000	-.722**	.168	-.614**	.259	.517**	.526**
		Sig. (2-tailed)	.054		.000	.327	.000	.127	.001	.001
		N	36	36	36	36	36	36	36	36
	Nitrate	**Correlation Coefficient**	-.423*	-.722**	1000	.201	.616**	-.179	-.402*	-.407*
		Sig. (2-tailed)	.010	000		.239	000	296	.015	.014
		N	36	36	36	36	36	36	36	36
	Nitrite	**Correlation Coefficient**	.162	.168	.201	1.000	-.213	.448**	.065	.049
		Sig. (2-tailed)	.345	.327	.239		.212	.006	.708	.775
		N	36	36	36	36	36	36	36	36
	DO	**Correlation Coefficient**	-360*	-.614**	.616**	-.213	1.000	-.276	-.527**	-.517**
		Sig. (2-tailed)	.031	.000	.000	.212		.103	.001	.001
		N	36	36	36	36	36	36	36	36
	Chlorophyll	**Correlation Coefficient**	.314	.259	-.179	.448**	-.276	1.000	.301	.270
		Sig. (2-tailed)	.063	.127	.296	.006	.103		.074	.111
		N	36	36	36	36	36	36	36	36
	Total zooplankton	**Correlation Coefficient**	.199	.517**	-.402*	.065	-.527**	.301	1.000	.994**
		Sig. (2-tailed)	.151	.001	.015	.708	.001	.074		000
		N	36	36	36	36	36	36	36	36
	Total copepods	**Correlation Coefficient**	.250	.526**	-.407**	.049	-.517**	.270	.994**	1.000
		Sig. (2-tailed)	.142	.001	.014	.775	.001	.111	000	
		N	36	36	36	36	36	36	36	36

****** Correlation is significant at the 0.01 level (2tailed)

***** Correlation is significant at the 0.05 level (2-tailed)

Table 2 a. Spearman's Rank correlation analysis depicts seasonal (NEM at M1, M3) correlation analysis between environmental factors and biological communities (metazooplankton and copepods) in Mandovi estuary.

M1 NEM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.0951	1						
Nitrate	-0.7539	-0.1045	1					
Nitrite	-0.5301	-0.8558	0.5929	1				
DO	-0.9121	-0.2014	0.9496	0.6766	1			
Chl *a*	0.0041	-0.9899	0.0961	0.8284	0.1507	1		
Total zooplankton	-0.3491	0.6234	0.6783	-0.1796	0.5044	-0.5902	1	
Total copepods	-0.3625	0.6289	0.6788	-0.1816	0.5102	-0.5985	0.9998	1

M3 NEM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	-0.3743	1						
Nitrate	0.0985	-0.9425	1					
Nitrite	0.0005	-0.1692	-0.0099	1				
DO	-0.9614	0.3653	-0.1531	0.2691	1			
Chl *a*	-0.3778	-0.7171	0.8652	0.1774	0.3603	1		
Total zooplankton	-0.0283	0.7589	-0.6780	-0.7272	-0.1305	-0.7417	1	
Total copepods	-0.0256	0.7562	-0.6756	-0.7293	-0.1337	-0.7412	1.0000	1

Table 2 b. Spearman's Rank correlation analysis depicts seasonal (NEM and SEM at M6, M1 respectively) correlation analysis between environmental factors and biological communities (metazooplankton and copepods) in Mandovi estuary.

M6 NEM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.6049	1						
Nitrate	-0.6289	-0.2776	1					
Nitrite	0.4298	0.9617	-0.0056	1				
DO	-0.4491	-0.9733	0.0550	-0.9986	1			
Chl *a*	-0.1064	-0.6895	-0.5023	-0.8577	0.8300	1		
Total zooplankton	0.5141	0.9329	-0.4943	0.8394	-0.8665	-0.4515	1	
Total copepods	0.5572	0.9237	-0.5491	0.8132	-0.8425	-0.4039	0.9977	1

M1 SWM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	-0.8149	1						
Nitrate	0.4486	-0.7294	1					
Nitrite	-0.3819	0.2596	0.4697	1				
DO	0.7999	-0.9995	0.7479	-0.2340	1			
Chl *a*	-0.2860	0.7748	-0.5807	0.2204	-0.7853	1		
Total zooplankton	0.2158	-0.4459	0.9351	0.7485	0.4689	-0.3157	1	
Total copepods	0.2287	-0.4727	0.9463	0.7282	0.4958	-0.3499	0.9993	1

Table 2 c. Spearman's Rank correlation analysis depicts seasonal (SWM at M3, M6) correlation analysis between environmental factors and biological communities (metazooplankton and copepods) in Mandovi estuary.

M3 SWM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.8086	1						
Nitrate	-0.6571	-0.7368	1					
Nitrite	0.7002	0.7023	-0.0898	1				
DO	-0.6773	-0.9664	0.5692	-0.7647	1			
Chl *a*	0.2699	0.6131	-0.8730	-0.1220	-0.5319	1		
Total zooplankton	0.7070	0.9866	-0.6746	0.6949	-0.9908	0.6266	1	
Total copepods	0.6698	0.9771	-0.6597	0.6797	-0.9914	0.6373	0.9987	1

M6 SWM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.9420	1						
Nitrate	-0.6308	-0.8540	1					
Nitrite	0.7076	0.4345	0.0966	1				
DO	0.0024	0.3045	-0.6762	-0.5575	1			
Chl *a*	0.8490	0.8127	-0.5928	0.4971	-0.1922	1		
Total zooplankton	0.8936	0.7246	-0.3081	0.8323	-0.4349	0.8940	1	
Total copepods	0.9187	0.7801	-0.3983	0.7818	-0.3582	0.9294	0.9950	1

Table 3. Spearman's correlation analysis results overall correlation between environmental factors and biological communities (metazooplankton and copepods) throughout the annual cycle in Zuari estuary.

			Temperature	Salinity	Nitrate	Nitrite	DO	Chlorophyll	Total Zooplankton	Total Copepods
Spearman's rho	Temperature	Correlation Coefficient	1.000	0.37	-.462**	-0.71	-.168	-.016	.142	.281
		Sig. (2-tailed)		.829	005	.679	.327	.927	.409	.097
		N	36	36	36	36	36	36	36	36
	Salinity	Correlation Coefficient	.037	1.000	-.457**	.217	-.445**	-.341*	.522**	.469**
		Sig. (2-tailed)	.829		.005	.203	.006	.042	.001	.004
		N	36	36	36	36	36	36	36	36
	Nitrate	Correlation Coefficient	-.462**	-.457**	1.000	.128	.145	-.021	-.290	-.347*
		Sig. (2-tailed)	.005	.005		.456	.398	.904	.086	.038
		N	36	36	36	36	36	36	36	36
	Nitrite	Correlation Coefficient	-.071	.217	.128	1.000	-.656**	.070	.299	.264
		Sig. (2-tailed)	.679	.203	.456		.000	.687	.077	.119
		N	36	36	36	36	36	36	36	36
	DO	Correlation Coefficient	-.168	-.445**	.145	-.656**	1.000	.319	-.456**	-.437**
		Sig. (2-tailed)	327	.006	.398	.000		.058	.005	.008
		N	36	36	36	36	36	36	36	36
	Chlorophyll	Correlation Coefficient	-016	-.341*	-.021	-.070	.319	1.000	-.068	.003
		Sig. (2-tailed)	.927	.042	.904	.687	.058		.693	.985
		N	36	36	36	36	36	36	36	36
	Total zooplankton	Correlation Coefficient	.142	.522**	-.290	.299	-.456**	-.068	1.000	.907**
		Sig. (2-tailed)	.409	.001	.086	.077	.005	.693		.000
		N	36	36	36	36	36	36	36	36
	Total copepods	Correlation Coefficient	.281	.469**	-.347*	.264	-.437**	.003	.907**	1.000
		Sig. (2-tailed)	.097	.004	.038	.119	.008	.985	.000	
		N	36	36	36	36	36	36	36	36

****** Correlation is significant at the 0.01 level (2-tailed). *****Correlation is significant at the 0.05 level (2-tailed)

Table 4 a. Spearman's Rank correlation analysis depicts seasonal (NEM at Z1, Z4) correlation analysis between environmental factors and biological communities (metazooplankton and copepods) in Zuari estuary.

Z1 NEM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.8722	1						
Nitrate	-0.7975	-0.4392	1					
Nitrite	-0.6991	-0.2785	0.8439	1				
DO	-0.9223	-0.9857	0.5740	0.3687	1			
Chl *a*	-0.2393	-0.3782	0.2805	-0.2752	0.4483	1		
Total zooplankton	-0.8637	-0.8862	0.6734	0.3225	0.9437	0.6859	1	
Total copepods	-0.8163	-0.8233	0.6904	0.2948	0.8953	0.7558	0.9923	1

Z4 NEM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	-0.9188	1						
Nitrate	-0.4490	0.2240	1					
Nitrite	0.3309	-0.3635	-0.7801	1				
DO	-0.9154	0.6846	0.5485	-0.1791	1			
Chl *a*	0.6094	-0.2470	-0.6655	0.0987	-0.8710	1		
Total zooplankton	-0.4578	0.3032	0.9803	-0.8872	0.4794	-0.5295	1	
Total copepods	-0.4483	0.2956	0.9793	-0.8902	0.4691	-0.5216	0.9999	1

Table 4 b. Spearman's Rank correlation analysis depicts seasonal (NEM and SEM Z7, Z1 respectively) correlation analysis between environmental factors and biological communities (metazooplankton and copepods) in Zuari estuary.

Z7 NEM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	-0.1637	1						
Nitrate	-0.4230	0.0578	1					
Nitrite	-0.2731	0.9674	0.3083	1				
DO	-0.2400	-0.8642	-0.1795	-0.8634	1			
Chl *a*	-0.3697	-0.1282	0.9824	0.1264	-0.0287	1		
Total zooplankton	0.6935	0.5849	-0.4227	0.4428	-0.7718	-0.5133	1	
Total copepods	0.7959	0.4563	-0.4465	0.3132	-0.6991	-0.5113	0.9881	1

Z1 SWM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.8944	1						
Nitrate	-0.3269	-0.5772	1					
Nitrite	-0.1234	-0.0280	0.6039	1				
DO	0.2609	-0.1622	0.2077	-0.5725	1			
Chl *a*	-0.3581	-0.6690	0.2548	-0.6004	0.7949	1		
Total zooplankton	0.0472	-0.3745	0.8296	0.1741	0.6967	0.5242	1	
Total copepods	-0.0279	-0.3843	0.1055	-0.7245	0.9334	0.9419	0.5327	1

Table 4 c. Spearman's Rank correlation analysis depicts seasonal (SWM at Z4, Z7) correlation analysis between environmental factors and biological communities (metazooplankton and copepods) in Zuari estuary.

Z4 SWM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.6556	1						
Nitrate	-0.6545	-0.0924	1					
Nitrite	0.7069	0.8402	0.0660	1				
DO	-0.7283	-0.8045	-0.0407	-0.9972	1			
Chl *a*	0.1838	-0.6213	-0.5364	-0.3423	0.2726	1		
Total zooplankton	0.9032	0.7698	-0.7054	0.5759	-0.5691	-0.0780	1	
Total copepods	0.9091	0.7768	-0.6964	0.5911	-0.5847	-0.0804	0.9998	1

Z7 SWM	Temperature	Salinity	Nitrate	Nitrite	DO	Chl *a*	Total zooplankton	Total copepods
Temperature	1							
Salinity	0.9293	1						
Nitrate	-0.5764	-0.2671	1					
Nitrite	0.7415	0.8833	0.1104	1				
DO	0.2434	-0.0381	-0.9048	-0.4687	1			
Chl *a*	0.9594	0.9464	-0.5346	0.6895	0.2844	1		
Total zooplankton	-0.3030	-0.3945	-0.3843	-0.7615	0.7360	-0.1195	1	
Total copepods	-0.3154	-0.1322	0.1842	-0.3558	0.1633	-0.0356	0.7233	1

Table 5a. One way ANOVA result showing the variation of environmental parameters between seasons of Mandovi estuary. F statistic and probability (p).

		Sum of Squares	df	Mean Square	F	Sig. (P)
Temperature	Between Groups	89.516	3	29.839	15.765	.000
	Within Groups	60.567	32	1.893		
	Total	150.083	35			
Salinity	Between Groups	2528.828	3	842.943	7.149	0.001
	Within Groups	3773.207	32	117.913		
	Total	6302.035	35			
Nitrate	Between Groups	283.549	3	94.516	2.518	0.76
	Within Groups	1201.294	32	37.54		
	Total	1484.843	35			
Nitrite	Between Groups	26.758	3	8.919	4.004	0.016
	Within Groups	71.277	32	2.227		
	Total	98.035	35			
DO	Between Groups	4006.533	3	1335.511	1.021	0.396
	Within Groups	41877.058	32	1308.658		
	Total	45883.59	35			
Chlorophyll	Between Groups	30.444	3	10.148	4.128	0.014
	Within Groups	78.665	32	2.458		
	Total	109.109	35			

Table 5b. One way ANOVA result showing the variation of environmental parameters between stations of Mandovi estuary. F statistic and probability (p).

		Sum of Squares	df	Mean Square	F	Sig. (P)
Temperature	Between Groups	3.401	2	10701	0.383	0.685
	Within Groups	146.682	33	4.445		
	Total	150.083	35			
Salinity	Between Groups	2752.721	2	1376.361	12.797	.000
	Within Groups	3549.314	33	107.555		
	Total	6302.035	35			
Nitrate	Between Groups	157.837	2	78.918	1.963	0.157
	Within Groups	1327.006	33	40.212		
	Total	1484.843	35			
Nitrite	Between Groups	20.201	2	10.101	4.282	0.022
	Within Groups	77.834	33	2.359		
	Total	98.035	35			
DO	Between Groups	13202.007	2	6601.003	6.665	0.004
	Within Groups	32681.583	33	990.351		
	Total	45883.59	35			
Chlorophyll	Between Groups	10.309	2	5.154	1.722	0.194
	Within Groups	98.800	33	2.994		
	Total	109.109	35			

Table 6. PERMANNOVA pairwise test showing the variation of environmental parameters between a pair of seasons and a pair of stations in Mandovi estuary. F statistic and probability (p).

PERMANOVA-Mandovi season-pairwise test

Groups	t	P(perm)	perms	P(MC)
FIM, NEM	1.6711	0.047	999	0.055
FIM, SIM	1.4619	0.171	997	0.167
FIM, SWM	1.5296	0.099	998	0.109
NEM, SIM	2.0464	0.018	999	0.019
NEM, SWM	2.7773	0.002	999	0.002
SIM, SWM	3.0694	0.003	998	0.004

PERMANOVA MANDOVI station –pairwise test

Groups	t	P(perm)	perms	P(MC)
nearmouth, midreach	1.1299	0.286	999	0.267
nearmouth, upstream	3.0885	0.001	998	0.002
midreach, upstream	2.4673	0.002	998	0.004

Table 7a. One way ANOVA result showing the variation of environmental parameters between seasons of Zuari estuary. F statistic and probability (p).

		Sum of Squares	df	Mean Square	F	Sig. (P)
Temperature	Between Graphs	46.199	3	15.400	6.158	0.002
	Within Graphs	80.027	32	2.501		
	Total	126.226	35			
Salinity	Between Graphs	724.008	3	241.336	1.213	0.321
	Within Graphs	6367.057	32	198.971		
	Total	7091.005	35			
Nitrate	Between Graphs	189.060	3	63.020	3.128	0.039
	Within Graphs	644.644	32	20.145		
	Total	833.704	35			
Nitrite	Between Graphs	8.195	3	2.732	1.866	0.155
	Within Graphs	46.844	32	1.464		
	Total	55.039	35			
DO	Between Graphs	1.463	3	0.488	1.270	0.301
	Within Graphs	12.290	32	0.384		
	Total	13.753	35			
Chlorophyll	Between Graphs	24.329	3	8.11	0.629	0.602
	Within Graphs	412.615	32	12.894		
	Total	436.944	35			

Table 7b. One away ANOVA result showing the variation of environmental parameters between stations of Zuari estuary. F statistic and probability (p).

		Sum of Squares	df	Mean Square	F	Sig. (P)
Temperature	Between Groups	7.233	2	3.617	1.003	0.378
	Within Groups	118.993	33	3.606		
	Total	126.226	35			
Salinity	Between Groups	5793.022	2	2896.511	73.638	.000
	Within Groups	1298.043	33	39.335		
	Total	7091.065	35			
Nitrate	Between Groups	61.525	2	30.763	1.315	0.282
	Within Groups	772.178	33	23.399		
	Total	833.704	35			
Nitrite	Between Groups	16.702	2	8.351	7.189	0.003
	Within Groups	38.337	33	1.162		
	Total	55.039	35			
DO	Between Groups	3.698	2	1.849	6.068	0.006
	Within Groups	10.055	33	0.305		
	Total	13.753	35			
Chlorophyll	Between Groups	81.757	2	40.878	3.798	0.033
	Within Groups	355.187	33	10.763		
	Total	436.944	35			

Table 8. PERMANNOVA pairwise test showing the variation of environmental parameters between (a) a pair of seasons and (b) a pair of stations in Zuari estuary. F statistic and probability (p).

Zuari PERMANOVA season-pairwise test

Groups	t	P(perm)	perms	P(MC)
FIM, NEM	1.6336	0.036	999	0.057
FIM, SIM	1.4711	0.125	994	0.146
FIM, SWM	1.6146	0.075	999	0.079
NEM, SIM	1.2609	0.173	999	0.219
NEM, SWM	1.9181	0.017	996	0.013
SIM, SWM	2.0516	0.006	999	0.014

PERMANOVA Zuari station wise -pairwise test

Groups	t	P(perm)	perms	P(MC)
nearmouth, midreach	2.4431	0.001	999	0.002
nearmouth, upstream	4.5556	0.001	999	0.001
midreach, upstream	3.383	0.001	996	0.001

Table 9. Species of copepods recorded in the distinct sampling sites of Mandovi estuary with respect to different seasons.

	M1				M3				M6			
	FIM	**NEM**	**SIM**	**SWM**	**FIM**	**NEM**	**SIM**	**SWM**	**FIM**	**NEM**	**SIM**	**SWM**
Copepod species	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Acartia Centrura	-	-	+	+	-	-	+	+	-	-	-	-
Acartia danae	-	-	+	+	-	-	+	+	-	-	-	-
Acartia erythraea	-	-	-	+	-	-	-	-	-	-	-	-
Acartia pacifica	-	-	+++	++	-	-	+++	++	-	-	-	-
Acartia sewelli	-	-	-	-	-	-	-	-	-	+	-	-
Acartia southwelli	-	-	-	-	+	+	-	++	++++	++++	++++	++
Acartia sp.	+	+	+	++	-	-	+	-	-	-	-	-
Acartia spinicauda	++	-	-	+	-	-	-	+	-	-	-	-
Acartia tropica	+	-	++	+	++	++	++	++	-	-	-	-
Acartiella gravelyi	-	-	-	-	-	+	-	+	-	-	-	-
Acartiella Keralensis	-	-	-	+	+	-	-	+	-	++	++	+++
Acartiella spp.	-	-	-	-	-	+	-	-	-	+	-	-
Acrocalanus gibber	++++	++++	++++	++	++++	++++	++++	++	++++	++++	+++	++
Acrocalanus gracilis	++++	+++	++++	++	+++	+++	++++	++	-	++++	++	++++
Acrocalanus longicornis	-	+	-	+	++	-	-	-	-	+	-	-
Acrocalanus monachus	-	+	-	-	-	-	-	-	-	-	-	-
Acrocalanus sp.	++	+++	++++	+++	+++	+++	++++	+++	-	+++	++++	++
Allodiaptomus mirabilipes	-	-	-	-	-	-	-	-	++++	-	+	++
Calanopia sp.	-	+	-	-	-	-	-	-	-	-	-	-
Canthocalanus pauper	-	-	-	-	-	+	-	-	-	-	-	-
Centropage furcatus	-	-	++	-	-	-	++	-	-	-	-	-

Continued …

Centropage sp.	-	+	+	-	-	-	++	+	-	-	-	+
Centropage tenuiremis	-	-	+	-	-	-	+	-	-	-	-	-
Copepod juveniles	++	++	+	+++	++++	++	+	+	+	++++	++++	+++
Corycaeus spp.	++	++	++	-	+	+	++	-	-	+	-	-
Cyclops sp.	-	-	-	-	-	-	-	-	+++	-	-	++
Diaptomus sp.	-	-	-	-	-	-	-	-	+++	++++	+++	++++
Eucalanus sp.	-	+	-	-	-	-	-	-	-	-	-	-
Euterpina acutifrons	+	++	++	++	+	+	++	+	-	++	-	++
Faranulla spp.	+	+	+	-	-	+	+	-	-	-	-	-
Heliodiaptomus cinctus	-	-	-	-	-	-	-	-	++	-	-	-
Lebidocera acuta	+	-	-	-	-	-	-	-	-	-	-	-
Lebidocera pectinata	-	+	-	-	-	+	-	-	-	-	-	-
Lebidocera sp.	+	+	++++	-	-	+	++++	-	-	-	-	-
Longipedia sp.	-	-	-	-	++	+	-	-	-	-	-	-
Macrosettella gracillis	-	-	+	-	-	-	+	-	-	-	-	-
Miccrosettela norvegica	++	+	-	-	+	-	-	-	-	-	-	-
Miccrosettela spp.	-	+	-	-	-	-	-	-	-	-	-	-
Oithona brevicornis	+	+	++	++++	++	+++	+	++++	-	+	+	-
Oithona plumifera	-	-	-	-	-	-	-	-	-	+	-	-
Oithona rigida	-	-	+	+	-	-	-	+	-	-	-	+++
Oithona sp.	-	++	+	++++	++	++++	++	+++	++	++	-	-
Onceae venusta	+	+	-	-	++	-	-	-	+++	-	-	-
Onceae conifera	-	-	+	-	-	-	+	-	-	-	-	-
Onceae sp.	-	+	-	+	-	+	-	-	-	+	-	+++
Paracalanus aculeatus	-	+++	++	+	+	++	++	++	-	+	+++	-

Continued …

Paracalanus parvus	++++	++++	+	++	++++	++	+	++++	+++	+++	+++	++
Paracalanus sp.	+	+++	++	+++	++	+	++	++	+	++	++	++
Pseudodiaptomus bowmini	+	+	-	++	+	++	-	++	-	+	-	-
Pseudodiaptomus jonesii	++	+	+	+	+	+++	+	++	-	+	+	+++
Pseudodiaptomus serricaudatus	++	+	-	+	+	-	-	+	-	-	-	+
Pseudodiaptomus sewelli	+	-	-	++	-	++++	-	+++	-	+	+	+
Pseudodiaptomus sp.	-	+	-	+	-	+	-	-	-	-	-	-
Sapphirina sp.	-	-	-	-	-	-	-	-	-	+	-	-
Temora sp.	++	-	+	-	-	-	+	-	-	-	-	-
Temora stylliferra	+	-	-	-	-	-	-	-	-	-	-	-
Temora turbinata	++	+	+	-	+	+	++	-	-	-	-	-

Table 10. Species of copepods recorded in the distinct sampling sites of Zuari estuary with respect to different seasons.

	Z1(2011-2012)				Z4(2011-2012)				Z7(2011-2012)			
	FIM	NEM	SIM	SWM	FIM	NEM	SIM	SWM	FIM	NEM	SIM	SWM
Copepod species	%	%	%	%	%	%	%	%	%	%	%	%
Acartia Centrura	+	-	-	++++	++++	++++	-	-	-	+++	-	-
Acartia erythraea	+	-	-	-	+++	-	-	-	-	-	-	-
Acartia juveniles	-	-	-	-	-	-	++	-	-	-	-	-
Acartia pacifica	-	-	++	+	++++	+	+	-	-	++++	-	++
Acartia sewelli	-	-	-	-	-	+	-	-	+	++++	-	-
Acartia sp.	-	-	-	+	-	++	++	++	-	-	-	-
Acartia spinicauda	-	-	+	-	-	-	+	-	-	-	-	-
Acartia tropica	-	-	++	++	+++	++++	++++	++	-	+++	++	+++
Acartiella gravelyi	-	+	-	+	-	+++	-	+	-	-	+++	++
Acartiella Keralensis	-	-	-	-	+++	+++	++	+++	+++	++++	+++	++
Acartiella sewelli	-	-	-	-	++	-	-	-	-	++	-	-
Acrocalanus gibber	++++	++++	++++	++++	+	+++	++++	++++	++++	++	+++	+++
Acrocalanus gracilis	+++	+++	++	++	++	++	+	+	++	+	+	-
Acrocalanus longicornis	-	+	+	-	++	-	-	-	-	-	-	-
Acrocalanus monachus	-	-	-	++	-	-	-	-	-	-	-	-
Acrocalanus sp.	++	++++	++++	++++	++++	++	++	++	-	+	++	+++
Allodiaptomus mirabilipes.	-	-	-	-	-	-	-	-	++	-	-	-
Centropage furcatus	-	-	++	+	-	-	-	-	-	-	+	-
Centropage sp.	++	-	+	-	-	-	-	-	-	-	+	-
Clausocalanus furcatus	-	-	-	+	-	-	-	-	-	-	-	-
Clausocalanus sp.	-	-	-	+	-	-	-	-	-	-	-	-

Continued …

Clytemnstra scutellata	-	-	-	-	-	-	-	-	-	-	-	-
Clytemnstridae	-	-	-	-	-	-	-	-	-	-	-	-
Copepod juveniles	+	++	+	+	++	++	++	++	++	++++	++++	++++
Corycaeus spp.	+	+++	++	++	-	-	+	-	-	-	-	-
Cyclop sp.	-	-	-	-	-	-	-	-	-	-	+	+++
Diaptomus sp.	-	-	-	-	-	-	-	-	++++	+	+++	++++
Euterpina acutifrons	++	+	++	++	+	-	+	-	-	-	-	-
Faranulla spp.	-	-	+	+	-	+	-	-	-	-	-	-
Lebidocera pectinata	-	-	+	-	-	-	-	-	-	-	-	-
Lebidocera sp.	-	+	+	-	-	-	-	-	-	-	-	-
Oithona brevicornis	++	+	-	+	-	-	+	+	-	-	-	-
Oithona plumifera	-	-	+	-	-	-	-	-	-	-	-	-
Oithona rigida	+	-	-	-	-	-	-	-	-	-	-	-
Oithona sp.	++	++	+	+++	+	+	++	+	-	+	-	-
Onceae venusta	-	+	+	+	++	-	-	-	-	-	-	-
Onceae sp.	-	++	+	+	+	-	+	+	-	-	-	+++
Paracalanus aculeatus	++	++++	+	+++	++	++++	+	++++	-	+	-	++
Paracalanus parvus	++	+++	++++	++	++++	++++	++++	++++	++	+	-	++++
Paracalanus sp.	-	++	++++	+++	++++	+	++	+	++	++	+++	++
Pseudodiaptomus bowmini	-	+	+	-	-	++	+	-	-	+	-	-
Pseudodiaptomus jonesii	++	-	+	-	++	+	+	+	++	+	++++	+
Pseudodiaptomus serricaudatus	+	-	-	-	+	-	-	-	-	+	-	+
Pseudodiaptomus sewelli	-	-	-	-	++	+++	++	-	-	-	+	+
Pseudodiaptomus sp.	-	-	-	-	-	++	-	+	+	+++	-	++
Temora stylifera	++	+	-	+	-	-	-	-	-	-	-	-
Temora turbinata	++	++	++	+	-	-	-	-	-	-	+	-

Table 11. Overall annual range of diversity index (H´), number of species (S), species richness (d) and evenness (J) for metazooplankton community in Mandovi estuary.

Stations	S	H'	J'	d
M1	15-19	3.1-3.4	0.80-0.84	2.1-2.8
Mean	17.3	3.3	0.81	2.5
SD	± 1.7	± 0.141	± 0.02	± 0.31
M3	13-18	2.7-3.48	0.74-0.91	1.8-2.5
Mean	15	3.2	0.82	1.988
SD	± 2.16	± 0.32	± 0.09	± 0.37
M6	Jun-17	2.2-3.4	0.75-0.9	0.9-3
Mean	12.5	2.9	0.83	2.3
SD	± 4.65	± 0.5	± 0.07	± 0.9

Table 12. Overall annual range of diversity index (H´), number of species (S), species richness (d) and evenness (J) for copepod community in Mandovi estuary.

Stations	S	H'	J'	d
M1	24-29	3.96-4.1	0.84-0.89	3.1-4.2
Mean	26.5	4.09	0.87	3.7
SD	± 2.08	± 0.09	± 0.02	± 0.43
M3	22-27	4.0-4.2	0.85-0.91	2.7-3.7
Mean	24.75	4.1	0.89	3.3
SD	± 2.21	± 0.08	± 0.03	± 0.45
M6	Nov-23	3.2-3.97	0.87-0.95	1.9-4.4
Mean	16.5	3.65	0.91	3.05
SD	± 5.19	± 0.37	± 0.03	± 1.2

Table 13. Overall annual range of diversity index (H´), number of species (S), species richness (d) and evenness (J) for metazooplankton community in Zuari estuary.

Stations	S	H'	J'	d
Z1	Dec-17	2.7-3.7	0.76-0.91	1.7-2.5
Mean	15.75	3.3	0.83	2.3
SD	2.5	0.44	0.07	0.38
Z4	Oct-18	2.3-2.66	0.63-0.78	1.39-2.5
Mean	12.75	2.6	0.71	1.8
SD	3.59	0.16	0.07	0.5
Z7	05-Oct	2.1-2.9	0.75-0.87	1.2-2.2
Mean	9.75	2.67	0.81	1.25
SD	2.06	0.38	0.05	0.43

Table 14. Overall annual range of diversity index (H´), number of species (S), species richness (d) and evenness (J) for metazooplankton community in Zuari estuary.

Stations	S	H'	J'	d
Z1	17-25	3.6-4.2	0.85-0.92	2.5-3.7
Mean	22	3.99	0.9	3.22
SD	± 3.83	± 0.24	± 0.03	± 0.49
Z4	16-22	3.8-4.2	0.8-0.96	1.99-3.05
Mean	19.75	3.8	0.88	2.68
SD	± 2.63	± 0.44	± 0.06	± 0.48
Z7	Nov-17	3.0-3.9	0.88-0.96	1.88-3.5
Mean	14.75	3.58	0.92	2.52
SD	± 2.63	± 0.37	± 0.03	± 0.7

Table 15a. SIMPER analysis showing the contribution of major metazooplankton groups for the differences among the groups in the sampling sites of Mandovi estuary. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 38.31	Group mouth	Group upstream				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Gastropod larvae	1.29	5.36	6.5	1.35	16.96	16.96
Cyclopoida	3.5	1.8	3.98	0.77	10.4	27.35
Decapoda larvae	1.71	3.42	3.66	1.04	9.56	36.91
Copepod Juveniles	2.23	2.37	2.39	1.52	6.25	43.16
Poecilostomatoida	1.21	1.52	2.13	2.02	5.57	48.73
Siphonophorae	1.26	0	2.11	0.98	5.5	54.22

Average dissimilarity = 38.10	Group midreach	Group upstream				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Cyclopoida	5.45	1.8	5.53	1.89	14.51	14.51
Gastropod larvae	4.29	5.36	4.8	1.17	12.61	27.12
Decapoda larvae	1.52	3.42	3.52	1.02	9.23	36.35
Polychaete larva	1.82	0.05	2.53	1.56	6.65	42.99
Copepod Juveniles	2.31	2.37	2.22	1.32	5.84	48.83
Barnacle nauplii	2.14	0.72	2.13	1.48	5.6	54.43

Average dissimilarity = 39.46	Group SIM	Group SWM				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Cyclopoida	3.19	5.8	5.37	0.78	13.61	13.61
Gastropod larvae	2.44	5.63	4.32	2.62	10.94	24.55
Decapoda larvae	2.06	4.13	3.79	0.83	9.59	34.14
Copepod Juveniles	1.14	2.85	3.27	1.73	8.29	42.43
Siphonophorae	1.65	0	2.43	0.95	6.15	48.58
Cladocerans	0.35	1.76	2.23	0.68	5.65	54.23

Table 15b. SIMPER analysis showing the contribution of copepod species in different sampling sites of Mandovi estuary. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 57.63	**Group mouth**	**Group upstream**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Acartia southwelli	0	7.55	6.44	2.26	11.17	11.17
***Diaptomus* sp.**	0	6.36	5.3	2.68	9.19	20.36
Acrocalanus gracilis	5.11	3.67	3.51	1.43	6.09	26.45
***Oithona* sp.**	3.33	1.26	3.14	0.94	5.45	31.9
Allodiaptomus mirabilipes	0	3.07	2.65	0.79	4.6	36.5
Oithona brevicornis	3.05	0.75	2.07	0.77	3.6	40.1

Average dissimilarity = 61.29	**Group FIM**	**Group SWM**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
***Oithona* sp.**	1.87	5.28	3.87	1.49	6.32	6.32
Paracalanus parvus	7.6	4.73	3.72	1.6	6.07	12.39
Oithona brevicornis	1.07	4.86	3.71	1.61	6.05	18.44
Acrocalanus gibber	7.34	2.93	3.61	2.19	5.88	24.33
Acartia southwelli	3.66	2.14	3.32	1.15	5.41	29.74
***Diaptomus* sp.**	1.47	3.33	3.2	0.93	5.22	34.95

Table 16a. SIMPER analysis showing the contribution of major metazooplankton groups for the differences among the groups in different sampling sites of Zuari estuary. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 43.88	**Group mouth**	**Group upstream**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Cladocerans	4.34	3.16	7.33	1.09	16.7	16.7
Copepod Juveniles	1.11	4.4	5.2	1.33	11.85	28.54
Pelecypoda larvae	2.69	1.07	3.95	1.07	9.01	37.56
Calanoida	9.31	8.4	3.15	0.78	7.17	44.73
Decapoda larvae	2.29	4.1	3.14	1.66	7.15	51.88
Harpacticoida	1.89	0	2.96	6.93	6.74	58.62

Average dissimilarity = 39.76	**Group SIM**	**Group SWM**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Cladocerans	0	5.83	9.04	1.2	22.73	22.73
Pelecypoda larvae	1.97	2.45	6.38	1.83	16.03	38.77
Decapoda larvae	5.92	2.91	5.91	0.68	14.85	53.62
Calanoida	9.98	7.87	3.54	0.59	8.9	62.53
Copepod Juveniles	3.04	1.37	2.79	0.68	7.02	69.54
Cyclopoida	1.09	1.42	1.88	1.79	4.72	74.26

Table 16b. SIMPER analysis showing the contribution of copepod species in different sampling sites of Zuari estuary. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 68.96	**Group mouth**	**Group upstream**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
***Diaptomus* sp.**	0	6.07	5.74	1.4	8.33	8.33
Acrocalanus gibber	9.3	4.24	4.65	2.92	6.74	23.03
Acartiella Keralensis	0	4.64	4.17	3.84	6.05	29.09
***Acrocalanus* sp.**	6.12	2.58	3.73	1.46	5.41	34.5
***Paracalanus* sp.**	4.54	2.83	2.95	1.22	4.28	38.77
Paracalanus aculeatus	3.58	0.67	2.85	1.41	4.14	42.91

Average dissimilarity = 60.44	**Group FIM**	**Group NEM**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Paracalanus aculeatus	1.78	5.28	3.75	1.42	6.21	6.21
Acartia Centrura	3.54	3.93	3.59	1.43	5.94	12.15
***Diaptomus* sp.**	3.33	0.37	3.51	0.72	5.81	23.83
Paracalanus parvus	4.16	5.1	3.5	1.41	5.79	29.62
Acartia tropica	2.24	4.14	3.24	1.15	5.36	34.98
***Acrocalanus* sp.**	3.83	4.19	3.11	1.52	5.15	40.13
Acartiella Keralensis	3.64	3.88	2.93	1.38	4.84	44.97

Average dissimilarity = 59.64	**Group FIM**	**Group SIM**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Paracalanus parvus	4.16	7.11	4.66	1.29	7.82	7.82
***Diaptomus* sp.**	3.33	1.46	4.19	0.83	7.03	14.85
***Paracalanus* sp.**	2.66	5.29	3.85	1.13	6.45	21.29
***Acrocalanus* sp.**	3.83	4.6	3.32	1.21	5.56	26.86
Acartia tropica	2.24	2.99	3.09	1.72	5.18	37.46
Pseudodiaptomus jonesii	2.27	3.72	2.82	0.99	4.72	42.18
Acartiella Keralensis	3.64	2.57	2.67	1.55	4.48	46.66

Table 17. Cumulative constrained percentage of the four axes extracted in the CCA analysis for **(a)** copepod communities in Mandovi estuary and **(b)** Zuari estuary.

Man copepod CCA

Factors	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues :	0.338	0.307	0.143	0.116
Species-environment relation:	35	66.9	81.7	93.7

Zoo copepod CCA

Factors	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues :	0.39	0.301	0.176	0.157
Species-environment relation:	32	56.6	71	83.9

Section B

3.b.1 Estuarine (Mandovi-Zuari) variability w.r.t. adjacent coastal and open waters of the Arabian Sea

To address horizontal variation of metazooplankton, sampling was carried out accordingly from different regions covering (estuarine, coastal and open ocean) sites of the Arabian Sea representing diverse ecosystems. Stations sampled include estuarine (Mandovi: M1, M3 and M6; Zuari: Z1, Z4 and Z7); Coastal (Inner shelf: G5; Outer shelf: G12) and open ocean (ASTS) sites. The details of the sampling sites are given in the chapter 2. Sampling in coastal and open waters of the Arabian Sea was carried out using Research Vessel Sindhu Sankalp (SSK) covering the seasonal cycle that is fall intermonsoon (FIM: October-November 2013-SSK 56), southwest monsoon (SWM: September 2014-SSK 69), and late northeast monsoon (NEM: March 2015-SSK 79). Metazooplankton samples from the coastal and open ocean sites collected from surface-oxic mixed layer of the water column are used for comparison to estuarine samples in this chapter. Sample were collected from 0-9 m from inner continental shelf station (G5) in all the three cruises whereas from outer continental shelf: (G12), 0-55 m oxic mixed layer was sampled during SSK 69 and 0-70 m during SSK 79 cruise. While, 0-40 m was sampled from the open ocean site (station: ASTS) during all the three cruises.

Sample analysis: Physico-chemical and biological parameters were collected and processed as given in chapter 3a.

Data analyses: Analysis of environmental and biological dataset was carried out as described in first section (chapter 3a).

3b.2 Results

3b.2.1 Hydrographical parameters

3b.2.1.1 SSK56-Fall inter monsoon (FIM)

Temperature (°C): Overall, the temperature varied from 25.64 to 30.58 °C (± 3.82 °C) at the stretch from estuarine to open ocean stations. Maximum temperature (30 °C) was observed at the mid estuarine station (Z4). Coastal stations (G5 and G12) revealed lower temperature (25.6-26.5 °C) compared to estuarine and open ocean stations (27.23-30.5 °C); (Table 18).

Salinity (PSU): Salinity changed drastically from estuarine to coastal and open waters of the Arabian Sea (0.04-36.87 ± 13.14 PSU). Highest salinity (36.87 PSU) was recorded at the open ocean station (ASTS). Spatially the average salinity of 16.64 (± 13.14 PSU) was observed in the estuarine system. Both, Mandovi and Zuari estuaries showed fluctuations in salinities from upstream (M6, Z7) to Mouth stations (M1, Z1). Mandovi (M3) and Zuari (Z4) revealed wildly different salinities (16.33 and 4.9 PSU respectively.

Dissolved oxygen (µM): All the estuarine, coastal and open ocean sites represented well saturated oxic water column (92- 325 µM). Among all the sampling sites upstream waters of Mandovi estuary (M6) revealed highly oxic (325 µM) waters and so in open ocean station (ASTS) 203 µM (Table 18).

Nitrate (µM): Mid estuarine (M3 and Z4) and upstream stations (M6 and Z7) of Mandovi and Zuari estuary revealed with high nitrate (3.61-4.41 µM) concentration compared to inner continental shelf station (below detectable limit; G5). But at outer continental shelf (G12) was much higher (9.97 µM) than at Open Ocean site (0.28 µM; ASTS); (Table 18).

Nitrite (µM): Most of the sampling sites during this period from estuarine to coastal and open waters showed lowest nitrite values (0.001 µM) except at mid estuarine (M3) site and upstream stations (M6) of Mandovi estuary (0.08-0.41 µM); (Table 18).

Chlorophyll *a* (µg L^{-1}): Chlorophyll *a* biomass in the estuarine system varied from 2.41-6.8 µg L^{-1} with higher values at near mouth region while, coastal inner continental shelf was more productive than outer continental shelf. Chlorophyll *a* concentration at the inner and outer continental shelf varied from 0.63-3.76 µg L^{-1}. On the other hand, Chlorophyll *a* level at open ocean was comparatively low (0.05 µg L^{-1}); (Table 18).

3b.2.1.2 SSK69-Southwest monsoon (SWM)

Temperature (°C): Spatially, sea surface temperature during this period varied between 25.16 and 29.56 °C (estuarine to coastal to open ocean sites). The average temperature of 27.69 °C (± 1.62 °C) was observed in the estuarine system while, the coastal site revealed average temperature (26.41 ± 0.99 °C). In the open ocean surface water was warmer (28.4 °C). Relatively lower temperature was recorded at near mouth (M1 and M2) station of Mandovi estuary (Table 19).

Salinity (PSU): Salinity during this period varied widely in the estuarine region (0.03-34.32 PSU). Comparatively low salinity waters prevailed in the estuarine system. Whereas, in coastal and open ocean water high salinity prevailed that salinity varied in a narrow range (35.17-36.94 PSU); (Table 19).

Dissolved oxygen (µM): The level of dissolved oxygen in the estuarine system varied from 61-264 µM with higher concentration in the Zuari estuary (154-264 µM). The inner continental shelf was with less DO (85.74 µM; G5) compared to outer continental shelf (161 µM; G12) while, open waters (ASTS) showed higher DO (185.7 µM; Table 19).

Nitrate (μM): Nutrient-NO_3^- showed wide spectrum of variation in the estuarine region. Nitrate concentration varied from 0.14-6.16 μM in the Mandovi estuary relatively larger than the Zuari estuary (0.17-3.53 μM). High nitrate concentration was recorded (2.89 μM) at the outer continental shelf station (G12) compared to inner continental shelf station (G5; 2.22 μM) and open ocean waters (ASTS; 1.96 μM); (Table 19).

Nitrite (μM): Low nitrite levels were documented at all the sampling sites (estuarine to open ocean) that varied from 0.03-0.34 μM. Amongst, the highest value was obtained at the inner continental shelf station (G5); (Table 19).

Chlorophyll *a* (μg L^{-1}): Comparatively higher chlorophyll *a* was noticed at most of the estuarine stations (4.59-6.18 μg L^{-1}) than the coastal (0.35-1.19 μg L^{-1}) and open waters (0.25 μg L^{-1}). Chlorophyll *a* as high as 6.18 μg L^{-1} was found at the near mouth station of Mandovi estuary (Table 19).

3b.2.1.3 SSK79 – Northeast monsoon (NEM)

Temperature (°C): Water temperature was relatively higher at most of the stations during this period. High temperature waters were recorded in estuarine system (Mandovi: 31.1 ± 0.9°C; Zuari: 31.8 ± 1°C) compared to coastal and that decreased gradually towards the oceanic region (ASTS: 27.31°C; Table 20).

Salinity (PSU): Overall, salinity was higher in this season as compared to other seasons. As expected, low salinity values were recorded in the estuarine system. Near fresh water prevailed in the upstream region of the Zuari estuary causing this estuary less saline (avg. 20 ± 14.7 PSU) than the Mandovi (avg. 29.1 ± 8.5°C). Inner continental shelf of coastal region was more saline (34.79 PSU) that gradually increased towards outer continental shelf (36.06 PSU) to open ocean (36.23 PSU) thereby showing a cumulative salinity trend towards the offshore region.

Dissolved oxygen (μM): DO concentration varied in the range of 155-228 μM in estuarine, coastal and open ocean waters. It also showed higher concentration in the upstream region of the estuary (~183.6-228.6 μM) compared to near mouth (~170 μM) and inner continental shelf site (G5; ~190 μM). Open Ocean station also revealed comparatively higher level of DO (~200 μM; Table 20). Overall, from estuarine to open ocean variation found to be roughly within 22 μM of DO.

Nitrate (μM): Most of the estuarine as well as inner continental shelf stations showed low nitrate concentration (maximum of 1.46 μM at upstream of Zuari estuary) compared to outer continental shelf (4.92 μM ;G12) and open ocean (Table 20).

Nitrite (μM): Overall, nitrite concentration in the water column remained low (maximum 0.5 μM at mid reach station of Mandovi estuary) irrespective of the region. Despite fluctuating values in-between estuarine, coastal and open ocean waters, a decreasing nitrite level was recorded from estuarine system to open ocean site (Table 20).

Chlorophyll *a* (μg L^{-1}): Spatially, chlorophyll *a* concentration also showed a decreasing trend from estuarine to coastal and open ocean site. During this period, the Zuari estuary revealed higher concentration 5.48-7.98 μg L^{-1} of chlorophyll *a* compared to other continental shelf and open ocean waters. The Zuari estuary found to be more productive (Chlorophyll a: 4.5 ± 2.8 μg L^{-1}) than the Mandovi estuary (1.0 ± 0.2 μg L^{-1}) with higher levels at the upstream region. However, open ocean and outer continental shelf showed comparable concentration (~0.1 μg L^{-1}), which were roughly six fold lower than the inner shelf coastal station (G5; Table 20). Overall, the range of chlorophyll *a* varied between 0.1 and 7.98 μg L^{-1} from estuarine to open ocean waters. Lowest chlorophyll *a* was recorded at the open ocean station (Table 20).

3b.2.2 Correlation between environmental factors and total metazooplankton

Spearman's correlation analysis between environmental factors and metazooplankton abundance are presented in the table 21. Temperature was significantly correlated with DO ($r= 0.591$; $p < 0.05$), while salinity showed significant negative correlation with DO ($r=-0.457$; $p < 0.05$). Also, chlorophyll *a* was negatively correlated with salinity ($r= -0.501$; $p < 0.01$). On the other hand, total metazooplankton as well as total copepod abundance revealed significant positive correlation ($r= 0.410$, $P < 0.05$; $r= 0.349$, $p < 0.05$) only with salinity (Table 21).

3b.2.3 Analysis of Variance (ANOVA)

This test is used to address the spatio-temporal variation of environmental factors occurred during study. The detail summary of environmental parameters is given in Table 5. Annual variation of specified environmental factors in the context of seasons and stations are described in Table 22.a and Table 22.b. Overall, with reference to season only temperature showed significant variation ($p= 0.001$; Table 22 a). Turkey's post hoc test defined that the temperature during the season NEM was significantly different from FIM and SWM. But, spatially there was no significant difference in temperature among all the stations. Salinity ($p < 0.01$) and chlorophyll *a* ($p < 0.01$) showed a significant difference among the stations spatially (Table 22 b).

3b.2.4 Metazooplankton community structure

Total metazooplankton and copepod density

Here, numerical density of metazooplankton and copepods (per $100m^{-3}$) is given seasonally. Numerical density is represented as $\log_{10}$ (x+1) transformed value and is represented in square bracket.

SSK 56 (FIM)

Total metazooplankton density (ind. $100m^{-3}$) varied from 88 to 903625 from estuarine to open ocean stations. The highest abundance was noticed at the mid estuarine regions particularly mid reach of the Zuari estuary (Z4). Among the coastal sites, the outer continental shelf station (G12) showed relatively less abundance (by two fold) compared to inner continental shelf (G5; 7330) whereas open ocean oxic mixed water column revealed abundance 19600 ind. $100m^{-3}$ compared to coastal strata (Fig. 16). Similarly, high copepod density (743590 ind. $100m^{-3}$) was also observed at the station Z4 of Zuari estuary contributed 82% of the total metazooplankton community. However, contribution of copepod to the total metazooplankton community was very low in estuarine system (Mandovi: avg. 26%; Zuari: avg. 40%) compared to coastal inner continental shelf (75%). Both at outer continental shelf and open ocean site, copepod contribution was sizably large. Thus, a general trend of increasing copepod contribution was observed from estuarine to open ocean region. For instance, copepod contribution was very less at most of the estuarine stations (M1-16 %, M3-13 %, M6-42 %, Z7-1%, and Z1-1%) as compared to other stations (Fig. 16).

SSK 69 (SWM)

During this time period, total metazooplankton abundance ranged from 140-3055556 ind. $100m^{-3}$ with increasing trend towards the open ocean site. As usual lowest abundance was observed in the estuarine region particularly at the upstream stations. Amongst, the river Mandovi was found to have numerically low population of metazooplankton than in the Zuari estuary. Spatially, pronounced increase in metazooplankton abundance was documented at the coastal inner continental shelf station (G5), which was mainly contributed by polychaete larvae (98%). Besides, the outer continental shelf (G12) and open ocean (ASTS) revealed higher abundance of

total metazooplankton groups compared to near shore and estuarine stations. The outer shelf and open ocean contributed about 77-84% of copepod abundance towards the total metazooplankton abundance (Fig. 17). Copepod contribution to the total metazooplankton was relatively lower at the upstream region (~52% at M6 and ~63% at Z7) which was lower than the near mouth estuarine region (69-86%), outer continental shelf and open ocean (77%) sites. Their lowest contribution (1.5%) was recorded at inner continental shelf region (G5; Fig. 17).

SSK 79 (NEM)

Total abundance of metazooplankton community during this period also spatially fluctuated between estuarine, coastal and open ocean stations. Highest abundance was recorded in high saline region that is at the mouth station of Zuari estuary (2724207 ind. $100m^{-3}$) and the lowest (7372 ind. $100m^{-3}$) was at the upstream station of (Z7). Amongst, the estuarine sites, upstream waters of only Zuari estuary (Z7) obtained low abundance compared to the other spatial sites. Interestingly, the metazooplankton community was dominated by poecilostomatoida copepods in both the coastal stations (inner continental shelf: G5; outer continental shelf: G12) and the open ocean sites (ASTS). 76% of poecilostomatoida copepods were at the inner continental shelf station than the outer shelf (39%) and open ocean sites (34%) (Fig. 18). Copepod community during this period maximum abundance at the mouth area of Zuari estuary contributing 74% to the metazooplankton community. Generally, estuarine mouth station revealed high copepod abundance that decreased at the coastal (G5 and G12) and open ocean stations (Fig. 18).

Metazooplankton community composition

SSK 56 (FIM)

Metazooplankton in the estuarine system during this period was relatively diverse particularly in the Mandovi estuary and was mainly dominated by larval form of decapods and gastropods apart from fish eggs. While in the inner continental shelf (G5) and outer continental shelf (G12), appendicularian, siphonophores and ostracods were predominant. On the other hand, decapods and ostracods together with polychaetes formed the major contributors to the metazooplankton community. Generally copepod community contributed higher to the total metazooplankton at outer continental shelf and open ocean region (Fig. 19 a).

In case of Zuari estuary, the dominant metazooplankton community was contributed by fish eggs at Z1 (85%), calanoid copepods at Z2 (59%) and Z4 (80%), while at upstream region (Z7) gastropod larvae were predominant forms (94%; Fig. 19 b). Calanoid copepods (36%), copepod juveniles (28%) and harpacticoida copepods (9.48%) were leading forms in the inner continental shelf of coastal region (G5). At outer continental shelf (G12), apart from calanoid copepods (32%), poecilostomatoid (26%) and copepod juveniles (26%) were main contributors to the community. Distinctly, poecilostomatoid (50%) and calanoid (26%) copepods dominated the metazooplankton community in the open waters (AST; Fig. 19 c).

Copepod species composition

A varying copepod contribution was noticed across the spatial stretches of Mandovi estuary. Mouth station was dominated by *Acrocalanus gibber* (54%) and *Acrocalanus gracilis* (24%) whereas the nearmouth station was dominated by *Acartia tropica* (26%), *Acrocalanus gibber* (12%), *paracalanus aculeatus* (12%) and *Oithona similis* (8%). Midreach area of estuary documented *Acrocalanus gibber* (24%) and 19% each of

Acrocalanus gracilis and *Acartia tropica*. In the Zuari estuary, the near mouth station (Z2) and mid reach station (Z4) revealed high copepod abundance compared to other reaches of the estuary. High copepod abundance at the station Z2 was contributed by *Paracalanus aculeatus* (11%), *Pseudodiaptomus bowmini* (17%), *Acartia* sp. (15%) whereas the midreach station Z4 was highly contributed by *Acrocalanus gibber* (29%), *Acrocalanus longicornis* (14%), and *Acartia tropica* (29%). Low contribution of *Acartiella keralensis* (2%) was recorded at the midreach and upstream station (Table 23). The inner continental shelf of the coastal station (G5) revealed the dominant copepod species as *Temora turbinata* (13%), *Euterpina acutifrons* (12%) and *Centropages furcatus* (10%). The outer continental shelf coastal station showed highest number of copepod species and was represented by *Onceae venusta* (11%), *Coryceaus* sp. (7%), *Acrocalanus gibber* (8%), *Paracalanus aculeatus* (6%) and *Acrocalanus monachus* (6%); (Table 23). On the other hand, *Euchaeta marina, Euchaeta indica*, *Euchaeta* sp. *Lebidocera Maduare*, *Pontellina plumata*, *Pleuromamma indica* and *Lucicutia* sp. were the distinct copepods found only in the open ocean regions compared to the coastal and estuarine sites. During this Season *Oncaea* spp. (43%), *Corycaeus* spp. (8%) showed their major contribution to the copepod community of the open ocean waters (ASTS; Table 23).

Metazooplankton community composition

SSK 69 (SWM)

Calanoida copepods were highly abundant at all the stations of the Mandovi estuary with the highest contribution of 81% at near mouth station (M2). Polychaete larvae (13%) were comparatively abundant at the mouth station whereas the gastropod larvae (9%) showed their dominance at the station M3 (Fig. 20 a). Similarly, calanoida copepods were abundant at all the spatial distinction area of Zuari estuary.

Comparatively fish eggs (21%) were the distinct dominant groups at the mouth station of Zuari estuary (Fig. 20 b).

There was a pronounced abundance of polychaete larvae (98%) noticed at the inner continental shelf coastal station (G5) while the outer continental shelf station (G12) was dominated by poecilostomatoida copepods (33%) and copepod juveniles (30%). The metazooplankton community at the open ocean station was contributed by poecilostomatoida copepods (29%), copepod juvenile (31%), ostracoda (15%) and calanoida copepods (14%) (Fig. 20c).

Copepod species composition

Distinctly *Pseudodiaptomus jonesii* and *Pseudodiaptomus sewelli* were observed at the midreach station of Mandovi estuary. *Acrocalanus gracilis* (31%) were dominant at the mouth area whereas *Acrocalanus gibber* showed their dominant contribution (59% and 40%) at the nearmouth and midreach stations (Table 24)

Zuari estuary found to have a higher abundance of copepods such as *Acrocalanus gibber* (34%), *Oithona* sp. (24%) followed by *Acrocalanus gracilis* (10%) at the mouth station. At the nearmouth station, *Acartia pacifica* were comparatively most abundant species followed by *Pseudodiaptomus bowmini* (19%) and *Pseudodiaptomus sewelli* (6%). *Acartiella keralensis and Acartia* sewelli were abundant at the midreach (18%) and upstream station (21%) representing low saline condition favouring copepod species. Moreover, *Acartia sewelli* contributed 3% and 13% of copepod community at the midreach station and the upstream stations. The upstream station represented distinct copepod species such as *Heliodiaptomus cinctus* (32%), *Heliodiaptomus contortus* (12%) and *Diaptomus* sp. which were present only at this station (Table 24). While, inner shelf waters of coastal site (G5) showed very low species abundance contributed by *Oncea conifera* (25%), *Euterpina acutifrons* (25%) and *Oithona*

brevicornis (25%). On the contrary, the outer shelf station revealed wide scale of species abundance at surface mixed water column. This site was mostly dominated by *Oncaea media* (19%), *Oncaea conifera* (9%), *Corycaeus* sp. (6%) and *Oncaea venusta* (4%). The open ocean station documented the copepods distribution such as *Onceae minuta* (9%), *Oncaea venusta* (9%), *Oncaea conifera* (6%), *Corycaeus* sp. (6%), *Euchaeta indica* (4%) and *Clausocalanus arcuicornis* (3%); (Table 24).

Metazooplankton community composition

SSK79 (NEM)

In the estuarine region the metazooplankton group composition was strongly dominated by calanoida copepods (53-71%), contribution of other copepods was low; cyclopoida (7-13%), poecilostomatoida (1.4%-20%) and harpacticoida copepods (1-3.9%) at all the spatial sites of Mandovi estuary (Fig. 21 a). Comparatively polychaete larvae (13%) were dominant at the near mouth station than the mouth, midreach and upstream waters. Pelecypoda larvae (7.8%) and gastropod larvae (5.5%) were documented prominently at the mid estuarine station. Cirripede larvae (12%) and decapod larvae (20%) were mostly documented at the upstream station compared to other stations. Similarly, the mouth station of the Zuari estuary revealed the dominance of calanoida copepods (65%) more than the cyclopoida copepods (9%), appendicularians (6.6%) and decapod larvae (11%). The near mouth station was highly dominated by decapod larvae (59%) followed by calanoida copepods (31%) whereas gastropod larvae (85.3%) were the dominant groups in the mid estuarine station. Comparatively calanoid copepods were the highest contributor to the metazooplankton groups (83%) followed by decapod larvae and polychaete larvae (Fig. 21b) while, poecilostomatoida copepods were highly dominant (75%) at the coastal inner continental shelf where fewer number of groups prevailed. But coastal outer continental shelf station contained again with 14

numbers of groups with the higher contribution of poecilostomatoida copepods (39%), other prominent forms were calanoida copepods (14%) and chaetognatha (13%). In case of the open ocean also poecilostomatoida copepods (34%) were dominating the metazooplankton community followed by calanoid copepods (23%), chaetognatha (9%), ostracoda (7%) and appendicularia (4%); (Fig. 21c).

Copepod species composition

Copepod species composition varied along the different spatial sites of Mandovi estuary. *Acrocalanus gibber*, *Acrocalanus gracilis*, *Paraclanus parvus* and *Paracalanus* sp. were dominant at all the three distinct stations of the estuary (Table 25). Moreover, *Oithona brevicornis* (15%) and *Corycaeus* spp. (22%) were abundant at the mouth and mid estuarine stations. In contrast, *Heliodiaptomus cinctus* (8%) and *Diaptomus* sp. (14%) were the distinct copepods found at the upstream station. Total of 27 species were noticed in the Zuari estuary where *Acrocalanus gibber*, *Acrocalanus gracilis, Paracalanus aculeatus* were abundant at mouth and near mouth stations of the estuary. A contrasting feature was noticed as *Acartia tropica* (91%) were solely dominant at this station. Distinctly *Acartiella* sp. (14%), *Alodiaptomus mirabilipes* (41%) *and Heliodiaptomus contortus* (6%) were noticed at the upstream station (Table 25). Poecilostomatoida copepods were abundant at the coastal inner continental shelf station with the contribution of *Oncaea conifera* (30%), *Oncaea venusta* (10%), *Oncaea* sp. (24%), *Oncaea media* and *Corycaeus* sp. (10%). The outer continental shelf coastal station was mainly contributed by *Corycaeus* spp. (17%), *Faranulla* spp. (16%), *Oncaea media* (8%), *Oncaea conifera* (9%), *Oncaea venusta* (9%), *Clausocalanus arcuicornis* (6%), *Nanocalanus minor* (5%), *Lucicutia flavicornis* and *Euchaeta indica* (4%). The copepod community in the open ocean waters was majorly contributed by

Corycaeus spp. (19%), *Oncaea venusta* (8%), *Oncaea conifera* (6%) and *Euchaeta indica* (5%); Table 25).

3b.2.5.Spatio-temporal variation of metazooplankton community

nMDS analysis based on Bray Curtis similarity formed three major clusters of metazooplankton groups (Fig. 22). Groups clustering were considered at 60% similarity which was confirmed in nMDS plot (Fig. 22). All the metazooplankton groups were distributed with reference to diverse seasons at distinct spatial sites reflecting spatio-temporal variation. Group 1 formed the sampling sites of all coastal (outer and inner continental shelf) and open ocean stations covering the Seasonal periods of FIM, NEM and SWM. The other two major groups were clustered with the estuarine stations where the one group clubbed with M6-SWM, Z2-SWM, M3-SWM, Z4-FIM, Z4-SWM, Z7-NEM and the other group clustered the stations of M1-NEM, M3-NEM, M6-NEM, M2-NEM, M1-SWM, and M2-SWM. The different groups were formed due to the dissimilarity in metazooplankton distribution pattern and is further explained during SIMPER analysis (Table 26). Across all habitats average dissimilarity was higher in between the Seasonal periods of FIM and NEM by the dissimilar contribution of calanoid copepods (16%), gastropod larvae (9%) and decapod larvae (8%); (Table 26a). Across all seasons spatial distinction was clearly noticed as the dissimilarity community pattern at mouth, near mouth, coastal and open ocean sites. The highest average dissimilarity of the community was noticed in between midreach and inner shelf station (79%) followed by upstream and outer shelf (71%) and upstream and open ocean (69%) stations (Table 26 b, c, d).

3b.2.6 Spatio-temporal distribution of copepod species

In case of copepod distribution nMDS analysis divided the copepod species abundance with reference to seasonal and spatial variation. In this context, two major groups were

clustered at 60% similarity (Fig. 23). Copepod species were distributed in various stations corresponding to different seasons proving the spatio-temporal differences. Among the two groups, all the coastal and open ocean sites formed one group while the other group reflected most of the estuarine stations (Mandovi and Zuari) covering the seasonal periods. The major clustering of groups is formed with the due dissimilarity of the copepod species pattern and is explained with the help of SIMPER analysis (Table 27). Across all the habitats the seasonal difference of copepod species was highest during FIM and NEM. The seasonal differences were responsible for the dissimilar contribution of copepod species such as *Acrocalanus gibber* (9%), *Acartia tropica* (5.8%), *Acrocalanus* sp. (5%), and *Acrocalanus gracilis* (4%); (Table 27 a). Across all the seasons, the habitat differences were summarized through the SIMPER analysis. It clearly indicated that the spatial differences are much higher than the seasonal differences. With reference to spatial variation of copepod species, the dominant species difference was noticed at the different ecological sites. The dissimilarity between open ocean and upstream (86%) contributed by *Acrocalanus gibber* (6%), *Oncaea* sp. (6%), *Corycaeus* sp. (5%), *Oncaea venusta* (5%), *Euchaeta indica* (4%) (Table 27b). 87% of average dissimilarity was observed between upstream and inner shelf stations which was contributed by *Oncaea conifera* (8%), *Acrocalanus gibber* (8%), *Euterpina acutifrons* (7%), *Acrocalanus longicornis* (6%), *Oithona brevicornis* (5%); (Table 27c).

The midreach of estuarine stations and open ocean showed 83% copepod species difference and were contributed by *Acartia tropica* (7%), *Acrocalanus gibber* (6%), *Oncaea* sp., *Corycaeus* sp., *Acrocalanus longicornis* (5%) and *Oncaea venusta* (5%); (Table 27 d). 83% of copepod species differences were observed between near mouth and inner shelf stations, contributed by *Acrocalanus gibber* (7%), *Oncaea conifera*

(7%), *Euterpina acutifrons* (6%), *Acrocalanus longicornis* (5%) and *Acartia tropica* (5%); (Table 27e). Moreover, 81% of average dissimilarity between mouth of estuary and outer shelf (coastal) station was contributed by *Acrocalanus gibber* (7%), *Corycaeus* sp. (5%), *Oncaea media* (5%), *Acrocalanus gracilis* (5%), *Oncaea venusta* (5%) and *Oncaea conifera* (4%); (Table 27f).

3b.2.7 Metazooplankton diversity

3b.2.7.1 Metazooplankton group diversity

During FIM, the numbers of metazooplankton groups (S) varied from 3-13. The lowest diversity values were recorded at the upstream station and the highest was at coastal region (inner continental shelf and outer continental shelf). Comparatively higher diversity (H') was noticed at coastal and open ocean station. Species richness (D) varied from 0.4-2.5 and the highest value was obtained at outer shelf station. The lowest species evenness (J') was found at the upstream station of Zuari estuary whereas the highest was at the near mouth and the coastal waters. In case of SWM, the numbers of groups varied from 5-15 with higher diversity index (H') of 1.3-2.9. Lower diversity were reflected at station (G5) and the upstream station (M6) while, highest group diversity was observed at the outer continental shelf station (G12), species richness (D) and species evenness (J') was very low at the coastal inner continental shelf station and midreach station. On the other hand, the numbers of groups during NEM varied between 3 and 17. Comparatively, highest group diversity was observed during this period (1.3-3.7) with maximum values at the outer continental shelf station. Also, the species richness (D) during this period varied from 0.3 -2.4 where, the highest value was recorded at the outer shelf station (G12) and the lowest value was at the mid estuarine station (Z4). Overall during NEM metazooplankton groups were evenly distributed as compared to FIM and SWM (Fig. 24).

3b.2.7.2 Copepod species diversity

During FIM, number of copepod species varied from 8-29 across all sampling sites. Highest copepod diversity (H´) was recorded at the outer continental shelf coastal station (4.5) followed by open ocean site (4.1). Similarly, copepod species richness and evenness was also higher at both these sampling sites. During SWM, species number varied in between 4-24. As in FIM, the outer continental shelf showed the maximum species diversity (4.2) than open ocean station (3.7). The coastal sites were also rich in species evenness (j´). Particularly the species richness was pronounced at the outer shelf station.

Overall, NEM encountered the higher numbers of copepod species (4-32), species diversity (H´) (1.3-4.7), species richness (d) (1.4-4.7) and species evenness (0.6-0.96) as compared to FIM and SWM. Also, open ocean, coastal, outer continental shelf and near mouth estuarine stations revealed higher diversity than the FIM and SWM (Fig. 25).

3b.2.8 Effect of environmental factors on metazooplankton groups and copepod species

CCA triplot with 2 axes explained 68.3 and 85.2% of relationship between metazooplankton group compositions and associated environmental factors from 11 sampling sites encompassing three seasonal cycles from estuarine, coastal and open ocean waters (Fig. 26). Eigen values represented first axis of 0.716 and 0.177 on second axis (Table 28a). The FS summary of CCA plot showed a significant correlation between salinity and the abundance of metazooplankton groups (Table 28b). The distribution of hydromedusae, ehinodermata larvae, chaetognatha, fish eggs, barnacle nauplii, appendicularia, siphonophorae, cyclopoida copepods, and decapod larvae found to be highly influenced by salinity. Moreover, chlorophyll *a*, dissolved oxygen and nitrate are the favourable factors for the spatio-temporal distribution of other

metazooplankton groups (Fig. 26). In case of copepod community, the CCA triplot of copepod species summarizes the influence of environmental attributes on their spatio-temporal species distribution (Fig. 27). The species association on the CCA ordination graph depicts a strong correlation with salinity, chlorophyll *a* ($p < 0.05$) and nitrate on the overall copepod species distribution (Table 29). Most of the copepod species as depicted in the figure 27 showed the close association of salinity. Distribution of copepod species such as *Acartiella keralensis*, *Acartia tropica*, *Acartiella gravelyi*, *Acartia sewellii*, *Pseudodiaptomus sewelli* and *Pseudodiaptomus jonesii* were found to be strongly influenced by chlorophyll *a*, dissolved oxygen and nitrate.

3b.2.9 Discussion

This chapter describe seasonal scale distribution and community composition of metazooplankton from a transect across the estuarine, coastal and open ocean sampling sites within the oxic mixed layer surface water column of the Arabian Sea. It is a small tropical/subtropical ocean basin experiencing regular fluctuations on an intra-annual scale between SWM and NEM (Madhupratap et al. 2001). This seasonal cycle is well known to affect the ecological pattern (including metazooplankton) in the estuarine and marine ecosystem of the Arabian Sea (Goswami, 1982; Madhupratp et al., 1992; Smith et al. 1998; Padmavati et al., 1998; Dalal et al., 2001). Seasonal variation of environmental conditions in Mandovi and Zuari estuaries is shown in earlier work of Qasim and Sengupta (1981). Likewise, seasonal variation of hydro-chemical parameters from the Arabian Sea is well described by Morrison et al. (1998). Additionally, spatio-temporal variation of environmental forcing impacting biological community of the Arabian Sea is reported in Campbell et al. (1998). Present study highlights significant variation of temperature with respect to Season transitions (FIM, SWM and NEM) whereas salinity and chlorophyll *a* were also the significant factors

for the contribution of observed spatially differences. From the observations on significant Seasonal variation of temperature indicates it is the favourable environmental factor in separating Seasonal variation in sampling sites, which may affects the biological community abundance. In the case of spatial distinction of environmental factors salinity and chlorophyll *a* were the possible reason for the distribution of site specific metazooplankton community and copepod species observed in estuarine, coastal and open ocean of the Arabian Sea.

nMDS (non-metric multidimensional scaling) plots followed by SIMPER analysis specifies the changing community pattern between FIM and NEM. It is well known that during SWM, coastal upwelling and Ekman pumping driven by the wind stress causes the high productivity along the south west coast of India (Madhupratap et al. 2001). Thus SWM supports high metazooplankton largely dominated by copepod abundance in coastal (outer shelf) and open ocean sites indicates the prominent seasonal variations in gross abundance compared to other seasons.

During present study, it was observed that the spatial variation has a greater affect on metazooplankton community, copepod species composition in particular than the seasonal variation. On a spatial scale, across all seasons, a prominent copepod species difference was observed with respect to varying patterns of ecological sites from estuaries to coastal and open ocean waters of the Arabian Sea. Copepods were the important contributors to the whole metazooplankton community underscoring that they are crucial for biodiversity studies because of their wide range of species. Further, spatial representation of copepod species revealed the habitat specific copepod species at different stations of the Arabian Sea. Variation of species patterns at estuarine, coastal and open ocean sampling sites provides a preliminary indication of considerable variability in species / group composition over this region. Copepod composition of

metazooplankton community also found to have environmental influence leading to spatio temporal variation across the studied region. Marked variation of salinity, temperature and chlorophyll *a* along the spatial scale determines the adaptations euryhaline and stenohaline copepod dominance that includes *Acartia tropica, Acrocalanus gibber, Acartia pacifica, Paracalanus aculeatus, Pseudodiaptomus jonesii, pseudodiaptomus sewelli, Acartiella keralensis* and *Acartiella gravelyi.*

The metazooplankton community can be broadly classified into herbivores, omnivores and carnivores or predators. Estuarine sites were mostly dominated by herbivores/omnivores metazooplankton groups including copepod species associated with low temperature and low salinity water compared to other coastal and open ocean sites with overall with high temperature and high salinity waters. The carnivorous forms such as hydromedusae, siphonophores, chaetognaths, appendicularia, gastropod larvae, and fish larvae showed their profound abundance with the increased salinity and temperature that prevailed in coastal and open ocean sites. Dominance of herbivores has been previously reported in the region during SWM (Madhupratap et al. 1992) associated with high primary productivity waters.

Herbivorous copepods, mostly calanoid copepods were commonly found in the study region (estuarine, coastal and open ocean sites). Omnivorous and carnivorous forms also prominently contributed to the metazooplankton assemblage. Estuarine sites mostly dominated herbivorous copepods as compared to coastal and outer shelf stations. In case of open ocean water of the Arabian Sea, appreciable numbers of carnivorous copepods were found. These carnivorous forms were composed of poecilostomatoida, and cyclopoida copepods are known to be important component of copepod community in the study site (Madhupratap et al. 1992; Padmavati et al. 1998). In the present study also, high abundance of poecilostomatoida copepods such as

Oncaea venusta, *Oncaea conifera*, *Oncaea media*, *Oncaea* sp. *Corycaeus* spp. and *Faranulla* spp. were recorded in the outer shelf and open ocean stations. Especially during NEM poecilostomatoida copepods such as *Oncaea conifera*, *Oncaea* sp., *Oncaea venusta* were highly abundant compared to other copepod species. This observation was quite similar with earlier observation made by Madhupratap et al. (1999) underlining that poicelostomatoida are ubiquitously found in coastal and offshore waters of the Arabian Sea. Under this scenario it may be predicted that these carnivorous copepods may switch over to feed on microzooplankton (commonly known as protozooplankton). Copepods are known to feed on wide range of autotrophic and heterotrophic protists (Yang et al., 2009). Metazooplankton (especially copepod) grazing could not only control protozooplankton but also can regulate phytoplankton population. However, it is important to note that metazooplankton are not the sole predators of protozoa. Carnivorous and omnivorous protozoans are known to prey on other planktonic protists as well (Dolan and Coats, 1991). Samuelsson et al. (2006), stated that ciliates are the dominant grazer of small heterotrophic flagellates. Also, ciliates and heterotrophic dinoflagellates were marked as a major predator of heterotrophic nano flagellates (Weisse, 1989, Weisse and Scheffel-Moser, 1991, Weisse, 1997). The prey and predator behaviour of metazooplankton and protozooplankton are playing a vital role in marine ecosystem functioning (Levinsen, 2000). Thus it is quite important to identify the different forms of protist communities as well as heterotrophic flagellates and ciliate communities associated in the different ecological sites of the Arabian Sea. Moreover, these protozooplanktonic communities are discussed with reference to varying oxygen strata associated in estuarine, coastal (inner shelf and outer shelf) and open ocean of Arabian Sea in the next chapter.

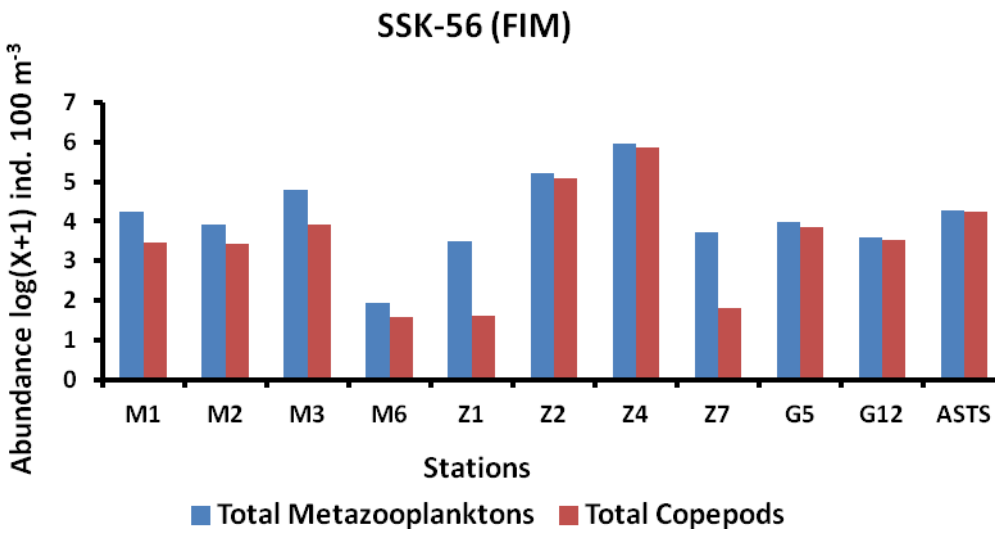


Figure 16. Representing the spatial variation of total metazooplankton and copepod abundance during FIM across different habitats from estuarine (M1, M2, M3, M6, Z1, Z2, Z4 and Z7), coastal: inner and outer continental shelf (G5, G12) and open ocean sites (ASTS). The abundance values were represented in the form of log (x+1) ind. 100 m^{-3}.

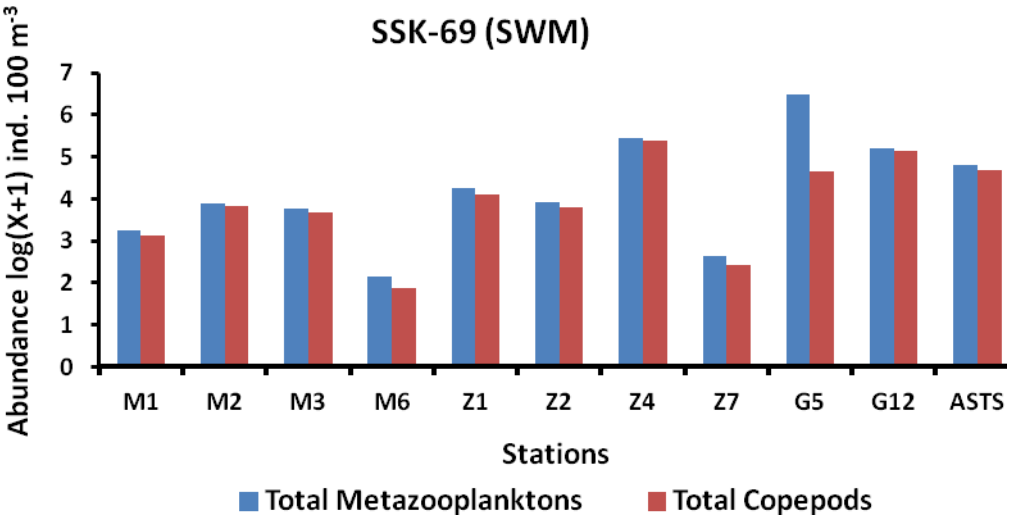


Figure 17. Representing the spatial variation of total metazooplankton and copepod abundance during SWM across different habitats from estuarine (M1, M2, M3, M6, Z1, Z2, Z4 and Z7), coastal: inner and outer continental shelf (G5, G12) and open ocean sites (ASTS). The abundance values were represented in the form of log (x+1) ind. 100 m^{-3}.

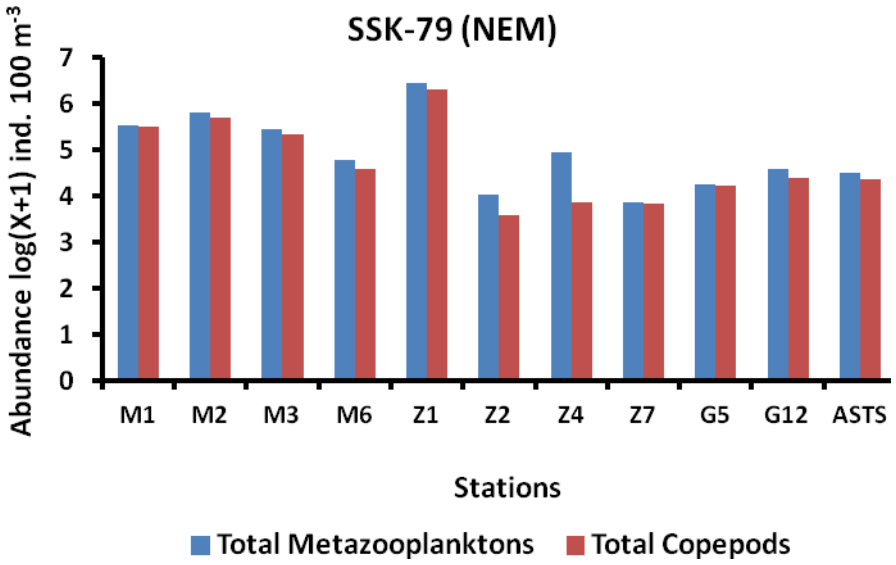


Figure 18. Representing the spatial variation of total metazooplankton and copepod abundance during NEM across different habitats from estuarine (M1, M2, M3, M6, Z1, Z2, Z4 and Z7), coastal: inner and outer continental shelf (G5, G12) and open ocean sites (ASTS). The abundance values were represented in the form of log (x+1) ind. 100 m^{-3}.

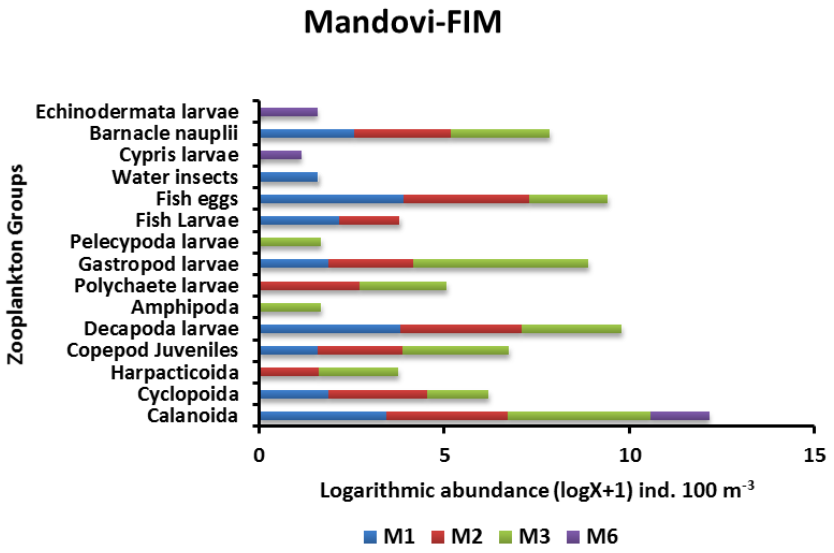


Figure 19 a. Variation of metazooplankton community composition in spatial sites of Mandovi estuary (M1, M2, M3, and M6) during the seasonal period of FIM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

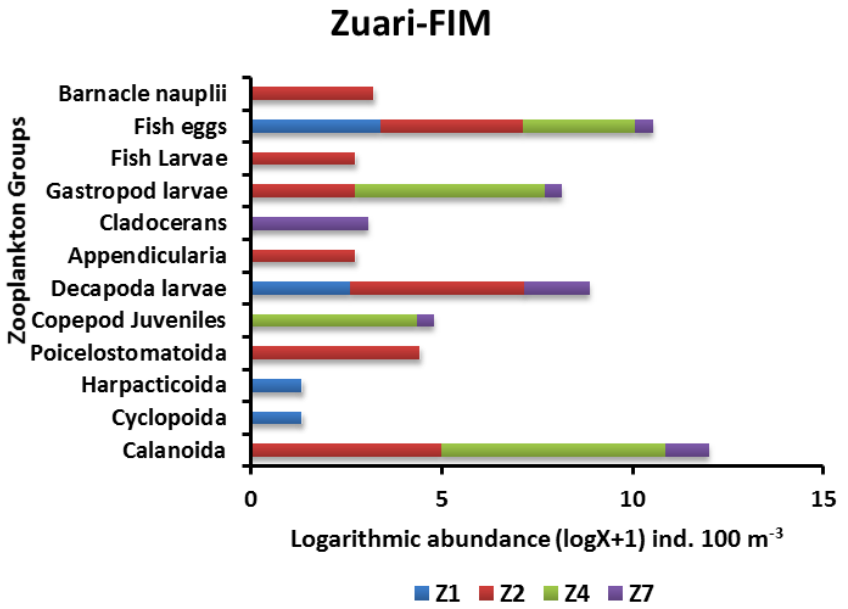


Figure 19 b. Variation of metazooplankton community composition in spatial sites of Zuari estuary (Z1, Z2, Z4, and Z7) during the seasonal period of FIM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

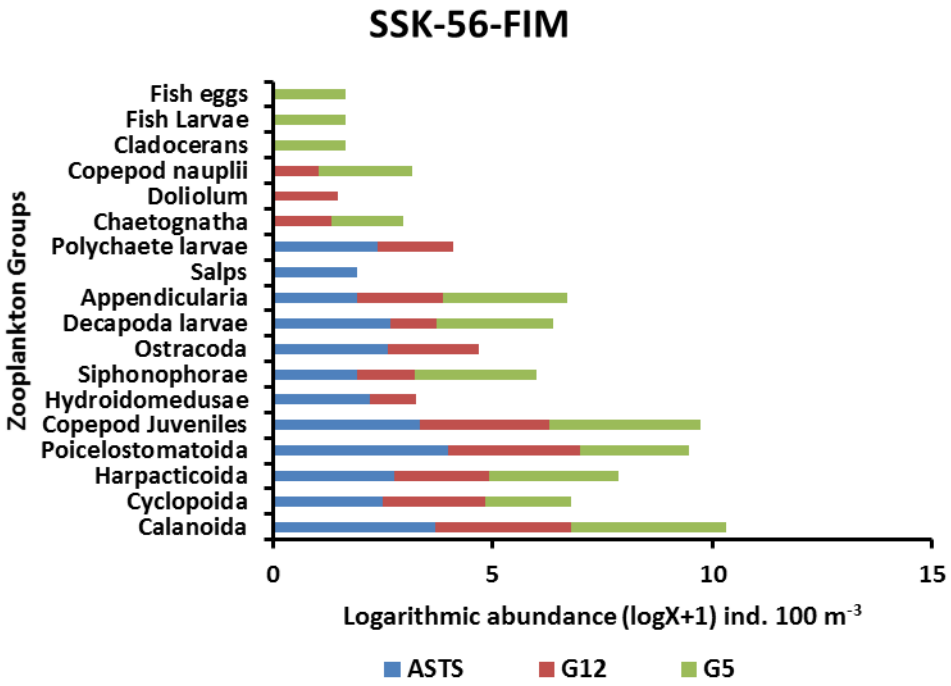


Figure 19 c. Variation of metazooplankton community composition in spatial sites of coastal (G5: inner continental shelf, G12: outer continental shelf) and open ocean waters (ASTS) during the seasonal period of FIM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

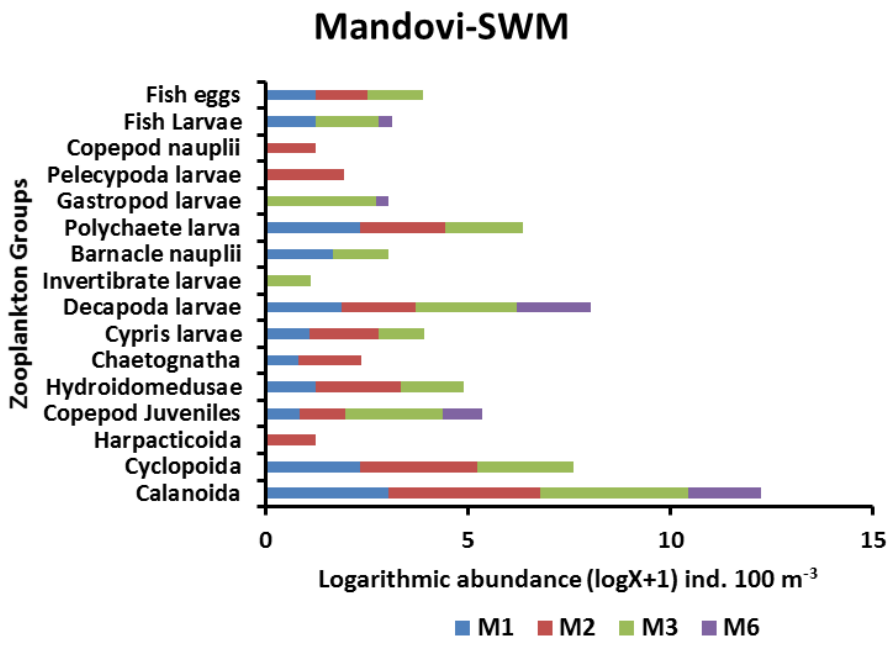


Figure 20 a. Variation of metazooplankton community composition in spatial sites of Mandovi estuary (M1, M2, M3, and M6) during the seasonal period of SWM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

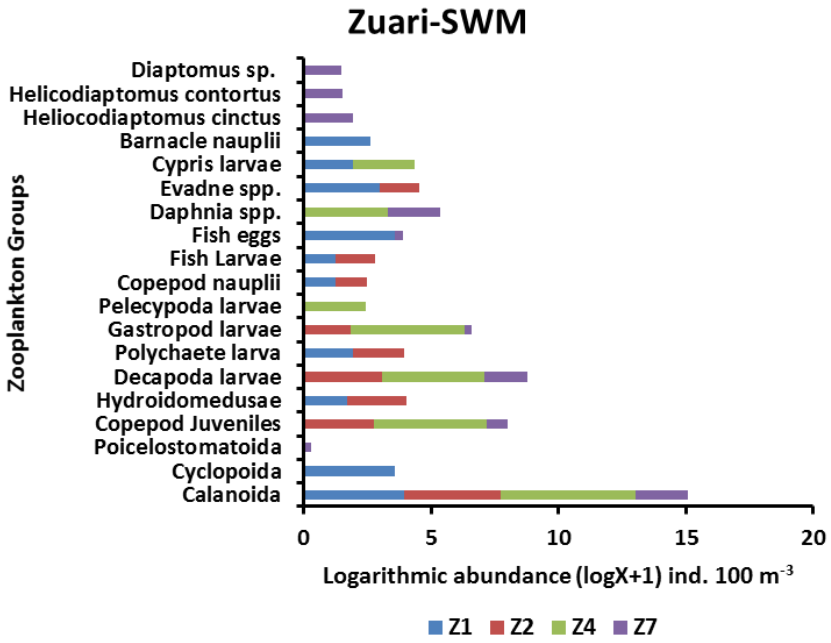


Figure 20 b. Variation of metazooplankton community composition in spatial sites of Zuari estuary (Z1, Z2, Z4, and Z7) during the seasonal period of SWM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

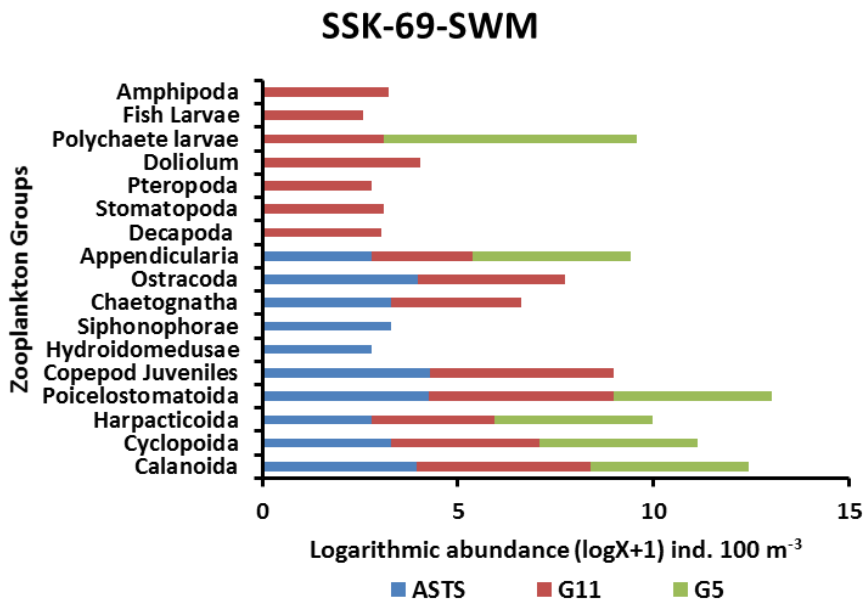


Figure 20 c. Variation of metazooplankton community composition in spatial sites of coastal (G5: inner continental shelf, G12: outer continental shelf) and open ocean waters (ASTS) during the seasonal period of SWM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

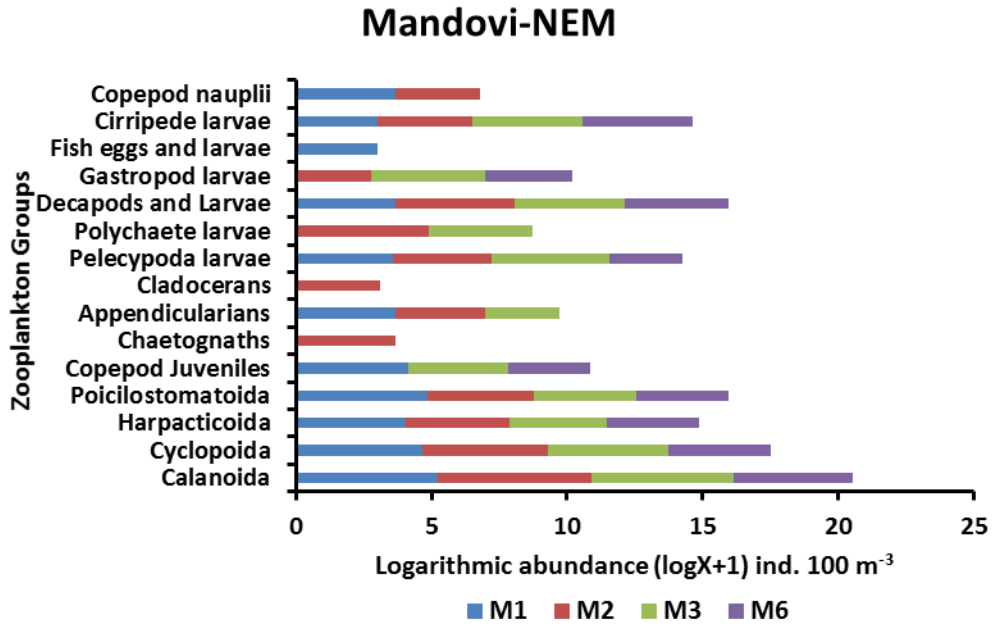


Figure 21 a. Variation of metazooplankton community composition in spatial sites of Mandovi estuary (M1, M2, M3, and M6) during the seasonal period of NEM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

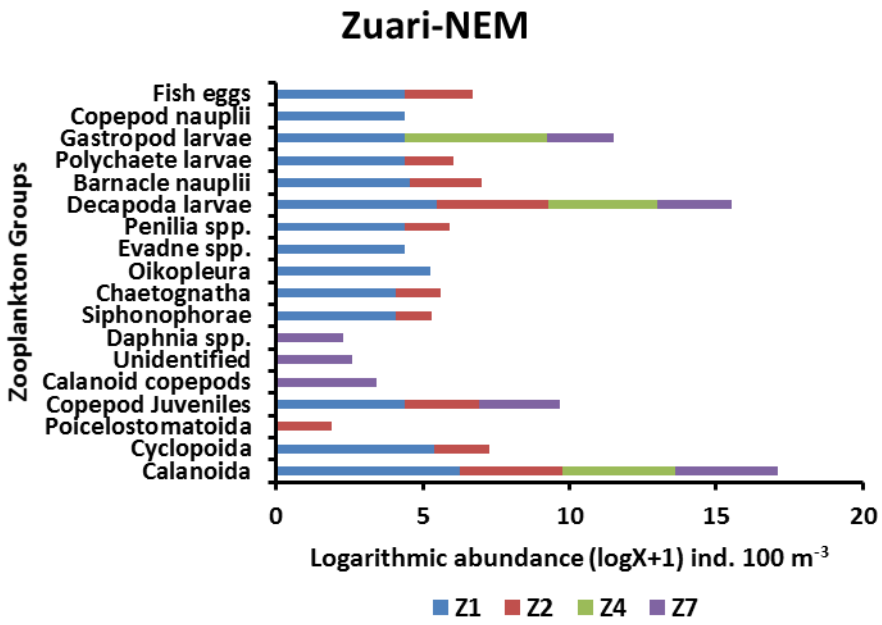


Figure 21 b. Variation of metazooplankton community composition in spatial sites of Zuari estuary (Z1, Z2, Z4, and Z7) during the seasonal period of NEM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

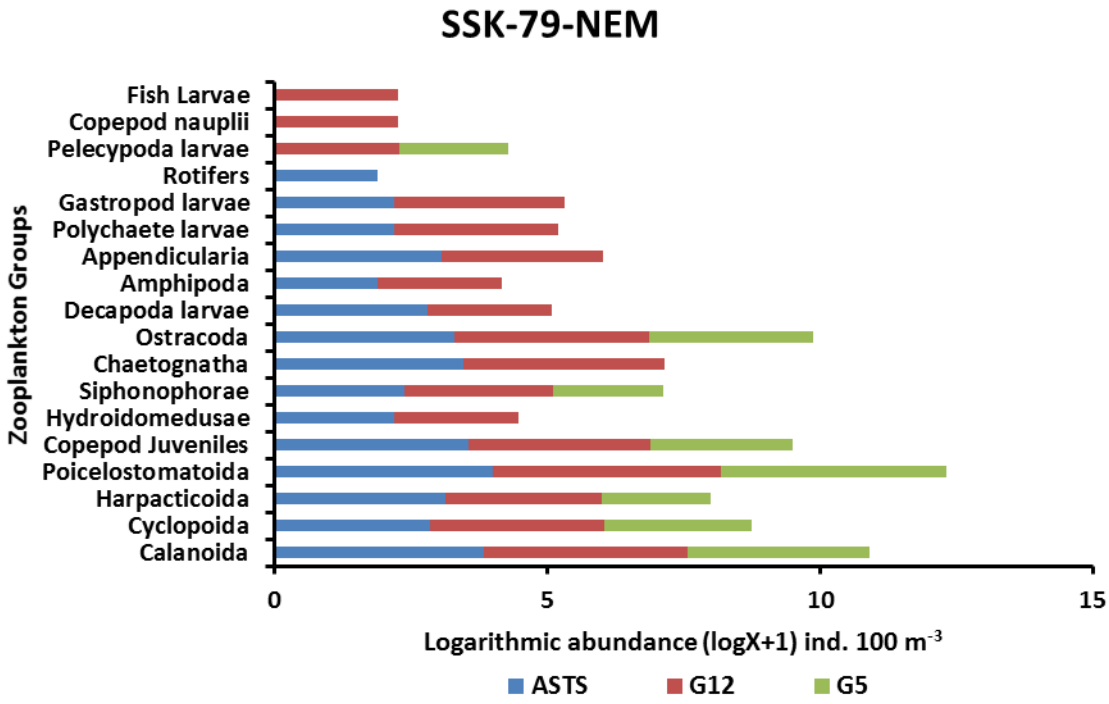


Figure 21 c. Variation of metazooplankton community composition in spatial sites of coastal (G5: inner continental shelf, G12: outer continental shelf) and open ocean waters (ASTS) during the seasonal period of NEM. Metazooplankton group abundance values were represented in the value of log (x+1) ind. $100m^{-3}$.

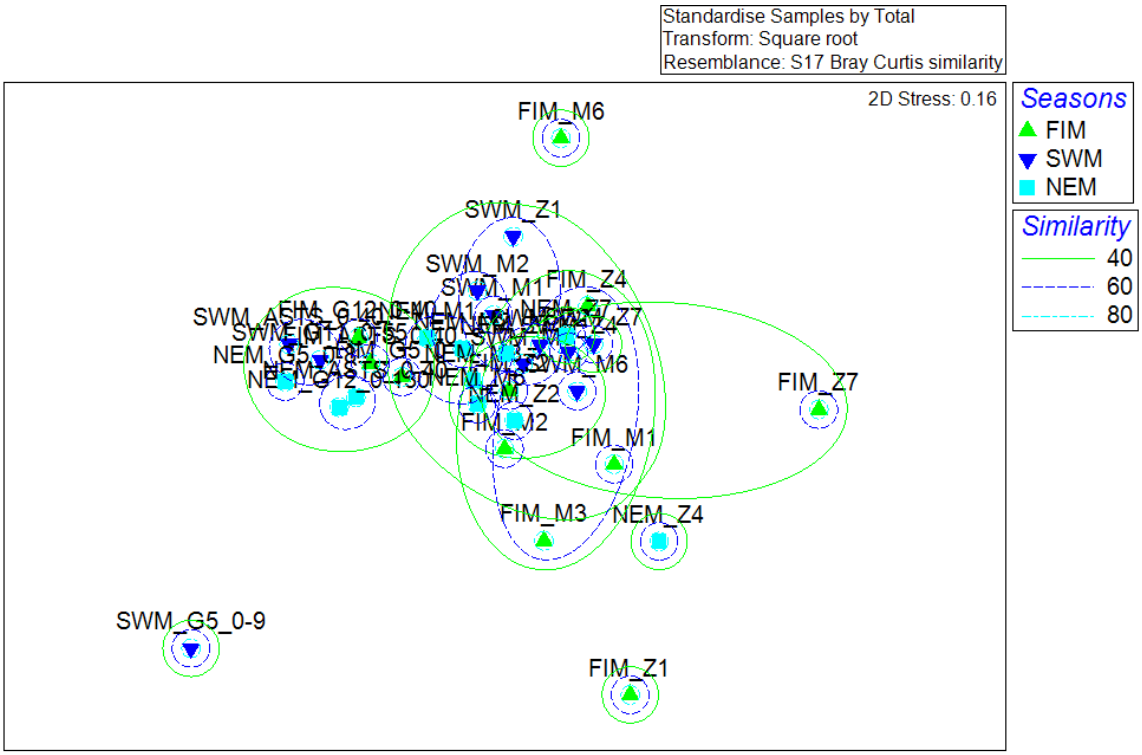


Figure 22. nMDS plots based on seasonal variation of metazooplankton group abundance in different sampling sites estuarine (M1, M2, M3, M6, Z1, Z2, Z4 and Z7), coastal (G5: inner continental shelf; G12: outer continental shelf) and open ocean sites (ASTS).

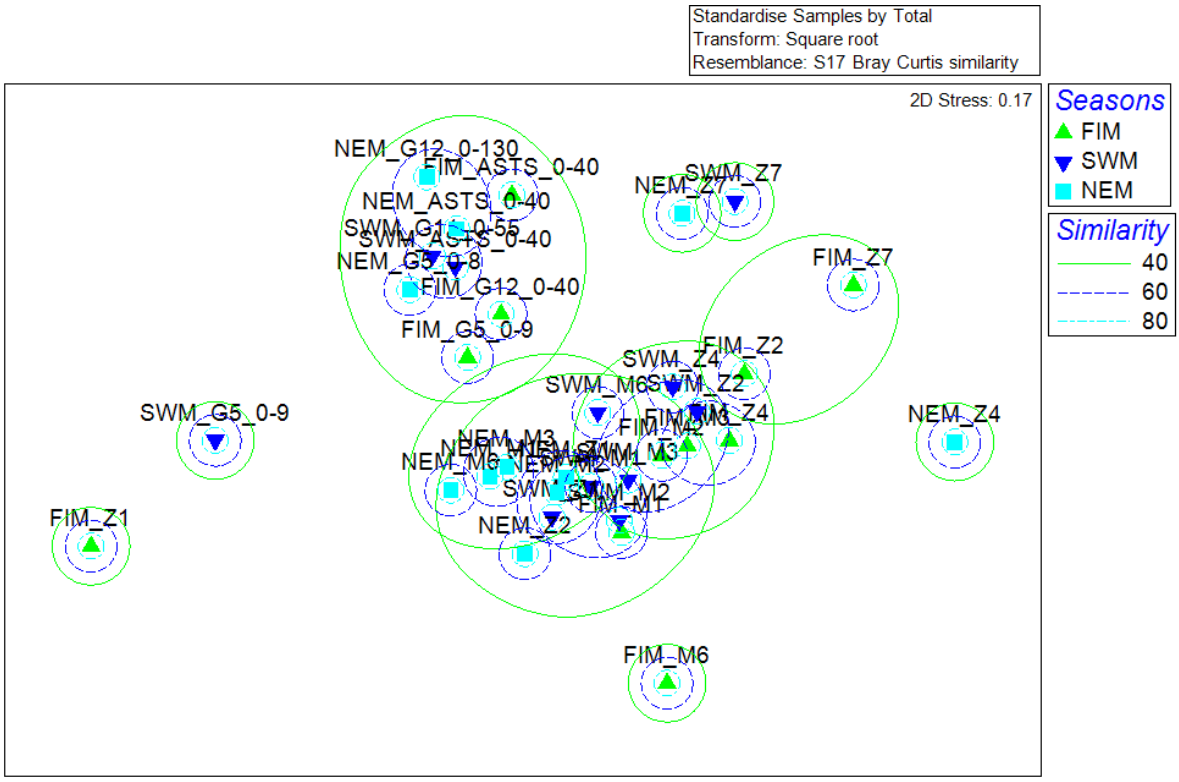


Figure 23. nMDS plots based on seasonal variation of copepod abundance in different sampling sites estuarine (M1, M2, M3, M6, Z1, Z2, Z4 and Z7), coastal (G5: inner continental shelf; G12: outer continental shelf) and open ocean sites (ASTS).

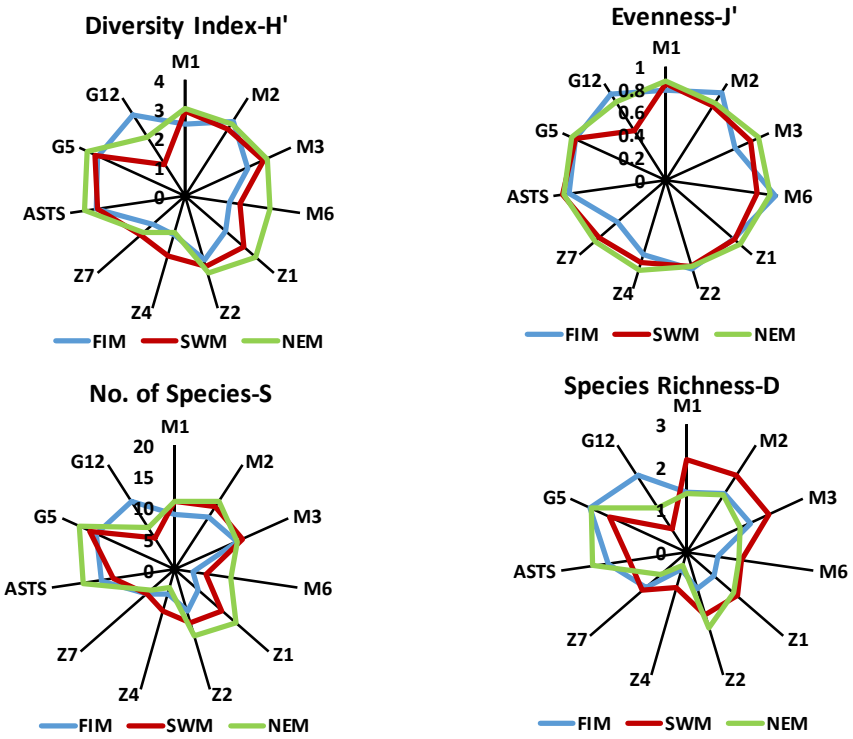


Figure 24. Seasonal and spatial variation of diversity index (H´), number of species (S), species richness (d) and evenness (J) for metazooplankton community in different habitats of estuarine (M1, M2, M3, M6, Z1, Z2, Z4 and Z7), coastal (G5: inner continental shelf; G12: outer continental shelf) and open ocean sites (ASTS).

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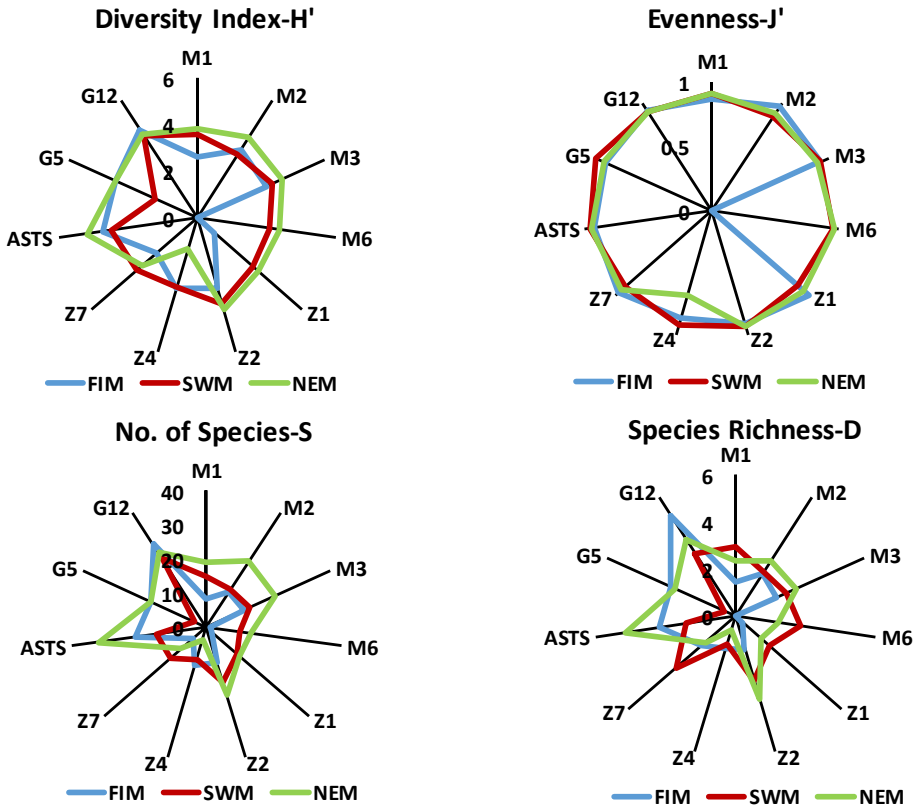

Figure 25. Seasonal and spatial variation of diversity index (H´), number of species (S), species richness (d) and evenness (J) for copepod community in different habitats of estuarine (M1, M2, M3, M6, Z1, Z2, Z4 and Z7), coastal (G5: inner continental shelf; G12: outer continental shelf) and open ocean sites (ASTS).

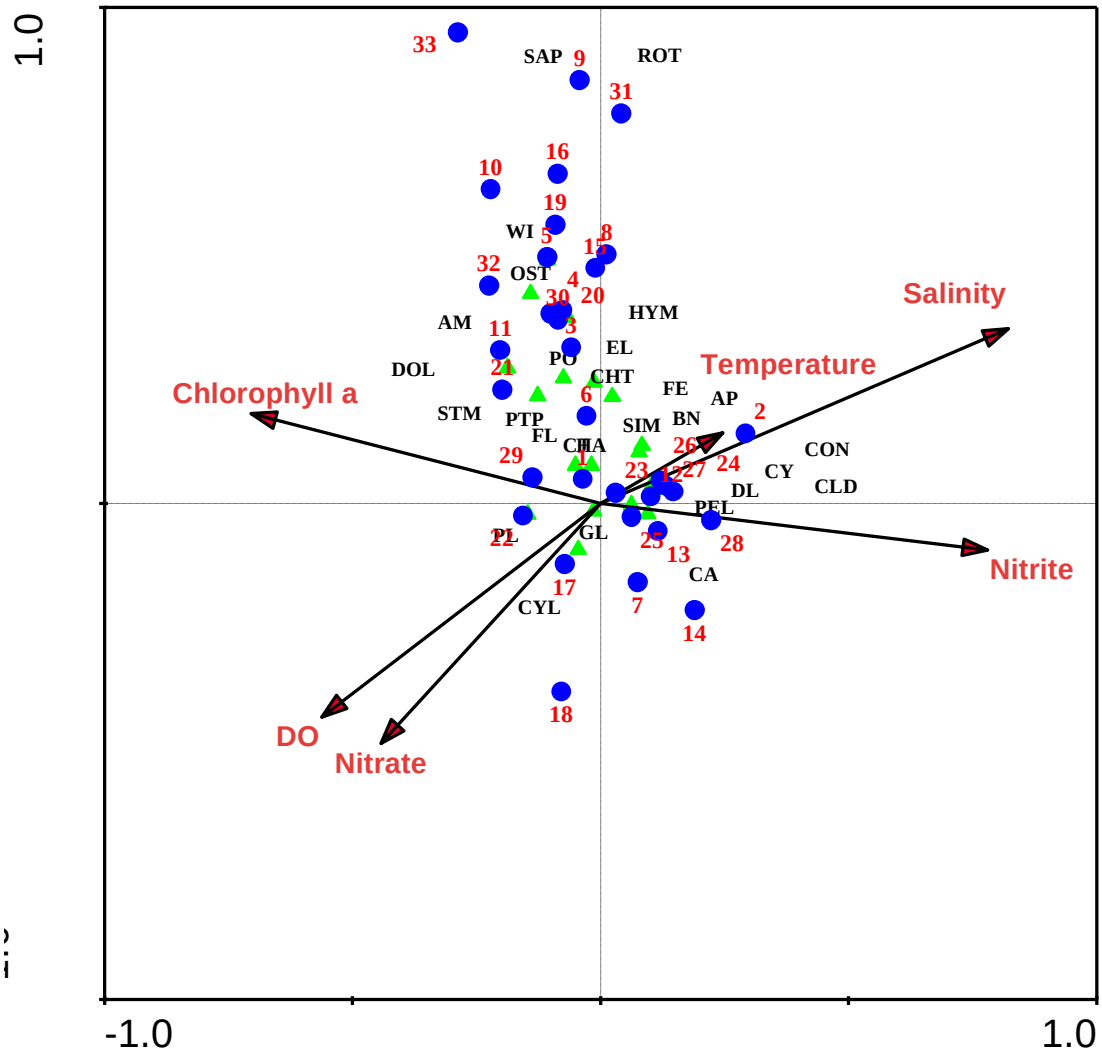

Figure 26. CCA triplot depicting environmental relationship of metazooplankton groups in different sampling sites of estuarine, coastal and open ocean stations corresponding to different seasonal periods.

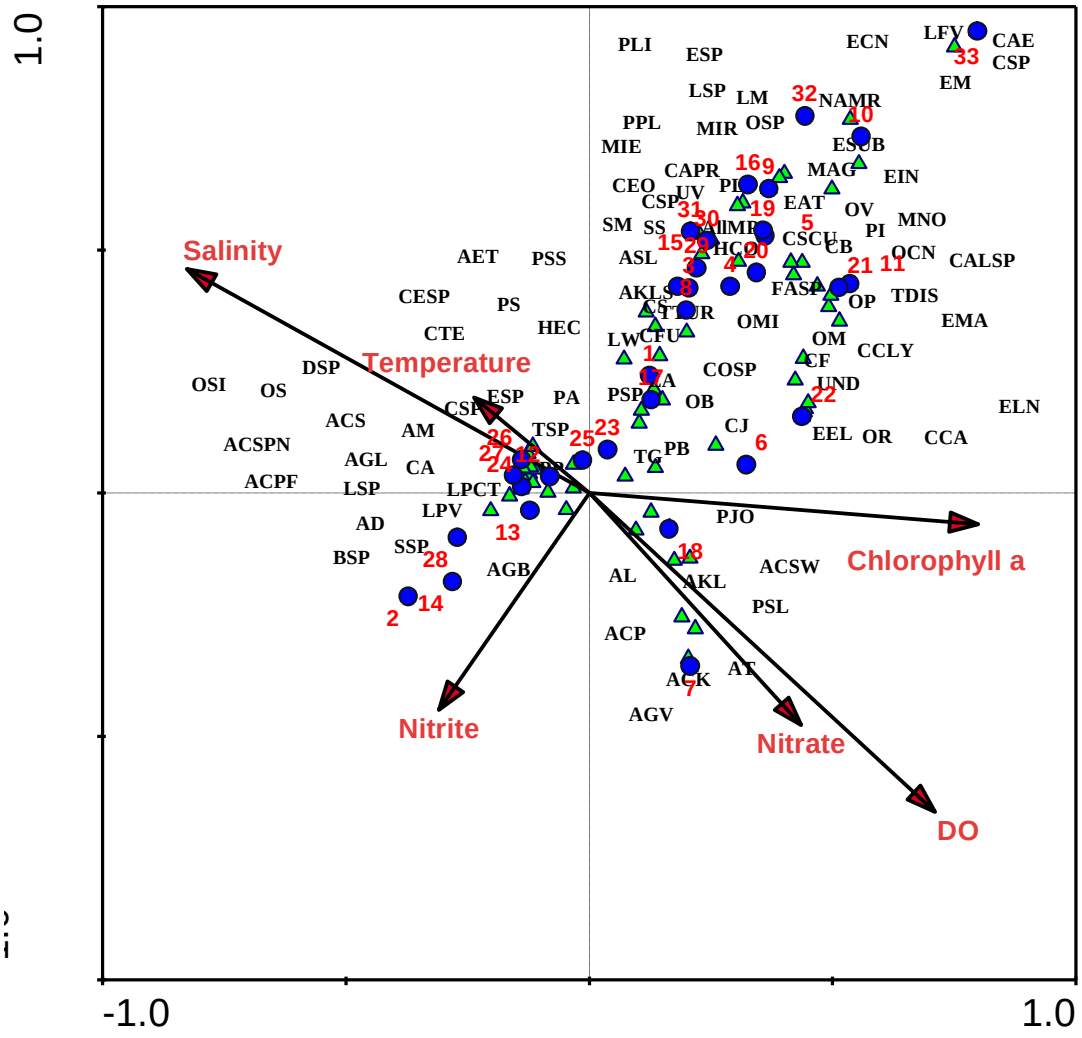

Figure 27. CCA triplot depicting environmental relationship of copepod abundance in different sampling sites of estuarine, coastal (inner and outer continental shelf) and open ocean stations corresponding to different seasonal periods.

Table 18. Detail analysis of environmental parameters at estuarine, coastal (inner and outer continental shelf) and open ocean waters of the Arabian Sea during the period of FIM (SSK56-2013).

SSK-56	Temperature (°C)	Salinity (PSU)	DO (µM)	Nitrate (µM)	Nitrite (µM)	Chl *a*(µg l-1)
ASTS(0-40)	28.45(±0.57)	36.87(±0.04)	203.64(±2.23)	0.28(±0.39)	BD	0.05(±0.08)
G12(0-40)	26.48(±3.78)	33.57(±1.05)	137(±85.74)	9.97(±14.73)	0.19(±0.37)	0.63(±0.15)
G5(0-9)	25.64(±3.82)	34.61(±1.13)	91.99 (±130.8)	BD	0.05(±0.03)	3.76(±1.72)
M1	27.43	27.93	161.12	1.41	0.03	3.60
M2	27.23	30.38	156.06	0.38	BD	4.19
M3	28.73	16.33	308.20	0.08	3.61	2.42
M6	28.87	0.08	325.58	0.41	4.19	3.46
Z1	28.93	26.10	158.15	1.63	0.23	4.68
Z2	28.76	27.40	145.74	0.79	0.19	6.81
Z4	30.58	4.91	180.87	3.68	0.02	5.70
Z7	29.88	0.04	246.08	4.41	0.01	4.22

Table 19. Detail analysis of environmental parameters at estuarine, coastal (inner and outer continental shelf) and open ocean waters of the Arabian Sea during the period of SWM (SSK69-2014).

SSK-69	Temperature (°C)	Salinity (PSU)	DO (µM)	Nitrate (µM)	Nitrite (µM)	Chl *a* (µg l-1)
ASTS(0-40)	28.40(±1.31)	36.94(±0.06)	185.78(±13.39)	1.96(±2.43)	0.17(±0.24)	0.25(±0.11)
G11(0-55)	27.11(±2.08)	35.66(±1.14)	160.77(±58.05)	2.89(±4.97)	0.16(±0.27)	0.35(±0.15)
G5(0-9)	25.71(±1.45)	35.17(±1.12)	85.74(±103.16)	2.22(±2.97)	0.34(±0.48)	1.19(±0.76)
M1	25.48	34.32	77.80	0.82	0.12	5.01
M2	25.16	34.20	61.22	0.14	0.04	6.18
M3	27.78	15.52	130.11	2.68	0.12	1.90
M6	28.47	0.05	225.14	6.16	0.08	0.58
Z1	27.31	31.70	154.64	0.17	0.03	4.59
Z2	28.79	18.63	163.79	1.05	0.14	4.59
Z4	29.56	2.02	211.81	1.78	0.12	4.84
Z7	28.99	0.03	264.12	3.53	0.08	3.06

Table 20. Detail analysis of environmental parameters at estuarine, coastal (inner and outer continental shelf) and open ocean waters of the Arabian Sea during the period of NEM (SSK 79 2015).

SSK-79	**Temperature (°C)**	**Salinity (PSU)**	**DO (µM)**	**Nitrate (µM)**	**Nitrite (µM)**	**Chl *a*(µg l-1)**
ASTS(0-40)	27.31(±0.002)	36.23(±0.01)	200.97(±0.13)	2.25(±3.16)	0.02(±0.01)	0.1(±0.02)
G12(0-130)	27.01(±2.46)	36.06(±0.54)	154.97(±63.86)	4.92(±7.30)	0.08(±0.13)	0.16(±0.15)
G5(0-9)	28.78(±0.05)	34.79(±0.003)	189.8 (±6.25)	0.22(±0.06)	0.21(±0.26)	0.645(±0.01)
M1	30.57	34.19	171.26	0.83	0.35	0.81
M2	30.48	34.12	172.05	0.92	0.37	0.86
M3	31.08	31.73	162.75	1.22	0.53	1.17
M6	32.37	16.42	183.62	0.89	0.12	1.08
Z1	30.53	32.45	195.23	0.44	0.02	1.66
Z2	31.51	29.81	172.63	0.60	0.33	5.48
Z4	32.38	17.60	181.00	0.10	0.02	3.07
Z7	32.69	0.13	228.63	1.46	0.09	7.98

Table 21. Spearman's correlation analysis results overall correlation between environmental factors and biological communities (metazooplankton and copepods) in varying sampling sites corresponding to different seasonal periods.

			Temperature	**Salinity**	**DO**	**Nitrate**	**Nitrite**	**Chl *a***	**Copepods**	**Total Zoo**
Spearman's rho	Temperature	Correlation Coefficient	1.000	-.536**	.591**	-.037	.151	.184	.204	.236
		Sig (2-tailed)		.001	.000	-.838	.402	.304	.256	.186
		N	33	33	33	33	33	33	33	33
	Salinity	Correlation Coefficient	-.536**	1.000	-.457**	-.325	.088	-.501**	.410*	.349*
		Sig (2-tailed)	.001		.008	.065	.627	.003	.018	.046
		N	33	33	33	33	33	33	33	33
	DO	Correlation Coefficient	.591**	-.457**	1.000	.310	-.142	-.171	-.042	-.049
		Sig (2-tailed)	.000	.008		.079	.430	.342	.816	.786
		N	33	33	33	33	33	33	33	33
	Nitrate	Correlation Coefficient	-.037	-.325	.310	1.000	.133	-.267	-.175	-.185
		Sig (2-tailed)	.838	.065	.079		.459	.134	.331	.303
		N	33	33	33	33	33	33	33	33
	Nitrite	Correlation Coefficient	.151	.088	-.142	.133	1.000	-.081	.117	.082
		Sig (2-tailed)	.402	.627	.430	.459		.654	.516	.651
		N	33	33	33	33	33	33	33	33
	Chl *a*	Correlation Coefficient	.184	-.501**	-.171	-.267	-.081	1.000	-.260	-.197
		Sig (2-tailed)	.304	.003	.342	.134	.654		.144	.273
		N	33	33	33	33	33	33	33	33
	Copepods	Correlation Coefficient	.204	.410*	-.042	-.175	.117	-.260	1.000	.925**
		Sig (2-tailed)	.256	.018	.816	.331	.516	.144		.000
		N	33	33	33	33	33	33	33	33
	Total Zoo	Correlation Coefficient	.236	.349*	-.049	-.185	.082	-.197	.925**	1.000
		Sig (2-tailed)	.186	.046	.786	.303	.651	.273	.000	
		N	33	33	33	33	33	33	33	33

Table 22a. Onaway ANOVA result showing the variation of environmental parameters between seasons of different estuarine, coastal (inner and outer continental shelf) and open ocean sampling sites. F statistic and probability (p).

		Sum of Squares	df	Mean Square	F	Sig.(p)
Temperature	Between Groups	50.045	2	25.022	9.103	.001
	Within Groups	82.464	30	2.749		
	Total	132.509	32			
Salinity	Between Groups	236.953	2	118.477	0.629	.540
	Within Groups	5654.397	30	188.48		
	Total	5891.35	32			
DO	Between Groups	7588.362	2	3794.181	1.155	.329
	Within Groups	98513.144	30	3283.771		
	Total	106101.506	32			
Nitrate	Between Groups	12.467	2	6.234	1.406	.261
	Within Groups	133.032	30	4.434		
	Total	145.499	32			
Nitrite	Between Groups	.044	2	0.022	1.211	.312
	Within Groups	.549	30	0.018		
	Total	.593	32			
Chl *a*	Between Groups	12.478	2	6.239	1.256	.299
	Within Groups	149.060	30	4.969		
	Total	161.538	32			

Table 22b. Onaway ANOVA result showing the variation of environmental parameters between different estuarine, coastal (inner and outer continental shelf) and open ocean sampling sites. F statistic and probability (p).

		Sum of Squares	df	Mean Square	F	Sig (p)
Temperature	Between Groups	60.765	10	6.076	1.863	.108
	Within Groups	71.744	22	3.261		
	Total	132.509	32			
Salinity	Between Groups	5275.129	10	527.513	18.833	.000
	Within Groups	616.221	22	28.010		
	Total	5891.350	32			
DO	Between Groups	55072.971	10	5507.297	2.374	.044
	Within Groups	51028.535	22	2319.479		
	Total	106101.506	32			
Nitrate	Between Groups	83.741	10	8.374	2.983	.016
	Within Groups	61.759	22	2.807		
	Total	145.499	32			
Nitrite	Between Groups	.143	10	.014	.699	.716
	Within Groups	.450	22	.020		
	Total	.593	32			
Chl *a*	Between Groups	101.568	10	10.157	3.726	.005
	Within Groups	59.970	22	2.726		
	Total	161.538	32			

Table 23. List of Copepod species in Mandovi –Zuari estuary, coastal (inner and outer continental shelf) and open ocean waters of the Arabian Sea during the period of FIM (SSK-56) 2013.

SSK56	**Mandovi_copepod(FIM)**				**Zuari_Copepod(FIM)**				**Cruise_Copepod (FIM)**		
	M1	**M2**	**M3**	**M6**	**Z1**	**Z2**	**Z4**	**Z7**	**G5_0-9**	**G12_0-40**	**ASTS_0-40**
Copepod species	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Acrocalanus sp.	-	-	-	-	-	-	-	-	+++	-	-
Acrocalanus gibber	++++	++++	++++	++++	-	++	++++	-	++	+++	-
Acrocalanus gracilis	++++	++	++++	-	-	++	+	-	+	++	++
Acrocalanus longicornis	-	-	++++	-	-	-	++++	-	+	+	-
Paracalanus aculeatus	+++	++++	+	-	-	++++	+	-	++	+++	+++
Paracalanus parvus	++++	++++	++	-	-	+++	++	-	+	++	+
Clausocalanus arcuicornis	++	++	-	-	-	-	-	-	+	++	-
Oithona sp.	-	+++	-	-	-	-	-	-	-	-	++
Oithona similis	++	+++	-	-	-	-	-	-	-	-	-
Oithona brevicornis	++	++	+	-	-	-	-	-	-	-	-
Oithona rigida	-	-	-	-	++++	-	-	-	-	-	-
Copepod juveniles	++	+++	+++	-	-	++++	++	++++	++++	++++	++++
Longipedia weberi	-	++	++	-	-	-	-	-	-	-	-
Pseudodiaptomus sewelli	-	-	++	-	-	+	+	-	-	-	-
Pseudodiaptomus bowmini	-	-	-	-	-	++++	-	++++	-	-	-
Acartiella Keralensis	-	-	++	-	-	-	++	-	-	-	-
Acartia chilkeinsis	-	++	++	-	-	-	++	++++	-	-	-
Acartia tropica	-	++++	++++	-	-	++++	++++	++++	-	-	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Continued …

Euterpina acutifrons	-	-	-	-	++++	-	-	-	++++	+	-
Acartia sp.	-	-	-	-	-	++++	++++	++++	-	-	-
Acartia pacifica	-	-	-	-	-	+	-	-	-	-	-
Tortanus gracilis	-	-	-	-	-	+	-	-	-	-	-
Acartiella gravelyi	-	-	-	-	-	-	+	-	-	-	-
Euchaeta spp.	-	-	-	-	-	-	-	-	-	-	++
Euchaeta indica	-	-	-	-	-	-	-	-	-	-	++
Euchaeta marina	-	-	-	-	-	-	-	-	-	-	+
Lucicutia spp.	-	-	-	-	-	-	-	-	-	-	+
Lebidocera Madurae	-	-	-	-	-	-	-	-	-	-	+
pontellina plumata	-	-	-	-	-	-	-	-	-	-	+
Eucalanus attenuatus	-	-	-	-	-	-	-	-	-	-	+
Pleuromamma indica	-	-	-	-	-	-	-	-	-	-	++
Pleuromamma spp.	-	-	-	-	-	-	-	-	-	-	++
Onceae sp.	-	-	-	-	-	-	-	-	+	++++	++++
Onceae venusta	-	-	-	-	-	-	-	-	++	++++	++
Onceae media	-	-	-	-	-	-	-	-	+	++	-
Coryceaus sp.	-	-	-	-	-	-	-	-	+	+++	+++
Faranulla sp.	-	-	-	-	-	-	-	-	-	+	++
Macrosettella gracillis	-	-	-	-	-	-	-	-	-	++	++
Miracia efferata	-	-	-	-	-	-	-	-	-	-	+
Paracalanus sp.	-	-	-	-	-	-	-	-	+++	++	-
Paracalanus indicus	-	-	-	-	-	-	-	-	-	++	-
Acrocalanus monachus	-	-	-	-	-	-	-	-	-	+++	-
Acartia danae	-	-	-	-	-	-	-	-	-	+	-
Candacia bradyi	-	-	-	-	-	-	-	-	-	+	-
Calanopia sp.	-	-	-	-	-	-	-	-	-	+	-
Centropage furcatus	-	-	-	-	-	-	-	-	+++	+	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Continued …

Eucalanus subcrassus	-	-	-	-	-	-	-	-	+	+	-
Subeucalanus mucronatus	-	-	-	-	-	-	-	-	-	+	-
Eucalanus sp.	-	-	-	-	-	-	-	-	-	+	-
Temora turbionata	-	-	-	-	-	-	-	-	++++	+	-
Temora discaudata	-	-	-	-	-	-	-	-	-	++	-
Oithona plumifera	-	-	-	-	-	-	-	-	++	+	-
Miccrosettela norvegica	-	-	-	-	-	-	-	-	-	+	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Table 24. List of Copepod species in Mandovi –Zuari estuary, coastal (inner and outer continental shelf) and open ocean waters of the Arabian Sea during the period of SWM (SSK-69) 2014.

SSK 69	Mandovi_Copepod_SWM				Zuari_Copepod_SWM				Cruise_Copepod_SWM		
	M1	M2	M3	M6	Z1	Z2	Z4	Z7	G5_0-9	G11_0-55	ASTS_0-40
Copepod species	%	%	%	%	%	%	%	%	%	%	%
Acrocalanus sp.	++	++	-	++++	++++	-	-	++	-	-	++
Acrocalanus gibber	++	++++	++++	++++	++++	++++	++++	++	-	-	++
Acrocalanus gracilis	++++	++++	++++	++	++++	++	++	+	-	+	-
Acrocalanus longicornis	-	-	+	-	++	++	++++	-	++++	++	++
Paracalanus sp.	++++	++	-	++++	++	-	-	-	-	+	-
Paracalanus aculeatus	+++	-	++	-	-	++	-	-	-	-	-
Paracalanus parvus	++	+	++	-	++	+++	++	+	-	++	-
Acartiella sp.	-	-	-	++	-	-	-	-	-	-	-
Acartia dannae	+	+	++	-	-	-	-	-	-	-	-
Acartia sp.	+	++	-	++	-	++	+++	++	-	-	-
Acartia pacifica	++	++	+++	++	+	++++	-	-	-	-	-
Acartia spinicauda	+	-	++	++	-	++	-	-	-	-	-
Acartia tropica	++	+++	++	-	-	++	-	-	-	-	-
Acartia sewelli	-	-	-	-	-	-	++	++++	-	-	-
Temora discaudata	+	-	-	-	-	-	-	-	-	-	-
Oithona sp.	++++	++	++	-	++++	-	-	-	-	+	++
Oithona brevicornis	++	-	-	-	-	-	-	-	++++	-	-
Oithona similis	-	+++	-	-	++	-	-	-	-	-	-
Oithona rigida	-	-	-	-	-	-	-	-	-	++	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Continued …

Oithona plumifera	-	-	-	-	-	-	-	-	-	+	-
Euterpina acutifrons	-	+	++	-	-	-	-	-	++++	+	-
Copepod juveniles	+	+	++	++++	-	+++	++++	++	-	++++	++++
Pseudodiaptomus sp.	-	-	-	+++	-	+	-	-	-	-	-
Pseudodiaptomus jonesii	-	-	++	-	+	+	++	++	-	-	-
Pseudodiaptomus sewelli	-	-	++	-	-	+++	-	-	-	-	-
Pseudodiaptomus bowmini	-	-	-	-	-	++++	+	+	-	-	-
Clausocalanus arcuicornis	-	-	-	-	+	++	-	-	-	++	++
Centropage furcatus	-	-	-	-	+	-	-	-	-	-	-
Acartiella Keralensis	-	-	-	-	-	++	++++	++++	-	-	-
Lebidocera sp.	-	-	-	-	-	+	-	-	-	-	-
Onceae sp.	-	-	-	-	-	-	-	+	-	-	++
Onceae venusta	-	-	-	-	-	-	-	-	-	++	+++
Onceae conifera	-	-	-	-	-	-	-	-	++++	+++	+++
Onceae media	-	-	-	-	-	-	-	-	-	++++	+++
Onceae minuta	-	-	-	-	-	-	-	-	-	-	++
Heliocodiaptomus cinctus	-	-	-	-	-	-	-	++++	-	-	-
Helicodiaptomus contortus	-	-	-	-	-	-	-	++++	-	-	-
Diaptomus sp.	-	-	-	-	-	-	-	++++	-	-	-
Euchaeta indica	-	-	-	-	-	-	-	-	-	++	++
Clytemnstra scutellata	-	-	-	-	-	-	-	-	-	-	++
Corycaeus sp.	-	-	-	-	-	-	-	-	-	+++	+++
Faranulla sp.	-	-	-	-	-	-	-	-	-	++	++

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Continued …

Euchaeta longicornis	-	-	-	-	-	-	-	-	-	+	-
Euchaeta marina	-	-	-	-	-	-	-	-	-	+	-
Clausocalanus furcatus	-	-	-	-	-	-	-	-	-	++	-
Calanoides carinatus	-	-	-	-	-	-	-	-	-	+	-
Undinula Darwini	-	-	-	-	-	-	-	-	-	+	-
Eucalanus elongatus	-	-	-	-	-	-	-	-	-	+	-
Clytemnstridae	-	-	-	-	-	-	-	-	-	+	-
Macrosettella gracillis	-	-	-	-	-	-	-	-	-	+	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Table 25. List of Copepod species in Mandovi –Zuari estuary, coastal (inner and outer continental shelf) and open ocean waters of the Arabian Sea during the period of NEM (SSK-79) 2015.

SSK 79	**Mandovi_copepod(NEM)**				**Zuari_Copepod(NEM)**				**Cruise_Copepod (NEM)**		
	M1	**M2**	**M3**	**M6**	**Z1**	**Z2**	**Z4**	**Z7**	**G5_0-8**	**G12_0-130**	**ASTS_0-40**
Copepod species	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Acrocalanus sp.	++	++	+++	++++	+++	+++	-	++++	++	-	++
Acrocalanus gibber	++++	++++	++++	++++	++++	++++	-	-	++	-	++
Acrocalanus monachus	++	-	++	-	++	++	-	-	-	-	-
Acrocalanus gracilis	++++	+++	++	++	++++	++++	+	-	+	+	++
Acrocalanus longicornis	++	-	++++	-	++	-	-	-	++	-	-
Paracalanus sp.	++	++	+++	++++	++	++	-	++++	+	-	-
Paracalanus aculeatus	-	+++	++	-	++++	+	-	-	+	-	+
Paracalanus parvus	+++	++++	++	-	++	++	-	++++	-	-	-
Pseudodiaptomus bowmini	-	+	-	-	+	-	-	-	-	-	-
Pseudodiaptomus jonesii	+	-	-	-	-	++	-	-	-	-	-
Pseudodiaptomus serricaudatus	++	+	+	-	-	+	-	-	-	-	-
Pseudodiaptomus sewelli	-	-	-	-	-	-	+++	-	-	-	-
Acartia sp.	+	++	+	++	-	+	-	++	-	-	-
Acartia danae	-	+	-	-	-	-	-	-	-	-	-
Acartia erythraea	-	-	+	-	-	-	-	-	-	-	-
Acartia pacifica	-	++	+	-	-	-	-	-	-	-	-
Acartia tropica	-	-	+	-	-	-	++++	-	-	-	-
Acartiella sp.	-	-	-	++	-	-	-	++++	-	-	-
Acartiella sewelli	-	-	-	-	-	-	+	++	-	-	-
Acartiella Keralensis	-	-	-	-	-	-	-	++	-	-	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Continued …

Totanus gracilis	-	+	-	-	-	-	-	-	-	-	-
Centropages sp.	+	+	-	-	-	+	-	-	-	-	-
Centropages orisini	-	-	-	-	-	-	-	-	-	-	+
Centropages furcatus	+	-	+	-	-	++++	-	-	++	-	-
Centropages tenuiremis	-	-	+	-	-	-	-	-	-	-	-
Eucalanus sp.	++	++	-	-	-	+	-	-	-	-	-
Temora turbinata	+	+	-	-	-	++	-	-	-	-	-
Temora sp.	-	-	+	-	-	-	-	-	-	-	-
Lebidocera sp.	-	-	-	-	++	-	-	-	-	-	-
Lebidocera Pavo	-	+	-	-	-	++++	-	-	-	-	-
Lebidocera pectinata	-	+	-	-	-	++	-	-	-	-	-
Clausocalanus arcuicornis	-	++	+	-	++	-	-	-	-	+++	++
Clausocalanus furcatus	-	-	-	-	-	-	-	-	++	-	++
Clausocalanus sp.	-	-	+	-	-	-	-	-	-	+	+++
Heliodiaptomus cinctus	-	-	-	+++	-	-	-	-	-	-	-
Diaptomus sp.	-	-	-	++++	-	-	-	-	-	-	-
Oithona sp.	+	++	++	++++	++++	-	-	-	++	++	++
Oithona brevicornis	++++	-	+++	-	-	-	-	-	++	++	-
Oithona rigida	-	-	-	-	-	-	-	-	-	-	-
Oithona similis	-	+++	-	-	-	++	-	-	-	-	-
Oithona plumifera	-	-	-	-	-	-	-	-	-	+	-
Cyclops sp.	-	-	-	++	-	-	-	-	-	-	-
Euterpina acutifrons	++	++	++	+++	-	-	-	-	+	-	-
Corycaeus sp.	++++	++	++	++	-	++	-	-	+++	++++	++++
Farranula spp.	+	+	-	++	-	-	-	-	-	++++	+
Copepod juveniles	-	-	-	-	++	+	-	++++	++	++++	++++

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Continued …

Sapphirina sp.	-	-	-	-	-	+	-	-	-	-	-
Bomolochus sp.	-	-	-	-	-	+++	-	-	-	-	-
Euchaeta sp.	-	-	-	-	-	-	-	-	-	+	+
Euchaeta indica	-	-	-	-	-	-	-	-	-	++	++
Euchaeta concinna	-	-	-	-	-	-	-	-	-	+	-
Euchaeta marina	-	-	-	-	-	-	-	-	-	+	+
Euchaeta longicornis	-	-	-	-	-	-	-	-	-	+	-
Onceae sp.	-	-	-	-	-	-	-	-	++++	+	++
Onceae venusta	-	-	-	-	-	-	-	-	++++	+++	+++
Onceae conifera	-	-	-	-	-	-	-	-	++++	+++	+++
Onceae media	-	-	-	-	-	-	-	-	+++	+++	++
Onceae minuta	-	-	-	-	-	-	-	-	-	-	++
Lucicutia flavicornis	-	-	-	-	-	-	-	-	-	++	+
pontellina plumata	-	-	-	-	-	-	-	-	-	-	+
Nanocalanus minor	-	-	-	-	-	-	-	-	-	++	++
Canthocalanus pauper	-	-	-	-	-	-	-	-	-	-	+
Undinula vulgaris	-	-	-	-	-	-	-	-	-	-	+
Subeucalanus mucronatus	-	-	-	-	-	-	-	-	-	-	+
Pleuromamma sp.	-	-	-	-	-	-	-	-	-	-	+
Microsetella rosea	-	-	-	-	-	-	-	-	-	-	++
Macrosettella gracillis	-	-	-	-	-	-	-	-	-	++	++
Euterpina acutifrons	-	-	-	-	-	-	-	-	-	-	+
Copilia sp.	-	-	-	-	-	-	-	-	-	-	+
Sapphirina stellata	-	-	-	-	-	-	-	-	-	-	++
Candacia sp.	-	-	-	-	-	-	-	-	-	+	-
Calanopia elliptica	-	-	-	-	-	-	-	-	-	+	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Continued …

Undinula Darwini	-	-	-	-	-	-	-	-	-	+	-
Eucalanus elongatus	-	-	-	-	-	-	-	-	-	+	-
Euclanus monachus	-	-	-	-	-	-	-	-	-	+	-
Allodiaptomus Mirabilipes	-	-	-	-	-	-	-	++++	-	-	-
Helicodiaptomus contortus	-	-	-	-	-	-	-	+++	-	-	-

'-' indicates absence of species; '+' indicates < 1%; '++' indicates 1 to 5%; '+++' indicates 5 to 10%; '++++' indicates >10%

Table 26a. SIMPER analysis showing the contribution of major metazooplankton groups for the differences among the groups in between FIM and NEM across all the habitats. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 71.33	**Group FIM**	**Group NEM**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Acrocalanus gibber	3.41	2.84	6.73	1.02	9.44	9.44
Acartia tropica	2.11	0.92	4.14	0.98	5.8	15.24
***Acrocalanus* sp.**	0.36	1.98	3.95	1.54	5.54	20.78
Copepod juveniles	2.87	1.2	3.59	1.36	5.03	25.81
***Paracalanus* sp.**	0.36	1.47	3.3	1.37	4.63	30.44
***Acartia* sp.**	1.38	0.51	3.12	0.78	4.37	34.81
Acrocalanus gracilis	1.57	1.92	2.86	1.09	4.01	38.81
Euterpina acutifrons	1.01	0.76	2.77	0.68	3.89	42.7
***Oithona* sp.**	0.37	1.34	2.55	0.88	3.58	46.28
***Coryceaus* sp.**	0.57	2.05	2.44	0.9	3.42	49.69
Paracalanus parvus	1.37	1.37	2.38	1.07	3.34	53.03

Table 26b. SIMPER analysis showing the contribution of major metazooplankton groups for the differences among the groups in between midreach of estuary and inner continental shelf stations across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 85.81	**Group upstream**	**Group open ocean**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Acrocalanus gibber	3.23	1.31	5.26	0.86	6.13	6.13
***Onceae* sp.**	0.1	3.27	5.12	1.3	5.97	12.1
***Coryceaus* sp.**	0.37	3.23	4.5	2.52	5.24	17.35
Copepod juveniles	1.9	4.59	4.47	1.19	5.2	22.55
Onceae venusta	0	2.53	4	3.55	4.66	27.21
Euchaeta indica	0	1.99	3.15	6.38	3.67	30.88

Table 26c. SIMPER analysis showing the contribution of major metazooplankton groups for the differences among the groups in between upstream of estuary and inner continental shelf stations across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 87.06	**Group upstream**	**Group inner shelf**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Onceae conifera	0	3.5	6.97	1.29	8.01	8.01
Acrocalanus gibber	3.23	1	6.6	0.75	7.58	15.58
Euterpina acutifrons	0.41	3.09	5.9	1.3	6.78	22.36
Acrocalanus longicornis	0	2.5	5.28	1.1	6.06	28.42
Copepod juveniles	1.9	2.53	4.62	1.3	5.3	33.73
Oithona brevicornis	0	2.11	4.6	0.87	5.29	39.02

Table 26d. SIMPER analysis showing the contribution of major metazooplankton groups for the differences among the groups in between upstream of estuary and open ocean stations across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 83.13	**Group mid reach**	**Group open ocean**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Acartia tropica	3.68	0	6.08	0.96	7.31	7.31
Acrocalanus gibber	4.48	1.31	5.36	2.12	6.45	13.76
***Onceae* sp.**	0	3.27	5.06	1.37	6.08	19.84
Copepod juveniles	1.72	4.59	4.53	1.35	5.46	25.29
***Coryceaus* sp.**	0.28	3.23	4.44	3.07	5.34	30.63
Acrocalanus longicornis	3.01	0.75	3.87	1.53	4.66	35.29
Onceae venusta	0	2.53	3.84	3.75	4.62	39.91
Euchaeta indica	0	1.99	3.02	7.65	3.63	43.54

Table 26 e. SIMPER analysis showing the contribution of copepod species in different habitats between near mouth of estuary and inner continental shelf stations across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 83.44	**Group near mouth**	**Group inner shelf**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Acrocalanus gibber	4.55	1	5.98	1.52	7.17	7.17
Onceae conifera	0	3.5	5.9	1.35	7.07	14.24
Euterpina acutifrons	0.27	3.09	4.92	1.41	5.89	20.13
Acrocalanus longicornis	0.22	2.5	4.16	1.11	4.99	25.12
Acartia tropica	2.39	0	4.06	1.12	4.87	30
Copepod juveniles	1.89	2.53	4.06	1.34	4.86	34.86
Paracalanus parvus	2.58	0.26	3.76	2.05	4.51	39.37
Oithona brevicornis	0.29	2.11	3.72	0.93	4.46	43.83
Acrocalanus gracilis	2.42	0.52	3.15	1.62	3.78	47.6

Table 26 f. SIMPER analysis showing the contribution of copepod species in different habitats between near mouth of estuary and outer continental shelf stations across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 80.86	**Group mouth**	**Group outer shelf**				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Copepod juveniles	0.48	4.72	6.36	2.69	7.86	7.86
Acrocalanus gibber	4.3	0.96	5.51	1.52	6.81	14.67
***Coryceaus* sp.**	0.78	3.08	4.27	2.67	5.28	19.95
Onceae media	0	2.81	4.25	1.98	5.25	25.2
Acrocalanus gracilis	3.53	0.99	4.18	2.16	5.17	30.37
Onceae venusta	0	2.76	4.1	4.73	5.07	35.44
Onceae conifera	0	1.96		1.35	3.68	39.12

Table 27 a. SIMPER analysis showing the contribution of copepod species in different seasonal periods between FIM and NEM across all the habitats. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 52.80	Group FIM	Group NEM				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Calanoida	4.86	6.16	8.49	1.11	16.08	16.08
Decapoda larvae	2.36	2.4	4.72	1.58	8.95	25.02
Gastropod larvae	1.73	1.7	4.39	0.81	8.31	33.33
Poecilostomatoida	1.63	2.8	3.87	0.93	7.32	40.65
Cyclopoida	0.79	1.97	3.62	1.16	6.85	47.5
Cladocerans	0.95	0.36	3.53	0.45	6.68	54.18
Copepod Juveniles	1.7	1.6	3.34	1.27	6.33	60.51
Fish eggs	1.43	0.26	3.22	0.73	6.1	66.62

Table 27 b. SIMPER analysis showing the contribution of copepod species in different habitats between upstream of estuary and open ocean across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 79.44	Group mid reach	Group inner shelf				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Polychaete larvae	0.47	3.3	13.28	0.86	16.72	16.72
Calanoida	6.78	3.35	13.2	1.41	16.62	33.34
Gastropod larvae	4.47	0	11.61	1.35	14.61	47.95
Poecilostomatoida	0.21	3.71	7.82	0.86	9.84	57.79
Decapoda larvae	2.34	0.73	7.72	1.18	9.72	67.5
Copepod Juveniles	1.66	2.28	6.59	1.96	8.3	75.8
Harpacticoida	0.24	1.48	3.08	1.32	3.88	79.68
Cyclopoida	0.77	1.08	2.8	2.29	3.53	83.21
Appendicularia	0.07	1.09	2.65	1.11	3.34	86.55
Siphonophorae	0	1.08	2.13	0.85	2.69	89.23
Ostracoda	0	0.79	1.72	0.58	2.16	91.39

Table 27 c. SIMPER analysis showing the contribution of copepod species in different habitats between upstream of estuary and inner continental shelf stations across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 69.41	Group upstream	Group open ocean				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Poecilostomatoida	0.51	6.08	12.86	2.8	18.53	18.53
Cladocerans	3.29	0	7.88	0.75	11.35	29.88
Calanoida	6.23	4.54	7.03	2.06	10.13	40.01
Copepod Juveniles	1.15	4.12	6.04	1.76	8.71	48.72
Ostracoda	0	2.61	5.08	2.43	7.32	56.04
Decapoda larvae	2.16	1	3.4	1.37	4.9	60.94
Cyclopoida	0.65	1.51	3.27	10.05	4.71	65.65
Chaetognatha	0	1.6	3.13	1.04	4.51	70.15
Echinodermata larva	1.31	0	3.09	0.45	4.46	74.61

Table 27 d. SIMPER analysis showing the contribution of copepod species in different habitats between midreach of estuary and open ocean across all the seasons. Av. Abund: average abundance; Av. Diss: average dissimilarity; Contrib: contribution.

Average dissimilarity = 75.00	Group upstream	Group inner shelf				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Calanoida	6.23	3.35	11.65	1.41	15.54	15.54
Poecilostomatoida	0.51	3.71	9.35	0.98	12.46	28
Cladocerans	3.29	0.23	8.69	0.91	11.59	39.59
Polychaete larvae	0	3.3	6.33	0.45	8.43	48.02
Copepod Juveniles	1.15	2.28	6.22	1.2	8.3	56.32
Decapoda larvae	2.16	0.73	5.76	1.55	7.68	64
Harpacticoida	0.39	1.48	4.14	1.55	5.52	69.52
Siphonophorae	0	1.08	3	1.17	4	73.52
Echinodermata larva	1.31	0	2.88	0.45	3.84	77.36
Cyclopoida	0.65	1.08	2.87	2.62	3.83	81.19

Table 28. Cumulative constrained percentage of the four axes extracted in the CCA analysis for **(a)** axis representation of Eigen values and axis contribution for association of metazooplankton communities in spatially distinct sites with reference to different seasons and **(b)** overall FS summary showing the correlation of environmental parameters and the group abundance ($p < 0.05$).

a)

Conditional Effects				
Variable	**Var.N**	**Lambda A**	**P**	**F**
Salinity	2	0.48	0.002	3.95
Nitrate	4	0.3	0.038	2.81
Chl a	6	0.22	0.032	2.05
DO	3	0.2	0.066	2.02
Temperature	1	0.14	0.194	1.32

b)

Conditional Effects				
Variable	**Var.N**	**LambdaA**	**P**	**F**
Salinity	2	0.51	0.001	5.66
Nitrite	5	0.2	0.084	2.24
Nitrate	4	0.05	0.618	0.59
Chl a	6	0.15	0.093	1.75
DO	3	0.05	0.621	0.58
Temperature	1	0.09	0.393	1.03

Table 29. Overall FS summary showing the correlation of environmental parameters and copepod species abundance in varying sampling sites with reference to different seasonal periods.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues :	0.716	0.177	0.065	0.048
species-environment relation:	68.3	85.2	91.4	96

Chapter 4

Ecological distribution of protist community in oxygen minimum zones of the Arabian Sea

4.1. Introduction

Protists, the unicellular eukaryotes, play a pivotal role in mediating biogeochemical processes in marine ecosystems (Caron et al., 2017). The substantial presence of these communities in diverse ecosystems has drawn considerable attention to studying their species variability on spatial scales (Lepere et al., 2013; Wu et al., 2017). Physicochemical environments along with biological interactions (competition and predation) are also known to influence species assemblage and their function (Webb et al., 2002). Sufficient information exist using traditional methods to understand protistan ecology (Azam et al., 1983; Sherr and Sherr, 2002). The metagenomics studies have further advanced insights into molecular protist identification (Zhou et al., 2015). Despite their ubiquity, information on some rare taxa of microbial communities is still rudimentary. The use of DNA sequence analysis has shown the complete classification of protistan taxa in natural ecosystems (Stoeck and Epstein, 2003; Doherty et al., 2007) and for their phylogenetic structure (Massana et al., 2006; Moreira et al., 2007). These studies are characterized by the demarcation of a gene library (graphical visualization of the number of sequences with operational taxonomic units; OTUs) to reveal community diversity and structure. Such a sequencing approach is an advanced tool to evaluate the taxonomic diversity of protists with the accuracy of taxonomic affiliation. Other molecular techniques are also prevalent for semi-quantitative assessment of microbial eukaryotic communities (Thiele et al., 2012).

The molecular diversity and community composition of protists are limited in the spatial demarcation of oxygen-depleted environments. Globally, high-throughput sequencing studies have profoundly impacted environmental surveys of microbial eukaryotes in unique ecosystems over a distinct geographic area (Filker et al., 2016; Kammerlander et al., 2015; Duret et al., 2015; Orsi et al., 2012). Marine oxygen minimum zones (OMZs) probably harbor protistan plankton communities because of their metabolic adaption to low-oxygen environments. Recent studies on protist diversity based on 18S rRNA gene sequences revealed a distinct pattern of eukaryotic groups present in the oxygen-deficient environments of world oceans (Jing et al., 2015; Parris et al., 2014; Stoeck and Epstein, 2003). The Arabian Sea OMZ, however, is unique among world oceanic OMZs, as limited information is available on the presence of microbial eukaryotes in these waters and their relation to the other metazooplankton groups. This is because of the small size and morphological peculiarities that make their identification to the correct taxonomic species difficult (Moon-van der Staay et al., 2001). An established eukaryotic tree is a valuable tool in understanding evolutionary lineages between flagellates from unicellular to multicellular organisms (Massana et al., 2002). It is therefore crucial to comprehend the degree of taxonomic variation of different protist groups to identify changing environments.

The present study is aimed to distinguish microbial protist community diversity linkages in spatially varying environmental settings using next-generation sequencing (NGS). Efforts were directed to identify site-specific eukaryotic protists and highlight their taxonomic group response in different oxygen regimes in the water column in the Arabian Sea. Statistical analysis was used to compare the abundance of protist communities to gain a better understanding of the community response to the surrounding environments. Thus, the study primarily aimed to explore the genetic

diversity of eukaryotic protists and their spatial distribution with the variation of oxygen regime. Further areas of interest were to determine the degree of similarity and dissimilarity between protist groups, and understand the environmental factors responsible for the partitioning and diversity of the protist community across varying biogeochemical regimes. The present studies could serve as a valuable baseline data outlining the molecular genetic diversity of microbial eukaryotic groups in the OMZ of coastal and estuarine regions.

4.2 Material and methods

4.2.1 Sample collection

Sampling sites were selected based on the variation in the oxygen regime from the estuarine to the perennial OMZ region through the coastal seasonal OMZ (the western Indian shelf) of the Arabian Sea. Samples were collected from four contrasting sites covered during Cruise SSK-56 on board the R/V Sindhu Sankalp from 18 October to 2 November 2013. The sampling sites were located in the open ocean (ASTS, 17°N-68°E), the shelf-edge away from the coast (G12, 15.24°N-72.98°E), a coastal (inner-shelf) station (G5, 15.50°N-73.67°E) and the estuarine region (Mandovi, 15.49° N-73.81°E and Zuari, 15.41°N-73.91°E) of the Arabian Sea (Fig. 28). In total, fourteen water samples were collected at four depths from ASTS (surface, oxic: 103 m, hypoxic: 134 m, upper suboxic: 190 m, lower suboxic), three depths from G12 (surface, oxic; 80 m, upper hypoxic: 120 m, lower hypoxic) and three depths from G5 (surface, oxic: 8 m, oxic: 24 m, anoxic). Surface and near-bottom waters were also collected from the estuarine stations, exhibiting oxic water column. The barrier of oxygen gradients was defined as per Naqvi et al. (2010): oxic (DO >62.5 µM), hypoxic (4<DO≤62.5 µM), suboxic (0<DO≤4 µM), and anoxic (0 µM).

Samples were collected using Niskin Bottles on a Rosette equipped with a CTD profiler (conductivity, temperature, depth) and a dissolved oxygen sensor. Vertical profiles of temperature and salinity in the water column were recorded from the CTD. Levels of dissolved oxygen (O_2) and nutrients (NO_3^- and NO_2^-) were measured onboard within a few hours of collection, following the titrimetric Winkler's method and the automated colorimetric procedures adapted for a SKALAR auto-analyzer, respectively (Grasshoff et al., 1983). One liter of the water sample was collected for chlorophyll analysis and immediately filtered through a GF/F filter. Chlorophyll *a* (Chl *a*) was extracted from the filters with 90% acetone for 24 hours, in the dark at -20° C and the fluorescence measured using a fluorometer (Turner Designs, Model no. 10-AU). Water samples of 1-5 liters were filtered through Durapore membrane filter paper (47 mm, 0.65 µm, Millipore, Germany) using a peristaltic pump for open ocean, shelf-edge, coastal and estuarine stations. Filters were placed in cryovials, preserved with 3 ml RNAlater (Ambion, Germany) and stored at -20° C for later DNA extraction.

4.2.2 Nucleic acid extraction, PCR and sequencing

Collected filter papers were cut into small pieces and shifted to a Lysis E-Matrix tube (MP Biomedicals, Germany). Then 600 µl RLT buffer and six µl b-mercaptoethanol were added and followed by shaking at 30 Hz for 45 s using a mixer mill (MM200, Retsch, Germany). The tubes were centrifuged at 1400 g for 3 m and the supernatant was collected. For the DNA extraction, we followed the protocol after the 4^{th} step given in the Qiagen's All Prep DNA/RNA Mini Kit Manual. Three replicates of each sample were extracted and pooled. The bulk DNA concentration was measured by NanoDrop 2000 (Thermo Scientific, USA). The V4 region of the 18s rDNA was amplified in triplicates with a set of universal PrimerTAReuk454FWD1 and TAReukREV3 given by Stoeck et al. (2010). The PCR mix contained 50–100 ng of DNA template in 50 µl

solution, 1 µl of Phusion High-Fidelity DNA polymerase (Finzymes, New England Biolabs, Ipswich, MA, USA), 1x Phusion Buffer (New England Biolabs, Ipswich, MA, USA), 200 µM each of deoxynucleotide triphosphate and 0.5 µM oligonucleotide primer. The PCR protocol started with the initial denaturation (30 s at 98° C) followed by 30 identical amplification cycles, denaturation (at 98° C for 10 s, annealing at 59° C for 10 s and extension at 72° C for 30 s) and a final extension at 72° C for 30 s. Three replicates of reactions for each sample were prepared to reduce PCR bias. Following the PCR process, agarose gel electrophoresis was conducted to purify the PCR products and the target bands of 400-500 bp checked with the help of a Qiagen gel extraction kit. Sequencing of purified V4 amplicons was performed on an Illumina Miseq platform by SeqIT, Kaiserslautern, Germany. A custom script was used to merge the paired-end reads produced from the same amplicon. Accessible sequence reads were deposited in the NCBI Sequence Read Archive under the Bioproject ID number PRJNA369134.

4.2.3 V4-amplicon data processing and OTU (operational taxonomic units) analysis

Raw paired-end Illumina reads were processed using the script spilt-libraries.py applied in QIIME v.1.8.0 (Caporaso et al. 2010). The phylotypes were clustered using Uclust (Edgar 2010) at different sequence similarity (100-90%). The length distribution of the tags was plotted in R (R Core Team 2012). For taxonomic classifications and statistical diversity, OTUs called at 97% sequence similarity were used (Nebel et al. 2011; Dunthorn et al. 2014). The core (the longest and thus most informative) sequence for each phylotype at 97% was extracted into a FASTA file. This file was analyzed with JAguc software (Nebel et al. 2011). JAguc employed BLASTn searches, with algorithm parameters adjusted for short (200–500 bp) reads (-m 7 -r 5 -q -4 -G 8 -E 6 -b 50). The custom script output files from QIIME's OTUpipe (seq_otus.txt) and JAguc

(the taxonomic tree for analyzed representative sequence) were merged to a biome file containing information about OTU IDs, the number of sequences per OTU and per sample as well as taxonomic affiliations. Non-target OTUs (metazoans and embryophytes) were excluded, and the resulting file, representing the total planktonic protists, was used for statistical analysis.

4.2.4 Statistical data analysis

Faction Rare profiles and Shannon index (alpha diversity) were determined using QIIME v.1.8.0 (Caporaso et al. 2010). For this purpose, data were first normalized and resampled 1000 times to account for uneven sample sizes (Logares et al. 2012). Similarity patterns of protist communities were visualized through non-metric-multidimensional scaling (nMDS) analysis based on the Bray-Curtis index which measures the relative abundance of OTU sequences representing higher taxon protist groups (Bray and Curtis 1957). A permutational multivariate analysis of variance (PERMANOVA) with two factors (habitats and oxygen gradients) was conducted to examine whether the significant variation of protist diversity was due to the partition of habitats (estuary, coastal, shelf edge and open ocean) or to different oxygen gradients (oxic, hypoxic, suboxic, and anoxic), or a combination of both (Anderson et al., 2008). The important environmental parameters for the distribution of the protist community were examined by the biota-environment (BIOENV) method (Clarke and Ainsworth, 1993), RELATE was performed and followed by a stepwise distance-based linear model permutation test (DistLM; McArdle and Anderson, 2001) to detect any significant relations of environmental parameters in support of multivariate variation of higher taxonomic groups of protist assemblages. The value of R^2 was used as a selection measure to show the best explanatory environmental variables in the model. Euclidean distance was applied as a resemblance criterion in the DistLM procedures.

Visualizations of results were produced with a distance-based redundancy analysis (dbRDA) (Anderson et al., 2008). All the statistical analyses were performed by the module of PRIMER V6 + PERMANOVA software.

4.3 Results

4.3.1 Hydrological parameters

The physical, chemical and biological properties of the water column at different sites in the Arabian Sea exhibited diverse environmental conditions. The water column of the sampling sites showed characteristic variation in their oxygen, temperature, salinity, nutrients, and chlorophyll *a* values. Both Mandovi and Zuari estuaries showed a well-mixed water column, a sign of normoxia. Estuarine stations did not indicate any hypoxic water column conditions, although O_2 levels remained below saturation level (Surface DO 161 µM and 156 µM in the bottom waters of the Mandovi estuary, and surface DO 158 µM and 145 µM at the bottom of the Zuari estuary). At the coastal sampling site (G5) O_2 varied from 153 µM (oxic) at the surface, 82 µM at 8 m (oxic) and an undetectable concentration at 24 m (bottom) indicating near anoxic conditions. The shelf edge station (G12) showed 197 µM at the surface (oxic), 14.6 µM at 80 m (hypoxic) and 6.9 µM at 120 m (hypoxic), whereas the open ocean station had 205 µM O_2 at the surface (oxic), 43 µM at 103 m (hypoxic), 4.1 µM at 134 m (suboxic) and 4.4 µM at 190 m (suboxic). Seasonal low oxygen condition was well established at the coastal site (G5) and at the shelf edge (G12) while permanent OMZ prevailed in the open Ocean (ASTS).

The water temperature in the estuary ranged from 27.2–28.9° C, in the coastal water column from 21.9–27.1° C, at the shelf edge from 18.1–29.1° C and in the open Ocean from 16.4–28.8° C. From all the sampling sites, the highest salinity was recorded in the open Ocean (36.9 PSU) and the lowest in the Mandovi surface waters (26.1 PSU).

Significant spatial variation in salinity was noticed from the estuarine sites to the open Ocean site. Maximum chlorophyll *a* was observed at the estuarine station and this gradually decreased from coastal to oceanic sites. The highest nitrite concentration (4 µM) was observed at OMZ core depth of the open Ocean, and the highest nitrate concentrations at 120 m depth at the shelf-edge station.

4.3.2 Sequencing statistics of V4 amplicon analyses

After the quality check, altogether 1395168 protistan V4 amplicons were obtained for taxonomic identification of protists at different sampling sites. Our target eukaryotic reads without singletons/doubletons produced 1387818 sequences, grouping into 12687 operational taxonomic units (OTUs) called at 97% sequence similarity. The highest (518676 reads) number of reads was obtained from the open Ocean, while the lowest (127621 reads) marked the shelf edge (Table 30). The rarefaction analysis established the saturated sampling profiles for OTUs called at 97% sequence similarity (Fig. 29). Out of total reads 54% of the target sequences showed sequence similarity of > 95% to their closest BLAST hit in the protist V4 18S rDNA database.

4.3.3 Protist diversity at the local scale (alpha-diversity)

In terms of alpha diversity estimates, estuarine communities appeared to be less diverse than the coastal, shelf edge and open Ocean stations. Among all the 14 sampling sites, the Shannon index varied from 1.01 in the estuarine station (MS) to 6.5 in the coastal station (G5–8 m). The statistical results clearly indicated that highest protist diversity occurred at 8 m depth in the coastal station and in the surface water of the open ocean station (Fig. 30). Comparatively, estuarine stations revealed very low diversity, in a range from 1–1.5, whereas the diversity ranged from 3.2–6.5 in the coastal and oceanic water column. The vertical diversity pattern of Mandovi and Zuari estuaries showed little variation based on higher taxonomic levels. Coastal water did not reveal a clear

diversity difference corresponding to oxygen gradients. Protistan OTU alpha diversity in the shelf edge and open Ocean showed a notable variation among the different depths of oxygen gradients (Fig. 30).

4.3.4 Taxonomic composition of protist communities in distinct spatial sites

In total, 22 Protistan phylogenetic groups, including Ciliophora, Dinophyceae, Unclassified Alveolates, Centroheliozoa, Choanoflagellida, Cryptophyta, Fungi, Haptophyceae, Picozoa, Rhizaria, Stramenopila, Telonema, Viridiplantae, Colpodellidae, Amoebozoa, Apusozoa, Dimorpha, Eccrinales, Ichthyosporea, Apicomplexa, Katablepharedophyta and Rhodophyta were identified at all the sampling sites in estuarine and open Ocean waters.

Estuarine sites were represented by Viridiplantae (91%–465389 OTU sequences), Stramenopiles (4%–20251 OTU sequences), Dinophyceae (3.8%–19291 OTU sequences) and Ciliophora (0.4%–2060 OTU Sequences). Estuary supported a higher abundance of Viridiplantae (85–98%) both in surface and near-bottom waters compared to neritic (G5 and G12) and oceanic waters (ASTS). Stramenopiles (3.2%) and Dinophyceae (7.17%) were at least 5–8 times more abundant in the bottom waters of the Mandovi estuary than at the surface (0.6–0.88%). Zuari estuary, on the other hand, supported four-fold higher abundance of Dinophyceae and Stramenopiles (4 % each) in surface waters than at the bottom (Fig. 31).

Overall, coastal waters showed the dominance of Dinophyceae (81.7%–190154 OTU sequences), Stramenopiles (12%–28141 OTU sequences) and Ciliophora (1.7%–4153 OTU sequences). With reference to oxygen gradients, the variation in the protist community between the oxic (surface and 8 m) and anoxic water column was insignificant. Dinophyceae remained the most abundant group both in oxic (83%–83422 OTU sequences) and anoxic (79%–23311 OTU sequences) zones (Fig. 31).

The community at the shelf-edge was dominated by Dinophyceae (88%–112025 OTU sequences), Rhizaria (8%–10543) and Stramenopiles (1.2%–1584 OTU sequences). Substantial variation of protist communities between oxic (surface water) and hypoxic water column was detected at this station. In the oxic water column, the dominant communities were Dinophyceae (95%), whereas 85% and 71% of the total protist community was represented by Rhizaria in the hypoxic water column (80 m and 120 m). Both hypoxic strata, however, showed unclassified Alveolates at 80 m (1.2%) and 120 m (1.5%). Remarkably, the hypoxic water column contained an approximately 200 times higher Rhizaria community than that at the surface (oxic) water (Fig. 31).

Overall, the open ocean water column was dominated by Dinophyceae (55.9%–290049 OTU sequences), Rhizaria (39.9%–206794 OTU sequences), unclassified Alveolates (2.7%–14061 OTU sequences), Ciliophora (0.4%–1882 OTU sequences), and Stramenopiles (0.3%–1550 OTU sequences). The surface waters of the open Ocean showed an abundance of Dinophyceae (94.8%–106495 OTU sequences), Rhizaria (2.2%–2451 OTU sequences), Haptophyceae (0.9%–1032 OTU sequences) and Stramenopiles (0.7%–788 OTU sequences). The hypoxic water column revealed Rhizaria (78%–134012 OTU sequences), Dinophyceae (13%–22910 OTU sequences) and unclassified Alveolates (8%–13654 OTU sequences). The suboxic strata at 134 m and 190 m were marked by distinctively different protist communities. Of these, the upper suboxic water column revealed Rhizaria (61.3%–67981 OTU sequences) and Dinophyceae (38%–42116 OTU sequences). Conversely, the lower suboxic water column was mainly represented by the Dinophyceae (96%–118528 OTU sequences) and less by Rhizaria (1.9%–2350 OTU sequences). The presence of Ciliophora, Choanoflagellida, Cryptophyta, and Fungi was noted in the deep OMZ core of the open ocean as compared to the oxic and hypoxic depths. Overall results suggest that the

protist communities (higher taxa) vary with the water strata at oxic, hypoxic and suboxic depths (Fig. 31).

4.3.5 Partitioning of protist diversity among spatially varying regimes

The pronounced differences in protist community composition in distinct biogeochemical regimes on higher taxonomic groups, was statistically confirmed at the OTU sequence level. In a nonmetric multidimensional scaling (nMDS-distance measured: Bray-Curtis similarity), the protist community clustered spatially and showed three major groups observed in distinct spatial habitats (Fig. 32). Stations from the open Ocean (AS–0 m), shelf edge (CS–0 m) and open ocean OMZ core (AS–190 m) clustered together, distinct from the other group consisting of AS–103 m, CS–80 m, AS–134 m and CS–20 m (Fig. 33). SIMPER analysis revealed the highest average dissimilarity (74%) between open ocean and estuary where Viridiplantae (38%) and Dinophyceae (25%) were the dominant contributors to the dissimilar community. Results of the analysis of the oxygen gradients showed the highest average community dissimilarity (64%) exhibited by the oxic and hypoxic water column, where Rhizaria (39%) and Viridiplantae (22%) were the largest contributors.

In relation to habitats and oxygen gradients distribution, the PERMANOVA community results based on the Bray-Curtis similarity revealed a significant difference (p = 0.003 and p = 0.011; Table 31a). As regards habitat distribution, pair wise PERMANOVA based on the Bray-Curtis similarity, results showed significant values of community difference between coastal and estuary (p = 0.009), shelf edge and estuary (p = 0.022) and open Ocean and estuary (p = 0.051). However, protist community composition between open Ocean and shelf edge, open Ocean and coastal, shelf edge and coastal showed no significant difference (Table 31b). The pairwise PERMANOVA test for oxygen gradients, on the other hand, revealed significant

community difference between oxic and hypoxic water columns. The community comparison in other oxygen gradients (oxic and suboxic, oxic and anoxic, hypoxic and suboxic, hypoxic and anoxic and suboxic and anoxic) showed no significant difference (Table 31c).

4.3.6 Environmental effects on partitioning diversity and community structure

RELATE analysis confirmed the enhanced correlation of environmental factors with the patterns of community structure in distinct spatial habitats. BEST analysis (BIOENV) revealed the importance of salinity, chlorophyll *a*, nitrate and nitrite on the abundance of higher taxa of the protist community. Following BEST analysis, distance-based linear model (DistLM) demonstrated that 87% of protist communities were influenced by five environmental factors. From among the six environmental factors, temperature, salinity, nitrate, nitrite, and chlorophyll *a* were the best fitting parameters to the model. The DistLM analysis identified the significant correlation of environmental variables ($p < 0.05$) with the distribution pattern of protist communities. Variables identified through the marginal test such as temperature ($p = 0.02$), salinity ($p = 0.009$) and nitrate ($p = 0.008$) showed significant correlations with the protist assemblages at different sites (Table 32). The influence of dissolved oxygen, however, was insignificant to protist distribution. The best fitting environmental variables obtained by the DistLM procedures using dbRDA plot (Fig. 44), indicated the primary importance of chlorophyll *a* association in the estuarine sites while salinity, nitrate and temperature influenced shelf edge, coastal and open ocean sites respectively.

4.4 Discussion

The present study has characterized protistan taxonomy in OMZs of the Arabian Sea using the metagenomics approach. Importantly, this is the first such report on genetic diversity of the protist community along spatial and oxygen gradients (OMZ sites) in

the Arabian Sea. The Illumina sequencing of the V4 amplicons of the 18S rRNA gene was used as a taxonomic sign which demonstrated a pattern of diversity with taxa previously unaccounted for when relying on identification under the microscope. Recent studies have shown similarly high genetic diversity in planktonic protists in OMZ sites of ETSP, ETNP and the Costa Rica coast (Parris et al. 2014; Duret et al. 2015; Jing et al. 2015). Attempt was made to investigate vertical community differences in the OMZ water column and compared the spatial distribution of the protist community under different oxygen gradients.

4.4.1 Importance of planktonic micro eukaryotes in varying ecological regimes

Ecosystems such as the estuary, coast, shelf edge, and open waters of the northern Arabian Sea are influenced by diverse environmental factors. They harbor an array of planktonic protists known to be the major functional components of pelagic food webs (Qasim and Gupta, 1981; Gifford et al., 2007; Strom et al., 2007). Both autotrophic and heterotrophic eukaryotic microbes have been reported to be present in the different ecological settings of the world ocean, which are prone to mediate food webs (Pernice et al., 2016; Massana et al., 2015). The ecological conditions of the Arabian Sea shelf and open waters are influenced by the presence of large rivers in the peninsular region that bring land runoff to the sea. The presence of two major estuaries, Mandovi and Zuari, also have a sizable impact on coastal waters which result in upwelling phenomena due to strong seasonal currents and monsoon land runoff to the sea. Upsurges of nutrient through the upwelling process results in planktonic blooms. As estuaries during different season are under tidal influence, this also causes sufficient changes in bio-geochemical regimes to result in high productivity in coastal waters. Earlier studies on large marine protists (gromiids), fungal diversity and picoplankton

revealed a diversified community structure in contrasting pelagic environments of the Arabian Sea (da Silva and Gooday, 2009; Jebaraj et al., 2010; Fuchs et al., 2005).

Oxygen depletion in the water column (hypoxic/suboxic conditions) is usually seen as an environmental stress leading to habitat compression, loss of fauna and energy diversions into microbial pathways in different marine ecological settings (Diaz and Rosenberg, 2008). However the Arabian Sea experienced coastal (seasonal) and open Ocean OMZ (permanent as well as seasonal) conditions (Naqvi et al., 2006), harboring Characteristic patterns of protist communities. The consumption of nutrients by autotrophic plankton is hampered by the prevailing turbidity of nearby coastal waters, caused by suspended particulates. Thus, the unutilized nutrients are carried towards the open coast, which probably helps the planktonic life in this region to flourish. Studies of these diverse communities in various ecological conditions would reveal vital facts on oceanic circulation including climate change.

4.4.2 Taxonomic distribution and diversity of the protist community

The eukaryotic organisms, Viridiplantae, belongs to the class of green algae and are mostly found as aquatics (without embryophytes) and land plants (embryophytes) (Becker and Marin, 2009; Cocquyt et al., 2009; Kim et al., 2014). The pronounced abundance of Viridiplantae observed in both Mandovi and Zuari could be the result of freshwater land runoff, common in these estuaries. It is confirmed in PERMANOVA (the pairwise test) where estuarine sites significantly differed from the neritic and oceanic water column in terms of Viridiplantae occurrence. SIMPER analysis also corroborated the relatively common Viridiplantae population in estuarine sites. A recent study on environmental metabarcoding in the Mersey estuary concluded that microbial plankton could be drivers in contrasting estuarine ecosystems (Lallias et al. 2015). These studies further described the influence of diversity patterns under

different salinity regimes (euryhaline, mesohaline and oligohaline). The taxonomic diversity of these eukaryote species reportedly declined from marine to fresh water systems (Lallias et al., 2015). The relatively low diversity of microbial eukaryotes in the Mandovi and Zuari estuaries also suggests that less saline waters mediate lower diversity than coastal open-sea ecosystems. Besides low salinity, environmental contaminants such as heavy metals and hydrocarbons could also be responsible for low microbial diversity and the presence of specific microbial eukaryotes in estuarine waters. The low bacterial diversity in the sediments of Zuari estuary reported in earlier studies was attributed to the presence of contaminants such as heavy metals, hydrocarbons and other anthropogenic inputs (Khandeparker et al., 2017).

Bottom anoxia and vertical oxygen gradients did not appear to greatly influence the prominent protist community at the coastal site, indicating free movement of the organisms throughout the water column. However, it is possible that few communities have evolved to thrive with low oxygen levels. The present study suggests that Dinophyceae were the dominant protist community in coastal, shelf edge and open ocean sites. The cosmopolitan nature of these organisms has been reported by many studies on rRNA based molecular signature (Duret et al., 2015; Edgcomb et al., 2011; Massana, 2011).

The hypoxic shelf edge water column community was characterized by a pronounced abundance of Rhizaria; their numbers were over 200 times higher than the surface (oxic) zone. Among the Rhizaria community, Polycystinea and Acantharea dominated the hypoxic strata at the shelf edge station. The deep open Ocean station also showed the dominance of Rhizaria groups in hypoxic and upper suboxic strata. Their aggregation in the OMZ could signify a cell sinking process through attachment to fecal matter or dead metazoan hosts from the surface (Turner, 2002; Parris et al., 2014).

As is believed, the eukaryotic community structure in OMZ influences the community present in the overlying photic zone. Earlier metagenomic studies also discovered a peak abundance of Acantharia and other radiolarians at the OMZ boundary (Parris et al., 2014; Edgcomb et al., 2011). Although oxygen levels were similar in the two suboxic depths of the open ocean (134 m and 190 m), the lower suboxic stratum sustained a higher proportion of Dinophyceae than did the upper suboxic zone. Here cell sinking from overlying waters plays a major role to sustain a higher abundance of Dinophyceae in the lower suboxic zone. This is also indicative of an accumulation of inactive or dead metazoan hosts. This is most commonly observed during the seasonal surface diatom bloom phenomenon, where the dead cells rapidly sink into the anoxic water column in the form of particulate organic matter (Parris et al., 2014).

Although the higher taxon groups which were identified in the contrasting spatial-ecological regimes did not differ greatly, their diversity and distribution patterns varied significantly. Substantial changes in oxygen and other environmental factors distinctly indicated an ecological partition across the sampling sites of estuarine, coastal, shelf-edge and open ocean sites on the spatial scale. These observations support the hypothesis that spatial patterns of genetic variability and the difference in oxygen gradients in OMZ sites in the Arabian Sea are directed by biogeochemical processes. In terms of habitat, estuarine sites showed significant differences compared to coastal, shelf edge and open ocean sites. However, as regards the oxygen gradients, only the communities in oxic and hypoxic strata differed significantly, whereas no significant changes were encountered in the communities of the strata of the oxic, anoxic and suboxic water column. Insufficient data from the anoxic and suboxic sampling sites when compared to hypoxic and oxic environments could reflect the discrepancies in community differences that were observed.

Statistical evaluations of the protist community structure revealed that the difference between habitats as well as oxygen gradients is strong indicators of community variability. The PERMANOVA test also showed significant differences between habitats as well as oxygen gradients. This study of spatial barriers in protist communities revealed a predictable pattern of beta-diversity as changes in habitat and oxygen gradients had a significant effect on the plankton community structure. At the regional scale, environmental factors could be responsible for structuring the planktonic protist communities. Heterogeneous community structure on a regional scale caused by differences in environmental factors was reported previously from OMZ of eastern tropical South Pacific (ETSP) off the coast of Chile (Parris et al., 2014), whereas in our analysis, the effects of spatial gradients in structuring plankton communities at a local scale were much more pronounced than the oxygen effects. It also revealed the spatial variation of community structure is determined by environmental factors at different spatial scales. This is the first such report to identify the whole protist community through NGS in this study region. The prevalence of the most abundant community at a particular site was determined by the characteristic environmental factors at the site which governs their site specific abundance. High abundance of a specific community is in agreement with Hubbell's Unified neutral theory of biodiversity and biogeography (Hubbell, 2001). This fact necessarily explains the diversity pattern of protists observed in estuarine, coastal, shelf edge and the open Ocean ecosystems of the present study.

The dynamic nature of the Arabian Sea is evident in the varying physical and biogeochemical properties which produce geographical partitioning. It is imperative, therefore, to understand the distribution pattern of oxygen and other environmental factors causing the community variation on a spatial scale. The results of BIOENV

analysis revealed a strong correlation between the environmental parameters (salinity, chlorophyll *a*, nitrate, and nitrite) and the abundance of the protist community.

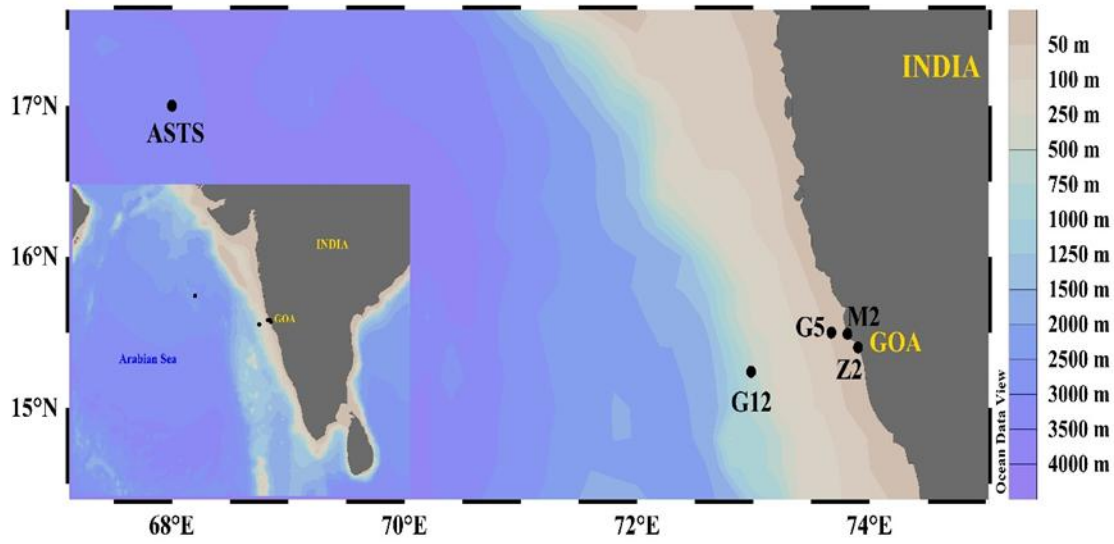

Figure 28. Map of the sampling sites located in different regions of the Arabian Sea ranging from estuarine to open ocean stations (Estuary: Mandovi-M2, Zuari-Z2; coastal: inner continental shelf -G5; outer continental shelf (Shelf edge) -G12; open ocean-ASTS).

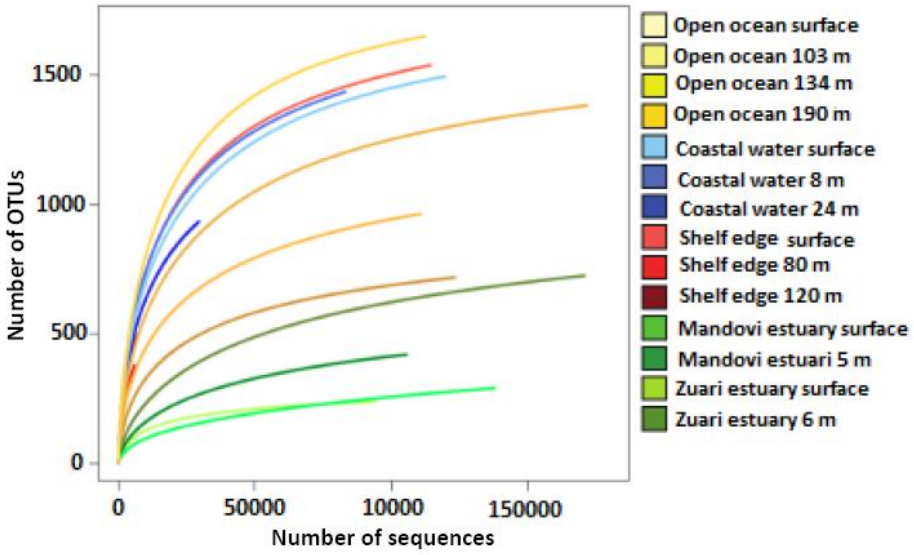

Figure 29. Rarefaction curves for the spatially different sampling sites, based on only target eukaryotes reads without singletons/doubletons. The profiles of the rarefaction curves specify near-saturation for all sampling sites.

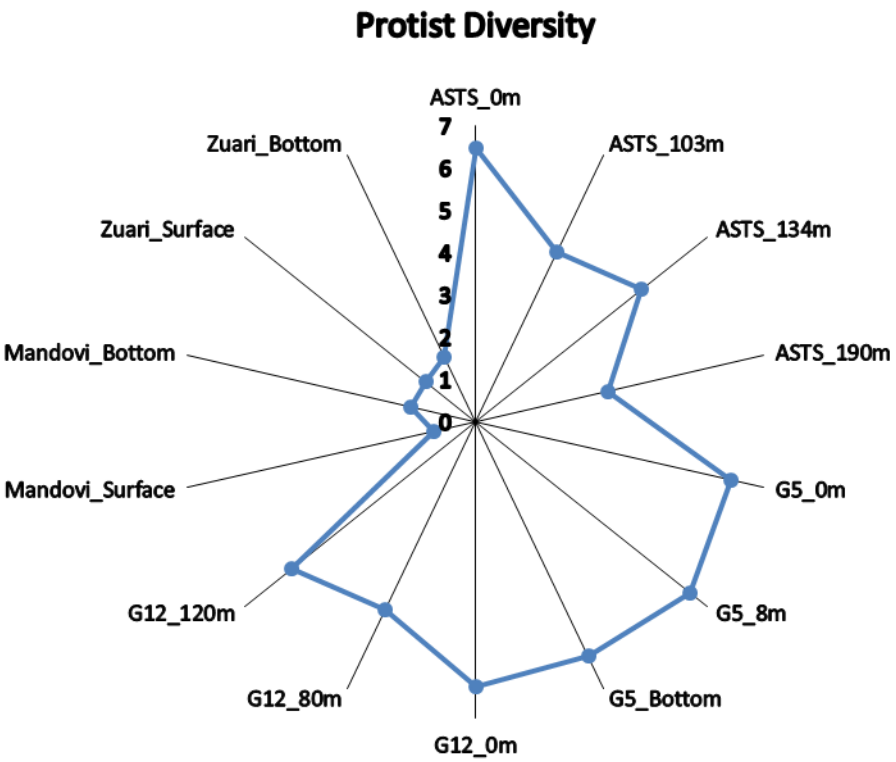

Figure 30. Radar plot to indicate the Shannon diversity index (0–7) for total protist diversity at the distinct sampling sites. Diversity values calculated are based on 97% similarity of protist OTUs.

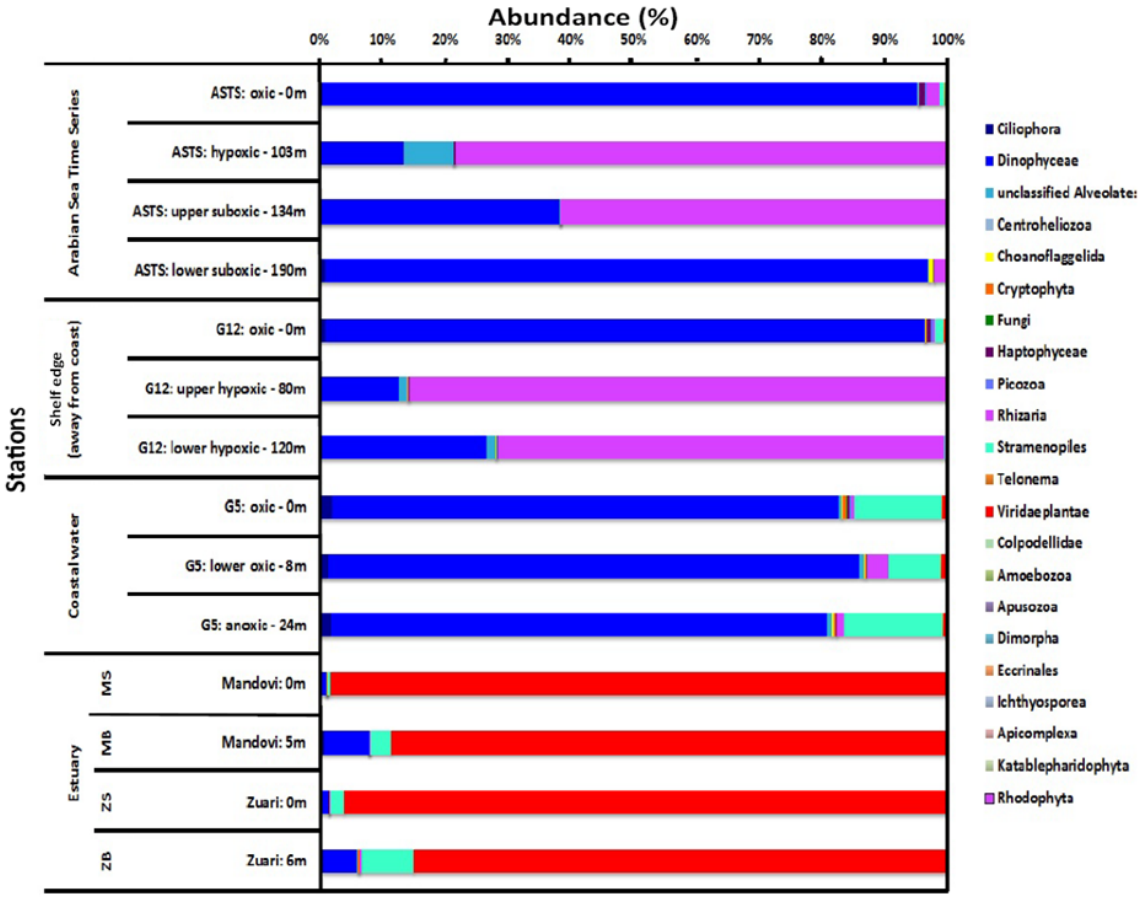

Figure 31. Total protist community composition in contrasting ecological sampling sites of the Arabian Sea.

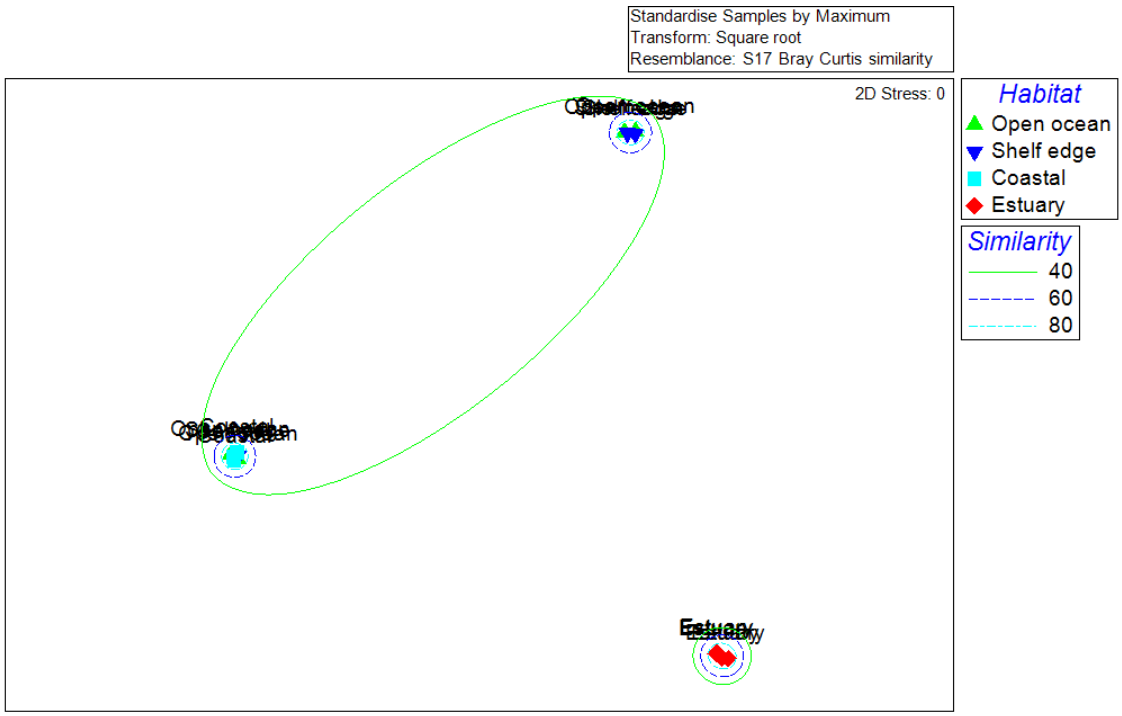

Figure 32. nMDS ordination based on total protist abundance according to the Bray-Curtis similarity index.

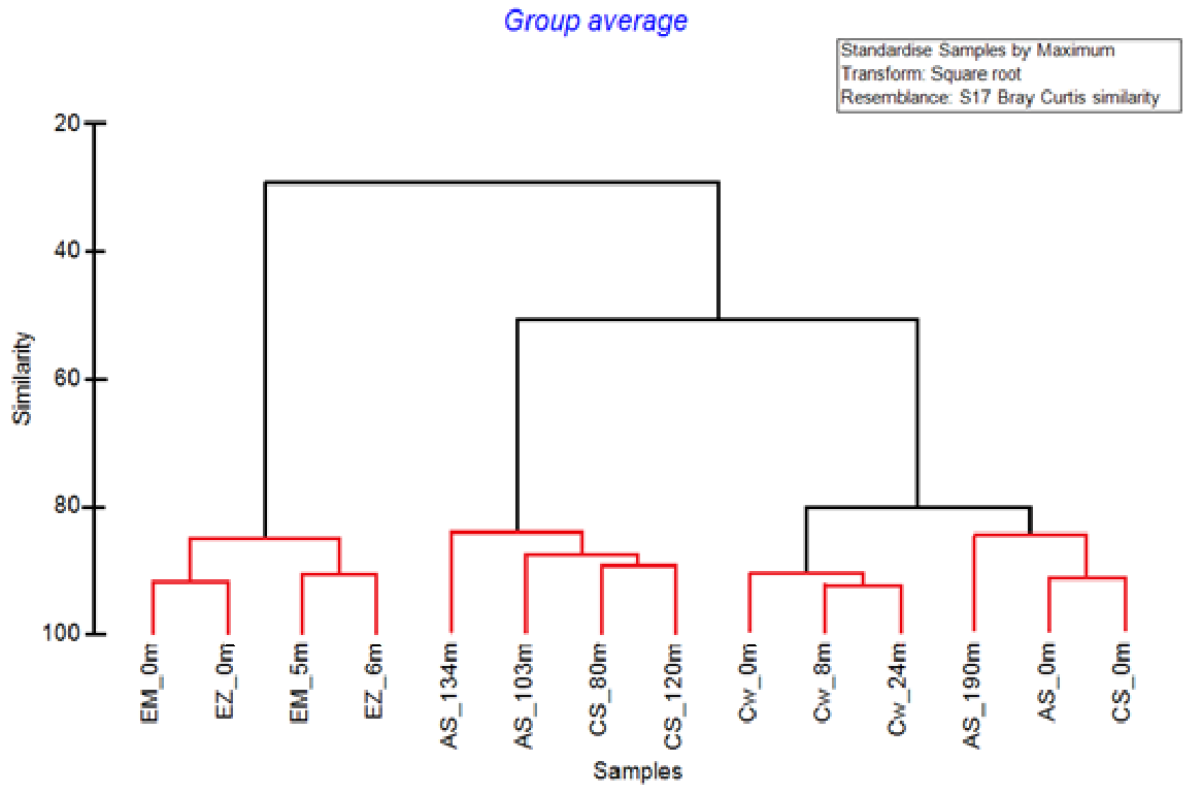

Figure 33. Cluster analysis based on total protist abundance with reference to the Bray-Curtis similarity index.

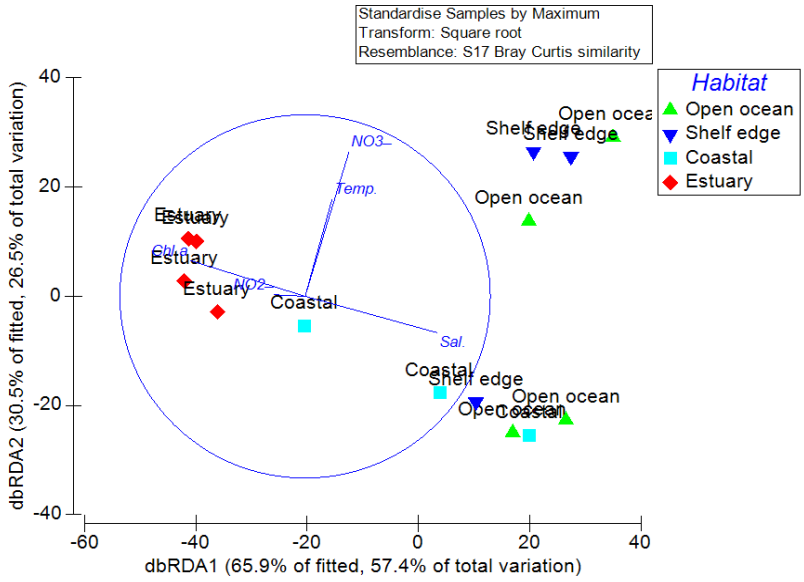

Figure 34. Distance-based redundancy (dbRDA) plot summarizing the DistLM model based on protist assemblage data and fitted environmental parameters (strength and direction of effect of the variable on the ordination plot). Axis legends include percentage of variation explained by the fitted model and percentage of community variation explained by the axis.

Table 30 An overview of Illumina sequencing data sets for the total protists in the open ocean, outer continental shelf, inner continental shelf and estuarine regions.

Sample ID	High quality target eukaryotes	High quality target eukaryotes without single and double tones
	Sequence nos. (%)	Sequence nos. (%)
ASTS (surface)	113609 (35)	112380 (34)
ASTS (103 m)	172875 (69)	171919 (68)
ASTS (134 m)	111497 (49)	110890 (49)
ASTS (190 m)	123886 (67)	123487 (67)
G12 (surface)	115635 (40)	114554 (39)
G12 (80 m)	5984 (5)	5849 (5)
G12 (120 m)	7364 (8)	7218 (8)
G5 (surface)	120655 (47)	119791 (47)
G5 (8 m)	84013 (48)	83207 (47)
G5 (24 m)	29819 (19)	29544 (19)
Mandovi (surface)	138079 (80)	137949 (79)
Mandovi (5m)	105881 (77)	105755 (77)
Zuari (surface)	94332 (75)	94240 (74)
Zuari (6m)	171539 (74)	171035 (73)

Table 31 Results of PERMANOVA analyses (Based on Bray-Curtis similarity measure) with reference to difference in habitats and oxygen gradients (a) results of Pairwise comparison PERMANOVA analysis with reference to habitats (b) results of Pairwise comparison PERMANOVA analysis with reference to oxygen gradients (c). Data were transformed; resemblance was calculated according to Bray and Curtis. The values ($P < 0.05$) reveals the significant differences (Perm: permutation; MC: Monte Carlo randomisation).

Groups	**df**	**P (perm)**	**Unique perms**	**P (MC)**
Habitats	3	0.003	912	0.0021
Oxygen gradients	3	0.011	999	0.011

(a)

Groups	**t**	**P (perm)**	**Unique perms**	**P (MC)**
Open ocean, Shelf edge	5.5	0.084	28	0.034
Open ocean, Coastal	0.7	0.596	291	0.626
Open ocean, Estuary	4.12	0.051	581	0.002
Shelf edge, Coastal	1.65	0.19	165	0.2249
Shelf edge, Estuary	6.52	0.022	105	0.004
Coastal, Estuary	8.25	0.009	105	0.001

(b)

Groups	**t**	**P (perm)**	**Unique perms**	**P (MC)**
Oxic, Hypoxic	6.27	0.001	992	0.001
Oxic, Suboxic	1.27	0.181	985	0.224
Oxic, Anoxic	0.53	0.86	798	0.827
Hypoxic, Suboxic	1.77	0.264	30	0.216

(c)

Table 32. Result of distance-based linear model (DistLM) analyses showing the influence of environmental parameters on the abundance of protist groups and Bray-Curtis similarity of square-root-transformed abundance.

Marginal Tests

Variable	**SS (trace)**	**Pseudo-F**	**P**	**Prop.**
Temperature	6000.2	5.405	0.02	0.31054
Salinity	6679.3	6.3398	0.009	0.34569
DO	3605.9	2.7533	0.089	0.18662
NO_3^-	6695.7	6.3637	0.008	0.34654
NO_2^-	1626.7	1.1031	0.239	8.4188
Chl *a*	3785.6	2.9239	0.084	0.195

SS: sum of squares; F: pseudo-F; P: p value; Prop: proprtion of explanation.

Chapter 5

5.1. Introduction

The autotrophic and heterotrophic microorganisms play key roles in cycling organic matter and are a driving force in the marine pelagic microbial food web (Sherr and Sherr, 1994; Legendre and Rivkin, 2009). While the vast majority of unicellular plankton ecology studies focused on pigmented protists (Irigoien et al., 2004; Ptacnik et al., 2008; Barton et al., 2010), heterotrophic plankton organisms are less well-characterised. Considering the only recently emphasised diversity and abundance of heterotrophic protists in ocean waters (de Vargas et al., 2015), balancing this disequilibrium will empower us to better understand the diversity, distribution and ecological role of unpigmented planktonic protists. Among the planktonic protists, heterotrophic flagellates (HF), a diverse group in terms of morphology and biogeochemical functions, play important ecological roles in predator-prey interactions in aquatic food webs (Weisse et al., 2016). In the pelagic microbial web, dissolved organic matter (DOM) serves as a substrate for bacterial growth, which, in turn, are the food source for heterotrophic nanoflagellates (HNF) and small ciliates (Azam et al., 1991). The HNF, smaller than 10 µm, are consumed by microflagellates and larger ciliates. This way, the microbial loop makes the DOM available to the filter-feeding zooplankton (Kopylov et al., 1981).

In suboxic and anoxic water columns, bacterivorous protists have profound implications on the growth and mortality rates of bacteria (Anderson et al., 2012). These bacteria play significant roles in ocean nutrient cycles through anaerobic processes, including the denitrification of ocean water and the production of potent greenhouse gases such as methane and nitrous oxide. A prerequisite for a concrete understanding of these fundamental processes in aquatic ecosystems is a basic knowledge of the key players involved in these processes. However, despite the importance of heterotrophic protists in ecosystem functioning, relatively

little is known about the diversity (molecular) in the largest oxygen-minimum zones (OMZ) of the oceans.

The Arabian Sea is home to one of the most pronounced perennial mesopelagic OMZs of our planet. Contrasting biogeochemical processes occurring in the open Ocean and coastal waters result in fundamentally different regimes in this oceanic region (Naqvi et al., 2006a). The large spatio-temporal variability arises primarily from monsoon-related processes (Naqvi et al., 2003). In general, the Arabian Sea is most productive during the southwest monsoon due to nutrient enrichment arising from upwelling events (Naqvi et al., 2003). Export of organic matter from the surface waters and its biological degradation contribute to the acute oxygen depletion observed in the mesopelagic zone (150–1,000 m); (Banse et al., 2014; Naqvi et al., 2006b). However, the oxygen deficiency in the open ocean differs from the one over the continental shelf. Most notably, the former is perennial, whereas the latter is seasonal, and sulphidic conditions develop only in coastal waters, but not in the open ocean (Naqvi et al., 2006a). An additional component of this oceanic water comes from a large number of small rivers discharging into the Arabian Sea from peninsular India, with their estuaries also experiencing large monsoon-driven changes. The Mandovi and Zuari estuary of Goa, typical of such estuaries (Qasim and Sen Gupta, 1981), exhibit biogeochemical and ecological characteristics and hydrological regime that are notably different from those of coastal and open ocean waters. The marked variation in salinity due to heavy precipitation and freshwater discharge during the monsoon mostly controls the distribution of plankton communities in estuarine waters (Madhu et al., 2007; Pednekar et al., 2011). Also, the turbulence of suspended particles at the freshwater-seawater interface results in maximum turbidity, which does not permit the complete utilisation of nutrients by phytoplankton. Therefore, the transport of unutilised nutrients to open coastal waters and of nutrients entrained through upwelling causes a high productivity over the inner and mid-shelf region (Naqvi et al., 2006b). As a result, this oceanic region is characterised by diverse biogeochemical regimes

and it is not surprising that multicellular zooplankton, fungi and bacteria have vastly different community structures in these heterogeneous pelagic environments (Jebaraj et al., 2010; Fuchs et al., 2005; Wishner et al., 2008; Morrison et al., 1999). The diversity of heterotrophic flagellates and ciliates in these regions of the Arabian Sea is poorly understood. Previous studies applied microscopic observations for the enumeration of ciliates, heterotrophic flagellates and thraustochytrids in the central and north-eastern Arabian Sea (Gauns et al., 1996; Garrison et al., 2000; Raghukumar et al., 2001). These studies were instrumental to demonstrate the general occurrence of specific taxon groups related to oxygen gradients in the water column. No attempts, however, were made to relate these taxon groups to specific environmental conditions. Moreover, microscopic studies are usually only successful in the identification of the most abundant and conspicuous protists, while smaller and low-abundant species often escape microscopic observations (McManus and Katz, 2009). In contrast, even though high-throughput sequencing strategies have their pitfalls, this approach is much more sensitive than microscopy and paints a more complete picture of protistan community structures (Stoeck et al., 2014). Accordingly, this approach has been widely used for studying protistan diversity in a variety of ecosystems, including oxygen-depleted environments (Stoeck et al., 2010; Jing et al., 2015; Parris et al., 2014; Duret et al., 2015), except in the Arabian Sea.

The present study was designed to better understand the spatial variations of heterotrophic flagellate and ciliate diversity in these water masses in different environmental settings of the Arabian Sea. Using high-throughput sequencing of heterotrophic marker genes (V4 region of the hypervariable SSU rDNA), attempt was made to analyse community structures of these functional protistan groups at open ocean sites, coastal regions and two estuaries.

5.2 Material and methods

5.2.1 Study sites and sample analyses

Samples for the present study were collected during the 56th cruise of R/V *Sindhu Sankalp*, during the period from 18th October 2013 to 2nd November 2013. The areas sampled included the open ocean (the Arabian Sea Time Series station, ASTS located at 17°N, 68°E; water depth 3,600 m), the outer continental shelf (station G12 located at 15.24°N, 72.98°E; water depth 160 m), the inner continental shelf (station G5 located at 15.50°N, 73.67°E; water depth 26 m), the Mandovi estuary (station M located at 15.49°N, 73.81°E; water depth 5 m) and the Zuari estuary (station Z located at 15.4°N, 73.9°E; water depth 6 m) (Fig. 35). Water samples were taken from four depths in the open ocean (surface-oxic, 103 m-hypoxic, 134 m-upper suboxic and 190 m-lower suboxic). Three depths were sampled over the outer shelf (surface-oxic, 80 m-upper hypoxic, 120 m-lower hypoxic). At station G5, samples were collected from three depths (surface-upper oxic, 8 m-lower oxic, and 24 m-anoxic). The estuarine stations were only sampled at the surface and close to the bottom, where oxic conditions prevailed (Table 33). The criteria used for defining the state of oxygenation were according to Naqvi et al. (2010): oxic (DO > 62 µM), hypoxic (< 62 µM), suboxic (< 4.5 µM) and anoxic (0 µM). Samples were collected using 10-litre Niskin bottles mounted on a Sea-Bird Electronics CTD (conductivity–temperature–depth)-Rosette system that also provided vertical profiles of temperature and salinity in the water column. Dissolved oxygen (O_2), nutrients (NO_3^-) and (NO_2^-) were measured on board the ship within a few hours of collection, following the titrimetric Winkler's method and the automated colorimetric procedures adopted for a SKALAR autoanalyser, respectively (Grasshoff et al. 1983). One litre of the sample was collected for chlorophyll analysis and immediately filtered through a GF/F filter. Chlorophyll *a* (Chl *a*) was extracted from the filters with 90% acetone for 24 hours in the dark at -20°C and fluorescence was measured using a fluorometer (Turner Designs, Model no. 10-AU).

For DNA analyses, plankton was collected from up to 5 litres of water on a Durapore membrane (47 mm, 0.65 µm, Millipore, Germany) using a peristaltic pump. Filters were

immediately placed into cryovials, preserved with 3 ml RNAlater (Ambion, Germany) and stored at −20°C until DNA extraction.

5.2.2 Total DNA extraction

The DNA was extracted directly from the Durapore membrane filters, using Qiagen's All Prep DNA/RNA kit according to the manufacturer's guidelines. Three replicates of each sample were extracted and pooled. Bulk DNA was quantified spectrophotometrically (NanoDrop 2000, Thermo Scientific, Wilmington, DE, USA); the detailed quantification for each sampling site is provided in Table 34. The DNA extracts were amplified with PCR primers specific for the hypervariable V4 region of the 18S rRNA gene (TAReuk454FWD1: 5′CCAGCA(G/C)C(C/T)GCGGTAATTCC3';TAReukREV3′5′ACTTTCGTTCTTGAT(C/T) (A/G)A-3′, Stoeck et al., 2010). The PCR reaction included 50–100 ng of DNA template in a 50 µl final volume with 1 µl of Phusion High-Fidelity DNA polymerase (Finzymes, New England Biolabs, Ipswich, MA, USA), 1x Phusion GC Buffer (New England Biolabs, Ipswich, MA, USA), 200 µM each of deoxynucleotide triphosphate and 0.5 µM oligonucleotide primer. The PCR protocol employed an initial denaturation (30 s at 9°C) followed by 30 identical amplification cycles, denaturation (at 98°C for 10 s, annealing at 59°C for 10 s and extension at 72°C for 30 s) and a final extension at 72°C for 30 s. Purified PCR products (Qiagen's MinElute Kit) were sent to SeqIT, Kaiserslautern, Germany, for library preparation and sequencing on an Illumina Miseq platform (2x250 bp paired-end).

5.2.3 Amplicon data processing and OTU (operational taxonomic units) analysis

The total raw sequences were denoised with Acacia (Bragg et al. 2012). Denoising, data cleaning and chimera checking were performed using QIIME (Caporaso et al. 2010). The quality reads were taken into account with particular barcodes and primers, having a minimum length of 300 bp. Phylotype clustering was performed using Uclust (Edgar 2010) at different sequence similarities (100–90%). The length distribution of the tags was plotted in R (R Core Team 2012). For taxonomic classifications and statistical diversity, OTUs at 97%

sequence similarity were used (Nebel et al. 2011; Dunthorn et al. 2014a). The core (=longest and thus most informative) sequence for each phylotype at 97% was extracted in a FASTA file, which was analysed with JAguc software (Nebel et al. 2011). The JAguc employed BLASTn searches, with algorithm parameters adjusted for short (200–500 bp) reads (-m 7 -r 5 -q -4 -G 8 -E 6 -b 50). The output files as a custom script QIIME's OTUpipe (seq_otus.txt) and JAguc (taxonomic tree for analysed representative sequence) were merged to a biome file containing information about OTU IDs, number of sequences per OTU and per sample as well as taxonomic affiliations. Non-target OTUs (metazoans and embryophytes) were excluded, and the resulting file was used for statistical analysis. From this total eukaryotic data set, we here analysed the HFs (genus-level, based on published articles, see Supplementary File S1) and ciliates as main contributors to the heterotrophic protistan plankton.

5.2.4 Statistical data analysis

Indices of alpha and beta diversity were calculated using QIIME v.1.8.0 (Caporaso et al., 2010). Shannon entropy was used to avoid the biased abundant taxa and to obtain a meaningful comparison of microbial eukaryotic diversity with reference to molecular datasets (Haegeman et al., 2013). For this interpretation, the Shannon index was converted to true diversity in the form of effective number of species (ENS = equally common species) (Jost, 2006). For this purpose, data were first normalised and resampled 1,000 times to account for uneven sample sizes (Filker et al., 2015). Jaccard distance dendrograms were drawn using the application of the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. Further multivariate statistical analysis was conducted on relative sequence abundance of HFs and ciliates, using the software package CANOCO 4.5 (ter Braak and Smilauer, 2002). Prior to statistical analysis, data were logarithmically (log x + 1) transformed to meet the normality assumptions. The relationship between community composition patterns and associated environmental gradients was investigated by canonical correspondence analysis (CCA). Prior to CCA, detrended correspondence analysis (DCA) was performed to

determine the variability within the dataset; the length of the first axis gradients for all data sets was > 2 standard deviation unit (SD), indicating the unimodal character of the data set. Due to the unimodal characteristics, CCA was performed (ter Braak and Smilauer, 2002). Data were run under a reduced model. Monte Carlo significance tests of the first coordination axes and all canonical axes together were performed using all available environmental factors. The correlations of individual HF and ciliate communities with the environmental factors were assessed through Spearman correlation analysis, using the software package SPSS 16.

5.2.5 Accession numbers

All sequences obtained from the study were deposited in the National Center for Biotechnology Information (NCBI), Sequence Read Archive (SRA), under accession numbers SRR5217061, SRR5217067, SRR5217068, SRR5217151 and SRR5217173.

5.3 Results

5.3.1 Environmental parameters

Environmental parameters at each sampling site are presented in Table 33. O_2 concentrations varied widely. At the open ocean station (ASTS), the concentrations were 205 µM at the surface (designated as oxic), 43 µM at 103 m (hypoxic), 4.1 µM at 134 m (suboxic) and 4.4 µM at 190 m (suboxic). The outer continental shelf station (G12) had 197 µM at the surface (oxic), 14.6 µM at 80 m (hypoxic) and 6.9 µM at 120 m (hypoxic). The inner shelf station (G5) had 153 µM O_2 (oxic) at the surface, 82 µM at 8 m (oxic) and an undetectable concentration close to the bottom (24 m), indicating anoxia. The two estuarine stations did not exhibit hypoxic conditions, although the O_2 levels were lower than the saturation values: 161 µM at the surface and 156 µM in near-bottom waters in the Mandovi estuary, and 158 µM at the surface and 145 µM in near-bottom waters in the Zuari estuary.

Chl *a* was conspicuously high in anoxic near-bottom waters at station G5 (3.48 µg l^{-1}). The shallow estuarine stations were well-mixed with similar Chl *a* levels in surface and near-bottom waters. Overall, in the oxic surface water, Chl *a* increased from the open ocean to the

estuaries, with the highest concentration (7.5 μg l^{-1}) observed in the surface water of the Mandovi estuary.

Nitrite (NO_2^-) had accumulated in the suboxic waters at ASTS, with the highest concentration of 4 μM at the lower suboxic depth (190 m). At the other stations, nitrite concentrations were low or below the detection limit. Nitrate (NO_3^-) was below the detection limit in surface waters, except for the two estuarine stations. The highest concentration (~19 μM) at the ASTS station was recorded at the upper hypoxic depth. The concentration was even higher (~25 μM) in the hypoxic bottom waters of station G12. Nitrate was absent in the water column at the inner shelf station (G5). The surface and near-bottom waters of two estuarine stations contained low amounts of nitrate, but well above the detection limit, with 2–3-fold higher concentrations at the surface (1.4–1.6 μM) than in the depth.

Surface salinity at the ASTS station (36.9 PSU) was considerably higher than at stations G12 (34.3 PSU) and G5 (34.2 PSU). The estuarine surface samples were characterized by brackish water. The hypoxic water at the ASTS was also more saline (36.2 PSU) than that over the outer continental shelf (34.5–34.6 PSU). However, the suboxic waters of the open ocean (35.7–35.8 PSU) and the anoxic water at G5 (35.3 PSU) had comparable salinities. A large salinity gradient existed between the surface (26.1–27.9 PSU) and near-bottom (27.3–30.3 PSU) waters in the estuaries, with more stratified conditions occurring in the Mandovi (salinity difference 2.4 PSU) than in the Zuari estuary (salinity difference 1.2 PSU).

Sea surface temperatures (SST) in the open Ocean and outer shelf stations were higher (29.1°C) than those in the inner shelf and estuarine stations (25.5–28.9°C). Hypoxic water at the ASTS was warmer (22.76°C) than the waters over the outer shelf (18.1–18.7°C). The deeper suboxic waters at ASTS were cooler (16.4–19.1°C). At the inner shelf station G5, temperature varied from 27.16°C at the surface to 21.89°C close to the bottom. There was little difference in the temperature recorded between surface and bottom waters at both estuarine systems, although the waters of the Zuari estuary were somewhat warmer (by

~1.5°C) than those of the Mandovi estuary. Entire profile data for temperature, salinity, dissolved oxygen, chlorophyll *a*, nitrate and nitrite are available in Supplementary File S3.

Sequence data overview

Low-quality reads and the sequences assigned to metazoans and embryophytes were removed from the total eukaryotic sample. After the removal of non-targets, a total of 1,395,168 sequences remained. Subsequently, the singletons and doubletons were removed, and the remaining 1,387,818 high-quality sequences represented target eukaryotes, accounting for 51% of total raw sequences. Of these, 152,858 reads (6%) represented heterotrophic flagellates (HF) and 7,853 reads (0.3%) were assigned to ciliates. The detailed distribution of HF and ciliate V4 SSU rDNA sequences for the sampling sites under study are listed in Table 35.

5.3.2 Community composition

The relative community compositions of heterotrophic flagellates (HF) and ciliates were evaluated in spatially distinct study areas.

Overall, 15 genera of the HF community were documented at the open ocean site, among which *Gyrodinium* (45%) and *Amoebophyra* (27%) were the dominant genera (Fig. 36). *Gyrodinium* was the dominant taxon (69% of HF) in oxic water, and accounted for 36% in hypoxic water. The suboxic strata (134 m and 190 m) supported a different HF community structure, with predominantly *Polykrikos* in the upper zone (35%) and the genus *Amoebophyra* (70%) in the lower zone. Interestingly, the genus *Monosiga* was present in the hypoxic (0.04%) and suboxic zones (0.3% at 134 m and 4% at 190 m) (Fig. 37).

The ciliate community at the open ocean station included 10 higher-level taxon groups, of which 53% were represented by Bryometopida; the lowest contribution was from Stichotrichia (0.7%) (Fig. 38). Oligotrichia was the dominant taxon (34%) in oxic waters, followed by Apostomatida (20%). Bursariomorphida was the dominant taxon (22%) in hypoxic water, whereas Scuticociliata (35%) was the most diverse subclass in the upper

suboxic water column. Bryometopida was dominant in terms of diversity (88% of total Ciliophora community) at the lower suboxic depth (Fig. 39).

Overall, the HF community in waters of the outer continental shelf station, represented by 16 genera, included *Gyrodinium* (77%), followed by *Amoebophyra* (18%). *Protaspis* and *Rhizaria,* the latter accounting for less than 1% of the HF community composition (Fig. 36). The oxic layer was dominated by the genus *Gyrodinium* (80%). Both the upper and lower hypoxic water columns were characterised by the dominance (55%) of the genus *Amoebophyra* (55%). Unclassified genera belonging to *Pfiesteriaceae, Monosiga* and *Abedinium* were present exclusively in hypoxic waters. The unclassified genus *Cryptomonas* (3%) was exclusive to the upper hypoxic water column (Fig. 37).

The ciliate community over the outer shelf was composed of 10 taxon groups, dominated by Oligotrichia (55%) and Choreotrichia (31%). Stichotrichia and Scuticociliatia only slightly (< 1%) contributed to this community (Fig. 38). Oxic waters were dominated by Oligotrichia (57%), followed by Choreotrichia (32%). Apostomatida (25 and 20%) and Astomatida (25 and 20%, respectively) were most diverse in the upper and lower hypoxic zones. Scuticociliata and Stichotrichia were restricted to the lower hypoxic water column (Fig. 39).

The HF community at the inner shelf station was mainly represented by the genera *Gyrodinium* (75%) and *Amoebophyra* (17%); (Fig. 36). Even though the oxygen regimes at this station were very similar at the surface and at a depth of 8 m, the HF community composition varied notably. In contrast, unclassified *Picozoa* showed a gradual decrease as oxygen level decreased with depth. The genus *Gyrodinium* was dominant throughout the water column (Fig. 37). The ciliate community at this shallow coastal station was dominated by Choreotrichia (61%) and Oligotrichia (23%). Astomatida was recorded in low abundance (< 1%) at this station (Fig. 38). The taxon group Choreotrichia showed a gradual increase with decreasing dissolved oxygen levels with depth, unlike Oligotrichia, which gradually decreased with the depletion of oxygen. Choreotrichia was abundant (64%) in near-bottom

water. Scuticociliata and Cyrtophoria were the distinct groups in lower oxic and anoxic waters (Fig. 39).

The water columns were oxygenated in the well-mixed and shallow Mandovi and Zuari estuaries. The HF community of the estuarine sites included the genera *Gyrodinium* (52%), *Amoebophyra* (32%), *Protaspis* (2%), *Halocafeteria* (5%), *Eudubosquella* (3%), unclassified *Cryptomonas* (1%) and unclassified *Picozoa* (1%) (Fig. 36). In the Mandovi estuary, the genus *Gyrodinium* (56%) was predominant in surface waters, which decreased by one order of magnitude in near-bottom waters (Fig. 37). In both estuaries, the HF community was less diverse in surface waters than at the bottom.

The ciliate community at the estuarine sites was mainly composed of Choreotrichia (34%), Oligotrichia (46%), Peritrichia (17%) and Haptoria (3%) (Fig. 38). Also, the ciliate community structure varied in the shallow water column. The surface waters of Mandovi were dominated by Oligotrichia (95%) and Prorodontida (4%), while in the bottom water, Oligotrichia (74%) and Choreotrichia (14%) were the major groups (Fig. 39). Surface waters at the Zuari estuary were dominated by Choreotrichia (62%) and Peritrichia (32%), while the bottom water had Peritrichia (38%), Choreotrichia (31%), Oligotrichia (26%) and Stichotrichia (5%); (Fig. 39).

5.3.3 Alpha diversity

In the open Ocean and over the outer continental shelf, higher HF diversity was observed in the hypoxic strata, compared to inner shelf station (Fig. 40). In the estuaries, higher diversity was recorded in near-bottom waters of the Zuari estuary compared to the surface waters at this site. In contrast, the Mandovi estuary harboured a higher HF diversity at the surface.

The lowest ciliate diversity was recorded for the lower suboxic layer at 190 m at the ASTS and the highest in the oxic and hypoxic waters at this open ocean site. Over the outer shelf, ciliate diversity was greater in the oxic water column compared to hypoxic waters. Over the

inner shelf, ciliate diversity was lower in the anoxic near-bottom waters. In the Mandovi and Zuari estuaries, ciliates were more diverse at the surface.

5.3.4 Partitioning of diversity

Jaccard distance matrices (beta diversity) showed different HF community clusters at various sites (Fig. 41). The UPGMA clustering, based on OTU abundance of sampling sites at different depths, identified four clusters, whereas the open ocean upper suboxic and lower suboxic communities formed one cluster with close similarity, unlike the hypoxic and oxic water column. The upper hypoxic waters over the outer continental shelf exhibited a close similarity with the lower hypoxic waters, but not with the oxic waters. At the inner shelf station, the upper oxic, lower oxic and anoxic strata formed a cluster showing no clear difference between the three strata. By contrast, the HF communities in the Zuari and Mandovi estuaries were quite different. As per Jaccard distance analysis, the surface and bottom waters in the Mandovi had a similar HF community, while in the Zuari estuary, different HF communities inhabited the surface and bottom waters (Fig. 41 a).

For the ciliate community, UPGMA analysis yielded three clusters where lower suboxic and oxic waters of the open ocean station were different from the hypoxic and upper suboxic waters, which formed one cluster. The upper and lower hypoxic waters over the outer shelf were not included in the cluster analysis due to low sequence numbers. High community similarity was noticed over the inner shelf among the upper oxic, lower oxic and anoxic waters. In the Zuari estuary, the bottom water community structure was distinct from that of the surface water and the waters of the Mandovi estuary (Fig. 41 b).

5.3.5 Impact of environmental variables on heterotrophic protist assemblages

The first two CCA axes explained 65% of the cumulative variance of HF communities (Fig. 42.a). Nitrate and salinity were significant explanatory environmental variables for the structuring of HF communities ($p < 0.05$; Table 36). Unclassified *Picozoa*, *Polykrikos*, *Podolampas* and *Amoebophyra* preferably occurred at low-oxygen sites of the open Ocean

and outer shelf stations. In waters of the inner shelf, unclassified Picozoa, *Pelagodinium* and *Warnoia* occurred at elevated dissolved oxygen concentrations and temperatures. *Protaspis*, *Halocafeteria* and unclassified *Cryptomonas* preferred lower salinities and occurred at sites with higher Chl *a* values. *Cercomonas* and *Planomonas* were positively related to Chl *a*, temperature and dissolved oxygen. The HNF genus *Monosiga* correlated more strongly with NO_3^- (r = 0.78; p = 0.001) preferring suboxic conditions of the open ocean water column (Supplementary file S2).

The first two CCA axes explained 66% of the cumulative variance of the ciliate communities. Chl *a* was significantly related to axis 1 and NO_2^- to axis 2 ($p < 0.05$; Table 37). Specifically, Haptoria, Peritrichia and Prorodontida correlated positively with Chl *a* at low-saline estuarine stations. Bryometopida, Scuticociliata, Stichotrichia and Colpodida were recorded in suboxic waters of the open ocean, which were characterised by high NO_2^- levels (Fig. 42 b). Cyrtophoria, Bursariomorphida, Astomatida and Apostomatida, were present in the open ocean samples (surface and upper hypoxic waters) and in all samples of the continental shelf, which were characterized by higher salinity and lower Chl *a*.

5.4 Discussion

5.4.1 Environmental selection and community structure

Autotrophic and heterotrophic organisms are getting more attention. Recently, taxonomic variations of flagellate and ciliate in OMZ waters were studied in the Eastern Tropical South Pacific (ETSP) and the Eastern Tropical North Pacific (ETNP) (Parris et al., 2014; Duret et al., 2015). The composition of heterotrophic flagellates and ciliates exhibited a patchy distribution in the pelagic and mesopelagic waters of the eastern Arabian Sea. In oxygenated waters, the heterotrophic dinoflagellate *Gyrodinium* showed its dominance at all sampling sites, except the subsurface waters of the Mandovi estuary during the fall inter-monsoon. This genus is a major component of heterotrophic community in oxygenated surface and subsurface waters due to its wide range of prey (picoplankton to large diatoms) and its

adaptability towards different environmental conditions (Jyothibabu et al., 2008). Other studies also concluded that this genus grazes actively on nano- and micro- phytoplankton cells (Gaines and Elbrachter, 1987). In the open ocean, the parasitoid dinoflagellate genus *Amoebophyra*, belonging to Syndiniales (alveolates), was dominant in the lower suboxic water at 190 m, close to the core of the OMZ. This observation agrees well with previous reports of the abundance of the Syndiniales within the OMZ of ETSP (Parris et al., 2014). *Amoebophyra* infects bloom-forming algae (Miller et al., 2012; Chambouvet et al., 2008), thereby regulating algal blooms. It also produces lumps of active dinospores, which could be the reason behind the dominance of this genus. Its high abundance in suboxic waters supports the earlier reported function as endoparasite of macrozooans and other protists (Chambouvet et al., 2008). This genus could be vertically transported along with the sinking organic particles or may exist within free-floating host eukaryotes. The high grazing pressure of predators on flagellates may be the reason for their lower abundance in oxic and hypoxic strata, and the limited grazing might account for the enhanced abundance within the OMZ core. The observed distribution patterns suggests that niche specialisation is co-influenced mainly by oxygen concentrations and biological interactions. Previous work on eukaryotic microbial communities in the ETNP noted the taxonomically different protists in the OMZ water column, although oxygen concentration was not the sole parameter influencing the community structure (Duret et al., 2015).

The distribution of the heterotrophic nanoflagellate genus *Monosiga* (Choanoflagellida) appears to be considerably influenced by low oxygen conditions. The gradual increase in the abundance of this genus from hypoxic to deeper suboxic depths in the open ocean suggests its preference for oxygen-depleted waters. The abundance of nanoflagellate genera at deeper suboxic depths may be supported by a rich bacterial biomass (Naqvi et al., 1993), in addition to lower grazing pressure from predators. Thus, the nanoflagellates may serve as important participants in the carbon cycling through the microbial loop. It should be noted that these

organisms were far less abundant in hypoxic waters over the outer shelf and completely absent at the inner shelf and estuarine stations, which shows that oxygen gradients create a boundary separating organisms that can adapt to different levels of oxygen (Borcard et al. 2004). Low oxygen marine waters support diverse microeukaryote communities with a complex pattern of taxonomic structures (Parris et al. 2014). Moreover, the positive correlation ($r = 0.78$; $p = 0.001$) of *Monosiga* abundance with NO_3^- indicates that this species is a potential denitrifier in the suboxic ambience. Other HF species showed either a weak or no correlation with NO_3^- and NO_2^-, which implies that they play no or an insignificant role in denitrification, and their distribution is not controlled by the vertical variability of NO_3^-/NO_2^- concentrations. There is, as yet, no experimental evidence of HF carrying out denitrification, and our study shows only a possibility of HF as denitrifier in the Arabian Sea OMZ.

Ciliates are one of the dominant groups of the heterotrophic community in the south-eastern Arabian Sea, and their abundance varies spatially during the late summer monsoon (Jyothibabu et al., 2008). In the present study, the dominance of ciliate taxonomic groups varied in different niches, with the dominance of Oligotrichia in the oxic water column of the open ocean, outer shelf and the Mandovi estuary. Choreotrichia was dominant in inner shelf waters and the Zuari estuary. Food availability in the oxic water column, as well as the other environmental factors such as temperature and dissolved oxygen, could be explanatory variables. The CCA identified Chl *a* as the most important parameter for the dominance of Peritrichia, Oligotrichia and Choreotrichia, many of which feed on smaller pigmented algae. In addition to Chl *a*, variations in dissolved oxygen and temperature further add to changes in the community structure in oxic waters at oceanic and inner shelf stations. In a recent study on global ocean ciliate plankton patterns, Gimmler et al. (2016) also identified these environmental parameters as important factors controlling the presence (and abundance) of numerous specific ciliate genera. Earlier studies in the Arabian Sea during the late summer monsoon reported that the dominance of ciliates at the inshore stations was promoted by the

abundance of pigmented flagellates and diatoms in the water column (Jyothibabu et al., 2008). As in the open Ocean and outer shelf stations, the Mandovi estuary (both at the surface and close to the bottom) was dominated by Oligotrichia. In the Zuari estuary, Choreotrichia dominated in the surface water, while Peritrichia was most abundant in the bottom water. These contrasting patterns between the two estuaries were possible because tidal currents in the Zuari are stronger than in the Mandovi estuary (Manoj et al., 2009).

The diversity of protists (heterotrophic flagellates as well as total ciliates) was high in the oxygen-deficient strata (hypoxic and suboxic waters) of the open ocean and inner shelf waters. Previous studies have shown that the particulate organic carbon (POC) content is highest during the southwest monsoon, which supports a high heterotrophic biomass in the Arabian Sea (Gauns et al., 2005). It is likely that heterotrophic protists favour low oxygen water, where the abundant organic matter promotes a high biomass of bacteria which, in turn, serve as a rich food source for HF and ciliates (Azam et al., 1983).

5.4.2 Partitioning of diversity

Jaccard incidence-based cluster analysis shows that the HF as well as the ciliate community exhibit a similar pattern in oxic, suboxic and anoxic waters of the inner shelf station, whereas waters over the outer shelf show different communities at the oxic and two suboxic depths. The open ocean station showed a higher variability in ciliate community composition across the oxygen gradient compared to the inner shelf and outer shelf stations. The observed community variation could be attributed to the effect of changing environmental conditions at intermediate distance scales (3–10,000 km), as suggested for microbes (Martiny et al., 2006). In the present study, a clear difference in environmental conditions was noticed from the oceanic to estuarine stations. Surprisingly, the two estuaries showed distinct patterns: while the Mandovi surface and bottom waters displayed a similar community structure, the Zuari estuary did not. This dissimilarity of heterotrophic communities (ciliate and flagellates) may be attributed to hydrographical differences (i.e. stronger tidal currents in surface than the

bottom water in the Zuari estuary); in addition, differences in runoff and the width of the mouths of these two estuaries may contribute to contrasting community patterns (Varma et al., 1975; Qasim and Sengupta, 1981). Among the ciliates, Bryometopida, Scuticociliata and Bursariomorphida dominated in the suboxic and hypoxic strata in the open ocean. The community pattern showed variability, presumably driven by changes in the ambient oxygen concentration. The high abundance of Bryometopida at a lower suboxic depth is apparently related to a high nitrite concentration. This dominant ciliate community may coincide with secondary nitrite maxima, which is a prominent feature in the suboxic waters of the Arabian Sea (Lam et al., 2011; Morrison et al., 1999). This indicates the role of ciliates in the denitrification process, as they are able to respire nitrate in the absence of oxygen, forming a nitrite peak. Earlier reports also suggested the freshwater ciliate *Loxodes* as a significant contributor to denitrification in eutrophic lakes (Aleya et al., 1992; Finlay, 1985). Overall, the ciliate community showed a positive correlation with NO_2^- in our study. However, on the individual species level, none of the ciliate species showed a strong correlation with NO_2^-. Oligotrichia showed a relatively strong correlation with DO (r = 0.64; p = 0.013) and a negative correlation with NO_3^- (r = -0.69; p = 0.006), which suggests its preference for oxygen-depleted waters. The overall abundance of ciliates within OMZ suggests that there could be substrates other than NO_3^- and NO_2^- which the ciliate community largely depends on. It is possible that the ciliates preferably consume denitrifying bacteria, which particularly reduces NO_2 and thus creates an impression of a weak correlation of ciliate abundance with NO_2. This further suggests that the denitrifying microbes were abundant in the intermediate layers of the OMZ water column (Jayakumar et al., 2009), thus acting as a plentiful food source for these ciliate communities. The sustainability of these communities implies that they are adapted to live in low-oxygen waters. Moreover, symbiotic associations between ciliates and bacteria are a common feature in oxygen-depleted waters (Gast et al., 2009; Edgcomb et al., 2011). In this symbiotic process, methanogens act as endosymbionts and make ciliates

adapt to the anaerobic environment (van Hoeck et al., 2000). Within hypoxic waters, the ciliate community composition varied notably among the sites. Besides oxygen, other environmental parameters, such as salinity, may also influence the community composition. The results of the CCA also suggest a strong influence of salinity on the distribution of Apostomatida, Astomatida and Bursariomorphida in hypoxic waters of the open ocean and outer shelf. Salt was identified as a major factor in relation to ciliate communities (Forster et al., 2012). The presence of Scuticociliata and Stichotrichia, albeit in lower numbers, in the lower hypoxic and upper hypoxic water column of the outer shelf suggests that they are also adapted to live in low-oxygen waters. Our observation agrees with earlier molecular surveys stating that scuticociliates are diverse and abundant members of the ciliate communities in oxygen-depleted marine habitats such as the Saanich Inlet, the Framvaren Fjord and the Cariaco Basin (Behnke et al., 2006; Edgcomb et al., 2011; Orsi et al., 2012).

At the inner shelf station, a clear difference in the community based on oxygen concentrations was not observed, even though this station was distinguished by the prevalence of a sulphidic condition in the bottom water. This lack of variability may be due to the shallow station depth, experiencing low levels of oxygen only for a short period of time (1-2 months). As the station is shallow, the sporadic wind-mixing events can introduce the surface community into the subsurface water; it is therefore likely that this community has evolved to adapt to anoxic bottom water. It is obvious that other biotic factors, particularly autotrophic picoplankton proliferating under low-oxygen conditions (Gauns M; unpublished data), may serve as the food source for these protist communities in coastal waters.

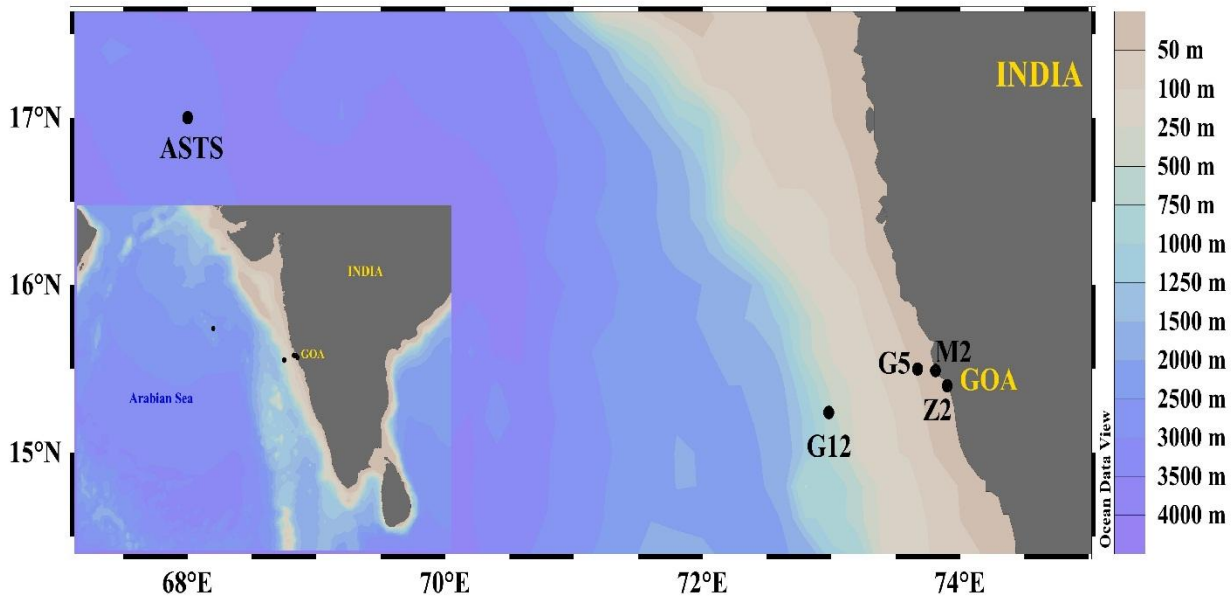

Figure 35. Map of the sampling sites located in different regions of the Arabian Sea ranging from estuarine to open ocean stations (Estuary: Mandovi-M2, Zuari-Z2; Open ocean-ASTS; Outer continental shelf-G12; Inner continental shelf-G5).

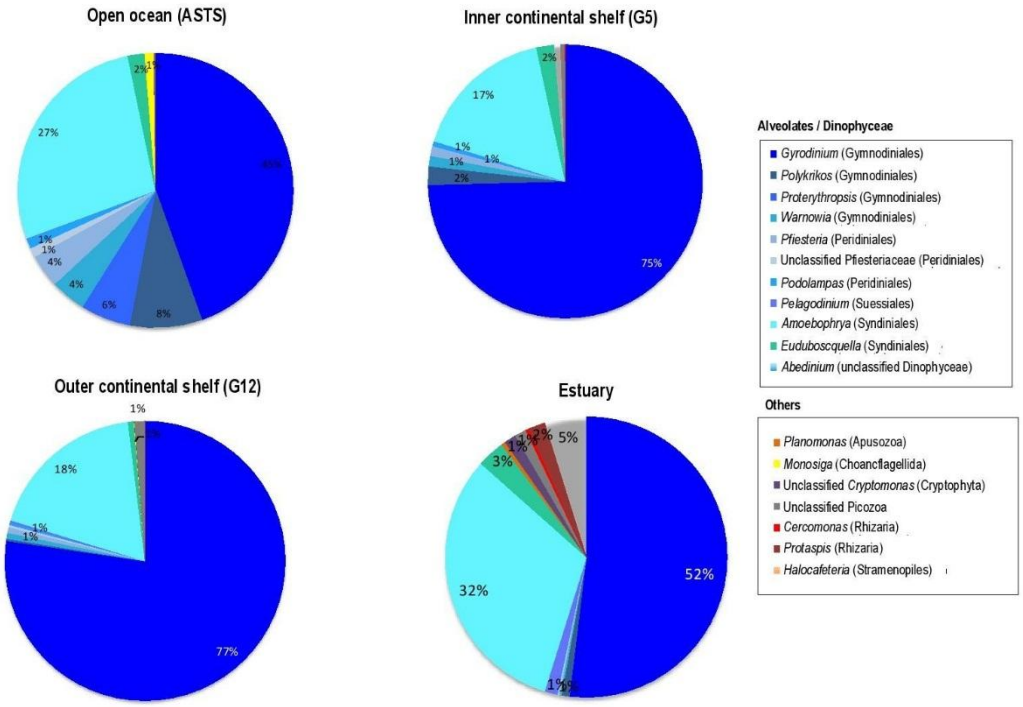

Figure 36. Taxonomic assignment (relative distribution) of heterotrophic flagellate (HF) OTUs at the investigated open ocean, continental shelf (inner and outer) and estuarine sites of the Arabian Sea.

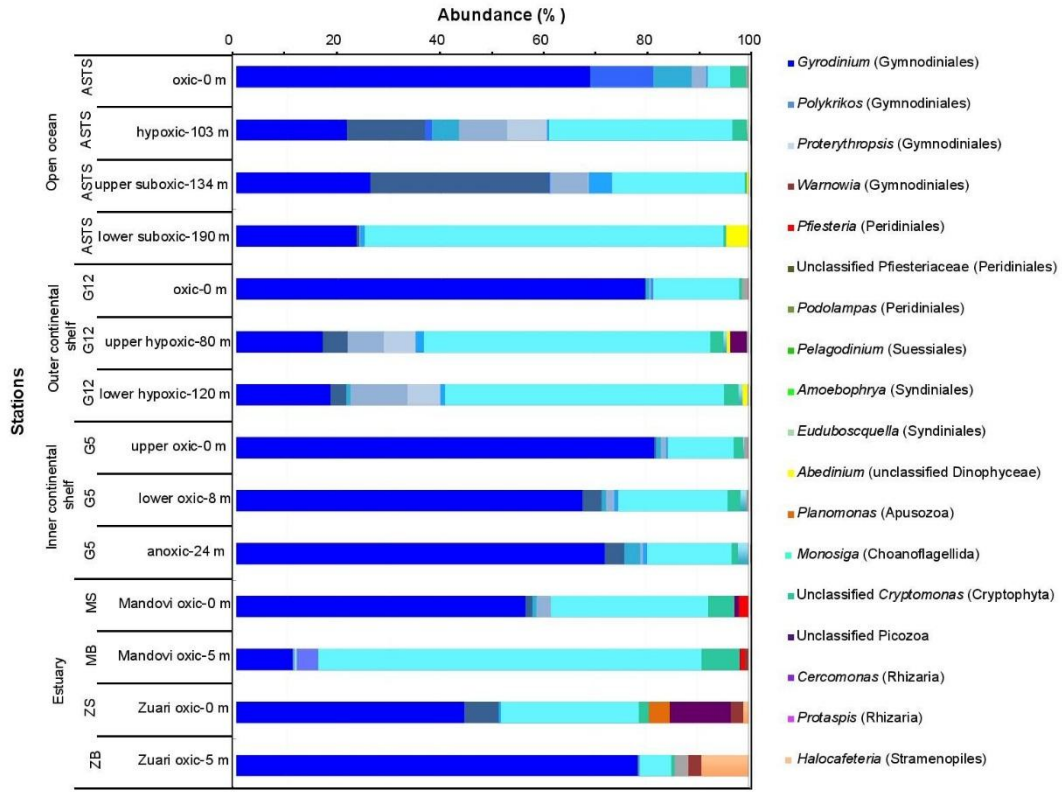

Figure 3

Figure 37. Heterotrophic flagellate community composition with reference to the degree of de-oxygenation at the sampling sites.

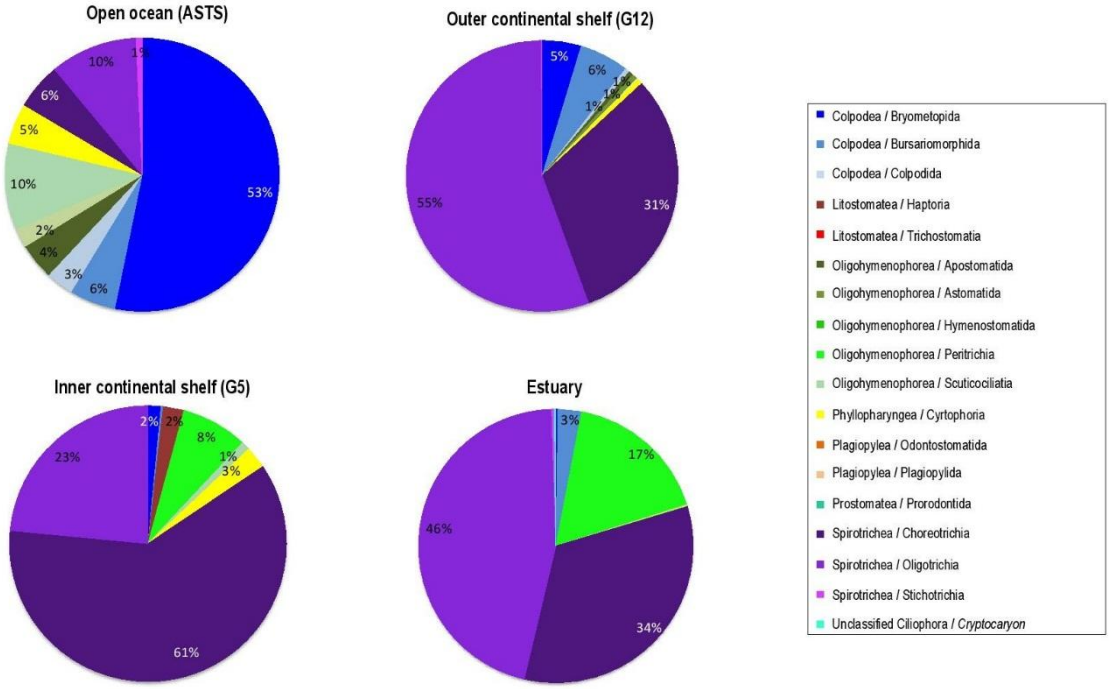

Figure 38. Taxonomic assignment (relative distribution) of ciliate OTUs at the investigated open ocean, continental shelf (inner and outer) and estuarine sites of the Arabian Sea.

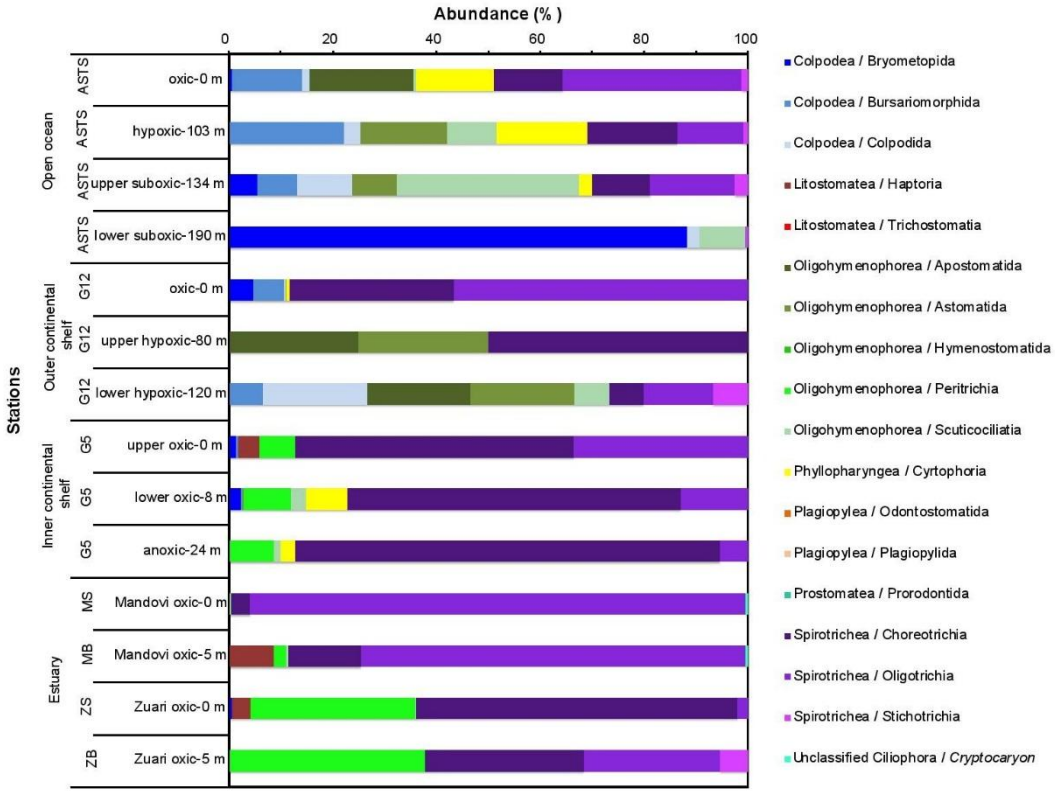

Figure 39. Ciliate community composition with reference to the degree of de-oxygenation at the sampling sites.

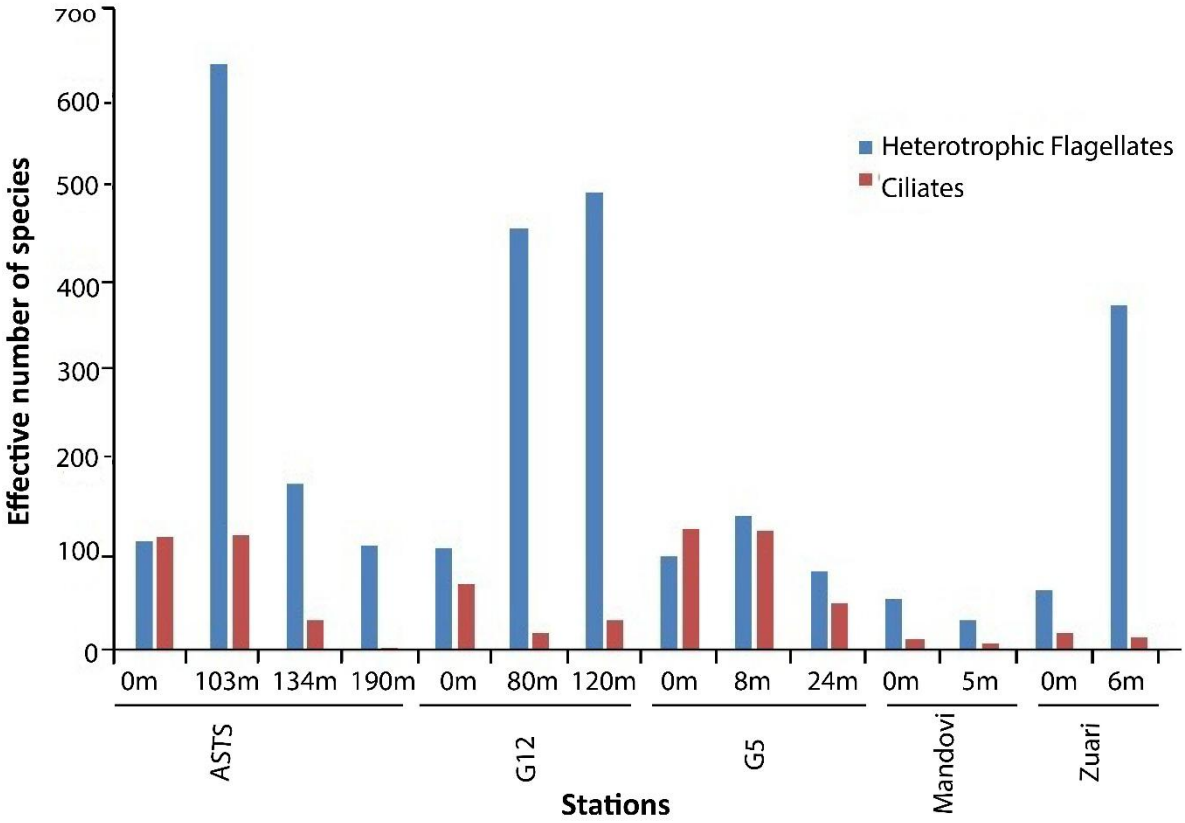

Figure 40. Shannon diversity index (Alpha diversity) for heterotrophic flagellates (HF) and ciliates at the sampling sites under study. Diversity values calculated are based on 97% similarity of ciliate and flagellate OTUs.

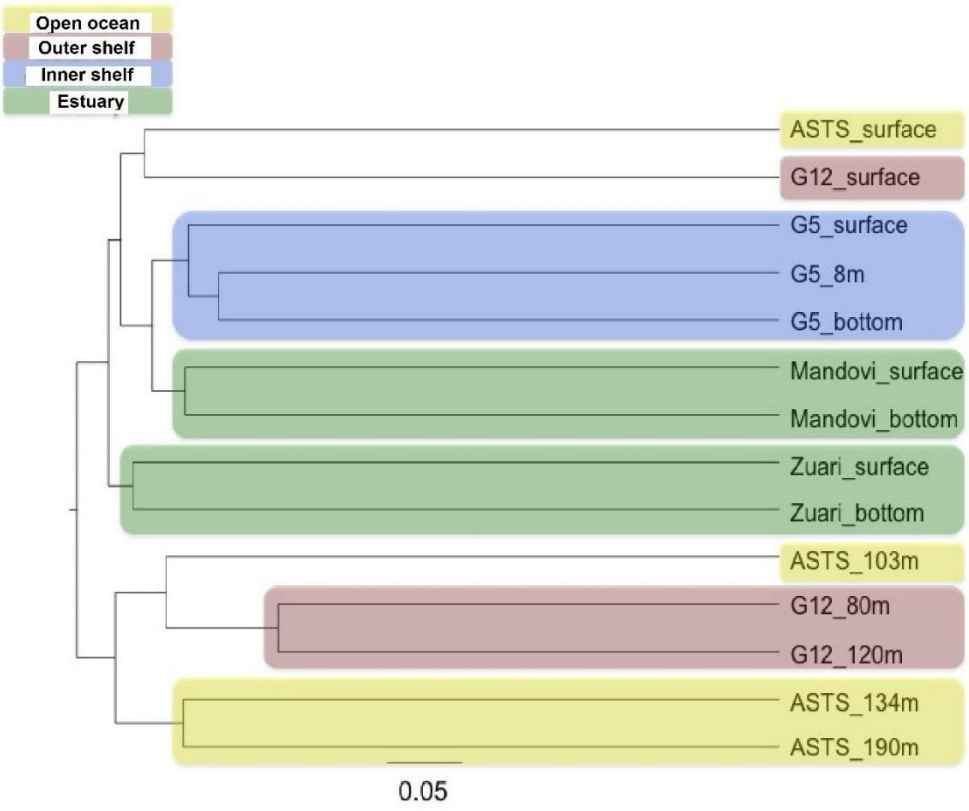

Figure 41 (a)

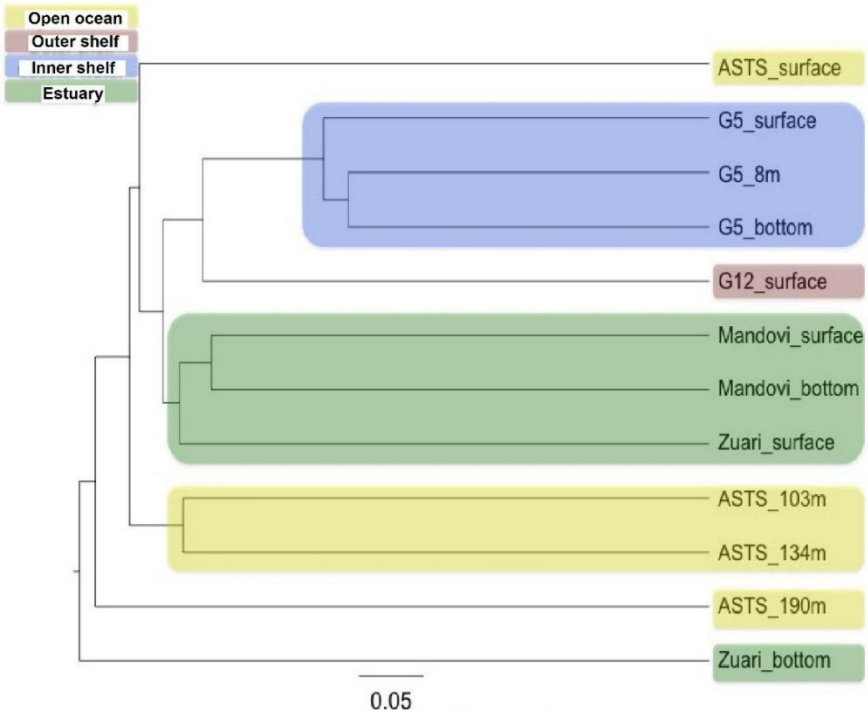

Figure 41. (b)

Figure 41. (a) UPGMA clustering of Jaccard beta diversity for heterotrophic flagellates (HF, OTUs called at 97% sequence similarity); (**b)** Clustering of Jaccard beta diversity for ciliates (OTUs called at 97% sequence similarity) in different regions of Arabian Sea.

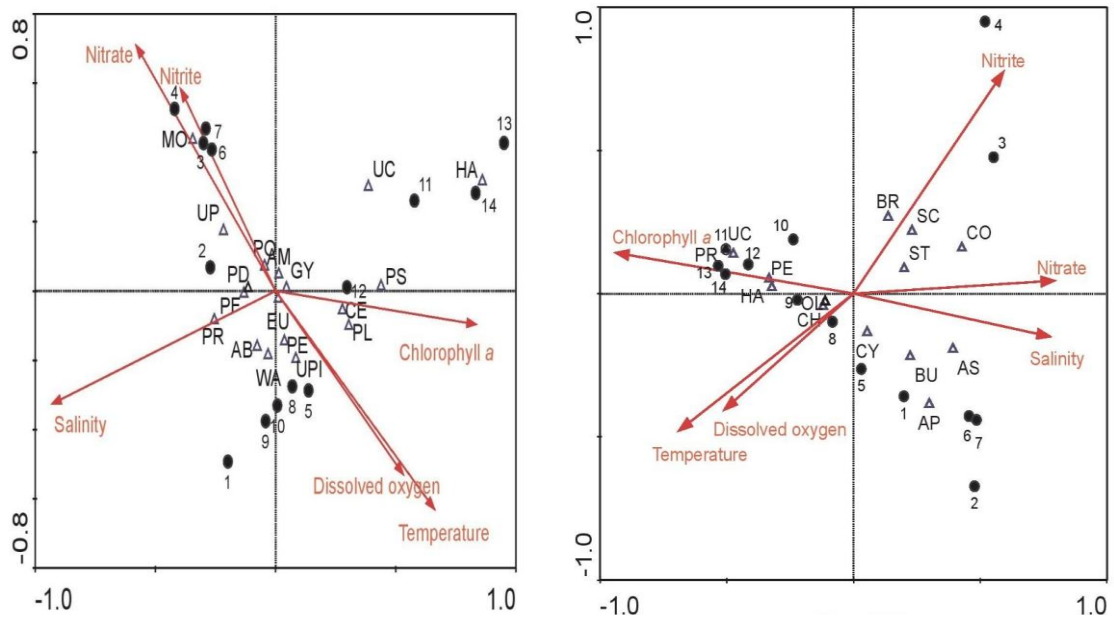

Figure 42. (a) **Figure 42. (b)**

Figure 42. Canonical correspondence analysis of (**a)** heterotrophic flagellates (HF) communities up to genera at four sites with respect to six environmental factors. HF communities represented as GY: *Gyrodinium*, PO: *Polykrikos*, PR: *Proterythropsis*, WA: *Warnowia*, PF: *Pfiesteria*, UP: Unclassified Pfisteriaceae, PD: *Podolampas*, PE: *Pelagodinium*, AM: *Amoebophyra*, EU: *Eduboscquella*, AB: *Abedinium*, PL: *Planomonas*, MO: *Monosiga*, UC: Unclassified *Cryptomonas*; UPI: Unclassified Picozoa, CE: *Cercomonas*, PS: *Protaspis*, HA: *Halocafeteria*; (**b)** Ciliate communities (subclasses) at 4 different sites with respect to six environmental factors. Ciliate communities are represented by BU: Bursariomorphida, CO: Colpodida, HA: Haptoria, AP: Apostomatida, AS: Astomatida, PE: Peritrichia, SC: Scuticociliata, CY: Cyrtophoria, PR: Prorodontida, CH: Choreotrichia, OL: Oligotrichia, ST: Stichotrichia, UC: Unclassified Ciliophora; 1 = Open ocean (ASTS-0m); 2 = Open ocean (ASTS-103 m); 3 = Open Ocean (ASTS-134 m); 4 = Open ocean (ASTS-190m); 5 = Outer continental shelf (G12-0m); 6 = Outer continental shelf (G12-80 m); 7 = Outer Continental shelf (G12-120 m); 8 = Inner continental shelf (G5-0 m); 9 = Inner continental shelf (G5-8 m); 10 = Inner continental shelf (G5-10 m); 11 = Mandovi (M-0 m); 12 = Mandovi (M-5 m); 13 = Zuari (Z-0 m); 14 = Zuari (Z-6 m); (a, b).

Table 33. Environmental parameters recorded at the four sampling sites: the open ocean (ASTS), outer continental shelf (G12), inner continental shelf (G5) and the Mandovi and Zuari estuaries.

Date and time	Station depths	Sampling stations	Temperature (°C)	Salinity (PSU)	Oxygen (µM)	Nitrate (µM)	Nitrite (µM)	Chlorophyll *a* (µg l^{-1})
2/11/13 3.50 AM	3600 m	ASTS (0 m)	28.85	36.903	205.3	0.55	BD	0.11
		ASTS(103 m)	22.76	36.268	43.0	18.92	0.08	0.08
		ASTS (134 m)	19.10	35.832	4.1	16.4	3.43	ND
		ASTS (190 m)	16.43	35.704	4.4	14.2	4.01	ND
19/10/13 10.09 AM	162 m	G12 (0 m)	29.16	34.319	197.7	BD	BD	1.00
		G12 (80 m)	18.78	34.623	14.6	22.87	BD	0.04
		G12 (120 m)	18.17	34.523	6.9	24.55	BD	0.02
18/10/13 9.21 AM	24 m	G5 (0 m)	27.17	34.233	153.6	BD	BD	2.54
		G5 (8 m)	25.54	34.858	82.4	BD	BD	4.98
		G5 (24 m)	21.90	35.357	0	BD	0.01	3.48
22/10/13 9.00 AM	5 m	M (0 m)	27.43	27.927	161.1	1.41	0.03	7.5
		M (5 m)	27.23	30.379	156.1	0.38	BD	7.12
23/10/13 9.40 AM	6 m	Z (0 m)	28.93	26.101	158.2	1.63	0.23	6.81
		Z (6 m)	28.76	27.395	145.7	0.79	0.19	6.61

Table 34. The water sample filtered and the bulk DNA was quantified spectrophotometrically (NanoDrop 2000) from the four sampling sites: the open ocean (ASTS), outer continental shelf (G12), inner continental shelf (G5) and the Mandovi and Zuari estuaries.

Sampling site	Sample filtered (L)	Measured DNA (ng/L)
ASTS (surface)	5	3.7
ASTS (103 m)	5	2.7
ASTS (134 m)	5	5.2
ASTS (190 m)	5	5.4
G12 (Surface)	2	16.2
G12 (80 m)	2	4
G12 (120 m)	2	2.3
G5 (surface)	2	8.6
G5 (8 m)	2	12.2
G5 (24 m)	2	7.3
M (surface)	1	8
M (5 m)	1	14.1
Z (surface)	1	8
Z (6 m)	1	7.5

Table 35. Overview of Illumina sequencing data sets for the open ocean, outer continental shelf, inner continental shelf and estuarine regions. % relates to total number of obtained raw sequences.

Sample ID	Raw sequences paired	High quality target eukaryotes	High quality target eukaryotes without single and double tones	High quality target heterotrophic flagellates (HF)	High quality target ciliates
		Sequence n (%)	Sequence n (%)	Sequence n (%)	Sequence n (%)
ASTS_surface	327354	113609 (35)	112380 (34)	30102 (9.2)	360 (0.1)
ASTS_103 m	252205	172875 (69)	171919 (68)	8975 (3.6)	126 (0)
ASTS_134 m	227350	111497 (49)	110890 (49)	11844 (5.2)	197 (0.1)
ASTS_190 m	185173	123886 (67)	123487 (67)	14910 (8.1)	999 (0.5)
G12_surface	290518	115635 (40)	114554 (39)	25442 (8.8)	879 (0.3)
G12_80 m	111567	5984 (5)	5849 (5)	308 (0.3)	8 (0.0)
G12_120 m	93441	7364 (8)	7218 (8)	792 (0.8)	15 (0.0)
G5_surface	254683	120655 (47)	119791 (47)	25813 (10.1)	1859 (0.7)
G5_8 m	176501	84013 (48)	83207 (47)	23232 (13.2)	879 (0.5)
G5_24 m	156937	29819 (19)	29544 (19)	7608 (4.8)	507 (0.3)
Mandovi_Surface	173529	138079 (80)	137949 (79)	218 (0.1)	733 (0.4)
Mandovi_5m	138098	105881 (77)	105755 (77)	1230 (0.9)	243 (0.2)
Zuari_surface	126545	94332 (75)	94240 (74)	406 (0.3)	937 (0.7)
Zuari_6m	233080	171539 (74)	171035 (73)	1978 (0.8)	111 (0.0)
Total	**2746981**	**1395168 (51)**	**1387818 (51)**	**152858 (6)**	**7853 (0.3)**

Table 36. CCA summary for heterotrophic flagellate (HF) communities with different environmental factors.

Marginal Effects			Conditional Effects		
Variable	Var. N	Lambda1	Lambda A	P	F
Salinity	2	0.19	0.19	0.001	4.41
Nitrate	4	0.14	0.12	0.001	3.12
Nitrite	5	0.1	0.07	0.047	2.14
Temperature	1	0.14	0.05	0.118	1.88
Dissolved oxygen	3	0.11	0.04	0.326	1.2
Chlorophyll *a*	6	0.15	0.03	0.529	0.84

Table 37. CCA summary for ciliate communities with different environmental factors.

Marginal Effects			Conditional Effects		
Variable	Var. N	Lambda1	Lambda A	P	F
Chlorophyll *a*	6	0.31	0.31	0.001	4.37
Nitrite	5	0.21	0.15	0.013	2.32
Nitrate	4	0.24	0.09	0.149	1.49
Temperature	1	0.21	0.08	0.27	1.3
Dissolved oxygen	3	0.16	0.06	0.345	1.13
Salinity	2	0.23	0.04	0.719	0.59

Chapter 6

6.1 Introduction:

It is well known that the heterotrophic flagellates (HF) play a significant role in the microbial food web, organic matter mineralization and biogeochemical cycling in the marine ecosystems (Sherr and Sherr, 2000). Ecologically important community including flagellates are known to vary with respect to space and time. Unravelling the mechanisms responsible for their patterns of diversity and species co-existence is one of the most important and challenging study in ecology (Brown, 1995; Gaston, 2000; Holyoak et al., 2005). Studying spatial variability of biodiversity is the best way to understand the impact of multiple environmental factors on community structure. These communities vary in their species composition and can be quite different among sites which are quite close geographically. The factors determining species composition and their variability in a certain region are the key to understand the environmental impact on site-specific community and their diversity (Weiher and Keddy, 1999). In recent years, ecologists have started to explore how evolutionary history is associated with community patterns. It reveals that certain species sharing a common phylogenetic history tend to have similar niches. Heat map analysis is a well-established approach in determining the communities that differ in their species composition owing to different in environmental settings. A heat map is a graphical representation of data where the individual values contained in a matrix are represented as colours (Zhao et al., 2014). There are many variations of heat map such as web heat map and treemap. Here, we focus on the biology heat map, which is typically used to represent the level of expression of genes across a number of comparable samples. Clusters of genes with similar or vastly different expression values are easily visible. Certainly, phylogenetics study attempts to use the information contained in a

phylogenetic relationship for understanding ecological and evolutionary processes explaining species distributions. In this study, I used heat map analysis in describing and interpreting patterns of HF communities based on their sites specific patterns of phylogenetic structure. This is to express community differences in composition or abundance in terms of metrics of phylogenetic diversity and composition.

6.2 Material methods

6.2.1 Sample analyses

Samples for the present study were collected during the 56th cruise of R.V.*Sindhu Sankalp*. The areas sampled is comprised of the open ocean (the Arabian Sea Time Series station ASTS located at 17°N, 68°E; water depth 3,600 m), outer continental shelf (station G12 located at 15.24°N, 72.98°E, water depth 160 m), inner comtinentalshelf (coastal station G5 located at 15.50°N, 73.67°E; water depth 26 m), the Mandovi Estuary (station M located at 15.49°N, 73.81°E; water depth 5 m) and the Zuari Estuary (station Z located at 15.4°N, 73.9°E; water depth 6 m); (Fig. 43). Water samples were taken from 4 depths in the open ocean with varying oxygen level i.e. surface (oxic zone), 103m (hypoxic zone, oxycline), 134m (upper OMZ, suboxic zone) and 190m (lower OMZ, suboxic zone). Three depths were sampled over the outer continental shelf i.e. surface (oxic zone), 80m (upper hypoxic zone), 120m (lower hypoxic zone). Over the inner continental shelf also samples were collected from 3 depths i.e. surface (upper oxic zone), 8m (lower oxic zone), 24m (anoxic zone) at station G5. The estuarine stations which were oxic sampling was done only at two depths i.e. near surface and close to the bottom. The criterion used for defining the oxygenation state of water column was given in Naqvi et al. (2010) i.e. oxic – (DO >62 µM), hypoxic (<62 µM), suboxic (<4.5 µM), and anoxic (0 µM).

6.2.2 Molecular analyses

Nucleic acid (rDNA) extraction, PCR, and sequencing were done in the same way as briefly described in Chapter 3 and 4.

6.2.3 Data analysis for phylogenetic composition

Phylogenetic composition of heterotrophic flagellates (HF) was accessed through a heat map analysis. The HF community composition was analyzed in two categories such as 1. Phylum level, and 2. Genus level. Heat maps were generated by the function of NMF package in R (a statistical package). I used "row" scaling function, which normalizes gene (18S rRNA) abundance data on the basis of Z scores that could be negative. The scaling is performed after row/column clustering. The colour scale with positive value indicates dissimilarity, whereas negative values show similarity. In all the figures heat maps describing the relative OTU abundance of the main lineages of HF communities across the different oxygen gradients of the open ocean (ASTS), outer continental shelf (G12), inner continental shelf shelf (G5), Mandovi (M) and Zuari (Z) estuarine systems.

6.3 Results

6.3.1 Open Ocean (ASTS)

Heat map analysis clearly showed the whole HF community in higher taxonomic groups of phylum at the surface and lower suboxic depth, was different from that at hypoxic and upper suboxic depths. In a brief note, the higher taxa alveolata, rhizaria and katablepharidophyta found at the surface were quite dissimilar from upper suboxic and lower suboxic depths. Also, the choanoflagellida community found at lower suboxic depth was different from oxic (surface) and hypoxic depths. Moreover, the hypoxic strata showed the stramenopiles, were most different from upper suboxic

depths (Fig. 44a). With reference to genus level, heat map analysis depicted that the HF community at the surface and lower suboxic depth were different from hypoxic and upper suboxic depths. The HF genera comprised of *Gyrodinium, Proterythropsis, Pelagodinium, Spumella, Picomonas, Warnowia, Ornithocerus, Thaumatomastigidae, Protoperidinium, Phalacroma, Eudubosquella* were found at the surface which were quite dissimilar from other depths in the hypoxic and suboxic zone. The genus like *Pfisteria, Protoperidinium, Ornithoceros, Picomonas, Spumella, Pelagodinium, Proterythropsis,* and *Gyrodinium* were common between hypoxic and upper suboxic depths (103m and 134m) (Fig. 44b).

6.3.2 Outer continental shelf (G12)

Over the outer continental shelf, most of the HF community at the surface water column was different from that at the upper and lower hypoxic depths. The higher taxa such as stramenopiles, katablepharedophyta and choanoflagellida found at upper hypoxic strata were different from the surface (oxic) water. The rhizaria, apusozoa and picozoa, found in both upper hypoxic and lower hypoxic strata, were quite different in the surface water (Fig. 45a).

The Genera like *Gyrodinium, Amoebophyra, Proterythropsis, Pelagodinium, Podolampas, Polykirkos, Thaumatomastigidae, Paraphysomonas, Sinophysis, Ornithocerus, Cercomonas, Protoperidinium, Lessardia, Massisteria, Spumella, Kofoidinium, Gotoius, Phalacroma, Allas, Picomona* and *Oxyphysis* represented close evolutionary linearity compared to oxic surface water. In contrast, different communities of *Pfistera* were observed between upper oxic and lower hypoxic water depth. The community composition varied across the depths (Fig. 45b).

6.3.3 Inner continental shelf (G5)

As per the heat map analysis, most of the HF community in the upper oxic (0m) and the lower oxic (8m) water were common which indicates close similarity among the higher taxon groups over a short distance compared to anoxic bottom water. For example, alveolata, picozoa, choanoflagellaida were less diversified at the bottom and the community compositions were different from two oxic depths. Remarkably, stramenopiles community composition at lower oxic depth were markedly different from upper and lower anoxic water depth (Fig. 46 a).

As per the heat map analysis, most of the HF community in the upper oxic (0m) layer showed close similarity with that at the lower oxic depth (8m) clearly due to close proximity compared to that in anoxic bottom water where community was dissimilar. For example, *Gyrodinium, Amoebophyra, Eudubosquella, Pfisteria, Pelagodinium, Sinophysis, Proterythropsis, Lessardia* were less diversified at the bottom water and their community composition were different from those at two oxic depths. Exceptionally, *Ornithocerus* species composition at upper oxic depth was markedly different than that the lower and anoxic water depth. Interestingly *Picomonas* species composition did not vary across the depths (Fig. 46b).

6.3.4 Mandovi estuary (M)

Overall, the whole HF community between the surface and bottom water were quite similar. In case of higher taxon groups e.g. Alveolata, Rhizaria, Katablepharidophyta, Stramenopiles, and Choanoflagellida community were different in both surface and bottom water. Exceptionally, Telonemida community was similar in at both the depths (Fig. 47a).

Crypthecodinium, Paraphysomonas, Ornithocerus, Polykrikos, Pfisteria communities at surface were highly dissimilar as compared to those in the bottom water. Most

communities such as *Amoebophyra, Eduboscquella, Pelagodinium,Thaumatomastigidae, Cercomonas, Podolampas, Eocercomonas,* and *Gyrodinium* were closely related in surface water and they were different in bottom water (Fig. 47b).

6.3.5 Zuari Estuary (Z)

Like in Mandovi estuary, there was quite similarity in the composition of HF community between the surface and bottom water but in close observation, *Alveolata, Stramenopiles, Rhizaria, Cryptophyta, Picozoa, Apusozoa, Choanoflagellida, Katablepharidophyta* and *Telonemida* communities were different between surface and bottom water (Fig. 48a).

Like wise, *Gyrodinium, Amoebophyra, Thaumatomastigidae, Allas, Cercomonas, Paraphysomonas, Pelagodinium, Eudubosquella, Ornithocerus, Oxyphysis, Neocercomonas, Massisteria, Picomonas* and *Crypthecodinium* communities were highly diversified in the bottom depth whereas *Helkesimastix, Podolampas, bodomorpha* and *Polykrikos* were highly diversified at the surface (Fig. 48b).

6.4 Discussion

In the present study, colour index of heat maps measures the clustering of higher taxonomic groups as well as genera of the heterotrophic flagellate communities on the phylogeny by comparing the distance between them in each sampling site. Here heat maps represents the phylogenetic distance by means of similarity or dissimilarity matrics, are observed through the two dimensional coloured plots. The colour codes indicates the degree of similarity and phylogenetic distance between the organisms. It is a well established approach for microbial community used by Cole et al. (2009).

The comparison of heterotrophic forms at different oxygen levels showed that the phylogenetic diversity is controlled by oxygen found at different sampling sites and

depths. Therefore, community composition differed between surface and oxygen deficient (hypoxic and suboxic) water column. Phylogenetic diversity of heterotrophic flagellates with respect to oxygen distribution has not been previously studied in the Arabian Sea. This is the first report from the region dealing with heterotrophic protist diversity across the oxygen gradients based on the NGS approach.

Recently, the effect of oxygen depletion on the variations of taxonomic flagellate has been studied in the eastern tropical South Pacific (ETSP) and the eastern tropical North Pacific (ETNP; Parris et al., 2014; Duret et al., 2015). Choanoflagellida is one of the groups mainly represented heterotrophic nanoflagellates (HNF) which prefers suboxic strata and the other communities such as apusozoa and cryptophyta which were closely related to the Wylezich et al. (2012) has described that choanoflagellates are adapted to hypoxic environments of Baltic Sea due to presence of derived mitochondria. It shows that choanoflagellida communities can thrive in low oxygen suboxic strata. Moreover, stramenopiles inhabiting hypoxic water column, are different from those in oxic water. This finding emphasizes advanced approach to understand the ecology of this community and their adaptation to low oxygen environments. To sum up, the phylogenetic composition of heterotrophic flagellates exhibited patchy distribution in the pelagic and mesopelagic waters of the eastern Arabian Sea as observed in the ETNP and ETSP (Duret et al., 2015; Parris et al., 2014).

As the diversity of heterotrophic flagellates was high within the oxygen deficient strata (hypoxic and suboxic waters) of the open ocean and coastal waters, the phylogenetic composition of HF communities revealed less diversity in outer continental shelf and estuarine waters. It is likely because most species and clades are dominant in open ocean and coastal water. On the other hand, very few species or clades characteristics

of outer shelf and estuarine sites were found in oxygen deficient sites of the open ocean and coastal sites.

A clear difference in environmental conditions was noticed from the oceanic to estuarine stations. Surprisingly, heterotrophic flagellate communities in the two estuaries yielded different phylogenetic relation. In a comparative note, higher phylogenetic variation was observed in Zuari estuary than in Mandovi estuary. This dissimilarity of Phylogenetic variation may be attributed to hydrographical differences (i.e. stronger tidal currents in surface than bottom water in the Zuari) differences in run-off and the width of the mouths which make the two estuaries behave differently (Varma et al., 1975; Qasim and Sengupta, 1981). Sites with different ecology may cause the difference in phylogeny of community. Therefore, this phylogenetic comparison could be useful in understanding the influence of environmental factors on certain ecosystems. Changing phylogenetic composition across diverse sites with different oxygen levels suggests that phylogeny represents evolutionary traits linked to ecological preference and the nature of surrounding environment.

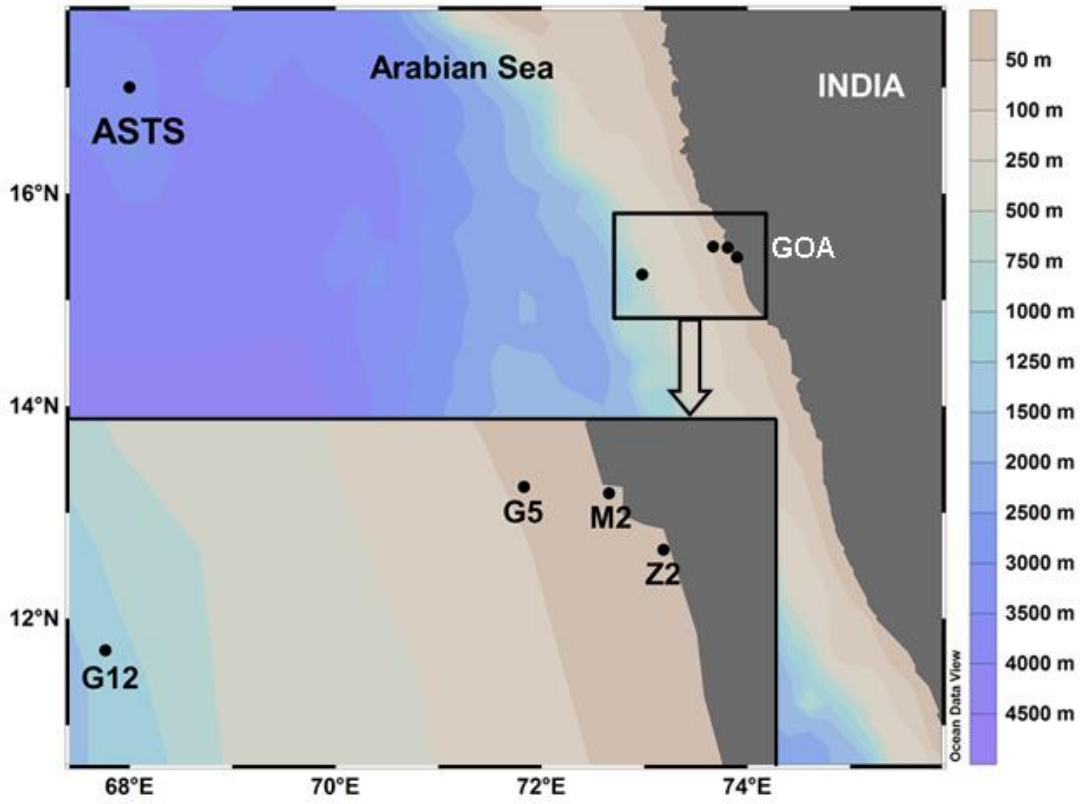

Figure 43. Map showing distinct sampling sites (open ocean: ASTS; outer shelf: G12; inner shelf: G5 and estuarine sites: M2 and Z2) of the Arabian Sea.

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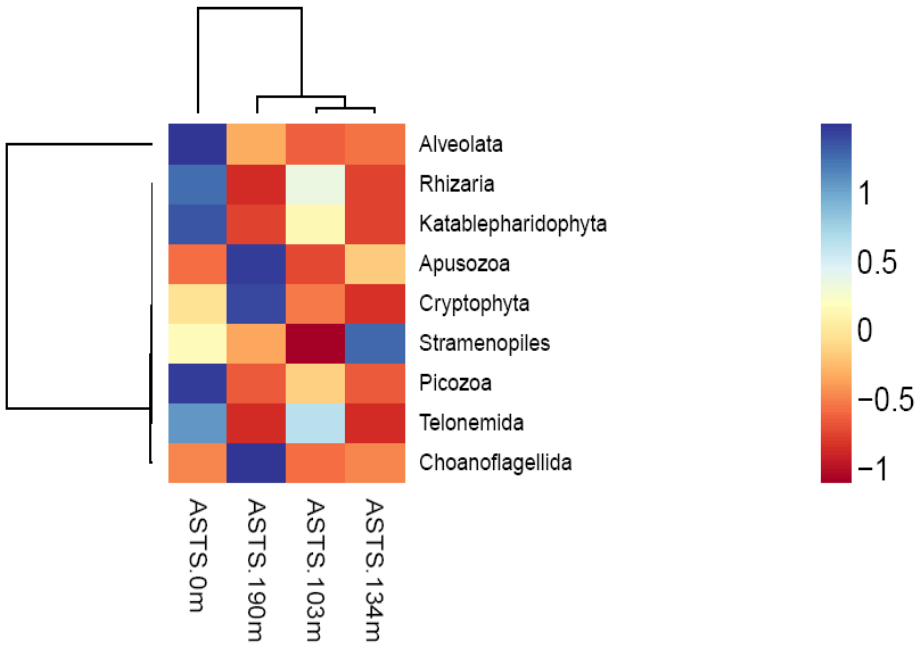

Figure 44 a. Heat map analysis showing HF community comparison in the higher taxon groups and related community composition at different oxygen gradients of the open ocean station (ASTS) based on 18sRDNA sequences.

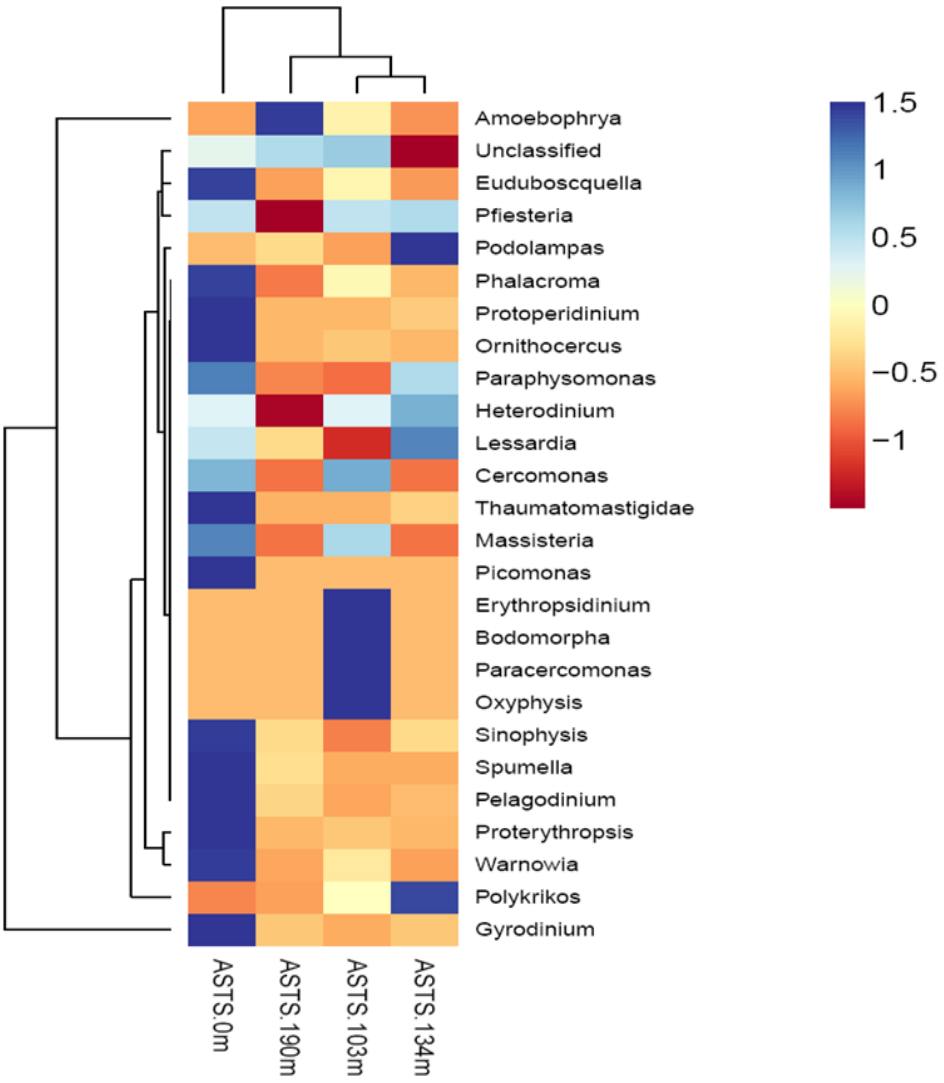

Figure 44 b. Heat map analysis showing HF community comparison in genus level and related genera composition at different oxygen gradients of the open ocean station (ASTS) based on 18sRDNA sequences.

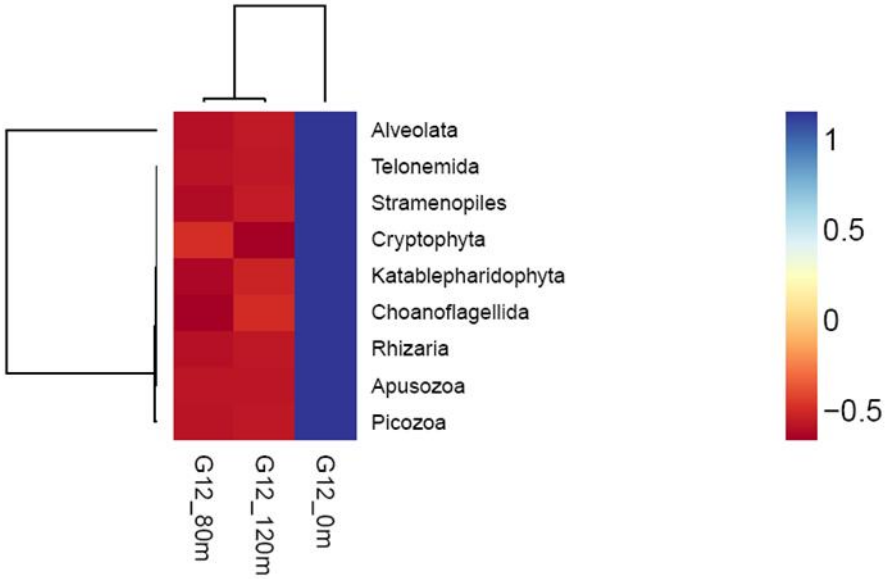

Figure 45 a. Heat map analysis showing HF community comparison in the higher taxon groups and related community composition at different oxygen gradients of the outer shelf (G12) station based on 18sRDNA sequences.

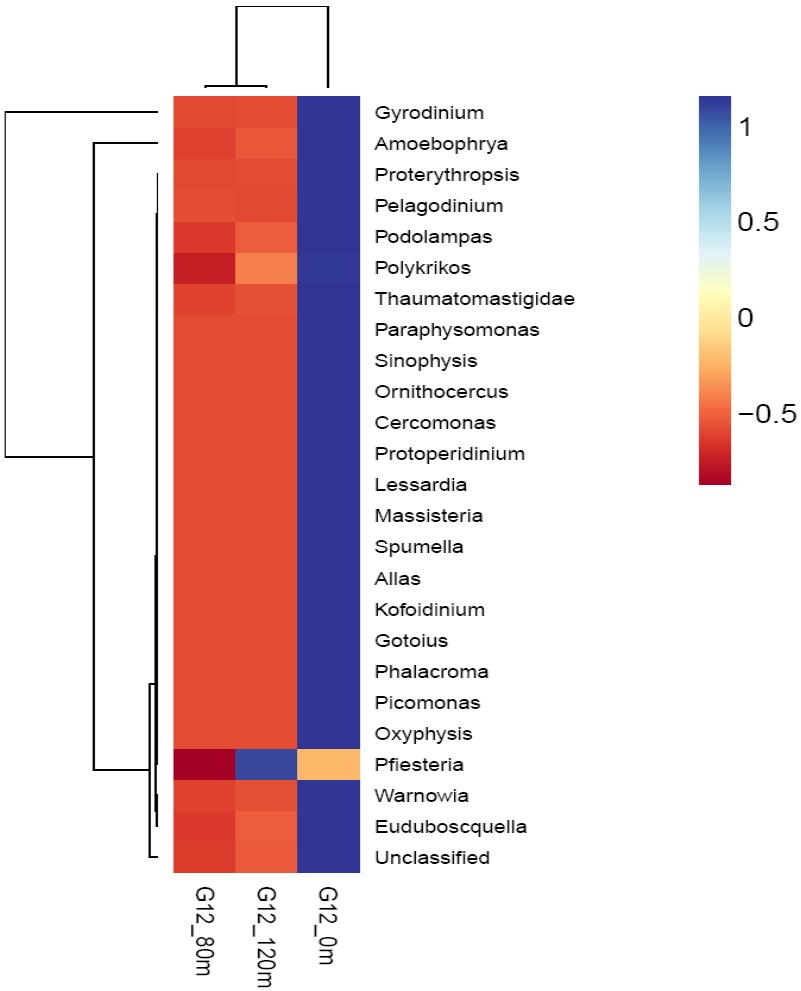

Figure 45 b. Heat map analysis showing HF community comparison in genus level and related genera composition at different oxygen gradients of the outer shelf station (G12) based on 18sRDNA sequences.

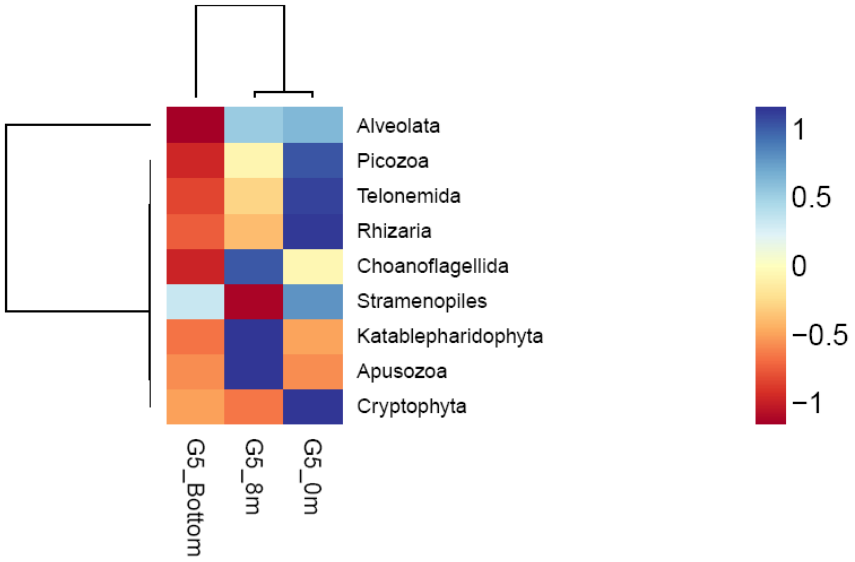

Figure 46 a. Heat map analysis showing HF community comparison in the higher taxon groups and related community composition at different oxygen gradients of the inner shelf station (G5) based on 18sRDNA sequences.

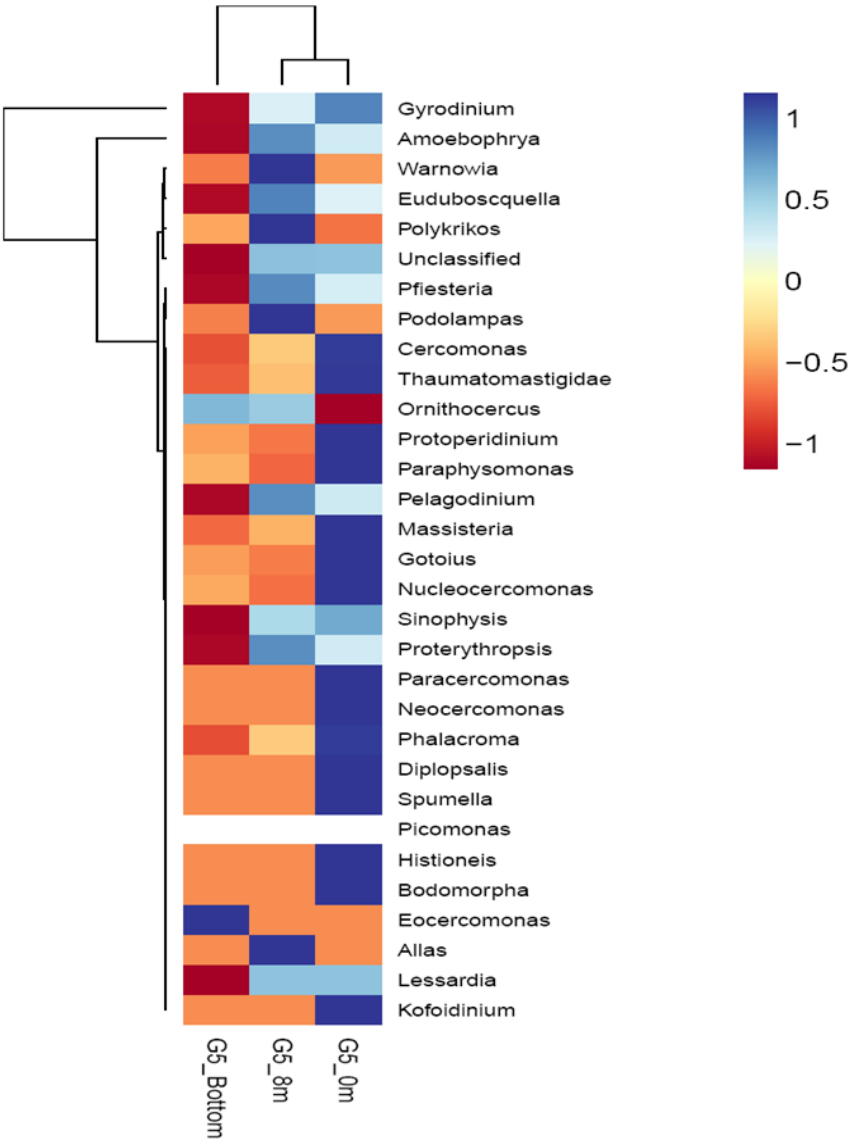

Figure 46 b. Heat map analysis showing HF community comparison in genus level and related genera composition at different oxygen gradients of the inner shelf station (G5) based on 18sRDNA sequences.

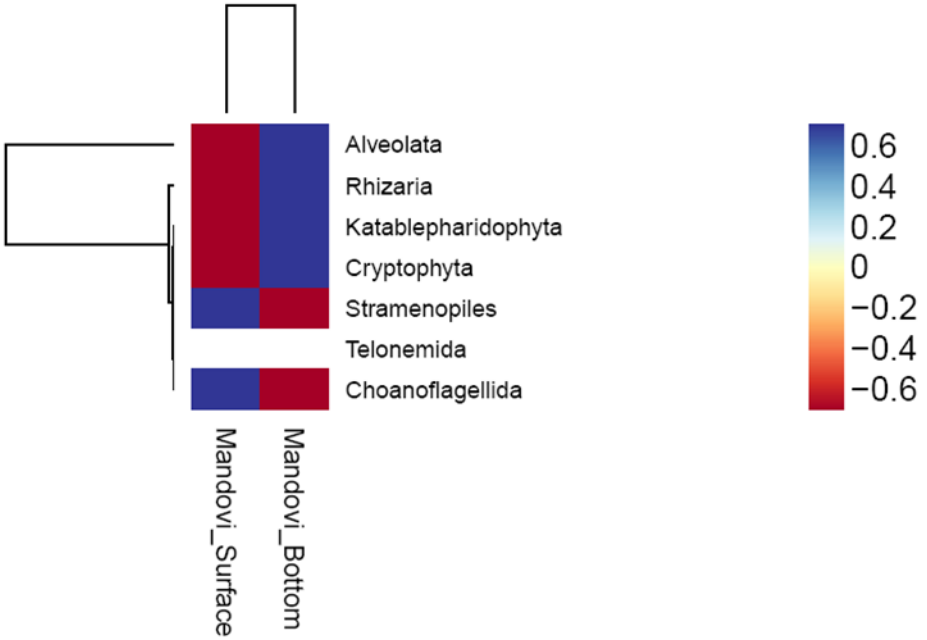

Figure 47 a. Heat map analysis showing HF community comparison in the higher taxon groups and related community composition at different oxygen gradients of Mandovi estuary based on 18sRDNA sequences.

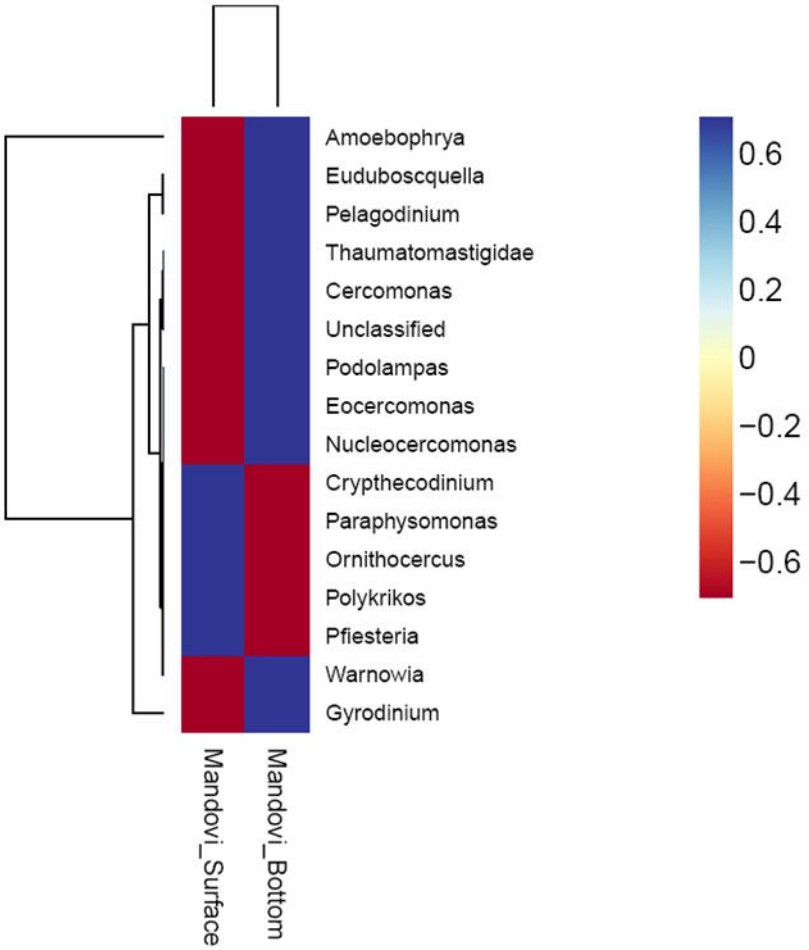

Figure 47 b. Heat map analysis showing HF community comparison in genus level and related genera composition at different oxygen gradients of Mandovi estuary based on 18sRDNA sequences.

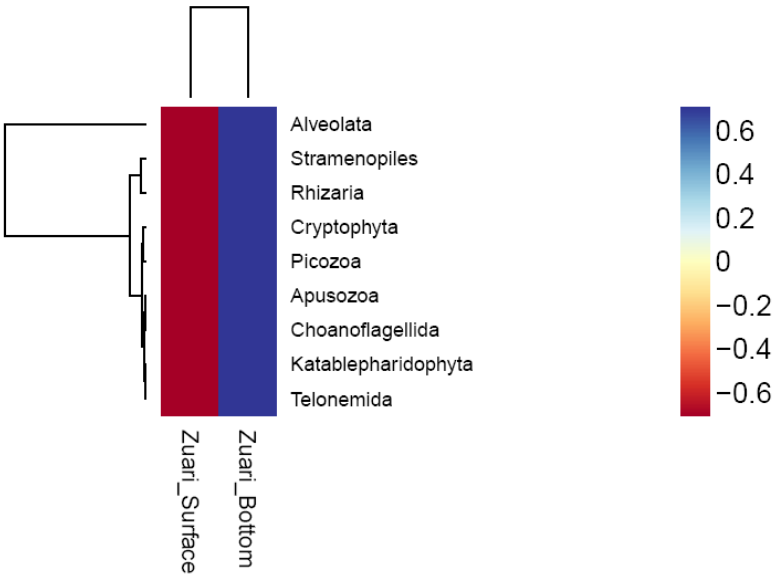

Figure 48 a. Heat map analysis showing HF community comparison in the higher taxon groups and related community composition at different oxygen gradients of Zuari estuary based on 18sRDNA sequences.

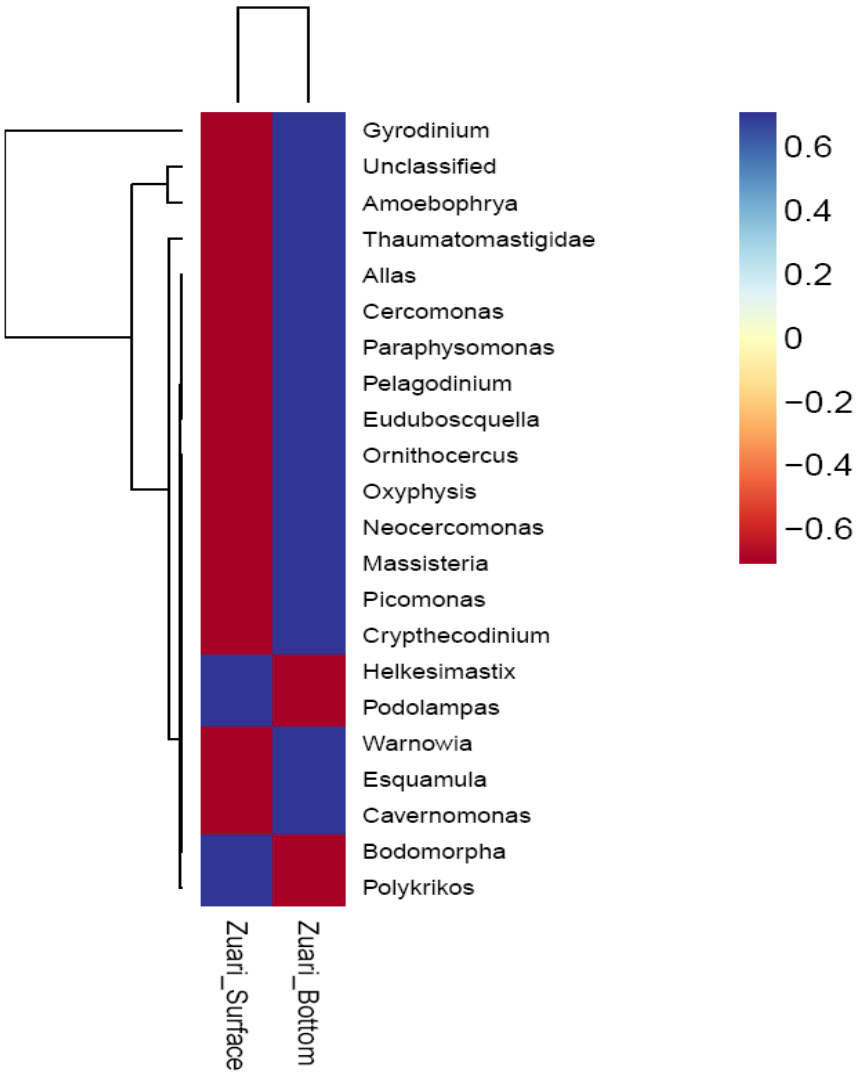

Figure 48 b. Heat map analysis showing HF community comparison in genus level and related genera composition at different oxygen gradients of Zuari estuary based on 18sRDNA sequences.

Summary and Conclusion

Arabian Sea and the associated estuarine systems on the west coast of India draws a special attention due to its unique features of climatic and biogeochemical processes. Oceanic subsurface waters and the coastal shelf waters of the eastern Arabian Sea are typical as waters of this region are severely stressed by either perennial / seasonal hypoxia, popularly known as the Oxygen minimum zones (OMZ). Such ecosystems with low level of oxygen can harm aquatic life (including plankton) and affect water quality and food chain. Thus, this work was performed to assess the relationship between environmental variables and community composition of zooplankton. In this thesis, the focus is laid on the differences in meta- and proto- zooplankton communities between estuarine, coastal and open water, to reveal interactions with the abiotic environment, which are in general understudied. Importantly, protist communitiy composition in the eastern Arabian Sea is addressed for the first time. For which, ecological high-throughput sequencing was applied to accurately estimate richness and composition of ciliats and flagellates in spatial dimensions of the Arabian Sea. Our basic ecological understanding would remain incomplete without this kind of measurements. The salient findings of this thesis have been presented below.

- In estuarine systems of Goa (Mandovi and Zuari estuaries), few estuarine copepods showed their wide adaptability in different parts of the estuary under different environmental conditions. Metazooplankton groups and copepod species abundance clearly showed the variability in their abundance in the three distinct regions (mouth, mid-estuarine and upstream) that changed with seasons and space.
- In the Mandovi estuary, the metazooplankton groups such as gastropod larvae, cyclopoida copepods and decapod larvae showed wide variation in percentage

composition between near mouth (M1) and upstream (M6) stations whereas cyclopoida copepods showed wide variation in percentage composition between mid-estuarine (M3) and upstream stations (M6). Likewise, on a temporal scale, cyclopida copepods, gastropod larvae and decapod larvae were contributed the maximum to the dissimilarity index between the seasons SIM and SWM. In addition, few of copepod species such as *Acartia southwelli*, *Diaptomus* sp. and *Allodiaptomus mirabillipes* were major contributors to the spatial scale dissimilarity between mouth and upstream station. While presence of *Oithona* sp., *Paracalanus parvus*, *Oithona brevicornis*, *Acrocalanus gibber*, *Acartia southwelli* and *Diaptomus* sp. led to the seasonal scale dissimilarity between FIM and SWM, and FIM and SWM.

- Also, in the Zuari estuary, the metazooplankton groups such as cladocerans, copepod juveniles, pelecypoda larvae, calanoida copepods and decapod larvae showed wide variation in percentage composition between mouth (Z1) and upstream station (Z7), and mouth (Z1) and midreach (Z4) station. Likewise, on a temporal scale, contribution of cladocerans, pelecypoda larvae, Decapod larvae and calanoida copepods to the total zooplankton depicted seasonal variation between SIM and SWM. The copepod species such as *Diaptomus* sp., *Acrocalanus gibber* and *Acartiella Keralensis* showed variation in percentage composition between the mouth and upstream stations. In temporal scale, *Paracalanus aculeatus*, *Acartia centrura*, *Diaptomus* sp., *Paracalanus parvus*, *Acartia tropica*, *Acartiella keralensis* contributed the maximum to the dissimilarity between FIM and NEM whereas, the maximum dissimilarity between FIM and SIM was contributed by *Paracalanus parvus*, *Diaptomus* sp., *Paracalanus* sp., *Acrocalanus* sp. and *Acartia* sp.

- Furthermore, the present study showed that the copepod species of Mandovi and Zuari estuaries are able to thrive under a wide range of salinity. The copepod species such as *Heliodiaptomus cinctus*, *Heliodiaptomus contortus*, *Allodiaptomus mirabillipes*, *Diaptomus* sp. *Acartiella gravelyi* and *Acartia sewelli* represented low saline species found in the upstream region of both the estuarine systems.
- The seasonal variation of copepod species and total metazooplankton abundance are regulated by the variation of salinity in the estuarine systems. Turkey's post hoc test revealed that the temperature during the NEM season was significantly different from that during FIM and SWM. However, no significant difference in temperature was recorded across the estuarine region. But, salinity ($p < 0.01$) and chlorophyll a ($p < 0.01$) were found to be significantly different among all the estuarine stations.
- Across all habitats, the metazooplankton groups such as calanoida copepods, gastropod larvae and decapod larvae showed a wide range of percentage composition with dissimilarity between FIM and NEM. The maximum average dissimilarity of the community was noticed between mid-estuarine and inner shelf station followed by upstream and outer shelf, and upstream and open ocean. The stations were classified into two groups i.e. coastal and open ocean sites as one group and estuarine stations (Mandovi and Zuari) as another group. In case of copepod species, *Acrocalanus gibber* (9%), *Acartia tropica*, *Acrocalanus* sp., and *Acrocalanus gracilis* found to be responsible for dissimilarity between FIM and NEM. Present study showed that the spatial differences are more significant than the seasonal differences. With reference to a spatial variation of copepod species, the variation in species dominance was noticed in different ecological sites. The dissimilarity between open ocean and upstream (86%) was influenced by

Acrocalanus gibber, *Onceae* sp., *Coryceaus* sp., *Onceae venusta* and *Euchaeta indica*. The 87% of average dissimilarity between upstream and inner shelf were influenced by *Onceae conifera*, *Acrocalanus gibber*, *Euterpina acutifrons*, *Acrocalanus longicornis* and *Oithona brevicornis*. The mid-estuarine stations and openocean showed 83% dissimilarity because of *Acartia tropica*, *Acrocalanus gibber*, *Onceae* sp., *Coryceaus* sp., *Acrocalanus longicornis* and *Onceae venusta*.

- The CCA ordination analysis depicts that salinity, nitrate and chlorophyll a ($p < 0.05$) are the major environmental parameters influencing the copepod species distribution in the study area. Most of the copepod species were influenced by salinity. Few species such as *Acartiella keralensis*, *Acartia tropica*, *Acartiella gravelyi*, *Acartia sewellii*, *Pseudodiaptomus sewelli* and *Pseudodiaptomus jonesii* were found to be influenced by chlorophyll a, dissolved oxygen and nitrate.
- In general, these results showed that temperature significantly influenced the metazooplankton distribution with respect to seasons whereas the salinity and chlorophyll a significantly influenced with respect to spatial scale variation of metazooplankton.
- The Protistan groups were identified (higher taxons) through the next generation sequencing approach in different regions of the Arabian Sea (estuary, coastal and open ocean). In total, 22 protistan phylogenetic groups, including Ciliophora, Dinophyceae, Unclassified Alveolates, Centroheliozoa, Choanoflagellida, Cryptophyta, Fungi, Haptophyceae, Picozoa, Rhizaria, Stramenopila, Telonema, Viridiplantae, Colpodellidae, Amoebozoa, Apusozoa, Dimorpha, Eccrinales, Ichthyosporea, Apicomplexa, Katablepharedophyta and Rhodophyta were identified from all the sampling sites of estuarine and open ocean waters.

- The maximum average dissimilarity (74%) between open ocean and estuaries were shown by Viridiplantae and Dinophyceae. In relation to oxygen gradients, the maximum average dissimilarity of protist was recorded from the oxic and hypoxic water column, which is contributed by Rhizaria and Viridiplantae communities. However, protist community composition between open ocean and outer shelf, open ocean and coastal (Inner and outer continental shelf) did not show any significant difference. The pairwise PERMANOVA test also did not show any significant difference of protist distribution on a spatial scale. The DistLM analysis of dbRDA plot and BIOENV results showed that chlorophyll a is a primary environmental variable influencing protist community in the estuarine systems, whereas, nitrate and temperature are influencing in the outer and inner continental shelf, and open ocean sites.
- The distribution of the heterotrophic nanoflagellate genus Monosiga (Choanoflagellida) appears to withstand low oxygen levels in the water column unlike other forms that are considerably affected by low oxygen conditions. In the oxygen-depleted water, heterotrophic nanoflagellates are possibly well supported by the availability of rich bacterial biomass at that depth. As a result, heterotrophic nanoflagellate abundance (Monosiga sp. - Choanoflagellida) from hypoxic to deeper suboxic waters was found to be higher in this study. In general, the oxygen gradients make a boundary which separate organisms to adapt in different levels of oxygen. It is worthy to state that heterotrophic nanoflagellates were very low in abundance in hypoxic waters over the outer shelf. Spatial differences in the beta (spatial) diversity between the open ocean, outer continental shelf, inner continental shelf and estuarine waters, experiencing different hydrography and biogeochemistry, reflected diverse communities of protists.

Overall, the present study provided insights into the baseline information, meta- and proto-zooplankton diversity and their relation to the environmental variables in estuarine to open ocean of the eastern Arabian Sea. The spearman's rank correlation (statistical analysis) clearly shows that association of environmental variables with metazooplankton community has diverged in the Mandovi and the Zuari estuary. This could be due to environmental factors; dissolved oxygen is the major environmental variable in the Zuari estuary whereas in the Mandovi estuary, salinity is the major environmental variable which controls the metazooplankton community distribution. These results reveal that the protist communities in the Arabian Sea are capable to survive even in oxygen-depleted waters (seasonal/perennial). Protist are important living organisms in the food chain and play an important role in biogeochemical cycling of carbon and nitrogen. Despite their impact of protistan grazing on the structure and function of the prokaryotic communities has not been examined yet from the region. Thus, present study emphasizes the distribution and composition of organisms such as ciliates and flagellates adapted at relatively low-oxygen level apart from ecology and their role in various microbial driven biogeochemical processes.

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Appendix: Common copepod species documented during the study

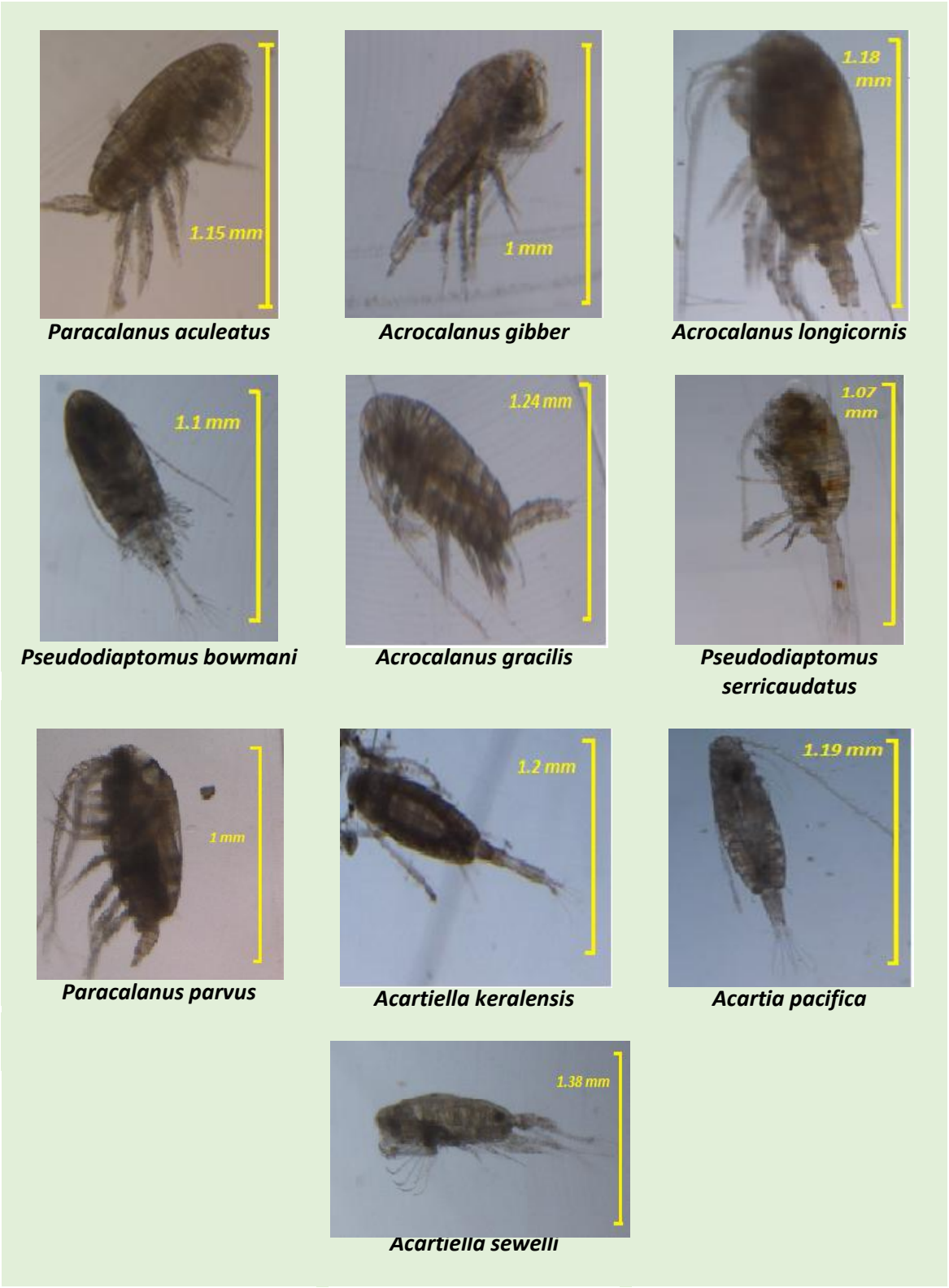

Fig.1. Common calanoida copepod species were identified in the estuarine system during the study.

Clausocalanus arcuicornis

Subeucalanus mucronatus

Pleuromamma indica

Euchaeta marina

Pontellina plumata

Temora turbinata

Temora discaudata

Euchaeta longicornis

Centropages orsinii

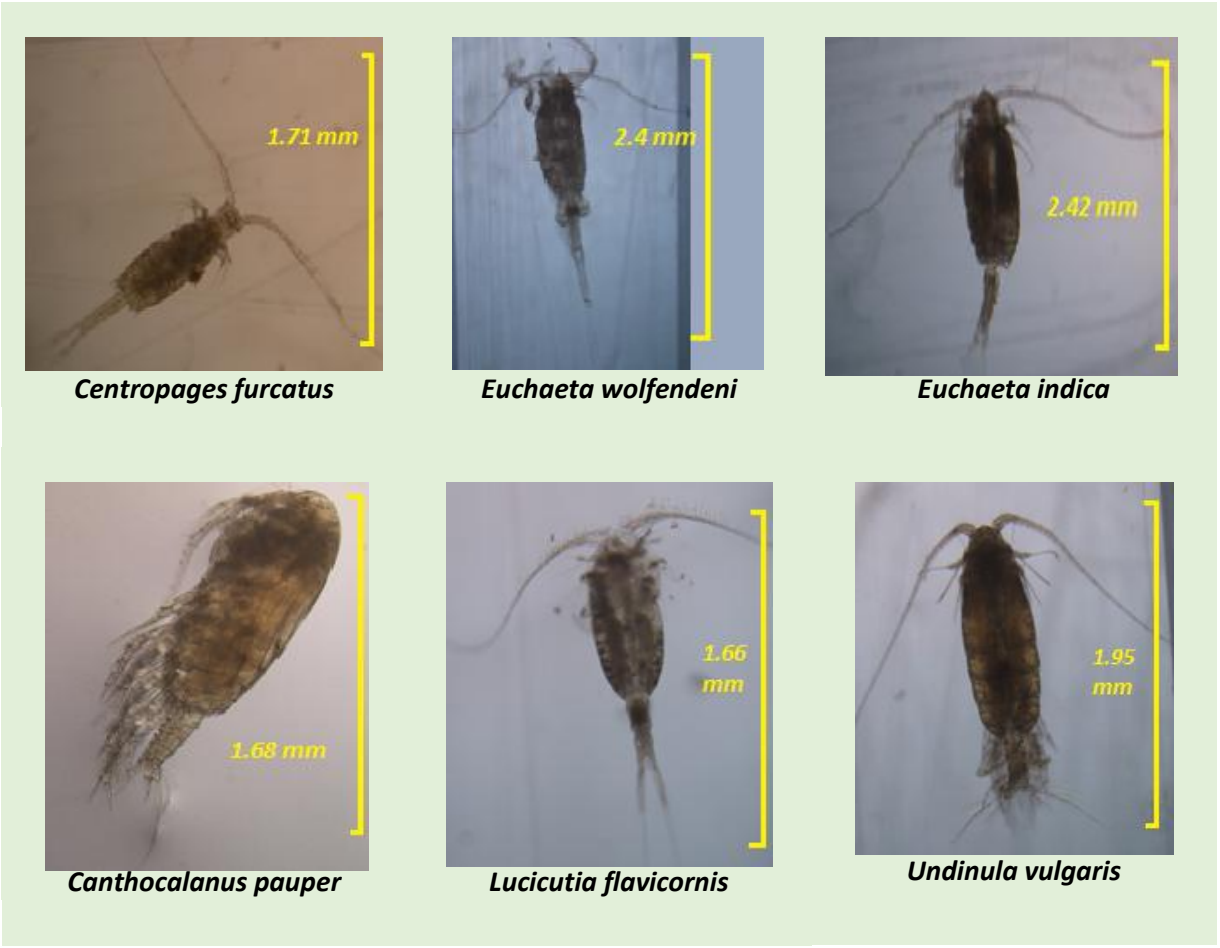

Fig.2. Abundant marine (coastal and open ocean) calanoida copepod species were identified during the study.

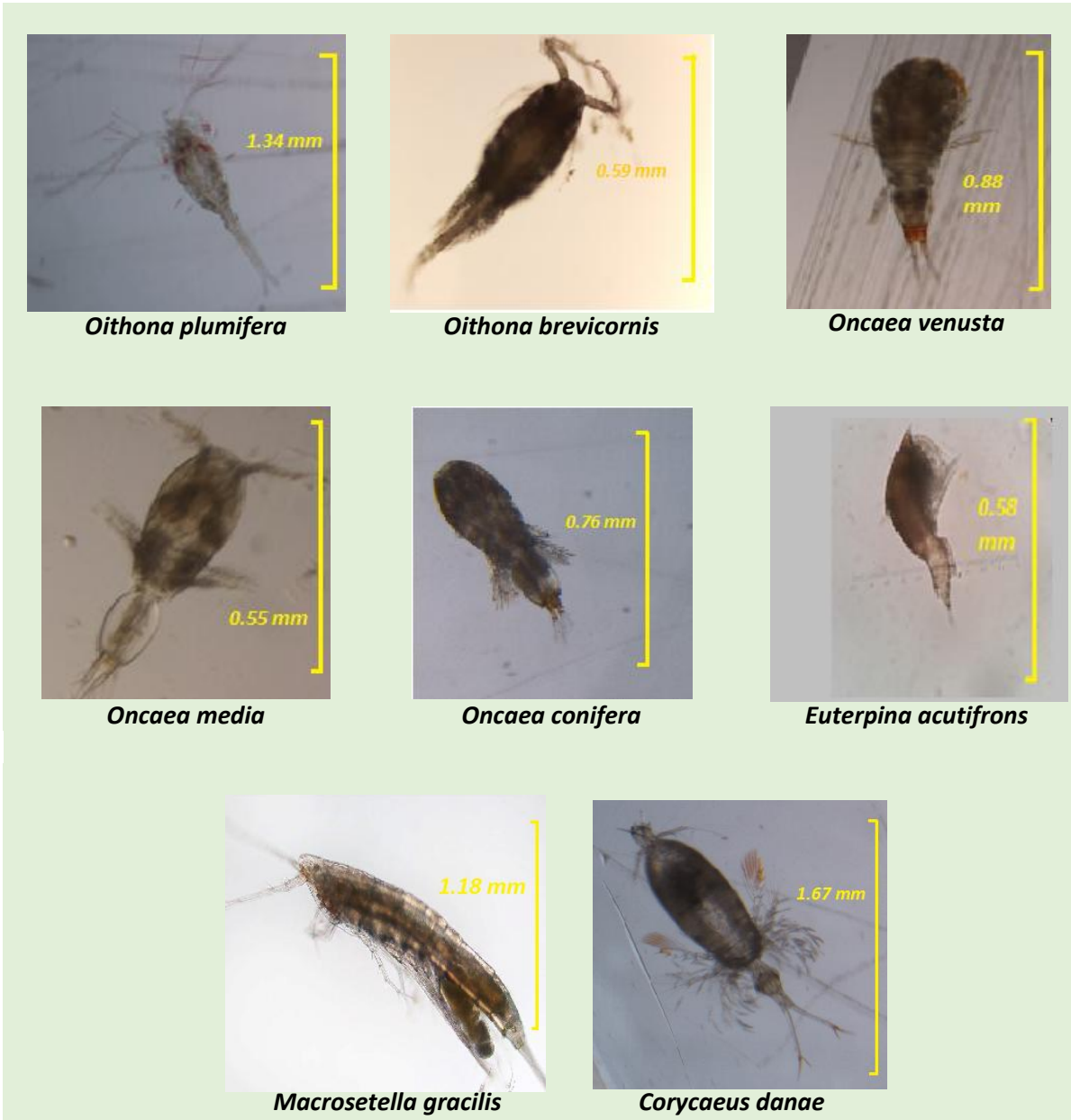

Fig. 3. Abundant Cyclopoida, Poicelostomatoda and Harpacticoida copepod species were identified in estuarine and marine systems during the study.

Publications

Priya Brata Das*, Mangesh Gauns, SWA Naqvi (2019). Ecological diversity of planktonic protists in spatial regimes of the Arabian Sea revealed through next-generation sequencing. Reg. Stud. Mar. Sci., 25, 100484. **(Enclosed).**

Priya Brata Das* (2017). Zooplankton assemblages in spatial stretches of a tropical estuary. IJRASET., 9, 1789-1799. **(Enclosed).**

Manuscript communicated

Priya Brata Das*, Mangesh Gauns, Alexandra Stock and SWA Naqvi. Environmental association of heterotrophic micro-eukaryotes in the varying biogeochemical regimes of the Arabian Sea, resovled via high-throughput sequencing. Environ. Sci. Pollut. R., (Under review- ESPR-D-19-03510).

Poster presentations

Priya Brata Das*, Mangesh Gauns, Alexandra Stock and SWA Naqvi. Next-generation sequencing approach: an insight into the spatial variation of ciliate diversity in the Arabian Sea. International Symposium on "Dynamics of the Indian Ocean: Perspective and Retrospective" at NIO, Goa, 2015. **(Enclosed).**

Mangesh Gauns*, Siby Kurian, Damodar Shenoy, **Priya Brata Das,** Ayaz Ahmed, Hema Naik, SWA Naqvi. Plankton process in the oxic-hypoxic boundary of Northern Indian Ocean. International Symposium on "Dynamics of the Indian Ocean: Perspective and Retrospective" at NIO, Goa, 2015.

Training Programme at Germany

Participated in one month training programme on Next generation sequencing analysis of protist (Heterotrophic nano-flagellates and ciliates) communities present in oxygen deficient sites of Arabian Sea at Kaiserslautern University, Germany. February 2014.

Regional Studies in Marine Science 25 (2019) 100484

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Ecological diversity of planktonic protists in spatial regimes of the Arabian Sea revealed through next-generation sequencing

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ARTICLE INFO

Article history:
Received 22 April 2018
Received in revised form 13 December 2018
Accepted 13 December 2018
Available online 21 December 2018

Keywords:
Arabian sea
Oxygen gradients
Eukaryotic microbes
Diversity
Environmental influence
Next-generation sequencing

ABSTRACT

Eukaryotic microbes inhabiting diverse ecological niches are capable of mediating biogeochemical shifts. Here, we studied the distribution patterns of protistan community in oxygen-deficient sites in the Arabian Sea and nearby estuarine waters. Protist diversity was quantified through Illumina Miseq sequencing of the V4 region of 18S rRNA gene amplicons. Overall, 12687 OTUs represented the diverse protist communities at various sampling sites such as the open ocean, outer shelf and inner shelf along the oxygen gradient. As per Alpha diversity estimation, estuarine communities were less diverse than the coastal, and open ocean sites. Multivariate analysis was applied to differentiate the community structure in estuarine, coastal and open ocean sites. The results indicated distinct community variation between oxic, hypoxic and suboxic water column at a comparatively deep sea station. However, the influence of dissolved oxygen was statistically insignificant for the protist distribution. The DistLM analysis suggests that the adaptation of protist communities across the spatial habitats could be significantly correlated with temperature, salinity, and nitrate. Moreover, chlorophyll *a* was the important environmental variable associated with the estuarine complex, whereas salinity, nitrate, and temperature influenced coastal and open ocean stations.

1. Introduction

Protists, the unicellular eukaryotes, play a pivotal role in mediating biogeochemical processes in marine ecosystems (Caron et al., 2017). This heterogeneous group is well known as a trophic link component in aquatic food webs and in nutrient cycling. Moreover, bacterivorous protists largely function as capable nutrient remineralizers (Wetzel, 2001). The substantial presence of these communities in diverse ecosystems has drawn considerable attention to studying their species variability on spatial scales (Lepere et al., 2013; Wu et al., 2017). Among various oceanic biogeochemical regimes, the oxygen minimum zones (OMZs) have profound effect on the distribution of marine metazoans and microbes. These prokaryotic and eukaryotic microorganisms facilitate carbon and nitrogen cycles in marine food webs (Medina et al., 2017). Oxygen depleted water causes shifting of microbial communities and their metabolic changes favored by altered biogeochemical processes which can lead to nitrogen loss by denitrification (Thampdrup et al., 2012). Physicochemical environments along with biological interactions (competition and predation) are also known to influence species assemblage and their function in the surrounding ecosystem (Webb et al., 2002). Sufficient information exists using traditional methods to understand protistan ecology (Azam et al., 1983; Sherr and Sherr, 2002). However, current era metagenomics studies have further advanced insights into molecular protist identification (Zhou et al., 2015). Despite their ubiquity, information on some rare taxa of microbial communities is still rudimentary. Indeed, the use of DNA sequence analysis has shown the complete classification of protistan taxa in some natural ecosystems (Stoeck et al., 2003; Doherty et al., 2007) and for their phylogenetic structure (Massana et al., 2006; Moreira et al., 2007). These studies are characterized by the demarcation of a gene library (graphical visualization of the number of sequences with operational taxonomic units; OTUs) to reveal community diversity and structure. Such a sequencing approach is an advanced tool to evaluate the taxonomic diversity of protists with the accuracy of taxonomic affiliation. Various other molecular techniques are also prevalent for semi-quantitative assessment of microbial eukaryotic communities (Thiele et al., 2012).

The molecular diversity and community composition of protists are limited by the spatial demarcation of oxygen-depleted environments. Globally, high-throughput sequencing studies have profoundly impacted environmental surveys of microbial eukaryotes in unique ecosystems over distinct geographic areas (Filker et al., 2016; Kammerlander et al., 2015; Duret et al., 2015; Orsi et al., 2012). Marine oxygen minimum zones (OMZs) probably harbor planktonic protist communities because of their metabolic adaption to low-oxygen environments. Recent studies on protist

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https://doi.org/10.1016/j.rsma.2018.100484
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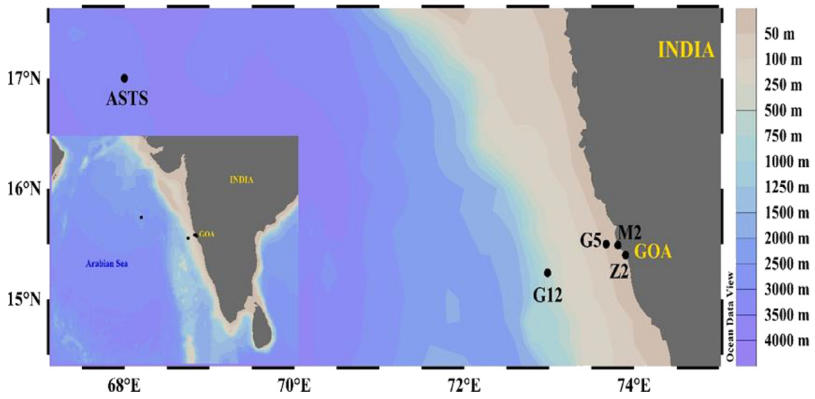

Fig. 1. Map of the sampling sites located in different regions of the Arabian Sea ranging from estuarine to open ocean stations (Estuary: Mandovi-M2, Zuari-Z2; coastal-G5; continental shelf-edge-G12; open ocean-ASTS).

diversity based on 18S rRNA gene sequences revealed a distinct pattern of eukaryotic groups present in the oxygen-deficient environments of world oceans (Jing et al., 2015; Parris et al., 2014; Stoeck et al., 2003). The Arabian Sea OMZ, however, is unique among world oceanic OMZs, as limited information is available on the presence of microbial eukaryotes in these waters and their relation to the other metazooplankton groups. This is because of the small size and morphological peculiarities that make their identification to the correct taxonomic species difficult (Moon-van der Staay et al., 2001). An established eukaryotic tree is a valuable tool in understanding evolutionary lineages between flagellates from unicellular to multicellular organisms (Massana et al., 2002). It is therefore, crucial to comprehend the degree of taxonomic variation of different protist groups to identify changing environments.

Our current study aimed to distinguish microbial protist community diversity linkages in spatially varying environmental settings using next-generation sequencing (NGS). Efforts were directed to identify site-specific eukaryotic protists and highlight their taxonomic group response in different oxygen regimes in the water column in the Arabian Sea. It seemed appropriate to assume that these varying environmental parameters such as dissolved oxygen, temperature, salinity, nitrate, nitrite and chlorophyll *a* dictate the genetic diversity between the estuarine, coastal, shelf-edge and open ocean ecosystems. Statistical analysis was used to compare the diversity of protist communities to gain a better understanding of the community response to the surrounding environments. Thus, the study primarily aimed to explore the genetic diversity of eukaryotic protists and their spatial distribution with the variation of oxygen regime. Further areas of interest were to determine the degree of similarity and dissimilarity between protist groups, and understand the environmental factors responsible for the partitioning and diversity of the protist community across varying biogeochemical regimes. The present study could serve as a valuable baseline source outlining the molecular genetic diversity of microbial eukaryotic groups in the OMZ of coastal and estuarine regions.

2. Material and methods

2.1. Sample collection

Sampling sites were selected based on the variation in the oxygen regime from the estuarine to the perennial OMZ region through the coastal seasonal OMZ (the western Indian shelf) of the Arabian Sea. Samples were collected from four contrasting sites covered during Cruise SSK-56 on board the R/V Sindhu Sankalp from 18 October to 2 November 2013. The sampling sites were located in the open ocean [Arabian Sea Time Series (ASTS), 17°N-68°E], the shelf-edge away from the coast (G12, 15.24°N-72.98°E), a coastal (inner-shelf) station (G5, 15.50°N-73.67°E) and the estuarine region (Mandovi, 15.49° N-73.81°E and Zuari, 15.41°N-73.91°E) of the Arabian Sea (Fig. 1). In total, fourteen water samples were collected at four depths from ASTS (surface, oxic: 103 m, hypoxic: 134 m, upper suboxic: 190 m, lower suboxic), three depths from G12 (surface, oxic; 80 m, upper hypoxic: 120 m, lower hypoxic) and three depths from G5 (surface, oxic: 8 m, oxic: 24 m, anoxic). Surface and near-bottom waters were also collected from the two stations near to estuarine mouth, exhibiting oxic water column characteristics. The range of oxygen gradients was defined as per Naqvi et al. (2010): oxic (DO >62.5 μM), hypoxic ($4<$DO≤ 62.5 μM), suboxic ($0<$DO≤ 4 μM), and anoxic (0 μM).

Samples were collected using Niskin bottles on a Rosette equipped with a CTD profiler (conductivity, temperature, depth) and a dissolved oxygen sensor. Vertical profiles of temperature and salinity in the water column were recorded from the CTD. Levels of dissolved oxygen (O_2) and nutrients (NO_3^- and NO_2^-) were measured onboard within a few hours of collection, following the titrimetric Winkler's method and the automated colorimetric procedures adapted for a SKALAR auto-analyzer, respectively (Grasshoff et al., 1983). One liter of the water sample was collected for chlorophyll *a* (Chl *a*) analysis and immediately filtered through a GF/F filter. Chl *a* was extracted from the filters with 90% acetone for 24 h, in the dark at −20 °C and the fluorescence measured using a fluorometer (Turner Designs, Model no. 10-AU). To study the protist taxonomy, water samples of 1–5 liters were collected and filtered through Durapore membrane filter paper (47 mm, 0.65 μm, Millipore, Germany) using a peristaltic pump for open ocean, shelf-edge, coastal and estuarine stations. Filters were placed in cryovials, preserved with 3 ml RNAlater (Ambion, Germany) and stored at −20 °C for later DNA extraction.

2.2. Nucleic acid extraction, polymerase chain reaction and sequencing

Collected filter papers were cut into small pieces and transferred to a Lysis E-Matrix tube (MP Biomedicals, Germany). Then 600 μl RLT buffer and six μl b-mercaptoethanol were added and followed by shaking at 30 Hz for 45 s using a mixer mill (MM200, Retsch, Germany). The tubes were centrifuged at 1400 g for 3 min, and the supernatant was collected. For the DNA extraction, we followed the protocol after the 4th step given in the Qiagen's All Prep DNA/RNA Mini Kit Manual. Three replicates of each sample were extracted and pooled. The bulk DNA concentration was measured by NanoDrop 2000 (Thermo Scientific, USA). The V4 region of the 18 s rDNA was amplified in triplicates with a set of universal PrimerTAReuk454FWD1 and TAReukREV3 given by Stoeck et al. (2010). The polymerase chain reaction (PCR) mix contained

50–100 ng of DNA template in 50 μl solution, 1 μl of Phusion High-Fidelity DNA polymerase (Finzymes, New England Biolabs, Ipswich, MA, USA), 1x Phusion Buffer (New England Biolabs, Ipswich, MA, USA), 200 μM each of deoxynucleotide triphosphate and 0.5 μM oligonucleotide primer. The PCR protocol started with the initial denaturation (30 s at 98 °C) followed by 30 identical amplification cycles, denaturation (at 98 °C for 10 s, annealing at 59 °C for 10 s and extension at 72 °C for 30 s) and a final extension at 72 °C for 30 s. Three replicates of reactions for each sample were prepared to reduce PCR bias. Following the PCR process, agarose gel electrophoresis was conducted to purify the PCR products and the target bands of 400–500 bp checked with the help of a Qiagen gel extraction kit. Sequencing of purified V4 amplicons was performed on an Illumina Miseq platform by SeqIT, Kaiserslautern, Germany. A custom script was used to merge the paired-end reads produced from the same amplicon. Accessible sequence reads were deposited in the NCBI Sequence Read Archive under the Bioproject ID number PRJNA369134.

2.3. V4-amplicon data processing and operational taxonomic units analysis

Raw paired-end Illumina reads were processed using the script spilt_libraries.py applied in QIIME v.1.8.0 (Caporaso et al., 2010). The phylotypes were clustered using Uclust (Edgar, 2010) at different sequence similarity (100%–90%). The length distribution of the tags was plotted in R (R Core Team, 2012). For taxonomic classifications and statistical diversity, OTUs called at 97% sequence similarity were used (Nebel et al., 2011; Dunthorn et al., 2014). The core (the longest and thus most informative) sequence for each phylotype at 97% was extracted into a FASTA file. This file was analyzed with JAguc software (Nebel et al., 2011). JAguc employed BLASTn searches, with algorithm parameters adjusted for short (200–500 bp) reads (-m 7 -r 5 — q — 4 — G 8 -E 6 -b 50). The custom script output files from QIIME's OTUpipe (seq_otus.txt) and JAguc (the taxonomic tree for analyzed representative sequence) were merged to a biome file containing information about OTU IDs, the number of sequences per OTU and per sample as well as taxonomic affiliations. Non-target OTUs (metazoans and embryophytes) were excluded, and the resulting file, representing the total planktonic protists, was used for statistical analysis.

2.4. Statistical data analysis

Rarefaction profiles and Shannon index (alpha diversity) were determined using QIIME v.1.8.0 (Caporaso et al., 2010). For this purpose, data were first normalized and resampled 1000 times to account for uneven sample sizes (Logares et al., 2012). Similarity patterns of protist communities were visualized through non-metric-multidimensional scaling (nMDS) analysis based on the Bray–Curtis index which measures the relative abundance of sequences representing higher taxon protist groups (Bray and Curtis, 1957). A permutational multivariate analysis of variance (PERMANOVA) with two factors (habitats and oxygen gradients) was conducted to examine whether the significant variation of protist diversity was due to the partition of habitats (estuary, coastal, shelf-edge, and open ocean) or to different oxygen gradients (oxic, hypoxic, suboxic, and anoxic), or a combination of both (Anderson et al., 2008).

The important environmental parameters for the distribution of the protist community was examined by the biota-environment (BIOENV) method (Clarke and Ainsworth, 1993), which produces a rank correlation between a similarity matrix obtained from the biota and the environmental variables. RELATE was performed and followed by a stepwise distance-based linear model permutation test (DistLM; McArdle and Anderson, 2001) to detect any significant relationships of environmental parameters in support of multivariate variation of higher taxonomic groups of protist assemblages. The value of R^2 was used as a selection measure to show the best explanatory environmental variables in the model. Euclidean distance was applied as a resemblance criterion in the DistLM procedures. Visualizations of results were produced with a distance-based redundancy analysis (dbRDA) (Anderson et al., 2008). All the statistical analyses were performed by the module of PRIMER V6 + PERMANOVA software.

3. Results

3.1. Hydrological parameters

The physical, chemical and biological properties of the water column at different sites in the Arabian Sea exhibited diverse environmental conditions. The water column of the sampling sites showed characteristic variation in their oxygen, temperature, salinity, nutrients, and chlorophyll *a* values. Both Mandovi and Zuari estuaries showed a well-mixed water column, a sign of normoxia. Estuarine stations did not indicate any hypoxic water column conditions, although O_2 levels remained below saturation level (Surface DO 161 μM and 156 μM in the bottom waters of the Mandovi estuary, and surface DO 158 μM and 145 μM at the bottom of the Zuari estuary). The coastal sampling site (G5) O_2 varied from 153 μM (oxic) at the surface, 82 μM at 8 m (oxic) and an undetectable concentration at 24 m (bottom) indicating near anoxic conditions. The shelf-edge station (G12) showed 197 μM at the surface (oxic), 14.6 μM at 80 m (hypoxic) and 6.9 μM at 120 m (hypoxic), whereas the open ocean station had 205 μM O_2 at the surface (oxic), 43 μM at 103 m (hypoxic), 4.1 μM at 134 m (suboxic) and 4.4 μM at 190 m (suboxic). Seasonal low oxygen condition was well established at the coastal site (G5) and at the shelf-edge (G12) while permanent OMZ prevailed in the open ocean (ASTS).

The temperature ranges in the estuarine water column ranged from 27.2–28.9 °C, in the coastal water column from 21.9–27.1 °C, at the shelf-edge from 18.1–29.1 °C and in the open ocean from 16.4–28.8 °C. From all the sampling sites, the highest salinity was recorded in the open ocean (36.9 PSU) and the lowest in the Mandovi surface waters (26.1 PSU). Significant spatial variation in salinity was noticed from the estuarine sites to the open ocean site. Maximum chlorophyll *a* concentration was observed at the estuarine station and the concentrations varied in between 6.61–7.12 μg l^{-1} whereas, it gradually decreased from coastal to oceanic sites.

The highest nitrite concentration (4 μM) was observed at OMZ core depth of the open ocean, and the highest nitrate concentrations at 120 m depth at the shelf-edge station (Supplementary File 1 and 2).

3.2. Sequencing statistics of V4 amplicon analyses

After the quality check, altogether 1395168 protistan V4 amplicons were obtained for taxonomic identification of protists at different sampling sites. Our target eukaryotic reads without singletons/doubletons produced 1387818 sequences, grouping into 12687 operational taxonomic units (OTUs) called at 97% sequence similarity. The highest (518676 reads) number of reads was obtained from the open ocean, while the lowest (127621 reads) marked the shelf-edge (Table 1). The rarefaction analysis established the saturated sampling profiles for OTUs called at 97% sequence similarity (Fig. 2). Out of total reads 54% of the target sequences showed sequence similarity of $>$ 95% to their closest BLAST hit in the protist V4 18S rDNA database.

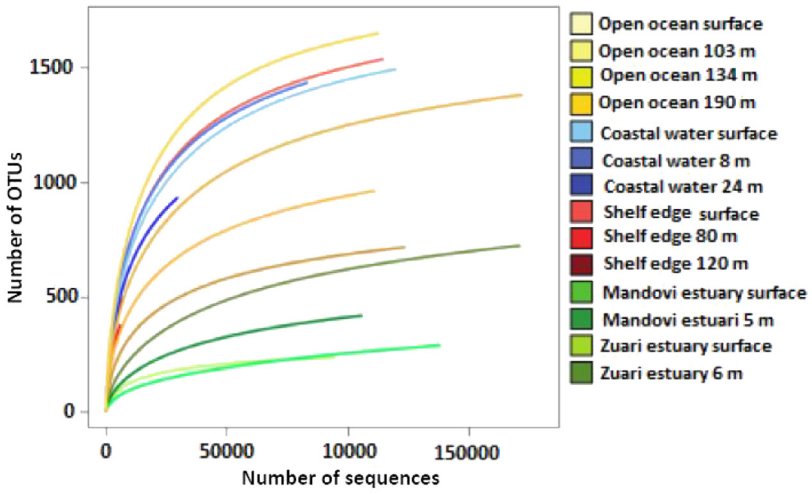

Fig. 2. Rarefaction curves for the spatially different sampling sites, based on only target eukaryotes reads without singletons/doubletons. The profiles of the rarefaction curves specify near-saturation for all sampling sites.

Table 1
An overview of Illumina sequencing data sets for the total protists in the open ocean (ASTS), continental shelf-edge (G12), coastal (G5) and estuarine regions (Mandovi and Zuari).

Sample ID	High quality target eukaryotes		High quality target eukaryotes without single and double tones	
	Sequence nos. (%)	OTU nos.	Sequence nos. (%)	OTU nos.
ASTS (surface)	113 609 (35)	2671	112 380 (34)	1652
ASTS (103 m)	172 875 (69)	2181	171 919 (68)	1384
ASTS (134 m)	111 497 (49)	1438	110 890 (49)	965
ASTS (190 m)	123 886 (67)	1062	123 487 (67)	719
G12 (surface)	115 635 (40)	2439	114 554 (39)	1540
G12 (80 m)	5984 (5)	495	5849 (5)	379
G12 (120 m)	7364 (8)	624	7218 (8)	502
G5 (surface)	120 655 (47)	2234	119 791 (47)	1496
G5 (8 m)	84 013 (48)	2136	83 207 (47)	1437
G5 (24 m)	29 819 (19)	1187	29 544 (19)	935
Mandovi (surface)	138 079 (80)	402	137 949 (79)	291
Mandovi (5 m)	105 881 (77)	534	105755 (77)	421
Zuari (surface)	94 332 (75)	320	94240 (74)	240
Zuari (6 m)	171 539 (74)	1152	171 035 (73)	726

3.3. Protist diversity at the local scale (alpha-diversity)

In terms of alpha diversity estimates, estuarine communities appeared to be less diverse than the coastal, shelf-edge and open ocean stations. Among all the 14 sampling sites, the Shannon index varied from 1.01 in the estuarine station (MS) to 6.5 in the coastal station (G5–8 m). The statistical results clearly indicated the highest protist diversity occurred at 8 m depth in the coastal station and in the surface water of the open ocean station (Fig. 3). Comparatively, estuarine stations revealed very low diversity, in a range from 1–1.5, whereas the diversity ranged from 3.2–6.5 in the coastal and oceanic water column. The vertical diversity pattern of Mandovi and Zuari estuaries showed little variation based on higher taxonomic levels. Coastal water did not reveal a clear diversity difference corresponding to oxygen gradients. Protistan OTU alpha diversity in the shelf-edge and open ocean showed a notable variation among the different depths of oxygen gradients (Fig. 3).

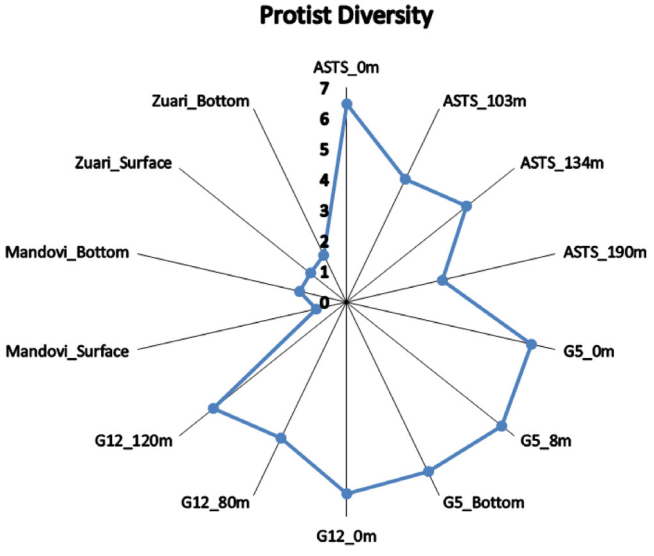

Fig. 3. Radar plot to indicate the Shannon diversity index (0–7) for total protist diversity at the distinct sampling sites. Diversity values calculated are based on 97% similarity of protist OTUs.

3.4. Taxonomic composition of protist communities in distinct spatial sites

In total, 22 Protistan phylogenetic groups, including Ciliophora, Dinophyceae, Unclassified Alveolates, Centroheliozoa, Choanoflagellida, Cryptophyta, Fungi, Haptophyceae, Picozoa, Rhizaria, Stramenopila, Telonema, Viridiplantae, Colpodellidae, Amoebozoa, Apusozoa, Dimorpha, Eccrinales, Ichthyosporea, Apicomplexa,

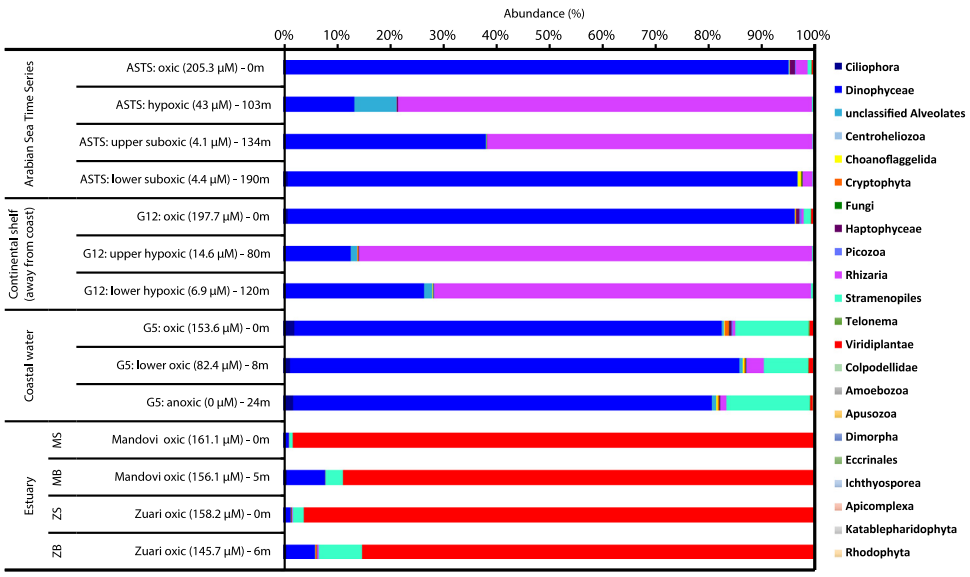

Fig. 4. Total protist community composition in contrasting ecological sampling sites of the Arabian Sea.

Katablepharedophyta and Rhodophyta were identified at all the sampling sites in estuarine and open ocean waters.

Estuarine sites were represented by Viridiplantae (91%–465389 sequences), Stramenopiles (4%–20251 sequences), Dinophyceae (3.8%–19291 sequences) and Ciliophora (0.4%–2060 sequences). In estuarine stations maximum number of sequences affiliates to Viridiplantae (85%–98%) both in surface and near-bottom waters as compared to neritic (G5 and G12) and oceanic stations (ASTS). Stramenopiles (3.2%) and Dinophyceae (7.17%) were more prominent in the bottom than the surface waters in Mandovi estuary (Fig. 4).

Overall, coastal waters showed the dominance of Dinophyceae (81.7%–190154 sequences), Stramenopiles (12%–28141 sequences) and Ciliophora (1.7%–4153 sequences). With reference to oxygen gradients, the variation in the protist community between the oxic (surface and 8 m) and anoxic water column was insignificant. Dinophyceae remained the most abundant group both in oxic (83%–83422 sequences) and anoxic (79%–23311 sequences) zones (Fig. 4).

The community at the shelf-edge was dominated by Dinophyceae (88%–112025 sequences), Rhizaria (8%–10543 sequences) and Stramenopiles (1.2%–1584 sequences). Substantial variation of protist communities between oxic (surface water) and hypoxic water column was detected at this station. In the oxic surface waters, the dominant communities were Dinophyceae (95%), whereas 85% and 71% of the total protist community was represented by Rhizaria in the hypoxic water column (80 m and 120 m). Remarkably, the hypoxic water column contained an approximately 200 times higher Rhizaria community than that at the surface (oxic) water (Fig. 4).

Overall, the open ocean water column was dominated by Dinophyceae (55.9%–290049 sequences), Rhizaria (39.9%–206794 sequences), unclassified Alveolates (2.7%–14061 sequences), Ciliophora (0.4%–1882 sequences), and Stramenopiles (0.3%–1550 sequences). The surface waters of the open ocean showed the dominance of Dinophyceae (94.8%–106495 sequences), whereas the hypoxic water column revealed Rhizaria (78%–134012 sequences), Dinophyceae (13%–22910 sequences) and unclassified Alveolates (8%–13654 sequences). The suboxic strata at 134 m and 190 m were marked by distinctively different protist communities. Of these, the upper suboxic water column revealed Rhizaria (61.3%–67981 sequences) and Dinophyceae (38%–42116 sequences). Conversely, the lower suboxic water column was mainly represented by the Dinophyceae (96%–118528 sequences) and less by Rhizaria (1.9%–2350 sequences). The presence of Ciliophora, Choanoflagellida, Cryptophyta, and Fungi was noted in the deep OMZ core of the open ocean as compared to the oxic and hypoxic depths. Overall results suggest that the protist communities (higher taxa) vary with the water strata at oxic, hypoxic and suboxic depths (Fig. 4).

3.5. Partitioning of protist diversity among spatially varying regimes

Across the distinct biogeochemical regimes, we observed remarkable differences in the protist community composition based on higher taxonomic supergroups. These variations in the protist community were statistically confirmed. In a nonmetric multidimensional scaling (nMDS-distance measured: Bray–Curtis similarity), the protist community clustered spatially and showed three major groups observed in distinct spatial habitats (Fig. 5). Stations from the open ocean (AS–0 m), shelf-edge (CS–0 m) and open ocean OMZ core (AS–190 m) clustered together, distinct from the other group consisting of AS–103 m, CS–80 m, AS–134 m and CS–20 m (Fig. 6). SIMPER analysis revealed the highest average dissimilarity (74%) between open ocean and estuary where Viridiplantae (38%) and Dinophyceae (25%) were the dominant contributors to the dissimilar community (Table 2). Results of the analysis of the oxygen gradients showed the highest average community dissimilarity (64%) was exhibited by the oxic and hypoxic water column, in this Rhizaria (39%) and Viridiplantae (22%) were the largest contributors.

In relation to habitats and oxygen gradients distribution, the PERMANOVA community results based on the Bray–Curtis similarity revealed a significant difference ($p = 0.003$ and $p = 0.011$; Table 3a). As regards to habitat distribution, pair wise PERMANOVA based on the Bray–Curtis similarity, results showed significant values of community difference between coastal and estuary ($p = 0.009$), shelf-edge and estuary ($p = 0.022$) and open ocean and estuary ($p = 0.051$). However, protist community composition between open ocean and shelf-edge, open ocean and coastal, shelf-edge and coastal showed no significant differences (Table 3b). The pairwise PERMANOVA test for oxygen gradients, on the other hand, revealed significant community differences between oxic and hypoxic water columns. The community comparison in other oxygen gradients (oxic and suboxic, oxic and anoxic, hypoxic and suboxic, hypoxic and anoxic and suboxic and anoxic) showed no significant differences (Table 3c).

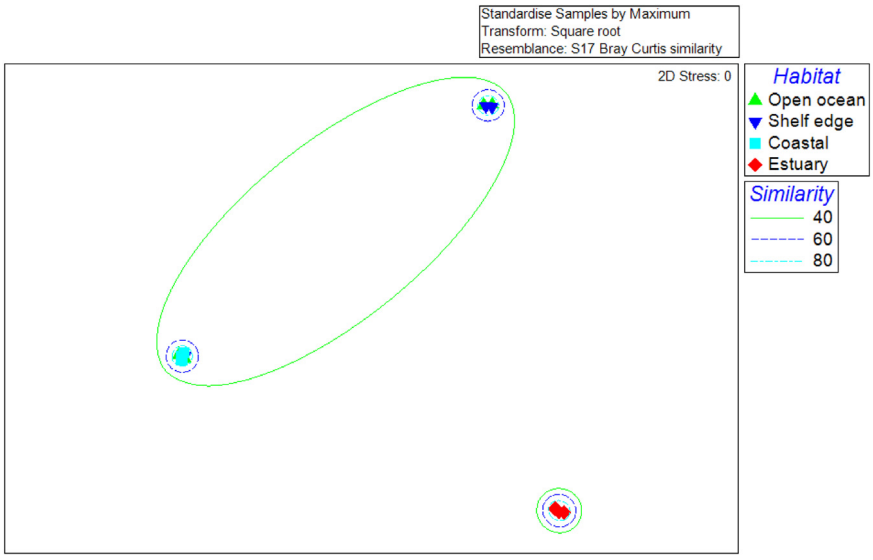

Fig. 5. nMDS ordination based on Bray–Curtis index of protist Illumina sequences analyzed in open ocean, continental shelf-edge, coastal and estuarine sampling sites.

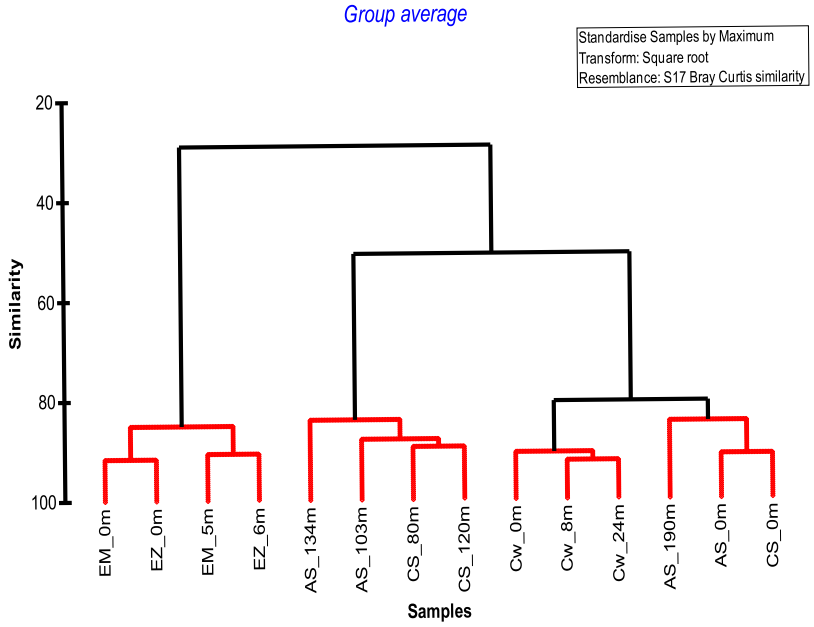

Fig. 6. Cluster analysis based on the abundance of protist Illumina sequences analyzed in the different sampling sites with reference to the Bray–Curtis similarity index (EM: Mandovi estuary; EZ: Zuari estuary; AS: open ocean; CS: continental shelf-edge; CW: coastal).

Table 2
Results of SIMPER analysis showing the difference of community contribution in the open ocean and estuary (Av. Abund: Average abundance; Av. Diss: Average dissimilarity; Diss/SD: Dissimilarity/Standard deviation).

Supergroups	Open ocean	Estuary				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Viridiplantae	0.4	10	28.22	9.97	38.14	38.14
Dinophyceae	8	1.87	18.53	2.05	25.04	63.18
Rhizaria	5.73	0.35	15.01	1.25	20.29	83.46
Stramenopiles	0.58	1.82	3.51	1.53	4.75	88.21
Unclassified Alveolates	1.08	0.11	2.74	0.78	3.7	91.91

3.6. Environmental effects on partitioning diversity and community structure

RELATE analysis confirmed the enhanced correlation of environmental factors with the patterns of community structure in distinct spatial habitats. BEST analysis (BIOENV) revealed the importance of salinity, chlorophyll *a*, nitrate and nitrite on the abundance of the higher taxa of the protist community. Following BEST analysis, distance-based linear model (DistLM) explained 87% of protist communities were influenced by five environmental factors. From among the six environmental factors, temperature, salinity, nitrate, nitrite, and chlorophyll *a* were the closest fitting

Table 3
Results of PERMANOVA analyses (Based on Bray–Curtis similarity measure) with reference to difference in habitats and oxygen gradients (a) results of Pairwise comparison PERMANOVA analysis with reference to habitats (b) results of Pairwise comparison PERMANOVA analysis with reference to oxygen gradients (c). Data were transformed; resemblance was calculated according to Bray and Curtis. The values ($P < 0.05$) reveals the significant differences (Perm: permutation; MC: Monte Carlo randomization)..

(a)

Groups	df	P (perm)	Unique perms	P (MC)
Habitats	3	0.003	912	0.0021
Oxygen gradients	3	0.011	999	0.011

(b)

Groups	t	P (perm)	Unique perms	P (MC)
Open ocean, Shelf-edge	5.5	0.084	28	0.034
Open ocean, Coastal	0.7	0.596	291	0.626
Open ocean, Estuary	4.12	0.051	581	0.002
Shelf-edge, Coastal	1.65	0.19	165	0.2249
Shelf-edge, Estuary	6.52	0.022	105	0.004
Coastal, Estuary	8.25	0.009	105	0.001

(c)

Groups	t	P (perm)	Unique perms	P (MC)
Oxic, Hypoxic	6.27	0.001	992	0.001
Oxic, Suboxic	1.27	0.181	985	0.224
Oxic, Anoxic	0.53	0.86	798	0.827
Hypoxic, Suboxic	1.77	0.264	30	0.216

Table 4
Results of distance-based linear model (DistLM) analysis showing the influence of environmental parameters on the prominence of protist groups and Bray–Curtis similarity of square-root-transformed abundance (SS: sum of squares; F: pseudo-F; P: p value; Prop: proportion of explanation).

Variable	SS (trace)	Pseudo-F	P	Prop.
Temperature	6000.2	5.405	0.02	0.31054
Salinity	6679.3	6.3398	0.009	0.34569
DO	3605.9	2.7533	0.089	0.18662
NO_3^-	6695.7	6.3637	0.008	0.34654
NO_2^-	1626.7	1.1031	0.239	8.4188
Chl *a*	3785.6	2.9239	0.084	0.195

parameters to the model. The DistLM analysis identified the significant correlation of environmental variables ($p < 0.05$) with the distribution pattern of protist communities. Variables identified through the marginal test such as temperature ($p = 0.02$), salinity ($p = 0.009$) and nitrate ($p = 0.008$) showed significant correlations with the protist assemblages at different sites (Table 4). The influence of dissolved oxygen, however, was insignificant to protist distribution. The best fitting environmental variables obtained by the DistLM procedures using dbRDA plot (Fig. 7), indicated the primary importance of chlorophyll *a* association in the estuarine sites while salinity, nitrate, and temperature influenced shelf-edge, coastal and open ocean sites, respectively.

4. Discussion

The present study attempts to characterize protistan diversity in OMZs of the Arabian Sea using the NGS amplicon sequencing approach. Importantly, this is the first such report on 18 s rRNA gene diversity of the protist communities along spatial and oxygen gradients (OMZ sites) in the Arabian Sea. We used Illumina sequencing of the V4 amplicons of the 18S rRNA gene as a taxonomic sign and demonstrated a pattern of diversity with taxa previously unaccounted for when relying on identification under the microscope. Recent studies have similarly shown high genetic diversity in planktonic protists in OMZ sites of Equatorial Tropical South Pacific (ETSP), Equatorial Tropical North Pacific (ETNP) and the Costa Rica coast (Parris et al., 2014; Duret et al., 2015; Jing et al., 2015). We tried to investigate vertical community differences in the OMZ water column and compared the spatial distribution of the protist community under different oxygen gradients.

4.1. Importance of planktonic micro eukaryotes in varying ecological regimes

Ecosystems such as the estuary, coast, shelf-edge, and open waters of the northern Arabian Sea are influenced by diverse environmental factors. They harbor an array of planktonic protists known to be the major functional components of pelagic food webs (Qasim and Gupta, 1981; Gifford et al., 2007; Strom et al., 2007). Both autotrophic and heterotrophic eukaryotic microbes have been reported to be present in the different ecological settings of the world ocean, which are prone to mediate food webs (Pernice et al., 2016; Massana et al., 2015). It is important therefore to understand the assemblages of the smallest eukaryotic microorganisms in the pelagic water column and their trophic roles in associated ecosystems. The ecological conditions of the Arabian Sea shelf and open waters are influenced by the presence of large rivers in the peninsular region that bring land runoff to the sea. The presence of two major estuaries, Mandovi and Zuari, also have a sizable impact on coastal waters which result in upwelling phenomena due to strong seasonal currents and monsoon land runoff to the sea. Upsurges of nutrient through the upwelling process results in planktonic blooms. As estuaries during non-monsoon season are under the tidal influence, this also causes significant changes in bio-geochemical regimes to result in high productivity in coastal waters. Earlier studies on large marine protists (gromiids), fungal diversity and picoplankton revealed a diversified community structure in contrasting pelagic environments of the Arabian Sea (da Silva and Gooday, 2009; Jebaraj et al., 2010; Fuchs et al., 2005).

Oxygen depletion in the water column (hypoxic/suboxic conditions) is usually seen as environmental stress leading to habitat compression, loss of fauna and energy diversions into microbial pathways in different marine ecological settings (Diaz and Rosenberg, 2008). However, the Arabian Sea experienced coastal (seasonal) and open ocean OMZ (permanent as well as seasonal) conditions (Naqvi et al., 2006), where the consumption of nutrients by autotrophic plankton is hampered by the prevailing turbidity of nearby coastal waters, caused by suspended particulates. Thus, the unutilized nutrients are carried towards the open coast, which probably helps the planktonic life in this region to flourish. These ecological processes result in harboring different patterns of protist communities. Future studies of these diverse communities in various ecological conditions would reveal vital facts on oceanic circulation including climate change.

4.2. Taxonomic distribution and diversity of the protist community

The eukaryotic organisms Viridiplantae, belong to the class of green algae these are mostly found as aquatics (without embryophytes) and land plants (embryophytes) (Becker and Marin, 2009; Cocquyt et al., 2009; Kim et al., 2014). The pronounced abundance of Viridiplantae observed in both Mandovi and Zuari could be the result of low salinity compared to other coastal sites. The reason for low salinity in these estuaries are mainly due to the land runoff. This result is confirmed in PERMANOVA (the pairwise test) where estuarine sites significantly differed from the neritic and oceanic water column in terms of Viridiplantae occurrence. SIMPER analysis also corroborated the relatively common Viridiplantae population in estuarine sites. A recent study on environmental metabarcoding in the Mersey estuary concluded that microbial plankton could be drivers in contrasting estuarine ecosystems (Lallias et al., 2015). These studies further described the influence of diversity patterns under different salinity regimes

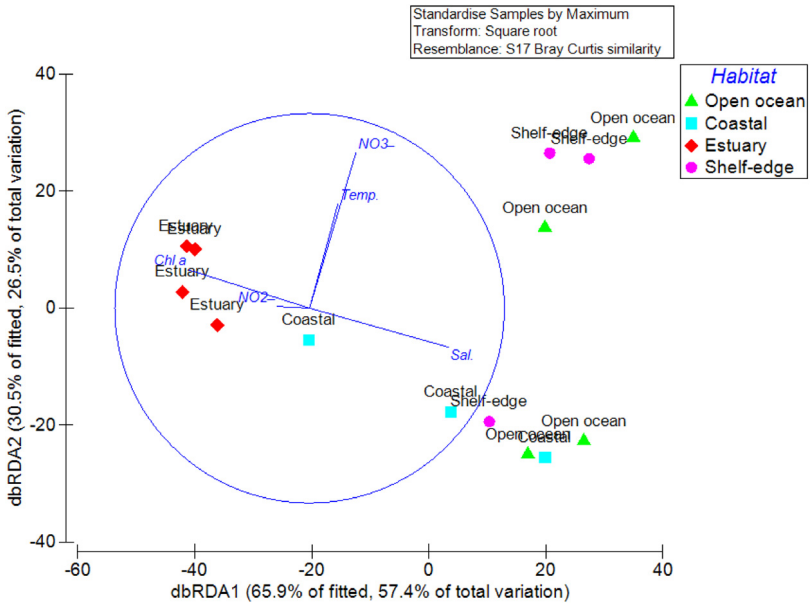

Fig. 7. Distance-based redundancy (dbRDA) plot summarizing the DistLM model based on protist assemblage data and fitted environmental parameters (strength and direction of effect of the variable on the ordination plot). Axis legends include percentage of variation explained by the fitted model and percentage of community variation explained by the axis.

(euryhaline, mesohaline and oligohaline). The taxonomic diversity of these eukaryote species reportedly declined from marine to fresh water systems (Lallias et al., 2015). The relatively low diversity of microbial eukaryotes in the Mandovi and Zuari estuaries also suggests that less saline waters mediate lower diversity than coastal open-sea ecosystems. Besides low salinity, environmental contaminants such as heavy metals and hydrocarbons could also be responsible for low microbial diversity and the presence of specific microbial eukaryotes in estuarine waters. Earlier studies have reported that Mandovi and Zuari estuarine waters were prone to release of heavy metals and petroleum hydrocarbons due to the anthropogenic additions from industrial discharges, boat traffic and barge building activities which contaminant the riverine waters (Pradhan and Shirodkar, 2011). Furthermore, heavy metals play an active role in controlling the eukaryotic community structure and their diversity pattern. In this context, the lowest eukaryotic diversity of Rio Tinto River, Spain was attributed to the presence of high heavy metal concentrations (Aguilera et al., 2006). In addition, low concentrations of petroleum hydrocarbons also affect the population dynamics of planktonic micro eukaryotes in estuarine and marine ecosystems (Nayar et al., 2005; Almeda et al., 2014).

Bottom anoxia and vertical oxygen gradients did not appear to greatly influence the prominent protist community at the coastal site, indicating free movement of the organisms throughout the water column. However, it is possible that few communities have evolved to thrive with low oxygen levels. It was observed that Dinophyceae was the dominant protist community in coastal, shelf-edge and open ocean sites. The cosmopolitan nature of these organisms has been reported by many studies on rRNA based molecular signature (Duret et al., 2015; Edgcomb et al., 2011; Massana, 2011).

The hypoxic shelf-edge water column was characterized by the prominence of Rhizaria community. Among the Rhizaria community, Polycystinea and Acantharea dominated the hypoxic strata at the shelf-edge station. The deep open ocean station also showed the dominance of Rhizaria groups in hypoxic and upper suboxic strata. Their aggregation in the OMZ could signify a cell sinking process through attachment to fecal matter or dead metazoan hosts from the surface (Turner, 2002; Parris et al., 2014). As was hypothesized, the eukaryotic community structure in OMZ was influenced by the community present in the overlying photic zone (Parris et al., 2014). Earlier metagenomic studies also discovered a peak abundance of Acantharia and other radiolarians at the OMZ boundary (Parris et al., 2014; Edgcomb et al., 2011). Although oxygen levels were similar in the two suboxic depths of the open ocean (134 m and 190 m), the lower suboxic stratum sustained a higher proportion of Dinophyceae than did the upper suboxic zone. Here cell sinking from overlying waters plays a major role to sustain a higher abundance of Dinophyceae in the lower suboxic zone. This is also indicative of an accumulation of inactive or dead metazoan hosts. This is most commonly observed during the seasonal surface diatom bloom phenomenon, where the dead cells rapidly sink into the anoxic water column in the form of particulate organic matter (Parris et al., 2014).

Although the higher taxon groups which were identified in the contrasting spatial-ecological regimes did not differ greatly, their diversity and distribution patterns varied significantly. Substantial changes in oxygen and other environmental factors distinctly indicated an ecological partition across the sampling sites of estuarine, coastal, shelf-edge and open ocean sites on the spatial scale. These observations support the hypothesis that spatial patterns of genetic variability and the difference in oxygen gradients in OMZ sites in the Arabian Sea are directed by biogeochemical processes. In terms of habitat, estuarine sites showed significant differences compared to coastal, shelf-edge and open ocean sites. However, as regards the oxygen gradients, only the communities in oxic and hypoxic strata differed significantly, whereas no significant changes were encountered in the communities of the strata of the oxic, anoxic and suboxic water column. Insufficient data from the anoxic and suboxic sampling sites when compared to hypoxic and oxic environments could reflect the discrepancies in community differences that were observed.

Statistical evaluations of the protist community structure revealed that the difference between habitats as well as oxygen gradients are strong indicators of community variability. The PERMANOVA test also showed significant differences between habitats as well as oxygen gradients. This study of spatial barriers in protist communities revealed a predictable pattern of beta-diversity as changes in habitat and oxygen gradients had a significant effect

on the plankton community structure. At the regional scale, environmental factors could be responsible for structuring the planktonic protist communities. Heterogeneous community structure on a regional scale caused by differences in environmental factors was reported previously from OMZ of ETSP off the coast of Chile (Parris et al., 2014), where, as in our analysis, the effects of spatial gradients in structuring plankton communities at a local scale were much more pronounced than the oxygen effects. Also, the spatial variation of community structure is determined by environmental factors at different spatial scales. This is the first such report to identify the whole protist community through NGS in this study region. The prevalence of the most abundant community at a particular site was determined by the specific environmental factors of that site. However, the earlier studies have reported that these micro eukaryotic protist communities are ubiquitously found over a global scale (Fenchel and Finlay, 2004; Finlay and Fenchel, 2004). High abundance of a specific community is in agreement with Hubbell's Unified neutral theory of biodiversity and biogeography (Hubbell, 2001). This fact necessarily explains the diversity pattern of protists observed in estuarine, coastal, shelf-edge and the open ocean ecosystems in the present study. The presence of a particular species in great abundance in the Arabian Sea OMZs could, therefore, also vary on a spatial scale with O_2 gradient supporting different sets of protist assemblages.

The dynamic nature of the Arabian Sea is evident in the varying physical and biogeochemical properties which produce geographical partitioning. It is imperative, therefore, to understand the distribution pattern of oxygen and other environmental factors causing the community variation on a spatial scale. The results of BIOENV analysis revealed a strong correlation between the environmental parameters (salinity, chlorophyll *a,* nitrate, and nitrite) and the abundance of the protist community. Moreover, environmental parameters showed wide variation between estuarine, coastal and oceanic waters. In this context, the estuarine community was different from the coastal and oceanic sites. However, dissolved oxygen did not exhibit a strong relationship with protist distribution. The DistLM analysis, on the other hand, showed a significant relation between variations in salinity, temperature, and nitrate, and protist community distribution.

5. Conclusions

This study investigated the dynamic nature of pelagic ecosystems with reference to protist community structure and environmental changes. Examination of spatially varying habitats exemplified by estuaries, coastal, outer shelf (shelf-edge) and open ocean revealed that environmental attributes largely govern the shift in protist community structure since NGS data analysis clearly showed the association of protist groups with different ecosystems. The ecological distribution of protist communities in the Arabian Sea, the first such report in this region to provide a systematic and reliable taxonomic hierarchy of the micro-nano eukaryotes present in the contrasting ecosystems and also could be a vital addition to global biogeographic diversity information. In view of the paucity of genetic data on protist diversity in oxygen deficient and estuarine sites, the present study serves as a valuable source of information on the adaptability of the protist community in diverse environmental settings in neritic and oceanic waters.

Acknowledgments

We thank the Director, CSIR-National Institute of Oceanography for supporting this work. P.B. Das is grateful to the Council of Scientific and Industrial Research (CSIR) for providing a doctoral research fellowship. We gratefully acknowledge Professor Dr. Thorsten Stoeck, HOD, Division of Ecology, and Kaiserslautern University, Germany for providing laboratory facilities and sharing expertise. We are also grateful to Dr. Hema Naik, Dr. Siby Kuriyan, Dr. Damodar Shenoy and Dr. Anil Pratihary for their support during this study. This work was carried out as a part of the Network Project PSC0108 funded by the CSIR and the SIBER program funded by the Ministry of Earth Sciences (MOES), Government of India. This is NIO's contribution no. 6335.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2018.100484.

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue IX, September 2017- Available at www.ijraset.com

Zooplankton Assemblages in Spatial Stretches of a Tropical Estuary

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Abstract: Present study embodies the spatial variations in zooplankton species dispersal and copepod community structure in the near mouth, away from the mouth, mid estuarine and upstream region of the Mandovi estuary. Overall 35 copepod species were identified in the 4 spatial demarcation of the estuary. Cluster analysis revealed the upstream zooplankton community were remarkably different from the near shore and mid estuarine regions. In the similar context, upstream water represented Diaptomus* sp.*, Acartiella* sp.*, Heliodiaptomus cinctus and Cyclops* sp. *abundantly while Paracalanus parvus, Paracalanus aculeatus and Oithona similis were characterising near mouth of the estuary. The mid estuarine location revealed higher abundance of Acrocalanus longicornis and Oithona brevicornis. The SIMPER analysis confirmed the influence of environmental factors on the zooplankton community distribution at a spatial distinction, where Salinity was the maximum contributor (93-94%) in differentiating these environmental settings.

Key words: Zooplankton assemblages, Estuarine partition, Environmental influence, Multivariate analysis, PRIMER

I. INTRODUCTION

An ecosystem is multiplexed with the association of biological communities and their respective environment [1]. The number of ecosystems vary with the geographical alternations and their function regulated by the environmental attributes. The multivariate tools have been used for the comparison of biotic community structure in different habitats and their significant association with surrounding physicochemical factors. A huge number of studies has debated the abundance and patterns of zooplankton community composition in ambient estuarine conditions [2, 3, 4]. Zooplankton is an important constituent in the aquatic food web, which plays a key role in the transfer of organic carbon from the autotrophs to higher trophic levels [5]. It is a measure of secondary productivity, and they respond to change in surrounding physical, chemical and biological parameters due to their short generation times (Anger, 2003; Bornet and Frid, 2004; Queiroga and Blanton, 2004) [6, 7, 8]. It is well known that the environmental factors mediated spatial distinction of estuarine regions influence the biological community variation [9]. Estuarine copepod distribution is governed by the interaction of physicochemical factors concerning their surrounding water masses.

In the current study multivariate methods are used to obtain possible cause, effect and relation among the zooplankton assemblages in four spatial distinctions of the Mandovi estuary, which split into near mouth (M1), away from the mouth (M2), mid estuarine (M4) and upstream stations (M6). Mandovi is one of the well-known estuaries in Goa on the west coast of India, which is experienced with seasonal as well as spatial variation of physical, chemical and biological factors. Salinity is one of the major criteria for the selection of euryhaline and stenohaline copepod communities associated with this estuarine system [3]. This estuary becomes saline dominated during the premonsoon period (Feb-May), and the well-mixed water column was in the estuarine system. The entry of sea water with the tidal variation regulates the flow of the Mandovi estuarine system. Mandovi river has an extension of 75 km, where the width of the mouth is 3.2 km, and the upstream narrows down to < 0.25 km [3]. Numerous studies have already discussed the zooplankton community structure in Mandovi estuary. However, these have not elaborated the association of environmental factors from near mouth to upstream water. During the current study, we have observed the distinguished zooplankton assemblages from near mouth to upstream waters of Mandovi estuary using multivariate analysis. Findings obtained during the investigation are considered vital because of identifying source responsible for changing biological assets in different environmental conditions.

II. MATERIAL METHODS

The sampling was carried out during the spring intermonsoon on 31st march 2015 at a stretch of four stations in Mandovi estuary (FIG. 1). The mechanised trawler was employed for the estuarine sampling. Zooplankton samples were collected from the near mouth (M1), away from the mouth (M2), mid estuarine (M4) and upstream stations (M6) using the Heron-Tranter net (mouth area 0.25 m^2 and mesh size of 200 µm) through horizontal hauls. The average station depth varied in between 5 and 15 m. The samples

were preserved in 4% buffered formalin. Depending on the sample concentration splitting (%) was determined through Folsom splitter. Total zooplankton and copepod numerical counts were calculated for the whole sample in the term of ind $100m^{-3}$. Surface water samples were collected for the analysis of important environmental factors such as temperature, salinity, nitrate, nitrite and dissolved oxygen concentration following standard protocols [10].

The statistical analysis includes multivariate analysis as cluster analysis and multidimensional scaling (MDS) through PRIMER v6 [11, 12].

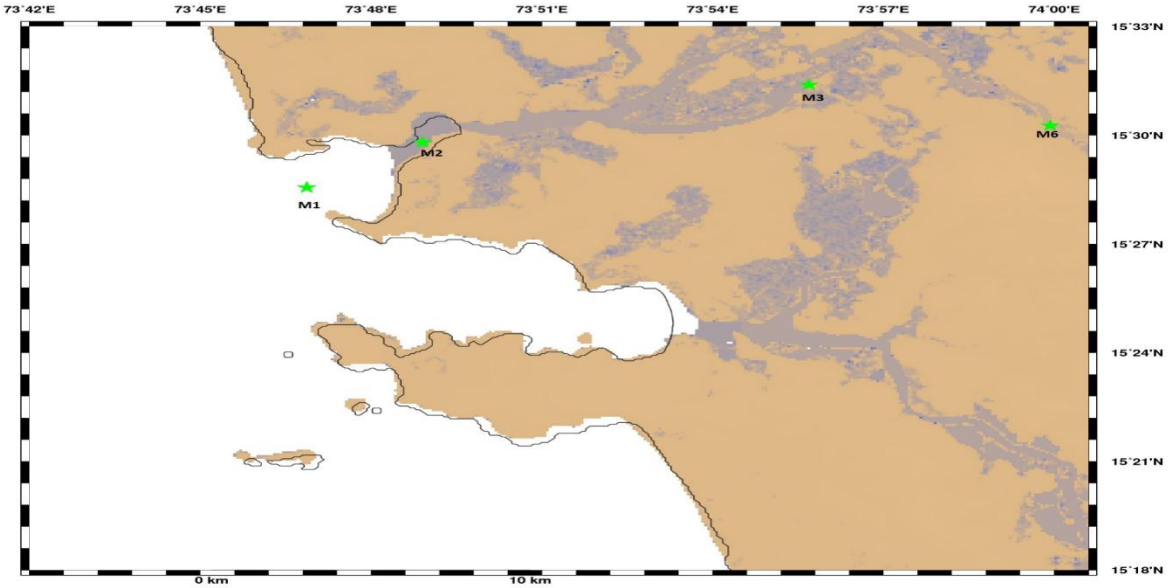

Fig. 1. Sampling locations represented the spatial distinction (near mouth: M1, away from the mouth: M2; mid estuarine: M3; upstream: M6) of the Mandovi estuary.

Zooplankton diversity values were also measured on the species abundance data using the same software. SIMPER analysis was used to identify the species discrimination. Moreover, Redundancy analysis (RDA) was performed by using CANACO 4.5 software to link zooplankton abundance with environmental attributes [13].

III. RESULTS

A. Environmental attributes

The detail measures of environmental variables in four different estuarine stations are given in a Table 1. The surface water temperature ranges from 30.5°C to 32.3°C at M1and M6. The highest temperature (32.4°C) was observed at M6, whereas lowest (30.48°C) was recorded at M2. Spatial variability of salinity in the Mandovi estuary appeals the explanation of freshwater discharge into the aquatic system. Measured salinity at M1 was highest (34.18 psu), whereas least salinity (16.42 psu) was recorded at M6. There were no much variation of dissolved oxygen concentration observed in between estuarine stations. The range of dissolved oxygen concentration from 3.8-4.1 µM was observed at all the sampling sites, where the upstream station revealed maximum (4.1 µM) and the minimum value (3.65 µM) was displayed at mid estuarine station M3. Among nutrients, high Nitrate concentration (1.21 µM) was noticed at M3 and low nitrite concentration (0.12 µM) was recorded at M6.

Table 1: Station-wise details of environmental factors in the Mandovi estuary.

Stations	Temperature (°C)	Salinity (psu)	DO (µM)	Nitrate (µM)	Nitrite (µM)
M1	30.5721	34.1888	3.84	0.83	0.345
M2	30.4842	34.1163	3.85	0.92	0.37
M3	31.0823	31.7251	3.65	1.215	0.53
M6	32.3669	16.4222	4.11	0.89	0.12

B. Zooplankton Species composition and abundance

Altogether 15 major groups were encountered in a stretch of estuarine stations from M1 to M6. Copepods were the most dominant group in the term of species richness and numerical abundance. Overall 35 species represented by four diverse groups of copepods were identified. Moreover, these copepods represent fourteen families along the spatial gradient of estuarine stations. Among all the families paracalanidae copepods revealed the highest contribution such as 48% at M1, 58% at M2 and M3, and 29% at M6. Details on family wise copepod abundance (ind $100m^{-3}$) and other major zooplankton groups are given in the Table 2. Moreover, species-wise copepod information was elaborated the copepod community distribution in four sampling points of the estuary (Table 3).

The total zooplankton abundance (ind $100m^{-3}$) was recorded at M1 was 340677 and 623766 was at the station M2. While the station M3 revealed 274618 ind $100m^{-3}$ and M4 showed 60098 ind $100m^{-3}$. At each station, copepod dominance was contributed by 94% at M1, 80% at M2, 76% at M3 and 65% at M6 (Table 4).

Table 2: Total zooplankton abundance (ind $100m^{-3}$) observed in the spatial distinction of Mandovi estuary, including copepods (family) and other zooplankton groups.

	M1		M2		M3		M6	
Copepods (family)	(ind $100m^{-3}$)	%	(ind $100m^{-3}$)	%	(ind $100m^{-3}$)	%	(ind $100m^{-3}$)	%
Paracalanidae	163446	48	359024	58	160000	58	17171	29
Pseudodiaptomidae	6892	2	5620	1	582	0.2	-	-
Acartiidae	985	-	38088	6	2909	1	937	2
Centropagidae	1969	1	624	0.1	1164	0.4	-	-
Tortanidae	-	-	624	0.1	-	-	-	-
Temoridae	985	-	624	0.1	582	0.2	-	-
Eucalanidae	4923	1	10615	2	-	-	-	-
Pontellidae	-	-	1249	0.2	-	-	-	-
Clausocalanidae	-	-	24976	4	2327	1	-	-
Diaptomidae	-	-	-	-	-	-	8585	14
Oithonidae	45292	13	43707	7	26764	10	5620	9
Cyclopidae	-	-	-	-	-	-	780	1
Tachidiidae	11815	3	6244	1	4073	1	2341	4
Corycaeidae	68923	20	8741	1	5818	2	2498	4
Copepod juveniles	14769	4	-	-	4655	2	1093	2
Other major groups								
Chaetognaths	-	-	4995	1	-	-	-	-
Appendicularians	4923	1	1873	-	582	0.2	-	-
Cladocerans	-	-	1249	-	-	-	-	-
Pelecypoda larvae	3938	1	4371	1	21527	8	468	1
Polychaete larvae	-	-	81171	13	6400	2	-	-
Decapods and larvae	4923	1	24976	4	10473	4	7024	12
Gastropod larvae	-	-	624	0.1	15127	6	1717	3
Fish eggs and larvae	985	-	-	-	-	-	-	-
Cirripede larvae	985	-	3122	1	11636	4	11863	20
Copepod nauplii	4923	1	1249	0.2	-	-	-	-
Total zooplankton	340677	100	623766	100	274618	100	60098	100

Table 3: Copepod species abundance (ind $100m^{-3}$) recorded in spatial reaches of the estuary.

	M1		M2		M3		M6	
Copepods (species)	(ind $100m^{-3}$)	%	(ind $100m^{-3}$)	%	(ind $100m^{-3}$)	%	(ind $100m^{-3}$)	%
Acrocalanus sp.	11815	3	21854	4	13964	5	4683	8
Acrocalanus gibber	71877	21	162341	26	32000	12	4995	8
Acrocalanus monachus	2954	1	-	-	4655	2	-	-
Acrocalanus gracilis	36431	11	43707	7	8727	3	1561	3
Acrocalanus longicornis	9846	3	-	-	78545	29	-	-
Paracalanus sp.	8862	3	18732	3	12218	4	5932	10
Paracalanus aculeatus	-	-	37463	6	2909	1	-	-
Paracalanus parvus	21662	6	74927	12	6982	3	-	-
Pseudodiaptomus bowmini	-	-	1873	-	-	-	-	-
Pseudodiaptomus jonesii	985	-	-	-	-	-	-	-
Pseudodiaptomus serricaudatus	5908	2	3746	1	582	-	-	-
Acartia sp.	985	-	11239	2	1164	-	468	1
Acartia danae	-	-	1873	-	-	-	-	-
Acartia erythraea	-	-	-	-	582	-	-	-
Acartia pacifica	-	-	24976	4	582	-	-	-
Acartia tropica	-	-	-	-	582	-	-	-
Acartiella sp.	-	-	-	-	-	-	468	1
Totanus gracilis	-	-	624	-	-	-	-	-
Centropages sp.	985	-	624	-	-	-	-	-
Centropages furcatus	985	-	-	-	582	-	-	-
Centropages tenuiremis	-	-	-	-	582	-	-	-
Eucalanus sp.	4923	1	10615	2	-	-	-	-
Temora turbinata	985	-	624	-	-	-	-	-
Temora sp.	-	-	-	-	582	-	-	-
Lebidocera pavo	-	-	624	-	-	-	-	-
Lebidocera pectinata	-	-	624	-	-	-	-	-
Clausocalanus arcuicornis	-	-	24976	4	582	-	-	-
Clausocalanus sp.	-	-	-	-	1745	1	-	-
Heliodiaptomus cinctus	-	-	-	-	-	-	3122	5
Diaptomus sp.	-	-	-	-	-	-	5463	9
Oithona sp.	985	-	12488	2	7564	3	5620	9
Oithona brevicornis	44308	13	-	-	19200	7	-	-

Oithona similis	-	-	31220	5	-	-	-	-
Cyclops sp.	-	-	-	-	-	-	780	1
Euterpina acutifrons	11815	3	6244	1	4073	1	2341	4
Corycaeus spp.	66954	20	8117	1	5818	2	1873	3
Farranula spp.	1969	1	624	-	-	-	624	1

Table 4: Order-wise copepod distribution in the spatial distinction of the estuary.

Zooplankton (ind $100m^{-3}$)	M1	%	M2	%	M3	%	M6	%
Calanoida	179200	53	441444	71	167564	61	26693	44
Cyclopoida	45292	13	43707	7	26764	10	6400	11
Harpacticoida	11815	3	6244	1	4073	1	2341	4
Poecilostomatoida	68923	20	8741	1	5818	2	2498	4
Copepod Juveniles	14769	4	-	-	4655	2	1093	2
Other Major Groups	20677	6	123629	20	65745	24	21073	35
Total zooplankton	340677	100	623766	100	274618	100	60098	100

The surface water at M1 station revealed the dominance of calanoida copepods (53%), followed by poecilostomatoida (20%), cyclopoida (13%), harpacticoida (3%) and other major zooplankton groups (6%) (Table 4). These groups were distributed such as appendicularians (1%), pelecypoda larva (1%), decapods (1%), fish larvae (985 ind $100m^{-3}$ < 1%), cirripede larvae (985 ind $100m^{-3}$ < 1%) and copepod nauplii (1%) (Table 2). Calanoida copepods were dispersed in the form of six families, and the dominant contributors were such as paracalanidae (48%), pseudodiaptomidae (2%), centropagidae 1% and eucalanidae 1%. The cyclopoida copepods only revealed oithonidae (13%), whereas harpacticoida copepods were found in the form of tachidiidae (3%) and poecilostomatoida were found in the form of corycaeidae (20%) (Table 2). Total zooplankton abundance (ind $100m^{-3}$) at M2 was 623766, which revealed the highest counts among other estuarine stations. Calanoid copepods revealed the highest contribution of 71 % followed by 7% cyclopoida, 1% harpacticoida and 1% poecilostomatoida (Table 4). There were 9 families contributed to the order calanoida were paracalanidae (58%), acartidae (6%), clausocalanidae (4%), eucalanidae (2%), pseudodiaptomidae (1%), pontellidae (0.2%), temoridae (0.1%), tortanidae (0.1%), centropagidae 0.1%. Cyclopoida contains only oithonidae (7%) and harpacticoida were distributed in tachidiidae (1%) and poecilostomatoida were found in the form of corycaeidae (1%). Other major groups of zooplankton contribute 20% of the whole population, of which chaetognaths were 4995 ind $100m^{-3}$-1%, pelecypodalarvae 4371 ind $100m^{-3}$-1%, polychaete larvae 81171 ind $100m^{-3}$-13%, decapod larva 24976 ind $100m^{-3}$- 4%, gastropod larva 624 ind $100m^{-3}$-0.1%, cirripede larvae 3122ind $100m^{-3}$-1%, copepod nauplii 1249ind $100m^{-3}$-0.2% (Table 2).

At mid estuarine station M3, zooplankton density covers 76% of copepods and 24 % other zooplankton groups. Out of 76% copepods calanoida contributed 61%, cyclopoida 10%, harpacticoida 1%, and poecilostomatoida 2% (Table 4). Calanoida copepods were found in the form of paracalanidae 50 %, pseudodiaptomidae 0.2 %, acartiidae 1%, centropagidae 0.4%, temoridae 0.2%, clausocalanidae 1%. Out of total copepods 10% oithonidae represented cyclopoida, 1% of tachidiidae represented harpacticoida, and 2% corycaeidae represented poecilostomatoida. Among other zooplankton groups, pelecypoda larvae formed 8% followed by gastropod larvae 6%, while both decapod and cirripede larvae contributed 4%. Moreover, polychaete larvae (2%) and appendicularians (0.2%) were associated with the total zooplankton population (Table2).

The total zooplankton density (ind $100m^{-3}$) in upstream water (M6) consists of 65% of copepods and 35% of other zooplankton groups. Among copepods, calanoida contributed up to 44%, followed by cyclopoida (11%), harpacticoida (4%), and poecilostomatoida (4%) (Table 4). Calanoida copepods were distributed by paracalanidae (29%), acartiidae (2%), diaptomidae 14%, whereas cyclopoida represented oithonidae (9%) and cyclopidae (1%). The contribution of 4% tachidiidae represented harpacticoida and also 4% corycaeidae represented poecilostomatoida (Table 4). Major contributors to other zooplankton groups were cirripede larvae (20%), pelecypoda larvae (1%), decapod larvae (12%), and gastropod larvae (3%) (Table 2).

C. Diversity and community structure

The diversity index values (Margalef richness, d; Shannon-Wiener, H'; Pielou's evenness, J') for total zooplankton community indicated less diversity variation in between near mouth and upstream stations. Comparatively away from the mouth station revealed the higher diversity and the least diversity was noticed in upstream region (Table 5).The station M1 revealed 90% of total zooplankton contributed by 19 copepod species and M2 represented 80% contributed by 23 species. While 22 species made up of 74% of total zooplankton abundance at M3 and 13 species represented 63% at M6 respectively (Table 3).It is observed that the universal pattern of zooplankton diversity was comparatively less at the upstream station than the near mouth and mid estuarine stations. In a surprise note, higher diversity was observed at M2 than M1.

The total zooplankton (copepods and other zooplankton groups) community structure was spatially changed with the influence of surrounding environmental factors. The results of hierarchical clustering displayed the grouping of sampling sites by linking in zooplankton abundance data for four sampling sites, representing near the mouth, away from near mouth, mid- estuary and upstream regions.

Table 5: Spatial observation of zooplankton diversity (Maragalef richness d; Shannon-Wiener, H´; Pielou's evenness, J´) in the stretch of the estuary are presented.

Sample	S	N	d	J'	H'(loge)	1-Lambda'
M1	16	29	4.444	0.8782	2.435	0.9142
M2	21	30	5.86	0.8533	2.598	0.9218
M3	16	29	4.437	0.8866	2.458	0.9154
M6	12	30	3.223	0.9412	2.339	0.9219

Data were square root transformed and then Bray-Curtis similarity was calculated to obtain dendrograms, which define the locations into 2 groups determined at 60% similarity. One group consists of near mouth (M1), away from the mouth (M2) and mid estuarine (M3) stations, while the second one represented upstream station (M6) as a distinct site (Fig. 2). The dendrogram revealing cluster analysis provided the convincing group of stations in relation to spatial distinction of the estuary and the same was displayed by MDS analysis (Fig. 3).The segregation of sampling sites was confirmed due to the differences in zooplankton community composition and their abundance in relation to change in environmental factors (salinity, temperature, dissolved oxygen and nutrients). The SIMPER analysis described the contribution of these environmental factors towards the dissimilarity pattern of sampling sites (Table 6a). The highest euclidian distance was observed in between the upstream and other 3 stations (near mouth, away from mouth and mid estuarine). Salinity was the highest contributor (93-94%) in differentiating these environmental settings. Moreover, the SIMPER analysis discriminated the copepod species with a particular biotic assemblage. The results described the dissimilarity of sampling sites determined by the contribution of zooplankton groups and copepod species (Table 6b). These are placed orderly by their average contribution to the average dissimilarity. The copepod species which are well discriminator of near mouth, mid estuarine and upstream stations are highlighted (Table 6b). Additionally, Redundancy analysis (RDA) was used to determine the association of different environmental factors with the zooplankton community distribution at different sampling sites. The results of RDA analysis clearly described the influence of environmental factors on the community distribution. It clearly indicates that salinity and temperature highly influence the distribution of zooplankton groups in the Mandovi estuary (Fig. 4).

Table 6a: SIMPER analysis of environmental factors in the different sites of Mandovi estuary.

	Group Near mouth	Group Away from the mouth			
Variable	Av.Value	Av.Value	Av.Sq.Dist	Contribution%	Cum.%
Nitrate	1.56	1.64	7.06E-03	82.5	82.5
Nitrite	1	1.04	1.36E-03	15.87	98.37
	Group Near mouth	Group Mid estuarine	Av.Sq.Dist	Contribution %	Cum.%
Temperature	9.46	9.9	0.195	44.49	44.49
Nitrate	1.56	1.96	0.159	36.25	80.74
Nitrite	1	1.29	8.29E-02	18.9	99.63
	Group Away from the mouth	Group Mid estuarine	Av.Sq.Dist	Contribution %	Cum.%
Temperature	9.45	9.9	0.198	54.9	54.9
Nitrate	1.64	1.96	9.91E-02	27.42	82.32
Nitrite	1.04	1.29	6.31E-02	17.45	99.77
	Group Near mouth	Group Upstream	Av.Sq.Dist	Contribution %	Cum.%
Salinity	10	7.12	8.28	94.21	94.21
	Group Away from the mouth	Group Upstream	Av.Sq.Dist	Contribution %	Cum.%
Salinity	10	7.12	8.28	94	94
	Group Mid estuarine	Group Upstream	Av.Sq.Dist	Contribution %	Cum.%
Salinity	10	7.12	8.28	93.27	93.27

Table 6b: The SIMPER analysis discriminated the copepod species with a particular biotic assemblage in different sampling sites of the estuary.

	Group Near mouth	Group Away from near mouth			
Species	Av.Abund	Av.Abund	Av.Diss	Contribution %	Cum.%
Oithona brevicornis	7.85	0	5.72	13.82	13.82
Corycaeus spp.	9.65	2.24	5.4	13.05	26.87
Paracalanus aculeatus	0	4.8	3.5	8.46	35.33

	Group Near mouth	Group Mid estuarine			
Species	Av.Abund	Av.Abund	Av.Diss	Contribution %	Cum.%
Corycaeus spp.	9.65	2.72	5.35	14	14
Acrocalanus longicornis	3.7	10	4.86	12.73	26.73
Acrocalanus gracilis	7.12	3.33	2.92	7.65	34.38
Acrocalanus gibber	10	6.38	2.79	7.31	41.69
	Group Away from near mouth	Group Mid estuarine			
Species	Av.Abund	Av.Abund	Av.Diss	Contribution %	Cum.%
Acrocalanus longicornis	0	10	8.1	17.07	17.07
Oithona brevicornis	0	4.94	4	8.44	25.52
Oithona similis	4.39	0	3.55	7.49	33
Paracalanus parvus	6.79	2.98	3.09	6.51	39.51
	Group Near mouth	Group Upstream			
Species	Av.Abund	Av.Abund	Av.Diss	Contribution %	Cum.%
Diaptomus sp.	0	9.6	6.16	11.33	11.33
Oithona sp.	1.17	9.73	5.5	10.11	21.44
Oithona brevicornis	7.85	0	5.04	9.27	30.71
Heliodiaptomus cinctus	0	7.25	4.66	8.56	39.27
Paracalanus sp.	3.51	10	4.17	7.66	46.93
	Group Away from near mouth	Group Upstream			
Species	Av.Abund	Av.Abund	Av.Diss	Contribution %	Cum.%
Diaptomus sp.	0	9.6	6.41	11.08	11.08
Heliodiaptomus cinctus	0	7.25	4.84	8.38	19.46
Oithona sp.	2.77	9.73	4.65	8.04	27.49
Paracalanus parvus	6.79	0	4.54	7.84	35.34
	Group Mid estuarine	Group Upstream			
Species	Av.Abund	Av.Abund	Av.Diss	Contribution %	Cum.%
Acrocalanus longicornis	10	0	7.04	11.41	11.41
Diaptomus sp.	0	9.6	6.76	10.95	22.36
Heliodiaptomus cinctus	0	7.25	5.11	8.28	30.64
Oithona sp.	3.1	9.73	4.67	7.57	38.21

In a brief note, salinity is a favourable environmental factor for most of the zooplankton groups at near mouth station M1, whereas high temperature and low salinity are the important environmental factor at the upstream station (M4).

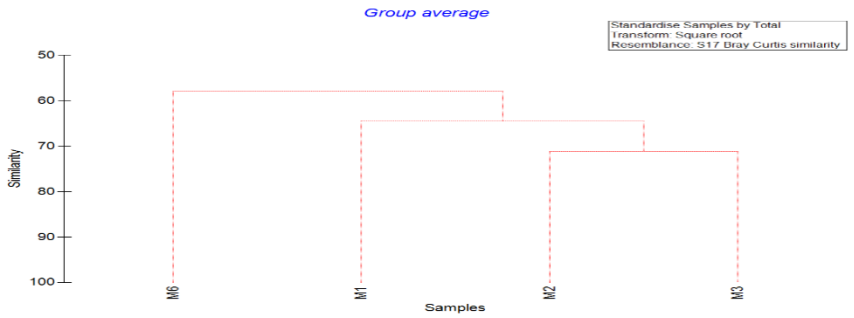

FiG. 2: Dendrogram of hierarchical clusters using group-average linkage of Bray-Curtis similarities based on transformed zooplankton datasets in 4 sampling sites (M1, M2, M3 and M4) of the Mandovi estuary.

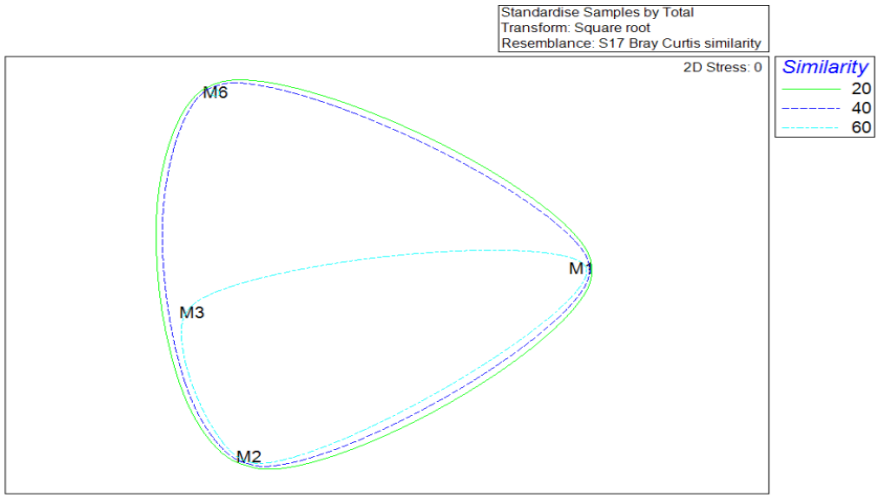

Fig. 3: Spatial separations in the estuary represented zooplankton assemblages based on the MDS (2D stress: 0) analysis.

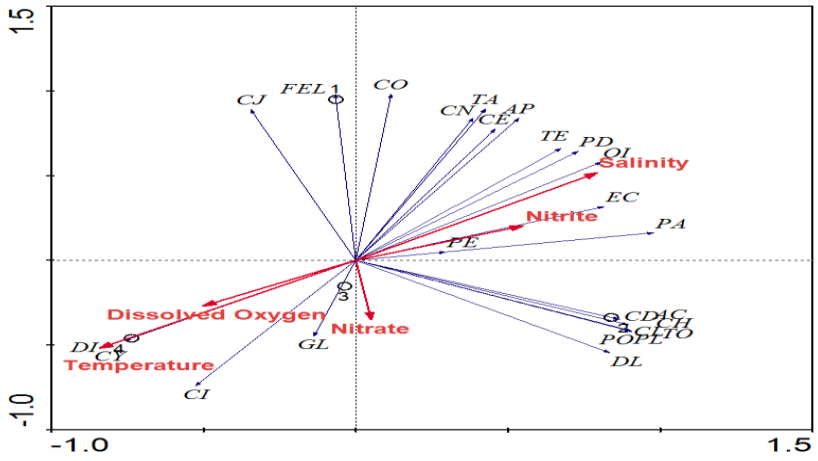

Fig.4: Redundancy analysis of Zooplankton communities at 4 sampling sites of the estuary. Zooplankton communities were represented as copepod families and other major groups of zooplankton, which are paracalanidae: PA, pseudodiaptomidae: PD, acartiidae: AC, centropagidae: CE, euchaetidae: EU, temoridae: TE, eucalanidae: EC, pontellidae: PO, calanidae: CA, clausocalanidae: CL, diaptomidae: DI, oithonidae: OI, cyclopidae: CY, tachidiidae: TA, oncedae: ON, corycaeidae: CO, copepod juveniles: CJ, chaetognaths: CH, appendicularians: AP, cladocerans: CD, pelecypoda larvae: PE, polychaete larvae: PL, decapods and larvae: DL, gastropod larvae: GL, fish eggs and larvae: FEL, cirripede larvae: CI and copepod nauplii: CN; Stations are represented as 1: near mouth of the estuary, 2: away from the mouth, 3: mid estuarine station, M6: upstream station.

IV. DISCUSSION

Zooplankton Assemblages are an important aspect of ecological research, which are quantified through statistical multivariate approaches (clustering and ordination approach) proves the association of biotic and abiotic factors in a particular ecosystem and their changes concerning space and time [14]. It is important to find the community pattern and their relation to surrounding environmental attributes, which defines the specific community structure in the array of aquatic ecosystems such as estuaries, the coastal and open ocean. In this study, we focused the variation of zooplankton community structure observed in different parts of the estuary and the reasonable explanation for their association in the ecosystem. In the same scenario, some earlier studies for zooplankton assemblages through the multivariate methods were well described in some estuarine waters [15, 16]. With reference to zooplankton abundance data and species assemblages, it is further discussed the varying pattern of most dominant copepod communities in different environmental settings of Mandovi estuary. In this context, upstream water represented *Diaptomus* sp., *Acartiella* sp., *Heliodiaptomus cinctus* and *Cyclops* sp. abundantly while *Paracalanus parvus*, *Paracalanusaculeatus* and *Oithona similis* were characterising near mouth of the estuary. The mid estuarine location revealed higher abundance of *Acrocalanus longicornis* and *Oithona brevicornis.*

Our research findings have shown a wide characteristic of zooplankton (copepod) assemblages in different parts of the estuary. This phenomenon of species difference leading to the diversity patterns of zooplankton in the estuarine ecosystem. Heterogeneity of environmental conditions in the contrasting waters of the Mandovi estuary [17]. Riverine water flow, salinity fluctuation in between the station, temperature and nutrient variability impinging on the photosynthetic productivity, which mediates the secondary production in the different parts of the estuary. These variations may be the controlling factors for the difference in zooplankton abundance and dominant copepod species assemblages [18]. The Arabian Sea is a dynamic ecosystem, where the Mandovi river flows towards the sea that establishes one of the well-marked estuaries on the west coast of India. In this study, the multivariate analysis explained the community alternation in the different parts of the estuary is to relate environmental attributes the zooplankton community. These analyses examine the influence of environmental variables on the community structure of the biota [19]. These statistical analysis showed the extent to which the environmental parameters related to their distribution and the possible reason of the biological association to their surrounding environment. From our study, it is fair to note that changes in salinity and temperature have a major effect on the biota. In this connection, we can assume that nMDS analysis clustered the community structure in three different groups where the near mouth, mid estuarine and upstream station showed different zooplankton assemblages.

V. CONCLUSION

We conclude that the total zooplankton abundance and copepod abundance could be affected by the variation of environmental attributes such as salinity, temperature and their spatial variability influence the species –specific associations (near mouth, away from the mouth, Mid estuarine and upstream) in the array of ecological environment. This is clearly reflect the association of environmental factors including spatial difference of riverine influx, coastal perturbance and circulation etc. are determined by the prevailing spatial regimes.

VI. ACKNOWLEDGEMENT

I am grateful to my Guide Dr. Mangesh Gauns for his valuable suggestions on this ecological investigation. Also, I acknowledge the trawler workers for their help in the net operation and collection of zooplankton samples from the different sampling sites.

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Next-generation sequencing approach: An insight into the spatial variation of ciliate diversity in the Arabian Sea

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Background of the Study

The next-generation sequencing (NGS) provides an advanced platform to investigate protist (ciliates) diversity in natural environments in a cost-effective manner. Aquatic ecosystems are regulated by energy transfer among the different trophic levels. The phototrophic and heterotrophic microorganisms play a key role in the cycling of organic matter in the sea by a Process of interlinked food chains between the consecutive trophic levels (Fenchel, T 1982).Arabian sea is a prominent site for bearing oxygen minimum zones (OMZ) in a temporary and permanent scale. So it is necessary to understand the response of microbial eukaryote diversity along the different oxygen gradients in spatial scale of Arabian sea.

Objective

Present study was to evaluate the ciliate community structure in different spatial sites of Arabian Sea (open ocean, continental shelf, coastal and estuarine waters) through Next-generation sequencing(NGS) approach.

Sampling sites

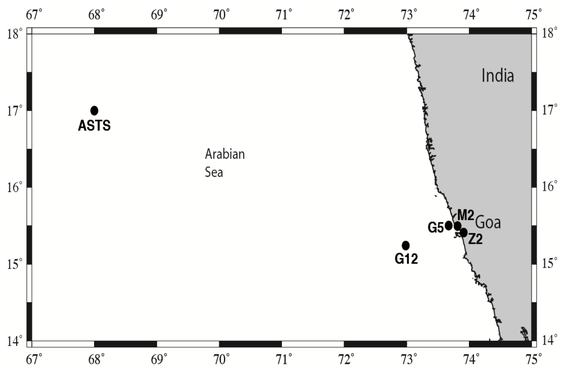

Fig 1:Sampling sites covering 4 spatial sites as openocean (ASTS), continental shelf (G12), coastal (G5) and interconnected estuarine waters (M2 and Z2).

Experimental method

Environmental sampling

↓

Filtration Dura pore membrane filter paper (0.65µ)

↓

PCR Amplification with Target V4 Primers

↓

NGS (illumina)

↓

Extraction of Genomic DNA

↓

Taxonomic Assignments (Ciliate diversity)

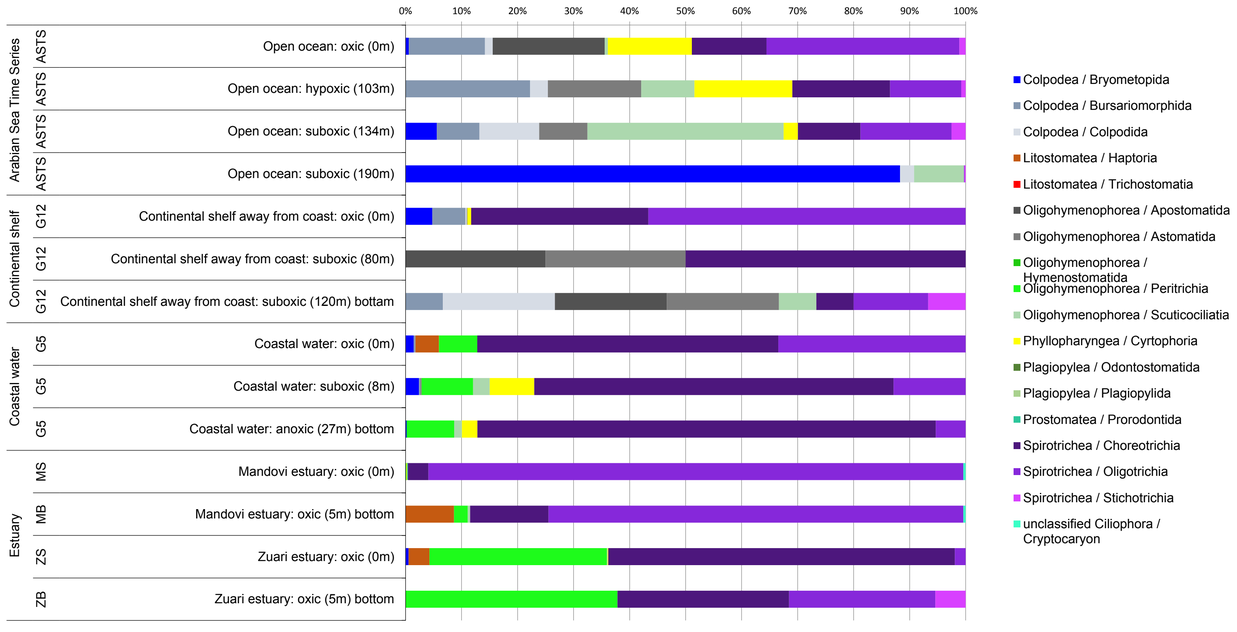

Fig 2: Taxonomic distribution of ciliate community detected by illumine sequencing. Only OTUs were considered for taxonomic assignment to the class/order level of 97%sequence similarity.

Amplification sequencing design

Construction of Illumina libraries and Illumina sequencing: The hyper variable V4 18SrDNA was amplified with primer pair followed by Stoeck et al., 2010. The PCR program also tailed as per Stoeck et al.2010.

Data processing

Denoising of amplicons (Acacia software)

↓

Chimera checking (QIIME analysis)

↓

Clustering

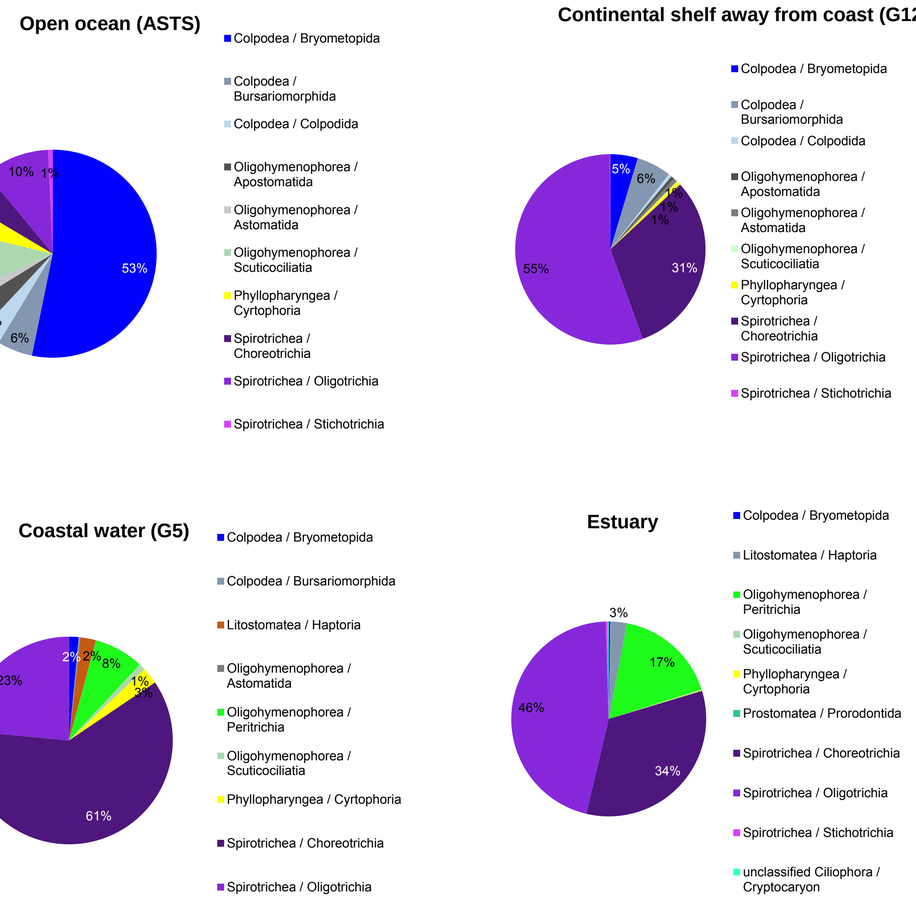

Fig 3: Spatial separation of ciliate community structure in Arabian sea.

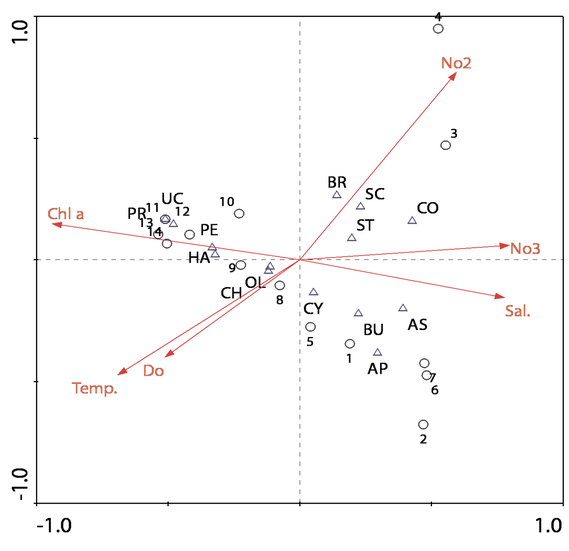

Fig 4: Canonical correlation analysis of ciliate taxonomic OTUs exploring relationships of ciliates with six environmental variables as nitrate, nitrite, dissolved oxygen, Chlorophyll a, Temperature, Salinity.

Research findings

- The results provide the base line data on ciliate community diversity in response to change in oxygen level.
- The reads of V4 region , derived via NGS, enable an evaluation of ciliate diversity in the open ocean, continental shelf, coastal and interconnected estuarine waters of the Arabian Sea.
- In the oxic region, Oligotrichia was abundant where as cyrtophoria was scarcely recorded.
- The suboxic zone was dominated by Bryometopida and Stichotrichia were the least abundant.
- From the CCA analysis, it is evident that coastal and estuarine stations are significantly related with chlorophyll a ($p<0.05$) where as open ocean station at suboxic depth of water column (134 m and 190 m) is significantly related with Nitrite ($p<0.05$) .
- V4 primer approach to identify the ciliate diversity is first of its kind from OMZ region of Arabian Sea.

Acknowledgement

We are thankful to Director NIO,Goa for the encouragement and financial support to visit Ecology Department of Kaiserslautern University, Germany for the molecular analysis of samples collected from the Arabian Sea. I am also grateful to CSIR for providing fellowships and funds towards my PhD workplan.

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