

Impact of fresh water influx on microzooplankton mediated food web in a tropical estuary (Cochin backwaters - India)

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Abstract

The diversity, abundance and biomass of microzooplankton in Cochin backwaters were studied for the first time during pre – summer monsoon to peak of summer monsoon (April to July 2003) to understand the impact of large freshwater influx. Microzooplankton abundance and biomass were highest during pre – summer monsoon (av. 3817 ind. L⁻¹ and 146 µgC L⁻¹) that declined with the onset (av. 2052 ind. L⁻¹ and 45 µg C L⁻¹) and peak (av. 409 ind. L⁻¹ and 10 µgC L⁻¹) summer monsoon. Species diversity, richness and evenness of microzooplankton also showed similar trends as that of abundance and biomass. Grazing experiment showed that microzooplankton consumes 43 ± 1% of the daily phytoplankton standing stock during the high saline condition (27.5). Low abundance of microzooplankton during summer monsoon period (1/9 of the pre - summer monsoon value) along with the concomitant occurrence of low mesozooplankton (1/8 times of pre summer monsoon value) suggests that there could be a general lack of planktonic grazers. This would result in a weak transfer of primary and bacterial carbon to higher trophic levels, eventually leaving behind much unconsumed basic food in the estuary during summer monsoon. Thus a major portion of the primary carbon either settles down or gets transported to the coastal regions during monsoon. High flushing of Cochin backwaters also facilitates faster removal of primary producers to the coastal regions during monsoon.

Keywords: microzooplankton; tropical estuary; freshwater influx; species diversity

Regional index terms: India, Kerala, Cochin backwaters

1. Introduction

Microzooplankton (20 - 200µm) constitutes a considerable portion of the zooplankton biomass in marine and estuarine environments (Porter *et al.* 1985; Pierce and Turner 1992). Consequently, there has been an upsurge of scientific interest on microzooplankton (MZP) worldwide. Due to small body size, MZP have higher weight specific physiological rates such as feeding, respiration, excretion and growth than large metazoans (Fenchel 1987; Verity 1985). Unlike meso and macrozooplankton, MZP are efficient in consuming pico and nanoplankton (Nival and Nival 1976; Johnson *et al.* 1979). Thus, MZP act as a trophic intermediate between pico/ nanoplankton and large zooplankton. Studies have further shown that MZP dominate among the grazers of tropical oceanic phytoplankton in the Atlantic (Burkill *et al.* 1993a; Variety *et al.* 1993), Indian (Burkill *et al.* 1993b), Pacific Oceans (Miller 1993) and also act as a significant food source for a variety of invertebrate and vertebrate predators (Robertson 1983; Stoeker and Egloff 1987; Stoeker and Capuzzo 1990; Fukami *et al.* 1999). MZP is also known to be a crucial component that transfers organic

carbon from heterotrophic bacteria to higher trophic levels (Azam *et al.* 1983). In the Arabian Sea, 'microbial loop' is significant in maintaining high mesozooplankton biomass throughout the year (Madhupratap *et al.* 1996).

Despite the potential ecological importance of MZP, detailed information on their diversity, abundance and biomass in Indian estuaries is lacking. Earlier studies were focussed on loricate ciliates (tintinnids) alone and information on aloricate ciliates and heterotrophic dinoflagellates from the region is rather absent. Available data records the presence of abundant community of tintinnids in Indian estuaries (Damodara Naidu and Krishnamurthy 1985; Godhantaraman 1994; Sujatha and Panigrahy 1999) and their distribution is controlled by factors such as salinity and phytoplankton standing stock (Godhantaraman 1994; Sujata and Panigrahy 1999).

Cochin backwaters (CBW), a tropical estuarine system located along the southwest coast of India, is influenced by heavy rain and fresh water influx during monsoon periods (June to September – summer monsoon, November to February - winter monsoon) in which most of the rain occurs during summer monsoon period. During the peak of summer monsoon (July – August) surface salinity reaches near zero values over most of the region (Madhupratap 1987). The present study in CBW is based on the following aspects: (a) knowledge on MZP community of Indian estuaries is limited (b) MZP community of CBW has not received a previous study (c) salinity is one of the most important factors that controls distribution of tintinnids and mesozooplankton in Indian estuaries. Therefore, the present investigation was made to assess the abundance and diversity of MZP in CBW with respect to environmental factors such as salinity, dissolved oxygen and chlorophyll *a*. An attempt has also been made to infer the influence of exceptionally low saline waters on MZP community during summer monsoon period and its implications on the food web.

2. Materials and Methods

2.1. Study area

The backwaters of Kerala are located along Lat. 9° 30' – 10° 10'N and Lon. 76° 15' – 76° 25'E that extent from Azhikode in the north to Alleppey in the south. It forms a complex system of shallow estuarine network running parallel to the coastline of Kerala. The backwater system has two permanent opening to the Arabian Sea – one at Cochin (renamed as Kochi) and the other at Azhikode. The Cochin barmouth is much wider and forms the main entrance to the Arabian Sea. The estuarine system around the city of Cochin and neighboring areas is known as CBW, the largest water body of its kind along the west coast of India. Six rivers (Pamba, Achancoil, Manimala, Meenachil, Periyar, and Muvattupuzha) with their tributaries along with several canals bring large volumes of freshwater into the backwater system of Kerala. Among these rivers, Periyar and Muvattupuzha discharge into the northern part of the backwater system and hence have an active influence on the prevailing salinity of Cochin backwaters. Tidal incursion from the Arabian Sea (tidal range of ~1m) contributes a regular flow of salt water, which diminishes considerably towards the head of the Cochin estuary (Madhupratap 1987).

The annual rainfall at Cochin is around 3200 mm, of which nearly 75% occurs during summer monsoon period (Qasim, 2003). The onset of summer monsoon and its duration may

vary from year to year. Normally, it occurs from June to September. During the peak of summer monsoon period (July/August) heavy rain occurs in the region (40 – 50 cm rainfall can occur in a few hours) (Qasim 2003). Salinity remains at near zero values over a large portion of the CBW during this period. In this ‘bar - built estuary’, seasonal effects of freshwater is readily visible in the prevailing salinities, which play an important role in the ecobiology of the system (Madhupratap 1987). During post monsoon period (October to January), the river discharge gradually diminishes and tidal influence gains momentum as the estuarine conditions change to a partially mixed type, weakening stratification (Menon *et al.* 2000). During pre summer monsoon period (March – May), fresh water input to Cochin backwater is minimum due to low rainfall over the region. Hence a gradient of salinity develops from the mouth to the head of the estuary and the lower reaches of the estuary behave as a section of the Arabian Sea (Madhupratap 1987; Menon *et al.* 2000). Since the backwater system is geographically located in the tropical region, seasonal variation of temperature at the surface is about 28 °C in summer monsoon period (June – September) and 30 °C in pre - summer monsoon period (March – May) (Madhupratap 1987).

In CBW, phytoplankton biomass and production remains largely constant all through the year although marked salinity variations arise seasonally as a result of heavy freshwater influx (Menon *et al.* 2000). Low saline water has insignificant effect on the growth and production of phytoplankton in this system (Qasim *et al.* 1972). However, a qualitative shift in phytoplankton composition is reported during extremely low saline conditions and small forms contribute to the majority of the standing stock and production all through the year (Menon *et al.* 2000; Qasim 2003). Interestingly, mesozooplankton standing stock and production varies seasonally with a minimum during summer monsoon period, which is 8 times lower compared to pre - summer monsoon (Madhupratap 1987). Low mesozooplankton standing stock during summer monsoon is known to be due to the osmotic problems produced by low saline waters.

Based on the above knowledge, samplings were carried out in CBW during pre - summer monsoon (April), onset of summer monsoon (June) and (c) peak of summer monsoon (July) in 2003. Eight stations were selected in the study area in which two stations (station 1 and 5) were located near the Cochin barmouth (Figure. 1). Stations 2, 3 and 4 were towards Periyar River estuary and 6, 7 and 8 were towards Muvattupuzha River estuary from the Cochin barmouth.

2.2. Abiotic factors and phytoplankton biomass (chlorophyll *a*)

Water samples were collected from surface (0.5m) using 5litre Niskin sampler (Hydro Bios). Salinity was measured using a digital salinometer (DIGI – AUTO, 3G Tsurumi Seiki, Japan, accuracy ± 0.001). Other parameters like dissolved oxygen, nitrate, phosphate and silicate in the environment were measured following standard procedures (Grasshoff *et al.* 1983). Water samples (500 ml) were filtered through GF/F filter papers and extracted the chlorophyll *a* content using 90% acetone. Measurement of chlorophyll *a* was carried out using spectrophotometer (Strickland and Parsons 1972).

2.3. MZP sampling and analysis

Water samples (2L) collected in black polythene bottles were brought to the laboratory and prefiltered through a 200 μm bolting silk to remove mesozooplankton and the filtered samples were collected again in another set of black polythene bottles. While doing these processes, utmost care was taken to cause minimum damage to the fragile MZP. Acid Lugols iodine (3%) was added to all the samples and kept for initial settling of the samples under gravity. After 48 hours, supernatant of the samples were decanted by siphoning through PVC tubing till $\sim 100\text{ml}$ volume remains in the carboy. One end of the tubing, which was immersed in the sample was tied with a $20\mu\text{m}$ Nitex screen for retaining all the organisms of $\geq 20\mu\text{m}$ size at the bottom of the carboy. Two days before the analysis by microscopy, initial sample concentrates (ca. 100 ml) were allowed to settle down again for 48 hours in a settling chamber. Supernatant of the settled samples were removed by siphoning process and concentrated to 10ml volume. Five sub - samples were taken from each sample concentrate in to a Sedgewick rafter-counting chamber and observed under an inverted microscope with phase contrast optics (100 – 400X). MZP in the samples were categorized broadly in to ciliates, heterotrophic dinoflagellates and crustacean larvae. Ciliates were further considered under two groups viz, loricates (organisms with lorica) and aloricates (organisms without lorica). Ciliates and heterotrophic dinoflagellates were identified up to the species level based on the literature (Kofoid and Campbell, 1939; Maeda 1986). Cell dimensions (μm) of protozoans were measured using calibrated micrometer to compute the biovolumes. The cell volume of tintinnid ciliates was assumed to be 50% of the lorica volume. Conversion of biovolumes into carbon was carried out by applying a factor of $0.19 \text{ pgC } \mu\text{m}^{-3}$ for ciliates and $0.14 \text{ pgC } \mu\text{m}^{-3}$ for dinoflagellates (UNESCO 1994). Crustacean larval forms were identified, enumerated and estimated the biomass following Berggreen et al,1988.

2.4. MZP herbivory

MZP herbivory is defined as the rate of grazing of phytoplankton organic carbon by MZP per unit volume of seawater. Measurement of MZP grazing was carried out at station 1 during pre - summer monsoon following the dilution technique (Landry and Hassett 1982). The aim of the experiment was to understand the role of MZP as grazers of phytoplankton in CBW when the system is high saline. 30 litres of water was collected from Station 1 (salinity 27.5) in polythene carbuoys and transported to the laboratory within 1 hour. The water was then gently filtered through a $200\mu\text{m}$ mesh to isolate the MZP fraction from larger predators. Although the screening of experimental samples through $200\mu\text{m}$ sieve may disturb large and fragile MZP, this process is widely used in MZP grazing experiments for discarding the mesozooplankton (Froneman and McQuaid 1997; Putland 2000, Stelfox - Widdicombe et al., 2004). Prey and predator free water was obtained by gently filtering half of the water collected through a $0.2\mu\text{m}$ polycarbonate filter. The prey and predator free water was then combined with unfiltered seawater to generate concentrations of 1, 0.75, 0.5 and 0.25 of the ambient concentration with duplicate bottles incubated at each concentration (total volume in each bottle is 5 litres). Incubation was carried out in the laboratory under natural light with temperature controlled at 24°C (mean day temperature). Before incubation was begun, a water sample was taken from each bottle of the dilution series to provide a measure of the initial chlorophyll *a* concentration. The corresponding bottles were sampled again (250ml) at the end of the incubation period (one day) for measuring the final chlorophyll *a* concentration and the measurements were carried out spectrophotometrically. Changes in the chlorophyll *a*

concentration over 1 day incubation were used to calculate the apparent phytoplankton growth rate, in each of the dilutions, assuming exponential growth:

$$\mu = k - cg = 1/t \ln [Pt/Po]$$

Where Po and Pt are the initial and final concentrations of chlorophyll a respectively; k is the instantaneous coefficient of phytoplankton growth, g the coefficient of grazing mortality and c is the dilution factor as the fraction of ambient seawater. The above formula convey that the μ is linearly related to the dilution factor c , that the negative slope is the grazing coefficient g and that the y - intercept is the phytoplankton growth rate k (Landry and Hassett 1982).

The proportion of initial chlorophyll a standing stock (Pi) turned over, as $\% d^{-1}$, by the microzooplankton was calculated according to the formula

$$Pi = 1 - e^{-g} * 100$$

2.5. Statistical analysis

2.5.1. Community indices

Diversity is a concise expression of how individuals of a community are distributed in subsets of groups. Diversity decreases when one or a few groups dominate in a community, when individuals of a more common group replace individuals of a rare group or when one or a few groups rapidly reproduce. To mathematically analyze and compare changes in aquatic communities due to environmental influence, species diversity can be used as one of the tools.

2.5.1.1. Shannon and Weaver species diversity index

$$H(S) = -\sum [p_i (\log_2 p_i)]$$

$P_i = n_i/n$ (proportion of the sample belonging to the i^{th} species).

2.5.1.2. Margalef's species richness index

$$D = (S-1)/\log_e^N$$

S = number of species

N = total number of individuals of all the species in the sample.

2.5.1.3. Heips species evenness index

$$E = e^{(H(S) - 1/S)}$$

H = species diversity in bits of information per individual.

S = the number of species

2.5.2. Cluster Analysis

Multilinkage cluster analysis was done by group linkage method for similarity between stations. Bray Curtis similarity index (Clifford and Stephenson 1975) is applied for the $\log_{10}(X+1)$ converted data of microzooplankton abundance (X indicate abundance of a species) and the similarity was determined at 60% level.

3. Results

3.1. General hydrography

During pre - summer monsoon, a marked salinity gradient (av. 16.5 ± 11.3) was present in the study area with high value near to the lower reaches of the estuary (Figure 2). Minimum salinity during the sampling (1psu) was found at station 3 and it was apparently due to its closeness to the River Periyar. With the onset of summer monsoon, salinity of the

study area declined from pre - summer monsoon (av. 2.1 ± 3.1). Many stations that were estuarine during pre - summer monsoon (stations 3, 6 and 8) showed lack of salinity during onset of summer monsoon (Figure 2). During peak summer monsoon, the entire study area was covered with freshwater (salinity 0.2 ± 0.4) and the maximum salinity (0.9) was found at station 1. Overall distribution of salinity during pre - summer monsoon and onset of summer monsoon showed marked gradients in the study area but such gradients were absent during peak summer monsoon due to enormous fresh water influx.

3.2. Dissolved oxygen and nutrients

Dissolved oxygen concentration was relatively higher during peak summer monsoon (av. $6.4 \pm 0.8 \text{ mg L}^{-1}$) compared with onset of summer monsoon (av. $5.8 \pm 1 \text{ mg L}^{-1}$) and pre - summer monsoon (av. $5.7 \pm 0.9 \text{ mg L}^{-1}$). Dissolved oxygen varied between stations during all the three samplings but it was fairly saturated (4 mg L^{-1}) in all the stations (Figure 2). High concentration of nitrate ($>3 \mu\text{M}$) was recorded in the environment during all the three samplings (Figure 2). Nitrate concentration was relatively higher in the study area during onset of summer monsoon (av. $20 \pm 9 \mu\text{M}$) compared with pre - summer monsoon ($11 \pm 5.9 \mu\text{M}$) and peak summer monsoon ($8.9 \pm 2.5 \mu\text{M}$). Nitrate concentration was more than $25 \mu\text{M}$ at many stations during onset of summer monsoon (Figure 2). Relatively higher concentration of phosphate was found during onset of summer monsoon (av. $2.8 \pm 3 \mu\text{M}$) and peak summer monsoon (av. $2.7 \pm 1.7 \mu\text{M}$) compared with pre - summer monsoon (av. $1.7 \pm 1.1 \mu\text{M}$) (Figure 2). Concentration of phosphate was $>1 \mu\text{M}$ in the study area except at station 4 there it was low ($0.7 \mu\text{M}$). Silicate concentration in the environment was always more than $10 \mu\text{M}$ during all the observations (Figure 2). Silicate concentration was considerably higher (av. $115.7 \pm 30 \mu\text{M}$ and $95.9 \pm 31.7 \mu\text{M}$) during onset of summer monsoon and peak summer monsoon, compared with pre - summer monsoon (av. $47.8 \pm 33.4 \mu\text{M}$).

3.3. Phytoplankton biomass

High concentration of chlorophyll *a* ($>1.5 \text{ mg m}^{-3}$) was present in the study area throughout the sampling periods and during pre - summer monsoon it was lower (av. $4.5 \pm 2.6 \text{ mg m}^{-3}$) compared with onset of summer monsoon (av. $5.4 \pm 3.9 \text{ mg m}^{-3}$) and peak summer monsoon (av. $6.9 \pm 5.5 \text{ mg m}^{-3}$) (Figure 3). Maximum chlorophyll *a* concentration during pre - summer monsoon (7.1 mg m^{-3}) was found at station 1 and in all other stations it was less than 5 mg m^{-3} . During onset of summer monsoon and peak summer monsoon, chlorophyll *a* distribution showed more marked spatial variations between stations (av. 5.4 ± 3.9 and $6.9 \pm 5.5 \text{ mg m}^{-3}$) compared with pre - summer monsoon. During peak of summer monsoon, maximum chlorophyll *a* during the study (18 mg m^{-3}) was found at station 7.

3.4. MZP abundance and biomass

Abundance of MZP was markedly higher during pre - summer monsoon (av. $3817 \pm 1893 \text{ ind. L}^{-1}$) compared with onset of summer monsoon (av. $2050 \pm 1623 \text{ ind. L}^{-1}$) and peak summer monsoon (av. $409 \pm 184 \text{ ind. L}^{-1}$) (Figure 3). During pre - summer monsoon, abundance of MZP was maximum (6080 ind. L^{-1}) at station 5. In general, station 3, 4 and 8 showed lower abundance than other stations. During onset of summer monsoon, maximum abundance (4400 ind. L^{-1}) was found at station 2. Stations 3, 4, 6 and 8 showed relatively lower abundance ($540, 660, 580$ and 640 ind. L^{-1} respectively) than other stations. During peak summer monsoon, abundance of MZP decreased markedly in the study area and maximum abundance during the period (650 ind. L^{-1}) was at station 6 (Figure 3). Biomass of MZP at different stations followed similar trend as that of numerical abundance. During the study, maximum biomass was found in pre - summer monsoon at station 5 ($146 \mu\text{gC L}^{-1}$)

where maximum abundance was recorded (Figure 3). Similar to abundance, average MZP biomass was markedly higher during pre - summer monsoon (av. $81 \pm 48 \mu\text{gC L}^{-1}$) than onset of summer monsoon (av. $45 \pm 38 \mu\text{gC L}^{-1}$) and peak summer monsoon (av. $10 \pm 5 \mu\text{gC L}^{-1}$).

3.5. Composition of MZP

Three distinct categories of organisms were present in the study area viz, ciliates, heterotrophic dinoflagellates and crustacean larvae. Their percentage contribution to the total abundance varied between stations and samplings. Generally, ciliates dominated the samples through out the study period (Figure 4). During pre - summer monsoon, percentage contribution of ciliates varied only marginally (av. $86.9 \pm 3.6 \%$). Crustacean larvae was the second dominant group (av. $8.3 \pm 3.6\%$) followed by heterotrophic dinoflagellates. During onset of summer monsoon, ciliates varied from (av. $82.3 \pm 7.4\%$) of the total abundance followed by crustacean larval forms (av. $13.6 \pm 9.7\%$) (Figure 4). Ciliates dominated during peak summer monsoon also ($70.6 \pm 15.1\%$) but, unlike pre - summer monsoon and onset of summer monsoon, heterotrophic dinoflagellates were the second dominant group (av. $21.5 \pm 16.1 \%$) during the period. Crustacean larvae were lowest in abundance during the period (av. $6.9 \pm 8.3 \%$) and were even absent at many stations (3, 4, 7 and 8). Relative dominance of loricates and aloricates were evident during all the three surveys (Figure 5). Loricate ciliates were maximum during peak summer monsoon (av. $70.8 \pm 10\%$) and minimum during pre - summer monsoon (av. $53.6 \pm 16.5\%$) whereas aloricates were maximum during onset of summer monsoon (av. $60.9 \pm 16.8\%$) and minimum during peak summer monsoon (av. $9.8 \pm 11.1\%$).

The microzooplankton in CBW was represented by 36 species of ciliates, 2 species of dinoflagellates and crustacean larvae (Table 1). Among ciliates, 22 species were loricates (tintinnids) and 14 species were aloricates. Heterotrophic dinoflagellates were represented by the genus *Peridinium* alone. Among ciliates, *Tintinnopsis* was the most common genus (16 species) and *Tintinnopsis lohmanni*, *T. beroidea*, *T. tubulosa* and *T. uruguayensis* were the most common species, found during all the three sampling. *Tintinnidium incertum* was also present during all the surveys and had maximum abundance during peak summer monsoon (Table 1). Among the eight species of aloricate ciliates found during the study, genus *Euplotes* was the most dominant one followed by *Strombidium*.

During pre - summer monsoon, MZP community was composed of 34 species of ciliates, 2 species of dinoflagellates and crustacean larvae (Table 1). Six species of ciliates viz, *Tintinnopsis lohmanni*, *T. beroidea*, *T. uruguaensis*, *Tintinnidium incertum*, *Dictyocysta seshahai*, were found in all the stations and the maximum number of species were found at station 5 (33) followed by station 1 (32). In onset of summer monsoon, of the 29 species of ciliates present in the study area, *Tintinnopsis beroidea* was common in all the stations and maximum number of species was at station 2 (26) followed by station 1 (25). During peak summer monsoon, MZP community was the lowest and represented by 8 species of ciliates with maximum at station 1 and 6 (7 each) followed by station 2 (5).

3.6. Diversity and similarity indices

Species richness of MZP was higher during pre - summer monsoon period (av. 2.7 ± 1.1) compared with onset of summer monsoon (av. 1.6 ± 1.3) and peak summer monsoon (av. 0.8 ± 0.6) (Table 2). Highest species richness of MZP was at station 5 (3.8). Species diversity

and species evenness were also highest during pre - summer monsoon (2.7 ± 1.1 and 3.3 ± 0.7 respectively). Maximum species diversity (4.8) during the study was at station 1, 6 and 7. Coefficient of variations of species richness, species diversity and species evenness were in the order of pre - summer monsoon <onset of summer monsoon <peak summer monsoon (Table 2).

During pre - summer monsoon, similarity between stations based on MZP abundance showed three clusters at 60% level. Largest cluster was formed between stations 1, 5, 6 and 7 (Figure 6). During onset of summer monsoon, two clusters of stations were formed with 4 stations each. Stations 1, 2, 5 and 7 formed the first cluster and remaining stations constituted the second cluster. During peak summer monsoon, three clusters of stations were found in which the largest cluster was formed between stations 1, 2, 6 and 7 (Figure 6).

3.7. Relationship of abiotic and biotic variables

Correlation between nutrients (nitrate, phosphate and silicate) and phytoplankton standing stock was insignificant relation ($P > 0.01$, $r < 0.3$, $n = 24$) and this could be due to the surplus nutrients present in the environment (Figure 7). Abundance of microzooplankton in the study area was significantly related with salinity ($P < 0.01$, $r = 0.88$, $n = 24$) (Figure 8). Interestingly, MZP abundance did not show significant relation with phytoplankton standing stock ($P > 0.01$, $r = 0.14$, $n = 24$) and dissolved oxygen ($P > 0.01$, $r = 0.28$, $n = 24$) (Figure 8).

3.8. Phytoplankton growth and microzooplankton grazing

Results of grazing experiment conducted at station 1 during April 2004 showed the trophic importance of MZP in the CBW. The net growth of phytoplankton and level of dilution from the grazing experiments are shown in figure 9. Specific growth of phytoplankton (K) during the experiment was 0.4 ± 0.02 and the grazing rate (g) was 0.6 ± 0.02 . Proportion of initial phytoplankton standing stock grazed (% per day) by MZP during the period was 43 ± 1 %.

4. Discussion

During pre - summer monsoon, high saline waters prevailed over the estuary due to the incursion of Arabian Sea waters and the lower reaches of CBW acts as a section of the Arabian Sea (Madhupratap 1987). However, in the upper reaches, freshwater influx of Periyar and Moovattupuzha rivers result in low saline waters. Therefore, a marked salinity gradient (0 - 32) was present between upper and lower reaches of CBW. During onset of summer monsoon, the increased fresh water influx reduced the salinity of the entire study area (av. 2.1 ± 3.1) compared with pre - summer monsoon (av. 16.5 ± 11.3). Normally, rain starts in June resulting in noticeable decrease in overall salinity of the system (Qasim 2003). During peak summer monsoon, due to heavy rain, salinity comes down to near zero values in the entire study area.

During the present study, high nitrate concentration ($>3\mu\text{M}$) indicates the eutrophic nature of the system. Relatively higher phosphate and silicate concentration during onset of summer monsoon (av. 2.8 ± 3 and $115.7 \pm 29\mu\text{M L}^{-1}$) and peak summer monsoon (av. 2.7 ± 1.7 and $95.9 \pm 31.7\mu\text{M}$) could be due to high river influx. Similarly, higher concentration of dissolved oxygen was found during peak summer monsoon (av. $6.4 \pm 0.8\text{mg L}^{-1}$) and onset of summer monsoon (av. $5.8 \pm 1\text{mg L}^{-1}$) compared to pre - summer monsoon (av. $5.7 \pm 0.9\text{mg L}^{-1}$).

¹) could be a result of high dissolution of oxygen in freshwater. The nitrate concentration in the study area was higher during the onset of summer monsoon (av. $20 \pm 9.1 \mu\text{M}$) compared with peak summer monsoon (av. 9 ± 2.4). This indicates that the high nitrate concentration in CBW during monsoon period is not exclusively brought by the river influx. Industrial and domestic sewage that reach directly in to CBW may also contribute significantly to the prevailing high nitrate concentration. Extensive human settlements along the coast of CBW produce large quantities of domestic sewage as well as agricultural wastes (Vijayan *et al.* 1976; Qasim 2003). The initial monsoonal rain, after the dry summer period (March – May), washes the adjacent land area and brings nitrate-enriched waters to CBW. As rain continues for longer period (later part of summer monsoon), concentration of nitrate in the land drainage also decreases resulting in low nitrate values. This kind of nitrate and phosphate enrichment is a general feature of Cochin estuarine system (Saraladevi, *et al.* 1983).

The presence of high concentration of chlorophyll *a* in the study area throughout the sampling periods (pre - summer monsoon - av. 4.5 ± 2.6 ; onset of summer monsoon - av. 5.4 ± 3.9 ; peak summer monsoon – av. 6.9 ± 5.5) indicated that the primary producers were always abundant in the system. This may be due to the high concentration of nutrients available in CBW through out the sampling. However, chlorophyll *a* was not positively correlated with the major nutrients (nitrate, phosphate and silicate) probably due to the exceedingly high concentration of these nutrients present at many stations. Higher average chlorophyll *a* concentration during peak summer monsoon compared with other sampling (pre - summer monsoon - 4.5mg m^{-3} , onset of summer monsoon - 5.4mg m^{-3}) showed that quantitatively phytoplankton community is not adversely affected by exceptionally low salinity. High phytoplankton biomass observed during peak summer monsoon could be due to the dominance of highly tolerant /low saline species of phytoplankton in the system. During summer monsoon period, phytoplankton community in the CBW undergoes a qualitative shift from that of pre - summer monsoon (Menon *et al.* 2000). During premonsoon period diatoms of the genera *Nitzschia*, *Skeletonema*, *Chaetoceros*, *Coscinodiscus* and *Pleurosigma* dominate the system. Species tolerant to low salinity such as *Skeletonema coastatum* and *Nitzschia closterium* continue to grow along with freshwater forms *Pledorina*, *Volvox*, *Pediastrum* and *Desmids* during monsoon period. Seasonally, *Nitzschia closterium* is found to be the most consistent species in CBW (Jayalakshmi *et al.* 1986). Another reason for relatively low chlorophyll *a* during pre - summer monsoon could be due to the grazing of abundant zooplankton (Madhuratap 1987).

In the present study, abundance and biomass of MZP was markedly higher during pre - summer monsoon compared with onset of summer monsoon and peak summer monsoon. Similarity between stations based on log (x+1) abundance of microzooplankton formed well-defined clusters of stations. High saline stations always formed a single cluster indicating that the MZP abundance in the system is controlled by salinity. Distinctly low species diversity, richness and evenness during summer monsoon suggest that only a few species are tolerant to near zero values of salinity. High coefficient of variations in species richness, species diversity and species evenness during pre - summer monsoon could be due to more gradient in salinity between stations during the period. Eight species of ciliates viz., *Tintinnopsis lohmanii*, *T. beroidea*, *T. tubulosa*, *T. uruguensis*, *Tintinnidium incertum*, *Leptotintinnus simplex*, *Euplotes vannus* and *Strombidinopsis cheshirii* were present during peak summer monsoon showing their wide range of tolerance to salinity. Salinity is reported to play a major

role in controlling the distribution of tintinnids in estuaries (Godhantaraman 1994; Sujatha and Panigrahy 1999). Influence of lower salinity on the distribution of mesozooplankton in CBW is also well established (Madhupratap and Haridas 1975, Madhupratap, 1987). In the present study, correlation between phytoplankton standing stock and microzooplankton biomass was insignificant due to the influence of immense freshwater during summer monsoon that would considerably decrease microzooplankton even when the phytoplankton biomass was high. Other environmental variables such as temperature and dissolved oxygen did not show any significant correlation with MZP abundance suggesting that these parameters do not control the abundance and distribution of MZP in this system

Dominance of ciliates in the MZP community is a general feature in estuarine systems worldwide. In the present study also ciliates contributed 71- 82% of the MZP community. Loricated ciliate present during all the surveys was maximum during onset of summer monsoon (82%) with the genus *Tintinnopsis* as the most dominant one. Globally, the genus *Tintinnopsis* is restricted to the estuarine and coastal regions possibly due to the requirement of the organism to incorporate the mineral flakes into their loricae. In culture, when deprived of suitable particles, *Tintinnopsis* produce a clear but much weaker lorica (Gold 1969; Gold and Morales 1975). Another advantageous characteristic of *Tintinnopsis* to survive in estuarine and coastal waters is the production of resting cysts and in shallow waters these cysts typically sink down and occur in the sediments (Krinsic 1987) and when appropriate conditions arrives, excystment and repopulation occurs very rapidly.

Abundance and diversity of tintinnids observed during the present study is higher than that observed in estuaries of east coast of India (Bahuda estuary in Orissa and the Pichavaram mangrove in Tamilnadu). In Bahuda estuary, abundance of tintinnids varied from 7 – 17 ind. L⁻¹ (Sujatha and Panigrahy 1999) and in Pichavaram mangrove it varied from 60 – 45 ind. L⁻¹ (Godhantaraman 1994). Though the abundance was low in these estuaries, the total number of species observed (22 species in Pichavaram mangrove and 23 species in Bahuda estuary) is comparable with the present study (22 species). CBW and the Pichavaram mangrove have many common species due to its closeness to CBW (Table 3). In the Zuary estuary (Goa), abundance of tintinnids was reported to be much higher (140 – 75760 ind. L⁻¹) when compared to Pichavaram mangrove, Bahuda estuary and CBW (20 – 6560 ind. L⁻¹). Furthermore, 18 out of 22 species of tintinnids found during the present study were reported earlier from the Zuary estuary (Table 3). Similarity in the abundance and composition of MZP in CBW and Zuary estuary could be due to their comparable hydrography under the influence of monsoon.

Aloricate ciliates inhabiting in Indian estuaries is rather poorly addressed. During the present study, abundance of aloricates increased from pre - summer monsoon (av. 33.3 ± 17.3%) to onset of summer monsoon (av. 36.3 ± 20.4%) and declined during peak summer monsoon (av. 9.8 ± 11.1). Species number were also more during pre - summer monsoon and onset of summer monsoon (13 species each) when compared with peak summer monsoon (2 species), which could be due to the inhibition of low salinity. This indicates that aloricate ciliates are quantitatively significant during non-monsoonal periods. Crustacean larvae showed a similar trend as that of aloricate ciliates with higher abundance during pre - summer monsoon (av. 8.3 ± 3.6) and onset of summer monsoon (av. 3.6 ± 9.7) compared with peak summer monsoon (av. 6.9 ± 8.3) due to their low survival during extremely low saline conditions. Low abundance of heterotrophic dinoflagellates (av. 3.9 ± 2%) throughout the

study period could be due to their preference to the coastal and oceanic regions. In general, dinoflagellate abundance in CBW is low due to the competitive advantages of diatoms in utilizing nutrients in eutrophic waters. Preponderance of diatoms in nutrient enriched environments is noticed many years back (Menzel *et al.* 1963, Qasim 1973) and in such conditions dinoflagellates fail to dominate. Dinoflagellates are known to be ‘slow growers’ which are more adapted to oligotrophic (stratified) waters (Cushing 1989).

Trophic importance of microzooplankton in CBW seems to be significant since most of the primary production (>70%) and phytoplankton standing stock (>60%) are contributed by nanoplankton (Qasim *et al.* 1974). The trophic dependency of mesozooplankton on MZP community is more significant in systems where small phytoplankton cells dominate (Cushing 1989). Grazing experiment during the present study showed that a significant portion of the phytoplankton standing stock (43%) is consumed by microzooplankton during pre - summer monsoon period. Tintinnid ciliates have been known to consume food of sizes as much as 45 – 50% of the lorica oral diameter (Splitter 1973) and hence are undoubtedly an important trophic link between detritus, bacteria, smaller phytoplankton and higher trophic levels (Rassoulzadegan *et al.* 1981, 1988). An earlier study on the food habits of tropical microzooplankton showed that most of the tintinnids prefer to feed on small phytoplankton (<30µm) (Godhantaraman 1997). Obviously, *Skeletonema costatum* and *Nitzschia closterium* having the size of <30µm form the bulk of the phytoplankton community in CBW (Menon *et al.* 2000) and hence naturally form an optimum food source for most of the tintinnids. Interestingly, in the secondary trophic level, copepods belonging to the family *Acartidae* form the bulk of the mesozooplankton population in CBW, (Madhupratap and Haridas 1975) and they ingest tintinnids at rates linearly related to the prey density. When phytoplankton were dominated by small diatom species (<10µm diameter), tintinnids in concentration exceeding 10³ organisms per litre can form an important item in diets of *Acartia*. (Robertson 1983). In laboratory experiments, addition of ciliates to the diet of copepod, *Acartia tonsa* enhanced the egg production indicating high nutritional value of the prey (Stoecker 1987a). Some other studies indicate that ciliates form an important diet of many gelatinous zooplankton, especially larval and post larval stages of ctenophore (*Mnemiopsis leidyi*) (Stoecker *et al.* 1987b) and for the medusae of *Aurelia aurita* (Stoecker *et al.* 1987c). Similarly, tintinnids are also known to be one of the most important food items of the first feeding stages of chaetognaths (Graziano 1989). In CBW, gelatinous zooplankton and chaetognaths are common during pre - summer monsoon (Madhupratap and Haridas 1975) and thus they may be utilising the abundant population of microzooplankton present during the period. Suspension feeding plankton in general, traditionally thought of as ‘herbivores’, in fact utilize the microbial food web in which microzooplankton is an important component (Sherr and Sherr 1988).

In CBW, the abundance and biomass of mesozooplankton are markedly low during summer monsoon period (Madhupratap and Haridas, 1975; Madhupratap, 1987). The present study shows that the abundance of microzooplankton also is very low during this period (8 times lower compared to pre - summer monsoon). Based on the current understanding on the planktonic interactions in aquatic systems, a simplified representation of the pelagic food web is shown in Figure 10. It is clear from the figure that if the major planktonic grazers such as micro and mesozooplankton are very low in abundance (as it occurs during summer monsoon in CBW), it would result in weak transfer of primary and bacterial carbon to higher trophic

levels eventually leaving much unconsumed basic food in the environment. Thus a major portion of the primary carbon during monsoon either settles down or gets transported to the coastal regions along with increased monsoon (Jomon and Kurup 1989). High concentrations of trace metals noticed in the coastal region of Arabian Sea during summer monsoon period has been attributed to the increased monsoonal supply of materials through CBW (Balachandran *et al.* 2003). Interestingly, the total organic detritus settling in CBW is reported to be higher during pre - summer monsoon period compared to summer monsoon (Qasim and Sankaranarayanan 1972) and could be a result of high residence time of the estuary and the high abundance of micro and mesozooplankton.

An earlier estimate on organic production of CBW (Qasim *et al.* 1970), shows a gross primary production of 272 – 292 g C m⁻² yr⁻¹ with an average of 280 g C m⁻² yr⁻¹ and the net production was 193 g C m⁻² yr⁻¹. The estimated annual consumption by zooplankton herbivores was only 30 g C m⁻² yr⁻¹, leaving a large surplus basic food (of about 163 g C m⁻² yr⁻¹) in the estuary. In the light of the present study, the ‘apparent wastefulness’ of primary production as suggested in the above study seems to be a result of over simplification of the food web. This is because, mesozooplankton with >300µm body size were only considered for estimating the biomass at the secondary level indicating that a considerable amount of the biomass of the small zooplankton (<300µm) were overlooked. The present study suggests that during high saline period, a considerable portion of the primary carbon is retained by MZP as their biomass that in turn increase the efficiency of the planktonic food web.

5. Conclusion

Present study evidences that an abundant community of microzooplankton inhabit in CBW (>10³ L⁻¹) during high saline conditions and seem to play a major role in the carbon cycling through the pelagic food web. However, during summer monsoon period, a major portion of the primary food in CBW may remain unutilized due to the low abundance of micro and mesozooplankton. Being a productive tropical estuary, both MZP and heterotrophic bacterial abundance may be significant factors contributing to the plankton food web through microbial loop. Unfortunately, knowledge on these factors are lacking for CBW. Future studies should address the bacterial and microzooplankton abundance, which would give new insight to the current understanding on the planktonic food web of CBW.

6. Acknowledgements

We express our thanks to the Director, National Institute of Oceanography for the facilities provided. This work form a part of ‘Ecosystem Modeling of Cochin backwaters (EMCB)’ and we are grateful to the Director, ICMAM – PD, Chennai for the financial support. We extend our thanks to all research students of the EMCB project for their valuable help during field collections and analysis of samples.

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Figure captions

Figure 1 - Station locations

Figure 2 – Distribution of environmental parameters

PSM – pre summer monsoon, OSM – onset of summer monsoon

PKSM – peak summer monsoon

Figure 3- Distribution of biological parameters

PSM – pre summer monsoon, OSM – onset of summer monsoon

PKSM – peak summer monsoon

Figure 4 – Percentage contribution of major components of MZP

Figure 5 – Relative occurrence of loricates and aloricates

PSM – pre summer monsoon, OSM – onset of summer monsoon

PKSM – peak summer monsoon

Figure 6 – Similarity between stations based on microzooplankton abundance

PSM – pre summer monsoon, OSM – onset of summer monsoon

PKSM – peak summer monsoon

Figure 7 – Correlation between chlorophyll *a* and major nutrients

Figure 8 – Correlation between MZP and major environmental variables

Figure 9 – Apparent growth rate Vs dilution factor

Figure 10 – A schematic picture of the planktonic food web in the CBW

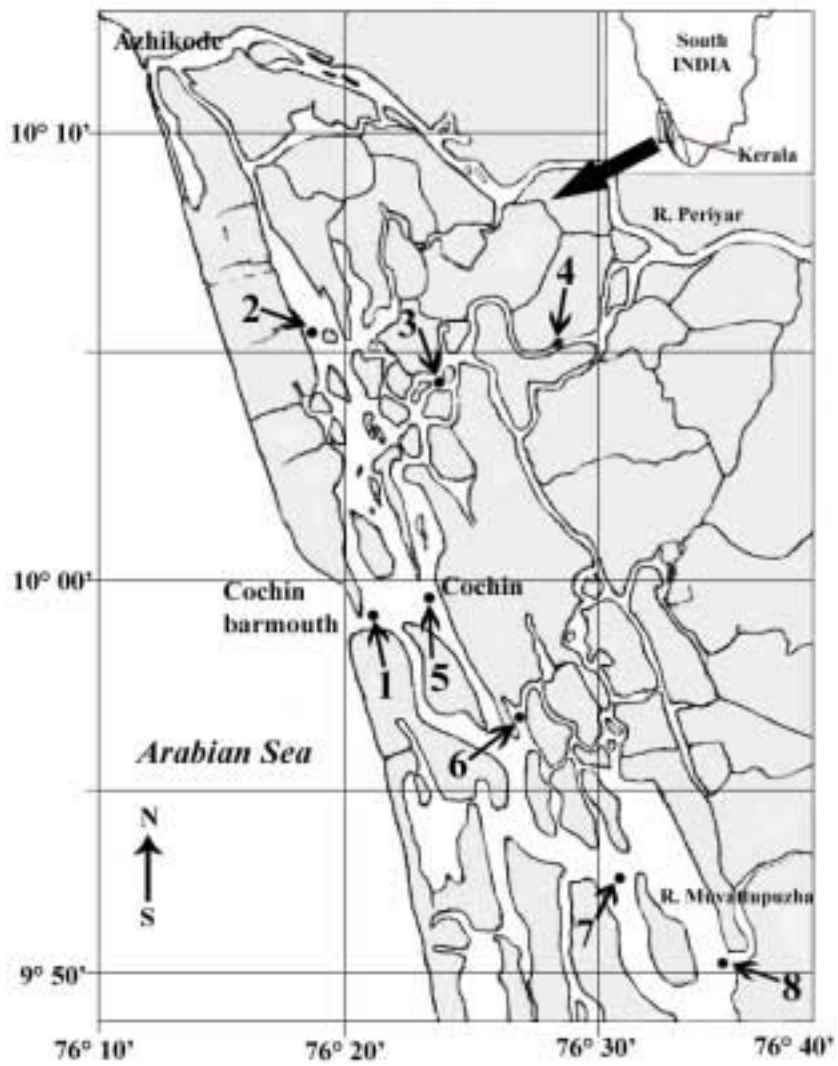
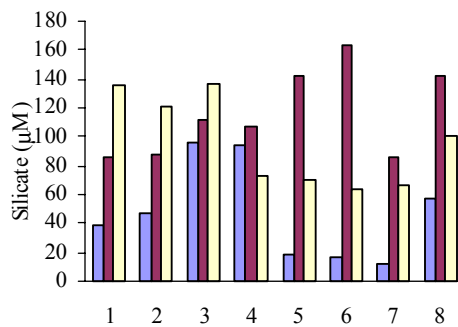
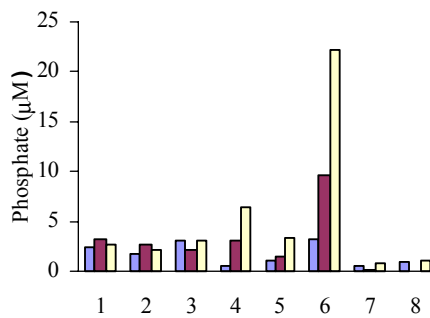
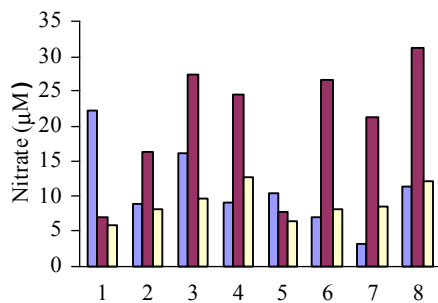
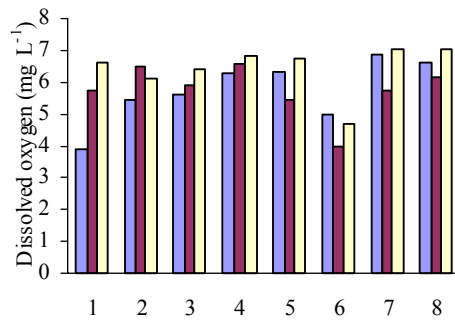
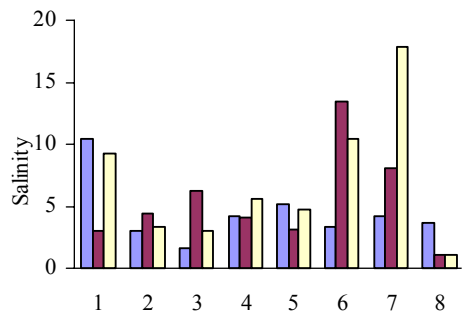


Figure 1



Stations

PSM OSM PKSM

Figure 2

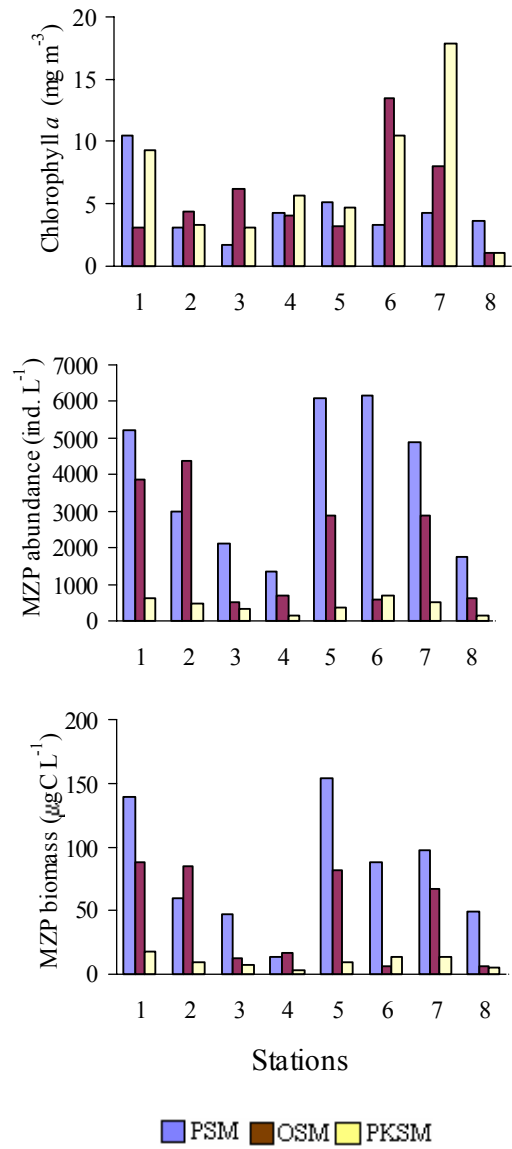


Figure 3

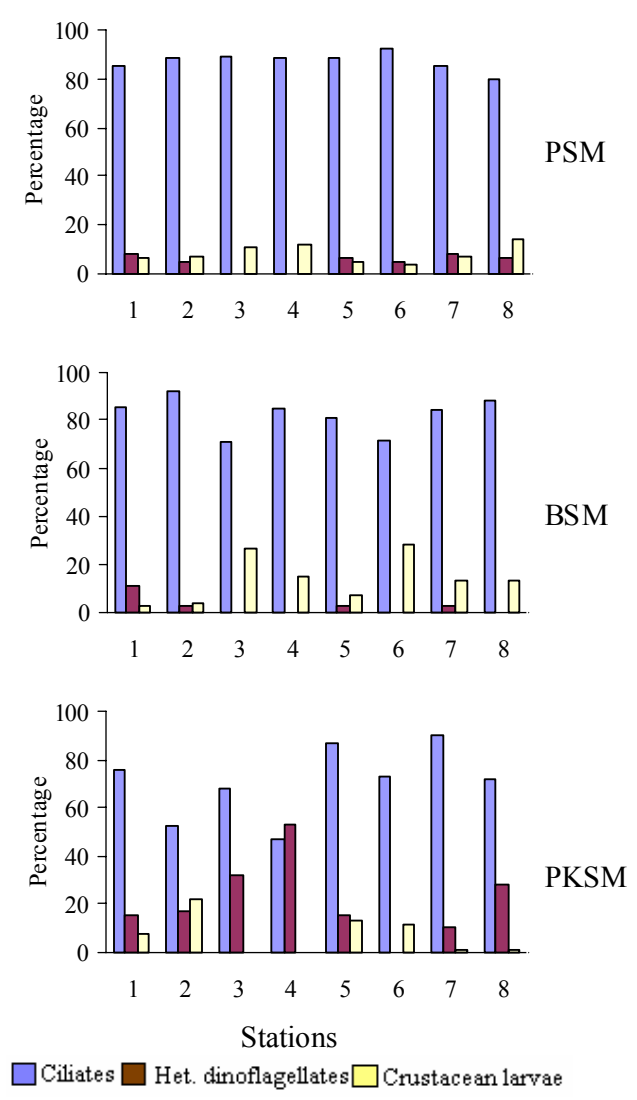


Figure 4

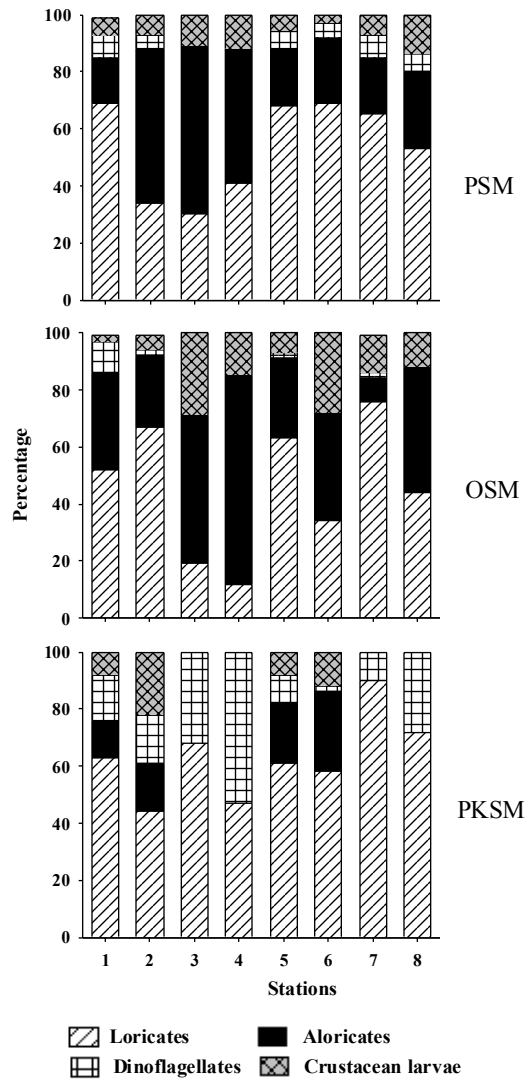


Figure 5

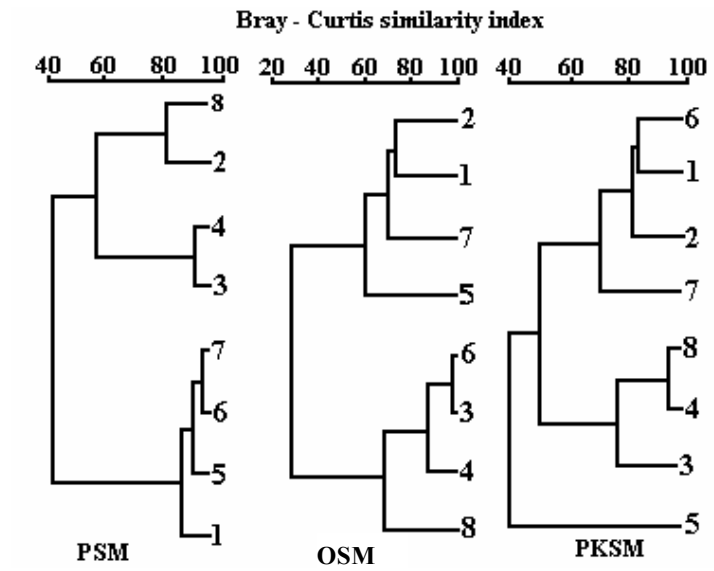


Figure 6

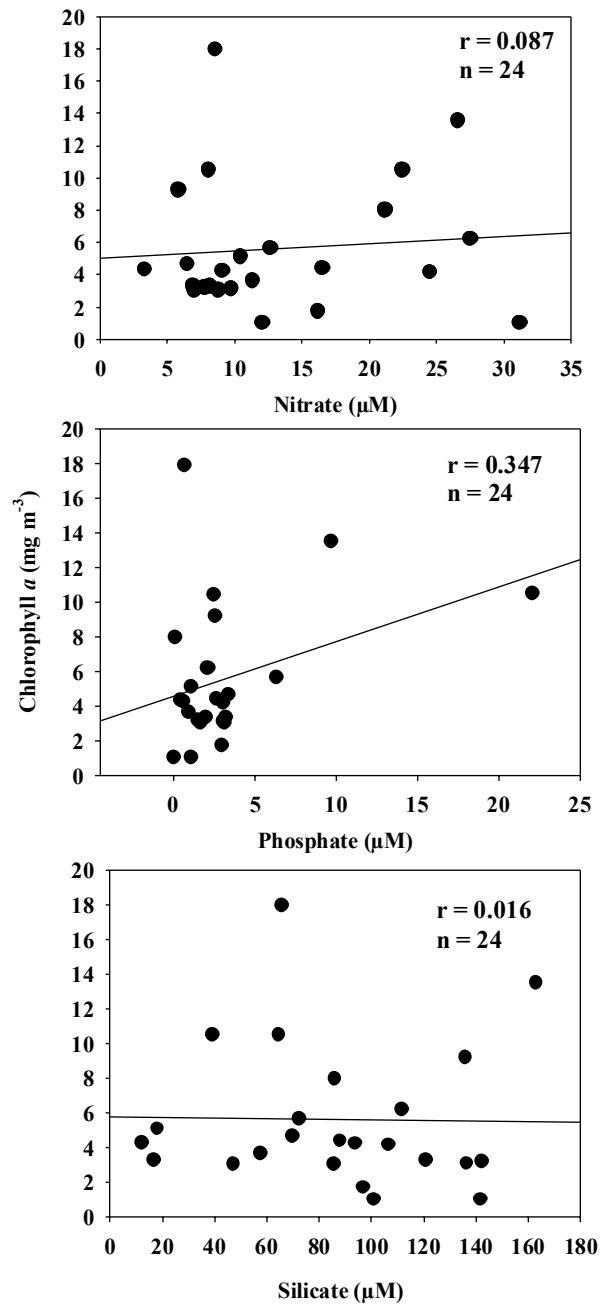


Figure 7

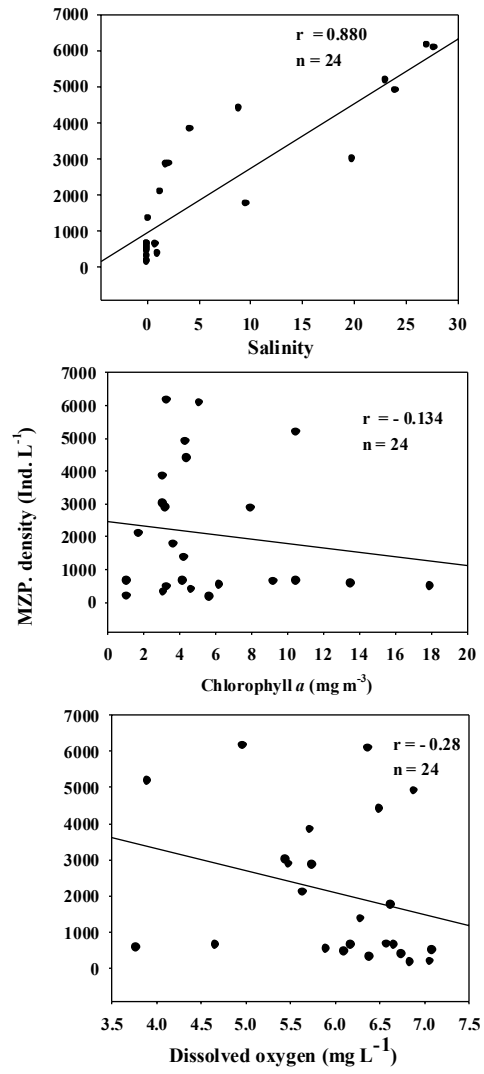


Figure 8

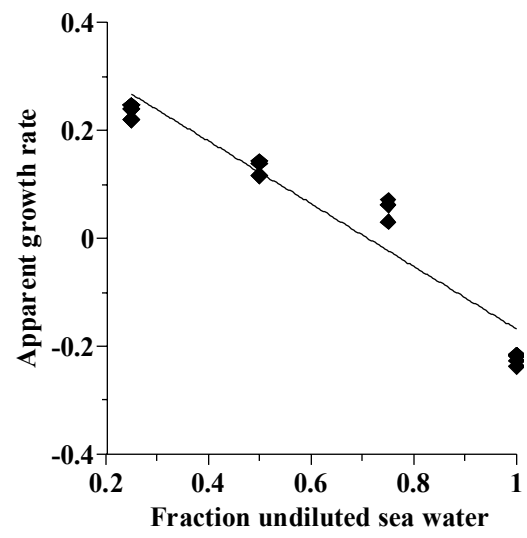


Figure 9

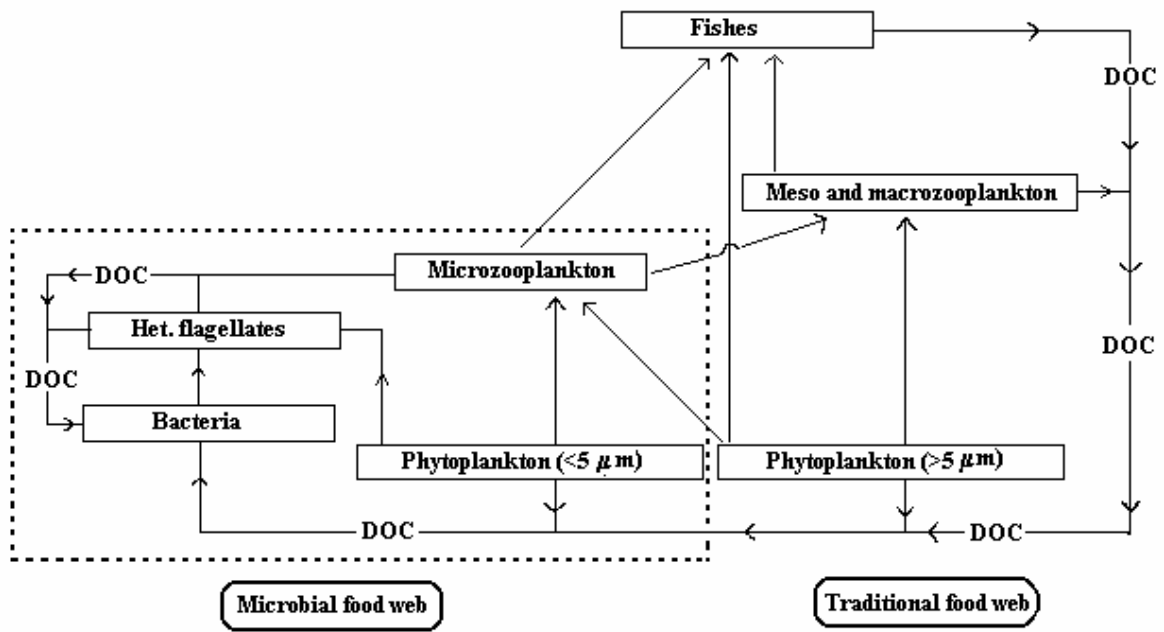


Figure 10

Species of MZP	PSM	OSM	PKSM
<u>Loricates</u>			
<i>Tintinnopsis lohmani</i>	148 ± 105	102 ± 118	6 ± 18
<i>T. beroidea</i>	230 ± 99	185 ± 105	50 ± 53
<i>T. bermudensis</i>	53 ± 62	100 ± 126	0
<i>T. directa</i>	108 ± 106	98 ± 146	0
<i>T. gracilis</i>	68 ± 85	85 ± 107	0
<i>T. tubulosa</i>	240 ± 188	115 ± 138	20 ± 28
<i>T. nucula</i>	95 ± 113	0	0
<i>T. uruguayensis</i>	183 ± 55	113 ± 150	45 ± 41
<i>T. tocaninensis</i>	58 ± 65	48 ± 68	0
<i>T. Kofoidii</i>	58 ± 65	58 ± 88	0
<i>T. glans</i>	125 ± 138	0	0
<i>T. cylindrica</i>	113 ± 121	20 ± 57	0
<i>T. butschli</i>	55 + 63	0	0
<i>T. rotundata</i>	50 ± 47	5 ± 14	0
<i>T. mortenseni</i>	18 ± 27	0	0
<i>T. radix</i>	80 ± 86	33 ± 62	0
<i>Tintinnidium incertum</i>	105 ± 40	80 ± 102	114 ± 32
<i>Leprotintinnus simplex</i>	233 ± 40	78 ± 117	13 ± 35
<i>L. nordquisti</i>	20 ± 57	-	0
<i>Dityocysta sehai</i>	100 ± 30	45 ± 62	0
<i>Epiplocylis undella</i>	68 ± 75	35 ± 66	0
<i>Eutintinnus lusus undae</i>	88 ± 72	0	0
<u>Aloricates</u>			
<i>Euplotes moebiusi</i>	138 ± 173	98 ± 55	0
<i>E. charon</i>	128 ± 149	103 ± 76	0
<i>E. vannus</i>	103 ± 139	73 ± 58	23 ± 42
<i>Stylonychia mytilus</i>	0	5 ± 14	0
<i>Oxytricha marina</i>	0	23 ± 64	0
<i>Lohmaniella spiralis</i>	50 ± 53	40 ± 56	0
<i>L. oviformes</i>	68 ± 78	20 ± 56	0
<i>Halteria chlorelligera</i>	33 ± 57	0	0
<i>Strombidium bilobum</i>	70 ± 61	23 ± 42	0
<i>S. conicum</i>	98 ± 55	18 ± 33	0
<i>S. sp.</i>	48 ± 35	18 ± 36	0
<i>Rimostrombidium sp.</i>	118 ± 110	43 ± 49	0
<i>Strobilidium minimum</i>	148 ± 107	78 ± 84	0
<i>Strombidinopsis cheshirii</i>	55 ± 79	58 ± 140	33 ± 48
<u>Dinoflagellates</u>			
<i>Peridinium ganii</i>	120 ± 83	58 ± 144	71 ± 36
<i>Peridinium sp.</i>	98 ± 107	38 ± 74	0
Crustacean larvae	257 ± 25	55 ± 87	35 ± 40

Table 1 – Species composition and abundance of MZP (ind. L⁻¹), PSM – pre summer monsoon, OSM – onset of summer monsoon, PKSM – peak summer monsoon

Stations	Species richness			Species diversity			Species evenness		
	PSM	BSM	PKSM	PSM	BSM	PKSM	PSM	BSM	PKSM
1	3.7	3.0	1.1	4.8	4.5	2.9	3.7	3.5	2.4
2	2.2	3.0	0.8	3.9	4.5	0.5	2.7	3.5	2.2
3	1.3	0.6	0.3	3.2	2.3	1.6	2.5	2.1	1.9
4	1.4	0.8	0.2	3.3	2.5	1.0	2.6	2.4	1.7
5	3.8	1.8	0.7	4.2	3.7	2.2	4.1	0.9	1.9
6	3.6	0.6	1.1	4.8	2.1	3.0	3.8	1.9	1.6
7	3.6	2.3	0.6	4.8	4.1	2.2	4.0	3.2	2.0
8	1.9	0.8	0.2	3.8	2.5	0.9	3.1	2.2	1.3
CV%	38.2	19.0	16.0	61.0	29.0	22.0	53.5	37.2	18.4

Table 2 – Diversity indices of MZP
PSM – pre summer monsoon, OSM – onset of summer monsoon
PKSM – peak summer monsoon

CBW	PM	BE	ZE
<i>Tintinnopsis lohmanii</i>	+	+	+
<i>T. beroidea</i>	+	+	+
<i>T. bermudensis</i>	+	+	+
<i>T. directa</i>	+	+	+
<i>T. gracilis</i>	+		+
<i>T. tubulosa</i>	+	+	+
<i>T. nucula</i>	+	+	+
<i>T. uruguayensis</i>	-	-	+
<i>T. tocantenensis</i>	+	+	+
<i>T. Kofoidii</i>	+	-	-
<i>T. glans</i>	+	-	+
<i>T. cylindrica</i>	+	+	+
<i>T. butschli</i>	-	+	+
<i>T. rotundata</i>	-	-	+
<i>T. mortenseni</i>	+	+	+
<i>T. radix</i>	+	-	-
<i>Tintinnidium incertum</i>	+	+	+
<i>Leptotintinnus simplex</i>	+	-	+
<i>L. nordquisti</i>	-	-	+
<i>Dityocysta sehahai</i>	-	+	+
<i>Epiplocylis undella</i>	-	-	-
<i>Eutintinnus lusus undae</i>	+	-	-

+ indicate presence - indicate absence

Table 3 – Comparison of species composition of tintinnids in CBW with other estuaries of India (PM - Pichavaram Mangroove, BE – Bahuda Estuary and ZE - Zuary Estuary)