Marine Pollution Bulletin 127 (2018) 726–732

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

Functional diversity of benthic ciliate communities in response to environmental gradients in a wetland of Yangtze Estuary, China

Yuan Xu[a], Xinpeng Fan[b], Alan Warren[c], Liquan Zhang[a,*], Henglong Xu[d]

[a] *State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, China*
[b] *School of Life Sciences, East China Normal University, Shanghai 200241, China*
[c] *Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK*
[d] *College of Marine Life Science, Ocean University of China, Qingdao 266003, China*

ARTICLE INFO

Keywords:
Ecosystem function
Estuarine wetland
Functional traits
Marine protozoans

ABSTRACT

Researches on the functional diversity of benthic ecosystems have mainly focused on macrofauna, and studies on functional structure of ciliate communities have been based only on trophic- or size-groups. Current research was carried out on the changing patterns of classical and functional diversity of benthic ciliates in response to environmental gradients at three sites in a wetland in Yangtze Estuary. The results showed that changes of environmental factors (e.g. salinity, sediment grain size and hydrodynamic conditions) in the Yangtze Estuary induce variability in species composition and functional trait distribution. Furthermore, increased species richness and diversity did not lead to significant changes in functional diversity due to functional redundancy. However, salt water intrusion of Yangtze Estuary during the dry season could cause reduced functional diversity of ciliate communities. Current study provides the first insight into the functional diversity of ciliate communities in response to environmental gradients.

Over the past twenty years, there has been an increasing body of literature demonstrating that classical diversity indices based on species identity and abundance provide an incomplete view of biodiversity as they do not take into account the functional differences among species (Bremner et al., 2003; Villéger et al., 2010). While functional diversity quantifies the value and range of species functional traits which affect ecosystem properties or processes (Bremner et al., 2006; Gagic et al., 2015). Studies on relationships of classical and functional diversity of macrobenthos suggest that their patterns across environmental gradients do not always follow the same trends (Paganelli et al., 2012; Villéger et al., 2010; Wan Hussin et al., 2012; Wong and Dowd, 2015). Therefore, an integrative approach combining both classical and functional diversity is likely to be more informative and provide a better understanding of general relationships between community structure and ecosystem functions (Gusmao et al., 2016; McGill et al., 2006; Villéger et al., 2011).

To date, studies on functional diversity of benthic fauna have mainly focused on the macrobenthos and the accumulation of more than ten years studies has generated a comparatively mature framework (MarLIN, 2006; Paganelli et al., 2012; Tillin et al., 2006). By contrast, little is known about the environmental factors regulating the functional diversity of ciliate communities in marine benthic ecosystems (Mazei and Burkovsky, 2006). Considering their high sensitivity to environmental change, wide distribution, large abundances, and key roles in microbial food webs, studies of their functional diversity are likely to enhance our understanding of benthic ecosystem function and improve our ability to assess environmental quality (Hamels et al., 2005).

Several pioneering studies have been carried out on trophic or body-size structure of ciliate communities (e.g. Xu et al., 2017). Although these studies have shown that functional structures can provide necessary understanding for ecological research and monitoring programs, allocating species to trophic- or size-groups reflects only one or two traits thereby limiting the information available for capturing a more comprehensive view of ecosystem function (Bremner et al., 2003). Thus, there is a need to investigate the functional diversity of ciliate communities by quantifying their functional traits following the framework suggested by Villéger et al. (2010).

The present study was carried out in the Yangtze Estuary to investigate the functional diversity of benthic ciliate communities in response to environmental gradients. The main aims were to assess: (1) how species composition and functional traits distribution of estuarine ciliate communities vary along environmental gradients; (2) how functional diversity is regulated by environmental factors, and; (3)

---

[*] Corresponding author.
E-mail address: lqzhang@sklec.ecnu.edu.cn (L. Zhang).

https://doi.org/10.1016/j.marpolbul.2017.12.068
Received 16 November 2017; Received in revised form 21 December 2017; Accepted 30 December 2017

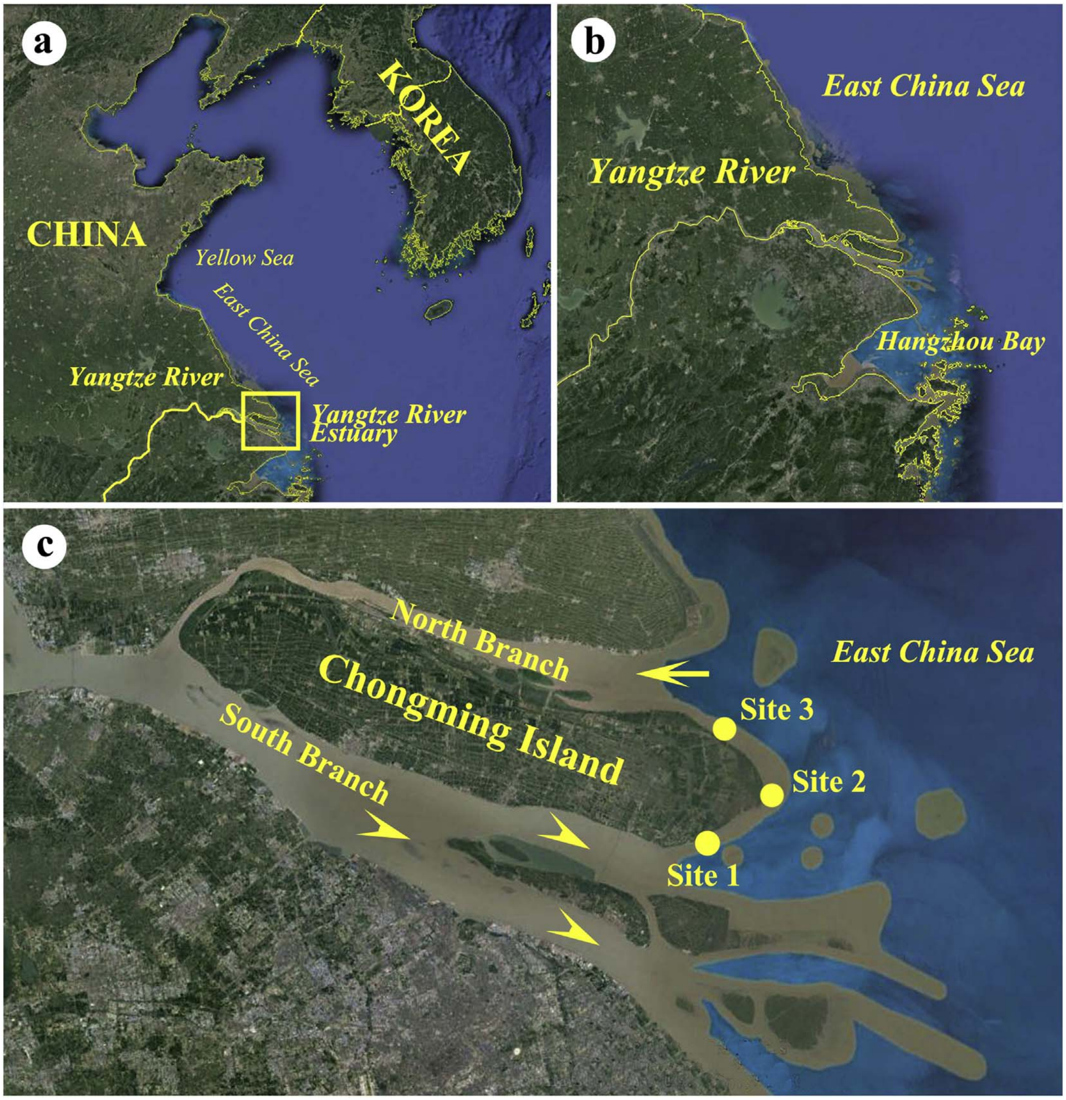

Fig. 1. Sampling sites in Yangtze Estuary, Shanghai, East China. Site 1 (31°27′1.88″N, 121°53′40.03″E) is close to the South Branch of the Yangtze Estuary and mostly influenced by the flow of fresh river water; Site 2 (31°30′35.00″N, 121°57′33.00″E) faces the East China Sea and is influenced by the mixing of fresh and salt water; Site 3 (31°34′46.00″N, 121°54′28.16″E) is close to the North Branch of the Yangtze Estuary and therefore mainly influenced by salt water. Arrowheads in (c) refer to the direction of freshwater flow coming from Yangtze River, and arrow in (c) indicates the direction of salt water intrusion from the East China Sea.

Table 1
Biological trait variables and categories used to describe functional diversity of benthic ciliate communities at Dongtan wetland on Chongming Island in the Yangtze Estuary.

Trait	Category
Feeding type	Bactivores
	Algivores
	Predators
	Parasite
Body size (measured as cell length)	Small (< 50 μm)
	Medium (50–150 μm)
	Large (> 150 μm)
Movement type	Attached to substrate
	Free-swimming
	Crawling
Respiration type	Anaerobic
	Aerobic
Body form	Dorso-ventrally flattened
	Cylindrical
Degree of flexibility	Cell nonflexible
	Cell flexible

whether combining functional and classical diversity indices can provide a deeper understanding of the relationships linking ciliate communities to their environment.

The Yangtze Estuary is influenced by a combination of fresh water discharge and tidal saline inflow and therefore has salinity gradients in both its North and South Branches (Fig. 1). The North Branch, located north of Chongming Island, is intruded by salt water, especially during the dry season when the river discharge is low (from November to April). The South Branch, located south of Chongming Island, is mainly influenced by the flow of fresh water down the river. Samples were collected at three typical sites with environmental gradients (e.g. salinity and sediment grain size) in the Dongtan wetland which is located at the eastern end of Chongming Island and is an internationally important conservation area for migratory bird (Fig. 1). Twenty replicate samples were collected at each site along a short transect in the mid-intertidal zone at low tide during 29th to 31th March 2015. The upper 2 cm of sediment was sampled using a 5 ml cut-off syringe (inner diameter 16 mm) with sharpened edges. Each sample was immediately fixed with an equal volume of glutaraldehyde (2% final concentration) until further processing. Ciliates were sampled, extracted and stained

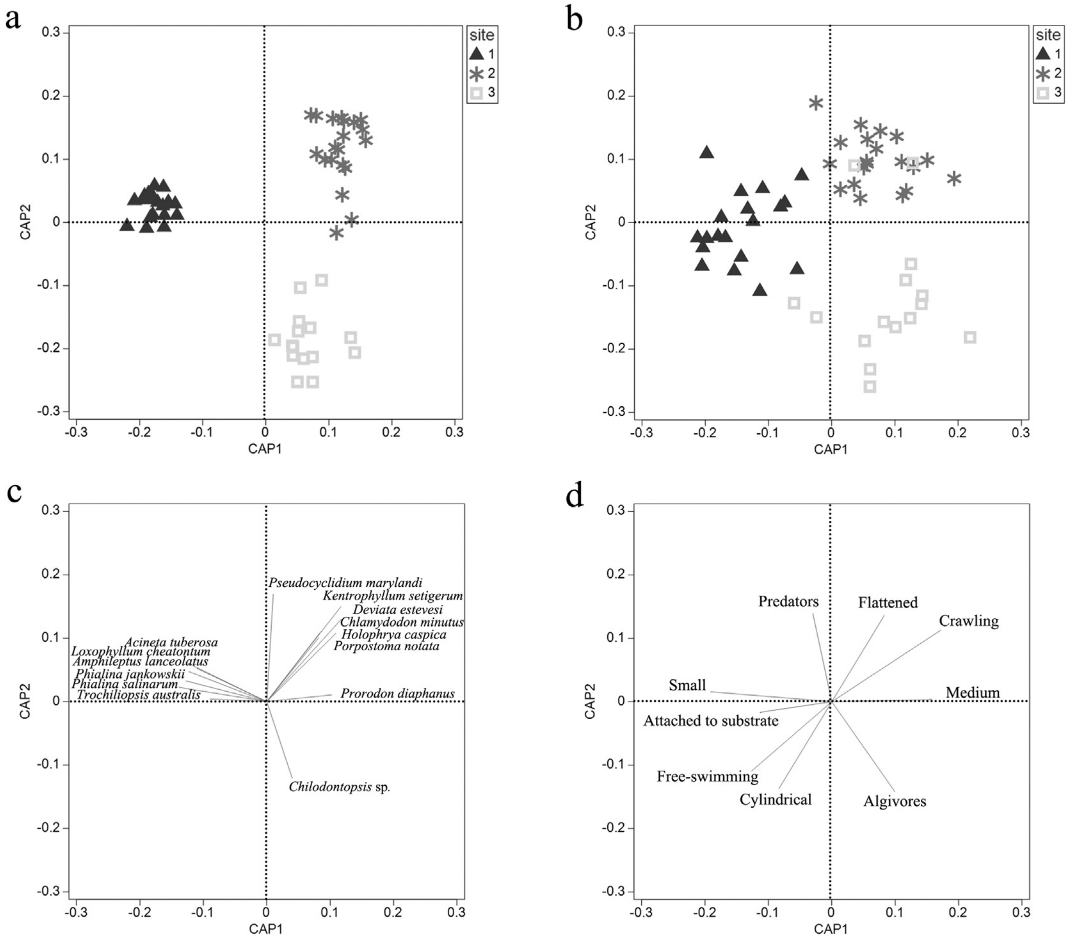

Fig. 2. Canonical analysis of principal coordinates on Bray-Curtis similarities from square root-transformed ciliate species abundance data with: correlations of 14 dominant species with CAP axes (a, c); Euclidean distances from log-transformed and normalised functional trait data with correlations of nine important trait categories with the CAP axes (b, d).

according to Xu et al. (2010). The stained ciliates were enumerated and identified using bright field microscopy (Olympus BX53) at 200 × to 1000 × magnifications. Identification was mainly according to Lynn and Small (2002). Salinity was measured in situ and three additional upper 2 cm-sediment samples were taken at each site for soil analysis. Grain size was determined with a laser size analyzer (BECKMAN COULTER LS13 320). The total organic carbon (TOC) and total nitrogen (TN) contents were determined (as % dry weight of the sediment) using a Vario EL CHNOS Elemental Analyzer (Ryba and Burgess, 2002).

Four classical diversity indices, i.e. Shannon-Wiener (*H′*), Simpson (*Simp*), Pielou's evenness (*J′*) and Margalef's richness (*d*) indices, were calculated using the submodule DIVERSE in the PRIMER v7.0.11 package (Clarke and Gorley, 2015).

Six biological traits sub-divided into 16 categories were selected according to functional diversity studies on macrobenthic invertebrates (Table 1; Bremner et al., 2006; Wong and Dowd, 2015). These traits reflect morphological characteristics (body size, degree of flexibility and body form), behaviour (feeding and movement type), and respiration type. Data on species traits were obtained both from our own observations and from the literature (Lynn, 2008). A fuzzy-coding procedure was used to account for an individual taxon displaying multiple trait categories, e.g. both bactivory and algivory within the feeding-type trait, according to Bremner et al. (2003). Four indices of functional diversity, i.e. Functional Richness (FRic), Functional Evenness (FEve), Functional Divergence (FDiv), and Rao's quadratic entropy index (RaoQ), were then calculated using the *dbFD* function in the “*FD*” R package (Laliberté et al., 2014).

The abundance data were square-root transformed and used to construct Bray-Curtis similarity matrices. Functional “traits by samples” data were log-transformed, normalised and used to construct Euclidean distances matrices. These matrices were used in the submodule CAP (canonical analysis of principal coordinates) of PERMANOVA to show differences of communities (Anderson et al., 2008). One-way analyses of similarities (ANOSIM) were used to determine the significance of differences between sites (Clarke and Gorley, 2015). The contribution of each species or biological trait to the dissimilarity between sites, as well as to the similarity within one site, was analysed using Similarity of percentages (SIMPER). CAP, ANOSIM and SIMPER were conducted using PRIMER v7.0.11 (Clarke and Gorley, 2015).

The ranges of physical-chemical parameters at the three sites are summarized in Supplementary Table S1. Among these variables, salinity showed major differences: site 1 was almost fresh water with salinity ranging from 0.3 psu to 0.7 psu, whereas sites 2 and 3 were brackish with salinity ranging from 7.7 psu to 9.7 psu and 17.4 psu to 20.0 psu, respectively. Sites 2 and 3 had a comparable sediment grain size which was finer than that for site 1. TOC and TN concentrations at sites 2 and 3 were higher than those at site 1. A total of 63 ciliates species belonging to nine classes, namely Armophorea, Heterotrichea, Litostomatea, Nassophorea, Oligohymenophorea, Phyllopharyngea, Plagiopylea, Prostomatea and Spirotrichea were identified at the three sites. Discriminating among samples was performed by CAP based on species abundance (Fig. 2a) or functional traits (Fig. 2b) and both indicated clear separation of the three sites. ANOSIM test revealed that there were significant differences among the three sites (R = 0.854,

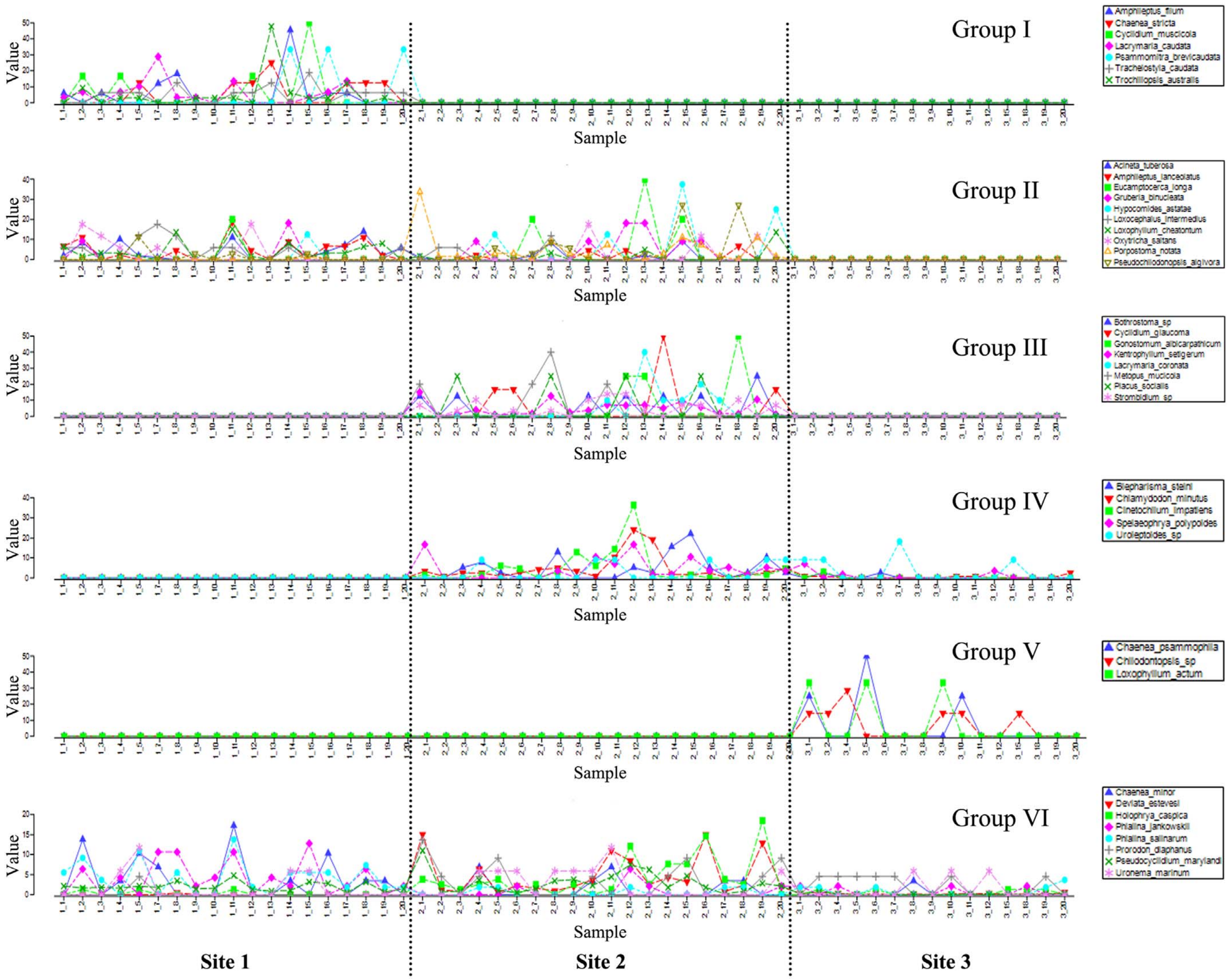

Fig. 3. Coherent species curves (line plots) of relative species abundances for groups I–VI showing the high species turnover between groups. Each species is standardised to total 100% across all samples of the three sites. Dotted lines refer to the separation of each site.

$P < 0.01$ for species abundance data and $R = 0.38$, $P < 0.01$ for functional traits data) and between each pair of sites ($P < 0.01$). Vector overlay of Pearson correlations of the 14 dominant species (as determined by the 80% cumulative contributions to the average Bray-Curtis similarity within each site using SIMPER analysis) or the nine important trait categories (as determined by the top five trait categories contributing to the average squared distance between each pair of sites using SIMPER analysis) with the CAP axes is shown in Fig. 2c, d.

The distribution of species revealed clear spatial patterns: except for 22 rare species which occurred in only one or two samples, all other species fell into six groups (I–VI) (Fig. 3). The comparison of each category of the six functional traits among three sites is shown in Fig. 4.

The spatial variation in four classical diversity indices (d, J', H', *Simp*) and four functional diversity indices (FRic, FEve, FDiv and RaoQ) are shown in Fig. 5, and the significance of differences analysed by ANOSIM is shown in Table 2.

Examination of species composition indicates how groups of species vary across the three sites: groups I, III and V tend to be more site-specific whereas groups II, IV and VI occurred either at two adjacent sites or at all three sites. Our results showed that ciliates do have species-specific salinity tolerances: group VI had a wider salinity tolerant range (ca. 0.5–18.7 psu) compared with other groups, while from group I to V there was a clear transition from freshwater species to brackish water species along the salinity gradient (Fig. 3).

The patterns in functional trait composition across the three sites showed clear functional turnover as in species composition. In our study, four functional traits, i.e. movement type, body size, body form and feeding type, contributed most in distinguishing the three sites. As shown in Fig. 2, two categories of movement type (attached and free-swimming) and cylindrical body form were more prevalent at site 1, whereas crawling movement and a flattened body form were more prevalent at sites 2 and 3. This implies that in coarser sediment environments, sessile and free-swimming benthic ciliates with cylindrical body form tend to be more dominant, whereas in finer sediment environments there are more ciliates possessing flattened body forms and a preference for crawling on substrates, which may be due to the hydrodynamic disturbances and interstitial spaces, since Hamels et al. (2004, 2005) indicated that intertidal ciliates tend to have a body form

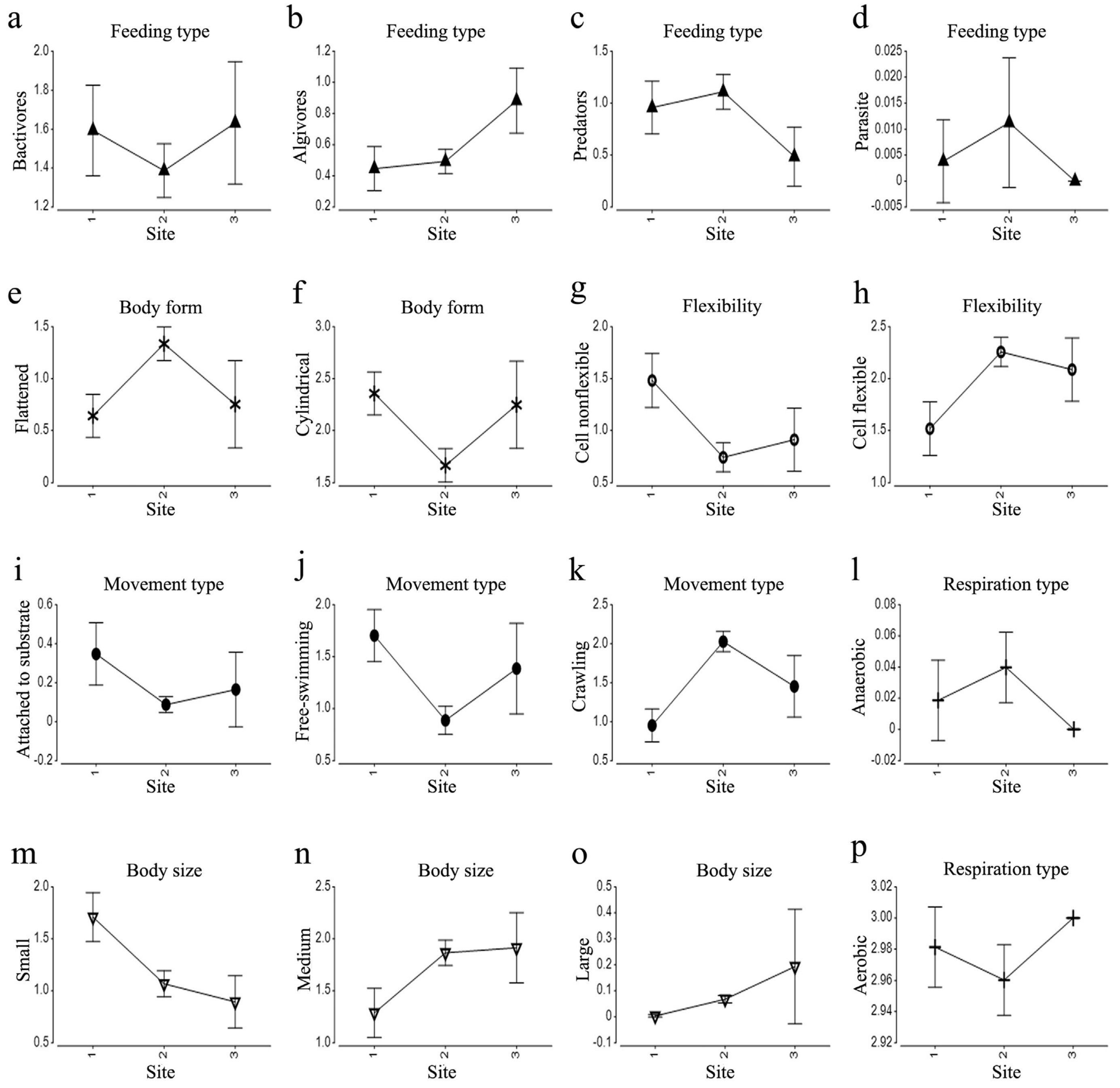

Fig. 4. Means plots of each biological trait category at the three sampling sites. Error bars denote 95% confidence intervals for those means.

adapted for the interstitial space in which they live.

The present study showed there was a clear increase in species richness (*d*) from site 1 to site 2, while FRic, FDiv and RaoQ did not differ significantly between these two sites. These results suggested that although there was a significant increase in species richness at site 2, functionally unique species did not increase in abundance relative to less unique species, and the newly appeared species at site 2 tended to be functionally redundant. Meanwhile, FRic, FDiv and RaoQ were all clearly reduced at site 3 suggesting a low degree of niche differentiation and high competition for resources among dominant species (Mason et al., 2005). In this rationale, the dominant species at site 3 were more functionally similar to each other than the dominant species inhabiting the other two sites. Significantly reduced functional diversity suggests that environmental constrains select coexisting species sharing similar functional traits, which leads to reduced ecosystem function and greater vulnerability to disturbance. Therefore, salt water intrusion at site 3 could have a negative impact on the ecosystem function of estuarine benthic ciliates which could serve as good bioindicators for detecting salt water intrusion into coastal waters.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2017.12.068.

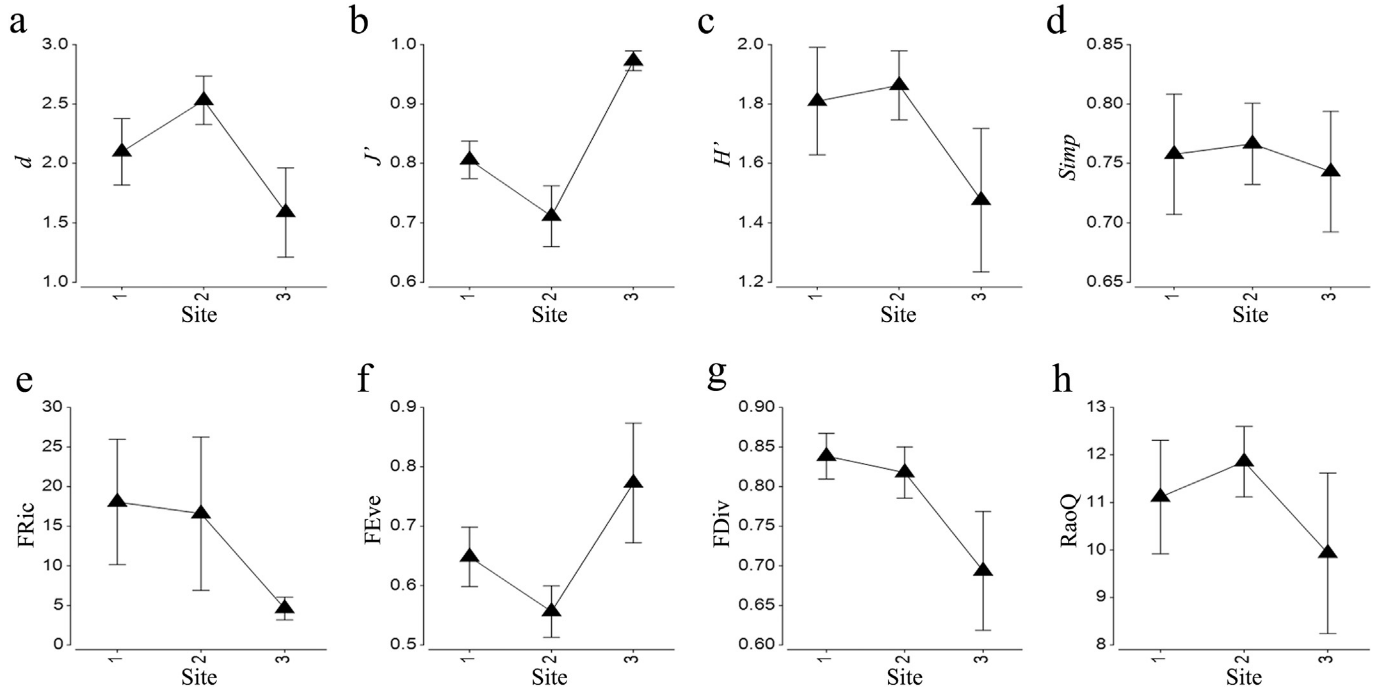

Fig. 5. Means plots of species richness (a), species evenness (b), Shannon-Wiener index (c), Simpson index (d) and functional richness (e), functional evenness (f), functional divergence (g), and Rao's quadratic entropy (h) at the three sampling sites. Error bars denote 95% confidence intervals for those means.

Table 2
ANOSIM analysis of variance of the four taxonomic diversity indices (richness *d*, evenness *J*′, Shannon-Wiener index *H*′ and Simpson index *Simp*) and the four functional diversity indices (Functional Richness FRic, Functional Evenness FEve, Functional Divergence FDiv and Rao's quadratic entropy index RaoQ).

Source	Species richness *d*		Species evenness *J*′		Shannon-Wiener index *H*′		Simpson index *Simp*	
	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value
Site 1 ∗ Site 2	0.085	0.039*	0.139	0.007**	0.01	0.528	0.03	0.873
Site 1 ∗ Site 3	0.151	0.012*	0.797	0.001**	0.158	0.016*	0.03	0.209
Site 2 ∗ Site 3	0.461	0.001**	0.765	0.001**	0.324	0.002**	0.081	0.073

Source	FRic		FEve		FDiv		RaoQ	
	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value
Site 1 ∗ Site 2	0.045	0.087	0.103	0.028*	0.011	0.528	0.009	0.303
Site 1 ∗ Site 3	0.278	0.003**	0.247	0.003**	0.298	0.001**	0.049	0.117
Site 2 ∗ Site 3	0.002	0.447	0.423	0.001**	0.208	0.004**	0.097	0.041*

Levels of significance are * $P < 0.05$, ** $P < 0.01$.

Acknowledgments

This work was supported by National Natural Science Foundation of China (project numbers: 31601843; 31672308).

References

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + for PRIMER Guide to Software and Statistical Methods. PRIMER-E Ltd., Plymouth.

Bremner, J., Rogers, S.I., Frid, C.L.J., 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. Mar. Ecol. Prog. Ser. 254, 11–25.

Bremner, J., Rogers, S.I., Frid, C.L.J., 2006. Matching biological traits to environmental conditions in marine benthic ecosystems. J. Mar. Syst. 60, 302–316.

Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E Ltd., Plymouth.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E.M., Steffan-Dewenter, I., Emmerson, M., Potts, S.G., Tscharntke, T., Weisser, W., Bommarco, R., 2015. Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. Proc. R. Soc. B Biol. Sci. 282, 20142620.

Gusmao, J.B., Brauko, K.M., Eriksson, B.K., Lana, P.C., 2016. Functional diversity of macrobenthic assemblages decreased in response to sewage discharges. Ecol. Indic. 66, 65–75.

Hamels, I., Sabbe, K., Muylaert, K., Vyverman, W., 2004. Quantitative importance, composition, and seasonal dynamics of protozoan communities in polyhaline versus freshwater intertidal sediments. Microb. Ecol. 47, 18–29.

Hamels, I., Muylaert, K., Sabbe, K., Vyverman, W., 2005. Contrasting dynamics of ciliate communities in sandy and silty sediments of an estuarine intertidal flat. Eur. J. Protistol. 41, 241–250.

Laliberté, E., Legendre, P., Shipley, B., 2014. FD: Measuring Functional Diversity From Multiple Traits, and Other Tools for Functional Ecology. R Package Version 1.0–12.

Lynn, D.H., 2008. The Ciliated Protozoa: Characterization, Classification and Guide to the Literature, 3rd ed. Springer, Dordrecht.

Lynn, D.H., Small, E.B., 2002. Phylum Ciliophora Doflein, 1901. In: Lee, J.J., Leedale, G.F., Bradbury, P.C. (Eds.), An Illustrated Guide to the Protozoa, 2nd ed. Society of Protozoologists, Lawrence, Kansas, pp. 371–656.

MarLIN, 2006. BIOTIC - Biological Traits Information Catalogue. Marine Life Information Network, Marine Biological Association of the United Kingdom, Plymouth.

Mason, N.W.H., Mouillot, D., Lee, W.G., Wilson, J.B., 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos 111, 112–118.

Mazei, Y.A., Burkovsky, I.V., 2006. Patterns of psammophilous ciliate community structure along the salinity gradient in the Chernaya River estuary (the Kandalaksha Gulf, the White Sea). Protistology 4, 251–268.

McGill, B.J., Enquist, B.J., Weiher, E., Westoby, M., 2006. Rebuilding community ecology from functional traits. Trends Ecol. Evol. 21, 178–185.

Paganelli, D., Marchini, A., Occhipinti-Ambrogi, A., 2012. Functional structure of marine benthic assemblages using biological traits analysis (BTA): a study along the Emilia-Romagna coastline (Italy, North-West Adriatic Sea). Estuar. Coast. Shelf Sci. 96, 245–256.

Ryba, S.A., Burgess, R.M., 2002. Effects of sample preparation on the measurement of organic carbon, hydrogen, nitrogen, sulfur, and oxygen concentrations in marine sediments. Chemosphere 48, 139–147.

Tillin, H.M., Hiddink, J.G., Jennings, S., Kaiser, M.J., 2006. Chronic bottom trawling alters the functional composition of benthic invertebrate communities. Mar. Ecol. Prog. Ser. 318, 31–45.

Villéger, S., Miranda, J.R., Hernández, D.F., Mouillot, D., 2010. Contrasting changes in taxonomic vs. functional diversity of tropical fish communities after habitat degradation. Ecol. Appl. 20, 1512–1522.

Villéger, S., Novack-Gottshall, P.M., Mouillot, D., 2011. The multidimensionality of the niche reveals functional diversity changes in benthic marine biotas across geological time. Ecol. Lett. 14, 561–568.

Wan Hussin, W.M.R., Cooper, K.M., Barrio Froján, C.R.S., Defew, E.C., Paterson, D.M., 2012. Impacts of physical disturbance on the recovery of a macrofaunal community: a comparative analysis using traditional and novel approaches. Ecol. Indic. 12, 37–45.

Wong, M.C., Dowd, M., 2015. Patterns in taxonomic and functional diversity of macrobenthic invertebrates across seagrass habitats: a case study in Atlantic Canada. Estuar. Coasts 38, 2323–2336.

Xu, K., Du, Y., Lei, Y., Dai, R., 2010. A practical method for Ludox density gradient centrifugation combined with protargol staining for extracting and estimating ciliates in marine sediments. Eur. J. Protistol. 46, 263–270.

Xu, G., Yang, E., Xu, H., 2017. Environmental divers of heterogeneity in the trophic-functional structure of protozoan communities during an annual cycle in a coastal ecosystem. Mar. Pollut. Bull. 121, 400–403.