



Identifying bioindicators across trait-taxon space for assessing water quality in marine environments

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ABSTRACT

The response units of protozoan communities, based on a community-weighted mean (CWM) dataset across trait-taxon space, were investigated in order to determine their utility as bioindicators of marine water quality. From a total of 17 functional categories of seven biological traits, three functional response units (FRUs) were identified at correlation levels of > 0.75 . FRUs 1 and 3 generally dominated the communities in more polluted areas during warm seasons, while FRU2 appeared to prefer less polluted waters and dominated the communities in spring and winter. Correlation analysis demonstrated that the CWM values of FRUs 1 and 3 were significantly positively correlated to the concentrations of chemical oxygen demand (COD), whereas those of FRU2 were negatively correlated to COD. Across taxon-function space, 16 species were identified as potential bioindicators of water quality. These results suggest that redundancy analysis across trait-taxon space is a useful tool for identifying indicators of environmental quality.

1. Introduction

Methodologies for identifying bioindicators in taxon space are commonly subject to a high signal-to-noise ratio due to functional redundancy of co-occurring species in communities (Menge et al., 1994; Chapin et al., 1995; Frost et al., 1995; Clarke and Warwick, 1998; Gray et al., 1998; Zhong et al., 2014). Determination of the functional redundancy is a useful tool for reducing these signal-to-noise ratios by identifying the changeable response units using functional compensation or competition in community-based monitoring programs (Gray et al., 1998; Zhong et al., 2014; Xu et al., 2017). This can be achieved by analyzing functional trait resolution (e.g., Hewitt et al., 2008; Paganelli et al., 2012). Such analyses have been widely utilized for summarizing spatial/temporal patterns of ecosystem functioning and processes (Lavorel et al., 2008; Mokany et al., 2008; Vandewalle et al., 2010; Xu et al., 2017).

Ciliated protozoa (ciliates), have long been used to evaluate environmental quality status (Song et al., 2009), and also have several advantages as bioindicators compared to metazoa, including their shorter life cycles, high abundances, ease of sampling and sensitivity to environmental change (e.g., Coppellotti and Matarazzo, 2000; Xu et al., 2014; Zhang et al., 2014; Liu et al., 2015). Bioassessment using ciliates have traditionally been carried out based on full-species datasets,

significantly limiting their use due to the effects of high signal-to-noise ratios (Jiang et al., 2014; Xu and Xu, 2016; Xu et al., 2014, 2016). Thus, we propose an approach for identifying potential indicators for assessment environmental quality by using redundancy analysis across trait-taxon space. This can be achieved by determining functional response units (FRUs) within community-weighted mean (CWMs) data, and linking traits with taxon spaces.

In this study, a redundancy analysis across trait-taxon spaces was performed for bioassessment using ciliates and other protozoa in Chinese coastal waters of the Yellow Sea. The objectives of our study were: (1) to identify the FRUs within the protozoan trait data; (2) to analyze the co-occurring species associated with each FRU; (3) to reveal the relationships between FRUs and environmental conditions; and (4) to determine potential species indicators for assessing marine water quality.

2. Materials and methods

2.1. Data collection

Four sampling sites were selected in coastal waters of the Yellow Sea, specifically the Jiaozhou Bay area near Qingdao, northern China. Each site had different levels of water quality due to discharges from

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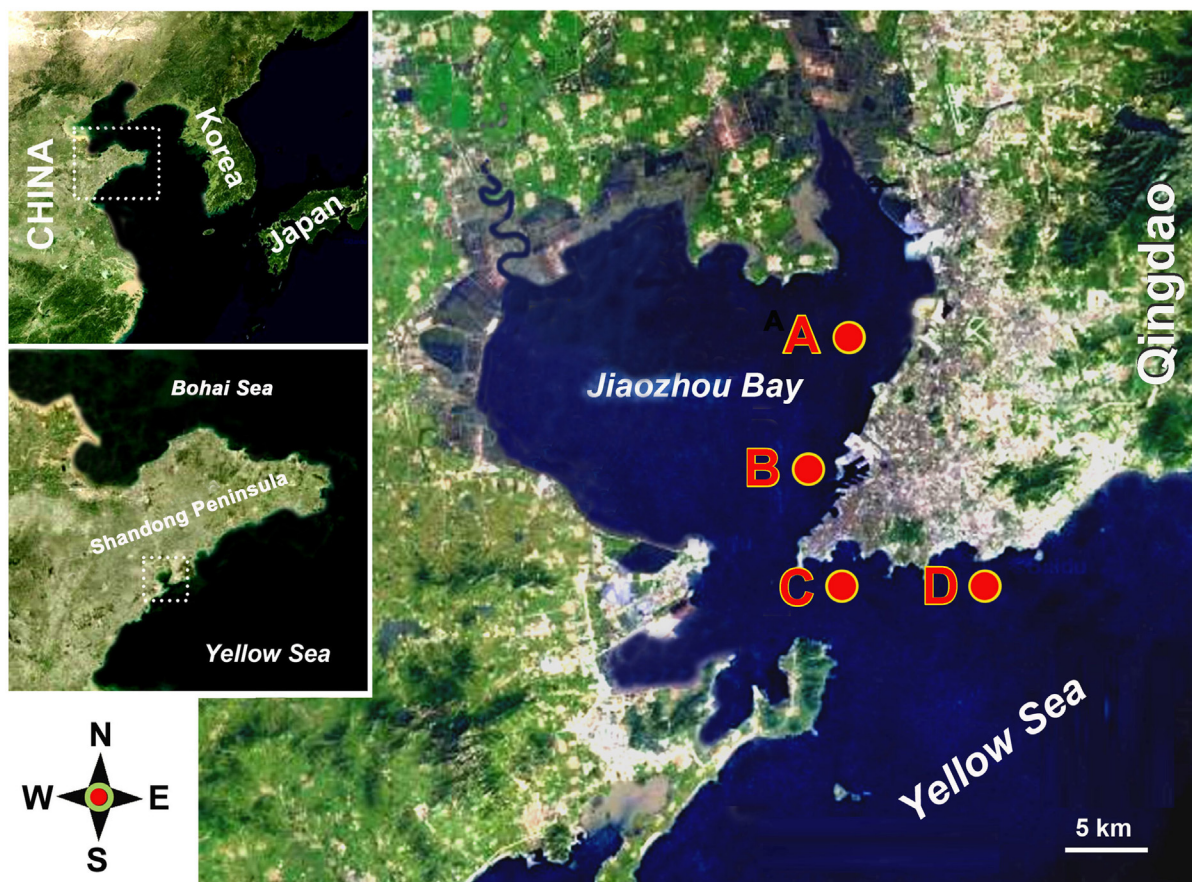


Fig. 1. Sampling sites in coastal waters of the Yellow Sea, near Qingdao, northern China

ivers and local point sources of pollution. Site A was in the most heavily polluted area of Jiaozhou Bay due to domestic sewage and industrial discharges entering the bay via several rivers; site B was in a moderately polluted area due to minor discharges entering the bay via a small river; site C was in a slightly polluted area near the mouth of the bay; site D was in a clean water area outside of the bay (Fig. 1, A–D).

Monthly samples were collected during a 1-year period (August 2011–July 2012) using glass microscope slides as artificial substrates (Xu et al., 2011). In brief, at each sampling station two frames, holding a total of 20 glass slides, were immersed at a depth of 1 m and left for 14 days in order to allow colonization by protozoa to take place. Upon collection the slides were transported to the laboratory with in situ water for examination using an inverted bright field light microscope. Ten replicate slides were examined for each site on each occasion.

Taxonomic identification of ciliate species was based on the published references (e.g., Song et al., 2009). The taxonomic scheme used was according to Lynn (2008).

Physico-chemical variables such as salinity (Sal), pH, dissolved oxygen (DO), and transparency were measured in situ using appropriate sensor. For measuring the following variables, 1-l water sample was collected using water sampler from 1 m depth at each station and preserved in ice box in dark condition and transported to the laboratory within 4 h. Concentrations of chemical oxygen demand (COD), ammonium nitrogen ($\text{NH}_4\text{-N}$), nitrate nitrogen ($\text{NO}_3\text{-N}$), nitrite nitrogen ($\text{NO}_2\text{-N}$) and soluble reactive phosphate (SRP) according to “Standard Methods for the Examination of Water and Wastewater” (APHA, 1992).

2.2. Data analysis

A fuzzy-coding trait system was established and used to calculate the community-weighted means (CWM) of biological trait data from the

original species data (Bremner et al., 2003; Lepš et al., 2011). In the trait system, seven biological traits sub-divided into 17 categories which were required for the community functioning of protozoa, were chosen as functional variables. These included basic morphological features such as body size and shape, degree of flexibility, sociability (e.g., motile, sessile or colonial) and behavioural characteristics (e.g., feeding strategy, food resource and movement) (Table S2). The biological traits were scored ranges 0 to 3, where each score categorized based on species affinities such as small body size scoring as 3 (small), 0 (medium) and 0 (large). Functional groupings of these variables were conducted following the published literature (e.g., Pratt and Cairns Jr, 1985; Song et al., 2009; Zhang et al., 2012) and direct observation. The CWM dataset was calculated using the *dbFD* function, which is a distance based trait matrix species biomass against their biological trait used for measuring functional diversity, in the “*FD*” R package (Laliberté et al., 2014).

The redundancy analysis and multivariate ordinations (nMDS) on the CWM data were carried out using the PRIMER v7.0.13 package. The peeling procedure, which was programmed using the function BVSTEP of the routine BEST, was used to exhaustively select the best matching subsets from the full functional variables within the CWM dataset by discarding the former selections (Zhong et al., 2014; Clarke and Gorley, 2015). The first subsets with > 0.75 correlations to the full dataset were identified as functional response units (FRUs) of the communities (Xu et al., 2017). The spatial patterns of FRUs were summarized using the bootstrap average analysis. The contour boundaries of four stations (bootstrap average regions) were ordinated by metric MDS, which was created from 75 bootstraps per group with 95% confidence intervals (Clarke and Gorley, 2015). The significance of correlations between each FRU and the full CWM matrix was tested using the routine RELATE (Clarke and Gorley, 2015).

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