

A comparative study on microzooplankton communities in two tropical monsoonal estuaries

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Abstract

We studied on microzooplankton (MZP) communities from two major rivers, the Mandovi and the Zuari estuarine system of Goa on central west coast India. Despite both estuaries having a common point of discharge (eastern part of the Arabian Sea) and origin (in the Western Ghats), the Zuari estuary retains its marine condition; in contrast, the Mandovi estuary becomes a nearly freshwater condition for most parts of the year. The essential components of the marine food web, the MZP showed substantial seasonal variation driven by hydrographical parameters. Canonical correspondence analysis (CCA) depicts that prevailing differential environmental variables such as salinity, chlorophyll a , and dissolved oxygen influence the MZP assemblages in the tropical estuarine systems, which lead to the dominance of heterotrophic dinoflagellates (~80%) in the Zuari estuary for most parts of the year and dominance of loricate ciliates (~67%) in the Mandovi estuary. During monsoon, the seasonal intrusion of low oxygen water from the coastal region directs the dominance of heterotrophic dinoflagellates (22 to 80%) in these estuarine systems, particularly in the Zuari estuary. Whereas, prevailing high saline waters during the pre-monsoon (23 to 62%) and the post-monsoon (34 to 55%) supported the loricate ciliates dominated MZP community. The higher abundance of MZP in the upstream during the pre-monsoon and euhaline region of the downstream compared to the upstream (oligohaline) during monsoon and post-monsoon seems to be supported by microbial food (pico and nano-plankton), which warrants further investigation.

Keywords: Microzooplankton, estuary, dissolved oxygen, seasonal variation, west coast of India.

Introduction

Estuaries are transitional zones between freshwater and marine environments and are influenced by physical, chemical, and biological variations (Lancelot and Muylaert, 2011; Paerl, 2009). Tropical estuaries are biogeochemical hotspots with higher primary production, organic matter, and nursery grounds for aquatic organisms (Ching, 2015; Baran, 2000; Cloern et al., 2014). Environmental variability in the estuaries is driven by daily tidal fluctuation and seasonal changes in the freshwater flow (Costa et al, 2009; Miranda et al., 2002; Findlay et al., 1996) that modifies water characteristics. Thus, plankton communities in the estuarine systems vary spatially and temporally due to the seasonal changes in the hydrographical parameters (Naidu et al., 1977).

Microzooplankton (MZP) are heterotrophic microplankton (20 – 200 μm in size) and comprised of protozoan and metazoan, e.g. ciliates, heterotrophic dinoflagellates, crustacean nauplii, and other metazoans (Porter et al., 1985; Marshall, 1973). They are an important component of microplankton in the seas, estuaries, and coastal waters (Beers and Stewart, 1971; Pace and Orcutt, 1981; Banse, 1982; Sherr et al., 1986) playing three major roles as a consumer of bacteria, heterotrophic flagellates, and phytoplankton (mainly pico- and nano- size), prey for larger zooplankton (copepods and cladocerans) and remineralizer of essential nutrients (Haris et al., 2000). Thus, MZP are one of the important components of the marine food web.

Along the west coast of India, except Cochin backwaters, the studies on MZP are fragmentary in all of the estuaries (Gauns et al., 2015; Jyothibabu, 2006). These studies show that seasonal variation of the MZP is significantly influenced by hydrographical parameters and only a few species that can sustain in low saline conditions (Jyothibabu, 2006). However, there is a lack of information on the seasonal dynamics of MZP in the Mandovi estuary, west coast of India. Though the Mandovi and Zuari estuaries are located adjacent to each other and connected through the Cumbarjua canal (Pednekar et al., 2011), the freshwater discharge from Zuari and Mandovi estuaries vary significantly during monsoon and post-monsoon. The Zuari estuary retains its marine condition and experiences seasonal hypoxic waters from the coast (Sankararnarayanan and Jayaraman, 1972; Naqvi et al., 2000; Naik et al., 2020). The Mandovi estuary becomes a nearly freshwater condition for most parts of the year (Dehadrai, 1970; Goswami and Singbal, 1974) and is not influenced by low coastal oxygen. Hence, an attempt has been made to compare the seasonal and spatial variation of MZP between the Zuari and the Mandovi estuaries (west coast of India) concerning hydrographical parameters.

Materials and Methods

The Zuari and Mandovi estuaries located in the coast of Goa, west coast of India (lat: 15°, 29.429'N and long: 74°, 06.250' E) are connected to the Arabian Sea. They are connected with the Cumbarjua canal in the upstream regions at distances of 14km and 11km, respectively (Goswami and Singbal, 1974). These estuaries' ecology is mainly influenced by salinity, particularly during the monsoon season, when hydrographical conditions change significantly. The Zuari estuary retains its marine nature, and the Mandovi estuary experiences freshwater conditions (Dehadrai, 1970). To understand the hydrographically driven variation of MZP in this tropical estuarine system, the present seasonal and spatial study was carried out during the year of 2013 in three different seasons, viz. pre-monsoon (February-May), monsoon (June-September), and post-monsoon (October-January). The rainfall during pre-monsoon, monsoon and post monsoon (2013) was recorded as 26.9, 3498.4 and 159.6 mm respectively (Kaur and Purohit, 2014). Based on salinity gradients (Day, 1964), eight stations were selected from downstream to upstream, viz euhaline (Z1 and M1), polyhaline (Z2 and M2) meshohaline (Z3 and M3), and oligohaline (Z4 and M4) (Supplementary table.S1; Fig.1).

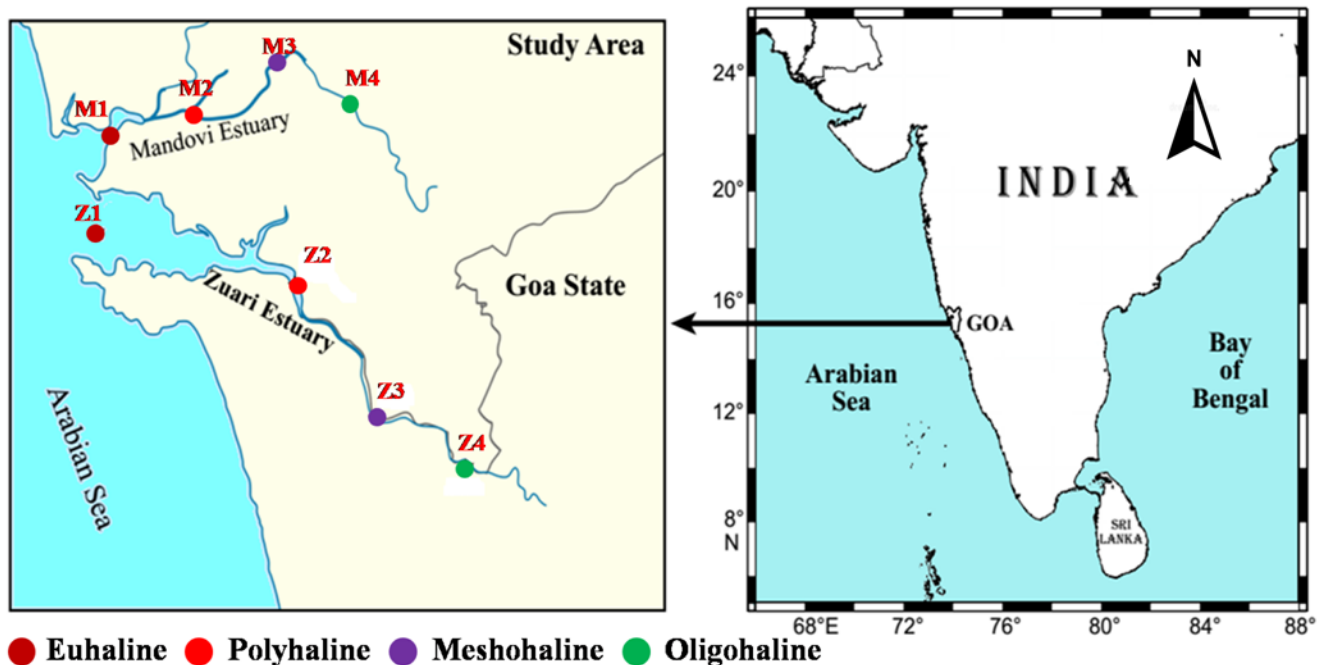


Fig.1. Map of the study area showing the locations of sampling stations in the Zuari and Mandovi estuaries.

Hydrographical parameters such as surface water temperature, salinity, and dissolved oxygen were recorded using portable CTD (SBE 19plus V2 SeaCAT Profiler). For estimation of phytoplankton biomass (Chlorophyll *a*), 1 L of water was filtered through 47-mm GF/F filters (0.7 µm pore size). Further, the samples were incubated in dark for 24 h at -20° C for extraction purposes using 10 ml of 90% acetone and analyzed by fluorometry (JGOFS, 1994). For the MZP taxonomic studies, 1 L surface water samples were collected with a Niskin water sampler and preserved with 1% acid Lugol's solution (JGOFS, 1994). After 24hrs, the collected samples were concentrated to 10ml by siphoning supernatant through 20µm mesh. 1 ml of the concentrated sample was placed in a Sedgwick-Rafter counting chamber and analyzed under the inverted microscope (Nikon Eclipse Ti-U) at 400x magnification.

Statistical analysis was performed by using various statistical software packages. Diversity indices such as species richness (*d*), Shannon-Weiner diversity (*H'*), and evenness (*J*) were calculated by using Primer (Ver. 6.1) software package. CCA analysis was performed by using statistical software Past - UiO (Ver. 2.17).

Results

Hydrography

During the study period, the surface water temperature ranged from 26-31°C in both estuaries. Salinity ranged from 0.02 to 34.8 psu with the high saline condition (34.8 and 34.7 psu) during the pre-monsoon and at downstream stations (euhaline: Z1 and M1), while low saline condition (0.02 and 0.04 psu) prevailed during the monsoon and at upstream stations (oligohaline: Z4 and M4). Moreover, the optimum salinity was recorded (~17-29 psu) at downstream and upstream regions during monsoon and pre-monsoon seasons, respectively (Table.1).

Table.1. Hydrography and chlorophyll *a* data of the Zuari and Mandovi estuarine systems (Mean \pm SD).

Hydrography and chlorophyll <i>a</i>	Zuari estuary			
	Z1	Z2	Z3	Z4
Temperature (°C)	25.8 – 28.9 27.4 \pm 1.5	27.9 – 30.5 29.1 \pm 1.4	27.8 – 30.7 29.1 \pm 1.5	27.5 - 30.3 28.5 \pm 1.6
Salinity (psu)	31.5 – 34.8 33.4 \pm 1.7	3.0 – 29.0 16.9 \pm 13.1	0.1 – 19.2 9.0 \pm 9.6	0.6 - 2.8 1.2 \pm 1.5
Do (ml L ⁻¹)	3.9 – 4.2 4.1 \pm 0.1	4.0 – 5.1 4.4 \pm 0.6	3.6 – 5.1 4.5 \pm 0.8	4.3 - 5.6 5.1 \pm 0.6
Chl <i>a</i> (µg L ⁻¹)	1.9 – 2.6 2.4 \pm 0.4	1.3 – 5.9 3.6 \pm 2.3	2.8 – 8.9 5.0 \pm 3.4	1.3 – 1.9 4.4 \pm 3.3
Hydrography and chlorophyll <i>a</i>	Mandovi estuary			
	M1	M2	M3	M4
Temperature (°C)	26.4 – 29.2 27.5 \pm 1.5	26.5 – 29.1 27.5 \pm 1.4	27.4 -30.5 29 \pm 1.6	26.9 – 30.9 28.5 \pm 2.1
Salinity (psu)	23.2 – 34.8 30.4 \pm 6.2	17.5 – 34.3 27.4 \pm 8.8	0.1 – 29.0 14.1 \pm 14.5	0.1 – 21.8 8.5 \pm 11.7
Do (ml L ⁻¹)	3.1 – 3.9 3.7 \pm 0.4	3.6 – 3.9 3.7 \pm 0.1	3.2 – 5.3 4.3 \pm 1.0	3.6 – 5.3 4.6 \pm 0.8
Chl <i>a</i> (µg L ⁻¹)	0.7 – 2.8 1.5 \pm 1.2	0.8 – 4.4 2.3 \pm 1.9	0.7 – 4.2 2.5 \pm 1.8	0.6 – 3.6 2.4 \pm 1.6

The dissolved oxygen varied from 3.2 to 5.5 ml L⁻¹. High (5.5 and 5.3 ml L⁻¹) values of dissolved oxygen were recorded during the monsoon and at upstream stations (oligohaline: Z4 and M4) and the low values (3.6 and 3.2 ml L⁻¹) were during the pre-monsoon and at midstream stations (mesohaline: Z3 and M3). Chlorophyll-*a* concentrations varied from 0.6 to 8.9 µg L⁻¹. High (8.9 and 2.6 µg L⁻¹) values of Chlorophyll-*a* were recorded during the pre-monsoon at midstream stations (mesohaline: Z3 and M3) and low (1.3 and 0.6 µg L⁻¹) values were recorded during the monsoon at upstream stations (oligohaline: Z4 and M4).

Abundance, diversity, and composition of microzooplankton

The MZP abundance was recorded in the range of 90-1060 cells L⁻¹ with high abundance during the pre-monsoon (360-1060 cells L⁻¹) followed by monsoon (90-400 cells L⁻¹) seasons. During the pre-monsoon season, the abundance of MZP was higher in the upstream (oligohaline: Z4 and M4) stations of both the estuaries (580 to 1060 cells L⁻¹). Whereas, during the monsoon and the post-monsoon seasons, the abundance of MZP was higher (300-400 cells L⁻¹, 220-280 cells L⁻¹) at the downstream stations (euhaline: Z1 and M1; Fig.2).

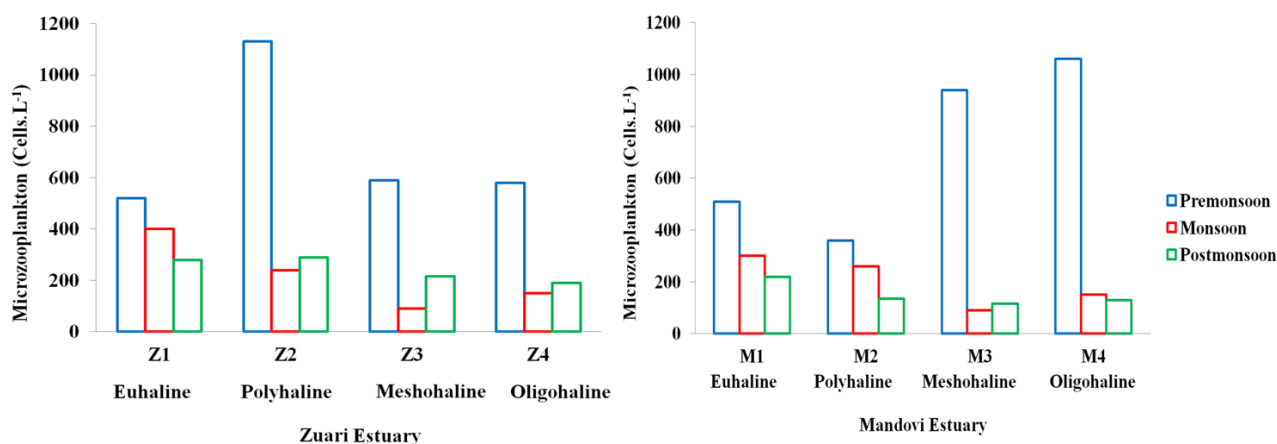


Fig.2. Microzooplankton abundance in the Zuari and Mandovi estuaries during pre-monsoon, monsoon and post-monsoon.

In the present study, a total of 28 species and 17 genera of MZP were recorded, and four major groups were identified, which include loricate ciliates (tintinnids), aloricate ciliates, heterotrophic dinoflagellates, and copepod nauplii. The Zuari estuary comprised of 15 genera and 26 species (Table.2), whereas 14 genera and 24 species were recorded from the Mandovi estuary (Table.2). Results show that MZP abundance and diversity among these estuaries did not vary significantly.

Table.2. List of microzooplankton species from the Zuari and Mandovi estuarine systems *indicates the species occurrence in the Zuari and Mandovi estuarine systems.

Microzooplankton	Euhaline		Polyhaline		Mesohaline		Oligohaline	
	Z1	M1	Z2	M2	Z3	M3	Z4	M4
Loricata ciliates								
<i>Amphorella acuta</i> Daday, 1887	*	*		*	*			
<i>Codonellopsis ostenfeldi</i> (Schmidt, 1902) Kofoid & Campbell, 1929	*	*	*	*		*		*
<i>C. tessellata</i> Kofoid and Campbell, 1929	*	*	*	*	*	*		*
<i>Codonellopsis</i> sp. Jorgensen 1924	*	*	*	*	*	*		*
<i>Dadaiella gaymedes</i> (Entz, 1884) Kofoid & Campbell, 1929	*	*						

<i>Dictyocsta</i> sp. Ehrenberg, 1854	*			*		*		*
<i>Eutintinnus apertus</i> Kofoid & Campbell, 1929				*				*
<i>Eutintinnus lusus undae</i> Entz, 1885		*						
<i>Leptotintinnus nordquisti</i> (Brandt, 1906) Kofoid & Campbell, 1929	*	*	*	*	*	*	*	*
<i>Stenosemella</i> sp. Jorgensen 1924	*	*	*	*	*		*	
<i>Tintinnopsis beroidea</i> Stein, 1867	*	*	*	*	*	*	*	*
<i>T. butschli</i> Daday, 1887	*							
<i>T. cylindrica</i> Daday, 1887		*	*			*	*	
<i>T. directa</i> Hada, 1932			*					*
<i>T. karajacensis</i> Brandt, 1896		*	*	*	*	*	*	*
<i>T. radix</i> Imhof, 1886			*		*		*	
<i>T. uruguayensis</i> Balech, 1948	*		*		*	*	*	*
Aloricate ciliates								
<i>Lohmanniella oviformis</i> Leegaard, 1915	*						*	
<i>L. spiralis</i> Leegaard, 1915			*	*		*	*	*
<i>Lohmaniella</i> sp. Leegaard, 1915			*				*	
<i>Strombidium conicum</i> (Lohmann, 1908) Wulff, 1919	*		*		*		*	*
<i>Strombidium</i> sp. Claparède & Lachmann, 1859							*	
Heterotrophic Dinoflagellates								
<i>Dinophysis caudate</i> W.S.Kent,1881		*						
<i>Noctiluca scintillans</i> (Macartney) Kofoid & Swezy, 1921	*							
<i>Podolampas</i> sp. Stein, 1883	*							
<i>Prorocentrum micans</i> Ehrenberg, 1834	*	*	*	*	*			*
<i>Protoperidinium breve</i> (Paulsen) Balech, 1974	*	*	*	*	*	*	*	*
<i>P. depressum</i> (Bailey, 1854) Balech, 1974	*	*		*	*	*		*
<i>P. divergens</i> (Ehrenberg) Balech, 1974	*	*		*		*		*
<i>P. granii</i> (Ostenfeld) Balech, 1974	*	*	*	*	*	*	*	*
<i>P. latistriatum</i> (Balech, 1958) Balech, 1974	*	*	*	*	*	*	*	*
<i>P. ovatum</i> Pouchet, 1883	*	*		*	*	*	*	*
<i>P. pellucidum</i> Bergh, 1881					*			*
<i>P. steinii</i> (E.G.Jørgensen) Balech 1974	*	*	*			*	*	*
<i>Protoperidinium</i> sp. Bergh, 1881		*				*	*	*
<i>Pyrophacus</i> sp. Stein, 1883	*	*			*	*	*	

In the Zuari estuary, loricate ciliates (23-62%) followed by heterotrophic dinoflagellates (7-50%) dominated the MZP during the pre-and post-monsoon seasons; the trend was different during the

monsoon period wherein heterotrophic dinoflagellates (42-80%) dominated over the loricate ciliates (7-21%). In the Mandovi estuary, contrary, loricate ciliates (27-67%) followed by heterotrophic dinoflagellates (22-46%) dominated the monsoon and the post-monsoon while heterotrophic dinoflagellates (14-73%) followed by loricate ciliates (23-44%) prevailed during pre-monsoon. In general, the dominant groups of the MZP community during most parts of the year varied among these estuarine systems, the loricate ciliates (~67%) dominant in Mandovi, while dinoflagellates (~80%) were dominated in Zuari estuary. Moreover, the aloricate ciliates were recorded only during the pre-monsoon season, and their abundance (2-21%) was even lower, then copepod nauplii (4-44%) (Fig.3).

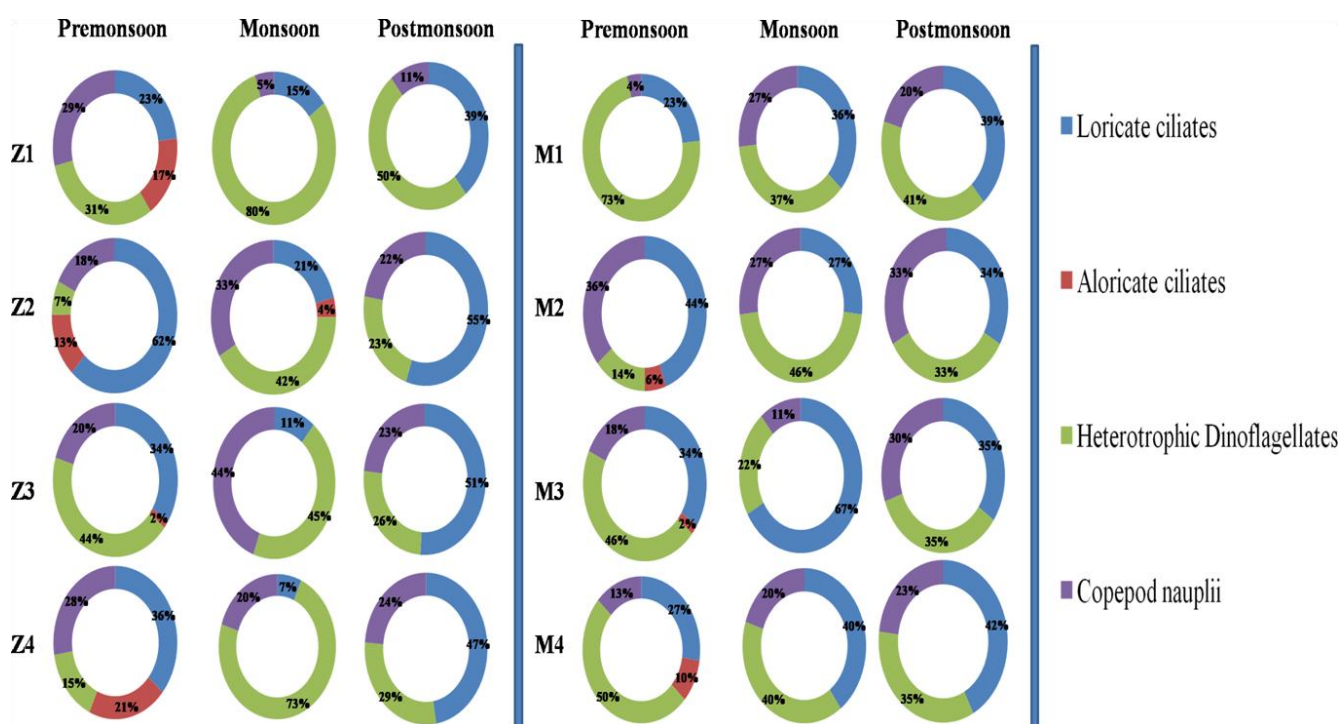


Fig.3. Microzooplankton composition in the Zuari and Mandovi estuaries during pre-monsoon, monsoon and post-monsoon.

The most dominant species in these estuarine systems include 5 species of loricate ciliates (*Dictyocysta* sp., *Leptotintinnus nordquisti*, *Stenosemella* sp., *Tintinnopsis beroidea*, and *T.uryguayensis*) and 3 species of heterotrophic dinoflagellates (*Protoperidinium breve*, *P.granii*, and *P.latistriatum*).

Statistical analysis

Canonical correspondence analysis (CCA)

The relationships between the MZP and the environmental factors were studied by (CCA analysis) with particular attention to the seasons and the stations. Figures 4 and 5 show that the first and the second ordination axis of CCA analysis of individual variables were correlated with the MZP assemblages. During the pre-monsoon and the post-monsoon seasons, the MZP assemblages in the downstream (euhaline and polyhaline) stations of both the estuaries (Z1, M1, Z2, and M2) were mainly influenced by temperature, salinity, and chlorophyll-*a*. While in the upstream (mesohaline and oligohaline) stations of both the estuaries (Z3.M3, Z4, and M4) were mainly influenced by dissolved oxygen, salinity, and Chlorophyll-*a* during the monsoon and the post-monsoon seasons (Fig.4 & 5; Supplementary table.S2-S9). In addition, spearman's rank correlation also performed and the results reveals that positive and/or negative correlations of MZP with hydrographical paramaters ($r = -0.5, 0.4, 0.5$) such as dissolved oxygen, salinity, and Chlorophyll-*a* (Supplementary table.S10 &11).

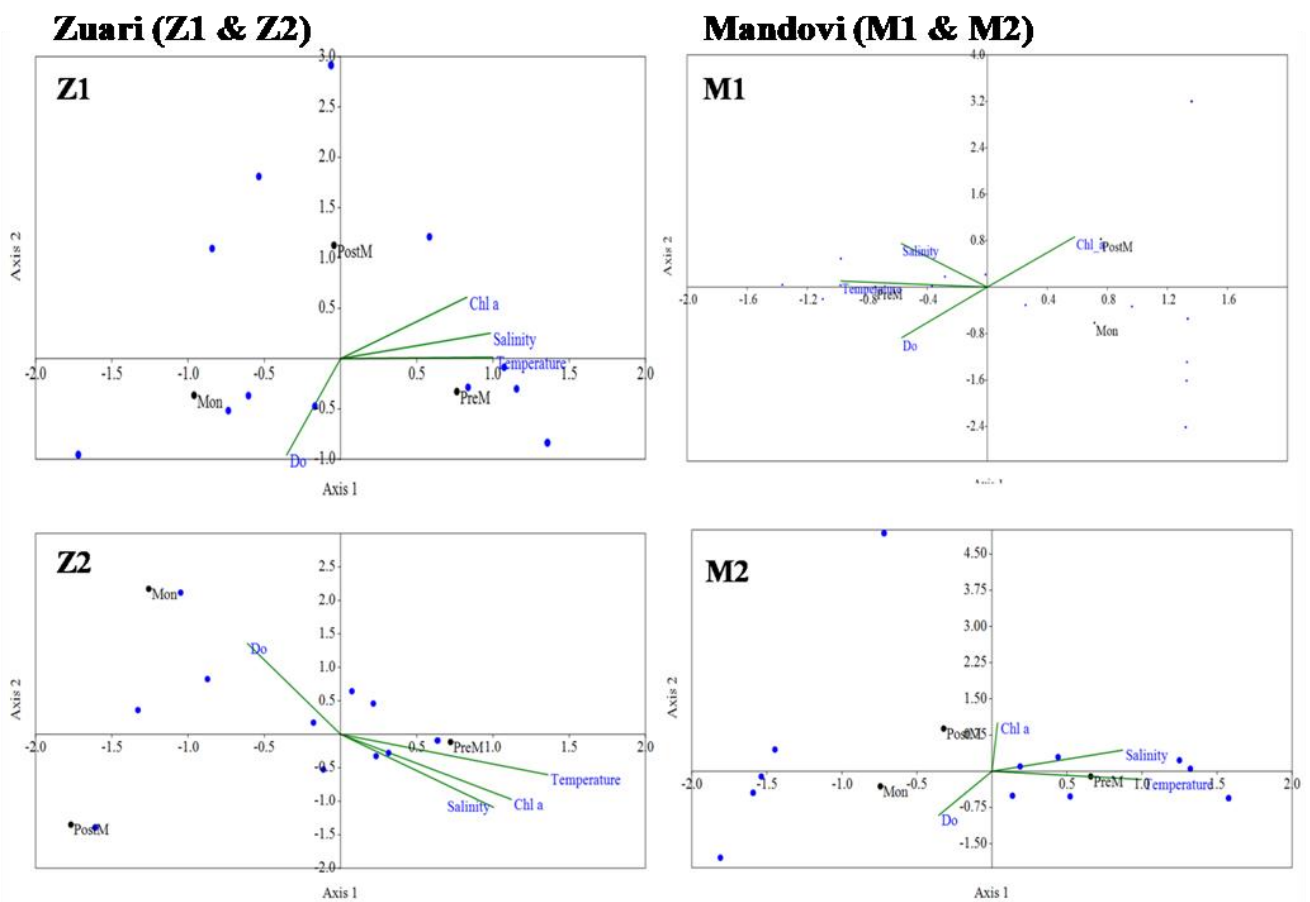


Fig.4. Results of canonical correspondence analysis from the Zuari and Mandovi estuaries covering pre-monsoon, monsoon and post-monsoon.

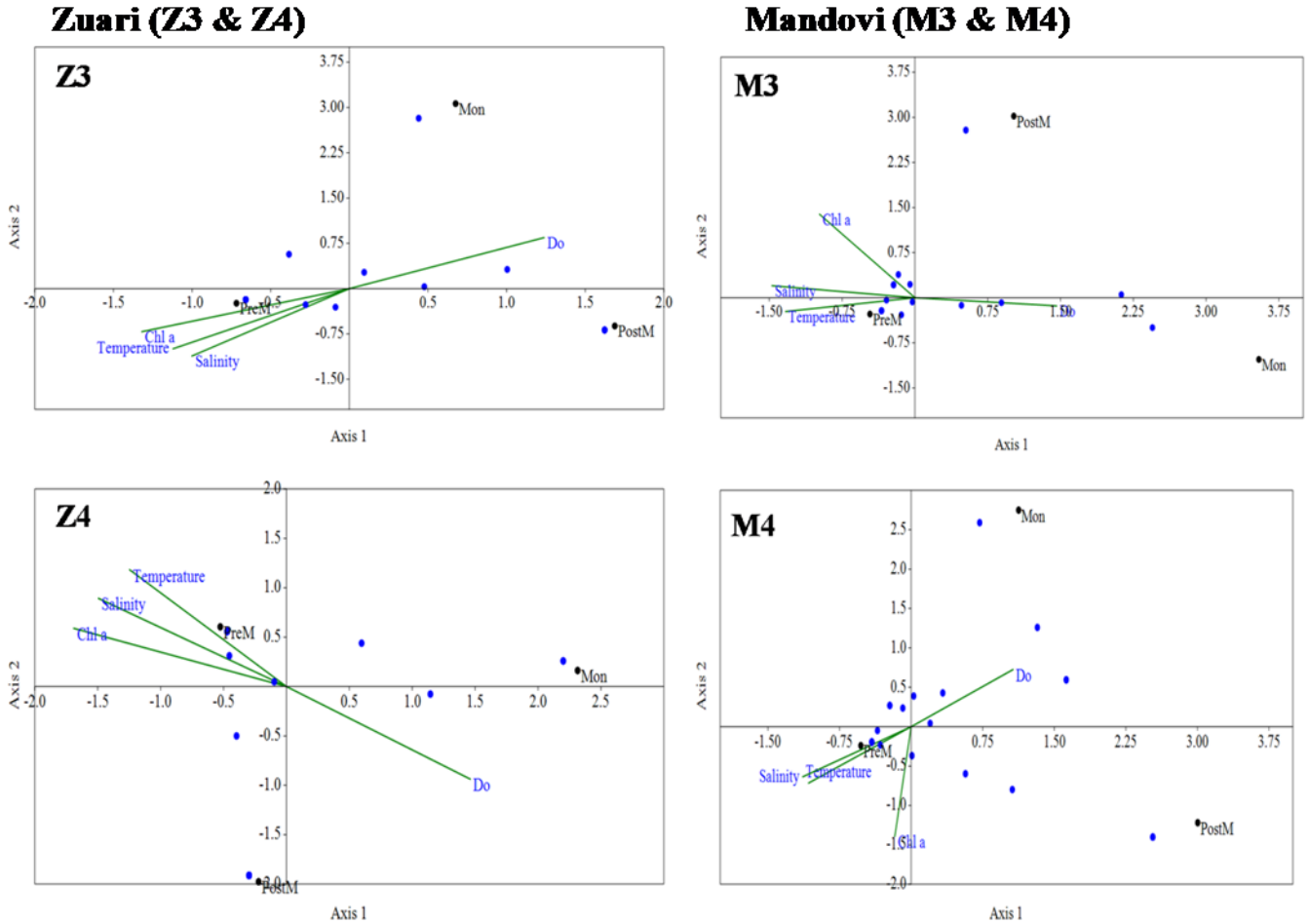


Fig.5. Results of canonical correspondence analysis from the Zuari and Mandovi estuaries covering pre-monsoon, monsoon and post-monsoon.

Diversity indices

Diversity indices such as species richness (d), Shannon-Weiner diversity (H'), and evenness (J) were calculated by using statistical software Primer (Ver.6.1). In the Zuari estuary, the number of MZP species (S) varied between 5 and 14 (Table.3). The maximum (14) number of species were recorded at the downstream and midstream stations (polyhaline and mesohaline: Z2 and Z3). Whereas, the minimum (5) number of species was recorded at midstream and upstream stations (mesohaline and oligohaline: Z3 and Z4). The Species diversity (H') varied between 2.2 and 3.8. The maximum (3.8) diversity was recorded at a downstream station (euhaline: Z1) and the minimum (2.2) diversity was recorded at an upstream station (oligohaline: Z4). The species richness varied between 1.2 and 3.7. The maximum (3.7) richness was recorded at a downstream station (euhaline: Z1) and minimum (1.2) richness was recorded at an upstream station (oligohaline: Z4). The evenness value ranged

between 0.95 and 0.98. The maximum (0.98) evenness was recorded at a downstream station (euhaline: Z1) and a minimum (0.95) was recorded at an upstream station (oligohaline: Z4).

Table.3. Number of species (S), species diversity (H'), species richness (d) and evenness (J) of microzooplankton from the Zuari and Mandovi estuarine systems (Mean \pm SD).

Stations	S	H'	D	J'
Z1	10-12	3.3-3.8	2.2-3.7	0.96-0.98
	12.7 \pm 3.1	3.5 \pm 0.3	2.8 \pm 0.8	1.0 \pm 0.0
M1	9-20	3.0-4.2	1.9-4.6	0.96-0.98
	14.7 \pm 5.5	3.7 \pm 0.6	3.3 \pm 1.3	1.0 \pm 0.01
Z2	6-14	2.4-3.7	1.4-2.7	0.95-0.97
	10.7 \pm 4.2	3.2 \pm 0.7	2.3 \pm 0.8	1.0 \pm 0.01
M2	8-14	2.9-3.7	1.8-3.5	0.98
	10.3 \pm 3.2	3.3 \pm 0.4	2.5 \pm 0.9	1.0 \pm 0.002
Z3	5-14	2.3-3.7	1.3-2.9	0.97
	9.7 \pm 4.5	3.1 \pm 0.7	2.2 \pm 0.8	1.0 \pm 0.004
M3	7-16	2.8-3.9	1.9-3.3	0.97-0.99
	12 \pm 4.6	3.4 \pm 0.6	2.8 \pm 0.8	1.0 \pm 0.01
Z4	5-12	2.2-3.5	1.2-2.7	0.95-0.97
	9.3 \pm 3.8	3.0 \pm 0.7	2.1 \pm 0.8	1.0 \pm 0.01
M4	11-16	3.4-3.9	2.7-3.5	0.97-0.99
	13.7 \pm 2.5	3.7 \pm 0.2	3.1 \pm 0.4	1.0 \pm 0.01
Previous study from the Zuari estuary (Gauns 2000)	Loricata ciliates (tintinnids)	0.77-3.04	0.1-2.34	0.22-1

In Mandovi estuary, the number of MZP species varied between 7 and 20 (Table.3). The maximum (20) number of species was recorded at a downstream station (euhaline: M1). Whereas the minimum (7) number of species was recorded at midstream station (mesholine: M3). The Species diversity (H') varied between 2.8 and 4.2. The maximum (4.2) number of species was recorded at a downstream station (euhaline: M1) and the minimum was recorded at midstream station (meshohaline: M3). The species richness varied between 1.8 and 4.6. The maximum (4.6) richness was recorded at a downstream station (euhaline: M1) and minimum (1.8) was recorded at a downstream station (polyhaline: M2). The evenness value ranged between 0.96 and 0.99. The maximum (0.99) evenness was recorded at midstream and upstream stations (mesohaline: M3 and oligohaline: M4) and the minimum (0.96) was recorded at a downstream station (euhaline M1).

Discussion

In the present study, the number of species and the abundance of MZP were recorded to be relatively higher in the Zuari estuary (26 species, 90-1130 cells L⁻¹) than the Mandovi estuary (24 species, 90-

1060 cells L⁻¹). The Zuari estuary is highly influenced by seawater, and it is very diverse with a high number of species and plankton biomass than the Mandovi estuary (Goswami and Sigbal, 1974). The high abundance of MZP during the pre-monsoon (580 to 1060 cells L⁻¹) and the monsoon (300 to 400 cells L⁻¹) are due to the availability of phytoplankton biomass in the estuarine systems (Chl *a*: 0.7-8.9 µg L⁻¹ and Chl *a*: 0.6-2.8 µg L⁻¹). The surface water primary productivity of the Mandovi and the Zuari estuaries were also reported to be higher during the pre-monsoon (11.41-46.75 mgC/m³/hr) and monsoon seasons (7.52-24.30 mgC/m³/hr) (Kumari et al., 2002). In addition, the availability of dissolved organic carbon in these estuarine systems supports the high bacterial abundance (primary productivity) during the pre-monsoon and the monsoon seasons (Gauns, 2000, 2015). The low abundance of MZP during post-monsoon could be because of zooplankton grazing pressure and limited availability of primary productivity and also because of high numbers of carnivorous zooplankton species such as *Oithona* sp., and *Corycaeus* sp. (zooplankton) that prevail in these estuaries during the post-monsoon season (Dalal and Goswami, 2001). During the pre-monsoon season, the higher abundance of MZP (360 to 1060 cells L⁻¹) at upstream stations (oligohaline: Z4 and M4) of both estuaries are due to well-mixed saline water incursion (Sooria et al., 2015; Jyothibabu et al., 2006). In the Zuari estuary, the abundance of *Synechococcus* (picoplankton) also reported being high during non-rainy seasons due to high salinity, temperature, and solar radiation and low abundance during monsoon season due to turbidity and low solar radiation (Rajaneesh and Mitbavkar, 2013). During monsoon and post-monsoon seasons, the higher abundance of MZP (300-400 cells L⁻¹) at downstream than the upstream stations believed to be due to the availability of bacteria and heterotrophic nano-plankton in the downstream regions; and poor adaptation in freshwater condition and availability of insufficient prey in the upstream regions (Sooria et al., 2015). Due to the availability of dissolved organic carbon (DOC) in the Zuari estuary, the bacterial abundance in the estuarine system is reported to be higher in the monsoon season (Gauns, 2000, 2015). These results suggest that the MZP abundance in these estuaries is mainly influenced by the microbial loop, which is functioning through the availability of pico- and nano-plankton communities as food. Additionally, observed spatial variation of MZP in different seasons could be the reason for optimal salinity (~17-29 psu) condition in the upstream (during pre-monsoon) and downstream (during monsoon) regions of the estuarine systems. Gauns (2000) reported that the spatial variation of MZP abundance in the Zuari estuary is regulated not only by food availability but also due to the optimal salinity condition (~15-20 psu) and other environmental variables.

The dominance of loricate ciliates (34-55%) during the post-monsoon is due to the favorable temperature, salinity, and high chlorophyll condition (Dibyendhu et al., 2014). Moreover, their dominance in these estuarine systems could be due to the availability of fine sand grains and mineral particles through river runoff. Tintinnids require fine grains and mineral flakes for constructing their loricae and are capable of accumulating biogenic and non-biogenic particles on their loricae (Henjes and Assmy, 2008; Elangovan and Padmavati, 2017). Moreover, the dominant loricate ciliates in these estuarine systems such as *Dictyocysta* sp., *Leprotintinnus nordquisti*, *Stenosemella* sp., *Tintinnopsis beroidea*, and *Tintinnopsis uryguayensis* were reported elsewhere (the southern Korea coastal waters) as biological indicators of warm water (Kim et al., 2012) and indicators of eutrophication in Indian coastal waters (Rakshit et al., 2017; Sivasankar et al., 2018). The previous study from the Zuari estuary also reported that a higher abundance of loricate ciliates (1800-75760 org.L-1) and heterotrophic dinoflagellates (100-16100 org.L-1) during post-monsoon and monsoon (Gauns, 2000). In the monsoon, the dominance of dinoflagellates (22-80%) is due to their strong response to turbulence and difficult to separate factor-like nutrient and light (Estrada and Berdalet, 1998). Most of the heterotrophic dinoflagellates in the marine environment are mixotrophic and have diverse feeding mechanisms (Jyothibabu et al., 2003; Jeong et al. 2010; Caron, 2016). Heterotrophic dinoflagellates act as mixotrophy by kleptochloroplastidy process, which involves not only feeding/digestion of algae but also retain the algal chloroplasts in a photosynthetically active condition (Caron, 2016). Secondly, the estuarine system (particularly Zuari estuary) is known to experience intrusion of cold upwelled (low oxygen) water (Sankaranarayanan and Jayaraman, 1972) from the coastal region with phytoplankton community largely dominated (72%) by dinoflagellates (Devassy, 1983). The dominance of dinoflagellates (eukaryotes) is due to the ability to survive and maintain their biogeochemical process in hypoxic and other stressful environments (Caron and Countway, 2009; Rocke et al., 2013). Moreover, many protists (MZP) are thermal tolerance with the range of $\geq 40^{\circ}\text{C}$, and the dinoflagellates are habitually found in warmer/stratified waters (Martinez, 1980; Sittenfeld et al., 2002; Caron and Hutchins, 2013; Barton et al., 2016). Recent studies suggest that the MZP (dinoflagellates) is better adapted to future climate change warming and could alter the fundamental relationship with their prey. Thus, the MZP growth rate and grazing pressure are at the same rate as phytoplankton production (Chen et al., 2012; Caron and Hutchins, 2013).

Metazoans like copepod nauplii were also significantly (4-44%) contributed to the total microzooplankton community composition in these estuarine systems. Copepod nauplii can feed efficiently on a wide range of prey sizes from nano- to micro-zooplankton such as nanoflagellates, phototrophic nanoflagellates, dinoflagellates, diatoms, and ciliates (Rodriogo et al., 2011; Uye &

Kasahara, 1983). Moreover, their abundance and specific intake rate of nano- to micro-zooplankton is higher than mesozooplankton (White and Roman, 1992) suggested that they play an important role between classical and microbial food webs (Berggreen, 1988; Turner & Roff, 1993). The low abundance of aloricate ciliates in these estuarine systems could be the reason of grazing pressure by the predators such as metazoan (copepod nauplii) and less availability of nanoplankton (Gomez, 2007; Booth et al., 1993).

CCA analysis depicted that the changes in the hydrographical parameters (primarily: Salinity, Chlorophyll *a* and dissolved oxygen) in different seasons lead to variations in the MZP composition and their abundance in these estuarine systems. It was reported that due to the environmental condition, MZP communities change their taxonomic composition and abundance (Godhantaraman, 2002). Diversity indices show that most parts of the year the MZP communities in these estuarine systems are fitted healthier in the downstream (euhaline) regions than the upstream (oligohaline) regions.

Conclusion

Tropical estuaries are most productive ecosystems and nursery grounds for many aquatic organisms. Studying the MZP communities in tropical estuaries are important due to their significant role in marine pelagic food web. Our study reveals that distinct hydrographical nature of the Zuari (Saline) and Mandovi (fresh water) estuaries was reflected on MZP composition. As a result, dominance of heterotrophic dinoflagellates in the Zuari and loricate ciliates in the Mandovi estuarine systems was recorded. The spatial variation of MZP abundance in these estuarine systems is seems to be supported by microbial food web (pico and nano-plankton). However, how efficiently the microbial food web mediated energy would be utilized by larger metazoans and higher tropic organisms under climate change warming are indefinite. Hence, further investigation is needed for a better perceptive of food web dynamics in the tropical estuarine systems.

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Competing interests

The authors declare that they have no competing interests.

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Supplementary tables.

Table.S1 Details of stations in the Zuari and Mandovi estuarine systems

Zuari estuary stations and depth	Mandovi estuary stations and depth
Z1 - 10 m	M1 - 8 m
Z2 - 3 m	M2 - 6 m
Z3 - 4 m	M3 - 4 m
Z4 - 3 m	M4 - 3 m

Table. S2 CCA analysis Axis values – St.Z1 (Zuari Estuary)

Axis	Eigenvalue	%
1	0.55843	59.42
2	0.38137	40.58
	Axis 1	Axis 2
<i>Amphorella acuta</i>	1.35679	-0.8358
<i>Codonellopsis ostenfeldi</i>	-0.0623012	2.91578
<i>Codonellopsis tessellata</i>	-0.0623012	2.91578
<i>Codonellopsis</i> sp.	-0.604228	-0.367692
<i>Dadayiella gaymedes</i>	-0.0623012	2.91578
<i>Dictyocysta</i> sp.	-0.0623012	2.91578
<i>Leprotintinnus nordquisti</i>	-0.535992	1.80998
<i>Stenosemella</i> sp.	-0.0623012	2.91578
<i>Tintinnopsis beroidea</i>	1.15407	-0.29986
<i>Tintinnopsis butschli</i>	1.35679	-0.8358
<i>Tintinnopsis uruguayensis</i>	-0.0623012	2.91578
<i>Strombidium conicum</i>	1.35679	-0.8358
<i>Lohmaniella oviformes</i>	1.35679	-0.8358
<i>Noctiluca scintillans</i>	-1.72022	-0.954507
<i>Podolompus</i> sp.	-1.72022	-0.954507
<i>Porocentrum micans</i>	-0.735962	-0.515869
<i>Protopteridinium breve</i>	0.582742	1.21052
<i>Protopteridinium depressum</i>	1.07297	-0.0854837
<i>Protopteridinium divergens</i>	-1.72022	-0.954507
<i>Protopteridinium granii</i>	-0.168445	-0.471716
<i>Protopteridinium latistriatum</i>	-0.842498	1.09447
<i>Protopteridinium ovatum</i>	1.35679	-0.8358
<i>Protopteridinium stenii</i>	-0.0623012	2.91578
<i>Pyrophacus</i>	-1.72022	-0.954507
Copepod nauplius	0.836227	-0.284933
PreM	0.762581	-0.326667
Mon	-0.960949	-0.363494
PostM	-0.0434375	1.12594
Temperature	0.998993	0.0141344
Salinity	0.980907	0.251985
Do	-0.352519	-0.954965
Chl a	0.827555	0.609212

Table. S3 CCA analysis Axis values – St.Z2 (Zuari Estuary)

Axis	Eigenvalue	%
1	0.46222	63.63
2	0.26424	36.37
	Axis 1	Axis 2
<i>Codonellopsis ostenfeldi</i>	-1.60909	-1.39202
<i>Codonellopsis tessellata</i>	0.635728	-0.09223
<i>Codonellopsis</i> sp.	0.635728	-0.09223
<i>Dictyocysta</i> sp.	-1.60909	-1.39202
<i>Leptotintinnus nordquisti</i>	-1.329	0.362134
<i>Stenosemella</i> sp.	-1.60909	-1.39202
<i>Tintinnidium apertus</i>	0.635728	-0.09223
<i>Tintinnopsis beroidea</i>	0.232811	-0.32553
<i>Tintinnopsis cylindrical</i>	0.635728	-0.09223
<i>Tintinnopsis directa</i>	0.635728	-0.09223
<i>Tintinnopsis karajacensis</i>	0.074186	0.64394
<i>Tintinnopsis radix</i>	-1.60909	-1.39202
<i>Tintinnopsis uruguayensis</i>	0.315039	-0.27792
<i>Strombidium conicum</i>	0.635728	-0.09223
<i>Lohmaniella spiralis</i>	0.635728	-0.09223
<i>Lohmaniella</i> sp.	-1.0489	2.11629
<i>Porocentrum micans</i>	0.214571	0.459897
<i>Protoperidinium breve</i>	-0.87308	0.825113
<i>Protoperidinium granii</i>	-1.60909	-1.39202
<i>Protoperidinium latistriatum</i>	-1.60909	-1.39202
<i>Protoperidinium stenii</i>	-0.11255	-0.5255
Copepod nauplius	-0.17785	0.175
PreM	0.721074	-0.11514
Mon	-1.25783	2.17236
PostM	-1.76874	-1.34917
Temperature	0.905526	-0.40201
Salinity	0.668584	-0.72705
Do	-0.4068	0.903291
Chl a	0.746218	-0.64724

Table. S4 CCA analysis Axis values – St.Z3 (Zuari Estuary)

Axis	Eigenvalue	%
1	0.47989	72
2	0.18658	28
	Axis 1	Axis 2
<i>Amphorella acuta</i>	-0.658846	-0.18115
<i>Codonellopsis tessellata</i>	-0.278474	-0.26501
<i>Codonellopsis</i> sp.	-0.658846	-0.18115
<i>Dictyocysta</i> sp.	1.62339	-0.68431
<i>Leprotintinnus nordquisti</i>	0.476377	0.0322712
<i>Stenosemella</i> sp.	1.62339	-0.68431
<i>Tintinnopsis beroidea</i>	1.62339	-0.68431
<i>Tintinnopsis karajacensis</i>	-0.658846	-0.18115
<i>Tintinnopsis radix</i>	1.62339	-0.68431
<i>Tintinnopsis uruguayensis</i>	1.62339	-0.68431
<i>Strombidium conicum</i>	-0.658846	-0.18115
<i>Porocentrum micans</i>	-0.658846	-0.18115
<i>Protoperidinium breve</i>	1.00379	0.317868
<i>Protoperidinium depressum</i>	-0.658846	-0.18115
<i>Protoperidinium granii</i>	-0.658846	-0.18115
<i>Protoperidinium latistriatum</i>	-0.0882874	-0.30694
<i>Protoperidinium ovatum</i>	-0.383883	0.569707
<i>Protoperidinium pellucidum</i>	-0.658846	-0.18115
<i>Pyrophacus</i>	0.441007	2.82228
Copepod nauplius	0.094039	0.271131
PreM	-0.718057	-0.242121
Mon	0.675445	3.06369
PostM	1.68774	-0.618048
Temperature	-0.747177	-0.663365
Salinity	-0.667523	-0.743463
Do	0.825276	0.563338
Chl a	-0.879279	-0.474824

Table. S5 CCA analysis Axis values – St.Z4 (Zuari Estuary)

Axis	Eigenvalue	%
1	0.41634	52.16
2	0.38191	47.84
	Axis 1	Axis 2
<i>Dictyocysta</i> sp.	2.19987	0.259624
<i>Leptotintinnus nordquisti</i>	-0.396908	-0.499739
<i>Stenosemella</i> sp.	-0.297472	-1.91139
<i>Tintinnopsis beroidea</i>	-0.454083	0.311962
<i>Tintinnopsis cylindrical</i>	-0.471484	0.559002
<i>Tintinnopsis karajacensis</i>	-0.297472	-1.91139
<i>Tintinnopsis radix</i>	-0.297472	-1.91139
<i>Tintinnopsis uruguayensis</i>	-0.297472	-1.91139
<i>Strombidium conicum</i>	-0.471484	0.559002
<i>Strombidium</i> sp.	-0.471484	0.559002
<i>Lohmaniella oviformes</i>	-0.471484	0.559002
<i>Lohmaniella spiralis</i>	-0.471484	0.559002
<i>Lohmaniella</i> sp.	-0.471484	0.559002
<i>Protoperidinium breve</i>	1.14376	-0.0758878
<i>Protoperidinium granii</i>	-0.297472	-1.91139
<i>Protoperidinium latistriatum</i>	0.597057	0.439251
<i>Protoperidinium ovatum</i>	2.19987	0.259624
<i>Protoperidinium stenii</i>	-0.297472	-1.91139
<i>Protoperidinium</i> sp.	-0.471484	0.559002
<i>Pyrophacus</i>	-0.297472	-1.91139
Copepod nauplius	-0.0971387	0.0477291
PreM	-0.525978	0.605037
Mon	2.31423	0.163012
PostM	-0.22141	-1.97565
Temperature	-0.623516	0.59125
Salinity	-0.746905	0.446138
Do	0.730186	-0.468194
Chl a	-0.845132	0.294591

Table. S6 CCA analysis Axis values – St.M1 (Mandovi Estuary)

Axis	Eigenvalue	%
1	0.55843	59.42
2	0.38137	40.58
	Axis 1	Axis 2
<i>Amphorella acuta</i>	1.35679	-0.8358
<i>Codonellopsis ostenfeldi</i>	-0.0623012	2.91578
<i>Codonellopsis tessellata</i>	-0.0623012	2.91578
<i>Codonellopsis</i> sp.	-0.604228	-0.367692
<i>Dadayiella gaymedes</i>	-0.0623012	2.91578
<i>Dictyocysta</i> sp.	-0.0623012	2.91578
<i>Leprotintinnus nordquisti</i>	-0.535992	1.80998
<i>Stenosemella</i> sp.	-0.0623012	2.91578
<i>Tintinnopsis beroidea</i>	1.15407	-0.29986
<i>Tintinnopsis butschli</i>	1.35679	-0.8358
<i>Tintinnopsis uruguayensis</i>	-0.0623012	2.91578
<i>Strombidium conicum</i>	1.35679	-0.8358
<i>Lohmaniella oviformes</i>	1.35679	-0.8358
<i>Noctiluca scintillans</i>	-1.72022	-0.954507
<i>Podolompus</i> sp.	-1.72022	-0.954507
<i>Porocentrum micans</i>	-0.735962	-0.515869
<i>Protopteridinium breve</i>	0.582742	1.21052
<i>Protopteridinium depressum</i>	1.07297	-0.0854837
<i>Protopteridinium divergens</i>	-1.72022	-0.954507
<i>Protopteridinium granii</i>	-0.168445	-0.471716
<i>Protopteridinium latistriatum</i>	-0.842498	1.09447
<i>Protopteridinium ovatum</i>	1.35679	-0.8358
<i>Protopteridinium stenii</i>	-0.0623012	2.91578
<i>Pyrophacus</i>	-1.72022	-0.954507
Copepod nauplius	0.836227	-0.284933
PreM	0.762581	-0.326667
Mon	-0.960949	-0.363494
PostM	-0.0434375	1.12594
Temperature	0.998993	0.0141344
Salinity	0.980907	0.251985
Do	-0.352519	-0.954965
Chl a	0.827555	0.609212

Table. S7 CCA analysis Axis values – St.M2 (Mandovi Estuary)

Axis	Eigenvalue	%
1	0.411	70.28
2	0.17379	29.72
	Axis 1	Axis 2
<i>Amphorella acuta</i>	1.57694	-0.557381
<i>Codonellopsis</i> sp.	1.24893	0.227259
<i>Codonellopsis ostenfeldi</i>	0.440324	0.294452
<i>Codonellopsis tessellata</i>	0.136572	-0.506543
<i>Leprotintinnus nordquisti</i>	-1.59187	-0.445537
<i>Stenosemella</i> sp.	-0.719156	4.9351
<i>Tintinnopsis beroidea</i>	1.32182	0.0528942
<i>Tintinnopsis karajacensis</i>	-0.719156	4.9351
<i>porocentrum micans</i>	0.520671	-0.5201
<i>Protopteridinium breve</i>	-0.719156	4.9351
<i>Protopteridinium depressum</i>	-1.44642	0.451235
<i>Protopteridinium divergens</i>	-1.81005	-1.7907
<i>Protopteridinium granii</i>	-1.53733	-0.109248
<i>Protopteridinium latistriatum</i>	-0.719156	4.9351
<i>Protopteridinium ovatum</i>	-1.59187	-0.445537
<i>Lohmaniella spiralis</i>	1.57694	-0.557381
Copepod nauplius	0.187498	0.0990658
PreM	0.658064	-0.106735
Mon	-0.744084	-0.31106
PostM	-0.321786	0.883707
Temperature	0.994391	-0.170243
Salinity	0.869406	0.436486
Do	-0.354021	-0.910222
Chl a	0.0369464	0.994796

Table. S8 CCA analysis Axis values – St.M3 (Mandovi Estuary)

Axis	Eigenvalue	%
1	0.28423	65.83
2	0.14753	34.17
	Axis 1	Axis 2
<i>Codonellopsis</i> sp.	0.524867	2.7877
<i>Codonellopsis ostenfeldi</i>	0.479124	-0.126292
<i>Codonellopsis tessellata</i>	-0.139991	-0.283819
<i>Leptotintinnus nordquisti</i>	2.44657	-0.496403
<i>Tintinnopsis beroidea</i>	-0.220267	0.214033
<i>Tintinnopsis cylindrical</i>	-0.344456	-0.214912
<i>Tintinnopsis karajacensis</i>	0.890914	-0.0819821
<i>Tintinnopsis uryguayensis</i>	-0.344456	-0.214912
<i>Protoperidinium</i> sp.	-0.344456	-0.214912
<i>Protoperidinium breve</i>	-0.0259355	-0.0687505
<i>Protoperidinium depressum</i>	-0.344456	-0.214912
<i>Protoperidinium divergens</i>	-0.220267	0.214033
<i>Protoperidinium granii</i>	-0.29332	-0.0382874
<i>Protoperidinium latistriatum</i>	2.12628	0.0509476
<i>Protoperidinium oceanicum</i>	-0.220267	0.214033
<i>Protoperidinium ovatum</i>	-0.170592	0.385611
<i>Protoperidinium stenii</i>	0.524867	2.7877
<i>Pyrococcus</i> sp.	-0.344456	-0.214912
<i>Lohmaniella spiralis</i>	-0.344456	-0.214912
Copepod nauplius	-0.0507783	0.222606
PreM	-0.463801	-0.271394
Mon	3.54315	-1.02582
PostM	1.01817	3.02117
Temperature	-0.88771	-0.152495
Salinity	-0.981702	0.133365
Do	0.972527	-0.0902886
Chl a	-0.655086	0.925312

Table. S9 CCA analysis Axis values – St.M4 (Mandovi Estuary)

Axis	Eigenvalue	%
1	0.31357	63.79
2	0.178	36.21
	Axis 1	Axis 2
<i>Codonellopsis</i> sp.	0.0084553	-0.367139
<i>Codonellopsis ostenfeldi</i>	2.53187	-1.40072
<i>Codonellopsis tessellata</i>	0.026967	0.389711
<i>Leptotintinnus nordquisti</i>	2.53187	-1.40072
<i>Tintinnidium apertus</i>	0.717983	2.59108
<i>Tintinnopsis beroidea</i>	1.62492	0.59518
<i>Tintinnopsis directa</i>	-0.352635	-0.0482466
<i>Tintinnopsis karajacensis</i>	1.32261	1.26048
<i>Tintinnopsis uryguayensis</i>	1.05988	-0.797798
<i>Porocentrum micans</i>	-0.087575	0.23721
<i>Protoperidinium</i> sp.	-0.412113	-0.194876
<i>Protoperidinium breve</i>	1.62492	0.59518
<i>Protoperidinium depressum</i>	-0.223764	0.26945
<i>Protoperidinium divergens</i>	2.53187	-1.40072
<i>Protoperidinium granii</i>	-0.322902	-0.231417
<i>Protoperidinium latistriatum</i>	0.33134	0.428848
<i>Protoperidinium oceanicum</i>	-0.412113	-0.194876
<i>Protoperidinium ovatum</i>	0.569213	-0.596824
<i>Protoperidinium pellucidum</i>	-0.412113	-0.194876
<i>Protoperidinium stenii</i>	-0.223764	0.26945
<i>Strombidium conicum</i>	-0.412113	-0.194876
<i>Lohmaniella spiralis</i>	-0.412113	-0.194876
Copepod nauplius	0.198998	0.0421409
PreM	-0.5276	-0.239743
Mon	1.12627	2.7497
PostM	3.00243	-1.21791
Temperature	-0.716474	-0.476653
Salinity	-0.756819	-0.423355
Do	0.711145	0.483328
Chl a	-0.113707	-0.925224

Table. S10 Spearman's rank correlation (r) between environmental parameters and microzooplankton in the Zuari estuary

Zuari Estuary	Temperature	Salinity	Dissolved Oxygen	Chlorophyll <i>a</i>	Microzooplankton
Temperature	1				
Salinity	0.005892792	1			
Dissolved Oxygen	-0.435325271	-	1		
Chlorophyll <i>a</i>	0.840746301	0.018164408	-0.538567778	1	
Microzooplankton	0.285903931	0.294582145	-0.494567717	0.521939846	1

Table. S11 Spearman's rank correlation (r) between environmental parameters and microzooplankton in the Zuari estuary

Mandovi Estuary	Temperature	Salinity	Dissolved Oxygen	Chlorophyll <i>a</i>	Microzooplankton
Temperature	1				
Salinity	0.341746652	1			
Dissolved Oxygen	-0.348185911	-	1		
Chlorophyll <i>a</i>	0.270255966	0.200563884	-0.336680197	1	
Microzooplankton	0.866534204	0.41369118	-0.536235616	0.12105861	1