Chapter III
Seasonal Dynamics of Plankton Food Web in a Monsoonal Estuary and the Significance of Mesohaline Region

3.1. Introduction

Estuary, the transition zone of river and sea, facilitates carbon flux between terrestrial and marine ecosystems. They are dynamic principally due to short-term changes caused by tide and the seasonal changes brought by the regional climate (Madhupratap & Rao, 1979; Iriarte & Purdie, 1994). Tide causes changes in salinity and nutrients distribution, which potentially impact the spatial distribution of biological components (Madhupratap, 1987; Iriarte & Purdie, 1994; Kimmerer et al., 1998). On the other hand, due to seasonal changes, an estuary can exhibit significant variations in the distribution of physicochemical as well as biological components (Madhupratap, 1987; Jyothibabu et al., 2006). India has about 25 estuaries located along its 7500-km coastline (Qasim, 2003) of which those heavily influenced by the Southwest Monsoon (June–September) rainfall are referred to as monsoonal estuaries (Vijith et al., 2009). Out of the monsoonal estuaries of Indian western coast, Cochin backwater is the largest and considered as a unique tropical ecosystem due to its highly dynamic nature. The backwater is constantly influenced by mixed semidiurnal tide with a maximum range of about 1m and all the environmental parameters fluctuate according to this. Magnitude of variation is not consistent and depends up on time of the year (Qasim & Gopinathan, 1969).

The two pronounced seasons in the ecosystems of tropical continental margins are spring intermonsoon (dry period) and south west monsoon (wet period). As a tropical ecosystem Cochin estuary is also controlled by the same seasonal contrast. Low freshwater inflow from rivers allows active salinity incursion in to the estuary from adjacent Arabian Sea during spring intermonsoon (Madhupratap, 1987). During monsoon the backwater transforms into a freshwater lake except near the inlet region due to the heavy fresh water input (Madhupratap, 1987; Qasim 2003). The heavy rainfall causes drastic changes in hydrography as the total fresh water inflow becomes several orders of magnitude larger than the estuarine volume and hence the name monsoonal estuary (Vijith et al., 2009).
Chapter III

The current hypothesis related to the food web dynamics of Cochin Backwater is that there is a general weakening of food web in monsoon due to the low relative abundance of grazers in the fresh water dominated system (Jyothibabu et al., 2006, Jyothibabu et al., 2015) and there is a substantial amount of unconsumed carbon at primary level owing to the reduction in phytoplankton grazers (Madhu et al., 2007). Studies also confirm that monsoonal flooding wipes out most of the organisms which thrives in high salinity and only a few organisms tolerant to low salinity are able to thrive in the middle and upper reaches (Madhupratap & Haridas 1975; Madhupratap et al., 1987). But when we carefully examine, these studies lead us towards an inevitable re-evaluation of the current hypothesis. On land and off shore sediments in the Laccadive basin indicate that Cochin estuary was originated during tertiary and quaternary period (Menon et al., 2000). It is also proven that endemic species and cosmopolitan species occurring in mixohaline areas could develop ‘physiological races’ through evolution (Kinne, 1964; Menon & Nair, 1967). Therefore, estuary must harbour various organisms which are highly adapted to its current hydrological characteristics and hence weakening of food web during monsoon has to be re-examined.

Moreover, marine ecosystems function studies are prone to ecological fallacies due to the highly dynamic nature of system and the limitations in the currently available methodologies (Weisse et al., 2016). In order to avoid this, a passable data analysis of all the ecological components in both spacial and temporal scale is essential. The literature on the hydrobiology of Cochin backwater consists of isolated studies on heterotrophic bacteria, phytoplankton, microzooplankton, and mesozooplankton (Madhupratap, 1987; Jyothibabu et al., 2006; Madhu et al., 2007; Thottathil et al., 2008). But integrated information on various functional components of the plankton food web is absent. Hence it is necessary to check the existing hypothesis in a time series manner which provides a continuous picture of spatial and temporal variation in the food web. Considering this, the present chapter provides a complete analysis of comprehensive seasonal time series data of plankton food web of Cochin backwater. Accordingly, the objectives of the chapter can be outlined as:

➢ To characterize the dynamics and distribution of different functional component of plankton food web of Cochin backwater during two major contrasting seasons – Spring Intermonsoon and Southwest monsoon.
To understand the variation in food web existing in different ecological regions of backwater based on a comprehensive seasonal time series data

3.2. Materials and Methods

3.2.1. Study Area

The Cochin backwater is a complex shallow estuary (average depth 4 m), located parallel to the coastline of India between 9° 30′–10° 10′ N and 76° 15′–76° 25′ E (Fig: 3. 1). It extends around 75 km along the coastline and has two permanent inlets to the Arabian Sea — the southern inlet located at Kochi and the northern at Azhikode. There are seven rivers bringing water to the estuary out of which the major ones are Periyar and Muvatupuzha.

3.2.2. Sampling strategy

Three hourly time series sampling was conducted in four locations in the Cochin backwater during the spring intermonsoon (March 2009) and the southwest monsoon (September 2009) periods. Out of four sampling locations along the salinity gradients in the Cochin backwater (L1 to L4), two locations each represented the downstream (L1- Azhikode and L2- Kochi) and the upstream (L3- Arookkutty and L4- Thanneermukkom) regions (Fig: 3.1). During the seasonal sampling, field measurements began at 0900 hours and ended at 0900 hours the next day (24 h). Water samples for various environmental and biological parameters were collected every three hours from the surface waters (0.5 m) using a Niskin sampler.

3.2.3. Physico-chemical parameters

Tide in all the four time series locations was measured using tide gauges, and readings were taken at every 10 min. Surface salinity was measured using a digital salinometer (Make TSK). Dissolved oxygen was estimated by the Winkler’s method. Dissolved inorganic nutrients nitrate (NO₃), phosphate (PO₄), and silicate (SiO₄) were measured following standard colorimetric techniques (Grasshoff et al., 1983).
**3.2.4. Biological parameters**

**Picoplankton**

Water samples (10 ml) were preserved in glutaraldehyde and processed for estimating picoplankton. Samples prefILTERED through 3-μm sterile glass filters, to remove larger particles, were used to quantify autotrophic and heterotrophic picoplankton (Porter & Feig, 1980). The heterotrophic picoplankton (HPP) or heterotrophic bacteria sample was stained with 4’6-diamidino-2-phenylindole (DAPI) whereas autotrophic picoplankton (APP) samples were processed without any staining.

The samples (2ml) for autotrophic and heterotrophic picoplankton were separately passed through 0.2 μm black nucleopore filters and mounted in immersion oil. The slides were examined under an Olympus BX 53 epifluorescence microscope equipped with an image analyzer (progRes Capture Pro 2.6) under UV excitation for DAPI and blue excitation for phototrophic components. The microscopic analysis was carried out as soon as the slide was prepared and, in any case, not later than a few hours.
of its preparation (Bloem et al., 1986). This approach ensured the preservation of autofluorescence of photosynthetic pigments in the samples. The carbon biomass of autotrophic and heterotrophic picoplankton was estimated based on the conversion factors presented by Garrison et al. (2000).

**Nanoplankton**

Water samples (15ml) preserved in glutaraldehyde were very gently prescreened through 20 μm bolting silk to discard particles >20μm size. The filtrate was stained with 1.65 μg ml⁻¹ proflavin hemisulfate and filtered through 0.8 μm pore sized Nucleopore filter (Haas, 1982). This filter is mounted in immersion oil and analysed under epifluorescence microscope not later than a few hours of its preparation (Bloem et al., 1986). All organisms between 2 and 20 μm body sizes that fluoresced green under blue illumination were considered as heterotrophic nanoplankton (HNP) or heterotrophic nanoflagellates. The phototrophs were separated from the heterotrophs by the presence of red or red-orange auto fluorescence of photosynthetic pigments. The counts of heterotrophic (HNP) and autotrophic nanoplankton (ANP) were taken using the uys0image analyzer. The carbon biomass of these components was measured based on their body dimensions using the image analyzer and their biovolume calculated by assuming appropriate geometrical shapes (Garrison et al., 2000). The mean biovolume was extrapolated to the total counts at each location to obtain the total biovolume of the nanoplankton fraction. The conversion of biovolume to organic carbon was carried out based on the numerical conversion factors of Garrison et al. (2000).

**Microzooplankton and mesozooplankton**

Water samples (1L) for microzooplankton were gently pre-filtered through a 200μm bolting silk, preserved in acid Lugol’s and stored in black polythene bottles. After 48 h of gravity settling, the water sample was concentrated to ~100 ml and again allowed to settle under gravity in a settling chamber for 48 h. The settled samples were observed under an inverted microscope with an image analyzer (Olympus IX 51). The microzooplankton community was broadly grouped into ciliates, heterotrophic dinoflagellates, and crustacean larvae. Ciliates and heterotrophic dinoflagellates were identified up to the species level based on available literature (Kofoid & Canmpbell, 1939; Subrahmanyan, 1971; Maeda, 1986; Krishnamurty et al., 1995). The mesozooplankton was collected using a working party net (mesh size 200 μm, mouth
area 0.28 m\(^2\)). The net was towed horizontally just below the water surface for 10 min. A digital flow meter (Hydro Bios, model 438110) was attached across the net opening to estimate the amount of water filtered to collect the sample. The mesozooplankton biomass was measured following the standard displacement volume method after removing large detrital particles (Harris et al., 2000). The displacement volume of zooplankton was converted into dry weight using a factor of 0.075 g dry wt.ml\(^{-1}\) and then to carbon biomass following the standard conversion factor of Madhupratap et al. (1981).

3.2.5. Statistical treatments

Analysis of variance

Standard statistical treatments were used to analyse the significance of tidal as well as seasonal variation on various hydrographic and biological parameters. First, the environmental and biological data were tested for their normal distribution and homogeneity. For data with the normal distribution, parametric analysis of variance (ANOVA) with Tukey’s HSD post hoc test was used to compare the significance. In the case of data with clumped distribution, nonparametric ANOVA (Kruskal-Wallis) with Dunn’s post hoc test analysed the significance of differences. The tests of normality, parametric and nonparametric ANOVA were carried out in XL stat pro-software package.

Cluster/SIMPROF and NMDS

Cluster/SIMPROF and NMDS were used to segregate the observations of different parameters into clusters based on their similarity/homogeneity. The data or observation in one cluster indicates their similarity or homogeneity whereas, their placement in different clusters shows the dissimilarity or heterogeneity. The data of plankton food web components were initially standardized and log (X+1) transformed to normalize the differences in numerical abundance (Clarke & Warwick, 2001). The Euclidean distance matrix based on group average method was used to understand the spatial grouping of observations during different seasons.

Dominant species index

The dominant species index is used to find out the most common and numerically abundant species in each group of observations or locations. Dominant species of ciliates and heterotrophic dinoflagellates in each location during the spring
intermonsoon and southwest monsoon were calculated using the standard equation (Yang et al., 1999; Lee et al., 2009; Lin et al., 2011).

\[ \text{Yi} = (\frac{N_i}{N}) \times f_i \]

Where Yi is the dominance of species i, Ni is the number of individuals of species i in all locations, N is the number of individuals of all species in all locations, and fi is the frequency of locations at which species i occurs. The species with Yi value ≥0.02 were considered as dominant species.

**Redundancy analysis**

The interrelationships between the plankton components and their environmental variables were analyzed by redundancy analysis (RDA) models (CANOCO 4.5). Initially, the data was analyzed using detrended correspondence analysis (DCA) to select the appropriate ordination technique. The result of DCA showed axis gradient length <2, suggesting that linear multivariate RDA was suitable for the present case (Birks, 1998; Leps and Smilauer, 2003) with species correlation scaling as ordination scores. The biological variables were log transformed prior to the analysis. Partial RDA was also carried out to find out the environmental parameters contributing more to the explained variation in the biological components. The ordination significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) (p<0.05). The results of the RDA are presented in the form of triplots in which the time series samples are displayed by points and environmental variables by arrows. Arrows for species abundance and environmental variables indicate the direction in which the corresponding parameters increase (Leps & Smilauer, 2003).

**3.3. Results**

**3.3.1. Hydrography- Spring Intermonsoon**

The changes in tidal phase and salinity distribution in the Cochin backwater (L1–L4) during the spring intermonsoon period have been presented in Fig: 3.2. The average tidal height in the inlet region was 0.7 m, which decreased toward the upstream (0.5 m). The tidal rhythm was distinct in salinity distribution, more prominently downstream; euhaline waters dominated in the downstream sites and mesohaline waters upstream (Fig: 3.2a). The highest and lowest salinity values were recorded at L1 downstream (av. 29.15 ± 2.78) and L4 upstream (av. 9.94 ± 0.02), respectively (Table: 3.1). In all locations, the highest/lowest salinity coincided with the highest/lowest tidal
amplitude. The tidal phase in the upstream sites showed a time lag from that in the downstream sites, and so was the salt intrusion. Salinity showed minor tidal variation in all the sampling locations, but significant spatial variation was observed between the upstream and the downstream (Table: 3.1 & Fig: 3.2b). The dissolved oxygen concentration was generally high in the entire study area with higher values in the mesohaline upstream region as compared to the downstream. The dissolved oxygen was the highest at L4 in the upstream region (av. 5.96 ± 0.15 mg l\(^{-1}\)) and the lowest at L1 downstream (av. 3.87 ± 0.18 mg l\(^{-1}\)). The tidal variation of dissolved oxygen was minor in all the study locations (Table: 3.1 & Fig: 3.2c). Nitrate (NO\(_3\)) concentration was remarkably high in the entire study area and showed minor tidal variations except in L1, in the downstream region (Table: 3.1 & Fig: 3.2d). The distribution of PO\(_4\) showed an increasing trend towards the downstream whereas the trend exhibited by SiO\(_4\) concentration was vice versa (Table: 3.1 & Fig: 3.2 e). While the tidal variation in PO\(_4\) was significant only in the downstream locations, SiO\(_4\) variation was significant at L2 and L3 (Table: 3.1 & Fig: 3.2 f). The spatial difference in PO\(_4\) and SiO\(_4\) was significant between the upstream and the downstream (Table: 3.1). Overall trend in the distribution of physicochemical parameters showed significant spatial variations between the downstream and the upstream (Fig: 3.2 a & b.).

Table: 3.1. Spatial distribution of environmental parameters related to tide (ANOVA) during spring intermonsoon. Mean and coefficient of variations (in parentheses) are presented (*P<0.05, significant tidal variation)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Spring Intermonsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
</tr>
<tr>
<td>Salinity</td>
<td>29.15 (0.10)</td>
</tr>
<tr>
<td>DO (mg l(^{-1}))</td>
<td>3.87 (0.15)</td>
</tr>
<tr>
<td>NO(_3) (μM)</td>
<td>16.93 (0.68) *</td>
</tr>
<tr>
<td>PO(_4) (μM)</td>
<td>1.60 (0.59) *</td>
</tr>
<tr>
<td>SiO(_4) (μM)</td>
<td>17.70 (0.39)</td>
</tr>
</tbody>
</table>
Fig: 3.2. Distribution of physicochemical variables in the Cochin backwaters during spring intermonsoon (a) The variation in tidal height during the observation; (b) the salinity variation with respect to the tidal phase. The salinity distribution is set as the background in subsequent panels with white contour lines representing (c) dissolved oxygen (DO), (d) nitrate (NO$_3$), (e) phosphate (PO$_4$), and (f) silicate (SiO$_4$).

3.3.2. Hydrography – Southwest Monsoon

The Cochin backwater was heavily influenced by freshwater, which caused low tidal amplitude in the upstream region (Fig: 3. 3a). The tidal height was noticeably low in the downstream regions (0.5 m), which decreased further toward the upstream (0.2 m). The large freshwater influx led to a drastic drop in salinity and an increase in the duration of low tide. The average surface salinity was significantly low in the entire stretch of the study area (0.10 to 8.66 ppt). Relatively high saline/mesohaline conditions
(av. 8.62 – 8.66 ppt) were found downstream while extremely low saline conditions (av. 0.1 – 2.11 ppt) were encountered upstream (Table: 3.2). Due to high advection of freshwater from the upstream, there was a phase lag in tidal propagation and the salinity intrusion in this area was also very weak (Fig: 3.3b). Even though the tidal variation in salinity was between oligohaline to mesohaline ranges, these variations were large in all the locations except L4 (Table: 3.2 & Fig: 3.3b). Similarly, the spatial variation in salinity was significant between all the locations except L1 and L2 in the downstream and L3 and L4 in the upstream area (Table: 3.2 & Fig: 3.3b). The dissolved oxygen concentration was generally high in the entire study area with the highest at L4 and the lowest at downstream (Table: 3.2 & Fig: 3.3c). The tidal variation of dissolved oxygen during the study period was infinitesimal in all the study locations (Table: 3.2 & Fig: 3.3d). The NO$_3$ concentration was generally high in the study area. The highest and lowest values of NO$_3$ were found in L1 and L3, respectively (Table: 3.2). The tidal fluctuation of NO$_3$ was small in all locations, whereas spatial variation in its distribution was significant (Table: 3.2 & Fig: 3.3d). The distribution of PO$_4$ and SiO$_4$ also showed a clear spatial trend; the former increased downstream and the latter upstream (Fig: 3.3e & 3.3e). The tidal variation of PO$_4$ and SiO$_4$ in the study area was small in all locations (Table: 3.2). The overall trend in distribution of all physicochemical parameters except salinity showed low tidal variations. On the other hand, there was a more prominent spatial variation in hydrographic parameters between the downstream and the upstream (Table: 3.2).

**Table: 3.2. Spatial distribution of environmental parameters at various stations according to tide (ANOVA) during southwest monsoon. Mean and coefficient of variations (in parentheses) are presented (*P<0.05, significant tidal variation)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Southwest Monsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
</tr>
<tr>
<td>Salinity</td>
<td>8.62 (0.60) *</td>
</tr>
<tr>
<td>DO (mg l$^{-1}$)</td>
<td>4.23 (0.30)</td>
</tr>
<tr>
<td>NO$_3$ (μM)</td>
<td>31.30 (0.08)</td>
</tr>
<tr>
<td>PO$_4$ (μM)</td>
<td>1.40 (0.40)</td>
</tr>
<tr>
<td>SiO$_4$ (μM)</td>
<td>42.79 (0.07)</td>
</tr>
</tbody>
</table>
Fig. 3.3: Distribution of physicochemical variables in the Cochin backwaters during southwest monsoon. (a) The variation in tidal height during the observation; (b) the salinity variation with respect to the tidal phase. The salinity distribution is set as the background in subsequent panels with white contour lines representing (c) dissolved oxygen (DO), (d) nitrate (NO$_3$), (e) phosphate (PO$_4$), and (f) silicate (SiO$_4$).
Fig: 3.4. (a) Schematic diagram of the plankton food web in the Cochin backwaters. The subsequent panels represent the photomicrographs of (b) APP - autotrophic picoplankton (c), HPP - heterotrophic picoplankton (d), ANP - autotrophic nanoplankton (e), HNP - heterotrophic nanoplanckton (f), MZP - microzooplankton, and (g) MSP - mesozooplankton/copepods. The abbreviations AMP represent autotrophic Microplankton and DOC represents dissolved organic carbon.
3.3.3. Biological parameters

The Plankton Food web

A schematic picture of a typical plankton food web in an estuarine system is presented in Fig: 3. 4a. The subsequent panel presents the photomicrographs of the plankton components quantified during the present study (Fig: 3. 4b – g). The distribution of various plankton components in relation to the salinity ingress and egress associated with tidal action during spring intermonsoon and southwest monsoon has also been presented in Fig: 3.5 and Fig: 3.6. Detailed information on the abundance and distribution of each plankton components in the temporal and spatial environmental settings in the study area is presented in the following sections.

Picoplankton

The tidal and spatial variation of autotrophic picoplankton and heterotrophic picoplankton during spring intermonsoon is presented in Table: 3.3 & Table: 3.4. Similarly, the abundance of autotrophic and heterotrophic picoplankton in relation to changes in salinity during the spring intermonsoon is presented in Fig: 5a & b. The abundance of autotrophic picoplankton was higher upstream as compared to the downstream. The autotrophic picoplankton abundance (Table: 3.3) and biomass (Table: 3.4) were the highest at L4 (av. 3.46×10⁷ l⁻¹ and av. 8.65 mg C m⁻³) and the lowest at L1 (av. 1.80 × 10⁷ l⁻¹ and av. 4.5 mg C m⁻³). In all study locations, autotrophic picoplankton showed only low tidal variation (Table: 3.3) whereas, their spatial variation was significant between the downstream and the upstream locations (Table: 3.3). The abundance (Table: 3.3) and biomass (Table: 3.4) of heterotrophic picoplankton showed relatively high values in the downstream sites. The heterotrophic picoplankton abundance (Table: 3.3) and biomass (Table: 3.4) were the highest at L1 (av. 2.20 × 10⁹ l⁻¹ and av. 24.2 mg C m⁻³) and the lowest at L4 (av. 1.53×10⁹ l⁻¹ and av. 16.8 mg C m⁻³). The tidal variation in the abundance of heterotrophic picoplankton was significant only in the downstream locations (Table: 3.3). The spatial variation of heterotrophic picoplankton was significant between the downstream and the upstream sampling sites (Table: 3.3).

During the southwest monsoon, the abundance and biomass of autotrophic picoplankton were higher downstream as compared to the upstream (Table: 3.3). The
abundance of autotrophic picoplankton and heterotrophic picoplankton in relation to changes in salinity during the southwest monsoon is presented in Fig: 6a & b. The abundance (Table: 3.3) and biomass (Table: 3.4) of autotrophic picoplankton were the highest at L2 (av.1.52 \times 10^7 \text{ l}^{-1} \text{ and av. 0.25 mg C m}^{-3}) and the lowest at L4 (av. 0.2 \times 10^7 \text{ l}^{-1} \text{ and av. 0.1 mg C m}^{-3}). The heterotrophic picoplankton abundance (Table: 3.3) and biomass (Table: 3.4) during the southwest monsoon was noticeably higher downstream as compared to upstream. The highest heterotrophic picoplankton abundance and biomass were found at L2 (av. 1.26 \times 10^9 \text{ l}^{-1} \text{ and av. 13.9 mg C m}^{-3}) and the lowest at L4 (av. 0.80 \times 10^9 \text{ l}^{-1} \text{ and av. 7.92 mg C m}^{-3}). The tidal variation in autotrophic picoplankton and heterotrophic picoplankton was found to be minor in all locations during the southwest monsoon, whereas, their spatial variation was significant between the upstream and the downstream locations (Table: 3.3 & Table: 3.4).

Table: 3.3. Seasonal and spatial distribution of biological parameters (ANOVA). Mean and coefficient of variations (in parentheses) are presented (*P<0.05, significant tidal variation)[APP - autotrophic picoplankton, HPP- heterotrophic picoplankton, ANP- autotrophic nanoplankton, HNP - heterotrophic nanoplankton, MZP - microzooplankton]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Spring Intermonsoon</th>
<th>Southwest Monsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
<td>L2</td>
</tr>
<tr>
<td>Numerical abundance (No. L^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APP (\times 10^7)</td>
<td>1.80 (0.59)*</td>
<td>2.46 (0.34)</td>
</tr>
<tr>
<td>HPP (\times 10^9)</td>
<td>2.20 (0.64)*</td>
<td>2.14 (0.61)*</td>
</tr>
<tr>
<td>ANP(\times 10^7)</td>
<td>0.76 (0.33)</td>
<td>2.40 (0.66)*</td>
</tr>
<tr>
<td>HNP(\times 10^6)</td>
<td>1.64 (0.46)</td>
<td>1.26 (1.03)*</td>
</tr>
<tr>
<td>MZP(\times 10^6)</td>
<td>1.11 (0.29)</td>
<td>1.93 (0.38)</td>
</tr>
</tbody>
</table>
Table: 3.4. Seasonal and spatial distribution of biomass (ANOVA). Mean and coefficient of variations (in parentheses) are presented (*P<0.05, significant tidal variation)[APP - autotrophic picoplankton, HPP- heterotrophic picoplankton, ANP- autotrophic nanoplankton, HNP - heterotrophic nanoplankton, MZP – microzooplankton, MSP- mesozooplankton]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Spring Intermonsoon</th>
<th>Southwest Monsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (mg Cm$^{-3}$)</td>
<td></td>
</tr>
<tr>
<td>APP</td>
<td>4.50 (0.59)*</td>
<td>6.15 (0.34)</td>
</tr>
<tr>
<td>HPP</td>
<td>24.20 (0.64)*</td>
<td>23.54 (0.5)*</td>
</tr>
<tr>
<td>ANP</td>
<td>162.49 (0.33)</td>
<td>513.12 (0.66)*</td>
</tr>
<tr>
<td>HNP</td>
<td>10.12 (0.46)</td>
<td>7.77 (1.03)*</td>
</tr>
<tr>
<td>MZP</td>
<td>121.41 (0.29)</td>
<td>211.1 (0.38)</td>
</tr>
<tr>
<td>MSP</td>
<td>4.90 (1.12)*</td>
<td>2.12 (1.18)*</td>
</tr>
</tbody>
</table>

Fig: 3.5. Spatial distribution of functional components in the plankton food web during spring intermonsoon. The salinity distributions set as the background in all panels with white circles representing the distribution of (a) APP autotrophic picoplankton ($\times 10^7$ no. $\Gamma^{-1}$) (b) HPP heterotrophic picoplankton ($\times 10^8$ no. $\Gamma^{-1}$).
Fig: 3.6. Spatial distribution of functional components in the plankton food web during southwest monsoon. The salinity distributions set as the background in all panels with white circles representing the distribution of (a) APP autotrophic picoplankton \((\times10^7 \text{ no. l}^{-1})\) (b) HPP heterotrophic picoplankton \((\times10^9 \text{ no. l}^{-1})\).

Nanoplankton

During the spring intermonsoon, the abundance and biomass of autotrophic nanoplankton showed an increasing trend towards the upstream (Table: 3.3). The abundance of autotrophic nanoplankton and heterotrophic nanoplankton in relation to the changes in salinity during the spring intermonsoon is presented in Fig: 3.7a & 7b. The autotrophic nanoplankton abundance (Table: 3.3) and biomass (Table: 3.4) were the highest in L3 (av. 3.02 \(\times\) \(10^7\) l\(^{-1}\) and av. 645.7 mg C m\(^{-3}\)) while the lowest was observed in L1 (av. 0.76 \(\times\) \(10^7\) l\(^{-1}\) and av. 162.5 mg C m\(^{-3}\)). During the period, the tidal variation of autotrophic nanoplankton was found significant at L2 and L3 (Table: 3.3 & 3.4). The abundance and biomass of heterotrophic nanoplankton were significantly higher in the mesohaline upstream as compared to the downstream sites (Fig: 3.7 b). Their abundance (Table: 3.3) and biomass (Table: 3.4) were the highest in L3 (av. 2.6 \(\times\) \(10^6\) l\(^{-1}\) and av. 16.4 mg C m\(^{-3}\)) and the lowest in L2 (av. 1.26 \(\times\) \(10^6\) l\(^{-1}\) and av. 7.8 mg C m\(^{-3}\)) (Table: 3.3).
During southwest monsoon, the autotrophic nanoplankton distribution was almost irregular when presented in the distribution graph (Fig: 3.8a & Table: 3.3). During the study period, autotrophic nanoplankton showed low tidal variation in all locations (Table: 3.3), but their spatial variation was significant between L1 and the upstream sites (Table: 3.3). The spatial difference in heterotrophic nanoplankton during the southwest monsoon showed noticeably higher values downstream as compared to upstream (Fig: 3.8b). Their abundance (Table: 3.3) and biomass (Table: 3.4) was the highest in L2 (av. $1.25 \times 10^6$ n.l$^{-1}$ and av. 7.1 mg C m$^{-3}$) and the lowest in L3 (av. $0.43 \times 10^6$ n.l$^{-1}$ and av. 2.7 mg C m$^{-3}$). The heterotrophic nanoplankton distribution also showed significant spatial difference between the upstream and downstream regions whereas their tidal variation was significant only upstream (Table 3.3). The abundance of autotrophic and heterotrophic nanoplankton showed prominent seasonal variations upstream, but only minor variations downstream (Table: 3.3).

**Fig: 3.7. Spatial distribution of functional components in the plankton food web during the spring intermonsoon.** The salinity distribution is set as the background in all panels with white circles representing the distribution of (a) ANP - autotrophic nanoplankton ($\times10^7$ no. l$^{-1}$), and (b) HNP - heterotrophic nanoplankton ($\times10^6$ no. l$^{-1}$).

**Fig: 3.8. Spatial distribution of functional components in the plankton food web during southwest monsoon.** The salinity distribution is set as the background in all panels with white circles representing the distribution of (a) ANP autotrophic nanoplankton ($\times10^7$ no. l$^{-1}$), and (b) HNP heterotrophic nanoplankton ($\times10^6$ no. l$^{-1}$).
Microzooplankton and Mesozooplankton

During the spring intermonsoon, microzooplankton abundance and biomass was noticeably higher upstream than downstream (Fig: 3.9 a). The highest and lowest abundance and biomass were recorded in L3 (av. $3.11 \times 10^4$ l$^{-1}$ and av. 340 mg C m$^{-3}$, respectively) and L1 (av. $1.11 \times 10^4$ l$^{-1}$ and av. 121 mg C m$^{-3}$, respectively). The tidal variation in microzooplankton abundance and biomass was minor in all locations (Table: 3.3 & 3.4) whereas, their spatial variation was significant between the upstream and the downstream locations (Table: 3.3 & 3.4). During the southwest monsoon, the abundance and biomass of microzooplankton community was noticeably low in upstream than downstream (Fig: 3.9c). The microzooplankton showed highest abundance and biomass at L1 (av. $1.98 \times 10^4$ l$^{-1}$ and av. 216 mg C m$^{-3}$) while the lowest was observed at L4 (av. $0.48 \times 10^4$ l$^{-1}$ and av. 52.50 mg C m$^{-3}$). The tidal variation in microzooplankton abundance was minor in the entire study area (Table: 3.3), whereas, the spatial variation was significant between the upstream and downstream sites (Table: 3.3 & 3.4). The seasonal variation in abundance of microzooplankton was large in the upstream location but, insignificant in the downstream (Table: 3.3 & 3.4).

High mesozooplankton biomass was found throughout the study area during the spring intermonsoon with an increasing trend toward the upstream (Table: 3.4; Fig: 3.9b). The highest mesozooplankton biomass was recorded at L4 (av. $11.7 \pm 1.3$ mg C m$^{-3}$), followed by L3 (av. $8.02 \pm 0.96$ mg C m$^{-3}$) in the upstream region. The mesozooplankton biomass over 24-h time series sampling showed significant tidal variations in all the locations (Table: 3.4). On the other hand, the spatial variation in mesozooplankton biomass distribution was significant only between the upstream and the downstream sites (Table: 3.4). During the southwest monsoon, the mesozooplankton biomass is significantly lower than that of the spring intermonsoon.
Relatively high mesozooplankton biomass was found in the downstream region during the southwest monsoon as compared to upstream (Fig. 9d); the highest was observed in L1 (av. 0.48 ± 0.5 mg C m\(^{-3}\)) and the lowest in L3 (av. 0.24 ± 0.96 mg C m\(^{-3}\)) (Table: 3.4). The variation in mesozooplankton biomass over 24-h time series sampling showed large fluctuations in the upstream sites while it was small in the case of downstream locations (Table: 3.4). The spatial variation in mesozooplankton biomass was found to be significant only between L1 and the upstream locations (L3 and L4) (Table: 3.4). Large seasonal variation in mesozooplankton biomass was evident in the upstream locations as compared to downstream (Table: 3.4).

**Fig: 3.9. Spatial distribution of Micro and Meso zooplankton.** The salinity distribution is set as the background in all panels with white circles representing the distribution of (a, c) MZP microzooplankton (×10\(^4\) no. l\(^{-1}\)) and (b, d) MSP mesozooplankton biomass (ml 100 m\(^{-3}\)). a&b represents distribution during spring intermonsoon period while c & d represents distribution during southwest monsoon period.

**Segregation of environmental and plankton variables**
The result of NMDS/SIMPROF analyses of hydrographic parameters during the spring intermonsoon is presented in Fig: 3. 10. Based on the spatial distribution of major physicochemical parameters (salinity, nitrate, phosphate, silicate, and dissolved oxygen) during the spring intermonsoon, three minor clusters and two major clusters were identified (Fig. 3. 10a & 3.10b). The minor clusters 1, 2, and 3 sequentially represented the mesohaline, mesohaline-high saline (polyhaline), and high saline (euhaline) waters in various locations during the time series observations. In subsequent panels (Fig: 3.10c–i), the quantitative data of salinity, silicate, and plankton food web components are superimposed on spatially clustered time series observations. The quantitative difference in parameters between the mesohaline upstream and euhaline downstream during the spring intermonsoon are presented in Fig.3.10. It is clear that there was a noticeable increase in the abundance of autotrophic picoplankton, autotrophic nanoplankton, heterotrophic nanoplankton, microzooplankton, and mesozooplankton in the upstream mesohaline regions (L3 and L4) as compared to the downstream euhaline region (L1 and L2).

The spatial distribution of hydrographic parameters measured during the southwest monsoon segregated using NMDS/SIMPROF is depicted in Fig: 3.11. Two major clusters of observations were segregated for the southwest monsoon based on the distribution of physicochemical parameters (Fig. 3.11a, b). The clusters 1 and 2 represented the mesohaline and oligohaline waters, respectively, in various sampling locations during the time series measurements. In the subsequent panels (Fig: 3.11 c – i), the quantitative data of salinity, silicate and plankton food web components are superimposed on spatially clustered time series observations. It was possible in these figures to distinguish the oligohaline upstream and mesohaline downstream regions during the southwest monsoon. The abundance of autotrophic picoplankton, heterotrophic picoplankton, heterotrophic nanoplanlkton, microzooplankton and mesozooplankton was noticeably high in the downstream mesohaline regions as compared to the upstream oligohaline region.
Fig: 3.10. (a) Cluster and (b) NMDS plots presenting the segregation of locations/observations based on the distribution of physicochemical parameters during the spring intermonsoon. The subsequent panels show physicochemical NMDS plots overlaid with the bubbles of (c) salinity, (d) silicate, (e) APP (f) ANP (g) HPP (h) HNP (i) MZP (j) MSP for visualizing their distribution based on spatially assembled observations.
Fig: 3.11. (a) Cluster and (b) NMDS plots presenting the segregation of locations/observations based on the distribution of physicochemical parameters during the southwest monsoon. The subsequent panels show physicochemical NMDS plots overlaid with the bubbles of (c) salinity, (d) silicate, (e) APP, (f) ANP, (g) HPP, (h) HNP, (i) MZP, (j) MSP for visualizing their distribution based on spatially assembled observations.
3.3.4. Interrelationships of environmental parameters and plankton components

Redundancy analysis (RDA) clearly demarcated the spatial difference and dynamics in environmental parameters during the sampling periods and also presented how they influence the food web components (Fig: 3.12). The RDA full model in which salinity, silicate, phosphate, dissolved oxygen, and nitrate were considered as environmental variables showed that they together explained 52.1 and 57.9% of the
variance in plankton components during the spring intermonsoon and the southwest monsoon, respectively. The RDA partial model, with salinity as the foremost variable and silicate and dissolved oxygen as co-variables, showed that the major variable alone could explain 32% of the variance in biological parameters during both seasons. Monte Carlo test showed that all the ordinations attempted in the RDA analyses are significant (F=4.915, P=0.006) in spring intermonsoon and in southwest monsoon (F=5.215, P=0.008). The prevalence of high salinity in the downstream sites was evident in the triplot. During the spring intermonsoon, the downstream was polyhaline (18–30 ppt) or euhaline (>30) whereas the upstream was mesohaline (5–18 ppt). During southwest monsoon, the upstream was limnohaline (<0.5) and oligohaline (0.5–5 ppt) and the downstream mesohaline (5–18 ppt). An inverse relationship between dissolved oxygen and silicate with salinity was evident as they increased with a decrease in salinity during both seasons. Though the overall pattern during both seasons showed an increasing trend in salinity toward downstream, the salinity values during the spring intermonsoon were significantly higher than those observed during the southwest monsoon. During both seasons, the upstream region was characterized by higher silicate and dissolved oxygen associated with the river influx whereas the downstream locations had higher phosphate concentration associated with saline waters intrusion. It is clear in RDA that changes in the salinity gradients make a noticeable difference in the distribution of most of the plankton functional components. During the spring intermonsoon, autotrophic picoplankton, microzooplankton, heterotrophic nanoplankton, and autotrophic nanoplankton increased toward the upstream sites (Fig. 3.12a). On the other hand, during the southwest monsoon, autotrophic picoplankton, heterotrophic nanoplankton, microzooplankton, and mesozooplankton were noticeably high downstream (Fig. 3.12b). Even though the autotrophic nanoplankton density distribution showed an irregular fluctuation, RDA confirmed their high density orientation towards the upstream during southwest monsoon.

Fig: 3.12. RDA triplot showing the distribution and interrelationships of environmental and biological parameters during (a) spring intermonsoon and (b) southwest monsoon. The overlaid attribution contours (pink dotted line and values) represent the spatial distribution of salinity and its relationship with other environmental and biological components. The sampling locations (1–4) and the time series observations in each of these locations are displayed by small red filled circles.
For example, points 1.1–1.9 represent the nine time series observations carried out at location 1. Different plankton functional components and environmental parameters are displayed by arrows; the blue dotted arrows indicate the former, and the black arrows indicate the latter.

3.4. Discussion

3.4.1. Temporal and spatial variations in hydrography

Being a monsoonal estuary, the Cochin backwater is characterized by large seasonal salinity fluctuation caused by the alternating dry (spring intermonsoon) and rainy (southwest monsoon) periods (Madhupratap, 1987; Qasim 2003). The semidiurnal
mixed tides play a dominant role in spatial distribution of salinity in the Cochin backwater during the spring intermonsoon whereas large freshwater influx from the upstream dominates over tidal forcing during the southwest monsoon (Qasim & Gopinathan, 1969; Srinivas et al., 2003). The maximum tidal height in the Cochin backwater observed during the present study was 0.7 m during the spring intermonsoon period, which indicates the low tidal amplitude/microtidal behavior of the system. The time lag in the tidal phase upstream is a general feature of the Cochin backwater due to its vastness, about 50-km stretch from the Kochi inlet to the L4 site upstream (Shivaprasad et al., 2013). During the spring intermonsoon, the river influx into the Cochin backwater becomes the seasonal lowest, which favours active salinity incursion into the system through the inlets (Qasim, 2003; Jyothibabu et al., 2006); this, in turn causes the highest seasonal salinity observed in the Cochin backwater during the spring intermonsoon. The high nutrient concentration observed throughout the Cochin backwater is a typical feature of the system irrespective of seasons (Qasim, 2003; Jyothibabu et al., 2006). The seven rivers that empty into the study area are responsible for the high concentration of silicate whereas several non-point sources also contribute to the elevated nitrate levels (Sankaranarayanan & Qasim, 1969; Saraladevi et al., 1983; Jyothibabu et al., 2006). The phosphate concentration in the Cochin backwater was the seasonal highest during the spring intermonsoon due to high salinity during the period, which aids the desorption of phosphate from the suspended particles (Reddy & Sankaranarayanan, 1972; Martin et al., 2008). During spring intermonsoon, the distribution of physicochemical parameters in most of the study locations showed relatively minor tidal variations, whereas the spatial difference between the locations in the downstream and the upstream was large which point towards a clearcut difference in the ecology of these regions (Fig: 3.2 & Table: 3.1). During the southwest monsoon, due to heavy rainfall, freshwater occupied a major part of the Cochin backwater (Madhupratap, 1987; Jyothibabu et al., 2006). This seasonal physiographic feature of the study region was clear in the present study also. Large seasonal variation in salinity was evident in all the study locations (Fig: 3.3 & Table: 3.2). The enormous freshwater influx during the southwest monsoon caused low tidal amplitude in the Cochin backwater. Due to increased freshwater influx and the resulting low salinity, the concentration of dissolved oxygen in the entire study area was the seasonal highest during the southwest monsoon. The NO\textsubscript{3} concentration was high during the southwest monsoon, contributed both by the river influx and non-point sources (Qasim, 2003;
Jyothibabu et al., 2006). The SiO$_4$ concentration was the seasonal highest during the southwest monsoon assisted by the increased river influx during the period. This caused large seasonal fluctuation in the availability of silicate in the Cochin backwater (Table: 3.2). The overall trend in distribution of physicochemical parameters showed low tidal variations of all parameters except the salinity. On the other hand, the spatial variations in most of the hydrographic parameters between the downstream (L1 and L2) and the upstream (L3 and L4) sites were significant (Table: 3.1 & Table: 3.2). Most of the hydrographic parameters during both seasons showed minor tidal variations as compared to their spatial and seasonal variations. Low tidal variations of the physicochemical parameters in most of the study locations can be attributed to the low tidal amplitude in the system. The study showed that, during the spring intermonsoon, the downstream region was polyhaline (18–30 ppt) and euhaline (>30 ppt), whereas, the upstream area was mesohaline (5–18 ppt). During the southwest monsoon, the upstream was limnohaline (<0.5 ppt) and oligohaline (0.5–5 ppt) and the downstream mesohaline (5–18 ppt). These spatial shifts in salinity regimes during the two seasonal sampling caused changes in the distribution of biological components. The autotrophic picoplankton, heterotrophic nanoplankton, microzooplankton, and mesozooplankton showed a clear seasonal shift from the upstream during the spring intermonsoon to the downstream during the southwest monsoon. This indicates the spatial shift in the abundance of planktonic grazers in the Cochin backwater during the two seasons. Conversely, irrespective of seasons, the autotrophic nanoplankton was higher in the upstream.

3.4.2. Ecology and dynamics of the plankton food web

The present study exhibited that both autotrophic and heterotrophic forms of picoplankton and nanoplankton are abundant in monsoonal estuaries. The trophic interaction in a plankton food web becomes effective when both prey and consumers become abundant and coexists in time and space (Landry & Fagerness, 1988; Garrison et al., 2000; Calbet and Landry, 2004; Landry et al., 2008). While considering the spatial distribution of plankton components in the Cochin backwater during the spring intermonsoon, the upstream mesohaline regions seem to have a more efficient plankton food web as compared to the downstream due to close coupling between plankton consumers and their potential prey (Fig: 3.9 & Fig: 11a). The hydrography of the Cochin backwater changes drastically during the southwest monsoon due to enormous
fresh water influx from rivers that feed the upstream region (Madhupratap, 1987). The present study also emphasizes the drop-in consumer abundance in the upstream locations during the southwest monsoon, which makes the spatial distribution of predator and prey discrete. For example, autotrophic nanoplankton density was higher in upstream in both season but during southwest monsoon the predator population, mesozooplankton was largely concentrated towards the downstream which lead to a weak predator prey interaction which inturn results in a weak linear food chain. It is proven that the major size fraction of primary producers in the linear food chain of Cochin backwater belongs to autotrophic nanoplankton (Kumaran & Rao, 1975; Gopinathan, 1975; Menon et al., 2000; Qasim, 2003; Madhu et al., 2007; Madhu et al., 2010). Therefore, the major reason for the presence of unconsumed carbon in Cochin backwater during southwest monsoon was found to be due to the spatial mismatch in the prey and predator population which was particularly prominent in linear food chain.

But in the case of microbial food web, the abundance of all the plankton components – prey and predator organisms (APP, HPP, HNP & MZP) – showed a clear spatial displacement from upstream to downstream along with the shifting mesohaline region as the season changes from spring intermonsoon to southwest monsoon (Fig 3.12). Thus, it can be assumed that there is a spatial shift in the active microbial food web region from upstream to downstream during southwest monsoon. In spite of the spatial shift, the orientation of both predator and prey organisms in the same ecological region (downstream) showed the presence of an efficient microbial food web in southwest monsoon also. It is noticeable that in the existing studies, the low abundance of prey and predator organisms in southwest monsoon led to the conclusion that reduction in number reduces the efficiency of the food web which results in its weakening. But efficiency of a food web is a combination of different factors like abundance, rate of resource utilization and rate of conversion of utilized resource into biomass. To find out the change in efficiency of the food web of Cochin backwater it is essential to consider how all these factors changes during both seasons. Unfortunately, the available studies which address the growth and grazing rate of lower size fraction was conducted only during spring intermonsoon season due to the assumption that efficiency of microbial food web decreases during southwest monsoon. (Jyothibabu et al., 2006; Madhu et al., 2007; Jyothibabu et al., 2015). In the present observation the close coupling between the predator and prey organisms in the downstream mesohaline
region during southwest monsoon indicated an active microbial food web region in monsoon as well. The other factors like grazing rate and carbon transfer from lower trophic level to the higher trophic level of microbial food web is addressed in the following chapters.

Badylak et al. (2007) indicated that autotrophic picoplankton is the numerically abundant primary producer in Tampa Bay Estuary even though they were not dominated in case of biovolume. Sincy in 2005 identified that Cochin backwater is rich in unicellular cyanobacterial genera. Present observation also shows that autotrophic picoplankton is numerically abundant in Cochin backwater. Sherr and Sherr (1994) showed that heterotrophic nanoplanckton is the dominant grazer of both heterotrophic and autotrophic picoplanckton in the marine environment. In the present observation, the spatial distribution of autotrophic picoplankton and its grazers suggest the efficient utilization of autotrophic picoplankton crop in the backwater irrespective of the season even though the active consumption zone differs (Fig: 9 – 11). Therefore, even when the linear food chain weakens due to the spatial disparity in predator and prey population, microbial food web is able to pump carbon to the higher trophic levels particularly in the mesohaline patches of the estuary during southwest monsoon.

3.5. Conclusion

In agreement with previous studies there was a general reduction in the numerical abundance of all planktonic components during southwest monsoon. Temporal variation of the parameters within the tidal cycle was insignificant. Spatial difference and segregation in plankton food web components except autotrophic nanoplanckton were very clear in the Cochin backwater during both sampling periods. There was a seasonal spatial shift in the mesohaline environment and all the plankton components showed an affinity to mesohaline environment. This indicates a clear spatial shift in the region of active plankton food web (region shows close coupling between plankton consumers and their potential prey) in the Cochin backwater between the seasons which can have applications in designing the seasonal food web models for monsoonal estuaries.

According to the present study the major reason for the presence of unconsumed carbon in Cochin backwater during monsoon could be explained based on the spatial mismatch in the prey and predator population which was particularly prominent in
linear food chain. The higher the autotrophic nanoplankton density (the most abundant primary producer population of Cochin backwater) in upstream despite the orientation of its predator population (MSP) towards the downstream lead to a weak linear food chain in monsoon. There was a spatial shift in the active microbial food web region from upstream to downstream during southwest monsoon as well. Dissimilar to the results of previous works (Jyothibabu et al., 2006; Jyothibabu et al., 2015), in spite of the spatial shift the orientation of both predator and prey organisms in the same ecological region (downstream) showed the presence of an efficient microbial food web in southwest monsoon also. The spatial distribution of autotrophic picoplankton and its grazers (heterotrophic nanoplankton and microzooplankton) suggest the efficient utilization of autotrophic picoplankton crop in the backwater irrespective of the season (even though the active consumption zone differs). The more detailed predator prey interaction with special reference to autotrophic picoplankton is addressed in the following chapter.