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# Identifying functional species pool of planktonic protozoa for discriminating water quality status in marine ecosystems

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ABSTRACT

It is cost-effective protocol to identify a functional species pool for marine bioassessment by removing redundant species from a raw dataset. The feasibility of functional species pool for discriminating water quality status was studied based on a dataset of 120 samples of ciliated protozoa. From the full 60-species dataset of the whole ciliate communities, a 35-species subset was identified as a functional species pool, the species number, abundance and biodiversity indices of which were significantly correlated with those of the full species dataset. The spatial pattern of the subset was significantly related to the changes in nutrients soluble reactive phosphates (SRP), nitrate/nitrite nitrogen ($NO_3$-N/$NO_2$-N) and ammonium nitrogen ($NH_4$-N). Four indices of the taxonomic diversity ($\Delta$), taxonomic distinctness ($\Delta^*$), average in taxonomic distinctness ($\Delta^+$) and the variation in taxonomic distinctness ($\Lambda^+$) based on this small species pool were significantly correlated with the changes of nutrients $NO_3$-N and/or ($NH_4$-N). The paired indices $\Delta^+$ and $\Lambda^+$ showed a clear decreasing trend of departure from the expected taxonomic pattern. These findings suggest that the 35-species functional species subset may be used as a feasible functional surrogate of ciliated protozoan assemblages for community-based bioassessment in marine ecosystems.



## 1. Introduction

Ciliated protozoa are a primary component of eukaryotic microbiota and play an important role in the functioning of the microbial food web in aquatic ecosystems (Jiang et al., 2011; Xu et al., 2011a,b). With their short generation times, rapid response to environmental changes, they have been widely used as a bioindicator for assessing water quality status at both species and community levels in aquatic ecosystems, especially freshwater biotopes (e.g., Ismael and Dorgham, 2003; Jiang et al., 2007; Shi et al., 2012). Previous works have demonstrated that the community pattern of ciliated protozoa can be used to discriminate the water quality status in Jiaozhou Bay, northern China, and that the taxonomic distinctness indices based on this fauna may be is a useful indicator of eutrophication bioassessment (Xu et al., 2011a,b; Jiang et al., 2011, 2014).

Multivariate analyses have proved to be a powerful tool for revealing the differences among community patterns on spatial and temporal scales and for illustrating how these patterns vary along gradients of environmental stress (Jiang et al., 2011; Xu et al., 2011a, 2014). Based on these methodologies, however, the analyzing outputs of community-based ecological research and monitoring program are usually influenced by the species redundancy (e.g., Micheli and Halpern, 2005; Ellingsen et al., 2007). Although taxonomic sufficiency has proved to be suitable approach for identification of potential surrogates for a community, studies on the utility of a functional species pool of a full species set in a ciliated protozoan community for bioassessment has yet to be carried out (Ellis, 1985; Stark et al., 2003; Xu et al., 2011a,b; Jiang et al., 2014).

In this study, a multivariate approach, step-best-matching analysis, was used to identify a functional species pool from a full set of species. A dataset of protozoa used was collected for assessing water quality status in coastal waters of the Yellow Sea, near Qingdao, northern China from August 2011 to July, 2012. The objective of this study was to determine a feasible species pool of the ciliated protozoa for marine bioassessment.

## 2. Materials and methods

### 2.1. Dataset collection

Five sampling sites were selected in Jiaozhou Bay (Fig. 1). Station A was slightly stressed area. Station B was located in a severely

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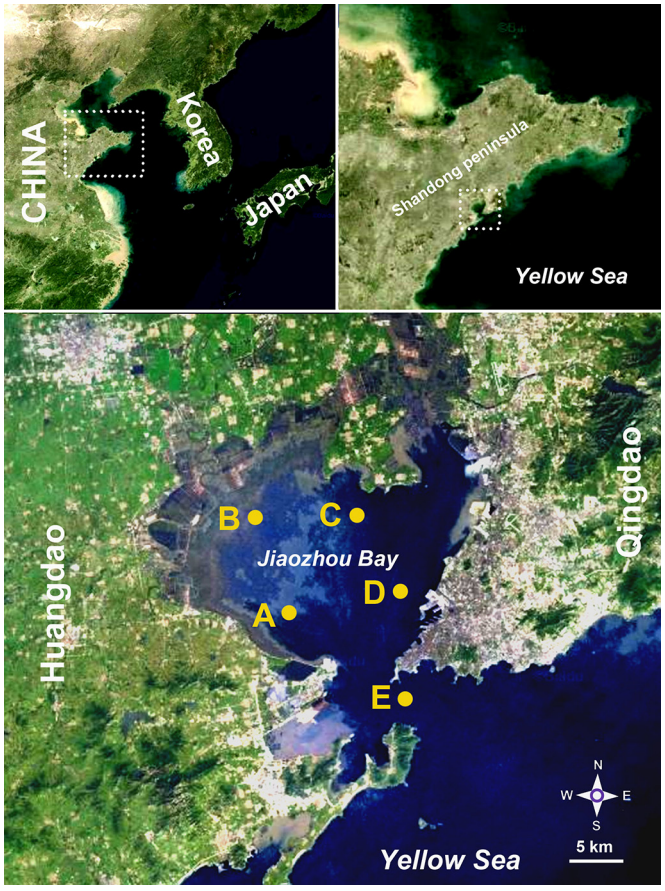


**Fig. 1.** Sampling stations in Jiaozhou Bay, near Qingdao, northern China. Station A was slightly stressed area. Station B was located in a severely stressed area that was polluted by organic pollutants, nutrients and heavy metals from both domestic sewage and industrial discharges. Station C is a heavy polluted area mainly due to mariculture activities. Station D was a moderately contaminated by both organic and heavy-metal pollutants. Station E was the least polluted area.

stressed area that was polluted by organic pollutants, nutrients and heavy metals (e.g., Pb, Zn) from both domestic sewage and industrial discharges. Station C is a heavy polluted area mainly due to mariculture activities. Station D was a moderately contaminated by both organic and heavy-metal pollutants (e.g., Cr, Cu). Station E was the least polluted area (Fig. 1) (Marine Environmental Monitoring Center, 1992).

A total of 120 datapoints were collected biweekly at a depth of 1 m from each sampling station during a 1-year cycle (June 2007–May 2008). Sampling strategy, sample processes, enumeration, species identification and measurement of environmental parameters were followed that described by Jiang et al. (2011, 2014). The taxonomic scheme used was according to Lynn (2008).

### 2.2. Data analysis

Dominance index ($Y$) of species in each sample was calculated using the following formula:

$$Y = \frac{n_i}{N} f_i$$

where $n_i$ is the number of individuals of species $i$; $f_i$ is the frequency of species $i$ that occurred in a sample and $N$ is the total number of species.

Species diversity (Shannon–Wiener $H'$), evenness (Pielou's $J'$) and richness (Margalef $D$) indices were computed following the equations:

$$H' = \sum_{i=1}^{s} P_i(\ln P_i)$$

$$J' = \frac{H'}{\ln S}$$

$$D = \frac{S-1}{\ln N}$$

where $H'$ = observed diversity index; $P_i$ = proportion of the total count arising from the $i$th species; $S$ = total number of species; and $N$ = total number of individuals.

The taxonomic diversity ($\Delta$), taxonomic distinctness ($\Delta^*$), average taxonomic distinctness ($\Delta^+$) and variation in taxonomic distinctness ($\Lambda^+$) indices of microperiphyton fauna were computed using the following the equations:

$$\Delta = \frac{\sum\sum_{i<j}\omega_{ij}x_i x_j}{N(N-1)/2}$$

$$\Delta* = \frac{\sum\sum_{i<j}\omega_{ij}X_i X_j}{\sum\sum_{i<j}X_i X_j}$$

$$\Delta^+ = \frac{\sum\sum_{i<j}\omega_{ij}}{S(S-1)/2}$$

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

where $x_i$ ($i = 1, 2, \ldots, S$) is the abundance of the $i$th species; $\omega_{ij}$ is the "distinctness weighting" given to the path length linking species $i$ and $j$ ($i < j$); $S$ is the number of species; and $N$ is the total number of individuals in the sample (Warwick and Clarke, 1995).

The distinctness weightings were according to Warwick and Clarke (1995) in context of the phylum Ciliophora: $\omega = 1$ (species in the same genus), 2 (same family but different genera), 3 (same order but different family), 4 (same class but different order) and 5 (same phylum but different class). The distinctness of two species connected at the highest taxonomic level was set equal to 100 (Warwick and Clarke, 2001). A regional master list was compiled using the data from Song et al. (2009), in which a total 75 planktonic ciliate species was recorded from local areas of the Yellow Sea, near Qingdao, China. This master list was used for testing the departure of the ciliate samples from the expected taxonomic pattern.

The step-best-matching analysis was used to exhaustively select the best matching subsets that were significantly correlated with the matrix of the full species abundance dataset from the same dataset by discarding the former selections using the submodule BVSTEP in the PRIMER package (v7.06). The initial selection was define as best 1, second selection after discarding the first selection as best 2, third selection after discarding the first and second selection as best 3, ..., and last selection with significant correlation with abiotic matrix after peeling out selections $1-n$ from full species set as best $n$. Among these selections, a smallest subset of species was made up as a functional species pool with sufficient information of the full species set (Clarke and Gorley, 2015).

The spatial community patterns among the four sampling stations were discriminated using the submodule CAP (canonical analysis of principal coordinates) of PERMANOVA+ for PRIMER on Bray–Curtis similarities from the transformed species-abundance data (Xu et al., 2014). Differences between groups of samples were

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