

## Use of planktonic protists for assessing water quality in Jiaozhou Bay, northern China

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### Summary

Spatial patterns of planktonic protist communities were studied for assessing water quality in Jiaozhou Bay, northern China, from June 2007 to May 2008. Samples were collected biweekly at five sampling sites with a gradient of environmental stress. A range of physico-chemical parameters were also measured in order to determine water quality. Multivariate/univariate analyses demonstrated that: (1) the spatial patterns of planktonic protist communities represented significant differences among the five sites; (2) the spatial variations in protist community structures correlated with environmental variables, especially the nutrients nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and soluble reactive phosphates (SRP); and (3) species diversity indices significantly correlated with nutrients (e.g.,  $\text{NO}_3\text{-N}$ ). The results suggest that planktonic protists may be used as bioindicators of marine water quality.

**Key words:** bioassessment, water quality, marine ecosystem, planktonic protists

### Introduction

Planktonic protists are primary components of microplankton fauna and play a number of important roles in aquatic ecosystems including those as producers, primary consumers and secondary consumers, in particular being actively involved at various functional levels in channeling the flow of energy and elements in the microbial food webs (Cairns et al., 1972; Pitta et al., 1998; Shen, 2001; Liu et al., 2008; Jiang et al., 2011a, 2011b). Autotrophic protists are responsible for the bulk of primary production in most aquatic habitats; protozoan consumers transfer the production of algae and bacteria to higher trophic levels (Jiang et al., 2011b). With their small size, high reproduction rates, and close contact with the

surrounding environments, they may react more quickly to environmental changes than most of other eukaryotic organisms (Pitta et al., 1998; Ismael and Dorgham, 2003). Thus, protists are increasingly used as favourable bioindicators for assessing water quality (Pitta et al., 1998; Liu et al., 2008; Xu et al., 2008, 2010a, 2011b).

Jiaozhou Bay is a semi-enclosed basin, Qingdao, northern China. It covers an area of about 390 km<sup>2</sup> with an average depth of 7 m and is connected to the Yellow Sea via a narrow opening about 2.5 km wide. In recent decades, Jiaozhou Bay has been increasingly impacted by anthropogenic activities both in and around the bay (e.g., industry, agriculture and aquaculture) and as a consequence it is subject to pollution and/or eutrophication events (Fan and Zhou, 1999; Shen, 2001; Liu et al., 2005).

Although there have been a number of recent investigations on plankton community dynamics in Jiaozhou Bay (Shen, 2001; Liu et al., 2008), spatial patterns of planktonic protist community structures to assess water quality have yet to be investigated.

In the present study, a 1-year cycle survey on planktonic protist communities was conducted in Jiaozhou Bay, Qingdao, northern China, from June 2007 to May 2008. Our main objectives were: (1) to document the planktonic protist community structures at five sampling sites with contrasting environmental conditions; and (2) to determine relationships between planktonic protist communities and environmental variables.

## Material and methods

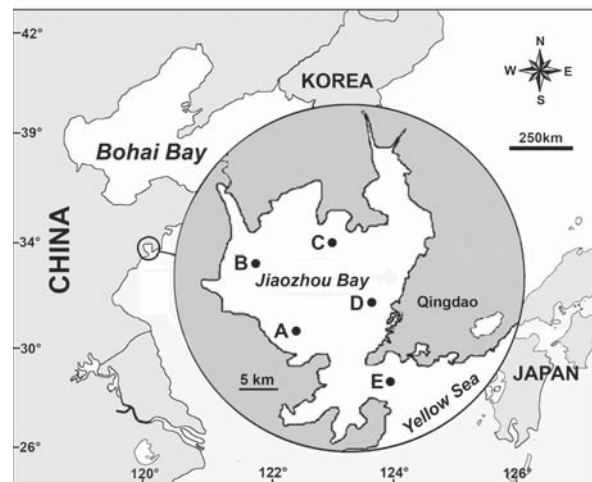
### STUDY SITES

Five sampling sites were located in Jiaozhou Bay ( $35^{\circ} 58' \text{ N} \sim 36^{\circ} 18' \text{ N}$ ,  $120^{\circ} 04' \text{ E} \sim 120^{\circ} 23' \text{ E}$ ), which is surrounded by the city of Qingdao, northern China (Fig. 1). The sites were chosen in areas with contrasting environmental conditions and anthropogenic impacts. Based on previous records, site B was known to be in the most heavily stressed area, the pollution being mainly in the form of organic pollutants and nutrients from domestic sewage and industrial discharge. Site C was fairly heavily polluted due mainly to intensive mariculture activities and the circulation of inshore waters from site B. Site D was moderately polluted by minor discharges from the Haipo and Licun rivers. Site A was only slightly polluted because it was located relatively distant from the rivers entering the bay. Site E was the least polluted area at the mouth of this bay (Marine Environmental Monitoring Center, 1992; Shen, 2001; Liu et al., 2005).

### SAMPLING AND SAMPLE PROCESSING

A total of 24 samplings were carried out biweekly from June 2007 to May 2008. All water samples were collected at a depth of 1 m. For quantitative studies and identification, 1000 ml seawater samples were fixed with acid Lugol's Iodine solution (2% final concentration, volume/volume) and settled for 48 h resulting in 30 ml of concentrated sediment (Utermöhl, 1958). Protist identification and enumeration were conducted following the methods outlined by Xu et al. (2008) and Jiang et al. (2011a, 2011b).

Salinity (Sal), pH, chlorophyll *a* (Chl *a*) and dissolved oxygen concentration (DO) were



**Fig. 1.** Sampling sites in Jiaozhou Bay, northern China. A, site A near Huangdao; B, site B near the mouths of Yang and Dagu rivers; C, Site C near mariculture area; D, site D near the mouths of Haipo and Licun rivers; E, site E at the mouth linking the bay with the Yellow Sea.

measured *in situ*, using a multi-parameter sensor (MS5, HACH). Samples for nutrient analyses were preserved immediately upon collection by placing at  $-20^{\circ} \text{ C}$  in the dark. Soluble reactive phosphate (SRP), ammonium nitrogen ( $\text{NH}_3\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and nitrite nitrogen ( $\text{NO}_2\text{-N}$ ) were determined using a UV-visible spectrophotometer (DR-5000, HACH) according to the 'Standard Methods for the Examination of Water and Wastewater' (APHA, 1992)

### DATA ANALYSIS OF SAMPLES

Margalef's index (species richness) of samples was computed following the equation:

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

where  $D$  = Margalef's index;  $S$  = total number of species;  $N$  = total number of individuals.

Multivariate analyses of spatial variations in planktonic protist communities were analyzed using the PRIMER v6.1 package (Clarke and Gorley, 2006). The separate clusters and multidimensional scaling (MDS) ordinations of five sites were used to summarize the spatial variation of abiotic factors and biotic communities on Euclidean distance and Bray-Curtis similarity from data of 120 samples, respectively (Clarke and Gorley, 2006). All data were subjected to log-transformations before analyzing.

The spatial environmental status of the five sampling sites was summarized using the principal

**Table 1.** Physical-chemical parameters of the five sampling sites in Jiaozhou Bay during the one-year cycle from June 2007 to May 2008 (mean values for a total of 24 samples from each sampling site).

| Parameters                                     | Sampling sites |              |              |              |              |
|--|----------------|--------------|--------------|--------------|--------------|
|  | A              | B            | C            | D            | E            |
| pH   | 8.20 ± 0.23    | 8.25 ± 0.23  | 8.23 ± 0.26  | 8.02 ± 0.26  | 7.86 ± 1.73  |
| Sal  | 31.29 ± 1.18   | 29.29 ± 5.59 | 30.38 ± 2.02 | 30.51 ± 2.13 | 29.46 ± 5.21 |
| Chl <i>a</i> (µg l <sup>-1</sup> )             | 1.52 ± 4.78    | 0.96 ± 1.63  | 1.52 ± 2.31  | 1.04 ± 1.35  | 1.00 ± 1.80  |
| DO (mg l <sup>-1</sup> )                       | 8.44 ± 1.65    | 8.99 ± 1.98  | 8.86 ± 1.54  | 8.05 ± 2.14  | 8.66 ± 1.56  |
| NH <sub>3</sub> -N (mg l <sup>-1</sup> )       | 0.12 ± 0.08    | 0.20 ± 0.16  | 0.22 ± 0.17  | 0.81 ± 0.74  | 0.19 ± 0.18  |
| NO <sub>3</sub> -N (mg l <sup>-1</sup> )       | 0.41 ± 0.29    | 0.51 ± 0.33  | 0.48 ± 0.37  | 0.41 ± 0.37  | 0.41 ± 0.33  |
| NO <sub>2</sub> -N (mg l <sup>-1</sup> )       | 0.02 ± 0.02    | 0.05 ± 0.07  | 0.07 ± 0.11  | 0.05 ± 0.04  | 0.04 ± 0.06  |
| SRP (mg l <sup>-1</sup> )                      | 0.19 ± 0.15    | 0.19 ± 0.15  | 0.16 ± 0.09  | 0.23 ± 0.17  | 0.12 ± 0.11  |
| DIN (mg l <sup>-1</sup> )                      | 0.55 ± 0.32    | 0.75 ± 0.44  | 0.77 ± 0.47  | 1.27 ± 1.05  | 0.76 ± 0.46  |
| DIN + SRP (mg l <sup>-1</sup> )                | 0.74 ± 0.32    | 0.94 ± 0.46  | 0.93 ± 0.50  | 1.49 ± 1.12  | 0.76 ± 0.49  |
| NO <sub>n</sub> -N (mg l <sup>-1</sup> )       | 0.43 ± 0.30    | 0.55 ± 0.38  | 0.55 ± 0.42  | 0.46 ± 0.39  | 0.46 ± 0.36  |
| NO <sub>n</sub> -N + SRP (mg l <sup>-1</sup> ) | 0.62 ± 0.33    | 0.74 ± 0.39  | 0.72 ± 0.42  | 0.69 ± 0.47  | 0.58 ± 0.38  |

Notes: Chl *a* – chlorophyll *a*; DO – dissolve oxygen; DIN – dissolved inorganic nitrogen; Sal – salinity; NO<sub>n</sub>-N – sum of NO<sub>3</sub>-N and NO<sub>2</sub>-N; SRP – soluble active phosphate.

component analysis (PCA) based on log-transformed/normalized abiotic data (Clarke and Gorley, 2006). The submodule BIOENV was used to explore potential relationships between abiotic parameters and biotic data. The significance of biota-environment correlations was tested using the routine RELATE (Clarke and Gorley, 2006).

Univariate correlation analyses were carried out using the statistical program SPSS v16.0. Data were log-transformed before analyses (Xu et al., 2008).

## Results

### ENVIRONMENTAL VARIABLES

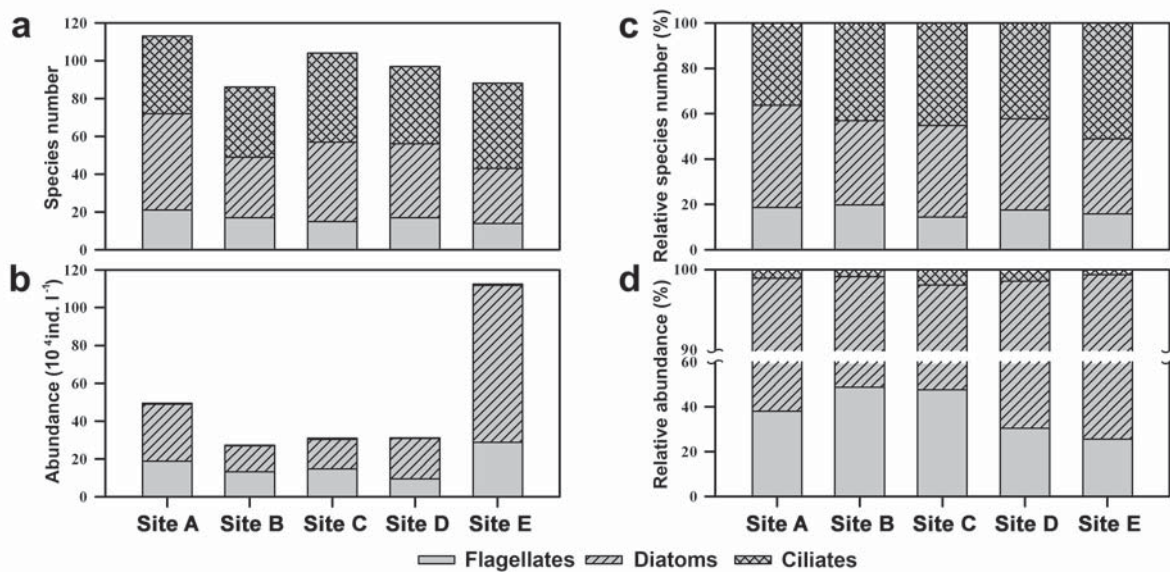
Environmental variables of the five sampling sites over the 12-month period were summarized in Table 1. Among these, pH showed minor differences at all sampling sites. Salinity ranged from 29.46 ‰ to 31.29 ‰ with lowest average values at site B and highest at site A. The concentrations of DO were usually higher than 8 mg l<sup>-1</sup> at all five sites, with the minimum average value at site D (8.05 mg l<sup>-1</sup>) and the maximum at site B (8.99 mg l<sup>-1</sup>). The highest concentrations of Chl *a* occurred at sites A and C, the lowest at site B. Concentrations of NO<sub>3</sub>-N, NO<sub>n</sub>-N (sum of NO<sub>3</sub>-N and NO<sub>2</sub>-N) and of NO<sub>n</sub>-N in combination with SRP were generally highest at sites B and C and lowest at sites E and

A, although DIN, SRP and sum of DIN and SRP reached maximum values at site D, mainly due to the primary contributor NH<sub>3</sub>-N (Table 1).

### SPATIAL PATTERNS OF SPECIES NUMBER AND ABUNDANCE OF PLANKTONIC PROTIST COMMUNITIES

A total of 164 protist species were recorded, comprising 75 diatoms, 25 flagellates and 64 ciliates. The average values of species number and abundance of planktonic ciliate communities at each of the five sampling sites are shown in Fig. 2. The average species count showed a maximum value at site A and a minimum at site B (Fig. 2a). However, the average abundances were highest at site E and lowest at site B (Fig. 2b).

The planktonic protist communities at the five sampling sites represented clear spatial patterns in both relative species numbers and abundance (Fig. 2c, d). It is noteworthy that at all five sites ciliates and diatoms were the primary contributors to the taxonomic diversity of planktonic protist communities (Fig. 2c). In terms of the relative abundances, flagellates and diatoms were the primary contributors and two structural types of communities can be recognized: (1) those dominated by flagellates and diatoms with the former being the primary contributor (e.g., sites B,



**Fig. 2.** Spatial variations in species number (a), abundance (b), relative species number (c) and relative abundance (d) of planktonic protist communities at five sites in Jiaozhou Bay from June 2007 to May 2008.

C); (2) those dominated by flagellates and diatoms with the latter being the primary contributor (e.g., site A, D and E) (Fig. 2d).

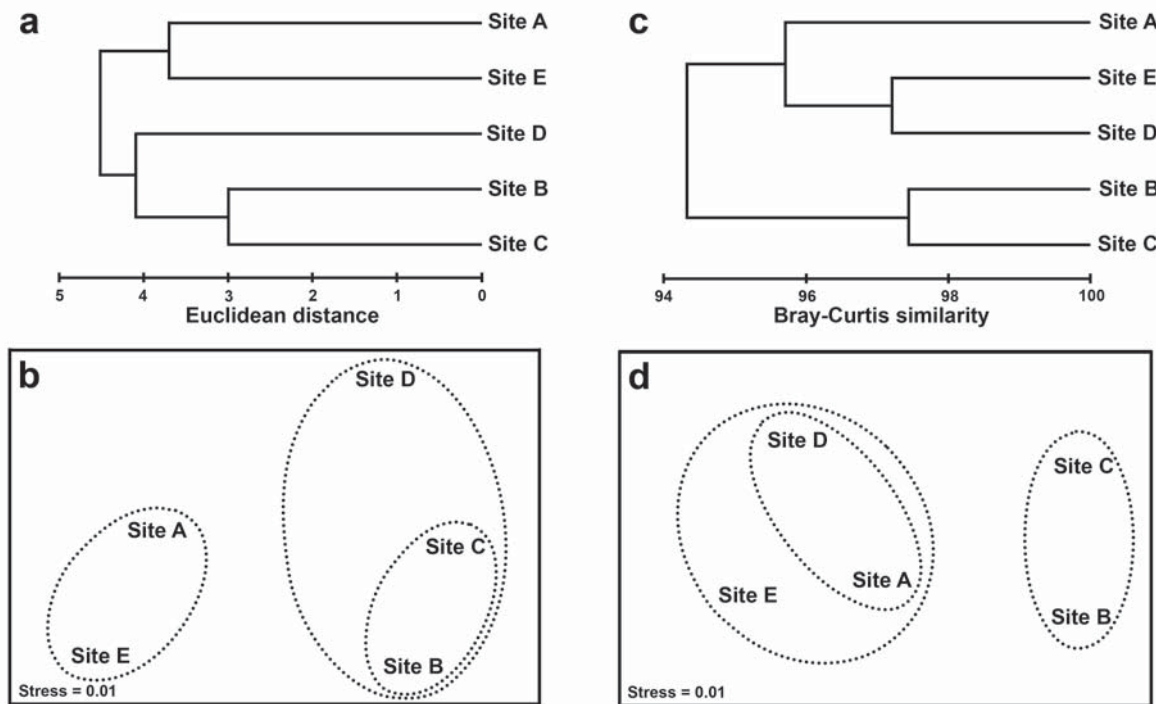
#### CORRELATION BETWEEN PLANKTONIC PROTIST DIVERSITY AND PHYSICO-CHEMICAL VARIABLES

The relationships among the five sampling sites based on data for environmental variables and for planktonic protists were summarized by clustering analyses and multidimensional scaling (MDS) ordinations in Fig. 3. The dendrograms were constructed using Euclidean distance from log-transformed abiotic data for the environmental variables (Fig. 3a, b) and on Bray-Curtis similarities from log-transformed species-abundance data for the planktonic protists (Fig. 3c, d). These multivariate approaches revealed that the spatial patterns of the protist communities were consistent with those of the environmental variables. In both cases there were high similarities between the two most polluted sites (B and C) that were separated from the two less polluted sites (A and E). As an exception, the moderately polluted site D was more similar to the latter pair than to the former in protist community (Fig. 3c, d), contrary to the environmental data (Fig. 3a, b). Furthermore, RELATE analysis revealed that there was a statistically significant though very low positive correlation between spatial variations in planktonic protist abundances and changes of environmental variables ( $R = 0.209$ ;  $P = 0.001$ ).

For all five sites, the correlations between protist abundances and environmental variables were analyzed by the routine BIOENV and summarized in Table 2. The results showed that the best matching with the protists occurred in case of the combination of salinity,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and SRP. It was also notable that the nutrients  $\text{NO}_3\text{-N}$  and SRP were the only two variables included in all correlations (Table 2).

PCA plotting with vectors for both structural parameters (individual abundance, the number and richness of species) of protist communities and physico-chemical variables is shown in Fig. 4. The two principal components, explaining 74.7% of the total spatial environmental variability, discriminated the five sites in a similar pattern that was shown by the clustering analysis and MDS (Figs 4 and 3a, b). It is noteworthy that species numbers and richness were correlated with the vectors for the physico-chemical variables (e.g.,  $\text{NH}_3\text{-N}$  and SRP) toward site D, while species abundances toward sites A and E and showing no correlations with nutrient parameters. The vectors of other variables (e.g.,  $\text{NO}_3\text{-N}$  and DO) pointed toward sites B and C (Fig. 4).

Moreover, Spearman correlation analysis showed that the species number and Margalef's index of protists, diatoms and flagellates were positively correlated with physico-chemical variables, in particular the nutrients ( $P < 0.01$ ), whereas the ciliate assemblages failed to reveal significant correlations with nutrients (Table 3).



**Fig. 3.** Cluster analysis and multidimensional scaling (MDS) ordinations for spatial changes of environmental status (a and b) on Euclidean distance from log-transformed environmental data, and of protist communities (c and d) on Bray-Curtis similarities for species-abundance data of five sampling sites in Jiaozhou Bay during the period from June 2007 to May 2008.

**Discussion**

Multivariate analyses are more sensitive than univariate analyses, and therefore it is fairly useful for analyzing variations of communities on spatial and temporal scales, as well as in illustrating how these communities vary along gradients of environmental stresses (Clarke and Ainsworth, 1993; Jiang et al., 2007; Hourston et al., 2009; Xu et al., 2011a, 2011b, 2011c, 2011d). In our study, both cluster analyses, MDS ordination and principle component analyses revealed a clear spatial pattern of environmental status. For example, the less polluted sites (A and E) were separated from the more polluted sites (B, C and D). Otherwise, clustering analyses and MDS ordination demonstrated that spatial patterns of planktonic protist communities were significantly consistent with spatial changes of water quality. Furthermore, the correlation analyses revealed that the spatial variations in the protist community structures correlated with environmental variables, especially nutrients nitrate nitrogen (NO<sub>3</sub>-N) and soluble reactive phosphates (SRP). These findings suggest that the spatial variations in planktonic protist community structures basically reflect the

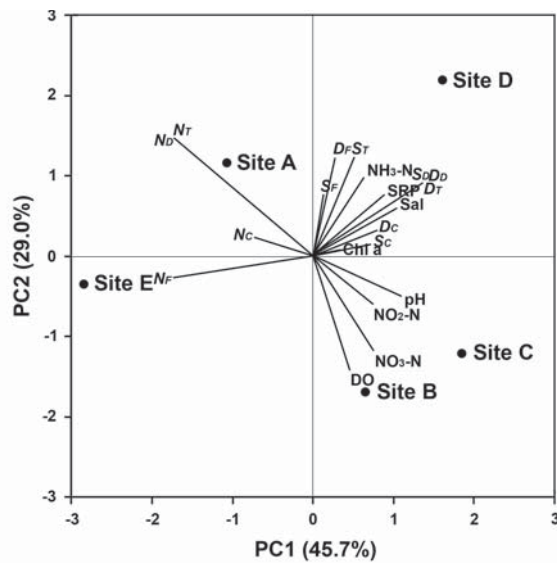
environmental status and may be used as a potential bioindicator in monitoring marine water quality.

Species richness and diversity are commonly employed in community investigations and amenable to simplify statistical analyses and used as indicators to evaluate water quality (Ismael

**Table 2.** Correlation (BIOENV) analysis showing the 8 best matches of environmental variables with spatial variations in protist abundances at five sampling sites in Jiaozhou Bay from June 2007 to May 2008.

| Rank | Abundance-environment |  |          |
|------|-----------------------|--|----------|
|      | <i>R</i>              | Variables  | <i>P</i> |
| 1    | 0.162                 | Sal, NO <sub>3</sub> -N, NO <sub>2</sub> -N, SRP | 0.01     |
| 2    | 0.161                 | Sal, NO <sub>3</sub> -N, SRP                     | 0.01     |
| 3    | 0.159                 | NO <sub>3</sub> -N, NO <sub>2</sub> -N, SRP      | 0.01     |
| 4    | 0.157                 | Tem, Sal, NO <sub>3</sub> -N, SRP                | 0.01     |
| 5    | 0.157                 | Tem, NO <sub>3</sub> -N, NO <sub>2</sub> -N, SRP | 0.01     |
| 6    | 0.155                 | Tem, Sal, NO <sub>3</sub> -N, SRP                | 0.01     |
| 7    | 0.154                 | pH, NO <sub>3</sub> -N, NO <sub>2</sub> -N, SRP  | 0.01     |
| 8    | 0.153                 | pH, Sal, NO <sub>3</sub> -N, SRP                 | 0.01     |

Notes: *R* = Spearman correlation coefficient. Tem = temperature. See Table 1 for other abbreviations.



**Fig. 4.** Principal component analysis (PCA) plotting based on log-transformed abiotic data of five sites. Axes 1 and 2 respectively accounted for 45.7% and 29.0% of the total variation present.  $S_T$ : total species number of protists;  $S_F$ : species number of flagellates;  $S_D$ : species number of diatoms;  $S_C$ : species number of ciliates;  $N_T$ : total abundance of protists;  $N_F$ : abundance of flagellates;  $N_D$ : abundance of diatoms;  $N_C$ : abundance of ciliates;  $D_T$ : total species richness of protists;  $D_F$ : species richness of flagellates;  $D_D$ : species richness of diatoms;  $D_C$ : species richness of ciliates.

and Dorgham, 2003; Connell, 1978; Magurran, 1991; Xu et al., 2011e, 2011f). In general, the higher the values, the higher the water quality, a notable exception being running waters with low organic pollution (Ismael and Dorgham, 2003). Subsequent theories have suggested that, at intermediate levels of disturbance, diversity (e.g., species richness) is highest and, depending on the starting point of the community in relation to existing eutrophication, increasing levels of eutrophication may result in either an increase or decrease in diversity. The changes in diversity can therefore only be assessed by comparisons between sites along a spatial contamination gradient or with historical data (Connell, 1978). In our study, results demonstrated that the species number and Margalef's index of protists, diatoms and flagellates were positively correlated with nutrients (e.g.,  $\text{NO}_3\text{-N}$ ). However, those of ciliates failed to reveal significant correlations with nutrients. These results are consistent with previous reports on the use of micro-organisms as indicators of water quality (Shen, 2001; Liu et al., 2008; Jiang et al., 2011a, b;

Ismael and Dorgham, 2003).

In summary, the spatial patterns of planktonic protist communities represented significant differences among the five sites, and the spatial variations in protist community structures correlated with environmental variables. Furthermore, species diversity indices significantly correlated with nutrients. Thus, we suggest that planktonic protist communities might be used as a helpful bioindicators for assessing water quality of marine ecosystems. However, further investigations on a range of marine habitats and over extended time periods are needed in order to verify this conclusion.

## Acknowledgements

This work was supported by "The Natural Science Foundation of China" (project number: 41076089), the Darwin Initiative Programme (Project No. 14-015) which is funded by UK Department for Environment, Food and Rural Affairs and partially supported by the Center of Biodiversity Research, King Saud University, Saudi Arabia. We thank Prof. Weibo Song, Laboratory of Protozoology, Ocean University of China (OUC), China, for his helpful discussions and Dr. Xinpeng Fan, Ms. Jiamei Jiang and Xumiao Chen, Laboratory of Protozoology, OUC, China, for their help with sampling and sample processing.

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**Table 3.** Correlations (Spearman analysis) between environmental variables and ciliate species number (S), abundance (N) and species richness (D) of planktonic protists at five sites in Jiaozhou Bay from June 2007 to May 2008.

|                | pH      | Sal    | Chl <i>a</i> | DO      | NH <sub>3</sub> -N | NO <sub>3</sub> -N | NO <sub>2</sub> -N | SRP    | DIN     | DIN+SRP | NO <sub>n</sub> -N | NO <sub>n</sub> -N+SRP |
|----------------|---------|--------|--------------|---------|--------------------|--------------------|--------------------|--------|---------|---------|--------------------|------------------------|
| S <sub>T</sub> | 0.146   | -0.020 | 0.150        | 0.146   | 0.068              | 0.199*             | 0.015              | 0.028  | 0.186*  | 0.167   | 0.192*             | 0.180*                 |
| S <sub>F</sub> | 0.049   | -0.068 | 0.176        | -0.004  | 0.117              | 0.266**            | -0.040             | 0.125  | 0.216*  | 0.222*  | 0.251**            | 0.277**                |
| S <sub>b</sub> | 0.286** | 0.015  | 0.070        | -0.135  | 0.152              | 0.359**            | 0.218*             | 0.160  | 0.278** | 0.270** | 0.369**            | 0.338**                |
| S <sub>c</sub> | 0.081   | 0.033  | 0.069        | 0.337** | -0.053             | -0.015             | -0.066             | -0.090 | 0.019   | -0.008  | -0.024             | -0.040                 |
| N <sub>T</sub> | 0.097   | 0.034  | 0.412**      | 0.537** | -0.007             | 0.021              | -0.036             | -0.107 | -0.006  | -0.012  | 0.011              | 0.002                  |
| N <sub>F</sub> | 0.048   | 0.028  | 0.348**      | 0.346** | -0.031             | 0.079              | -0.026             | -0.049 | 0.006   | 0.010   | 0.072              | 0.077                  |
| N <sub>b</sub> | 0.092   | 0.038  | 0.366**      | 0.602** | -0.008             | -0.021             | -0.030             | -0.085 | -0.013  | -0.033  | -0.033             | -0.051                 |
| N <sub>c</sub> | 0.100   | -0.025 | 0.197*       | 0.320** | -0.038             | 0.042              | -0.039             | -0.034 | 0.019   | 0.021   | 0.034              | 0.044                  |
| D <sub>T</sub> | 0.149   | -0.027 | 0.087        | 0.060   | 0.078              | 0.216*             | 0.033              | 0.054  | 0.202*  | 0.182*  | 0.212*             | 0.199*                 |
| D <sub>F</sub> | 0.041   | -0.091 | 0.159        | -0.027  | 0.104              | 0.254**            | -0.066             | 0.092  | 0.194*  | 0.198*  | 0.237**            | 0.256**                |
| D <sub>b</sub> | 0.264** | -0.009 | 0.028        | -0.227* | 0.150              | 0.358**            | 0.223*             | 0.182* | 0.275** | 0.273** | 0.370**            | 0.347**                |
| D <sub>c</sub> | 0.063   | 0.046  | 0.046        | 0.340** | -0.053             | -0.037             | -0.082             | -0.097 | 0.008   | -0.020  | -0.048             | -0.063                 |

Notes: \* $P < 0.05$ ; \*\* $P < 0.01$ ; see Table 1 and Figure 3 for other abbreviations.

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