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Use of biofilm-dwelling ciliate communities to determine environmental quality status of coastal waters

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HIGHLIGHTS

- Biofilm-dwelling ciliate communities showed significant differences among four stations.
- The ciliate communities were significantly correlated with environmental variables.
- Five dominant ciliate species were significantly correlated with nutrients or COD.
- Species richness index was significantly correlated with the nutrient NO_3–N and DO.
- Biofilm-dwelling ciliates might be an indicator for bioassessment in coastal waters.

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ABSTRACT

It has increasingly been recognized that the ecological features of protozoan communities have many advantages as a favorable bioindicator to evaluate environmental stress and anthropogenic impact in many aquatic ecosystems. The ability of biofilm-dwelling ciliate communities for assessing environmental quality status was studied, using glass slides as an artificial substratum, during a 1-year cycle (August 2011–July 2012) in coastal waters of the Yellow Sea, northern China. The samples were collected monthly at a depth of 1 m from four sampling stations with a spatial gradient of environmental stress. Environmental variables, e.g., salinity, dissolved oxygen (DO), chemical oxygen demand (COD), nitrate nitrogen (NO_3–N), ammonium nitrogen (NH_4–N) and soluble reactive phosphates (SRP), were measured synchronously for comparison with biotic parameters. Results showed that: (1) the community structures of the ciliates represented significant differences among the four sampling stations; (2) spatial patterns of the ciliate communities were significantly correlated with environmental variables, especially COD and the nutrients; (3) five dominant species (*Hartmannula angustipilosa*, *Metaurostylopsis* sp.1, *Discocephalus ehrenbergi*, *Stephanopogon minuta* and *Pseudovorticella paracratera*) were significantly correlated with nutrients or COD; and (4) the species richness measure was significantly correlated with the nutrient NO_3–N. It is suggested that biofilm-dwelling ciliate communities might be used as a potentially robust bioindicator for discriminating environmental quality status in coastal waters.

1. Introduction

Ciliated protozoa have been proved to have many advantages in bioassessment of many aquatic ecosystems (Cairns et al., 1972; Cairns and Henebry, 1982; Xu et al., 2005; Jiang et al., 2007; Shi et al., 2012). Because of their short life cycle and delicate pellicles, they may response more rapidly to environmental changes than any metazoa (Coppellotti and Matarazzo, 2000; Xu et al., 2002; Ismael and Dorgham, 2003; Kchaou et al., 2009). Many ciliates can tolerate what would be extremes of environmental conditions to macrofauna (Cairns and Henebry, 1982; Xu et al., 2011a,b). Thus, ciliated protozoa have widely been used as favorable bioindicator for assessing water quality (Xu et al., 2011a; Jiang et al., 2013a,b).

As a primary component of the periphytic microfauna, biofilm-dwelling or periphytic ciliates play an important role in the functioning of microbial food webs, thus aroused increasing interests in various aquatic ecosystems (Bryers and Characklis, 1982; Eisenmann et al., 2001; Fischer et al., 2002; Weitere et al., 2003; Kathol et al., 2009; Norf et al., 2007, 2009a,b; Xu et al., 2011c,d). With ease of sampling, relative immobility, availability of user-friendly taxonomic references and standardized methodologies for temporal and spatial comparisons, the periphytic ciliates have widely been accepted as robust indicators to evaluate environmental stress and anthropogenic impacts in many aquatic ecosystems, especially the freshwater environments (Strüder-Kypke, 1999; Morin et al., 2008, 2010; Risse-Buhl and Küsel, 2009; Xu

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et al., 2005; Kralj et al., 2006; Jiang et al., 2007; Norf et al., 2007, 2009a,b; Mieczan, 2010). So far there have been several studies on community patterns and colonization dynamics of marine biofilm-dwelling ciliates (Railkin, 1998; Gong et al., 2005; Xu et al., 2009a, 2012a,b,c; Zhang et al., 2012a,b, 2013). As regards their ability to assess marine water quality status, however, little information was well documented.

A 1-year baseline survey of biofilm-dwelling ciliate community patterns was conducted using an artificial substratum (glass slides) in coastal waters of the Yellow Sea, near Qingdao, northern China from August 2011 to July, 2012. The main objectives of this study were: (1) to document the taxonomic composition and community structure of biofilm-dwelling ciliate communities over a 1-year period; (2) to reveal the spatial variations in ecological features of the ciliate communities with contrasting environmental conditions; and (3) to determine the ability of the ecological features to evaluate environmental quality status based on the ciliate communities in coastal waters.

2. Methods

2.1. Study area and data set collection

Four sampling stations were selected in coastal waters of the Yellow Sea, near Qingdao (population 7×10^6), northern China according to their environmental status and types of pollution, based on the marine water quality standards of China (Fig. 1, A–D). The four stations were located in areas with contrasting environmental conditions and anthropogenic impacts. Based on previous records, station A was known to be in the 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 was moderately polluted area in Jiaozhou Bay by minor discharges from a small river entering the bay; station C was only slightly polluted area near the mouth of Jiaozhou Bay, and relatively distant from the rivers entering the bay; station D was the least polluted area which was out of this bay and more distant from the river discharges (Fig. 1) (Jiang et al., 2011; Xu et al., 2011a).

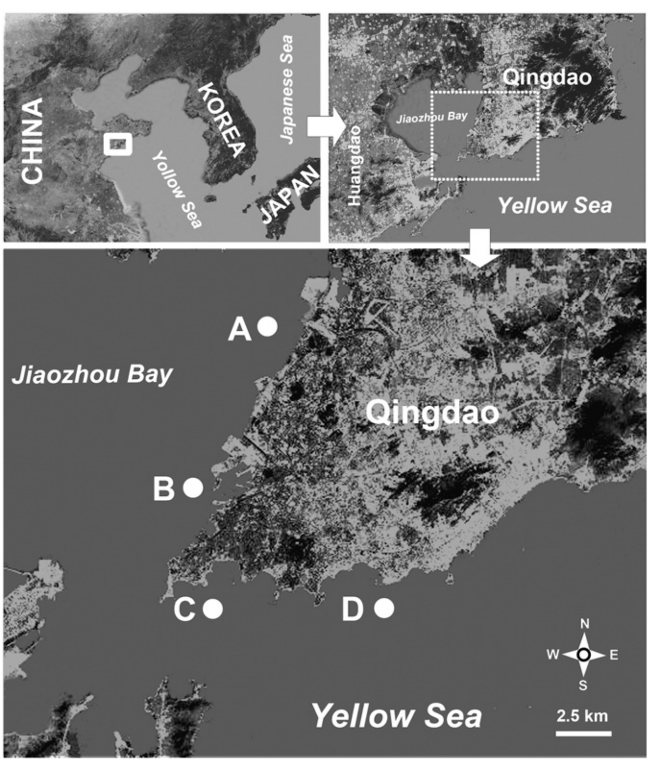

Fig. 1. Sampling stations in coastal waters of the Yellow Sea, near Qingdao, northern China. A: station A, heavily stressed area in Jiaozhou Bay, the pollution being mainly in the form of organic pollutants and nutrients from domestic sewage and industrial discharge from several rivers; B: station B,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

A total of 12 samplings were carried out monthly at the four stations from August 2011 to July 2012. The glass slide systems were designed, deployed, anchored, and sampled as described by Xu et al. (2009a,b). A total of 960 glass slides (2.5×7.5 cm) were used as artificial substrata for collecting the ciliates at a depth of 1 m below the water surface. For each sampling station, two PVC frames were used to hold the 20 slide replicates and all of slides were collected at the exposure time of 14 days. The glass slides were transferred into Petri dishes containing water from the sampling station and then stored in a cooling box before transporting to the laboratory within 2 h for identification and enumeration (Xu et al., 2009a, 2012a,b).

Ciliate identification and enumeration were conducted following the methods outlined by Xu et al. (2009a,b). Protargol staining was performed for species identification (Berger, 1999). Taxonomic classification of ciliates was based on the published references to keys and guides such as Song et al. (2009). The taxonomic scheme used was according to Lynn (2008).

The enumeration of ciliates *in vivo* was conducted at a 100-fold magnification under an inverted microscope as soon as possible within 4–8 h after sampling in order to prevent significant changes in species composition (Xu et al., 2009a,b). For recovering all species colonizing the glass slides, whole slide (17.5 cm^2) was examined to record both occurrences and individual abundances, using bright field illumination. As for counting the numbers of very high density species, 20 fields of view per slide were randomly chosen. The average abundance of ciliates (ind cm^{-2}) was calculated from 20 glass slide replicates (Xu et al., 2011c; Zhang et al., 2013).

A 500 ml water sample for nutrient and chemical oxygen demand (COD) analyses was collected simultaneously at a depth of 1 m from each sampling station. All water samples were preserved immediately upon collection by placing at −20 °C in the dark. The measurements of dissolved inorganic nitrogen (nitrate nitrogen NO_3–N, nitrite nitrogen NO_2–N and ammonium nitrogen NH_3–N), soluble reactive phosphate (SRP) and COD were carried out according to the "Standard Methods for the Examination of Water and Wastewater" (APHA, 1992). Water temperature (T), salinity (Sal), pH and dissolved oxygen (DO) were recorded with WTW Multi 3500i sensor, and transparency (Tra) was measured *in situ* using a transparent scale.

2.2. Data analyses

Species diversity (Shannon–Wiener H'), evenness (Pielou's J') and richness (Margalef D) indices are commonly employed in community-level investigations and are suitable for simple statistical analyses (Ismael and Dorgham, 2003). The three indices were computed following the equations:

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

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

$$D = (S-1)/\ln N$$

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

Multivariate analyses of spatial variations in the ciliate communities were analyzed using the PRIMER v6.1.16 package