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# Can annual cyclicity of protozoan communities reflect water quality status in coastal ecosystems?



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#### ABSTRACT

With many advantages, many ecological parameters of protozoan communities have been successfully used as a useful bioindicator for bioassessment of water quality in Chinese marine waters. However, as regard the response of the annual cyclicity of protozoan communities to seasonal environmental stress, a further investigation was needed. In this study, the cyclicity of annual variations in community patterns of biofilm-dwelling protozoa was studied based on an annual dataset. Samples were monthly collected, using glass slide method, at four stations, within a pollution gradient, in coastal waters of the Yellow Sea, northern China during a 1-year period. The cyclicity patterns of the microbiota represented a significant spatial variation among four stations. The low value of cyclicity coefficients occurred in heavily polluted area, while the high values were in less stressed areas. Correlation analysis showed that the cyclicity measure was significantly related to environmental variables ammonia, transparency and dissolved oxygen. Thus, it is suggested that the annual cyclicity of protozoan communities may be used as a potential bioindicator of bioassessment in marine ecosystems.

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#### 1. Introduction

In marine ecosystems, protozoa are a primary contributor to microperiphyton communities in terms of both composition and abundance, and play an important role in the functioning of microbial food webs by mediating the flux of carbon and energy from bacteria, pico- and nano-algae to high trophic levels (Finlay and Esteban, 1998; Jiang et al., 2011; Xu et al., 2014; Wang et al., 2016). With short growth cycle, rapid response to environmental changes, high tolerance to a large range of environmental conditions and easy of sample processing, protozoa have proved to be robust in indication of environmental quality status (Cairns, 1979; Jiang et al., 2011; Gomiero et al., 2013; Xu et al., 2014; Feng et al., 2015).

So far, our previous investigations have demonstrated the feasibility of protozoan communities for bioassessment of water quality in marine ecosystems. Furthermore, our recent studies have revealed that the ecological parameters based on protozoan communities, such as species richness, taxonomic distinctness, body-size distinctness and dispersion measures can be used as useful bioindicators of water quality status in marine ecosystems

# (Jiang et al., 2011, 2013a,b; Xu et al., 2015, 2016; Xu and Xu, 2016).

However, as regard the feasibility of protozoan annual cyclicity in indication of water quality status, little information was known.

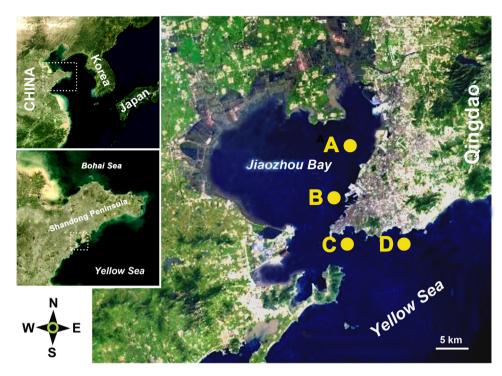
In this study, the cyclicity of annual variations in biofilm-dwelling protozoan communities were studied based on a dataset from coastal waters of the Yellow Sea, northern China. Our aims of this study were to demonstrate the feasibility of annual cyclicity in bioassessment of water quality status in marine ecosystems. This may provide a new ecological parameter for indication of marine water quality, using protozoa.

#### 2. Materials and methods

#### 2.1. Study areas and sample collection

Our study areas were located in coastal waters of the Yellow Sea, near Qingdao city, northern China (Fig. 1A–D). Four stations were selected along a pollution gradient: station A was a heavily stressed area in Jiaozhou Bay with the pollution mainly in the form of organic pollutants from several rivers; station B was moderately polluted area in Jiaozhou Bay, with minor discharges from a small river; station C, slightly polluted area near the mouth of Jiaozhou Bay; and station D, relatively clean area which was out of this bay.

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**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 in 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 10 sampling events were conducted monthly, using microscopy glass slides as an artificial substratum, at each station during a 1-year cycle of August 2011–July 2012 (except for February and March). Thus, a total of 40 samples were collected at four stations with two replicates after the exposure time of 14 days (Xu et al., 2014).

Species identification and enumeration were carried out following the methods described by Xu et al. (2011). For identification of species, the references such as Song et al. (2009), Fan et al. (2010) and Jiang et al. (2010) were used.

Water temperature (*T*), salinity (Sal), pH and dissolved oxygen (DO) were detected using WTW Multi 3500i sensor, while the values of transparency (Tra) were measured *in situ* using a transparent scale. The concentrations of nutrients ammonium nitrogen (NH<sub>4</sub>-N), nitrate nitrogen (NO<sub>3</sub>-N), nitrite nitrogen (NO<sub>2</sub>-N), and soluble reactive phosphate (SRP) were obtained using the "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association, APHA, 1992).

#### 2.2. Data analyses

Multivariate analyses were done using the software package PRIMER v7.0.10 (Clarke and Gorley, 2015). The Bray–Curtis similarity matrices were computed on fourth root transformed species-abundance data, while the Euclidean distance matrices for environmental variables were obtained from log-transformed/normalized abiotic data (Clarke and Gorley, 2015). Metric multidimensional scaling (nMDS) ordinations were used to show the trajectory of temporal dynamics of the samples, while the spatial patterns of protozoan distribution and annual water quality status were discriminated by the routine Bootstrap Average (Clarke and Gorley, 2015). The cyclicity was detected using the routine RELATE (Mantel test) with the significance at the *P* value < 0.05 level (Clarke and Gorley, 2015).

#### 3. Results

#### 3.1. Environmental conditions

The average values of environmental conditions at four sampling stations are showed in Fig. 2. There was a clear variation in these nine environmental variables among four stations. For example, the average values of transparency and DO represented an increasing trend, while those of NH<sub>4</sub>-N showed a clear decreasing trend from station A to D. The concentrations of NO<sub>3</sub>-N and SPR were low at stations C and D, and high at stations A and B.

#### 3.2. Annual dynamics and cyclicity

The mMDS ordinations, with bubble plots of species number and individual abundance, on the annual dynamics in protozoan communities were summarized in Fig. 3. In terms both species number and abundance, the trajectories of temporal variations of the samples were different among four stations (Fig. 3). For example, the values of abundances in August showed an increasing trend, while those in September were decreased from station A to D (Fig. 3)

Correlation analysis indicated that there was a significant annual cyclicity correlation in the trajectory of the temporal variation at all four stations. The correlation coefficients showed an increasing trend from station A to D (Fig. 4).

#### 3.3. Relationships between biotic and abiotic parameters

Bootstrap average analyses showed that there was a similar spatial pattern in both biota and abiota (Fig. 5). Furthermore, Spearman correlation analysis revealed a significant correlation between cyclicity correlation coefficients and environmental variables, such as NH<sub>4</sub>-N (R = -1.000; P < 0.05), DO and transparency (R = 1.000; P < 0.05).

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