



# Diversity, distribution and polymorphism of loricate ciliate tintinnids along Hooghly estuary, India

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## Abstract

The choreotrich ciliate tintinnid (Ciliophora: Tintinnida) community was assessed from nine sampling sites ( $n=108$ ) along the Hooghly estuary, east coast of India during June 2012 to May 2013. A total of 32 tintinnid species (3 core, 12 seasonal and 17 perennial species) were recorded from agglomerated form, dominated by *Tintinnopsis* sp., contributing ~62 % of total tintinnid population. The community was predominated by *Tintinnopsis beroidea* followed by *Tintinnidium primitivum*, *Leprotintinnus simplex*, *Tintinnopsis tubulosa* and *T. minuta*. The maximum abundance of tintinnids (1287 ind.  $L^{-1}$ ) and species diversity ( $H' = 2.87$ ) was recorded during post monsoon season (January) coinciding with high concentration of diatoms (6720 cells  $L^{-1}$ ). In contrast, both numerical density (55 ind.  $L^{-1}$ ) and species diversity ( $H' = 1.21$ ) values were minimum during monsoon in association with low phytoplankton density (2200 cells  $L^{-1}$ ). The large size tintinnids mainly composed of Codonellidae (55%) and Tintinnidiidae (30%) were the most dominating in this estuarine ecosystem. The result of ANOVA showed significant variation between species abundance and months ( $F = 2.21$ ;  $P \leq 0.041$ ). K-dominance curves were plotted against log rank  $k$ , showed species dominance over the investigated sites. The CCA map revealed a clustering of core species with chl  $a$  and dissolved oxygen which were found to be the most important factor controlling the distribution and seasonal patterns of tintinnids. The changes in lorica morphology for three dominant tintinnids are directly related to their unique adaptive strategies to the variations of temperature and salinity.

The study provided exhaustive information of microzooplankton which enhances our understanding of their interactions in a tropical estuarine system and of immense importance in the context of maintaining its ecological and economic stability.

**Keywords:** *Tintinnid, polymorphism, seasonal variations, loricate ciliate, Hooghly estuary*

## Introduction

The loricate ciliate tintinnids are planktonic spirotrich ciliates (body size 20-200  $\mu m$ ) and differ from other ciliates by the possession of vase-shaped shell (lorica), which consists of particles collected from the surrounding water and cemented together. 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. With their

short life cycle and delicate pellicles, they may respond more quickly to environmental changes than any metazoa (Ismael and Dorgham, 2003). Tintinnids are important components of the aquatic ecosystem and play a crucial role in transferring elements and energy from low trophic levels (e.g., pico- and nano-phytoplankton) to high (e.g., copepods) (Corliss, 2002). Although there have been a number of recent investigations on bio-assessment using ciliated protozoa, the ability of marine tintinnids for discriminating water quality status bio-assessment have yet to be studied (Jiang *et al.*, 2011; Xu *et al.*, 2011, 2014). The present study has been undertaken with the following objectives: (1) To investigate the spatio-temporal distribution and biodiversity of tintinnids in relation to hydrological conditions in Hooghly estuary and (2) To determine the polymorphic features of tintinnid for assessment of ecological characteristics and species assemblage in the estuarine system.

## Material and methods

### Study area

The Hooghly estuary (21° 31' N–23° 30' N and 87° 45' E–88° 45' E) is a globally important tropical, meso-macrotidal, positive estuary with a complexity of environmental conditions. The large tidal variations, irregular coastal geometry, the presence of islands and the presence of navigational channels separated by shallow zones make the flow quite complicated. Due to the typical patterns of rainfall being restricted to only about 3 months during a year in the basin, the dry season flows in this estuary and its tributaries is only a fraction of the total annual flow. 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 (Khan, 1995). 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 ~1700 mm). Hence, the climate of the area was chiefly influenced by the monsoon season were 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 (Chugh, 2009). Nine sampling sites, almost equidistant from each other, have been chosen (Fig. 1) along the stretches of this estuary (covering ~140km) on the basis of different environmental stress, tidal environments, wave energy fluxes and distances from the sea (Bay of Bengal). The sites can be sub-divided into 3 distinct ecological zones on the basis of salinity regime, namely, Fresh water zone [Barrackpore (S<sub>1</sub>), Dakhineswar (S<sub>2</sub>), Babughat (S<sub>3</sub>) and Budge Budge (S<sub>4</sub>); Brackish water zone [Nurpur (S<sub>5</sub>) and Diamond harbour (S<sub>6</sub>)] as well as Estuarine zone [Lot 8

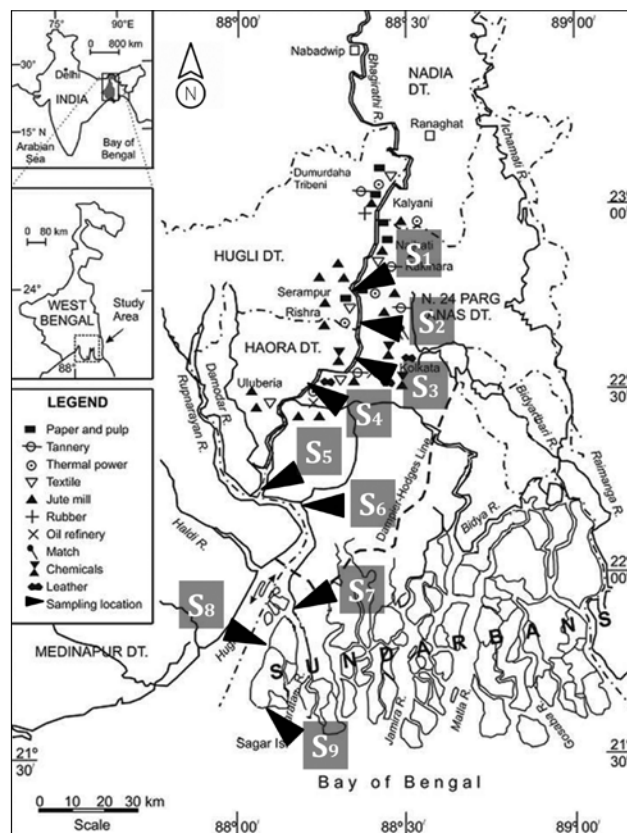


Fig. 1. Sampling sites

(S<sub>7</sub>), Phuldubi (S<sub>8</sub>) and Gangasagar (S<sub>9</sub>)]. Global positioning system (GPS) was used to fix the geographic position of the sampling sites, which have a mean elevation of 13.7-16.7 m, belonging to a lower deltaic plain experiencing intense semidiurnal tides and wave action.

### Collection, preservation and analysis of samples

Surface water samples were collected from nine sampling sites on monthly basis during high tide in the morning hours. The environmental variables such as water temperature and salinity were recorded in situ with the help of a celsius thermometer (0-110°C, Mercury) and refractometer respectively. Winkler's titrimetric method (Strickland and Parsons, 1972) was followed for the estimation of dissolved oxygen (DO) and biochemical oxygen demand (BOD<sub>5</sub>; difference between DO of 100% saturated water on day 1 and after 5 days incubation). Turbidity, pH and inorganic nutrients (nitrate, phosphate and silicate) were measured by water analyzer 371 (Systronics). To analyze the chlorophyll *a* (chl *a*) concentration (mg m<sup>-3</sup>), 1000 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 refrigerator for 24 hours. Later chl *a* concentration was measured spectrophotometrically adopting the procedure of Strickland and Parsons (1972).

For collection of tintinnids, 1000 ml water was collected from each sampling site in pre-cleaned plastic bottles and fixed with 2% acid Lugol's iodine solution and stored in a cool dark place until analyses (Dolan *et al.*, 2002). In the laboratory, water samples were placed in measuring cylinders of 1000 mL with 2 special outlets at the level of 500 ml and 250 ml respectively which were blocked by clumps and incubated for at least 48 h (Godhantaraman, 2002). After that when almost all the plankters were settled at the bottom of the cylinder, the clumps were opened and the water from the upper portion of the measuring tube was allowed to flow out without disturbing the last 250 ml sample and kept incubated for another 24 h. Finally the sample was concentrated to a volume of 25 mL by siphoning out from rest of the volume and stored in a sterile storage vial (Godhantaraman, 2002). From the remaining 25 ml, 0.1 ml of well-mixed concentrated sample was taken and analyzed on a microscope slide. 1 ml sample has been used for identification of tintinnid and counted under a phase contrast microscope at 40× magnification (NIKON Trinocular Microscope, Model E-200). For getting biometry of each species, minimum 20 individuals were examined for morphological measurements and the species identification was carried out following published literatures (Kofoid and Campbell, 1929, 1939; Zhang *et al.*, 2012). The dimensions of about 30 individual cells for each taxon were measured and average biovolume of each taxon was estimated from appropriate geometric shapes. The Lorica Oral Diameter (LOD) is an important taxonomic characteristic of tintinnid as they use lorica for ingestion of food (Krs̃inic', 2010a). Lorica volume ( $LV, \mu\text{m}^3$ ) was calculated and transformed to biomass using the regression equation (Verity and Langdon, 1984): Biomass ( $\mu\text{g C L}^{-1}$ ) =  $444.5 + 0.053 LV$ . Production rate ( $P, \mu\text{g CL}^{-1} \text{ day}^{-1}$ ) was estimated from biomass ( $B, \mu\text{g CL}^{-1}$ ) and empirically determined specific growth rate ( $g, \text{day}^{-1}$ ):  $P = B \times G$ . Multiple regression equation of Müller and Geller (1993) was used for estimation of growth rate of tintinnid: Growth rate =  $1.52 \ln T - 0.27 \ln CV - 1.44$ ; where T is the temperature ( $^{\circ}\text{C}$ ) and CV is the cell volume ( $\mu\text{m}^3$ ).

### Statistical analysis

Species diversity (Shannon-Wiener,  $H'$ ), evenness (Pielou's,  $E'$ ) and richness (Margalef,  $R'$ ) indices are commonly calculated in community-level tintinnid investigations by using the following equations:

$$H' = - \sum_{i=1}^s P_i (\ln P_i)$$

$$E' = H / \ln S$$

$$R' = (s-1) / \ln N$$

where s is total number of species in the community,  $P_i$  is the proportion of s made up of the  $i$ th species and N is the total number of individuals (Xu *et al.*, 2008). Data have been log

transformed [ $\log_{10}(n + 1)$ ] to determine the uniform variability between rare and frequently occurring species (Clarke and Warwick, 1994). The Pearson's correlation coefficient was calculated to determine the significant correlation within environmental variables and species abundance data (Sokal and Rohlf, 1981). Two-way ANOVA was performed to establish the seasonal and site-specific variation of species distribution by adopting the statistical software MINITAB 13. Cumulative dominance curves ( $k$ -dominance) and Canonical Correspondence Analysis (CCA) were performed by PRIMER v6.0 and XLSTAT 2014 respectively.

## Results and discussion

### Changes in environmental variables

The surface water temperature ranged from  $18^{\circ}\text{C}$  to  $32.5^{\circ}\text{C}$ , with a mean of  $28.7 \pm 3.5^{\circ}\text{C}$ , showing maximum and minimum at Budge budge (S4) and Phuldubi ( $S_9$ ) respectively. Maximum salinity (25 psu) was recorded at Gangasagar ( $S_9$ ) during summer (June) and minimum (0.22 psu) at Dakhineswar ( $S_2$ ) during monsoon period (November). Generally, changes in the salinity in the brackishwater habitats such as estuaries, backwaters and mangroves are due to the influx of freshwater from land run off, caused by monsoon or by tidal variations. The recorded higher values could be attributed to the low amount of rainfall, higher rate of evaporation and also due to neritic water dominance (Rajasegar, 2003). The surface water pH was remained alkaline throughout the study period at all the stations with an average value of  $7.67 \pm 0.11$ , being maximum in Lot 8 (S7) (8.06) during post-monsoon and minimum (7.24) at Nurpur (S5) during pre-monsoon. Total dissolved solids (TDS) recorded lowest value ( $1.44 \text{ mg L}^{-1}$ ) during post monsoon at Nurpur (S5) and highest ( $3.25 \text{ mg L}^{-1}$ ) during monsoon at Lot 8 (S7). The mean dissolved oxygen value recorded was  $5.11 \pm 0.23 \text{ mg L}^{-1}$ , being highest ( $5.72 \text{ mg L}^{-1}$ ) during pre-monsoon at Gangasagar (S9) and lowest ( $4.06 \text{ mg L}^{-1}$ ) during post-monsoon in Lot 8 (S7). Distribution of nutrients is mainly based on the season, tidal conditions and freshwater flow from land source. Nitrate concentration showed a wide range ( $15.27$ - $28.45 \mu\text{mol L}^{-1}$ ) of seasonal variation with an annual mean of  $19.22 \pm 2.67 \mu\text{mol L}^{-1}$ . Total phosphate exhibited similar trend of distribution during the study period with average concentration of  $1.05 \pm 0.15 \mu\text{mol L}^{-1}$  throughout the period. Regeneration and release of total phosphorus from bottom mud into the water column by turbulence and mixing also attributed to the higher monsoonal values ( $1.59 \mu\text{mol L}^{-1}$ ) (Chandran and Ramamoorthy, 1984; Rakshit *et al.*, 2016). The concentration of silicate was recorded maximum ( $96.3 \mu\text{mol L}^{-1}$ ) at Gangasagar during post-monsoon, might be due to turbulent nature of water where bottom sediment might have been exchanged with overlying water in this estuarine environment. The low silicate concentration ( $58.35 \mu\text{mol L}^{-1}$ ) in Barrackpore

(S<sub>1</sub>) during post-monsoon could be attributed to its uptake by phytoplankton for their biological activity (Ramakrishnan *et al.*, 1999). Chl *a* concentration showed maximum value of 2.59 mg m<sup>-3</sup> at Phuldubi (S<sub>9</sub>) during pre-monsoon and minimum (0.96 mg m<sup>-3</sup>) during monsoon at Barrackpore (S<sub>1</sub>) and this could be due to anthropogenic effects as evidenced from its positive correlation with salinity and may also due to freshwater discharges from the rivers (dilution), causing turbidity and less availability of light (Godhantaraman, 2002).

### Seasonal changes in distribution pattern of tintinnid

The tintinnid community consists of 32 species with 11 genera and 8 families, dominated by 3 core species (present in substantial number almost throughout the year) such as *Tintinnopsis beroidea*, *Tintinnidium primitivum* and *Leprotintinnus simplex* followed by 17 perennial and 12 seasonal species (Table 1). The tintinnid was primarily

Table 1: Site-specific abundance of tintinnid species (n=32) considering nine sampling sites (S1-S9) along Hooghly Estuary

Species	Fresh water zone (0.13-0.3 psu)				Brackish water zone (1.1-6 psu)		Estuarine zone (7.5-26 psu)		
	S1	S2	S3	S4	S5	S6	S7	S8	S9
<i>Tintinnopsis beroidea</i>	**	**	**	**	***	***	***	**	***
<i>Tintinnopsis tubulosa</i>	**	***	**	**	**	**	**	*	**
<i>Tintinnopsis minuta</i>	*	*	*	**	*	*	**	*	*
<i>Tintinnopsis bermudensis</i>	*	*	**	*	-	-	-	**	-
<i>Tintinnopsis karajacensis</i>	*	*	*	*	-	-	*	*	*
<i>Tintinnopsis lohmani</i>	-	-	*	*	*	*	*	**	*
<i>Tintinnopsis lobiancoi</i>	*	-	-	-	*	*	*	*	*
<i>Tintinnopsis umula</i>	*	*	-	-	-	-	*	*	-
<i>Tintinnopsis parva</i>	-	*	-	-	*	-	-	-	-
<i>Tintinnopsis tentaculata</i>	-	-	-	-	-	-	-	*	*
<i>Tintinnopsis parvula</i>	-	-	-	-	-	-	-	-	*
<i>Tintinnopsis nucla</i>	-	*	-	-	-	-	-	-	*
<i>Tintinnopsis directa</i>	*	-	-	-	-	-	-	-	*
<i>Tintinnidium primitivum</i>	**	**	***	**	**	**	**	**	**
<i>Tintinnidium incertum</i>	-	-	*	-	-	*	-	-	-
<i>Leprotintinnus nordqvisti</i>	*	-	-	-	-	*	-	-	-
<i>Leprotintinnus simplex</i>	***	***	***	**	**	**	**	**	**
<i>Tintinnopsis gracilis</i>	-	-	*	-	*	*	*	-	-
<i>Dadayiella ganymedes</i>	-	*	-	*	*	-	*	-	-
<i>Favella ehrenbergii</i>	*	*	-	*	-	-	-	-	*
<i>Tintinnopsis turbo</i>	-	-	-	-	-	*	-	*	-
<i>Tintinnopsis acuminata</i>	-	-	-	-	-	-	-	-	*
<i>Tintinnopsis nana</i>	-	-	-	-	*	-	-	-	-
<i>Metacylis</i> sp.	-	-	-	-	-	-	*	-	-
<i>Eutintinnus</i> sp.	-	-	-	-	-	-	*	-	-
<i>Helicostomella</i> sp.	-	-	-	-	-	-	*	-	-
<i>Tintinnopsis rotundata</i>	-	-	-	-	-	-	*	-	-
<i>Tintinnopsis cylindrica</i>	-	-	-	-	-	-	-	*	**
<i>Tintinnopsis radix</i>	-	-	-	-	*	-	**	-	*
<i>Stenosomella ventricosa</i>	*	*	*	*	*	*	*	*	*
<i>Codonellopsis schabi</i>	-	-	-	-	-	-	*	-	*
<i>Wangiella dicollaria</i>	-	-	-	-	-	-	-	-	*

Species abundance (ind. L-1): \*<sup>†</sup> ≤100; \*\*<sup>†</sup> = 100-1000; \*\*\*<sup>†</sup> ≥ 1000

grouped into agglomerated and non-agglomerated forms where agglomerated forms were dominated by the genera *Tintinnopsis* (22 species). The dominance of *Tintinnopsis* in estuarine and coastal water in India (Godhantaraman, 2002) and other environments such as Jiaozhou Bay (Feng *et al.*, 2015) and Zhangzi Island (Ying *et al.*, 2013) was mainly due to their unique flexible adaptive strategies by which they reached their maximum abundance during the four days of the study period. *T. beroidea*, the dominant tintinnid in the Hooghly estuary (Rakshit *et al.*, 2014, 2016) as well as in other estuaries and coastal waters (Kamiyama and Tsujino, 1996), is considered as 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. The Non-agglomerated genera (*Favella* sp., *Dadayiella* sp., *Eutintinnus* sp., *Metacylis* sp. and *Helicostomella* sp.) contributed 16 % of the total tintinnid community. The abundance was ranged from 55 ind. L<sup>-1</sup> in August 2012 (monsoon) at Dakhineswar (S<sub>2</sub>) to 1287 ind. L<sup>-1</sup> in January 2013 (post monsoon) at Gangasagar (S<sub>9</sub>) coinciding with high concentration of diatoms (6720 cells L<sup>-1</sup>). It has been stated that tintinnid biomass decreases in brackishwaters due to the effect of the osmotic stress, which only very euryhaline species can support (Muylaert *et al.*, 2000). Species abundance increased in late post monsoon, when water temperature was at its low and the residence time of the water was sufficient to retain planktonic populations within the estuary. In this season, tintinnid were the most abundant taxa, peak abundance of which is closely matched those of total species individuals. The overall environmental condition of estuary favored the presence of large-size tintinnid (mainly from Codonellidae and Tintinnidiidae), could be attributed by the persistence of *Tintinnopsis* spp (Rakshit *et al.*, 2014, 2016). However, these two families contributed between 55% and 30% respectively of total ciliate community in this region. Rest of the families (Codonellopsidae, Dictyocystidae, Xystonellidae, Tintinnidae, Metacylididae, Coxliellidae) contributing ~15-20% of total tintinnid abundance. The biomass of the loricate ciliates was minimum (0.004 µg C L<sup>-1</sup>) during monsoon and maximum (2.764 µg C L<sup>-1</sup>) during pre-monsoon months. In general, most of the species were smaller in size fraction (lorica length < 65 µm) and the contribution of these size categories to biomass was large (Godhantaraman, 2002). Minimum and maximum daily production rate was ranged from 0.04 µg C L<sup>-1</sup> day<sup>-1</sup> during post-monsoon to 3.54 µg C L<sup>-1</sup> day<sup>-1</sup> during pre-monsoon.

#### *Changes in lorica dimension (polymorphism) 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 lorica

oral diameter (LOD) (Dolan, 2010). The lorica length and LOD of all three tintinnid (*T. beroidea*, *T. primitivum* and *L. simplex*) reflected decrease in the size of the individual species with decreasing temperature and salinity. The smallest lorica of *T. beroidea* (lorica length = 41.85 µm and LOD = 10.10 µm), *T. primitivum* (lorica length = 35.44 µm and LOD = 8.45 µm) and *L. simplex* (lorica length = 82.66 µm and LOD = 24.43 µm) were attributed in fresh water (salinity < 0.3) sites (S<sub>2</sub> and S<sub>3</sub> mainly) during post-monsoon (February) when the temperature was below 20 °C and salinity of 0.14-0.25 psu. But in pre-monsoon period, large size of *T. beroidea* (lorica length = 73.90 µm and LOD = 23.60 µm), *T. primitivum* (lorica length = 64.50 µm and LOD = 18.86 µm) and *L. simplex* (lorica length = 122.45 µm and LOD = 33.76 µm) were noticed in estuarine zone (S<sub>7</sub>, S<sub>8</sub> and S<sub>9</sub>) when the temperature reached over 30 °C and salinity range 11-18 psu. Low salinity during early post-monsoon may also be responsible for the small size of *T. beroidea*. In the tintinnid populations, the geometric distribution of LOD size-class abundance is most simply attributable to the availability of prey concentration and size given the close relationship between LOD size and prey exploited by tintinnids (Dolan, 2010). Thus, characterization of tintinnid communities simply in terms of LODs can provide information on the ecological characteristics of the species assemblage.

#### *Contribution of small-sized tintinnid to the total community*

An overall dominance and diversity of the small-sized tintinnid (lorica length < 76 µm) belonging to the genera *Tintinnopsis* sp., *Tintinnidium* sp., *Codonellopsis* sp., *Wangiella* sp., *Eutintinnus* sp., *Metacylis* sp. and *Helicostomella* sp. was pronounced, accounting ~66% of the total tintinnid abundance (Fig. 2a). The influence and dominance of small-sized fraction on estuarine tintinnid community has also been reported from Shenhu Bay of China during autumn due to reduced prey size as well as by a seasonal succession of the phytoplankton community (Wang *et al.*, 2014). The lorica length and lorica oral diameter for small-sized form were varied from 22.76-73.90 µm and 10.10-40.20 µm respectively. *T. beroidea* and *T. primitivum* together represent 62.6% of the small-sized tintinnid abundance (Fig. 2b). Some previous studies suggested that the lorica dimensions of tintinnid, especially the oral dimensions, showed positive and significant correlations with the size of phytoplankton prey (Dolan, 2010). It was also reported that tintinnids mainly feed on nano-sized prey, preferably nanoflagellates (Balkis, 2004). The predominance of small-sized tintinnid over the large-sized form (lorica length > 76 µm) was evident and shares a high abundance throughout the estuary, might be attributed by environmental variables (Chl *a*, salinity and turbidity) as well as by biotic interaction.

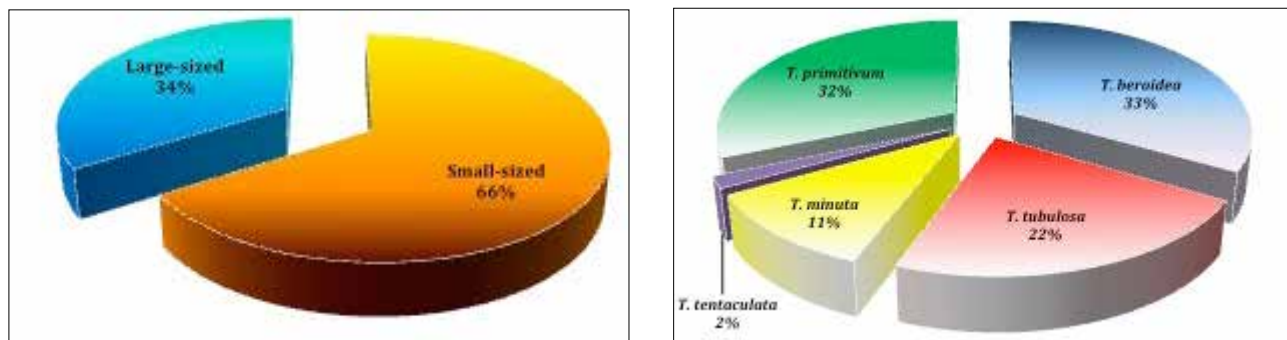


Fig. 2: Percentage contribution of (a) small and large-sized tintinnids and (b) five most dominant small-sized tintinnid along Hooghly Estuary

### Interaction between planktonic ciliate biodiversity and abiotic parameters

As evidenced from the correlation matrix values, the dominant tintinnids exhibited significant correlations in majority of the cases with pH, turbidity and chl a. Some species showed strong positive correlations with DO and BOD while others are negatively correlated with turbidity and nutrient concentration. *T. beroidea* showed a significant positive correlation with temperature ( $r = 0.894$ ;  $P \leq 0.05$ ), salinity ( $r = 0.765$ ;  $P \leq 0.05$ ) and chl a ( $r = 0.947$ ;  $P \leq 0.01$ ) indicating its high adaptability and sustainability with a broad range of salinity as well as temperature. Again, two other core species like *T. primitivum* ( $r = 0.811$ ;  $P \leq 0.05$ ) and *L. simplex* ( $r = 0.781$ ;  $P \leq 0.05$ ) showed positive correlation with chl a.

The CCA map revealed the clustering of 3 core species (*T. beroidea*, *T. primitivum* and *L. simplex*) with Chl a as well as dissolved oxygen which were found to be the most important factors controlling the distribution, tolerating a wide range of salinity and most of them reached their higher abundances at the mouth of the estuary (Fig. 3). On the other hand, two seasonal

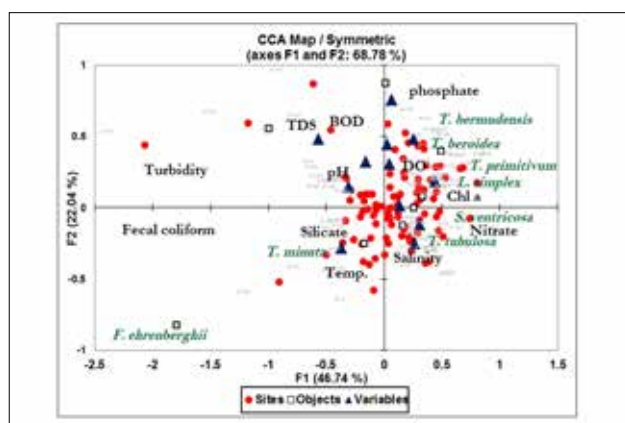


Fig.3: Canonical Correspondence Analysis (CCA) ordination diagram of tintinnid taxa considering the dominant tintinnids for 9 sampling sites and the environmental variables

species *T. tubulosa* and *S. ventricosa*, clustered positively with water temperature, salinity and the nutrients which were found to get a high concentration during monsoon and post monsoon due to heavy rainfall and freshwater input. *T. minuta* and *F. ehrenbergii* are totally separated from rest of the group and are less stimulate to environmental fluctuations. The sites were clustered together each other in terms of both environmental variables and tintinnid species assemblage.

Two-way ANOVA was performed within sites and months considering the studied tintinnid species. The abundance of core species, *T. beroidea* ( $F = 1.17$ ;  $P = 0.032$ ), *T. primitivum* ( $F = 1.61$ ;  $P = 0.019$ ) and *L. simplex* ( $F = 3.79$ ;  $P = 0.000$ ) was significantly varied within months, depending on different environmental stresses. The persistence and occurrence of *T. tubulosa* ( $F = 0.87$ ;  $P = 0.025$ ), *T. minuta* ( $F = 2.03$ ;  $P = 0.050$ ) and *S. ventricosa* ( $F = 10.01$ ;  $P = 0.000$ ) were significantly varied within different sampling sites.

K-dominance curves, the cumulative percentage (i.e. the percentage of total abundance made up by the *k*-th 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. 4). The most elevated curve showed the lowest diversity at Nurpur ( $S_5$ ). The dominance of tintinnid species between stations was found to be similar in reference to species rank. A dominance >80% was found only at  $S_5$  at species rank 8, while at brackish and estuarine sites  $S_6$ - $S_9$ , dominance >80% was reached at species rank 9 and fresh water sites  $S_1$ - $S_4$  showed >80% dominance at species rank 10 or more.

### Comparison with other studies in Indian coastal waters

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), 47 tintinnid had been identified with abundance range of 2- 420 ind. L<sup>-1</sup>, whereas, in Cochin Backwaters (Jyothibabu *et al.*, 2006)

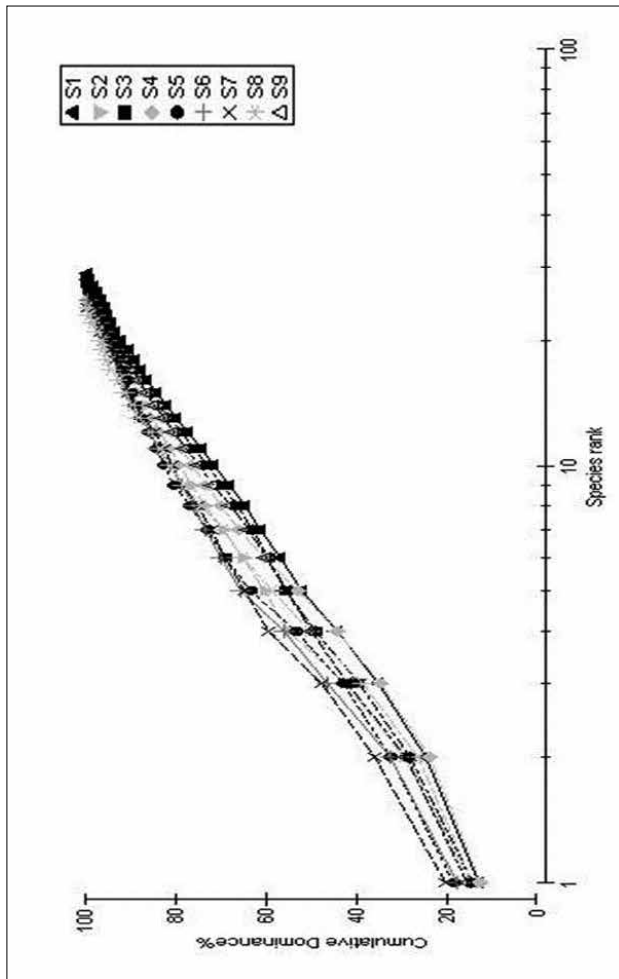


Fig. 4. *K*-dominance curves of tintinnid species (x axis logged) for 9 sampling sites ( $S_1$ - $S_9$ ) along Hooghly Estuary, where the dominance (>80%) was reached at species rank 8 (site  $S_5$ ); species rank 9 (site  $S_1$ - $S_4$ ) & species rank 10 or more (site  $S_6$ - $S_9$ )

and Central and eastern Arabian Sea (Gaus *et al.*, 1996), 22 genera (409- 6080 ind.  $L^{-1}$ ) and 30 genera (130-700 ind.  $L^{-1}$ ) respectively were documented. Recently, in coastal regions of Sundarban wetland formed at the estuarine phase of Hooghly, Naha Biswas *et al.* (2013) recorded 32 tintinnid species with an abundance of 75-1050 ind.  $L^{-1}$ . However, present investigation also documented 32 species in this estuarine phase with the mean abundance of 55-1287 ind.  $L^{-1}$  which could be attributed mainly due to high Chl *a* throughout the year as discussed earlier.

The study illustrates a well-defined tintinnid assemblage representing the "fingerprint" of a specific ecological zone of the estuary, characterized by a set of environmental variables. In addition, tintinnid diversity is also influenced by various biological factors (predators, resilience of dominant tintinnids etc.) as well as by anthropogenic factors (algal bloom, festival bathing and idol immersion etc.) of each sampling site. Small-

sized tintinnid (lorica length <76  $\mu m$ ) was found to be dominated over the large-sized and this might be favored by environmental variables (low Chl *a*, salinity and high turbidity) as well as biotic interaction (e.g. reduced prey size). These findings suggest that the taxonomic diversity and seasonal variation of tintinnid communities may be served as a useful tool with scientifically operational value in assessment of estuarine environments.

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## References

- Balkis, N. 2004. Tintinnids (Protozoa: Ciliophora) of the Büyükçekmece Bay in the Sea of Marmara. *Sci. Mar.*, 68: 33-44.
- Chandran, R. and K. Ramamoorthy. 1984. Hydrobiological studies in the gradient zone of the Vellar estuary- Nutrients, Mahasagar. *Bull. Nat. Inst. Oceanogr.*, 17: 133-140.
- Chugh, R. S. 2009. Tides in Hooghly River, *Hydrol. Sci. J.*, 6, 10-26.
- Clarke, K. R. and R. M. Warwick. 1994. Change in Marine Communities. Plymouth Marine Laboratory: 144pp.
- Corliss, J. O. 2002. Biodiversity and biocomplexity of the protists and an overview of their significant roles in maintenance of our biosphere. *Acta Protozool.*, 41: 199-220.
- Dolan, J. R., H. Claustre, F. Carlotti, S. Plounevez, T. Moutin. 2002. Microzooplankton Diversity: relationship of tintinnid ciliate with resources, competitors and predators from the Atlantic Coast of Morocco to the Eastern Mediterranean. *Deep Sea Res. Pt.*, 49:1217-1232.
- Dolan, J. R. 2010. Morphology and Ecology in Tintinnid Ciliates of the Marine Plankton: Correlates of Lorica Dimensions. *Acta Protozool.*, 49: 235-244.
- Feng, M., W. Zhang, W. Wang, G. Zhang, T. Xiao and H. Xu. 2015. Can tintinnids be used for discriminating water quality status in marine ecosystems? *Mar. Pollut. Bull.*, 101(2): 549-555.
- Gaus, M., R. Mohanraju and M. Madhupratap. 1996. Studies on macrozooplankton from central and eastern Arabian Sea. *Curr. Sci.*, 71: 874-877.
- Godhantaraman, N. 2002. Seasonal variations in species composition, abundance, biomass and estimated production rates of tintinnids at tropical estuarine and mangrove waters, Parangipettai, southeast coast of India. *Indian J. Mar. Sci.*, 36: 161-171.
- Ismael, A. A. and M. M. Dorgham. 2003. Ecological indices as a tool for assessing pollution in El-Dekhaila Harbour (Alexandria, Egypt). *Oceanol.*, 45: 121-131.
- Jiang, Y., H. Xu, K. A. S. Al-Rasheid, A. Warren and X. Hu. 2011. Planktonic ciliate communities in a semi-enclosed bay of Yellow Sea, northern China: annual cycle. *J. Mar. Biol. Assoc. U.K.*, 91: 97-105.
- Jyothibabu, R., N. V. Madhu, P. A. Maheswaran, C. R. Asha Devi, T. Balasubramanian and K. K. C. Nair. 2006. Environmentally-related of symbiotic associations of heterotrophic dinoflagellates with cyanobacteria in the Bay of Bengal. *Symbiosis*, 42:51- 58.
- Kamiyama, T. and M. Tsujino. 1996. Seasonal variation in the species composition of tintinnid ciliates in Hiroshima Bay, the Seto Inland Sea of Japan. *J. Plankton Res.*, 18: 2313-2327.
- Khan, R. A. 1995. Ecology of the Hugli-Matla estuarine system, Estuarine Ecosystem series, Part 2: Hugli-Matla estuary, 417-464.
- Kofoed, C. A. and A. S. Campbell. 1929. A conspectus of the marine and freshwater Ciliata belonging to the sub-order Tintinninea with descriptions of new species principally from the Agassiz Expedition to the eastern tropical Pacific, 1904-1905. University of California Publication in Zoology, 1-403.
- Kofoed, C. A. and A. S. Campbell. 1939. Reports on the scientific results of the expedition to the Eastern tropical Pacific. *The Ciliata: The Tintinninea*, Bulletin of the Museum of Comparative Zoology, Harvard, 1-473.
- Kršini, F. 2010a. Tintinnids (Tintinnida, Choreotrichia, Ciliata) in the Adriatic Sea, Mediterranean. Part I. Taxonomy. Split, Croatia: Institute of Oceanography and

- Fisheries; Dalmacija papir, Split. kurzer Diagnose der neuen Arten. *Ergebn. Atlant. Ozean Planktonexped. Humboldt-Stift*, 3:1-33.
- Müller, H. and W. Geller. 1993. Maximum growth rates of aquatic ciliate protozoa: The dependence on body size and temperature reconsidered. *Archiv. fuer Hydrobiol.*, 126: 315–327.
- Muylaert, K., R. Van Mieghem, K. Sabbe, M., Tackx and W. Vyverman. 2000. Dynamics and trophic roles of heterotrophic protists in the plankton of a freshwater tidal estuary. *Hydrobiol.*, 432: 25–36.
- Naha Biswas, S., N. Godhantaraman, D. Rakshit and S. K. Sarkar. 2013. Community composition, abundance, biomass and productive rates of Tintinnids (Ciliata: Protozoa) in the coastal regions of Sundarban Mangrove wetland, India. *Indian J. Geo- Mar. Sci.*, 42(2): 163-173.
- Rajasegar, M. 2003. Physico-chemical characteristics of the Vellar estuary in relation to shrimp farming. *J. Environ. Biol.*, 24: 95-101.
- Rakshit, D., S. N. Biswas, S. K. Sarkar, B. D. Bhattacharya, N. Godhantaraman and K. K. Satpathy. 2014. Seasonal variations in species composition, abundance, biomass and production rate of tintinnids (Ciliata: Protozoa) along the Hooghly (Ganges) River Estuary, India: A multivariate approach. *Environ. Monit. Assess.*, 186 (5): 3063–3078.
- Rakshit, D., P. S. Ganesh and S. K. Sarkar. 2016. Choreotrich ciliate tintinnid (Protozoa: Ciliophora) in a tropical meso-macrotidal Estuary, eastern part of India. *Regional Stud. Mar. Sci.*, 3: 89-100.
- Ramakrishnan, R., P. Perumal and P. Santhanam. 1999. Spatio-temporal variations of hydrographical features in the Pichavaram mangroves and Mohi aqua farm, Southeast coast of India. In: Proceedings in International Seminar for Application in Hydrogeochemistry, Annamalai University, Chidambaram, Tamil Nadu, India, 197-203.
- Sokal, R. R. and J. F. Rohlf. 1981. *Biometry*. In: The principles and practice of statistics in biological research, (2nd eds.), W. H. Freeman and Company, San Francisco: 859pp.
- Strickland, J. D. H. and T. R. Parsons. 1972. *Practical Handbook of Sea Water Analysis*. Bull. No. 167. In: Fish Research Board, Canada, Ottawa.
- Verity, P. G., and C. Langdon. 1984. Relationship between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. *J. Plankton Res.*, 6:859–868.
- Wang, Y., W. Zhang, Y. Lin, W. Cao and L. Zheng. 2014. Phosphorus, Nitrogen and Chlorophyll-a Are Significant Factors Controlling Ciliate Communities in Summer in the Northern Beibu Gulf, South China Sea. *PLoS ONE*, 9(7): e101121.
- Xu, H., Y. Jiang, K. A. Al-Rasheid, S. A. Al-Farraj and W. Song. 2011a. Application of an indicator based on taxonomic relatedness of ciliated protozoan assemblages for marine environmental assessment. *Environ. Sci. Pollut. Res.*, 18: 1213–1221.
- Xu, H., W. Song, A. Warren, K. A. S. Al-Rasheid and S. A. Al-Farraj. 2008. Planktonic protist communities in a semi-enclosed mariculture pond: structural variation and correlation with environmental conditions. *J. Mar. Biol. Assoc. U.K.*, 88: 1353–1362.
- Xu, H., W. Zhang, Y. Jiang and E. J. Yang. 2014. Use of biofilm-dwelling ciliate communities to determine environmental quality status of coastal waters. *Sci. Total Environ.*, 470: 511–518.
- Ying, Y., Z. Wuchang, W. Shiwei and X. Tian. 2013. Abundance and biomass of planktonic ciliates in the sea around Zhangzi Island, Northern Yellow Sea. *Acta Ecol. Sinica*, 33: 45-51.
- Zhang, W., M. Feng, Y. Yu, C. Zhang and T. Xiao. 2012. *An Illustrated Guide to Contemporary Tintinnids in the World*. Science Press, Beijing (In Chinese).