Seasonal variations in species composition, abundance, biomass and production rate of tintinnids (Ciliata: Protozoa) along the Hooghly (Ganges) River Estuary, India: a multivariate approach

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Received: 6 June 2013 /Accepted: 23 December 2013 \odot Springer Science+Business Media Dordrecht 2014

Abstract The study is the first documentation of seasonal variations in species composition, abundance and diversity of tintinnid (Ciliata: Protozoa), in relation to water quality parameters along the stretch of the Hooghly (Ganges) River Estuary (HRE), eastern coastal part of India. A total of 26 species (22 agglomerated and 4 non-agglomerated) belonging to 8 genera has been identified from 8 study sites where Tintinnopsis (17 species) represented the most dominant genera, contributing up to 65 % of total tintinnid community followed by Tintinnidium (2 species), Leprotintinnus(2 species) and Dadayiella, Favella, Metacylis, Eutintinnus and Helicostomella (each with solitary species). The maximum $(1,666 \text{ ind.} \text{ln}^{-1})$ and minimum $(62 \text{ ind.} \text{ln}^{-1})$ abundance of tintinnids was recorded during post-monsoon and monsoon, respectively. A distinct seasonal dynamics in terms of biomass (0.005–2.465 µg C Γ^{-1}) and daily

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production rate (0.04–3.13 µg C l^{-1} day⁻¹) was also noticed, accounting highest value during pre-monsoon. Chlorophyll a and nitrate were found to be potential causative factors for the seasonal variations of tintinnids as revealed by a stepwise multiple regression model. The result of ANOVA showed a significant variation between species abundance and months $(F=2.36, P\leq 0.05)$. k-dominance curves were plotted to determine the comparison of tintinnid dominance between the investigated stations. Based on a principal component analysis (PCA), three main groups were delineated with tintinnid ciliates and environmental parameters. The changes in lorica morphology in terms of temperature and salinity, recorded for three dominant species, provided information on the ecological characteristics of the species assemblage in this estuarine system.

Keywords Tintinnids Community structure . Biomass and production rate . Seasonal cycle . Hooghly River Estuary

Introduction

The ecological importance of microzooplankton (MZP) in the pelagic food webs of the world's oceans has long been recognized. These organisms often constitute a significant component of the plankton community in many marine environments (Relevante et al. [1985;](#page-15-0) Burkil et al. [1987\)](#page-14-0). Microzooplankton has long been

thought to be a major consumer of smaller particles unavailable to meso- and macrozooplankton (Linley et al. [1983;](#page-15-0) Gifford [1991\)](#page-14-0). Microzooplankton also acts as a significant food source for a variety of invertebrate and vertebrate predators (Fenchel [1987;](#page-14-0) Stoecker and Egloff [1987](#page-15-0); Godhantaraman [2001,](#page-14-0) [2002](#page-14-0); Godhantaraman and Uye [2001\)](#page-14-0) and constitutes an important link in transferring pico- and nanoplankton production to higher trophic levels. The importance of ciliates was initially associated mainly with the microbial loop and corresponding microbial web, but now, there is increasing evidence that these protists are also a crucial part of the herbivorous web, consuming a wide spectrum of particle sizes from bacteria to large diatoms and dinoflagellates as well as other ciliates. As a consequence, in the past decades, much research effort has been devoted to finding the factors affecting ciliate abundance and distribution and their trophic behavior in different environments. Tintinnids are planktonic choreotrich ciliates forming the most distinctive and dominant microzooplankton group, characterized by their possession of a tubular or vase-shaped shell or lorica whose architecture forms the basis of classic taxonomic schemes. Lorica morphology is not only a valuable taxonomic characteristic but has also been linked to ecological characteristics of tintinnids especially in terms of feeding activity. Many reports summarizing tintinnid species revealed some general biogeographic patterns, i.e. taxon association with cold, temperate and warm water masses or identification of cosmopolitan, neritic, boreal, austral or warm water taxa (Souto [1981;](#page-15-0) Pierce and Turner [1994](#page-15-0); Alder [1999](#page-14-0)). Despite the potential ecological importance of MZP, detailed information on their diversity, abundance and biomass in Indian estuaries is still lacking. Therefore, the present investigation was carried out to assess the abundance and diversity of MZP along the Hooghly (Ganges) River Estuary in the context of environmental parameters.

Materials and methods

Study sites

West Bengal. In lower reaches, it is further joined by several tributaries like, Ajoy-Damodar, Rupnarayan and Haldi (Khan [1995\)](#page-14-0). Due to the typical patterns of rainfall being restricted to only about 3 months during a year in the basin, the dry season flow in the "Ganga" and its tributaries is only a fraction of the total annual flow. The HRE is one of the most important tropical estuaries with a complexity of environmental conditions. The area is characterized by shallow waters which are constantly mixed by wind and tidal currents. The tides in the estuary are always strong and variable. The onset and duration of this twice-a-day phenomenon differ considerably from day to day and from season to season (Khan [1995](#page-14-0)). The velocity of the current varies considerably with the state of the tides and the season in this estuarine system. Both the flood and ebb currents go up to 6 knots during high spring tides (Chugh [2009\)](#page-14-0). This highly turbid estuary allows a scarce light penetration due to a great amount of organic and inorganic suspended materials. The mean monthly rainfall showed that more than 74 % of the annual rainfall occurred during monsoon months (mean annual rainfall \sim 1,700 mm). Hence, the climate in the area which is chiefly influenced by the monsoon season is classified as pre-monsoon (March–June), dry season with occasionally higher temperature, monsoon (July–October) accompanied by heavy rainfall and post-monsoon (November–February) characterized by lower temperatures and lower precipitations (Khan [1995](#page-14-0)).

Eight sampling sites, almost equidistant from each other, have been chosen along the stretches of the HRE (covering \sim 140 km) on the basis of different environmental stress, tidal environments, wave energy fluxes and distances from the sea (Bay of Bengal) (Fig. [1](#page-2-0)). The sites can be subdivided into three distinct ecological zones on the basis of salinity regime, namely, freshwater zone [Barrackpore (S_1) , Dakhineswar (S_2) , Babughat (S_3) and Budge Budge (S_4)]; brackish water zone [Nurpur (S_5) and Diamond Harbour (S_6)] and estuarine zone [Lot 8 (S_7) and Gangasagar (S_8)] (as shown in Table [1\)](#page-3-0). These sites have a mean elevation of 45–55 ft belonging to a lower deltaic plain experiencing intense semidiurnal tides and wave action with a mesomacrotidal setting (3–6-m amplitude).

Collection and preservation of samples

The study was conducted on monthly basis along the lower stretches of the HRE during March 2012 to

Fig. 1 Location of eight sampling sites (S_1-S_8) along the Hooghly River Estuary showing three distinct ecological zones as well as the Sundarban mangrove wetland

February 2013. Surface water samples were collected monthly from eight selected sites during high tide in morning hours. Environmental parameters such as water temperature (degrees Celsius) were taken immediately in the field by a Celsius thermometer (0–110 \degree C, mercury), whereas salinity, turbidity, total dissolved solids (TDS) and pH were measured by Water Analyzer 371 (Systronics). Dissolved oxygen (DO), biological oxygen demand (BOD) and inorganic nutrients (nitrate and silicate) were measured following standard methods (Strickland and Parsons [1972\)](#page-15-0). Total phosphorus (dissolved and particulate) was analysed by the persulfate digestion method (as discussed by Gales et al. [1966](#page-14-0)). Fecal coliform was estimated by the most probable number (MPN) technique using an A1 media broth (Vanderzant and Splittstoesser [1992](#page-15-0)). To analyse the chlorophyll a concentration (milligrams per cubic metre),

1,000 ml water was collected and filtered onto glass fiber (Whatman GF/F filter paper). The extract was prepared in 90 % acetone and was kept in a refrigerator for 24 h. Later, chlorophyll *a* concentration was measured spectrophotometrically adopting the procedure of Strickland and Parsons [\(1972\)](#page-15-0). Water Quality Index (WQI) was calculated by an NSF water quality index calculator (Oram [2013\)](#page-15-0). The calculation is based on eight water quality parameters such as temperature, pH, DO, BOD, turbidity, total phosphate, nitrate and fecal coliform.

For tintinnids, 1,000 ml of surface water samples was collected by pre-cleaned plastic bottles and immediately preserved with Lugol's solution (2 % final concentration, v/v) and stored refrigerated in darkness except during transport and settling (Dolan et al. [2002](#page-14-0)).

Table 1 Geographical and ecological description of eight sampling sites (S_1-S_8)

In the laboratory, the water samples were concentrated to a volume of 25 ml by settling in a measuring cylinder of 1,000 ml with two special outlets (Godhantaraman [2002\)](#page-14-0) for 48 h. From the last 25 ml, the sample was taken drop by drop with a micropipette on a glass slide for quantitative and qualitative analysis by a phase contrast microscope at a magnification of×40. Three aliquots of each sample were counted, and the mean value was considered. Abundance was expressed as individuals per litre. Tintinnids were identified on the basis of lorica morphology described by Kofoid and Campbell [\(1929,](#page-14-0) [1939\)](#page-14-0) and Marshall [\(1969\)](#page-15-0). Both the shape and size of the lorica including the oral diameter and the total body length were considered as these parameters vary from species to species. Lorica length and oral diameter were measured, and volumes were calculated by assigning standard geometric configurations. Biomass was calculated as the total bio-volume (lorica volume) of the tintinnid community for each sampling date. Lorica volume (LV, cubic micrometres) was converted to body carbon weight or biomass using the following regression equation: body carbon weight or biomass= 444.5+0.053LV (Verity and Langdon [1984\)](#page-15-0). Production rate $(P, \text{ micrograms of } C \text{ per litre per day})$ was estimated from biomass $(B, \text{ micrograms of } C \text{ per litre})$ and empirically determined specific growth rate (g, per day): P=B×G. Multiple regression=1.52lnT−0.27lnCV−1.44, where T is the temperature (degrees Celsius) and CV is the

cell volume (cubic micrometres) proposed by Müller and Geller [\(1993\)](#page-15-0) for ciliates was used.

Phytoplankton samples were collected by a phytoplankton net with a 20-μm mesh size. The nets were trawled on the surface water for about 20 min, and collected samples were immediately preserved in 4 % buffered formalin and taken to the laboratory for further analyses. An aliquot sample of 1 ml was taken in a Sedgewick-Rafter counting cell for quantitative as well as qualitative analyses (Kellar et al. [1980](#page-14-0)) and examined under a binocular microscope. The identification of phytoplankton was done following standard taxonomic monographs such as diatoms by Desikachary [\(1987\)](#page-14-0), dinoflagellates by Subramanian ([1968](#page-15-0)) and green and blue-green algae (cyanobacteria) by Fristch [\(1935\)](#page-14-0).

Data analyses of samples

The community structure in the context of three indices, namely, species diversity (H') , evenness (E') and richness (R') of tintinnids, was calculated as follows: $H'=-\sum S_i=P_i(\ln P_i)$, $E'=H/\ln(S)$ and $R'=(s-1)/\ln(N)$, where P_i =proportion of the total count arising from the *i*th species, S =total no. of species and N =total no. of individuals (Xu et al. [2008\)](#page-15-0). Data were transformed using the $log_{10} (n+1)$ function to allow the less abundant species to exert same influence on the calculation of similarities (Clarke and Warwick [1994\)](#page-14-0). Correlations

were calculated using Pearson 's correlation coefficient (Sokal and Rohlf [1981](#page-15-0)) to analyse the relationships among all the variables for each site. Hierarchical cluster analysis, one-way ANOVA and multiple stepwise regression analysis were performed to establish the relationships between the biotic and abiotic factors and variation between months and sites using the statistical software Minitab 13. The index of dominance of phytoplankton and tintinnids was done by using the formula $Y_i=(N_i/N)\times f_i$ where $Y_i=$ Index of Dominance, $N_i=$ no. of individual species, N=total no. of all species and f_i =frequency of individual species. Cumulative ranked curves (k-dominance curves), which were obtained by PRIMER 6, were used to compare tintinnid dominance separately from the species number. k-dominance curves were plotted on the cumulative ranking of species in order of importance in terms of abundance. To gain insight into the spatial coincidences in the distribution of tintinnids in relation to environmental variables involved, principal component analysis (PCA) was performed by using the XLSTAT 2013 software.

Results and discussion

Hydrological characteristics

The spatio-temporal distribution of hydrological parameters was observed at all the study sites (as shown in Table 2). The surface water temperature ranged from 16.5 to 33 °C, being the highest in the month of May and the lowest in December. Generally, the temperature is influenced by the intensity of solar radiation, evaporation, freshwater influx and cooling and mix up with ebb and flow from adjoining neritic waters (Prabu et al. [2008](#page-15-0)). Maximum salinity (22.5 p.s.u.) was recorded at Gangasagar (S 8) during summer and minimum (0.13 p.s.u.) at Babughat (S_3) during the monsoon period. The recorded higher values could be attributed to the low amount of rainfall, higher rate of evaporation and also neritic water dominance (Rajasegar [2003](#page-15-0)). Hydrogen ion concentration (pH) in surface waters remained alkaline throughout the study period at all the stations, being maximum (8.3) in monsoon at Gangasagar (S_8) and minimum (7.8) during summer at Barrackpore (S_1) . Turbidity showed a wide range of variation (2 –16 NTU), being lowest during postmonsoon with a gradually increasing trend towards pre-monsoon followed by monsoon. TDS recorded the

at eight study sites Table 2 Annual pooled mean values and standard deviation of hydrological parameters at eight study sites **Table 2.** Annual pooled mean values and standard deviation of hydrological parameters

lowest value (114 mg l^{-1}) during early monsoon and highest $(1,980 \text{ mg } 1^{-1})$ during pre-monsoon months. The dissolved oxygen value was recorded highest $(5.72 \text{ mg } l^{-1})$ during monsoon and lowest $(3.36 \text{ mg } l^{-1})$ during pre-monsoon. The observed high values during monsoon might be due to the cumulative effect of higher wind velocity coupled with heavy rainfall and the resultant freshwater mixing (Das et al. [1997](#page-14-0)). Season-wise observation of DO values showed an inverse relationship against pH concentration. Values of biochemical oxygen demand ranged from 0.58 to 2.66 mg l^{-1} during premonsoon and post-monsoon, respectively. Nitrate concentration showed a wide range $(3.02-28.99 \text{ }\mu\text{mol } \text{ } 1^{-1})$ of seasonal variations during pre-monsoon and early monsoon, respectively. An abrupt increase of silicate concentration (127.76 µmol 1^{-1}) was recorded during pre-monsoon at Diamond Harbour (S_6) . The low silicate concentration (46.77 µmol l^{-1}) in Gangasagar (S₈) could be attributed to its uptake by phytoplankton for their biological activity (Ramakrishnan et al. [1999](#page-15-0)). Total phosphate exhibited a similar trend of distribution during the study period with an average concentration of 0.88 µmol l^{-1} throughout the year. Among the biotic parameters, chlorophyll a (chl a) showed a maximum value (4.44 mg m⁻³) at Gangasagar (S₈) during postmonsoon and a low value (0.93 mg m⁻³) during monsoon at Barrackpore (S_1) , and this could be due to anthropogenic effects as evidenced from its positive correlation with salinity and may also be due to freshwater discharges from the rivers (dilution), causing turbidity and less availability of light (Godhantaraman [2002](#page-14-0)). A relatively high concentration of chl a in Gangasagar (S_8) coincides with a multispecies algal bloom formed by two centric diatoms, i.e. Coscinodiscus radiatus and Chaetoceros lorenzianus, and one pennate diatom Thallassiothrix frauenfeldii during January 2013. Fecal coliform loading was abruptly high (7,898 MPN/100 ml) during monsoon in Lot $8(S_7)$ and low (2,220 MPN/100 ml) during pre-monsoon in Nurpur (S_5) . Values of WQI for the eight sampling sites are in the range of 42–66, indicating bad to medium water quality during the study period. The value was low during monsoon followed by post-monsoon and pre-monsoon as shown in Table 3.

Community composition and distribution of microzooplankton

The microzooplankton community consists of 26 species of tintinnid (8 genera and 6 families) followed by 2

Table 3 Water Quality Index (WQI) of eight sites showing water quality characteristics throughout the year [according to Water Quality Index legend: Excellent (90–100), Good (70–90), Medium (50–70), Bad (25–50), Very bad (0–25); Yisa et al. [2012\]](#page-15-0)

Sites	Water Quality Index (WQI) value	Water quality
S_1	52	Medium
S_2	46	Bad
S_3	42	Bad
S_4	61	Medium
S_5	66	Medium
S_6	44	Bad
S_7	50	Medium
S_8	58	Medium

species of copepod nauplii (*Acartia* sp. and *Oithona* sp.) and solitary species of rotifer (Brachionus sp.). The tintinnid community was dominated by three core species (present in substantial number almost throughout the year) such as Tintinnopsis beroidea, Tintinnidium primitivum and Leprotintinnus simplex followed by 10 seasonal and 13 occasional species. The ciliates were primarily grouped into agglomerated and nonagglomerated forms where the former constituted the major part both qualitatively (22 species) and quantitatively (84 % of total species). Agglomerated forms were dominated by the genera Tintinnopsis (17 species) followed by Tintinnidium (2 species), Leprotintinnus (2 species) and Helicostomella (1 species). Tintinnopsis is predominant due to their more flexible adaptive strategies as endorsed by Aleya [\(1991\)](#page-14-0) and Reynolds [\(1997](#page-15-0)). Another advantageous characteristic of Tintinnopsis to survive in estuarine and coastal waters is the production of resting cysts which typically sink down in shallow waters and occur in the sediments (Krinsic [1987](#page-14-0)), and when appropriate condition arrives, excystment and repopulation occur very rapidly. Nonagglomerated genera include Favella sp., Eutintinnus sp., Metacylis sp. and Dadayiella sp., each comprising single species and occupying 16 % of the total community. Site-specific abundance of tintinnids along with their morphology (lorica length and lorica oral diameter) has been described in Table [4.](#page-7-0) Microphotographs of dominant tintinnid species from each site have been depicted in Fig. [2](#page-8-0).

The persistence of T. beroidea, T. primitivum and L. simplex as the most abundant and ubiquitous species along the HRE reflects their eurythermal (17–33 °C) and euryhaline (0.2–22 p.s.u.) nature, although they reached their highest abundance in the brackish water sites $(S₅$ and $S₆)$. Among those three species, T. primitivum showed its peak individual abundance $(833 \text{ ind.}1^{-1})$ during pre-monsoon at Diamond Harbour (S_6) . The density of two core species, viz., T. beroidea and L. simplex, was as high as 400 and 416 ind.l⁻¹ at Nurpur (S_5) during monsoon and pre-monsoon, respectively. Besides, T. beroidea, the dominant tintinnid in this estuary as recorded in other estuaries (Kamiyama and Tsujino [1996](#page-14-0); Laybourn-Parry et al. [1992;](#page-14-0) Leakey et al. [1993](#page-14-0); Pierce and Turner [1994](#page-15-0)), is considered a picoplanktivorous species. The dominance of this agglutinated species appears to be related to the availability of particles to construct the lorica in addition to the presence of its preferred food (Urrutxurtu et al. [2003\)](#page-15-0).

Distinct differences in tintinnid distribution were observed between freshwater zone, brackish water zone and estuarine zone along the HRE depending on trophic conditions in relation with the environmental variables. Peak density (1,666 ind.l⁻¹) and diversity (H °=1.41) of tintinnids were found to be associated with the high concentration of diatoms $(5,520 \text{ cells } 1^{-1})$, and such coeval presence of these two organisms was reported by Lebour [\(1922](#page-14-0)) from Plymouth River Island, UK. This suggests that the tintinnid might affect phytoplankton abundance with respect to the relationship of prey and predator. In addition, high density and diversity of tintinnid were supported by a high diversity of phytoplankton (diatoms and dinoflagellates) during the postmonsoon period. Their spatial distribution coincided well with that of tintinnids with which they showed a significant and positive correlation ($r=0.865, P\leq 0.001$).

The distribution and abundance of tintinnids varied remarkably due to seasonal environmental fluctuations. An overall scenario of tintinnid abundance for all the eight sites indicates a high value during the postmonsoon season followed by pre-monsoon and monsoon (Fig. [3](#page-9-0)). Higher mean abundance and diversity were encountered at brackish water sites, namely, Nurpur (S_5) and Diamond Harbour (S_6) , respectively, where a distinct lower density was recorded in the high-energyzone Gangasagar (S_8) than the low-energy-zone Lot 8 (S_7) . This might be attributed to the presence of a high concentration of small suspended particles in the water which interfere with the filter-feeding mechanism of the tintinnids (Laybourn-Parry et al. [1992](#page-14-0)). However, four species, namely, Tintinnopsis tentaculata, Tintinnopsis parvula, Tintinnopsis acuminata and Tintinnopsis directa, were exclusively found at estuarine site, Gangasagar (S_8) , and this might be due to the migration of neritic species into the site during high tide (Godhantaraman [1994](#page-14-0)). The existence and dominance of Tintinnopsis bermudensis, Tintinnopsis urnula, Leprotintinnus nordqvisti and Metacylis sp. during premonsoon; Tintinnopsis parva, T. tentaculata, T. parvula, Tintinnopsis turbo and Tintinnidium incertum during monsoon and Tintinnopsis nucula, T. directa, Tintinnopsis nana, Helicostomella sp. and Eutintinnus sp. during post-monsoon season are worth mentioning. It is evident that there is a sharp difference in tintinnid species distribution between high-saline and low-saline zones, as this factor is reported to play the most crucial role for their distribution in estuaries (Godhantaraman [1994](#page-14-0) and Sujatha and Panigrahy [1999](#page-15-0)). An overall species diversity index (H') was found to be high during post-monsoon and low during pre-monsoon. Species richness (R') value showed an almost opposite trend with diversity indices. However, species evenness (E') indicates almost a similar trend throughout the year in all the sites (as shown in Table [5,](#page-9-0) Fig. [4](#page-9-0)). Maximum average species diversity (1.41) and evenness (0.47) were noticed in Diamond Harbour (S_6) , whereas their lowest values were recorded in Barrackpore (S_1) . Distinct differences in tidal effects and wave fluxes might influence the community index values in these two sites.

Naked ciliates showed very low abundance throughout the study, contrasting with the pattern usually described for tropical coastal waters (Pierce and Turner [1994](#page-15-0)). The occurrence of rotifers and some copepod nauplii of Acartia sp. and Oithona sp. was marked at Diamond Harbour (S_6) and Nurpur (S_5) , indicating the preference of these organisms for lower salinity.

Seasonal variation of abundance, biomass and production rate of tintinnid ciliates

Awide range of seasonal variations in tintinnid abundance was observed with maximum value $(\sim 1,666 \text{ ind.} \text{T}^{-1})$ during post-monsoon (January) at Nurpur (S_5) and minimum (~62 ind.l−¹) during monsoon (September) at Diamond Harbour (S_6) . A sharp increase in population density was recorded when surface water temperature and salinity were low, suggesting that mixing had occurred. Such response of tintinnid assemblages to mixing events has been frequently reported for both coastal and offshore waters (Thompson et al. [1999;](#page-15-0) Modigh and Castaldo [2002](#page-15-0)). The lower abundance during monsoon was

Table 4 A detailed account of tintinnid species recorded in the sampling sites

Family	Species	Status	Maximum abundance $(\text{ind.} \mathbb{I}^{-1})$	Site	Month	Lorica length LOD (μm)	(μm)
Codonellidae	Tintinnopsis beroidea	Core	400	Nurpur	August 2012	13.85-63.9	$4.2 - 21.6$
	T. tubulosa	Seasonal	333	D. Harbour	January 2013	14.9-51.97	$4.6 - 16.8$
	T. minuta	Seasonal	500	Lot 8	January 2013	$8.59 - 20.3$	$2.6 - 9.7$
	T. bermudensis	Seasonal	83	Budge Budge	April 2012	34.8	8.8
	T. karajacensis	Occasional	200	Lot 8	April 2012	$24.9 - 40.1$	$7.4 - 10.6$
	T. lohmani	Seasonal	166	Gangasagar	August 2012	$23.1 - 56.32$	$12.9 - 19.2$
	T. lobiancoi	Seasonal	333	Nurpur	January 2013	33.84-61.94	$9.84 - 16.3$
	T. urnula	Occasional 83		Lot 8	June 2012	22.9	5.3
	T. parva	Occasional 83		Nurpur	July 2012	16.8	3.4
	T. tentaculata	Occasional 62		Gangasagar	July 2012	32.88	9.75
	T. parvula	Occasional 50		Gangasagar	July 2012	41.69	18.3
	T. nucula	Occasional 50		Dakhineswar	November 2012	22.24	10.1
	T. turbo	Occasional	20	D. Harbour	September 2012	37.63	14.5
	T. acuminata	Occasional 12		Gangasagar	October 2012	41.1	22.65
	T. directa	Seasonal	75	Gangasagar	February 2013	42.3	21.45
	T. nana	Occasional	25	Nurpur	December 2012	24.56	15.7
	T. gracilis	Seasonal	100	Babughat	March 2012	39.7	9.34
Tintinnidiidae	Tintinnidium incertum	Seasonal	83	D. Harbour	October 2012	31.1	6.3
	T. primitivum	Core	833	D. Harbour	January 2013	$22.5 - 57.33$	$5.3 - 11.10$
	Leprotintinnus simplex Core		416	Nurpur	January 2013	23.58-74.7	$8.75 - 19.3$
	L. nordqvisti	Occasional	75	D. Harbour	May 2012	38.3	9.2
Tintinnidae	Dadayiella ganymedes	Seasonal	83	Nurpur	February 2013	32.4-35.66	$7.1 - 12.09$
	Eutintinnus sp.	Occasional	10	Lot 8	November 2012	53.4	13.8
Metacylididae	Metacylis sp.	Occasional	50	Lot 8	April 2012	28.3	37.25
Coxliellidae	Helicostomella sp.	Occasional 15		Lot 8	December 2012	41.55	16.4
	Ptychocylididae Favella ehrenbergii	Seasonal	83	Dakhineswar	March 2012	55.7-71.9	$17.65 - 28.6$

evidently due to the incursion of freshets commencing from July to October 2013, resulting in dilution of salinity (0.14–7.85 p.s.u.) and other environmental situations like fast currents and high turbidity (11–16 NTU) (Sarkar et al. [1985\)](#page-15-0). As a result, water temperature, salinity and chl a concentration decreased largely with increased turbidity as endorsed by Godhantaraman ([2002](#page-14-0)) from Pichavaram mangrove regions, southeast coast of India. Species richness runs almost proportionally with species abundance in each month. However, the temperature appeared to be the main factor responsible for the seasonal dynamics and distribution of tintinnid populations. Thus, the early winter peak occurred when the water temperature did not exceed 24 °C (Posta [1963;](#page-15-0) Hedin [1975](#page-14-0)).

A distinct seasonal succession in tintinnid community structure was observed in this estuarine system. Some tintinnid species appeared on a remarkably seasonal basis, with the eurythermal species Favella ehrenbergii and Dadayiella ganymedes standing out particularly in freshwater site, Dakhineswar (S_2) , and brackish water site, Nurpur (S_5) , respectively, during post-monsoon. The winter peak was mainly concerned due to the dominance of Tintinnopsis minuta, Tintinnopsis lohmani, Tintinnopsis lobiancoi, T. primitivum and L. simplex, indicating their thermophilic nature. However, F. ehrenbergii was recorded during the post-monsoon period (January 2013) with a density of 83 ind.l⁻¹ during the occurrence of multispecies bloom (C. radiatus, C. lorenzianus and T. frauenfeldii) in January 2013 in Gangasagar (S_8) ; complete exclusion of tintinnids was registered except only for a large-sized tintinnid F. ehrenbergii which was able to withstand due to its very large lorica dimension (531.1 μm in length and 151.2 μm in lorica oral diameter (LOD)). The

Fig 2 Microphotographs of representative tintinnid species of diverse lorica structures recorded from the Hooghly River Estuary. a T. karajacensis, b T. beroidea, c L. simplex, d T. parvula, e Metacylis sp., f T. turbo and g T. minuta

species can be able to graze on a large-sized phytoplankton (732.8–13,069-μm surface area) during the bloom (Barría de Cao et al. [1997](#page-14-0)) conditions but this was difficult for the rest of the tintinnid population. The abundance of Tintinnopsis karajacensis and Tintinnopsis gracilis was relatively high during monsoon (July–August) exclusively at Lot 8 (S_7) and Nurpur (S_5) , respectively. The existence of T. *lohmani* and T. lobiancoi during monsoon as well as in postmonsoon season was confined at three sites in the lower estuarine stretch, namely, Diamond Harbour (S_6) , Lot 8 (S_7) and Gangasagar (S_8) . Two successful species, namely, Tintinnopsis tubulosa and T. minuta, showed almost year-round existence with the highest concentration during pre- and post-monsoon. A large number of smaller-sized tintinnids during winter months might be related to favourable temperature and salinity regimes of water. The biomass of the loricate ciliates was minimum (0.005 µg C 1^{-1}) during monsoon and maximum $(2.465 \mu g C l^{-1})$ during pre-monsoon months (as shown in Table [6](#page-10-0)). In general, most of the species were smaller in size fraction (lorica length ≤ 65 µm) and the

Fig 3 Seasonal variations in mean abundance of tintinnids along the Hooghly River Estuary

contribution of these size categories to biomass was large (Godhantaraman [2002\)](#page-14-0). Minimum and maximum daily production rates ranged from 0.04 μ g Cl⁻¹day⁻¹ during post-monsoon to 3.13 µg C l^{-1} day⁻¹ during premonsoon (Table [7](#page-10-0)).

Changes in lorica morphology of tintinnid

Lorica dimension of tintinnids exhibited morphological adaptability in compliance with the fluctuating environmental conditions. Diverse ecological characteristics appear to be related to dimensions of lorica and most precisely with LOD (Dolan [2010\)](#page-14-0). Polymorphism is very much pronounced in the species T. beroidea, T. primitivum and L. simplex based on temperature and salinity. The lorica length and LOD of all three ciliates reflected a decrease in the size of the individual species with decreasing temperature and salinity. The smallest lorica of T. beroidea (13.85 μm in length and 4.2 μm in LOD), T. primitivum $(22.5 \mu m)$ in length and $5.3 \mu m$ in LOD) and L. simplex $(23.58 \mu m)$ in length and $8.75 \mu m$ in LOD) was attributed to freshwater (salinity ≤ 0.3 p.s.u.) sites (S₂ and S₃ mainly) during post-

 \rightarrow Species diversity (H") \rightarrow Species evenness(E") \rightarrow Species richness (R")

Fig 4 Monthly variations of tintinnid community structure in the context of species diversity, species evenness and species richness

monsoon (February) when the temperature was below 20 °C and salinity of 0.14–0.25 p.s.u. But during the pre-monsoon period, maximum lorica sizes of T. beroidea (63.9 μm in length and 21.6 μm in LOD), T. primitivum $(57.33 \mu m)$ in length and 11.1 μ m in LOD) and L. simplex (74.7 μ m in length and 19.3 μ m in LOD) were noticed in the estuarine zone $(S_7 \text{ and } S_8)$ when the temperature reached over 30 °C and the salinity ranged between 11 and 18 p.s.u. Characterization of tintinnid communities simply in terms of LODs can provide information on the ecological characteristics of the species assemblage.

Occurrence of tintinnids in relation to environmental factors

As evidenced from the correlation matrix values in Table [8](#page-11-0), the dominant tintinnids showed significant correlations in majority of the cases with water temperature and nutrients. Some species showed a strong positive correlation with DO and BOD, while others are negatively correlated with turbidity and nutrient concentration. Dominant species like T. beroidea was

Table 5 Mean value of tintinnid abundance and community indices recorded in all the sites throughout the year

Parameters	Mar 2012	Apr 2012	May 2012	June 2012	July 2012	Aug 2012	Sept 2012	Oct 2012	Nov 2012	Dec 2012	Jan 2013	Feb 2013
Abundance $(ind.1^{-1})$	232	302	432	263	227	255	167	239	302	388	672	501
Species diversity (H')	1.13		1.16	1.14	1.19	1.24	1.3	1.3	1.33	1.25	1.41	1.45
Species richness (R')	8.46	9.58	8.29	8.27	8.29	6.9	7.64	7.48	7.3	6.68	7.25	6.82
Species evenness (E')	0.38	0.33	0.38	0.38	0.4	0.41	0.44	0.43	0.44	0.42	0.47	0.48

Sites	Mar 2012	Apr 2012	May 2012	June 2012	July 2012	Aug 2012	Sept 2012	Oct 2012	Nov 2012	Dec 2012	Jan 2013	Feb 2013
S_1	0.011	0.003	0.015	0.052	0.105	0.028	0.048	0.035	0.049	0.064	0.069	0.061
S ₂	0.183	0.046	0.006	0.011	0.018	0.058	0.049	0.103	0.037	0.044	0.053	0.131
S_3	0.023	0.02	0.014	0.037	0.057	0.044	0.034	0.075	0.08	0.045	0.007	0.616
S_4	0.007	0.093	0.039	0.003	0.064	0.025	0.015	0.008	0.077	0.029	0.124	0.595
S_5	0.026	0.067	0.067	0.064	0.005	0.069	0.047	0.022	0.023	0.013	0.284	0.237
S ₆	0.026	0.012	2.465	0.015	0.238	0.102	0.209	0.059	0.069	0.03	0.353	0.073
S_7	1.427	0.04	0.03	0.018	0.064	0.04	0.095	0.06	0.032	0.025	0.135	0.08
S_8	0.29	0.05	0.033	0.2	0.101	0.255	0.027	0.04	0.076	0.065	0.06	0.242

Table 6 Month-wise variation of tintinnid biomass (micrograms of C per litre) at all the sampling sites (S_1-S_8)

negatively correlated with turbidity $(r=-0.657)$, $P \leq 0.05$), indicating its poor density in highly turbid condition. Again, two other core species like T. primitivum and L. simplex showed a positive correlation with water temperature ($r=0.634$, $P\leq 0.05$) and nitrate concentration ($r=0.699$, $P\leq 0.05$), respectively. Species like *T. minuta* showed a negative correlation with nitrate but positively correlated with total phosphorus concentration. This suggests that spatiotemporal succession of species was strongly influenced by environmental variables during the period of investigation. An overall species abundance showed a significant positive correlation with diversity ($r=0.765$, $P\leq 0.05$) and negatively correlated with richness ($r=-0.801, P\leq 0.05$), indicating that the existence of a particular species can lower the diversity as well as the population density in that site. Species number and abundance showed strong positive correlations for all sampling sites ($r=0.981$, $P\leq 0.001$). One-way ANOVA was performed for each sample to test the variation between species and between months, taking into consideration the numerical abundance of all the species. The resulting output showed a significant variation between species abundance and months $(F=2.36,$ $P=0.014$), but no variation was observed between abundance and sites.

The dominance indices (Y_i) for T. beroidea was found to be maximum (2.96) at Babughat (S_3) followed by L. simplex (2.81) at Barrackpore (S_1) and T. primitivum (2.68) at Budge Budge (S_4) , indicating their abundance as well as existence throughout the year in this estuarine system. The other two less frequent ciliates T. tubulosa (1.68) and T. *minuta* (1.10) showed their main existence in Diamond Harbour (S_6) and Budge Budge (S_4) , respectively.

A dendrogram of the species distribution was plotted using group-average clustering from the logtransformed species abundance data of eight dominant species documented from the eight study sites (Fig. [5\)](#page-11-0). A single cluster was formed by three monsoon species (T. lohmani, T. lobiancoi and T. gracilis) followed by five outliers among which the first three species T. beroidea, T. primitivum and L. simplex were found

Sites	Mar 2012	Apr 2012	May 2012	June 2012	July 2012	Aug 2012	Sep 2012	Oct 2012	Nov 2012	Dec 2012	Jan 2013	Feb 2013
S_1	0.05	0.07	0.08	0.19	0.48	0.1	0.22	0.2	0.21	0.18	0.27	0.35
S_2	0.4	0.15	0.05	0.08	0.08	0.24	0.23	0.39	0.16	0.08	0.55	0.53
S_3	0.11	0.1	0.08	0.15	0.24	0.17	0.21	0.319	0.27	0.11	0.045	0.3
S_4	0.3	0.24	0.25	0.05	0.27	0.12	0.06	0.05	0.29	0.08	0.24	0.45
S_{5}	0.08	0.05	0.41	0.27	0.66	1.57	0.14	0.13	0.09	0.04	0.44	0.44
S ₆	0.1	0.07	0.44	0.07	0.61	0.33	0.64	0.23	0.25	0.06	0.31	0.44
S_7	3.13	0.16	0.07	0.11	0.19	0.16	0.39	0.23	0.19	0.09	0.71	0.58
S_8	0.63	0.2	0.17	0.79	0.35	0.75	0.11	0.2	0.28	0.2	0.36	1.02

Table 7 A comparative accounts of tintinnid production rate (micrograms of C per litre per day) during the study period

Species	Temp.	Salinity	pH	Turbidity	TDS	DO	BOD	chl a	NO ₃	TP	SiO ₄
T. beroidea	-0.088	-0.156	-0.509	-0.657	0.107	0.178	0.184	0.013	0.366	-0.299	0.568
T. minuta	0.387	0.074	0.164	0.495	0.552	-0.193	0.015	-0.417	$-0.961*$	$0.778*$	0.131
T. lohmani	$0.693*$	0.607	$0.693*$	-0.136	$0.730*$	$0.762*$	-0.076	0.241	-0.408	0.445	0.134
T. lobiancoi	0.19	0.036	0.384	-0.208	0.217	$-0.709*$	$0.793*$	-0.05	0.057	0.502	0.558
T. primitivum	$0.634*$	-0.59	0.178	0.129	-0.517	0.107	0.601	-0.593	0.235	0.077	0.415
L. simplex	-0.456	-0.372	-0.593	-0.553	-0.444	-0.043	-0.098	0.057	$0.699*$	-0.682	0.243
T. gracilis	-0.02	-0.175	0.254	-0.285	0.135	0.685	$0.740*$	-0.271	0.16	0.33	$0.755*$

Table 8 Correlations between population density of seven tintinnid ciliates and hydrological parameters at eight sampling sites (S_1-S_8) along the Hooghly River Estuary

*P≤0.05

to be predominant in all the sites throughout the study period with maximum density during post-monsoon and other two (T. tubulosa and T. minuta) were available during pre- and post-monsoon.

k-dominance curves, the cumulative percentage (i.e. the percentage of total abundance made up by the kth most dominant plus all more dominant species), are plotted against log rank k to allow a better comparison of differences in tintinnid diversity between the investigated stations (Fig. [6\)](#page-12-0). The most elevated curve showed the lowest diversity at Barrackpore (S_1) . The dominance of tintinnid species between stations was found to be similar in reference to species rank. A dominance of >80 % was found only at S_1 at species rank 4, while at stations S_2 , S_3 and S_4 , a dominance of $>80\%$ was reached at species rank 5 and stations S_5-S_8 showed a>80 % dominance at species rank 6 or more.

To gain insight into the spatial coincidences in the distribution of tintinnids in relation to the environmental

Fig. 5 Dendrogram showing the clustering of eight dominant tintinnids recorded in the Hooghly River Estuary

variables (temperature, salinity, pH, turbidity, total dissolved solids, chlorophyll a, dissolved oxygen, biochemical oxygen demand, fecal coliform, nitrate, total phosphate and silicate concentration in surface water) involved, a PCAwas performed, which revealed three main groups (Fig. [7\)](#page-12-0). Group I represents some rarely available species (T. bermudensis, T. turbo, T. nucula, T. parva, T. urnula, T. gracilis, T. incertum, L. nordqvisti and D. ganymedes) attributed mainly to temperature, turbidity, nitrate, silicate and fecal coliform loading. These species appeared in low numbers, being more frequent in pre-monsoon and post-monsoon mainly. The second group was formed by T. beroidea, L. simplex, T. primitivum, T. minuta, T. tubulosa, T. lohmani, T. lobiancoi, F. ehrenbergii, T. directa and T. nana, probably regulated by chl a , salinity, pH and DO. This group was mainly formed by small-sized taxa, which reached some of the greatest abundances of the estuary. Group III is composed of seven tintinnid species (T. parvula, T. tentaculata, T. acuminata, T. karajacensis, Metacylis sp., Eutintinnus sp. and Helicostomella sp.) which were present only once throughout the study, mainly appearing at the seaward end of the estuary. Species in this group were mainly influenced by environmental variables such as phosphate, TDS and BOD.

Multiple stepwise regression model between abundance of eight dominant tintinnid and environmental factors revealed that chl a was found to be positively related in the majority of cases (eight) with density of total tintinnid. The next factor was nitrate, which found to be positively related in six cases. The best multiple regression models were produced by the post-monsoon species *T. minuta* ($R^2 = 92.9 \%$, $P = 0.002$) and the monsoon species *T. lobiancoi* ($R^2 = 90.6$ %, $P = 0.022$) as those species were best fitted with the environment in Fig. 6 k-dominance curves of tintinnid species (x-axis logged) for eight stations (S_1-S_8) in the Hooghly River Estuary, where the dominance (>80 %) was reached at species rank 4 (site S_1), species rank 5 (sites S_2 , S_3 and S_4) and species rank 6 or more (sites S_5 , S_6 , S_7 and S_8)

that specific season. Multiple regression models with other species were not significant, suggesting that the species can withstand in fluctuating environmental conditions. Regression analyses map between total tintinnid and total phytoplankton are given in Fig. [8.](#page-13-0) The abundance $(R^2 = 93.5 \%)$ and diversity $(R^2 = 82 \%)$ map revealed significant results, indicating that population density of tintinnid is dependent on phytoplankton

Fig. 7 Principal component analysis (PCA) map of tintinnid species in relation to environmental variables

Fig. 8 Regression analyses map showing a abundance, b diversity, c richness and d evenness between total phytoplankton and total tintinnid recorded in eight sites throughout the year

density which is the main prey for this tiny ciliates as endorsed by many workers. This suggests that tintinnid might affect phytoplankton abundance with respect to the relationship of prey–predator. In addition, high density and diversity of tintinnids were always supported by a peak diversity of phytoplankton (Naha Biswas et al. [2013\)](#page-15-0).

Comparison with other studies in Indian water

Tintinnid abundance and distribution have been studied by several workers in coastal and backwater system in India. In Parangipettai, southeast coast of India (Godhantaraman [2002\)](#page-14-0), 47 tintinnids had been identified with an abundance range of 2–420 ind.l⁻¹, whereas in Cochin Backwaters (Jyothibabu et al. [2006](#page-14-0)) and Central and eastern Arabian Sea (Gauns et al. [1996\)](#page-14-0), 22 genera $(409-6,080 \text{ ind.}1^{-1})$ and 30 genera $(130–700 \text{ ind.}^{-1})$ of tintinnids were documented, respectively. Recently, in the coastal regions of Sundarban wetland formed at the estuarine phase of the Hooghly River, Naha Biswas et al. [\(2013\)](#page-15-0) recorded 32 tintinnid species with an abundance of $75-1,050$ ind.l⁻¹. However, the present investigation documented comparatively less diversity (26 species) in this estuarine phase

with a mean abundance of 62–1,666 ind.^{1^{-1}} which could be attributed mainly due to high turbidity throughout the year as discussed earlier.

Conclusion

The study used a combined taxonomic and ecological approach of the microzooplankton dominated by tintinnids from the neritic eutrophicated Hooghly estuarine ecosystem and provides some new information on their spatio-temporal distribution and community structure in the context of environmental parameters. Welldefined tintinnid assemblages are evident, representing the fingerprint of the specific ecological zone, characterized by a set of environmental variation. The ubiquitous presence of three core tintinnid species, namely, T. beroidea, T. primitivum and L. simplex, exhibited seasonal variations of their oral diameter and lorica length which deserves special attention. Further investigations about the trophic relationship between ciliated and other microbial and metazoan components are required for the better understanding of their role in the microbial food webs. Differences in the occurrence and abundance of less abundant nauplii and rotifer suggest

adjustments in the microzooplankton community. There is a need for further studies on the diversity and abundance of pico- and nanoplankton to get a holistic view of the trophic level status in this estuarine environment.

Acknowledgments The research work was financially supported by the University Grants Commission (UGC), New Delhi, India [sanction no. F. no.: 40(388)/2011(SR)] in a project titled "Biodiversity of Microzooplankton along the lower stretch of Ganges (Hooghly) River, west Bengal with special emphasis on Tintinnids (Ciliata: Protozoa)". The first author is grateful to UGC for awarding him a research fellowship. Authors are greatly indebted to Dr. J. R. Dolan, from the Centre National de la Recherche Scientifique (CNRS), France for the identification as well as for the microphotographs of the tintinnids.

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