

Seasonal variations in abundance, biomass and grazing rates of microzooplankton in a tropical monsoonal estuary

MANGESH GAUNS^{1*}, SUNITA MOCHEMADKAR², SHRIKANT PATIL³, ANIL PRATHIHARY¹, S.W.A. NAQVI¹ AND M. MADHUPRATAP¹

¹ CSIR-National Institute of Oceanography, Dona Paula, Goa 403 004, India

² Directorate of Fisheries, Govt.of Goa, Dayanand Bandokar Marg- Panaji – Goa, India

³ Stazione Zoologica Anton Dohrn Napoli – Italy

*Corresponding author. E-mail:gmangesh@nio.org;

Tel: 91(0)832-2450 217; Fax: 91(0)832- 2450-602.

Seasonal abundance, composition and grazing rates of microzooplankton (20-200 μm) in the Zuari estuary were investigated to evaluate their importance in food web dynamics of a tropical monsoonal estuary. Average abundance of microzooplankton (organisms $\times 10^4 \text{ l}^{-1}$) during the three seasons were 0.44 (southwest monsoon), 1.13 (post-monsoon) and 0.96 (pre-monsoon). Protozoan (ciliates, heterotrophic dinoflagellates and sarcodines) accounted for most (96%) of the microzooplankton community, with micrometazoan (nauplii and copepodid stages of copepods, fish eggs, etc). being far less abundant. Among protozoans, ciliates (loricates and aloricates) were most numerous (69% of the total microzooplankton). Statistically significant ($P < 0.001$) co-variations of microzooplankton with other biological parameters such as chlorophyll *a* and bacterial biomass were observed. Salinity influenced microzooplankton distribution with an optimum range of 15-20.

Microzooplankton formed a large organic carbon pool, accounting for 24-40% of the total carbon in the living matter. Seasonally averaged microzooplankton biomasses were 22.3, 36.1 and 24.6 mmole C m^{-3} , respectively, during the southwest monsoon, post- and pre-monsoon periods, and were largely supported by non-living particulate carbon (detritus) particularly during the non-monsoon seasons.

Experimental studies revealed significant microzooplankton grazing on phytoplankton standing stock mainly (>60%) by the pico- and nano- fraction (<20 μm) for most part of the year. Phytoplankton growth rates (d^{-1}) ranged between 0.69 and 1.24. Microzooplankton grazing was estimated to consume 30-82% of the phytoplankton standing stock, and 58-97% of the daily primary production. Results of the present study highlight the role of the microzooplankton as an important consumer of phytoplankton production.

Keywords: Microzooplankton· bacteria· phytoplankton· mesozooplankton· food web· Zuari estuary

1. Introduction

Microzooplankton, the fauna in 20-200 μm -size range primarily consist of heterotrophic ciliates and dinoflagellates, with a smaller contribution from sarcodines and crustacean larval stages. They play a significant role in carbon flow in marine ecosystems (Gast, 1985; Pierce and Turner, 1992) by consuming 20-100% of the primary production (Riley, 1965; Beers and Stewart, 1970; Heinbokel and Beers 1979; Capriulo and Carpenter, 1983; Frost, 1991; Landry, et al., 1998). Micro zooplankton also directly ingest bacteria (Gast, 1985; Sherr and Sherr, 1987; Reid and Karl, 1990), and thus being an component of in the microbial loop act as trophic intermediaries between the bacterioplankton and larger mesozooplankton grazers (Hass and Webb, 1979; Gifford and Dagg, 1991) and in nutrient regeneration (Goldman et al., 1987; Probyn, 1987).

It is believed that due to the close coupling between microbial and microzooplankton components of the aquatic food webs, less organic carbon leaves the euphotic zone especially where microzooplankton form the mediator route for the uptake of organic carbon thereby influencing biogeochemical cycles (Gauns et al., 2005). Studies from the west coast of India (Madhupratap et al., 1992, 1996; Gauns et al., 1996; Gauns et al. 2005; Jyothibabu et al., 2008; AshaDevi et al., 2010) indicate that microzooplankton community plays a key role in the food web of the region. However, there has not been any study of microzooplankton in any of the estuaries except Cochin backwaters (Jyothibabu et al., 2006) along the west coast of India. Such studies are needed for a comprehensive understanding of their role in tropical estuarine systems. A round-the-year investigation on microzooplankton, along with other biological, chemical and physical parameters, in the Zuari estuary was carried out together with an experimental study for this purpose The present study tests the hypothesis that microzooplankton play a key role in maintaining higher standing stock of carbon in the tropical estuaries possibly by efficiently linking detritus into the food web..

2. Methods

2.1 Study location

The Zuari estuary in Goa is among the major estuaries along the west coast of India (Fig. 1). The southwest monsoon (hereafter referred to as the monsoon) plays a major role influencing the hydrographic characteristics of the Zuari estuary as in other estuaries in the region. The classification of data presented in this paper has been made considering 3 seasons, viz. monsoon (June–September); postmonsoon (October–January) and premonsoon (February - May). Large freshwater influx during the monsoon is a distinguishing feature of the west coast estuaries. In the Zuari estuary, for example, salinity is as low as 0.2 during the monsoon as against its maximum (32.9) recorded

during premonsoon (Qasim, 1979). The water column in the estuary is generally well mixed during most parts of the year, but during the peak monsoon period a strong salt wedge is formed extending about 10-12 kms upstream from the mouth of the estuary (Qasim, 1979). Accordingly, sampling strategy was modified during this period (see below). Further details about this estuary are given in Shetye et al. (2007).

Results of experimental studies conducted in 2006 and 2008 together with data collected monthly for one year (June 1996- May 1997) are used here. Water samples were collected from three stations viz., Z1, Z2 and Z3 situated about 15, 25 and 30 km upstream from the mouth of the estuary (Fig. 1). The mean depths during the high tide were about 8 m at Stn. Z1 and 4 m at Stns. Z2 & Z3. Sampling was restricted to about 1 m (1.5 m during the monsoon) below the sea surface in order to avoid the fresh water lens. All water samples, in duplicate, were obtained using 5-litre Niskin samplers (General Oceanics).

Samples for microzooplankton, mesozooplankton, phytoplankton and bacteria were collected following the JGOFS Protocols (UNESCO, 1994). Samples for chlorophyll *a* and nutrient analyses were kept in an icebox soon after collection and analyzed within 8-10 hr of sampling. Temperature was noted immediately after collection using a thermometer and salinity by ATAGO S/Mill-E refractometer. Particulate organic carbon (POC) and nitrogen (PON) were analyzed by a Perkin Elmer CHN analyzer. Nitrate was estimated colorimetrically using a Skalar analyzer (Grasshoff et al., 1983). The GF/F-filtered samples were analyzed for dissolved organic carbon (DOC) by the high temperature catalytic oxidation using a TOC-5000 analyzer (Shimadzu). Primary production (PP) and bacterial production (BP) rates were not measured during this study; instead, respective data sets on PP and BP were obtained from Devassy and Goes (1989) and De Souza (2002).

2.2. *Microzooplankton (MZP)*

A 5 litre water sample pre-screened through 200 μm mesh was gently siphoned out using a PVC tubing with its cod end fitted with a 20 μm Nitex screen for retaining MZP of $\geq 20 \mu\text{m}$ size. These concentrated samples were preserved in 2% acid Lugol's solution and 1% hexamine buffered formaldehyde. Strontium sulphate solution (2 mg l^{-1}) was added for preserving acantharians. Samples were also fixed separately with fresh chilled glutaraldehyde (0.3% final concentration) to differentiate auto- and heterotrophic forms based on autofluorescence technique (UNESCO, 1994).

Known volume of two replicates of the sample concentrate were then observed under an inverted microscope with phase contrast optics following Paranjape et al. (1985) at 100-400X magnification.

Microzooplankton were identified and assigned to the following five groups: loricate ciliates (tintinnids), aloricate ciliates, heterotrophic dinoflagellates, sarcodines and micrometazoans.

Based on morphology of individual specimens, geometric shapes were assigned to each taxon and biovolumes calculated. These volumes were then converted to carbon biomass through appropriate volume to organic carbon ratios (see below). While computing the biovolume of protozoans, additional mean cell shrinkage due to preservation was accounted for following Stoecker et al. (1994). The calculated biovolume was divided by 0.7 to make-up for 30% cell shrinkage due to preservation.

From lorica volume (LV, μm^3) the body weight carbon of a tintinnid (pg) was calculated using the equation of Verity and Langdon (1984). The cell volume of tintinnid ciliate was assumed to be 50% of lorica volume (Gilron and Lynn, 1989). The carbon biomass was converted from cell volume using a factor of 0.19 pg C μm^{-3} for aloricate ciliates (Putt and Stoecker, 1989) and 0.14 pg C μm^{-3} for dinoflagellates (Lessard, 1991). The carbon content of copepod nauplii was calculated from the body length (BL, μm) (Uye, personal communication, see also Uye et al., 1996). The amount of carbon required for microzooplankton community was then calculated based on gross growth efficiency. Protozoan microzooplankton being dominant forms, a value of 0.4 was used for calculation (Fenchel, 1987).

2.3. *Heterotrophic nanoflagellate (HNF)*

For studying the abundance of heterotrophic nanoflagellates, 50 ml of water sample was fixed in 2% glutaraldehyde. 4'-6-Diamidino-2-phenylindole (DAPI) and proflavin were added to 5–10 ml sub-samples to a final concentration of 5 $\mu\text{g ml}^{-1}$ each, allowed to stain for 5 minutes (Hass, 1982; Booth, 1993) and filtered through 0.8 μm black Nuclepore filters (Sherr and Sherr, 1983; Booth, 1993). Slides were prepared and held at 5°C in a darkened box until taken up for epifluorescence microscopy. Only unbroken well-defined individuals were counted and their biovolumes determined. The cell numbers were converted to carbon (pg C μm^{-3}) using a factor of 0.11 (Edler, 1979).

2.4. *Phytoplankton Cell Counts*

Water samples were fixed with 2% Lugol's iodine and preserved in 3% formaldehyde solution and stored in dark at room temperature until enumeration, which was done within one month of collection. A settling and siphoning procedure was followed to obtain 20-25 ml phytoplankton concentrate from a 250 ml sample. Two replicates of 1 ml each of these concentrates were examined with a stereoscopic binocular microscope at a magnification of 100-200X in a Sedgwick-Rafter plankton counting chamber.

2.5. *Chlorophyll a (Chl a)*

To measure the concentration of chl *a*, duplicate samples (1 l) were filtered through Whatman GF/F (nominal pore size 0.7 μm) under low vacuum. The chl *a* pigments were extracted for 24 hours in 10 ml of 90% acetone (Qualigens AR) in dark in a refrigerator. Samples were brought to room temperature and the fluorescence measured using a precalibrated fluorometer (Turner Designs). Chl *a* concentration was calculated from the fluorescence using appropriate calibration factor. Phytoplankton biomass (as carbon) was calculated by multiplying the chl *a* concentration by 50 (Banse, 1988).

A known volume of water sample (2-5 l) was passed through different pore size filters (200, 60, 20, 10 μm nylon and 0.7 μm GF/F) for the size-fractionated chl *a* analysis following the above procedure.

2.6. *Bacterial Abundance (TDC)*

Water samples (20 ml) were fixed with formaldehyde (2%) and refrigerated in the laboratory. Sub-samples of 2 ml were stained with DAPI and filtered onto black 0.2 μm pore size Nucleopore filters (Porter and Feig, 1980) and slides prepared. Bacteria were enumerated using UV excitation. A minimum of 20 fields were counted for each sample at 1000X in an Olympus BH2 epifluorescence microscope and cell numbers calculated following Parsons et al. (1984). Bacterial cell abundance was converted to carbon biomass using a value of 11 fg C cell⁻¹ (Garrison et al., 2000).

2.7. *Mesozooplankton (ZP)*

The mesozooplankton samples were collected using a Heron-Tranter net (mesh size 200 μm), having a mouth area of 0.25 m². The net was towed horizontally ~1 m below the surface for 5 minutes and the volume of water filtered was estimated with a flowmeter (General Oceanics). Immediately after the retrieval, the ZP samples were placed on an absorbent paper to remove excess water and their volume determined by the displacement method. All samples were fixed in buffered formalin (4% v/v) to examine their composition. Mesozooplankton biomass estimated as displacement volume was converted to dry weight (1 ml displacement volume = 0.075 g dry wt.) and to carbon (34.2% of dry wt.; Madhupratap et. al., 1981; Madhupratap and Haridas, 1990).

Due to large variability, the raw data were log-transformed for normalization and correlation was determined between the stations and seasons using the Microsoft Excel package.

2.8. Microzooplankton grazing

To determine microzooplankton grazing and growth rates of phytoplankton community, dilution experiments were carried out (Landry and Hassett, 1982) at the mouth of the Zuari estuary and in the adjoining Mandovi estuary (Fig. 1) during postmonsoon as micro-grazers attain maxima in this season. Water samples were collected from 1 m depth and pre-screened through 200 μm mesh to exclude mesozooplankton. Half of the water sample was filtered through 0.2 μm filter capsules to prepare dilution series 100%, 75%, 50%, 25% and 10% of the ambient concentration. Triplicates of each series were incubated for 24 hours in 2-litre acid-cleaned polycarbonate bottles. Initial and final subsamples were collected for Chl *a* measurements. Subsamples (1 l) were filtered onto 47 mm GF/F filter papers, extracted with 10 ml of 90% acetone for 24 hours at -20°C and analyzed fluorometrically with a Turner Designs fluorometer. Apparent growth rates were plotted as a function of dilution using the equation:

$$1/t \ln (N_t / N_0) = -g X + K$$

Where N_0 and N_t are the initial and final Chl *a* concentrations. Regression analyses of the data yielded slope and intercept corresponding to microzooplankton grazing rate (g) and instantaneous phytoplankton growth rate (K), respectively. Percent standing stock grazed (P_i) and potential primary production grazed (P_p) per day were calculated using the formulae (James and Hall, 1998):

$$P_i = 1 - e^{-g}$$

$$P_p = (e^k - e^{(k-g)}) / (e^k - 1).$$

3. Results

3.1. Physico-Chemical Parameters

The temperature at the three stations did not show very large variability (Fig. 2A). The highest temperatures was observed in the month of May; which along the stretch of the estuary varied from 33.4 (stn Z1), 34.4 (Stn Z2) and 33.7 $^{\circ}\text{C}$ (Stn Z3). Seasonally, largest variation was observed during premonsoon.

As expected, salinity exhibited large variability from a minimum of 1 at Z3, indicating nearly freshwater conditions, to a maximum of 32 at Z2 and Z1, indicating almost completely marine conditions (Fig. 2B). A general decrease in salinity from the mouth to upper reaches of the estuary was observed during all the three seasons. With the end of the monsoon, salinity increased steadily during postmonsoon and was the highest during premonsoon. During the monsoon and postmonsoon

periods, average salinity at Z1 was almost twice the value at the upstream station Z3. Salinity variations between the Z1 and Z3 stations were minimal during premonsoon.

The nitrate (NO₃-N) concentration at Z1, Z2 and at Z3 stations varied within the ranges 1.30-12.50 μM, 0.85-14.56 μM and 1-11.94 μM, respectively (Fig. 2C). The highest NO₃-N concentrations were recorded during monsoon (June-July) with secondary peaks occurring during October-November (Fig. 2C).

3.2. Biological Parameters

3.2.1. Microzooplankton.

Ciliates [loricates (tintinnids) and aloricate forms] and heterotrophic dinoflagellates were predominant microzooplankton. During the entire study, protozoans dominated the microzooplankton community, accounting for 96% of total counts. Six species, *Dictyocysta seshaiyai*, *Leprotintinnus nordequistii*, *Tintinnopsis beroidea*, *T. gracilis*, *T. uruguensis* and *Tintinnidium incertum* were common among the tintinnids. *Strombidium* spp and *Labeo* spp dominated the aloricate ciliates. *Protoperidinium* spp and *Gymnodium* spp, subjugated heterotrophic dinoflagellates, acantharians, the sarcodines and, nauplii and copepodid stages of copepods constituted the micrometazoans. Average contribution of ciliates [tintinnids (31%) and aloricate (38%)] was more than that of heterotrophic dinoflagellates (27%), sarcodines (1%) and micrometazoans (4%). Tintinnids, micro-metazoans and sarcodines exhibited maxima at stns Z1 and Z2 during monsoon and postmonsoon. The maxima in aloricate ciliates and heterotrophic dinoflagellates occurred at stns Z2 and Z3 during post- and premonsoon. Tintinnid diversity was higher during the non-monsoon periods and at near mouth stations. Optimum salinity for their occurrence was found to be between 15 and 25 (see below).

In the study area as a whole, the microzooplankton density (organisms x 10⁴ l⁻¹) recorded during the monsoon, post- and premonsoon periods varied within the ranges 0.014-1.50 (avg. 0.49 ± 0.55), 0.10-7.57 (avg. 1.13 ± 2.05) and 0.19-2.66 (avg. 1.09 ± 0.76), respectively (Fig. 3A), with an annual average of 0.90 (± 1.15). Spatially, they were higher at stn Z1 during monsoon and postmonsoon and at stn Z3 during premonsoon. During the former two seasons MZP were abundant at stn Z1, and at stn Z3 during the latter period. Their counts (x 10⁴) at the three stations varied from 0.19 to 7.57 at stn. Z1, from 0.014 to 7.57 at stn. Z2, and from 0.060 to 0.86 organisms l⁻¹ at stn. Z3 with averages of 1.23 (±2.02), 0.61 (±0.51) and 0.86 (±0.91), respectively. There were significant differences in microzooplankton abundance between months ($P < 0.001$) as compared to stations ($P > 0.01$).

3.2.2. Chlorophyll *a*

Chl *a* (mg m^{-3}) varied between 0.18 and 12.78 (0.26-8.6 at Z1; 0.36-12.74 at Z2 and 0.18-10.77 at Z3) with higher concentrations found during premonsoon. Peaks were recorded in March at the upstream stations and in April at Z1 (Fig. 3B). Lowest chl *a* was observed in the month of July at all the three stations. Its variation was found to be significant ($P < 0.01$) between the stations except during premonsoon.

3.2.3. Phytoplankton

Analyses of phytoplankton composition showed that diatoms (*Nitzschia*, *Ditylum*, *Thalassiosira* sp) dominated the community. Numerically they were more abundant at the upstream station during all the three seasons. Phytoplankton cell numbers ($\times 10^4$) varied from 0.04 to 5.56 l^{-1} during monsoon, from 0.06 to 6.24 l^{-1} during postmonsoon, and from 0.05 to 4.01 l^{-1} during pre-monsoon season with relatively higher abundance in postmonsoon. Seasonal peaks were recorded in August (monsoon), November (postmonsoon) and April (premonsoon) (Fig. 3C). Unlike the spatial variations, the phytoplankton cell counts showed significant seasonal variations ($P < 0.001$).

3.2.4. Heterotrophic nanoflagellate

The heterotrophic nanoflagellate (HNF) abundance ($\times 10^7$) varied from 0.29 to 11.65 l^{-1} with maxima in premonsoon (Fig. 3D). HNFs were more abundant at the mouth (Z1, 0.65- 11.65 l^{-1}) than at the upstream stations [Z2 (0.25- 9.45 l^{-1}) and Z3 (0.29- 10.51 l^{-1})]. Peaks were recorded in March at stns Z1 and Z2 and in August at stn Z3 (Fig. 3D). Spatial variation was not significant ($P > 0.05$) although cell numbers decreased from stns Z1 to Z3 unlike the highly significant seasonal variation.

3.2.5. Bacterial Abundance

Bacterial cell counts ranged from 0.19 to 4.44×10^9 cells l^{-1} (Fig. 3E) with higher counts during postmonsoon. Cell counts at stn Z2 were slightly higher (avg. 2.04, range: 0.42- $4.44 \times 10^9 \text{ l}^{-1}$) than at stn Z1 (avg. 1.83; range: 0.43-3.59) or stn Z3 (avg. 1.89, range: 0.19-3.7). Seasonal peaks were recorded during June, December and March (Fig. 3E). Statistically, bacterial cell numbers did not show significant spatial variation ($P > 0.05$) as compared to the temporal ($P < 0.01$) change.

3.2.6. Mesozooplankton

Mesozooplankton biomass fluctuated seasonally from 0.03 ml m^{-3} during monsoon to 1.9 ml m^{-3} during premonsoon with peaks in March-April (Fig. 3F). The mesozooplankton community was largely dominated by herbivores during monsoon and carnivores during other seasons. There was

persistence of ctenophores during most parts of the year. Monthly variation in ZP biomass was statistically significant ($P < 0.01$) similar to MZP biomass.

3.2.7. Size fractionated phytoplankton biomass (chl *a*)

Size fractionated chl *a* analysis showed a clear seasonal shift in the autotrophic community composition. The smaller fraction ($< 20 \mu\text{m}$) was dominant ($> 60\%$) throughout the year (Fig. 4).

3.2.8. Carbon biomass

The average standing stock of MZP recorded during the study period was $15.25 \text{ mmole C m}^{-3}$. The postmonsoon season was characterized by the highest average carbon biomass ($21.5 \text{ mmole C m}^{-3}$) followed by premonsoon ($18.7 \text{ mmole C m}^{-3}$) and monsoon ($5.5 \text{ mmole C m}^{-3}$). Among the individual groups, ciliates and dinoflagellates contributed substantially to MZP carbon standing stocks followed by micrometazoans and sarcodines (Table 1). Annually, ciliates (tintinnids and aloricates) accounted for 57-84% of the MZP biomass, whereas heterotrophic dinoflagellates, micrometazoans and sarcodines contributed 11-30, 5-26 and 0-2%, respectively. Within the ciliated protozoans, aloricate ciliates were more abundant (11-68%) than loricate ciliates i.e. tintinnids (15-46%). The average standing stock of aloricate ciliates was two folds higher than that of loricate ciliates. The carbon biomass of tintinnids and heterotrophic dinoflagellates increased with the corresponding increase in salinity from monsoon to premonsoon.

Carbon standing stocks of phytoplankton, bacteria, heterotrophic nanoflagellates ($< 20 \mu\text{m}$) and mesozooplankton were within the ranges 5.64-24.49, 2.85-3.74, 6.64-14.74 and 0.19-0.63 mmole C m^{-3} , respectively. Even though, biomasses of many groups were higher during premonsoon, total POC was higher during the monsoon-postmonsoon period (Table 1).

3.2.9. Microzooplankton grazing

Based on the experimental studies carried out at the mouth of the Zuari estuary, microzooplankton grazing rate was found to be 0.295 d^{-1} and the phytoplankton growth rate was 0.904 d^{-1} . Percent standing stock and potential primary production grazed by microzooplankton were calculated as ~ 34.0 and 57.6 , respectively (Fig. 5). The microzooplankton grazing rate and phytoplankton growth rate were much higher in the Mandovi estuary, 0.527 d^{-1} and 1.24 d^{-1} , respectively. Percent standing stock (69) and potential primary production (97) grazed by microzooplankton were accordingly higher as well. Further, nutrient enrichment experiment carried out close to the mouth of the Zuari estuary also showed high rates of phytoplankton growth (1.6 d^{-1}) with microzooplankton grazing on phytoplankton standing stock being around 84% (Sunita et al., 2013). These observations clearly

show that a large fraction of the autotrophic crop in the estuarine system is mobilized through microzooplankton grazing.

The ciliate (tintinnid) distribution with salinity is depicted in Fig 6A. The number of tintinnid species and occurrence of their swarms were found to be at maximum within the salinity range 15-25. At higher salinity (>25) the number of species and swarm formation also decreased. *Dictyocysta sheshayaii*, *Dictyocysta sp.*, *Tintinnopsis beroidea*, *T. gracilis*, *T. tubulosa*, *T. uruguensis*, *T. ventricosa*, *Tintinnidium incertum*, and *Stenosemella nucula* were recorded in a wide range of salinity. The occurrence of tintinnids was higher at lower chl *a* concentration ($\leq 8 \text{ mg m}^{-3}$, Fig 6B). The highest densities were at ≤ 2 and 6-8 mg m^{-3} of the chl *a* concentration. As many as 36 species were recorded at higher chl *a* and was dominated by *Codonellopsis ecaudata*, *C. shabi*, *Eutintinnus tennus*, *Leprotintinnus nordequistii*, *T. beroidea*, *T. butchii*, *T. dadayaii*, *T. directa*, *T. primitivum*, *T. tocantensis*, *T. tubulosa*, *T. uruguensis* and *Tintinnidium incertum*. On the other hand, distribution of tintinnids and bacterial abundance (Fig 6C) indicated maximum occurrence at bacterial cell concentration of $< 1 \times 10^9 \text{ l}^{-1}$. However, tintinnid species composition was found to be increasing above these concentrations and attained peak at $2-3 \times 10^9 \text{ l}^{-1}$. Similar trend was also observed with the species that formed swarms. At higher bacterial cell abundance ($> 3 \times 10^9 \text{ l}^{-1}$), both species composition and swam formation showed decreasing trend. *Codonellopsis ostenfoidii*, *Dictyocysta sheshayaii*, *Dictyocysta sp.*, *Leprotintinnus nordequistii*, *Stenosemella ventricosa*, *Tintinnopsis dadayaii*, *T. directa*, *T. gracilis*, *T. minuta*, *T. tubulosa*, *T. uruguensis*, *T. climacocyclis* and *Tintinnidium incertum* variations in bacterial cell counts. The distributions of tintinnids and nanoflagellate (Fig. 6D) suggest that the tintinnid occurrence, species composition and swarms formation were highest at a nanoflagellate concentration of $2-4 \times 10^7 \text{ l}^{-1}$ beyond which a decreasing trend was observed. *Codonellopsis ostenfoidii*, *Dictyocysta sheshayaii*, *Dictyocysta sp.*, *Stenosemella ventricosa*, *T. beroidea*, *T. butchii*, *T. amphora*, *T. fimbriata*, *T. gracilis*, *T. minuta*, *T. tubulosa*, *T. uruguensi* and *Tintinnidium incertum* occurred at wider range of nanoflagellate cell abundance.

Relationship of microzooplankton with Chl *a* and bacteria was highly significant ($p < 0.001$) than with HNF ($P > 0.01$) or ZP ($P > 0.05$). Similar level of significance was recorded with salinity ($p < 0.05$) compared to temperature. Microzooplankton abundance also varied significantly with the oxygen content and pH of water ($p < 0.05$).

4. Discussion

The microprotozoans, planktonic protists in the size range of approximately 20-200 μm are known to be a major functional component in pelagic food webs (Azam et al., 1983; Strom et al., 2007;

Gifford et al., 2007). These heterotrophic protists represent an important link of bacterial and microalgal biomass to higher trophic levels (Lee et al., 2007). In some systems such as Apalachicola Bay (Florida, USA), microzooplankton are known to consume on an average 10 times more phytoplankton productivity than the mesozooplankton community (Putland and Iverson, 2007). In the present study, based on experimental studies, potential primary production grazed by this community varied between 58 and 97%. Comparable primary production consumption by microzooplankton has been reported from the western Arabian Sea (Landry et al., 1998).

In the Zuari estuary, varying microzooplankton population with respect to relatively uniform temperature both spatially and seasonally signifies that temperature directly is not responsible for changes in microzooplankton population. Therefore, salinity (see Putland and Iverson, 2007) apart from food supply and predators could be responsible for the observed spatio-temporal changes in abundance. The study region is subjected to heavy rainfall and land runoff during June-September every year, which results in large decrease in salinity but an enrichment of nutrients. The maximum observed $\text{NO}_3\text{-N}$ concentration ($14.6 \mu\text{M}$) observed in the present study is higher than that observed by Devassy (1983) in the lower reaches of Zuari estuary during monsoon ($8 \mu\text{M}$). This in turn at times supports Chl *a* as high as 16 mg m^{-3} (M. Gauns, unpublished). It is understood that salinity variation in estuaries generally controls the species composition and succession of planktonic organisms (Madhupratap and Haridas, 1975). The amplitude of salinity fluctuation observed during this study (1-32) is well in agreement with those reported earlier by Dehadrai (1970) and Qasim and Sen Gupta (1981). Generally, higher abundance of MZP was associated with high salinity, which appears to govern significantly ($P < 0.01$) the MZP abundance and distribution. An optimum salinity range of 15-20 for microzooplankton (tintinnid) occurrence in the Zuari estuary, which occurs during the postmonsoon, results in high microzooplankton population and a decrease in autotrophic picoplankton biomass (Fig. 4) compared to other times of the year.

As pointed out earlier PP and BP data were obtained from earlier studies in the study region. The PP ($\text{mg C m}^{-2} \text{ d}^{-1}$) has been found to vary from 249 to 430 (Bhattathiri et al., 1976; Devassy, 1983, 1989). Devassy (1989) also recorded high surface production ($79 - 134 \text{ mg C m}^{-3} \text{ h}^{-1}$) in the Zuari estuary during post- and premonsoon seasons. Phytoplankton biomass in terms of chl *a* in the present study was in the range of 0.2 to 12.8 mg m^{-3} . Devassy and Goes (1989) also recorded wide fluctuations in chl *a* ($0.22 - 3.7 \text{ mg m}^{-3}$) from November to April and a sharp decline in the monsoon months. Similarly, chl *a* in the range of 0.56 - 11.86 mg m^{-3} was recorded by Bhargava and Dwivedi (1976) in the Mandovi-Zuari estuaries. This is again within the range recorded during the present study, indicating a cyclic variation of phytoplankton biomass in this estuary with peaks during pre-

monsoon when estuary is well mixed and the water is clear. Likewise, considerable variation in phytoplankton cell abundance has been noticed previously ranging from 3600 to 387500 cells l^{-1} (Bhattathiri et al., 1976; Devassy and Goes, 1989). The range of phytoplankton cell numbers observed in the present study (400 to 62000 cells l^{-1}) is comparable to that reported by Devassy and Bhargava (1978). A combination of variability in abundance of grazers (both micro- and mesozooplankton), availability of right type of nutrients and other factors (e.g. clear water column) required for phytoplankton growth could produce such a wide fluctuations in phytoplankton density in the estuary.

In general, microzooplankton (ciliates) are known to exert a key control over bacterial population (Gast, 1985; Sherr and Sherr, 1987; Putland, 2000; Sakka Hlaili, et al., 2008). Ciliate contribution to the total microzooplankton in the study area was quite high (~70%), which may play a significant role in the trophodynamics by effectively linking microbial biomass to higher (secondary/tertiary) trophic levels. Relatively high bacterial counts during post- and pre-monsoon seasons must have been supported by the higher DOC, measured during these periods (Table 1). It is likely that the blooms of *Trichodesmium erythraeum*, which occur every year with a marked periodicity from February to April (Devassy et al., 1978), may provide DOC and promote bacterial growth during this season. Surprisingly, the bacterial counts during postmonsoon were lower than during premonsoon, even though higher DOC pool occurred during the former season. We do not have experimental evidence from the Zuari estuary to support the observed mismatch. However, we feel that the observed lag may be because of the grazing pressure exerted by the predators (flagellates and/or ciliates; see below) or due to the lack of labile DOC for the bacteria to take up. Devassy and Goes (1989) found viable bacterial counts in the range of $0.2\text{-}0.4 \times 10^6 \text{ l}^{-1}$. These counts are lower by an order of magnitude than those reported by Ramaiah and Chandramohan (1992) from Dona Paula Bay (near the mouth of Zuari estuary).

Heterotrophic nanoflagellates (HNFs) form a group that has not been properly investigated in the Zuari estuary so far. They are known to play a very important role in linking bacteria to higher trophic levels (Sanders et al., 1992). For example, in Masan Bay (Korea) about 69% of bacterial production is being grazed by HNF (Lee et al., 2007). By consuming bacterial production and controlling bacterial abundance, HNFs occupy a key niche within microbial food webs and presumably impact strongly the structure and function of bacterial communities and energy fluxes. Some of the tintinnid ciliates like *Favella* sp., *Tintinnopsis lobiancoi* and *T. kofoidii* are known to ingest flagellates (Stoecker et al., 1981). These predator species are commonly found in the Zuari estuary. Thus, investigating mechanisms that regulate abundance of HNFs is important for

understanding bacterioplankton dynamics and the microbial food web. Grazing pressure exerted by ciliates (and environmental parameters like salinity) may play regulatory role on HNFs of the Zuari estuary.

Both micro- and nanoplankton biomasses are grazed upon by mesozooplankton (see review by Pierce and Turner, 1992). Mesozooplankton biomass recorded during the present study was comparable to that reported earlier from the Zuari estuary and also had similar seasonal pattern with lower biomass in monsoon (Goswami and Singbal, 1974, Goswami and Selvakumar, 1977; Selvakumar et al., 1980; Qasim and Sen Gupta, 1981; Padmavati and Goswami, 1996). Reduction of salinity is believed to be the primary factor for lower mesozooplankton biomass during the monsoon. However, in general, copepods dominate the zooplankton community forming as much as 66.2% of the total annual counts followed by decapods larvae (17.2%). Carnivorous forms like hydromedusae, siphonophores, ctenophores and chaetognaths usually occurred in the Zuari estuary during high salinity periods (Padmavati and Goswami, 1996).

4.1. *Microzooplankton and carbon flow in the Zuari trophodynamics*

Data from the present work on microzooplankton, HNFs, bacterial abundance and mesozooplankton and those on primary production (Devassy and Goes, 1989) and bacterial production (De Souza, 2002) are used to evaluate the role of microzooplankton in the food web of the Zuari estuary. Mesozooplankton biomass was separated into three categories viz. (a) herbivores (b) carnivores and (c) omnivores. This was done based on their percentage composition observed by Padmavati and Goswami (1996) in the Zuari estuary. Carnivorous forms such as hydromedusae, siphonophore, ctenophores and chaetognaths usually occur during high temperature and saline period and herbivorous like copepods belonging to genera *Undinula*, *Eucalanus*, *Cosmocalanus*, *Centropages* and *Temora* are found when the water temperature and salinity were low (Goswami and Padmavati, 1996; Padmavati and Goswami, 1996). Herbivorous mesozooplankton (HZ) in the study area are not strictly phytoplankton feeding but could be considered as mixed feeding type as they graze on phytoplankton as well as microzooplankton (Fornemann, 2001).

Large variations in both living carbon (LC= chlorophyll *a* + bacteria + nanoflagellates + microzooplankton + mesozooplankton) and nonliving carbon components [NLC=POC-LC] were observed in the present study. POC was higher during monsoon and postmonsoon seasons (Table 1). The NLC varied from 284-536 mmole C m⁻³. The microzooplankton carbon biomass accounted for 24-40% of living carbon component. Sizable contribution by HNFs (12-38%) is as compared to bacteria (6-13%) and mesozooplankton (~1%) is interesting underlining need for further research on

this group. Bacterial and HNF productions were respectively higher during post- and pre-monsoon seasons. As heterotrophic bacteria can utilize and grow on various kinds of organic matter in the marine ecosystem, in the Zuari estuary too they appear to assimilate organic matter quite efficiently and are useful as food for HNFs and other members of the microzooplankton community, which, in turn, are consumed by several larval stages of mesozooplankton. Seasonal disparity in their occurrences indicates close coupling between these two microbial components ($P < 0.001$; $n = 40$; $r = 0.68$) as observed in the waters of the Arabian Sea (unpublished data).

In order to understand the fate of carbon in the Zuari estuary, carbon requirement to sustain the observed standing stocks of microzooplankton was calculated based on the gross growth efficiency. The highest requirement of microzooplankton was during the postmonsoon period (Table 2). The analysis revealed that even if one assumes that all organic carbon of phytoplankton, HNFs and bacteria is consumed by microzooplankton with a growth efficiency of 0.4 (Fenchel, 1987), these living carbon components are individually insufficient to sustain such a high standing stock of microzooplankton particularly during post- and pre-monsoon seasons. In all probability, POC in discrete, nano-meter sized particles might serve as alternate food for MZP, which can assimilate this particulate carbon source. Verity (1986) found that in the Narragansett Bay tintinnid community growth rates increased when chl *a* and POC increased in the < 10 and or < 5 μm size ranges. In the study area also $> 60\%$ of the phytoplankton biomass (chl *a*) remained in smaller fraction (< 20 μm) for most part the year (Fig. 4), thereby supporting microzooplankton community. A review by Pierce and Turner (1992) also suggests that detritus may be an important for ciliates in both coastal and oceanic system. If one assumes that about 30% of the particulate organic carbon is lost either to the bottom or fluxed out of the estuary (Bhaskar et al., 2000), there is still enough POC in the estuary for exclusive “consumption” by MZP (Table 2). In addition, a considerable amount of the carbon required by microzooplankton might be supplied via the microbial food web, which is fueled by *in situ* dissolved organic carbon pool, which was also found to be high during the high salinity periods (Table 1). Further, a significant part of the food requirements of carnivorous and herbivorous mesozooplankton might be met from microzooplankton particularly during the post- and premonsoon seasons due to the dominance of carnivores as observed in the present study as well as by others (Goswamy and Padmavati, 1996). The best-known predators of ciliates are planktonic copepods. For example, copepods, especially *Acartia* spp has been found to feed selectively on tintinnids even when phytoplankton were abundant (Robertson, 1983, Turner and Graneli, 1991). In the subarctic waters of the Oyashio Current, a few tintinnid species showed large fluctuation in abundance that may be controlled by the copepods (Gomez, 2007). Both of these prey and predators

are preponderant in the study area. The work of Last (1978) suggests that tintinnids are consumed by marine fish larvae. Later, Stoecker and Govoni (1984) confirmed that small size fish larvae of 93 μm -5 mm mostly prefer tintinnids – *Favella* and the dinoflagellate - *Prorocentrum*, whereas larger larvae feed upon copepod nauplii. At times tintinnids form as much as 75% of the diet of certain size classes of fish species (Jenkins, 1987). The gut contents and faeces of a number of invertebrates and fish larvae show that microzooplankton form a significant portion (~31%) of their food (Godhantaraman, 2001).

In conclusion, the results of the present study from the Zuari estuary show that microzooplankton play an important role in the food web of the tropical estuarine systems. The Zuari estuary and probably all similar estuaries along the Indian west coast are largely dominated by smaller autotrophs for most part of the year. The ability of microzooplankton to take-up smaller food particles enables efficient linking of these autotrophs to higher trophic levels. The top-down control over food-webs seems to be dominant for most part of the year in the study area. Further, this study also points that non-living particulate carbon may be important in the nutrition of microzooplankton and overall net heterotrophy.

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Legends to figure

Fig. 1. Map showing the location of stations (filled circles) in Zuari estuary. Open circles indicate sites of experimental studies.

Fig. 2. Monthly variations in (A) temperature ($^{\circ}\text{C}$), (B) salinity (psu), (C) nitrate concentration (μM) of the Zuari estuary [Dotted lines indicate annual average], and (D) vertical distribution of temperature and salinity during representative month of the seasons (Courtesy: Mr. Sundar D., NIO-Goa).

Fig. 3. Monthly variations in abundances of different biological parameters in the Zuari estuary. (A) Microzooplankton, (B) Chlorophyll *a*, (C) Phytoplankton abundance, (D) Het. nanoflagellates, (E) Het. Bacteria, and (F) Mesozooplankton. Dotted lines indicate annual average.

Fig. 4. Average size fractionated phytoplankton biomass (chl *a*, in percentage) in the Zuari estuary during different seasons.

Fig. 5. The grazing effect of microzooplankton on phytoplankton biomass (Chl *a*) in the estuarine system during postmonsoon season [(A) Zuari, (B) Mandovi, and (C-D) nutrient enrichment experiment of the Zuari estuary].

Fig. 6. Distribution of ciliates (tintinnids) in relation to physical [(A) salinity] and other biological [(B) Chl *a*, (C) Het. bacteria and (D) Het. nanoflagellates] parameters in the Zuari estuary.

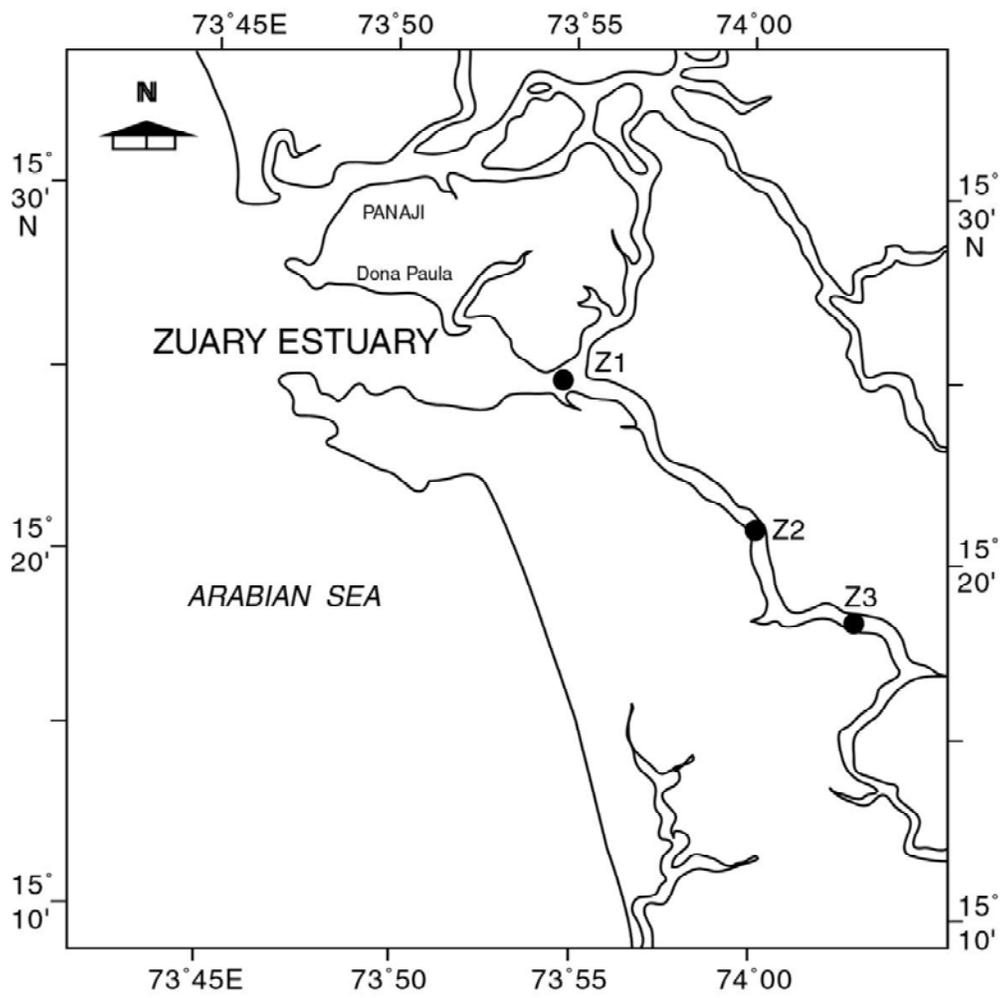


Fig. 1. Gauns Mangesh et al

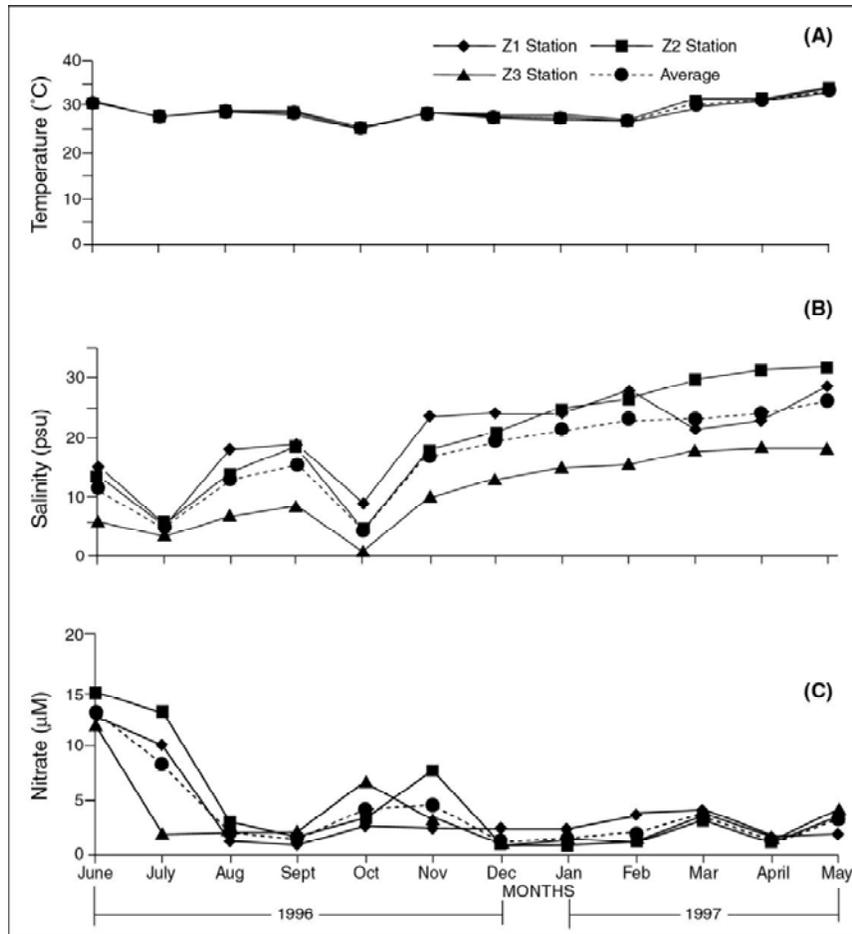


Fig. 2 (A-C). Gauns Mangesh et al.

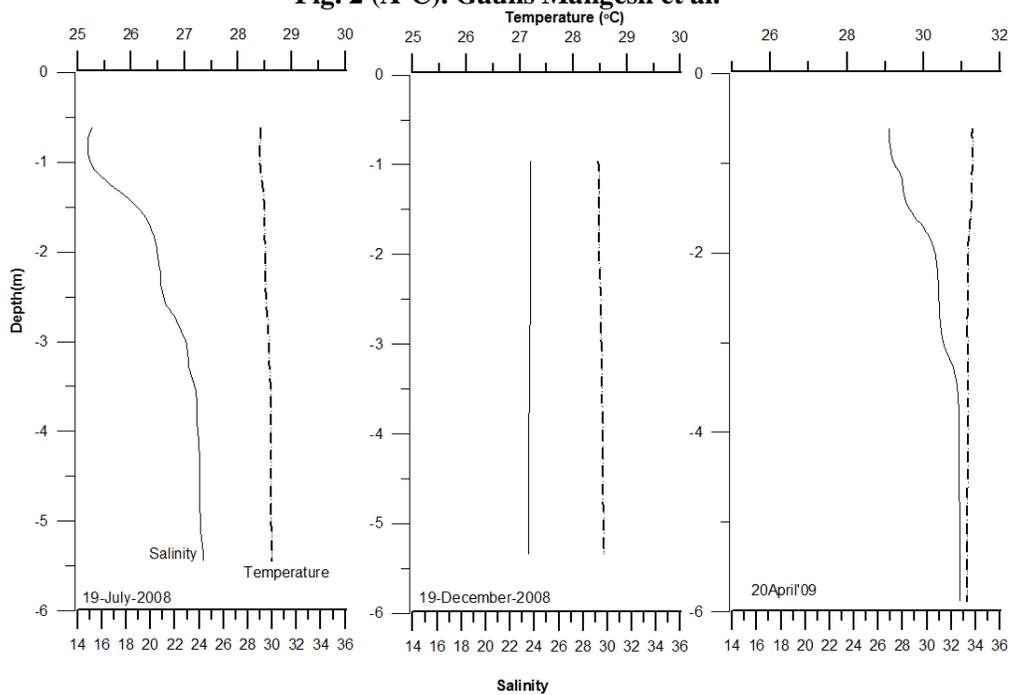


Fig. 2D. Gauns Mangesh et al.

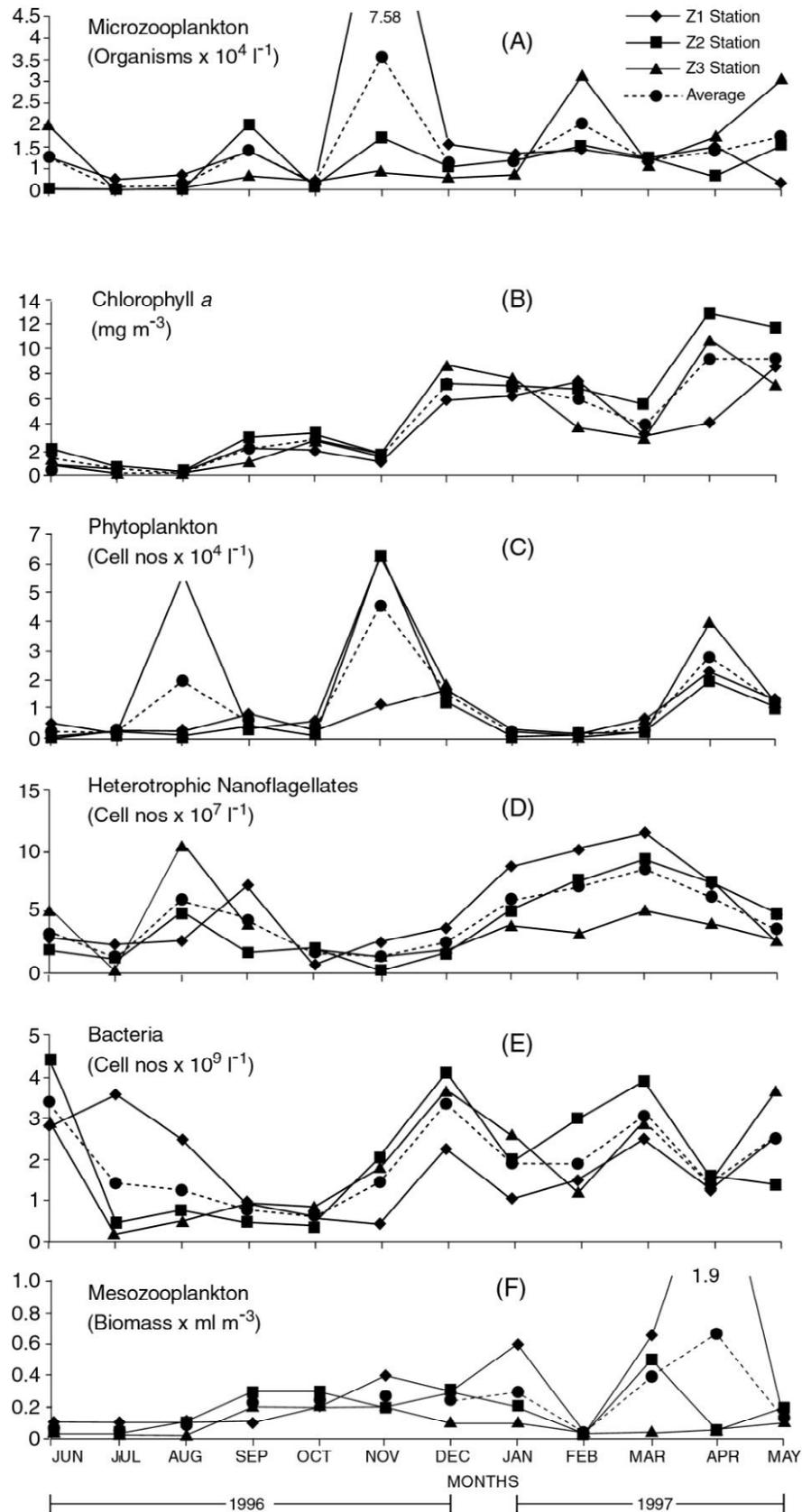


Fig. 3 (A-F). Gauns Mangesh et al.

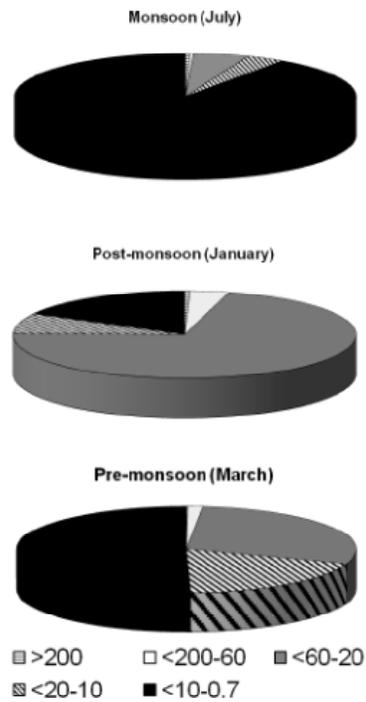


Fig. 4. Gauns Mangesh et al.

Fig. 4. Gauns Mangesh et al.

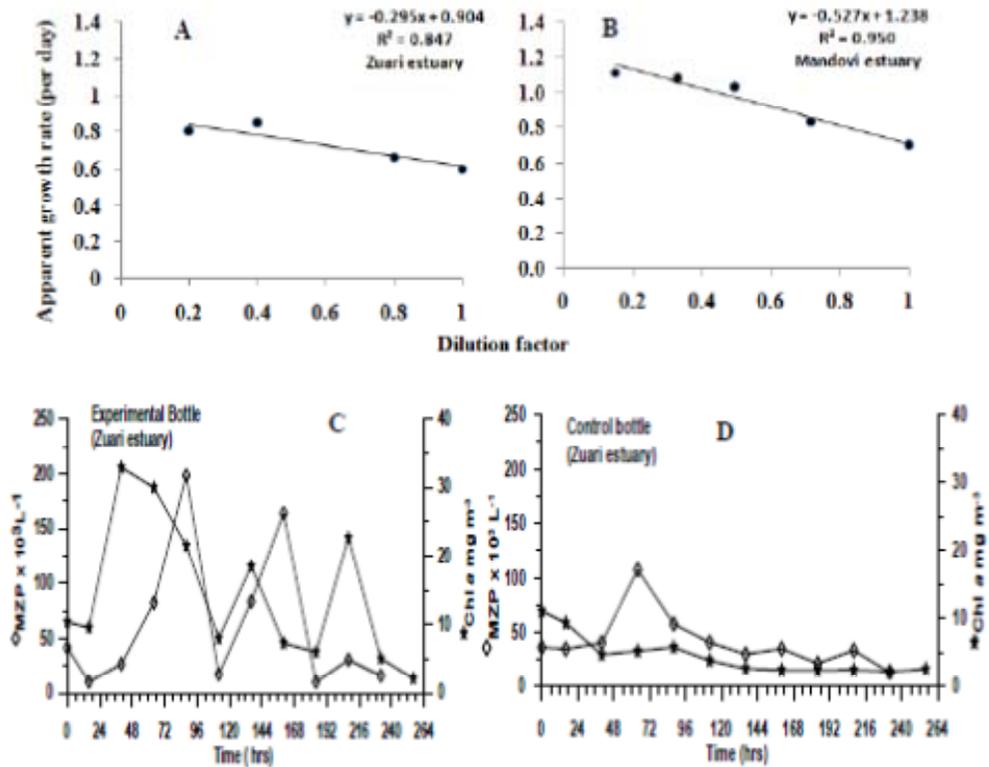


Fig. 5. Gauns Mangesh et al.

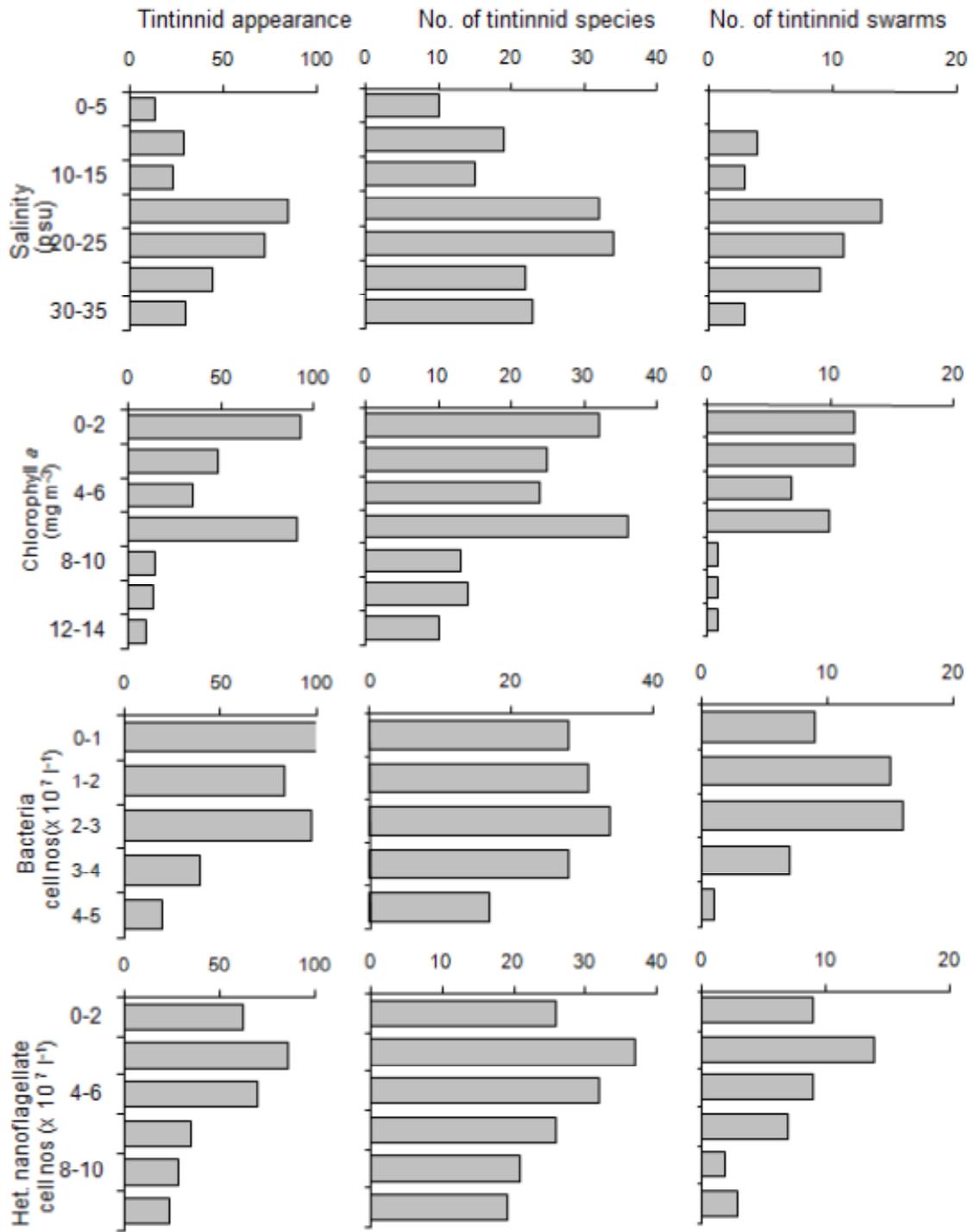


Fig. 6 Gauns Mangesh et al.

Table 1. Seasonal variability in carbon biomass (m mole C m^{-3}) of various biological parameters plus the production rates of bacterial and phytoplankton in the Zuari estuary

	Monsoon	Post monsoon	Pre monsoon
PRODUCTION ($\text{m mole C m}^{-3} \text{ d}^{-1}$)			
Phytoplankton	2.3	6.75	4.58
Bacteria	1.75	3.3	0.35
CARBON POOL (m mole C m^{-3})			
Chlorophyll a	5.64	22.57	24.49
Bacteria	2.85	3.03	3.74
Nanoflagellates (<20μm)	8.57	6.64	14.74
Microzooplankton	21.99	20.33	14.86
Tintinnid ciliates	2.20	2.90	4.30
Aloricate ciliates	0.50	9.49	0.61
Flagellates (>20 μm)	15.74	12.88	5.72
Sarcodines	0.20	0.07	0.00
Micrometazoans	3.35	1.23	3.37
Mesozooplankton	0.19	0.56	0.63
POC	559	556	346
DOC	52.00	93.00	75.00

Table 2. Living & nonliving component of organic carbon (m mole C m^{-3}) in the water column of Zuari estuary.

	Monsoon	Postmonsoon	Premonsoon
Total POC	559	556	346
Non living Carbon component	519.46	487.29	277.77
Living Carbon component	39.54	68.71	68.23
Requirement of living carbon component	83.48	114.65	107.98
Bacteria	5.70	6.06	7.48
Nanoflagellates	21.43	16.60	36.85
Microzooplankton	55.73	90.13	61.55
Mesozooplankton	0.63	1.87	2.10
POC available for flux out of water column (or for other activities)	419.62	385.75	203.42