Baseline

# 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

## 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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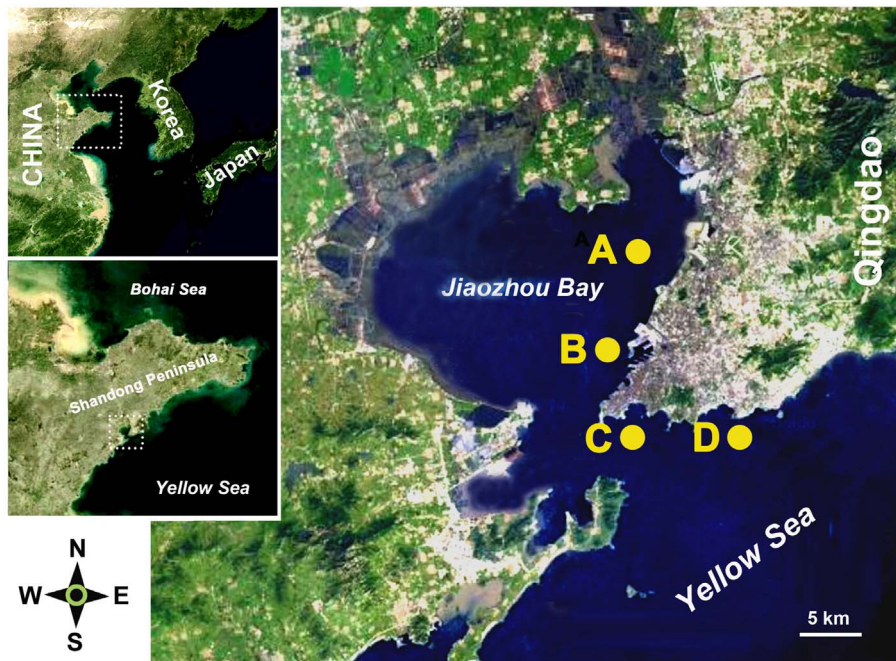
**Fig. 1.** Sampling stations in coastal waters of the Yellow Sea, near Qingdao, northern China. A: station A, heavily stressed area in Jiaozhou Bay, the pollution being mainly in the form of organic pollutants and nutrients from domestic sewage and industrial discharge from several rivers; B: station 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
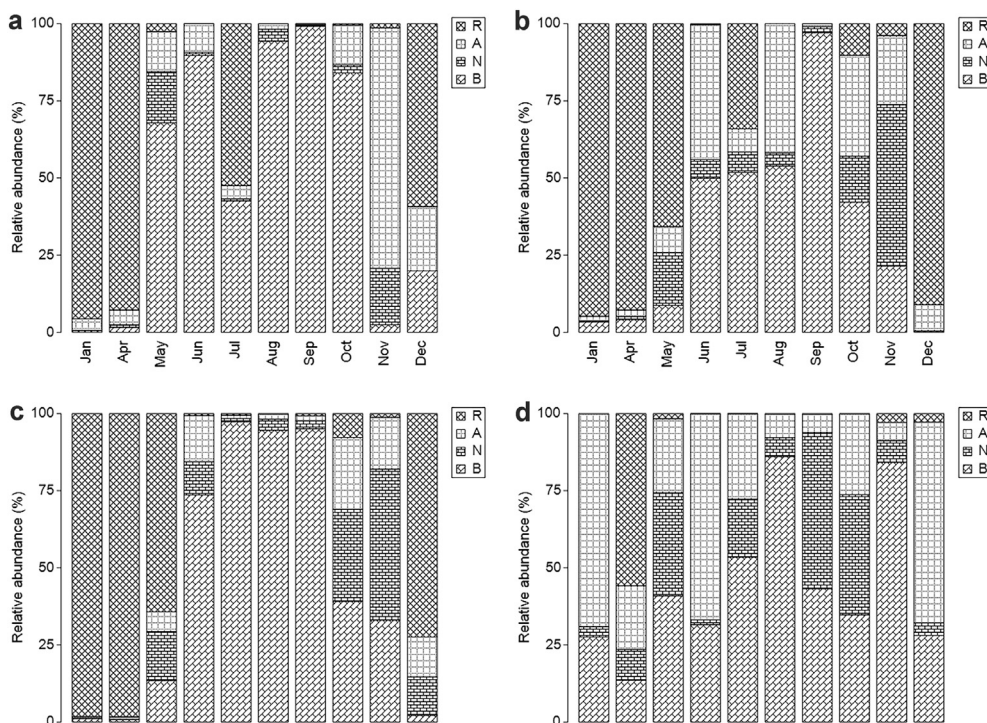


**Fig. 2.** Temporal variations in relative abundance of each trophic-functional group in protozoan communities during a 1-cycle at stations A (a), B (b), C (c) and D (d).