Original Articles

Use of functional distinctness of periphytic ciliates for monitoring water quality in coastal ecosystems

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A B S T R A C T

As trait-based ecological parameters, functional diversity/distinctness measures are robust parameters for biodiversity research by reducing "signal to noisy" ratios due to functional redundancy in a community, and have an advantage for bioassessment due to a statistical framework for testing departure from expectation by combining ecosystem functions and community structure. The effectiveness of four functional diversity/distinctness measurements for water quality assessment was based on a study of additional ciliate datasets for biological evaluation of coastal waters along the Yellow Sea in northern China. A fuzzy-coding trait system was used as a trait pool, which comprised six characters of feeding types, movement features, body size/form/flexibility, and sociability. Results showed that (1) the functional diversity (Δ_χ) significantly related to the spatial variation in water transparency; (2) the average functional diversity index (Δ_χ^+) and variation in functional distinctness (Λ_χ^+) represented a significant correlation with the changes in pH and nutrients, especially ammonium nitrogen, and soluble active phosphate; and (3) ellipse test for the paired indices (Δ_χ^+ and Λ_χ^+) revealed a clear departure from an expected functional trait pattern. According to our findings, it is recommended that the functional diversity/distinctness measures may be served as a robust bioindicator of water quality status in marine ecosystems.

1. Introduction

With the increasing anthropogenic impacts on global change, functional diversity/distinctness has proved to be a robust indicator to summarize community shaping and functioning and discriminate water quality status in a variety of aquatic environments (Somerfield et al., 2008; Zhong et al., 2017; Xu et al., 2018a,b). As trait-based ecological parameters, functional diversity/distinctness measures have an advantage for bioassessment due to a statistical framework for testing departure from expectation (Somerfield et al., 2008). Because of linking community structure and ecosystem functioning, this framework can significantly reduce the "signal to noise" ratios due to functional redundancy of common species that exists in a community (Schleuter et al., 2010).

So far, several approaches to measuring functional diversity have been proposed based on species multiple ecological and morphological traits (Bremner, 2008; Mouchet et al., 2010). In this sense, specific species traits are considered to be indicators of ecosystem functioning on the grounds that they are related directly or indirectly to ecosystem functions and processes (Bremner et al., 2003; Mason et al., 2005; Villéger et al., 2008). Previous investigations have demonstrated that

the functional traits of periphytic ciliates such as feeding types and body-size spectrum are significantly associated with water quality (e.g., Zhong et al., 2017). For example, bacterivores are commonly with small size and dominated in polluted waters while the predators are with large size and occur in clean water area (Zhong et al., 2017; Xu et al., 2018a,b). However, as regards the effectiveness of functional distinctness on discriminating water quality status using ciliated protozoa, little understanding was reported by Somerfield et al. (2008).

In this study, we attempted to address the functional diversity/ distinctness of periphytic ciliates in response to water quality status based on a dataset from Yellow Sea coastal waters. Our objectives of this study were to demonstrate the effectiveness of the functional distinctness of periphytic ciliates for monitoring water quality in marine ecosystems.

2. Materials and methods

2.1. Data collection

Selecting four stations (Fig. 1, A–D), with in a gradient of pollution in coastal waters of the Yellow Sea, northern China, were selected as
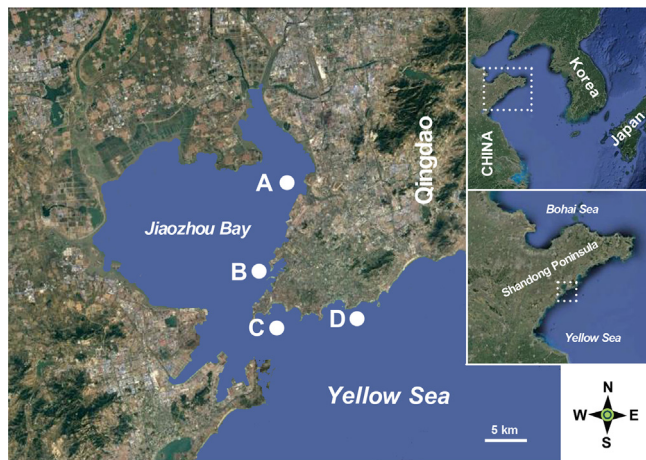

Fig. 1. Sampling stations in coastal waters of the Yellow Sea, near Qingdao, northern China. A–D, stations A–D.

our study areas. 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; station B, moderately polluted area Jiaozhou Bay by minor discharges from a small river entering the bay; station C, slightly polluted area near the mouth of Jiaozhou Bay and relatively distant from the rivers entering the bay; station D, relatively clean area which was out of this bay and more distant from the river discharges.

During the one-year period from August 2011 to July 2012, 40 samples were collected every month and microscope slides were used as the artificial base with a depth of 1 m by Xu et al. (2014).

Species identification and individual enumeration were carried out according to the methods described (Xu et al., 2014). For identification of species, the references such as Song et al. (2009) were used.

The enumeration of ciliates *in vivo* was carried out at a 100X magnification under an inverted microscope (Leica DM2500) within 24 h after sampling (Xu et al., 2009a,b). In order to recover all species colonizing the glass slides, one surface of an entire slide (17.5 cm^2) was examined using bright field illumination and occurrences were recorded. For the enumeration of individual abundances, 10 randomly chosen fields of view per slide were examined and the dominant ciliates were enumerated. The ciliate abundances were calculated for 10 glass slides to confirm the average abundance of ciliate individuals (ind cm^{-2}).

Water temperature (T), salinity (Sal), pH, chemical oxygen demand (COD) and dissolved oxygen (DO) were detected *in situ* using WTW Multi 3500i sensor. Concentrations of ammonium nitrogen (NH$_4$-N), nitrate nitrogen (NO$_3$-N), nitrite nitrogen (NO$_2$-N), and soluble reactive phosphate (SRP) were measured using the "Standard Methods for the Examination of Water and Wastewater" (APHA, 1992).

2.2. Functional traits and coding

The functional characteristics which were selected in the present study incorporated those functional traits (variables) that describe some of the major ecological functions with the strongest on energy flow in the ecosystem and are likely to be available for most of the species. Therefore, we used those functional traits common in the related literature on benthic ecosystems (Tillin et al., 2006; Michaud et al., 2006; Norling et al., 2007). As is known to all, these traits directly or indirectly, as an important indicators of the processes in aquatic ecosystems, such as crossing the sediment-water interface nutrient fluxes, irrigation and bioturbation, habitat modification, carbon recycling and sediment transport (Bremner, 2008). Seven major functional traits (variables) were incorporated in our study: two variables related to

nutrients and space resources dynamics ('adult mobility' and 'living position'), three variables connected to trophic structure ('feeding type', 'feeding apparatus' and 'food type'), one variable reflecting habitat modification ('habitat modificators'), as well as one variable related to bioturbation ('bioturbation') (Table S1). Filling in missing data was based on information of congeneric species as it was proposed by Tillin et al. (2006) and Papageorgiou et al. (2009).

The fuzzy coding trait system was used when a species represents more than one trait within each variable based on literatures such as Bremner et al. (2003) and Zhong et al. (2017).

The feeding type of ciliate species should be addressed, i.e., bacterivores (B), algivores (A), raptors (R) and non-selectives (N) (Pratt and Cairns, 1985; Song et al., 2009) and direct observation. Fuzzy encoding program was used in the cases of species representing a plurality of traits within each variable based on literatures such as Dimitriadis et al. (2012).

It should be noted that in the fuzzy coding system, The scoring range of 0 to 3 was adopted, with 0 expresses no affinity for the given trait category, 1 or 2 express partial affinity and 3 expresses total exclusive affinity. For example, *Euplotes vannus* feeds on both bacteria and microalgae but prefer the former, so it was coded 2 (bactivores), 1 (algivores), 0 (predators) for the trait "feeding type".

2.3. Data analyses

For functional diversity/distinctness measures, functional diversity (Δ_χ), functional distinctness (Δ^*_χ), average functional distinctness (Δ^+_χ) and variation in functional distinctness (Λ^+_χ) were used to conclude the functional distinctness of periphytic ciliates in react to environmental changes. All four indices were calculated logically by taxonomic diversity/distinctness measures following the four equations (Eqs. (1)–(4)):

$$\Delta_z = \left[\sum \sum_{i<j} \omega_{ij} x_i x_j \right] / [N(N-1)/2] \tag{1}$$

$$\Delta^*_\chi = \left[\sum \sum_{i<j} \omega_{ij} x_i x_j \right] / \left[\sum \sum_{i<j} x_i x_j \right] \tag{2}$$

$$\Delta^+_\chi = \left[\sum \sum_{i<j} \omega_{ij} \right] / [S(S-1)/2] \tag{3}$$

$$\Lambda^+_\chi = \left[\sum \sum_{i<j} (\omega_{ij} - \Delta^+) \right] / [S(S-1)/2] \tag{4}$$

where the abundance of the *i*th species is denoted by x_i ($i = 1, 2, …, S$); The total number of individuals in the sample is N in the sample; The "distinctness weight" given to the path length linking species *i* and *j* is ω_{ij} ($i < j$); The number of species is S (Xu and Xu, 2016; Xu et al., 2016).

It should be noted that the trait-resemblance matrix that was required for calculation of all four indices was generated the from the fuzzy coding trait data using the routine Textual/Similarity in the program PRIMER v7.013. Thus, no tree was used for computing all four indices unlike for taxonomic diversity/distinctness measures requiring a tree by their definition (Clarke and Gorley, 2015).

Ellipse tests for pair-wised indices (Δ^+_χ and Λ^+_χ) were conducted to signify the departure samples from an expected functional pattern using the routine TAXDTEST of the program PRIMER (Clarke and Gorley, 2015).

Before the analysis, logarithmically transform the data and use SPSS v22.0 statistical program to distinguish between the functional diversity/distinctness index and the environmental variables (Xu et al., 2014). The coefficients with a significance at the 0.05 level were