Marine Pollution Bulletin 121 (2017) 400–403

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

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Environmental drivers of heterogeneity in the trophic-functional structure of protozoan communities during an annual cycle in a coastal ecosystem

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ARTICLE INFO

Keywords:
Trophic-functional structure
Periphytic protozoa
Water quality
Bioassessment
Coastal ecosystems

ABSTRACT

Trophic-functional groupings are an important biological trait to summarize community structure in functional space. The heterogeneity of the tropic-functional pattern of protozoan communities and its environmental drivers were studied in coastal waters of the Yellow Sea during a 1-year cycle. Samples were collected using the glass slide method at four stations within a water pollution gradient. A second-stage matrix-based analysis was used to summarize spatial variation in the annual pattern of the functional structure. A clustering analysis revealed significant variability in the trophic-functional pattern among the four stations during the 1-year cycle. The heterogeneity in the trophic-functional pattern of the communities was significantly related to changes in environmental variables, particularly ammonium-nitrogen and nitrates, alone or in combination with dissolved oxygen. These results suggest that the heterogeneity in annual patterns of protozoan trophic-functional structure may reflect water quality status in coastal ecosystems.

Protozoa are primary consumers in microbial communities, feeding on bacteria and pico-/nano-algae and transferring the flux of elements and energy to metazoa in aquatic ecosystems (Patterson et al., 1989; Finlay and Esteban, 1998; Zhang et al., 2012). Protozoa are traditionally classified into five trophic-functional groups based on feeding strategy: bacterivores, algivores, saprotrophs, raptors, and non-selectives (Pratt and Cairns, 1985). Thus, the functional structure of the protozoan community can be summarized simply in trophic-functional trait space (Xu et al., 2010; Zhang et al., 2012; Jiang et al., 2013; Yang et al., 2016).

Protozoa have been widely used to assess water quality in both freshwater and marine environments due to their simple life cycles, easy sampling, and particular sensitivity to environmental changes relative to metazoa (e.g., Norf et al., 2009; Jiang et al., 2011; Kathol et al., 2011; Xu et al., 2014). However, a protozoan-based bioassessment is commonly conducted in taxon space (e.g., Sheldon et al., 1972; Kamenir et al., 2010; Jiang et al., 2011; Xu et al., 2014; Feng et al., 2015). Changes in environmental variables may significantly affect the trophic-functional pattern of protozoan communities (Pratt and Cairns, 1985; Norf et al., 2009). However, few reports have documented the environmental drivers of heterogeneity in the trophic-functional pattern of protozoan communities in marine ecosystems (Franklin and Mills, 2005; Wey et al., 2009; Früh et al., 2011).

In this study, the relationship between heterogeneity in the tropic-functional structure of protozoan communities and environmental variables was studied in coastal waters of the Yellow Sea. Our aims were: (1) to demonstrate heterogeneity in the spatial pattern of the community structure in functional space; (2) to explore the relationships between functional pattern and environmental conditions; and (3) to demonstrate the potential environmental drivers of heterogeneity in the functional structure of protozoan communities in marine ecosystems.

A total of 40 samples were collected monthly, using the artificial substratum (microscopy glass slides) method, at a depth of 1 m from four stations within a water pollution gradient during a 1-year cycle (August 2011–July 2012) (Xu et al., 2014) (Fig. 1).

Species were identified and individual species were enumerated according to the methods described by Xu et al. (2014). References, such as Song et al. (2009), were used to identify species.

Trophic-functional groupings of the ciliate species, which were comprised of bacterivores, algivores, raptors, and non-selectives, were performed according to Pratt and Cairns (1985), Song et al. (2009), and direct observations.

Water temperature, salinity, pH, chemical oxygen demand, dissolved oxygen (DO), ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), and soluble reactive phosphate (SRP) were measured in situ or according to the "Standard Methods for the Examination of Water and Wastewater" (APHA, 1992).

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http://dx.doi.org/10.1016/j.marpolbul.2017.06.012
Received 13 January 2017; Received in revised form 1 June 2017; Accepted 6 June 2017
Available online 16 June 2017
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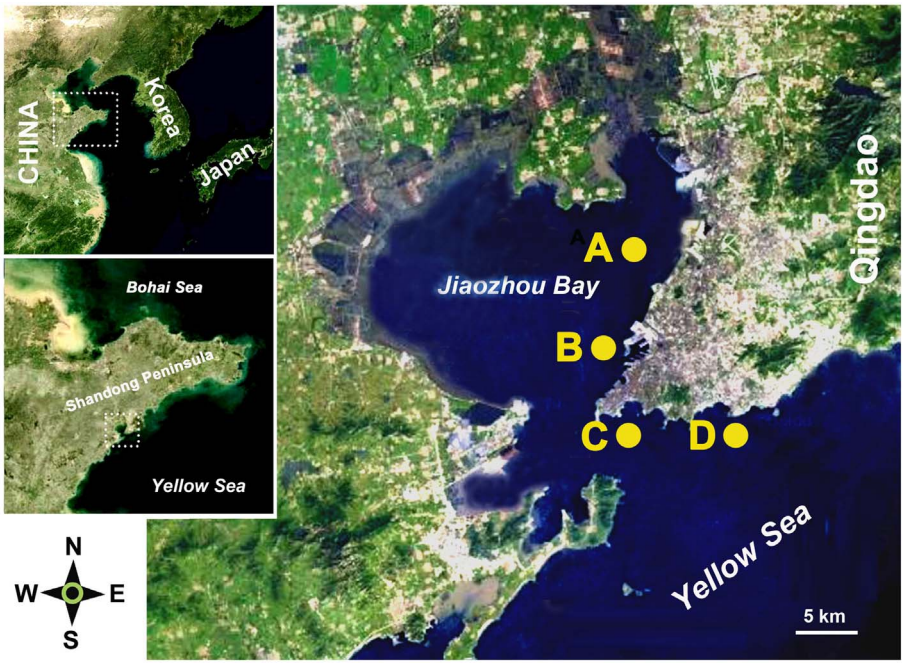

Fig. 1. Sampling stations in coastal waters of the Yellow Sea, near Qingdao, northern China. A: station A, heavily stressed area in Jiaozhou Bay, the pollution being mainly in the form of organic pollutants and nutrients from domestic sewage and industrial discharge from several rivers; B: station B, moderately polluted area Jiaozhou Bay by minor discharges from a small river entering the bay; C: station C, slightly polluted area near the mouth of Jiaozhou Bay and relatively distant from the rivers entering the bay; D: station D, relatively clean area which was out of this bay and more distant from the river discharges.

Table 1
Trophic-functional groupings of protozoa at the four sampling stations in coastal waters of the Yellow Sea, near Qingdao, northern China during the 1-year cycle (August 2011–July 2012).

Grouping	St A	St B	St C	St D	Total
A	45	55	46	48	66
B	30	28	31	25	36
N	15	20	19	17	26
R	13	12	15	11	16

A, algivores; B, bacterivores; N, non-selectives; R, raptors. St A–D: Stations A–D.

A multivariate approach was used to reveal spatial variations in the functional pattern of the communities, and their relationship to changes in environmental variables, by running the relevant routines in the PRIMER package (ver. 7.0.12). Bray–Curtis similarity matrices were computed among communities on fourth root transformed data, and the Euclidean distance matrices for environmental variables were obtained from log-transformed and normalized abiotic data (Clarke and Gorley, 2015). Metric multidimensional scaling (mMDS) ordination was used to show the trajectory of the temporal variation in functional structure, whereas the spatial patterns of protozoan functional structure and annual pattern of water quality status were shown by the routine bootstrap average (Clarke and Gorley, 2015). A second-stage (2STAGE) matrix-based analysis was used to summarize spatial variations in the

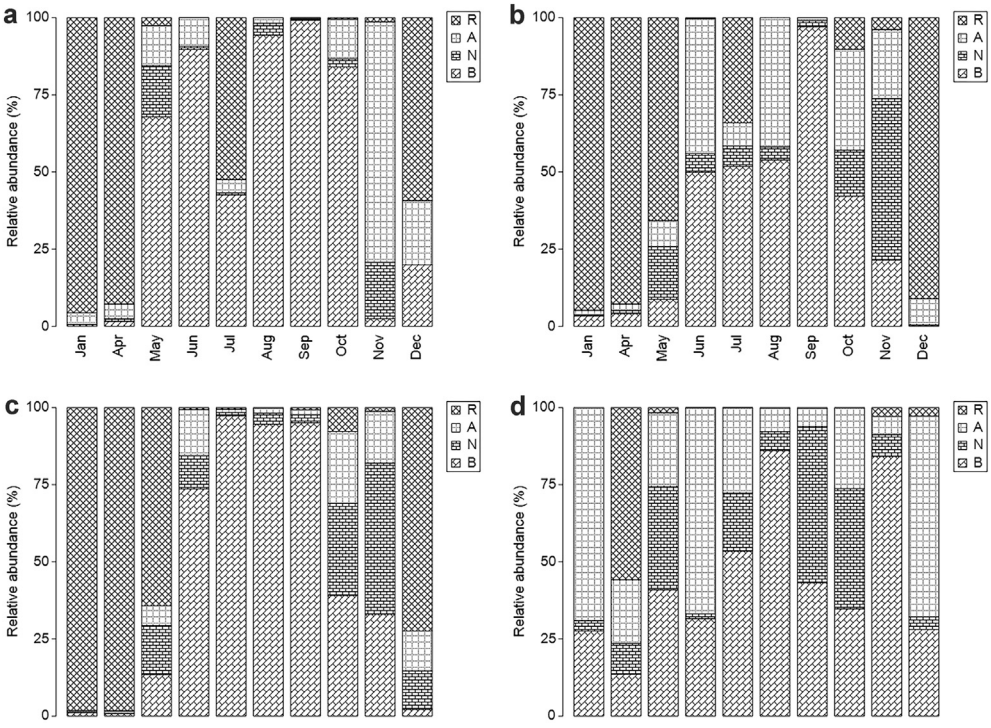

Fig. 2. Temporal variations in relative abundance of each trophic-functional group in protozoan communities during a 1-cycle at stations A (a), B (b), C (c) and D (d).

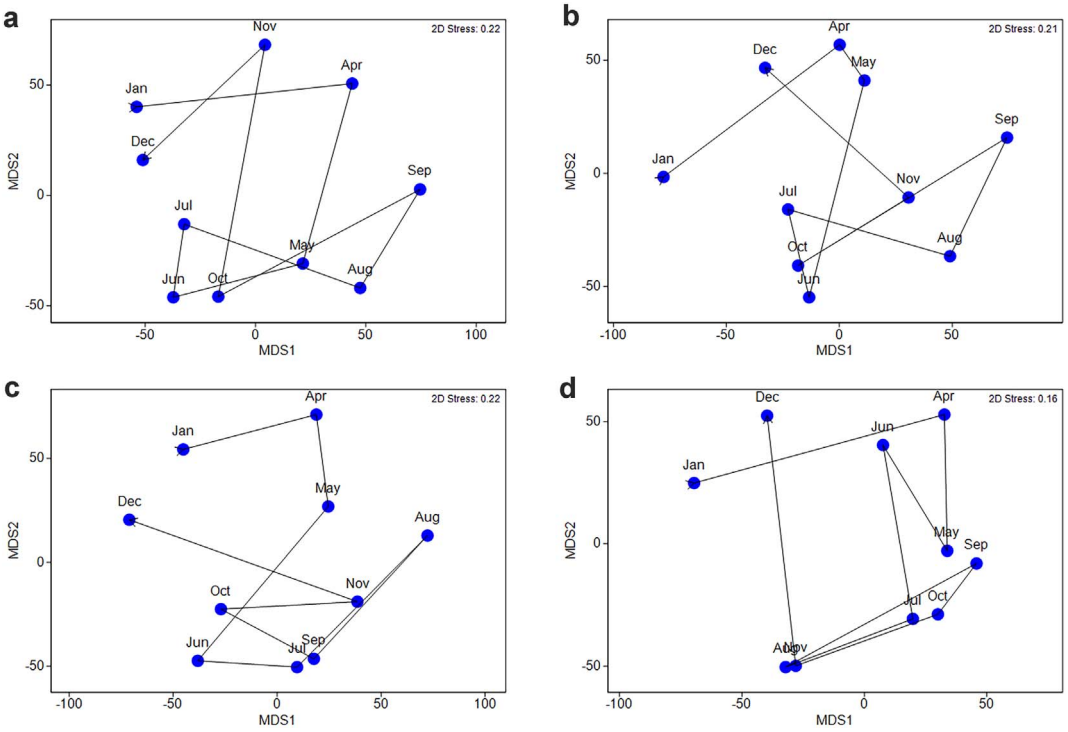

Fig. 3. Metric multidimensional scaling (mMDS) ordinations for protozoan communities at four stations A (a), B (b), C (c) and D (d), showing the dynamic trajectory of the trophic-functional structures of protozoan communities during a 1-year cycle.

annual pattern of protozoan communities, and in the annual variability of water quality conditions (Clarke and Gorley, 2015). The RELATE routine was used to evaluate the relationships among similarity matrices, and the BIOENV (biota-environment) routine was used to identify the close matches of environmental variables to spatial variations in the protozoan functional patterns (Clarke and Gorley, 2015).

The environmental conditions at the four sampling stations are summarized in Table S1. Of the nine environmental variables, the average NH_4-N values showed a clear decreasing trend from stations A to D, and those of NO_3-N and SRP were low at stations C and D, but high at stations A and B (Table S1).

The species list of the total 144 protozoa identified during the study period, and the trophic-functional groupings and occurrence at the four stations, are shown in Table S2. The numbers of species in each trophic-functional group at the four stations are summarized in Table 1.

The heterogeneity in the annual patterns of the functional structures, in terms of relative abundance, is shown in Fig. 2. The functional pattern showed clear spatial variation among the four stations (Fig. 2).

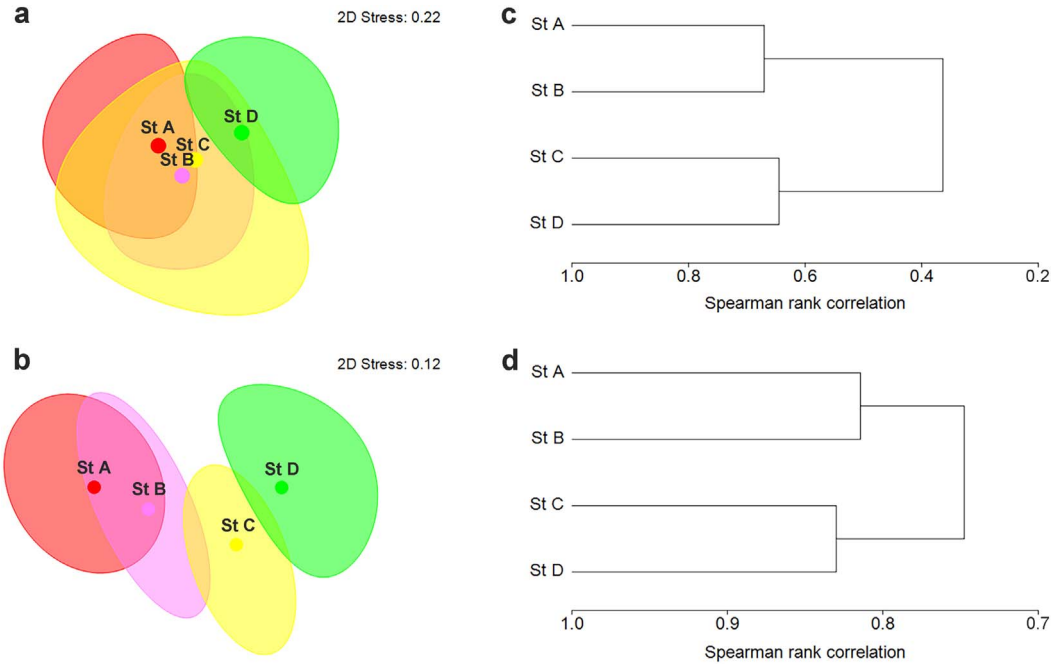

Fig. 4. Bootstrapped average analyses on biotic (a) and abiotic matrices (b), and second-stage clustering analyses for annual patterns of protozoan communities in terms of trophic-functional structure (c) and annual variations in water conditions (d), showing the spatial variations in biota and abiota among four sampling stations. St A–D: Stations A–D.

Table 2
Summary of results from biota-environment (BIOENV) analysis showing the 10 best matches of environmental variables with spatial variations in trophic-functional structures of the protozoa at four sampling stations in coastal waters of the Yellow Sea, near Qingdao, northern China during the study period.

Rank	Environmental variables	ρ value	*P* value
1	NH_4-N	0.926	0.05
2	NH_4-N, NO_3-N	0.926	0.05
3	DO, NH_4-N, NO_3-N, NO_2-N	0.915	0.05
4	DO, NH_4-N, NO_3-N, SRP	0.887	0.05
5	DO, NH_4-N, NO_3-N, NO_2-N, SRP	0.886	0.05
6	NH_4-N, SRP	0.881	0.05
7	DO, NH_4-N, NO_2-N, SRP	0.877	0.05
8	DO, NH_4-N, NO_3-N	0.868	0.05
9	NH_4-N, NO_3-N, SRP	0.857	0.05
10	DO, NH_4-N, SRP	0.852	0.05

ρ value, Spearman correlation coefficient; *P* value, statistical significance level. Sal, salinity; COD, chemical oxygen demand; DO, dissolved oxygen; SRP, soluble active phosphate; NO_3-N, nitrate nitrogen; NO_2-N, nitrite nitrogen; NH_4-N, ammonium nitrogen.

For example, mainly functional groups of raptors and bacterivores dominated the samples at station A (Fig. 2a), whereas the raptor, bacterivores, and non-selective groups dominated the communities at station D (Fig. 2d).

The mMDS ordination analyses showed that the trajectories of temporal variations in the functional structure differed among the four stations (Fig. 3).

Bootstrap average analyses revealed a similar spatial pattern between the biota and abiota (Fig. 4a and b). Furthermore, the spatial variation in the annual trophic-functional pattern was closely related to that of the environmental variables, based on clustering analyses for second-stage matrices of the biotic and abiotic data (Fig. 4c and d).

The RELATE analysis demonstrated a significant correlation between spatial variation in the annual distribution of protozoa and changes in environmental variables ($\rho = 0.657$, $P < 0.05$).

The multivariate BIOENV best matching analysis showed that nutrients, particularly NH_4-N and NO_3-N, alone or in combination with DO, were closely matched to spatial variations in the annual pattern of the functional structure (Table 2).

The "ecological effectiveness" of trophic-functional patterns to assess water quality status has not been well documented (e.g., San Martin et al., 2006; Xu et al., 2010; Zhang et al., 2012; Wang et al., 2016; Yang et al., 2016). In this study, we demonstrated clear spatial variation in the functional structures of protozoan communities. Multivariate analysis revealed that the spatial pattern of the protozoa was significantly correlated with changes in environmental conditions, particularly nutrients alone or in combination with DO.

In summary, the annual patterns of trophic-functional structures showed a significant difference on a spatial scale. This heterogeneity was significantly related to water quality status. Nutrients and DO might be potential environmental drivers that shape the functional pattern of the protozoan community.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.06.012.

Acknowledgements

This work was supported by "The Natural Science Foundation of China" (project numbers: 31672308 and 41076089), and grants from the K-AOOS Program (KOPRI; PM17040) funded by the MOF, Korea.

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