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Use of multiple functional traits of protozoa for bioassessment of marine pollution

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ABSTRACT

Ecological parameters based on multiply functional traits have many advantages for monitoring programs by reducing "signal to noise" ratios of observed species data. To identify potential indicators for bioassessment of marine pollution in function space, the functional patterns of protozoan communities and relationships with environmental changes were studied in coastal waters of the Yellow Sea during a 1-year period. The results showed that: (1) the spatial variability in functional trait distributions of the protozoa was significantly associated with changes in environmental variables, especially chemical oxygen demand (COD) and nutrients on spatial scale; (2) the functional traits, especially food resources and feeding type, were significantly correlated with COD and nutrients; and (3) the functional diversity indices were generally related to nutrients or COD. Based on the results, we suggest that the functional traits and diversity indices of protozoan communities may be used as more effective indicators for bioassessment of marine pollution.

1. Introduction

Biological trait analysis (BTA) has proved to be a useful tool to summarize community functioning and to measure the functional diversity in various types of marine ecosystems (Bremner et al., 2003; Micheli and Halpern, 2005; Villéger et al., 2010; Dimitriadis et al., 2012; Wong and Dowd, 2015; Gusmao et al., 2016). Compared with traditional analysis at species abundance/biomass resolutions, BTA can reveal the species trait distribution and the range of what organisms do in communities, and thus reduce the "signal to noise" ratios of observed species data in community-level monitoring programs (Mason et al., 2005; Petchey and Gaston, 2006; Villéger et al., 2008, 2010; Schleuter et al., 2010; Wan Hussin et al., 2012). Furthermore, functional diversity measures based on BTA can be allowed being categorized according to the component that they measure in the functional space (Bremner et al., 2006; Hewitt et al., 2008; Mouchet et al., 2010; Paganelli et al., 2012).

Protozoa play an important role in the functioning of microbial food webs by transferring carbon and energy from bacteria, pico and nino-plankton to high trophic levels in aquatic ecosystems (Finlay and Esteban, 1998; Kathol et al., 2011; Zhang et al., 2012; Zhong et al., 2014). With their global distribution and rapid responses to environmental changes, they have been used as useful bioindicator of water quality status (e.g., Xu et al., 2014). However, the previous work was commonly carried out at species abundance/biomass resolutions, and thus subject to high "signal to noise" ratios due to functional redundancy (Norf et al., 2009; Wey et al., 2009; Kathol et al., 2011; Zhang et al., 2012; Xu et al., 2014, 2016). As regards bioassessment using protozoan communities based on multiple biological traits, however, little was known although a number of investigations on benthic macrobenthos have done over the past ten years (Tillin et al., 2006; Paganelli et al., 2012).

In this study, functional trait distribution of protozoan communities and relationships with environmental changes were studied in coastal waters of the Yellow Sea. The aims of this study were to: (1) reveal the spatial variation in functional trait distribution of the protozoa and relationships with changes in prevailing environmental variables; (2) to demonstrate the relationship between functional diversity and water quality status; and (3) identify potential indicators for bioassessment of marine pollution using BTA.

2. Materials and methods

2.1. Study area and data collection

Four sampling sites (Fig. 1A–D), which were selected with different pollution/eutrophication levels, were located in coastal waters of the Yellow Sea. Ten samplings were monthly carried out using glass slide method during a 1-year period (August 2011–July 2012). A total of 40 samples were obtained for this monitoring program.

Samples processing were done according to the methods described by Xu et al. (2014). Protozoan species (mainly ciliates) identification of the protozoan species was according to the published references such as Song et al. (2009). It should be noted that rhizopods and flagellates were ignored in this study due to their low contribution to the communities.

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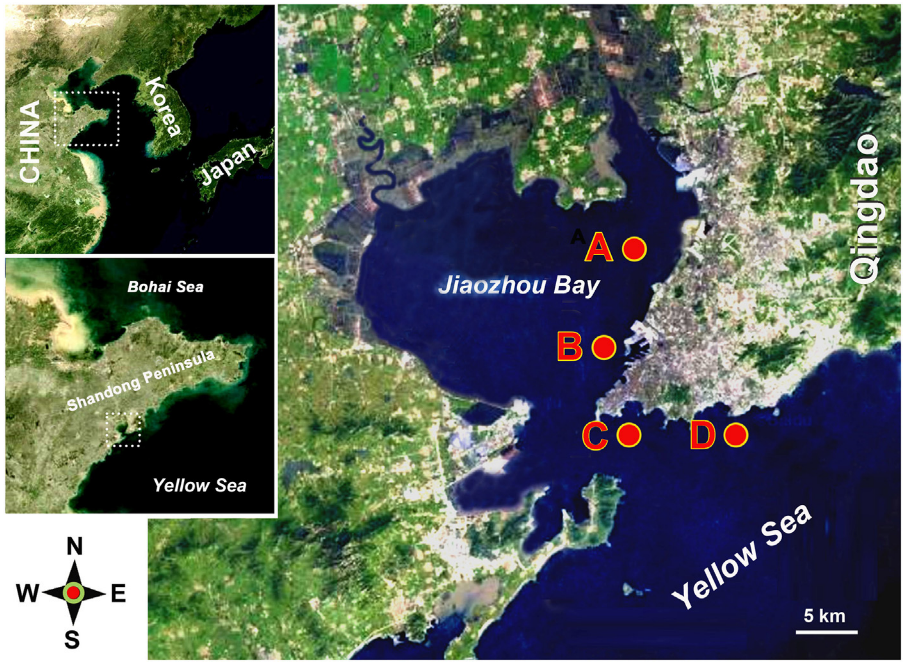

Fig. 1. Sampling sites in coastal waters of the Yellow Sea, near Qingdao, northern China. A: site A, heavily polluted area in Jiaozhou Bay mainly by organic pollutants and nutrients from domestic sewage and industrial discharge from several rivers; B: site B, moderately polluted area Jiaozhou Bay by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

Water temperature (T), salinity (Sal), pH, and dissolved oxygen (DO) were in situ detected using WTW Multi 3500i sensor, while concentrations of chemical oxygen demand (COD), and nutrients ammonium nitrogen (NH_4-N), nutrients nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N) and soluble reactive phosphate (SRP), were measured according to the "Standard Methods for the Examination of Water and Wastewater" (APHA, 1992).

2.2. Functional traits

The biological traits are selected according to published literatures such as Bremner et al. (2003), Michaud et al. (2006), Norling et al. (2007), Papageorgiou et al. (2009), Pacheco et al. (2010) and Wong and Dowd (2015).

Seven functional traits sub-divided into 17 categories were chosen for biological trait analyses (Table 1). These variables covered morphological characteristics (body size, degree of flexibility, body form and sociability) and behaviour (types of feeding, food resource and movement). Data of species traits were determined according to both published literatures (e.g., Lynn, 2008; Pratt and Cairns, 1985; Song et al., 2009; Zhang et al., 2012) and direct observation. A fuzzy coding procedure was used in cases where species represented multiple traits within each variable based on literatures such as Bremner et al. (2003) and Dimitriadis et al. (2012).

2.3. Data analyses

For summarizing functional pattern and calculating functional diversity indices, a "traits by samples" matrix, i.e., community-weighted means (CWM) of traits, was computed from "taxa abundance by samples" (Lepš et al., 2011; Ricotta and Moretti, 2011).

Five functional measures, functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional dispersion (FDis) and Rao's quadratic entropy index (RaoQ), were used as potential indicators of water quality status, which indicate a range of different aspects of the community functioning (Botta-Dukát, 2005; Mason et al., 2005; Lepš et al., 2006; Villéger et al., 2008; Laliberté and Legendre, 2010).

The CWM matrix and the five functional diversity indices were computed using the *dbFD* function in the "*FD*" R package (Laliberté et al., 2014).

Multivariate analyses for CWM and environmental data were performed using PRIMER v7.0.11 (Clarke and Gorley, 2015). The procedures CAP (canonical analysis of principal coordinates) and PERMANOVA were used to summarize spatial variations and to test the significant differences in functional structure of communities among four sites, respectively (Anderson et al., 2008). The procedure "bootstrap average analysis" was used to the spatial patterns of both biota and abiota. For both CWM data and environmental data, Euclidean distances matrices were obtained from log-transformed/normalized datasets. The routine RELATE was used to test the significance of the

Table 1
Biological traits and categories used to describe functional diversity of periphytic protozoan (mainly ciliate) communities in coastal waters of the Yellow Sea, northern China.

Traits	Categories
Body size (measured as cell length)	Small (<50 μm)
	Medium (50–150 μm)
	Large (>150 μm)
Body form	Dorsal-ventrally flattened
	Cylindrical
Degree of flexibility	Cell nonflexible
	Cell flexible
Sociability	Solitary
	Colonial
Feeding type	Bacterivores
	Algivores
	Predators
Food resource	Outside (from plankton)
	Inside (from periphyton)
Movement type	Sessile
	Vagile
	Free-swimming

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