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Insights into discriminating environmental quality status using taxonomic distinctness based on a small species pool of ciliated protozoa in marine ecosystems

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HIGHLIGHTS

- The order Tintinnida and genus *Strombidium* showed a low variability mainly at species level.
- The reduced species assemblage maintained full information of the whole ciliate community.
- The taxonomic diversity based on this species pool was significantly related to nutrients.
- The small species pool by present/absence data may be an indicator for bioassessment.

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ABSTRACT

The objective of this study was to determine the feasibility of developing a protocol for assessing marine water quality based on taxonomic relatedness within a small pool of planktonic ciliates. An annual dataset was compiled based on samples collected biweekly at five sites, with a gradient of environmental stress, during a 1-year cycle in Jiaozhou Bay, northern China. A total of 60 species, belonging to 17 genera 10 families, 5 orders and 2 classes of the phylum Ciliophora, were identified. Among five orders, Tintinnida showed a low variability mainly at species level whereas the other orders (especially Strombidiida and Choreotrichida, although with the exception of the genus *Strombidium*) represented a high variability at higher taxonomic ranks (e.g. family or order). Mantel analyses showed that spatial patterns of the ciliate assemblages, with tinitinnids and *Strombidium* spp. excluded, were significantly correlated with those of the total planktonic ciliate communities in terms of their response to environmental status. The average taxonomic distinctness (Δ^+) based on the small species pool was significantly negatively correlated with the changes in concentrations of nutrients ($P < 0.05$). Furthermore, the paired indices of Δ^+ and the variation in taxonomic distinctness (Λ^+) showed a clear departure from the expected taxonomic pattern. These findings suggest that it is possible to assess the status of marine water quality using the taxonomic relatedness within a small pool of planktonic ciliates.

1. Introduction

With increasing environmental stress, anthropogenic impacts, and the global decline in biodiversity, there is a pressing need for methods to assess environmental/ecological quality that are rapid, reliable and cost-effective. As species level identification usually involves high costs in terms of time, money and expertise, the search for suitable biotic surrogates that reflect species diversity patterns has become a priority in bioassessment planning (Stark et al., 2003; Terlizzi et al., 2008; Xu et al., 2011a, 2011b). Taxonomic sufficiency (TS) has proved to be a suitable alternative approach both for ecological monitoring programs and for biological conservation assessment (Ellis, 1985). Numerous investigations have demonstrated that a low taxonomic resolution may be used as a reliable surrogate of whole species assemblages in assessing responses to environmental stress, especially in cases where the impact is severe (Warwick, 1988; Stark et al., 2003; Terlizzi et al., 2008; Xu et al., 2011a, 2011b). An alternative approach of identifying suitable surrogates is to choose a single taxonomic or ecological group that reflects the sensitivity of the entire species assemblage to the environmental changes (Xu et al., 2011a, 2011b). Although the effectiveness of taxonomic/ecological surrogates has been reported for both metazoan and protozoan assemblages, studies on the utility of taxonomic relatedness within a small species pool of aquatic microfauna for bioassessment are yet to be carried out (Warwick, 1988; Vanderklift et al., 1996; Stark et al., 2003; Terlizzi et al., 2008; Shi et al., 2012; Xu et al., 2011a, 2011b).

Taxonomic relatedness measures have been widely used to evaluate marine biodiversity and environmental stress, largely due to their

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reduced sensitivity to habitat type and sampling effort, avoidance of species identifications, high sensitivity to environmental stress and anthropogenic impact, and the presence of a statistical framework for determining the significance of departure from expectation (Warwick and Clarke, 1995, 2001; Xu et al., 2011c). The utility of taxonomic distinctness indices based on presence/absence data for assessing environmental stress and anthropogenic impact has been demonstrated for both macrofauna and microfauna (Mouillot et al., 2005; Leonard et al., 2006; Somerfield et al., 2008; Prato et al., 2009; Xu et al., 2011c).

Ciliated protozoa are an important contributor to the microplankton communities and play a crucial role in the functioning of the microbial food web in most aquatic ecosystems (Jiang et al., 2007). Because of their short life cycles and delicate membranes, they generally respond more rapidly to environmental stress than metazoa (Coppellotti and Matarazzo, 2000; Jiang et al., 2011a, 2011b; Xu et al., 2011a, 2011b). Thus, they have been widely used as bioindicators for discriminating water quality status (Ismael and Dorgham, 2003; Jiang et al., 2007, 2011a, 2011b, 2012a). Jiang et al. (2011a, 2011b) reported that the spatial variations, both in community pattern and taxonomic distinctness, of planktonic ciliate assemblages in Jiaozhou Bay are significantly associated with environmental conditions. Furthermore, Xu et al. (2011c) revealed that the taxonomic distinctness of these planktonic ciliate communities is a useful indicator in response to anthropogenic impact and eutrophication. Recent investigations have demonstrated that non-loricate ciliate assemblages can be used as potential surrogates of total planktonic ciliate communities and that the loss of information using presence/absence resolution can be negligible (Xu et al., 2011a; Jiang et al., 2012b). However, determining the minimum size of a species pool that is sufficient for assessing water quality, based on the taxonomic distinctness of the ciliated microfauna, has received comparatively little attention (Xu et al., 2011a, 2011b).

In the present study, we investigated further the utility of taxonomic distinctness of the planktonic ciliate microfauna for the assessment of marine water quality in Jiaozhou Bay. The main aim was to determine the size and composition of the ciliate species pool that is sufficient for the reliable assessment of marine water quality in the bay based on the taxonomic distinctness of the ciliate microfauna.

2. Methods

2.1. Study area and dataset collection

Jiaozhou Bay (35°58′N–36°18′N, 120°04′E–120°23′E) is located in the western part of the Yellow Sea. It is a semi-enclosed basin with an area of about 390 km^2 and an average depth of about 7 m. It is surrounded by the city Qingdao and connects the South Yellow Sea via a narrow mouth. More than ten small seasonal streams empty into the bay most of which have become discharge canals carrying industrial and domestic waste wastewaters from Qingdao with varying water and sediment loads, and are important sources of external nutrients entering Jiaozhou Bay (Fig. 1). In recent decades there has been a deterioration in the water quality of the bay which is a serious concern both for the fisheries industries and for biodiversity conservation (Liu et al., 2008; Jiang et al., 2011a, 2011b; Xu et al., 2011c).

Five sampling sites were selected according to their environmental status and types of pollution, based on the marine water quality standards of China (Marine Environmental Monitoring Center, 1992). Site A was slightly stressed by pollutants (mainly nutrients) from inshore waters due to tidal circulation. Site B was selected as a severely stressed area contaminated by organic pollutants, nutrients and heavy metals (e.g., Pb, Zn) from domestic sewage and industrial discharges via two primary rivers. At site C there was heavy organic pollution mainly, from mariculture activities and the tidal circulation of inshore waters. Site D was moderately stressed by both organic and heavy-metal pollutants (e.g., Cr, Cu) from two rivers. Site E was located at the mouth of the bay, which was the least polluted area sampled (Fig. 1) (Marine Environmental Monitoring Center, 1992; Liu et al., 2005).

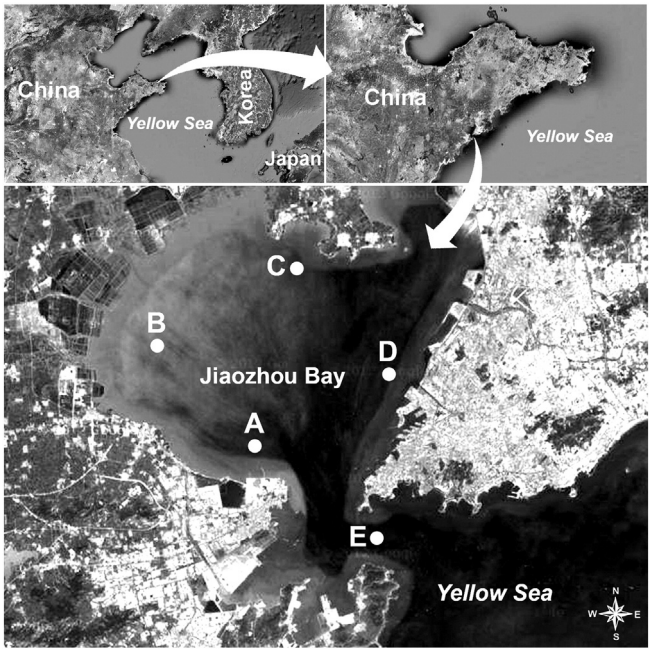

Fig. 1. Sampling stations in Jiaozhou Bay, northern China. A, site A, slightly stressed area, impacted by pollutants (mainly nutrients) from inshore waters due to tidal circulation; B, site B, severely stressed area, polluted by organic pollutants, nutrients and heavy metals (e.g., Pb, Zn) from domestic sewage and industrial discharges; C, site C, heavy organic pollution area with stress sources from mariculture activities and tidal circulation of inshore waters; D, site D, moderately stressed area with both organic and heavy-metal pollutants (e.g., Cr, Cu) from two rivers; and E, site E, the cleanest area.

A total of 24 samplings were carried out biweekly at a depth of 1 m from each sampling site during the period June 2007–May 2008. For quantitative and qualitative studies, 1000 ml water samples were fixed with Lugol's solution to a final concentration of 2% (volume/volume) and allowed to settle for 48 h resulting in 30 ml of concentrated sediment. For the enumeration of ciliates, a 0.1 ml aliquot of each concentrated sample was placed in a Perspex chamber and the ciliates were counted under a light microscope at a magnification of 400×. This was replicated five times, so for each sample a total of 0.5 ml of concentrated sample was counted yielding a standard error (SE) of <8% of the mean values of counts.

The measurements of dissolved inorganic nitrogen (NO_3–N, NO_2–N and NH_3–N), soluble reactive phosphate (SRP) and chlorophyll *a* (Chl *a*) were carried out using standard methods as described by Xu et al. (2008). Water temperature (Tem), salinity (Sal), pH and dissolved oxygen (DO) were recorded in situ with appropriate sensors (WTW).

Protargol staining was performed according to Montagnes and Humphrey (1998). Identification of ciliates was based on the published references to keys and guides such as Strüder-Kypke and Montagnes (2002) and Song et al. (2009). The taxonomic scheme used was according to Lynn (2008).

2.2. Data analysis of samples

Species diversity (H'), evenness (J') and species richness (D) were calculated as follows:

$$H' = -\sum_{i=1}^{S} Pi(\ln Pi)$$

$$J' = H'/\ln S$$