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Sampling frequency of ciliated protozoan microfauna for seasonal distribution research in marine ecosystems

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A B S T R A C T

Sampling frequency is important to obtain sufficient information for temporal research of microfauna. To determine an optimal strategy for exploring the seasonal variation in ciliated protozoa, a dataset from the Yellow Sea, northern China was studied. Samples were collected with 24 (biweekly), 12 (monthly), 8 (bimonthly per season) and 4 (seasonally) sampling events. Compared to the 24 samplings (100%), the 12-, 8- and 4-samplings recovered 94%, 94%, and 78% of the total species, respectively. To reveal the seasonal distribution, the 8-sampling regime may result in >75% information of the seasonal variance, while the traditional 4-sampling may only explain <65% of the total variance. With the increase of the sampling frequency, the biotic data showed stronger correlations with seasonal variables (e.g., temperature, salinity) in combination with nutrients. It is suggested that the 8-sampling events per year may be an optimal sampling strategy for ciliated protozoan seasonal research in marine ecosystems.

1. Introduction

Ciliated protozoa are an important component of microfauna and play a crucial role in the functioning of microbial food webs by mediating energy and elements from pico- and nano-planktonic productions to higher trophic levels in marine ecosystems (Sherr and Sherr, 1987; Caron and Goldmann, 1990; Gifford, 1991; Legendre and Rassouldezgan, 1995; Sime-Ngando et al., 1995; Elloumi et al., 2006; Jiang et al., 2011a, 2011b; Xu et al., 2014a, 2014b, 2015). Changes in distribution of ciliated protozoa may significantly affect other components of the aquatic food web, and thus may influence the species composition and community structure of both lower and higher organisms (Finlay et al., 1988; Dolan and Coats, 1990). Otherwise, ciliated protozoa have many advantages in water quality monitoring surveys, such as short life generations, more sensitive to environmental stress (Jiang et al., 2014a, 2014b; Xu et al., 2011d, 2014a, 2014b; Zhong et al., 2014). Thus, these microbial eukaryotes have widely been used as a useful bioindicator of bioassessment in aquatic environment (Coppellotti and Matarazzo, 2000; Ismael and Dorgham, 2003; Zhang and Wang, 2000; Jiang et al., 2013a, 2013b, 2014a, b; Xu et al., 2014a, 2014b).

So far, the development of fast and cost-effective procedures for sampling sufficiency has become a pressing issue for marine ecologists with the increase of environmental pollution and human impact (Warwick, 1988; Terlizzi et al., 2003; Xu et al., 2011a, 2011b, 2011c; Zhang et al., 2015). For this purpose, taxonomic sufficiency has been received increasing attention since environmental stress can be detected using coarse ecological and/or taxonomic resolutions with sufficient information of the full dataset (Ellis, 1985; Vanderklift et al., 1998; Olsgard and Somerfield, 2000; Dauvin et al., 2003; Mendes et al., 2007; Bertasi et al., 2009; Xu et al., 2011c; Zhang et al., 2015). These approaches are to identify a taxonomic/ecological group as a surrogate of the whole species dataset (Olsgard and Somerfield, 2000; Bertasi et al., 2009; Carneiro et al., 2010; Xu et al., 2011c; Zhang et al., 2015). Although the effectiveness of these approaches has been reported on both microbiota and metazoa, such studies have yet to be conducted on sampling frequency for seasonal distribution research of ciliated protozoan fauna in marine ecosystems.

In this study, an annual dataset of the ciliated protozoan communities with four sampling frequency strategies in a basin of the Yellow Sea, northern China, was studied based on a dataset from the Yellow Sea, northern China. The objective of our study was focused on determining a sufficient sampling frequency strategy for exploring seasonal distribution of the ciliated protozoa in marine ecosystems

2. Materials and methods

2.1. Dataset collection

The dataset was extracted at five sampling stations in a basin of the Yellow Sea, surrounded by Qingdao, northern China, during the study period of June 2007–May 2008 (Fig. 1). Four subsets were constructed with 24 (biweekly), 12 (monthly), 8 (bimonthly in each season) and 4 (seasonally: traditionally in February, May, August and November) sampling events, respectively.

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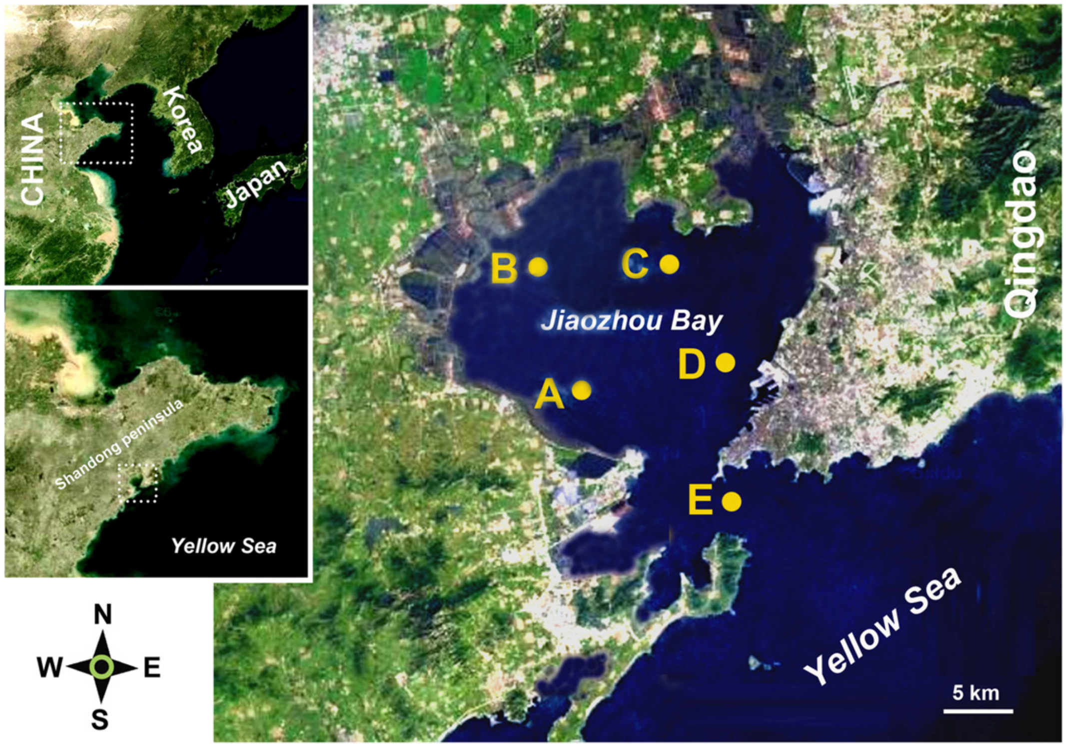

Fig. 1. Sampling stations in Jiaozhou Bay, northern China. A–E, sampling stations A–E.

The sample collection, processing and enumeration of the ciliated protozoa were conducted as described by Xu et al. (2011d). Species identification was done following to the published references and guides such as Song et al. (2009). The taxonomic scheme used was according to Lynn (2008).

For the abiotic data collection, water temperature (T), salinity (Sal), pH and dissolved oxygen (DO), NO_3-N, NO_2-N, NH_4-N, soluble reactive phosphate (SRP) and chlorophyll *a* (Chl *a*) were measured according the methods described by Xu et al. (2011d).

2.2. Data analysis

Species diversity (Shannon diversity) (H'), evenness (Pielou's evenness) (J') and richness (Margalef's richness) (D) of samplings were calculated as follows:

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

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

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

where Pi = proportion of the total count arising from the ith species; S = total number of species; and N = total number of individuals (Xu et al., 2014b).

The season patterns of the ciliated protozoan communities were summarized using canonical analysis of principal coordinates (CAP) based on the Bray–Curtis similarity matrices. The PERMANOVA test was used to signify the differences in community structure among/between four seasons. The significance of biota–environment correlations was signified using Mantel (RELATE) analysis, while the potential relationships between community patterns in species composition and the abiotic data were explored using biota–environment (BIOENV) matching analysis (Clarke and Gorley, 2015). Multivariate correlations between matrices by 24-, 12-, 8- and 4-sampling strategies were explored using RELATE analysis. All these analyses were carried out using the "PRIMER" (v7.09) software package (Clarke and Gorley, 2015; Anderson et al., 2008).

Univariate correlation analysis between biotic and abiotic variables was done using the software SPSS (v16.0).

3. Results

3.1. Environmental variables

The average values of seven environmental variables within each month were summarized in Table S1. Water temperature followed a clear seasonal pattern, ranging from 2.3 °C to 26.9 °C (mean 14.7 °C). Salinity was around 30.0 psu with a minimum value in September (23.7 psu) and a maximum in January (31.8 psu) over the 1-year cycle. The pH values ranged from 7.1 to 8.5, averaging 8.2. SRP ranged from 0.1 mg l^{-1} to 0.3 mg l^{-1} (mean value of 0.2 mg l^{-1}) with a minor peak in early August. The mean concentration of NH_4-N and NO_3-N peaked in late September, whereas low concentrations of NO_2-N were maintained throughout the year apart from a minor increase between July and September (Table S1).

3.2. Species composition and occurrence

In this dataset, 64 ciliate species were recorded (Table S2, Fig. 2a). Their seasonal pattern of species distribution by four sampling strategies was shown in Fig. 2b. Of the total 64 ciliate species that were identified by the biweekly samplings, the 12-, 8- and 4-sampling frequency recovered 60 (94%), 60 (94%) and 50 (78%) taxa, respectively. In the four subsets, 26, 19, 16 and 5 forms were distributed at all four seasons, and were defined as "common/dominant" species (Table 1).