Ocean Sci. J. (2018) 53(4):707–718
http://dx.doi.org/10.1007/s12601-018-0048-5

Article

Available online at http://link.springer.com
SpringerLink

Ocean Science Journal

pISSN 1738-5261
eISSN 2005-7172

Seasonal Shift in Community Structure of Periphytic Ciliates in Estuarine Waters in the Northern Bay of Bengal, Bangladesh

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Received 28 January 2018; Revised 21 June 2018; Accepted 22 July 2018

Abstract – To investigate the seasonal heterogeneity of the periphytic ciliate communities, a 1-year baseline survey was conducted in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh. A total of 54 ciliate species were recorded, including seven common and 14 dominant species. Maximum species number was in autumn whereas maximum abundance was in winter; the minimum for both occurred in summer. Multivariate analyses, i.e., canonical analysis of principal coordinates (CAP) and principal co-ordinates analysis (PCoA), revealed a clear seasonal heterogeneity of community structure and environmental variables. Multivariate correlation analysis (RELATE) demonstrated that the community structure of the periphytic ciliate communities was significantly correlated with environmental variables, and best matching analysis (BIOENV) indicated that heterogeneity of community patterns was mainly driven by water temperature, pH, dissolved oxygen, total dissolved solids and nutrients. Species richness and diversity peaked in autumn whereas species evenness peaked in summer. These results suggest that environmental conditions shape periphytic ciliate community structure, which is a potentially useful bio-indicator of estuarine water quality.

Keywords – community structure, estuarine habitats, Northern Bay of Bengal, periphytic ciliates, seasonal heterogeneity

1. Introduction

Ciliated protists (ciliates) are top predators in many microbial food webs and play an important role in transferring energy and materials from one trophic level to another in aquatic ecosystems (Corliss 2002; Morin et al. 2008; Xu et al. 2014, 2016; Zhong et al. 2017). Furthermore, due to their short life cycles, rapid generation times, high abundance, ubiquitous distribution, ease of collection and manipulation, and sensitivity to pollutants, ciliates are widely accepted as useful indicators of environmental quality (Corliss 2002; Xu et al. 2002, 2014; Kathol et al. 2009; Kchaou et al. 2009; Jiang et al. 2011; Payne 2013; Xu and Xu 2016). Properties of ciliate communities such as taxonomic distinctness, taxonomic/functional diversity, and body-size distinctness have been used as bio-indicators in ecological and monitoring research for about 100 years (Yang et al. 2016).

Periphytic ciliates colonize a wide variety of surfaces in aquatic ecosystems (Xu et al. 2002, 2009a, 2009b, 2011; Norf et al. 2007). Analysis of periphytic communities attached to natural substrates such as submerged plant material and inanimate objects is, however, problematic because such substrates are almost invariably opaque and render the ciliates difficult to observe by light microscopy. Consequently, artificial substrates such as glass microscope slides methods are commonly used for analysing periphytic ciliate communities following their immersion for a period of time to allow colonization to take place. The attached and surface-associated ciliates can then be observed, identified and enumerated *in vivo* using a light microscope (Gong et al. 2005; Xu et al. 2009a, 2009b, 2011). Several studies have demonstrated that community-based parameters (e.g., community structure, colonization dynamics) of periphytic ciliates and other protists

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*Corresponding author. E-mail: xuhl@ouc.edu.cn can be used for bio-assessment in both lentic and lotic ecosystems worldwide (Xu et al. 2002, 2014; Ismael and Dorgham 2003; Zhong et al. 2014, 2017; Wang and Xu 2015; Xu and Xu 2016; Abdullah et al. 2017).

Bangladesh coastal waters of the Bay of Bengal are subject to a wide range of environmental threats including climate change and pollution. Nevertheless, there is little knowledge and understanding of the effects of such threats to local biodiversity and ecosystem function.

In the present study, a 1-year baseline survey was conducted in estuarine waters in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh from January to December, 2017. The aims of the study were; (1) to analyse the community structure of the periphytic ciliate communities in the study area; (2) to reveal the seasonal patterns of periphytic ciliate communities in the estuary; and, (3) to assess the utility of periphytic ciliate community structure for assessing environmental quality status in an estuarine habitat.

2. Material and Methods

Study area selection

Samples were collected from the Karnaphuli River estuary (22°14.48'N–91°49.27'E: Garmin GPS-60), northern Bay of Bengal, Bangladesh (Fig. 1). The study area is ~5 m deep with a tidal interval of ~3 m, and, due to high siltation rates,

Fig. 1. Map of the sampling station in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh

average diaphaneity is less than 1 meter. The Karnaphuli River is one of the major rivers that discharge into the Bay of Bengal. Due to rapid industrialization and urbanization along the river, several types of pollutants discharge directly into the Karnaphuli estuary. Furthermore, there are several large industrial infrastructures in the Karnaphuli River estuarine area itself including oil refineries, cement clinkers, and an export processing zone. Recent studies have reported that the estuarine area is now a pollution hotspot and poses a significant threat to local aquatic biodiversity (Hossain et al. 2015; Islam et al. 2017).

Sample collection

Periphytic ciliates were collected following Xu et al. (2011). A PVC frame holding 20 glass slides (2.5 × 7.5 cm) was immersed for 14 days at a depth of 1 m below the water surface. The slides were collected along with *in situ* water and stored in a cooling box before being transported to the laboratory. Identification and enumeration of the periphytic ciliate communities were carried out as soon as possible and within 2-3 hours of collection (Xu et al. 2011). Samples were collected four times during the 12-month period of study (Jan. to Dec. 2017), one for each season.

Environmental variables measured *in situ* included: water temperature (Celsius scale thermometer); salinity, (refractometer); pH, DO and conductivity (WTW multi 3500i sensor); and transparency (transparent scale). Other variables were measured in the laboratory following standard methods (APHA 1992). For these, 1 litre of water was collected at 1 m depth and stored in a cooling box at ~20°C in the dark. The variables measured included water nitrite-nitrogen (N), soluble reactive phosphate (P), total dissolved solids (TDS) and water hardness.

Species identification and enumeration

Species identification and enumeration were carried out following the methods outlined by Xu et al. (2011). Taxonomic classification follows Song et al. (2009). Enumeration of periphytic ciliates was conducted *in-vivo* at 100-400X magnification using an inverted microscope. Species composition and abundances of periphytic ciliates are expressed as a percentage of the total species richness (%) and individuals per cm^2 (ind./cm^2) from randomly selected five glass slides in each sampling occasion, respectively.

Data analysis

The species diversity (Shannon-Winner, H'), species evenness (Pielou's, J') and species richness (Margalef, D) indices were used to summarize the biodiversity of the periphyic ciliate communities. These measures were computed using PRIMER package (v7.0.13) following the equations:

$$H' = -\sum_{i=1}^{S} Pi(\ln Pi)$$

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

$$D = (S-1)/\ln N$$

where, Pi = proportion of the total counted arising from the ith species; S = total number of species and N = total number of individuals.

Species contributions among the four seasons and within each season were computed using SIMPER analysis from square root-transformed species abundance data, whereas species distributions among the four seasons were summarized using clustering analysis on the matrix of 'index of association' from standardized presence/absence data (Anderson et al. 2008; Clarke and Gorley 2015). Shade plotting analysis was used to summarize the species composition among four seasons in terms of relative abundance. The seasonal heterogeneity of periphytic ciliate community structure was summarized using the sub-module CAP (canonical analysis of principal coordinates) on Bray-Curtis similarity matrices from square root-transformed species abundance data (Anderson et al. 2008; Xu et al. 2011). Seasonal variability of environmental variables was summarized using the sub-module PCoA (principal coordinates) from Euclidean distance on log(x+1) transformed/normalized data (Anderson et al. 2008). Multivariate correlation was analyzed by Mantel analysis (RELATE) and the relationships between biotic and abiotic parameters were determined using best matching analysis (BIOENV) in PRIMER v.7.0.13 + PERMANOVA add on (Anderson et al. 2008; Clarke and Gorley 2015).

Univariate analysis of Pearson correlation was conducted in order to identify potential environmental variables for seasonal heterogeneity of periphytic ciliates from log-transformed data.

3. Results

Seasonal variation in environmental variables

The average values of the environmental variables are shown in Table 1. Water temperature varied from 19 to 27°C; pH from 6.98 to 7.11; water transparency from 31 to 45 cm; water salinity from 7.5 to 11.5 psu; dissolved oxygen

Table 1. The seasonal variation in environmental parameters in the Karnaphuli River estuary, northern Bay of Bengal in Bangladesh. (Average ± Standard deviation)

Parameters	Winter	Spring	Summer	Autumn
WT (°C)	19 ± 0.71	25.5 ± 0.35	27 ± 0.35	27 ± 0.35
pH	7.11 ± 0.01	7.01 ± 0.01	6.98 ± 0.01	7.10 ± 0.01
Trans (cm)	45 ± 0.71	43 ± 0.35	39 ± 0.35	31 ± 0.71
Sal (psu)	11.5 ± 0.35	11 ± 0.01	9 ± 0.02	7.5 ± 0.07
DO (mg/l)	5.44 ± 0.01	5.88 ± 0.01	5.21 ± 0.01	4.98 ± 0.02
TDS (mg/l)	7.9 ± 0.01	9.11 ± 0.01	8.99 ± 0.01	7.55 ± 0.02
Cond	13 ± 0.35	11 ± 0.01	17 ± 0.35	14 ± 0.35
N (mg/l)	0.97 ± 0.01	0.75 ± 0.01	0.91 ± 0.01	0.84 ± 0.01
P (mg/l)	0.39 ± 0.01	0.33 ± 0.01	0.45 ± 0.01	0.41 ± 0.01
Hardness (mg/l)	550 ± 1.41	510 ± 7.07	432 ± 0.71	495 ± 0.71

WT, water temperature; Trans, Transparency; Sal, salinity; DO, dissolved oxygen; TDS, Total dissolved solids; Cond, Conductivity; N, nitrite-nitrogen; P, water soluble phosphate

from 4.98 to 5.88 mg/l; total dissolved solid from 7.55 to 9.11 mg/l; conductivity from 11 to 17; nitrite-nitrogen from 0.75 to 0.97 mg/l; phosphate-phosphorus from 0.33 to 45 mg/l; and water hardness from 432 to 550 mg/l (Table 1).

Taxonomic composition and species distribution

A total of 54 periphytic ciliate species were identified. Of these, seven were present in all four seasons and were defined as 'common' while 14 species that contributed to the top 10 ranked contributors in each of the four seasons by SIMPER analysis were defined as 'dominant'. It should be noted that three species occurred in only winter, four only in spring, four only in summer and one only in autumn. These were defined as 'endemic species with season'. The list of identified species, their average abundances, frequency of occurrence and distribution among four seasons are summarized in Table 2.

Based on species occurrences (present/absent), 33 species were present in winter, 34 in spring, 30 in summer and 33 in autumn (Fig. 2). In terms of relative abundance, 11 species were dominant in winter, 11 in spring, 14 in summer and 12 species in autumn and the contribution of each species to the total community (index of association) was > 25% (Fig. 2).

Seasonal heterogeneity of community patterns

In terms of species number and total abundance, periphytic ciliate communities revealed a clear seasonal heterogeneity (Fig. 3). For example, species numbers peaked in autumn and fell in winter but were similar in spring and summer (Fig. 3a). Highest abundance was recorded in winter and lowest was in summer (Fig. 3b). In terms of relative abundances, there was a clear seasonal heterogeneity of species succession (Fig. 4a). For example, among the 14 dominant species, two types of community patterns were identified; (1) *Epicarchesium abrae*, *Epistylis clampi* and *Pseudovorticella marina* decreased from winter to autumn whereas *Agnathodysteria littoralis*, *Conchacineta complatana*, *Loxophyllum jini*, *Pseudovorticella cylindrica*, *Zoothamnium marinum* and *Zoothamnium sinense* increased; (2) *Epicarchesium variable* and *Zootamnium parahentscheli* were primary contributors in spring and winter, respectively, while *Amphileptus aeschtae* and *Zoothamnopsis mengi* were primary contributors in winter and summer, respectively (Fig. 4b).

CAP analysis revealed seasonal variations among the periphytic ciliate communities (Fig. 5). The first axis of CAP (CAP1) separated the winter and spring samples (left) from those of summer and autumn (right). The second axis (CAP2) separated the winter and autumn samples (upper) from those of spring and summer (lower) (Fig. 5a).

Vector overlay of the 14 dominant species with CAP axis revealed that vectors of six species (i.e., *Zoothamnium marinum*, *Z. parahentscheli*, *Zoothamnopsis mengi*, *Epistylis clampi*, *Pseudovorticella marine* and *Epicarchesium abrae*) pointed towards the winter sample cloud, four species, (i.e., *Zoothamnium paraentzii*, *Z. sinense*, *Pseudovorticella cylindrica* and *Conchacineta complatana*) pointed towards the autumn sample cloud, three species (i.e., *Loxophyllum jini*, *Amphileptus aeschtae* and *Agnathodysteria littoralis*) pointed towards the summer sample cloud and one species, *Epicarchesium variable,* pointed towards the spring sample cloud (Fig. 5b).

The seasonal heterogeneity of biodiversity indices of the periphytic ciliate communities is summarized in Figure 6.

Table 2. List of periphytic ciliate communities recorded in estuarine waters in the northern Bay of Bengal, Karnaphuli estuary in Bangladesh including their average abundance, frequency of occurrences and distribution in four seasons during the study period

Species	Winter			Spring			Summer			Autumn		
	N	%	Dist	*N*	%	Dist	*N*	%	Dist	*N*	%	Dist
Agnathodysteria littoralis	+	**0**	–	+	**8.70**	+	+	**21.74**	+	++	**69.57**	+
Amphileptus aeschtae	+	**0**	–	+	**45.45**	+	+	**27.27**	+	+	**27.27**	+
Amphileptus houi	++	40.74	+	+	0	–	+	0	–	++	59.26	+
Apotrachelotractus variabialis	++	24.44	+	++	48.89	+	++	26.67	+	+	0	–
Chaenea vorax	++	80.95	+	+	0	–	+	0	–	+	19.05	+
Chlamydonella derouxi	+	0	–	+	0	–	+	36.36	+	+	63.64	+
Conchacineta complatana	+	**12**	+	+	**20**	+	+	**24**	+	++	**44**	+
Cyclotrichium taniguchii	+	0	–	+	0	–	+	100	+	+	0	–
Dysteria magna	++	87.5	+	+	0	–	+	0	–	+	12.5	+
Ephelota crustaceorum	++	92.59	+	+	0	–	+	0	–	+	7.41	+
Epicarchesium abrae	++	**61.90**	+	+	**23.81**	+	+	**14.29**	+	+	**0**	–
Epicarchesium variable	+	**12**	+	++	**68**	+	+	**0**	–	+	**20**	+
Epistylis clampi	++++	**70**	+	++	**12.73**	+	++	**17.27**	+	+	**0**	–
Flectacineta livadiana	+	0	–	+	100	+	+	0	–	+	0	–
Frontonia sinica	++	84.62	+	+	0	–	+	15.38	+	+	0	–
Hartmannula angustipilosa	+	0	–	+	100	+	+	0	–	+	0	–
Holophrya oblonga	+	0	–	+	0	–	+	100	+	+	0	–
Kentrophyllum verrucosum	+	0	–	+	0	–	+	0	–	+	100	+
Lacrymaria marina	+	27.27	+	+	27.27	+	+	0	–	+	45.45	+
Litonotus bergeri	+	42.86	+	+	0	–	+	0	–	+	57.14	+
Litonotus guae	+	11.11	+	+	0	–	+	88.89	+	+	0	–
Litonotus paracygnus	++	55.56	+	+	7.41	+	+	11.11	+	+	25.93	+
Loricophrya parva	+	0	–	+	0	–	+	100	+	+	0	–
Loxophyllum jini	+	**0**	–	+	**19.23**	+	+	**11.54**	+	++	**69.23**	+
Loxophyllum qiuianum	+	0	–	++	33.33	+	++	45.45	+	+	21.21	+
Loxophyllum rostratum	+	7.143	+	+++	55.71	+	++	18.57	+	++	18.57	+
Loxophyllum sinicum	+	0	–	+	8.33	+	+	0	–	++	91.67	+
Orthodonella apohamatus	++++	84.54	+	+	2.06	+	+	0	–	++	13.40	+
Orhtodonella gutta	+	0	–	+	0	–	+	100	+	+	0	–
Paramucophrya semisphaerica	+	0	–	+	23.53	+	+	58.82	+	+	17.65	+
Paraspathidium apofuscum	+	0	–	+	0	–	+	0	–	+	100	+
Paratetrahymena wassi	+	0	–	+	0	–	+	0	–	+	100	+
Pseudovorticella banatica	+	0	–	+	40	+	+	60	+	+	0	–
Pseudovorticella cylindrica	++	**34.38**	+	+	**15.63**	+	+	**15.63**	+	++	**34.38**	+
Pseudovorticella difficilis	+	0	–	+	0	–	+	28.57	+	+	71.43	+
Pseudovorticella lima	+	0	–	+	100	+	+	0	–	+	0	–
Pseudovorticella marina	+++	**63.08**	+	++	**20**	+	+	**12.31**	+	+	**4.62**	+
Pseudovorticella sinensis	++	70.83	+	+	29.17	+	+	0	–	+	0	–
Spirotontonia turbinata	+	0	–	+	44.44	+	+	22.22	+	+	33.33	+
Thuricola parafolliculata	+	0	–	+	0	–	+	33.33	+	+	66.67	+
Trachelotractus entzi	+	0	–	+	0	–	+	57.14	+	+	42.86	+
Trochilia petrani	+	0	–	+	100	+	+	0	–	+	0	–
Zoothamnium alrasheidi	+	22.22	+	++	77.78	+	+	0	–	+	0	–
Zoothamnium alternans	++	78.57	+	+	21.43	+	+	0	–	+	0	–
Zoothamnium commune	+	55.56	+	+	44.44	+	+	0	–	+	0	–
Zoothamnium dichotomum	+	23.53	+	+	35.29	+	+	0	–	+	41.18	+
Zoothamnium duplicatum	+	40	+	+	20	+	+	0	–	+	40	+
Zoothamnopsis liui	++	78.57	+	+	0	–	+	21.43	+	+	0	–
Zoothamnium marinum	+++	**29.21**	+	++	**25.84**	+	++	**20.22**	+	++	**24.72**	+
Zoothamnium maximum	+++	53.97	+	+++	46.03	+	+	0	–	+	0	–
Zoothamnopsis mengi	++	**55.56**	+	+	**22.22**	+	+	**22.22**	+	+	**0**	–
Zoothamnium paraentzii	++	**47.83**	+	+	**0**	–	+	**34.78**	+	+	**17.39**	+
Zoothamnium parahentscheli	+++	**84.85**	+	+	**15.15**	+	+	**0**	–	+	**0**	–
Zoothamnium pararbuscula	+	100	+	+	0	–	+	0	–	+	0	–
Zoothamnium sinense	++	**23.17**	+	++	**19.51**	+	++	**18.29**	+	+++	**39.02**	+
Zoothamnium xuianum	++	73.08	+	+	0	–	+	0	–	+	26.92	+

Text bold, dominant species; %, frequency of occurrences; Dist, Distribution (+, present; -, absent); *N*, abundance (ind./cm^2): > 150, +++++, 100-149, ++++, 50-99, +++, 49-10, ++, 9-0, +

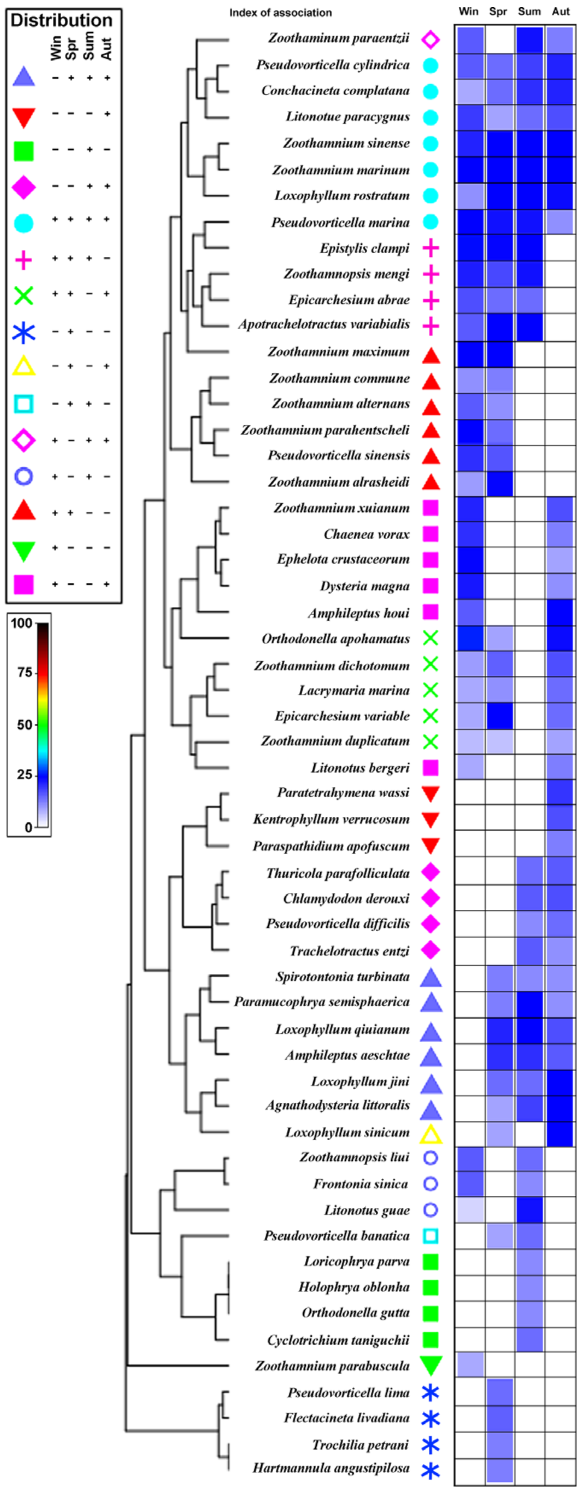

Fig. 2. Shade plot of the species distribution using group-average clustering on Bray-Curtis similarities on square root-transformed/standardized abundance data of each species within the periphytic ciliate communities among four seasons in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh. (Color shade 0–100, relative abundance; +, present; -, absent; Win, winter; Spr, spring; Sum, summer, Aut, autumn)

Highest species richness and diversity were in autumn (Fig. 6a, c) whereas highest species evenness was in summer (Fig. 6b). All the three indices were lowest in winter (Fig. 6).

Heterogeneity of community structure relation with environmental variables

The interrelationship between environmental variables and heterogeneity of community structure among four seasons were summarized by principal coordinates (PCoA) analysis on Euclidean distance from log(x+1) transformed/normalized data (Fig. 7). PCoA analysis revealed the heterogeneity of the environmental variabilities along with the periphytic ciliate communities for each season. The first axis of PCoA, PCoA1 accounted for 69.3% of total variation while PCoA2 accounted for 18.4% (Fig. 7a). Vector overlay of the environmental variables and community parameters of the periphytic ciliate communities revealed that abundance was significantly correlated with salinity, dissolved oxygen, transparency, hardness and nitrite-nitrogen and pointed towards winter, whereas species richness, evenness and diversity were correlated with water temperature and pointed towards autumn (Fig. 7b).

Multivariate correlation of community structure and environmental variables revealed a significant correlation between the periphytic ciliate community structure and environmental variables (correlation coefficient, $\rho = 0.357$, statistical significant level at 0.05).

Multivariate analysis of biota-environment (BIOENV) analysis revealed that the heterogeneity of community structure of periphytic ciliate communities was mainly driven by water temperature, DO, pH, and TDS, either individually or combined with salinity, transparency and water nutrients (Table 3). Species richness, evenness and diversity correlated with water temperature in autumn, whereas total abundance correlated with water transparency, salinity conductivity and phosphate in winter (Table 3).

Univariate Pearson correlation showed that community parameters and dominant species of periphytic ciliate communities were significantly correlated with water temperature, salinity, transparency, dissolved oxygen, nitrite and phosphate (Table 4). For example, of the 14 dominant species four (i.e., *Epicarchesium abrae, Epistylis clampi, Pseudovorticella marina* and *Zoothamnium parahentscheli*) were negatively correlated with water temperature; three (i.e., *Epistylis clampi, Pseudovorticella marina* and *Zoothamnium parahentscheli*) were positively correlated

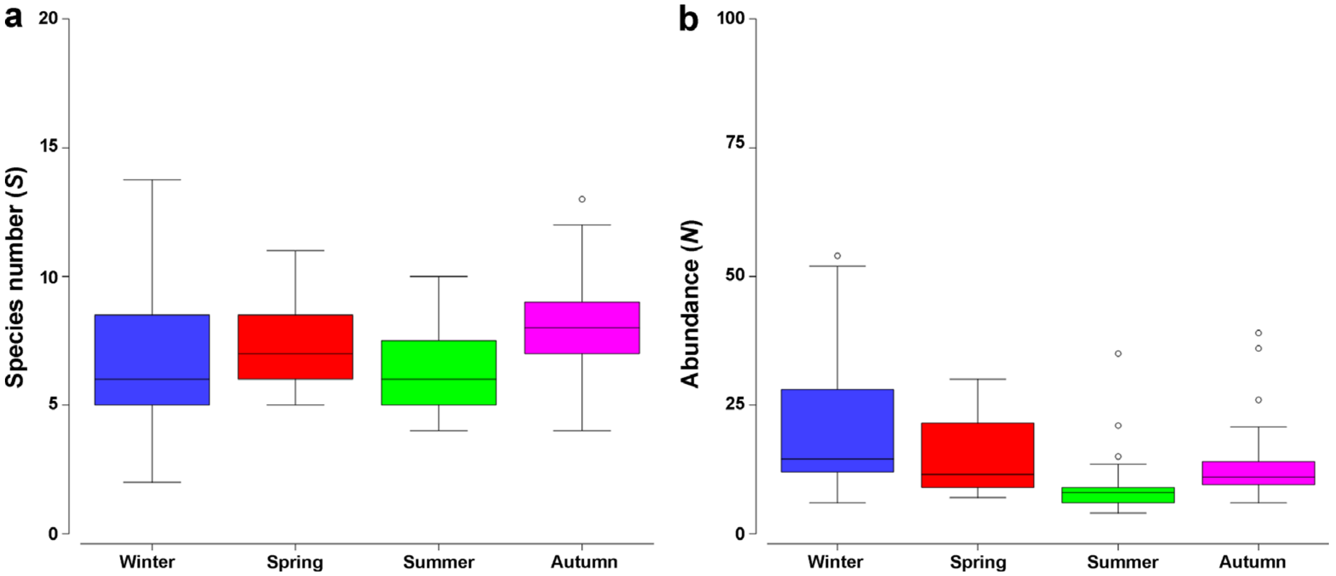

Fig. 3. Seasonal heterogeneity of species number (a) and abundance (b) of periphytic ciliate communities in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh

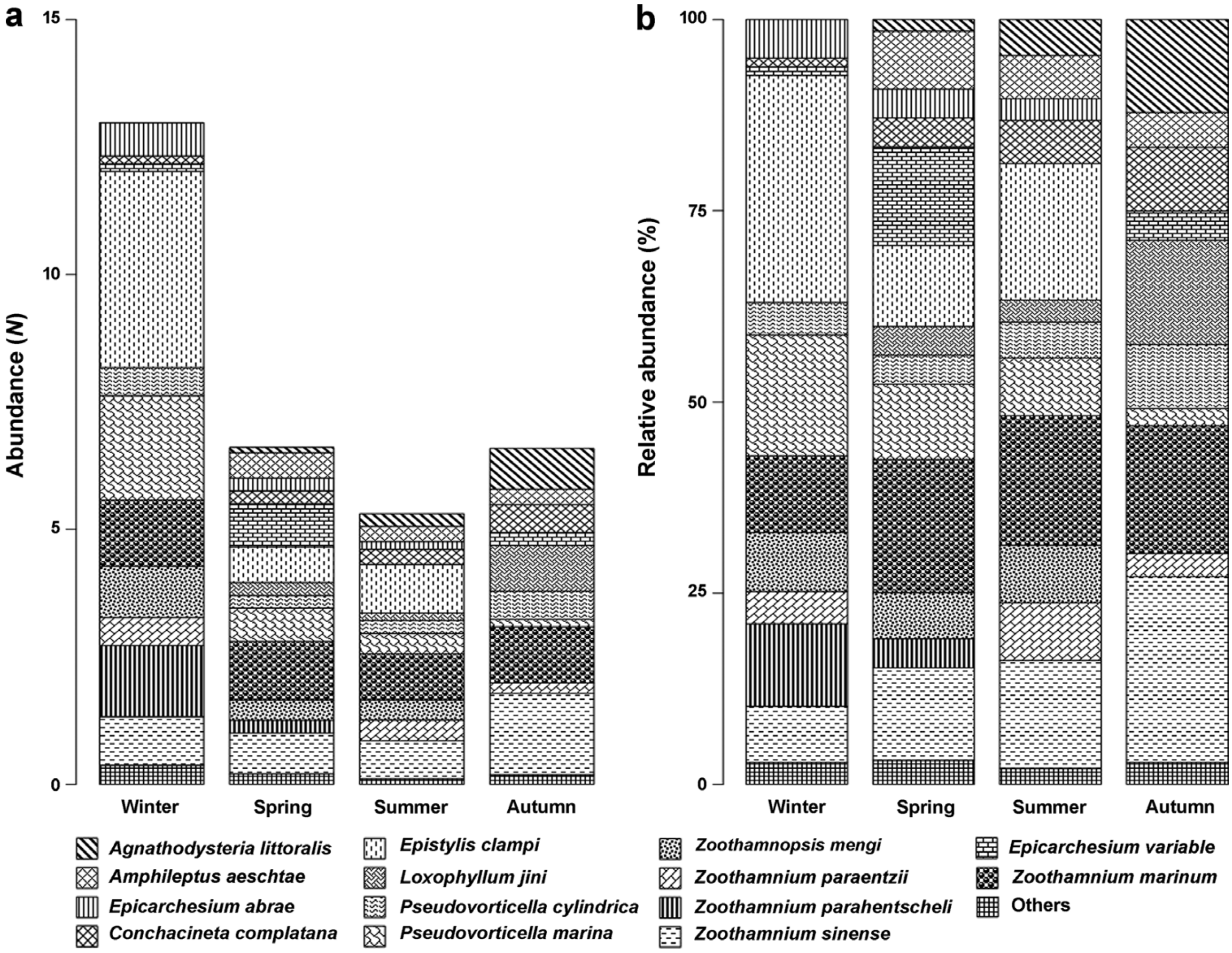

Fig. 4. Seasonal heterogeneity of abundance (a) and relative abundance (b) of 14 dominant species of periphytic ciliate communities in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh

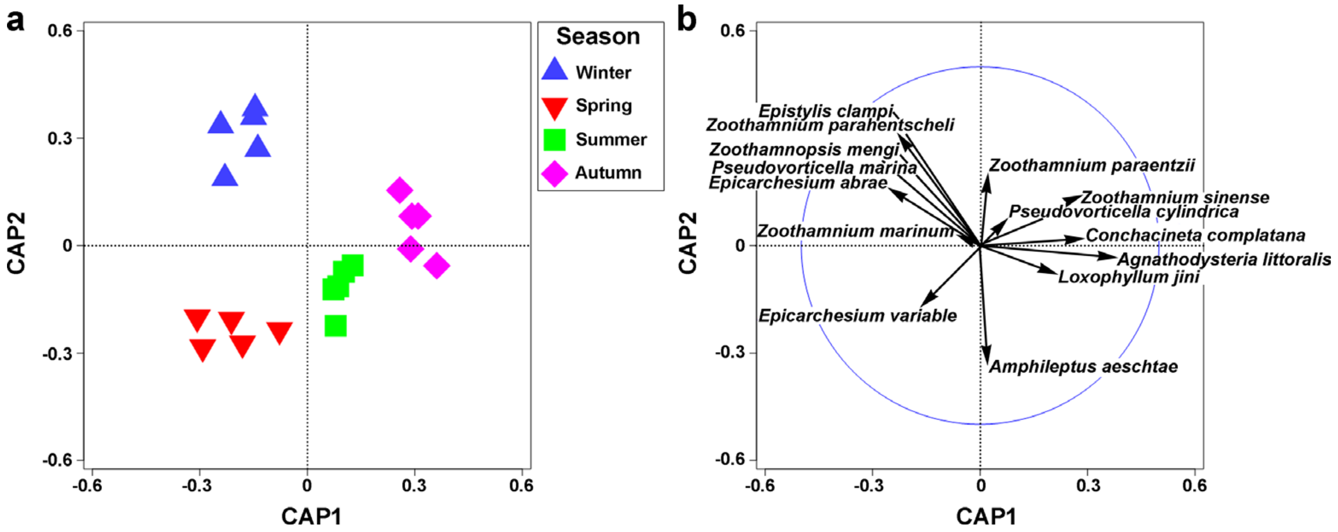

Fig. 5. Canonical analysis of principal coordinates (CAP) on Bray-Curtis similarities from square root-transformed species abundance data of periphytic ciliate communities (a), with correlations of the 14 dominant species with the CAP axes (b), showing the seasonal heterogeneity of community patterns

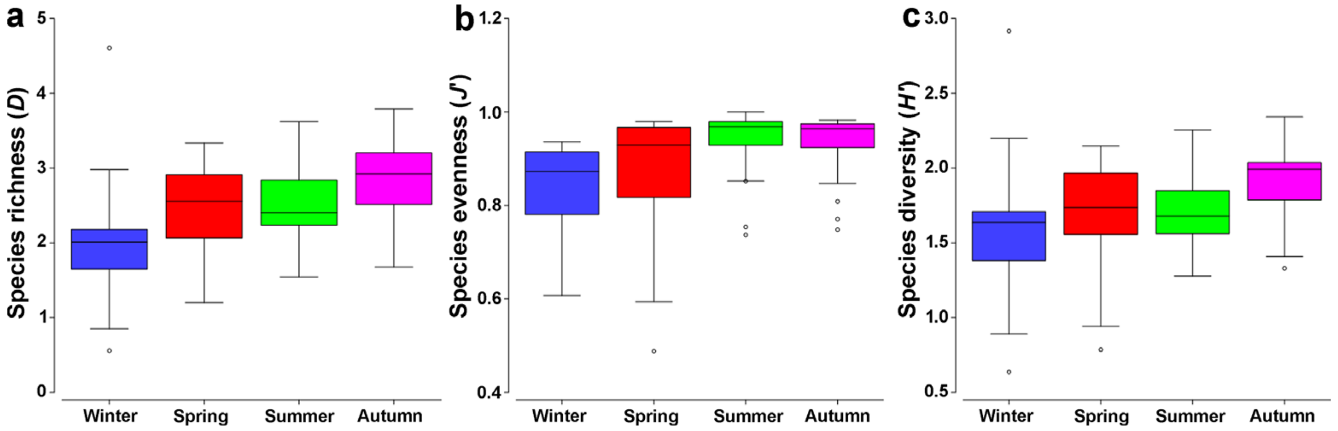

Fig. 6. Species richness (a), species evenness (b) and species diversity (c) of periphytic ciliate communities among four seasons in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh

with DO and water nitrite; one (*Zoothamnium marinum*) was positively correlated with transparency and salinity; and one (*Amphileptus aeschtae*) was negatively correlated with phosphate (Table 4). Among the community parameters, abundance showed significantly positive correlation with transparency and salinity, while species richness was positively correlated with water temperature (Table 4).

4. Discussion

Community based parameters e.g., abundance, species richness, evenness and diversity, are widely used to describe the ecological status or environmental quality of aquatic ecosystems (Ismael and Dorgham 2003; Xu et al. 2009a; Zhang et al. 2012; Abdullah Al et al. 2017; Wang et al. 2017). Heterogeneity of these parameters is often strongly correlated with habitat features such as water parameters, available food supply and light intensity, both on temporal and spatial scales (Xu et al. 2002, 2009a, 2009b; Zhang and Xu 2015; Abdullah et al. 2017). Several studies using glass slides as artificial substrates have reported that community parameters of periphytic ciliates can be accurately determined only with sufficient sampling size and effort (Xu et al. 2002, 2009b; Xu et al. 2011; Zhang and Xu 2015).

In the present study, a total of 54 species of periphytic ciliates were identified from a total of 80 glass slides that

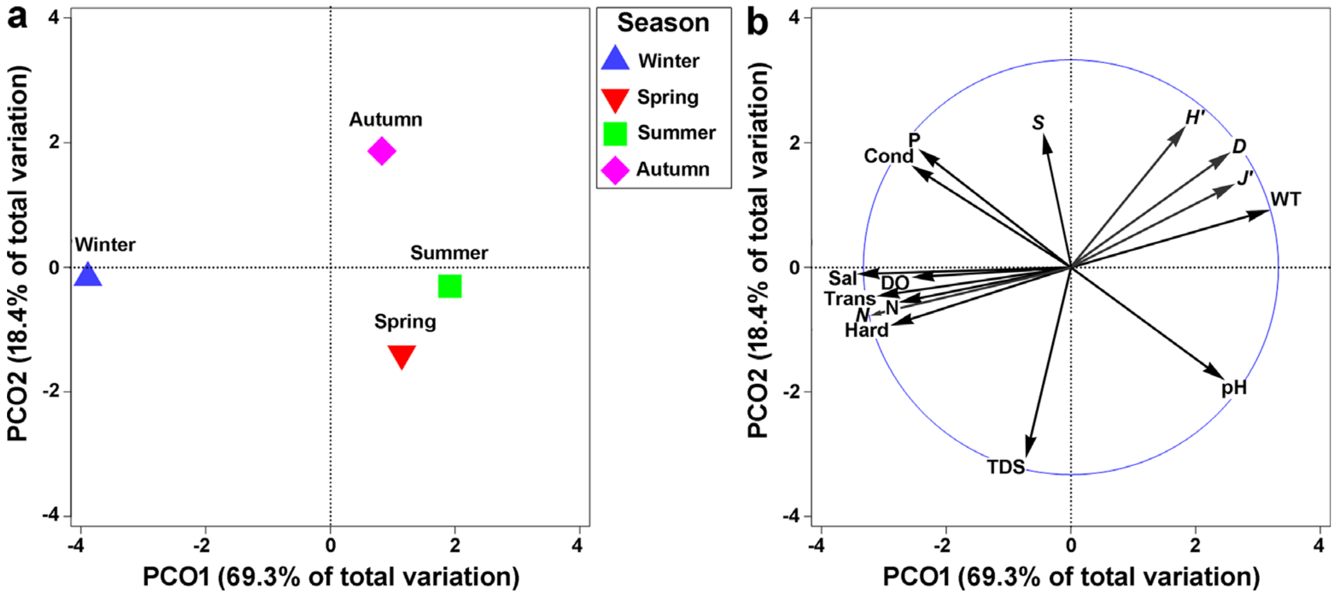

Fig. 7. Principal coordinates (PCoA) on Euclidean distance from log(x+1)/normalized-transformed environmental variables data (a), with correlations of the five community parameters with the PCoA axes (b), which together show the seasonal heterogeneity of community structure of periphytic ciliate communities with environmental variables in the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh

Table 3. Summary results from biota-environment best matching analysis (BIOENV) showing the 10 best matches of environmental variables with seasonal variations in the periphytic ciliate communities abundances during four seasons in estuarine waters in the northern Bay of Bengal, Karnaphuli estuary in Bangladesh during the study period

Rank	ρ value	Environmental variables
1	0.943	WT, pH, DO, N, TDS
2	0.943	WT, TDS, Cond
3	0.943	pH, Sal, TDS
4	0.943	Sal, TDS, Cond
5	0.943	TDS, Cond, Hardness
6	0.943	WT, pH, Trans, TDS
7	0.886	pH, TDS, N
8	0.886	pH, TDS, Hardness
9	0.829	WT, Trans, TDS
10	0.829	WT, Sal, TDS

See table 2 for all others elaborations. ρ value, Spearman correlation coefficient. Statistical significant level at 0.05 ($P < 0.05$)

were used as artificial substrates for seasonal (20 slides per season) sampling of the Karnaphuli River estuary, northern Bay of Bengal, Bangladesh. The species richness here was higher than that reported by Persoone (1968), who recorded 30 species in the Ostend harbor in Belgium, or by Gong et al. (2005) who reported 37 species in the Jiaozhou Bay, northern China. By contrast, Agamaliev (1974) reported 130 species in the Caspian Sea and Zhang and Xu (2015) identified 144 species in Jiaozhou Bay. There are likely to be several reasons for these differences including geographical locations, climate, environmental conditions and sampling methods employed. The Karnaphuli River estuary Bangladesh, for example, is heavily contaminated by various industrial effluents resulting in water with reduced pH and DO and increased BOD and metals (Hossain et al. 2015; Islam et al. 2017). These factors are likely to have resulted in a lower ciliate species count than might otherwise be expected.

Based on species distribution and abundances, clustering analysis revealed that highest species richness occurred in spring whereas maximum abundance was recorded in winter. The minimum of both was in summer. Furthermore, dominant species were significantly correlated with water parameters such as temperature, dissolved oxygen and nutrients. For example, among the 14 dominant species, four species were negatively correlated with water temperature, three were positively correlated with DO and nitrite-nitrogen and one was positively correlated with salinity and transparency and negatively with phosphate, respectively. These findings are consistent with those of Zhang and Xu (2015) who reported that species richness of periphytic ciliates peaked in either spring or autumn, correlating with water temperature, pH, DO and nutrients. Thus, these findings indicated that periphytic ciliates are potentially useful bio-indicators for monitoring water quality of estuarine ecosystems.

Table 4. Pearson correlations between average abundances of 14 dominant species, including community parameters of periphytic ciliate communities and environmental parameters during four seasons in estuarine waters in the northern Bay of Bengal, Karnaphuli estuary in Bangladesh during the study period

Species	WT	pH	Trans	Sal	DO	TDS	Cond	N	P
Agnathodysteria littoralis	.685	-.225	-.471	-.486	-.560	-.824	.166	-.598	.089
Amphileptus aeschtae	.793	.568	-.622	-.665	-.916	.224	-.593	-.893	**-.951***
Conchacineta complatana	.766	-.136	-.540	-.561	-.661	-.767	.076	-.695	-.039
Epicarchesium abrae	**-.983***	-.357	.852	.874	.923	.550	.412	.942	.438
Epicarchesium variable	.153	.027	.154	.098	-.388	.710	-.023	-.344	-.781
Epistylis clampi	**-.985***	-.394	.805	.838	**.983***	.344	.445	**.991****	.625
Loxophyllum jini	.666	-.314	-.350	-.381	-.602	-.647	.258	-.630	-.052
Pseudovorticella cylindrica	-.427	-.941	.589	.596	.525	-.450	.932	.495	.806
Pseudovorticella marina	**-.999****	-.469	.890	.913	**.960***	.448	.520	**.973***	.544
Zoothamnium marinum	-.767	-.813	**.974***	**.959***	.663	.430	.842	.679	.341
Zoothamnium mengi	-.950	-.198	.738	.768	.908	.528	.255	.926	.427
Zoothamnium paraentzii	-.648	-.212	.327	.385	.807	-.287	.237	.782	.870
Zoothamnium parahentscheli	**-.995****	-.575	.929	.949	**.962***	.388	.622	**.972***	.595
Zoothamnium sinense	.288	-.664	.010	-.008	-.190	-.699	.618	-.225	.353
Biodiversity parameters									
S	.024	-.831	.392	.354	-.048	-.224	.803	-.059	.177
N	-.897	-.795	**.994****	**.996****	.840	.343	.830	.849	.542
D	**.952***	.205	-.748	-.777	-.904	-.544	-.263	-.923	-.414
J'	.896	.505	-.945	-.942	-.764	-.693	-.553	-.793	-.228
H'	.829	-.075	-.539	-.573	-.786	-.571	.017	-807	-.274

Text bold, statistically significant values; *, Significant level at 0.05; **, Significant level at 0.01; *S*, species number; *N*, total abundance; *D*, species richness; *J'*, evenness; and *H'*, diversity. Please see table 1 for other elaborations

Multivariate CAP analysis and correlation (RELATE and BIOENV) analysis revealed that periphytic ciliate community structure correlated with water temperature, DO, salinity and transparency, either alone or in combination with nutrients (N and P). Multivariate approaches are more useful than univariate approaches for summarizing heterogeneity of community structure (Clarke and Gorley 2015; Zhang and Xu 2015; Abdullah et al. 2017). Based on these strategies, the results of the present study revealed that the periphytic ciliate community structure in the Karnaphuli River estuary was significantly shaped by water temperature, DO, salinity and transparency. This suggests that periphytic ciliate communities reflected water quality status and might be used as bio-indicators in community-based bio-assessment of estuarine habitats.

In the present study, species richness and diversity were recorded as highest in autumn and lowest in winter. Among the five community parameters, species richness was positively correlated with water temperature whereas species abundance was positively correlated with water salinity and transparency. These findings are consistent with previous reports that higher diversity indices represent better water quality as measured by levels of organic contamination (Magurran 1991; Ismael and Dorgham 2003). Biodiversity indices are therefore potentially useful bio-indictors of water quality.

In conclusion, periphytic ciliate community structure in the Kamaphuli River estuary varied significantly among the four seasons. Species richness and diversity peaked in autumn whereas species evenness peaked in summer. Community structure parameters showed clear seasonal variability in relation to environmental variables, primarily water temperature, pH, DO, TDS and nutrients. These findings suggest that the community structure of periphytic ciliates in the Kamaphuli River estuary is shaped by environmental conditions and is therefore potentially useful for assessing water quality status. Further studies on the community patterns of periphytic ciliates with a wider range of water parameters are needed in order to verify this conclusion.

Acknowledgements

This work was supported by the Chinese Scholarship

Council (CSC) under the Ministry of Education China one Excellent Master's (CSC no.: 2016GXY030) and one PhD (CSC no.: 2016GXY026) and financed by "The Natural Science Foundation of China" (project numbers: 31672308 and 41076089). We are grateful to Swan Nahid, Assistant Professor, Chittagong Veterinary and Animal Sciences University, Bangladesh for providing laboratory facilities, and Salina Sultana, Junior Instructor (Quality control), Department of Marine Fisheries, Marine Fisheries Academy (Fish Harbour Chittagong), Bangladesh for providing safety sampling station in their boat jetty. We also thank Ms. Wang Zheng for providing all necessary materials for sample collection, and Mr. Kausar Rahman, Mr. Kajemul Hasan Shahed and Mr. Rakibul Hoque for their help during sample collection.

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