Can tintinnids be used for discriminating water quality status in marine ecosystems?

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A B S T R A C T
Ciliated protozoa have many advantages in bioassessment of water quality. The ability of tintinnids for assessing water quality status was studied during a 7-year cycle in Jiaozhou Bay of the Yellow Sea, northern China. The samples were collected monthly at four sites with a spatial gradient of environmental pollution. Environmental variables, e.g., temperature, salinity, chlorophyll a (Chl a), dissolved inorganic nitrogen, soluble reactive phosphate (SRP), and soluble active silicate (SRSi), were measured synchronously for comparison with biotic parameters. Results showed that: (1) tintinnid community structures represented significant differences among the four sampling sites; (2) spatial patterns of the tintinnid communities were significantly correlated with environmental variables, especially SRSi and nutrients; and (3) the community structural parameters and the five dominant species were significantly correlated with SRSi and nutrients. We suggested that tintinnids may be used as a potential bioindicator for discriminating water quality status in marine ecosystems.

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1. Introduction

Ciliated protozoa have many advantages as a favorable bioindicator to evaluate environmental stress and anthropogenic impact in aquatic ecosystems (Cairns and Henebry, 1982; Jang et al., 2011, 2013a, 2013b; Xu et al., 2011a, b, c, 2014). With their short life cycle and delicate pellicles, they may respond more quickly to environmental changes than any metazoa (Coppellotti and Matarazzo, 2000; Ismael and Dorgham, 2003). Many ciliated microbiota can tolerate extremes of environmental conditions to macrofauna (Cairns and Henebry, 1982; Xu et al., 2011a, b). Thus, ciliated protozoa have been used as favorable bioindicators of water quality in many aquatic environments (e.g., Jang et al. 2011, 2013a, b; Xu et al., 2011a, 2014).

Tintinnids, as planktonic loricate ciliates, are important components of the aquatic ecosystem and play a crucial role in transferring elements and energy from low trophic levels (e.g., pico- and nano-phytoplankton) to high (e.g., copepods) (Corliss, 2002; Crawford et al., 1997; Montagnes and Lynn, 1989). So far, there have been several studies of marine planktonic ciliate assemblages (e.g., Dolan and Coats, 1990; Jang et al., 2011; Zhang and Wang, 2000; Dolan et al., 2012, 2013). As regards their relationships to marine water quality, however, little information was well documented (Dolan and Coats, 1990; Zhang and Wang, 2000, 2001). Although there have been a number of recent investigations on bioassessment using ciliated protozoa, the ability of marine tintinnids for discriminating water quality status bioassessment have yet to be studied (Jiang et al., 2011; Xu et al., 2011a, 2014).

A 7-year survey was conducted on tintinnid assemblages in Jiaozhou Bay from January 2006 to December 2012. The main objectives of this study were: (1) to document over a 7-year period the tintinnid community patterns at four sampling sites with contrasting environmental conditions; (2) to investigate the spatial variations in planktonic ciliate biodiversity; (3) to determine relationships between tintinnid communities and environmental variables.

2. Materials and methods

2.1. Study area and data collection

Jiaozhou Bay (35° 58′ N – 36° 18′ N, 120° 04′ E – 120° 23′ E) is a semi-enclosed basin, which is surrounded by the city of Qingdao, northern China. It covers an area of about 390 km² 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 impacted by anthropogenic activities both in and around the bay (e.g., industry, agriculture and aquiculture) and as a consequence it is subject to pollution and/or eutrophication events (Fan and Zhou, 1999; Liu et al., 2004; Sun et al., 2005). At least ten small seasonal streams, with varying water and sediment loads, empty into the bay. Most of these streams have become discharge trenches for industrial effluents and urban...
wastewaters from Qingdao city and are important sources of pollutants entering Jiaozhou Bay (Marine Environmental Monitoring Center, 1992; Shen, 2001; Liu et al., 2004, 2005). The principal routes by which the pollutants entered the bay were the Dagu and Yang Rivers. The Dagu River being the primary contributor with an annual average discharge of about 5.35 × 10⁸ m³ and an annual sediment load of 95.92 × 10⁶ t which accounted for 81% and 74% respectively of the total inputs into Jiaozhou Bay (Jiang et al., 2011, 2014; Xu et al., 2014).

Four sampling sites (Fig. 1) were selected in Jiaozhou Bay. The four sites were located in areas with contrasting environmental conditions and anthropogenic impacts. Based on previous records, Site 1 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 2 was fairly heavily polluted area near the mouth of this bay due mainly to intensive mariculture activities and the circulation of inshore waters from site 1. Site 3 was only slightly polluted but it was located relatively distant from the rivers entering the bay. Site 4 was least polluted area for more distance from the river discharges (Fig. 1) (Marine Environmental Monitoring Center, 1992).

A total of 84 cruises were carried out aboard the boat ‘Chuangxinbao’ at monthly intervals over a 7-year period from January 2006 to December 2012. The water depths of the Jiaozhou bay ranged from 2 to 64 m. All the samplings were carried out from a depth of about 1 m. For the tintinnid sampling, 30 l surface water was collected by a large volume water sampler (30 l) at each site. Immediately upon collection, water samples were concentrated to ~150 ml by pouring slowly and gently into a net (mesh size 20 μm) fixed with formalin solution. Subsamples of 20 ml from well-mixed concentrated samples were pipetted into a sedimentation chamber and settled for 24 h, and then Chl a concentrations were determined using a Turner Design Model-10 fluorometer that was calibrated with pure from Sigma (Strickland and Parsons, 1972).

Chl a concentrations were measured as index of phytoplankton biomass. A 500 ml water sample was collected at the surface layer of the site and filtered onto GF/F filters, which were preserved at ~ -20 °C in the dark until taken back to lab. The GF/F filters were extracted with 90% acetone at ~ -20 °C in the dark for 24 h, and then Chl a concentrations were determined using a Turner Design Model-10 fluorometer that was calibrated with pure from Sigma (Strickland and Parsons, 1972).

2.2. Identification and enumeration

Tintinnid enumeration was conducted according to the method of Utermöhl (1958). Subsamples of 20 ml from well-mixed concentrated samples were pipetted into a sedimentation chamber and settled for 24 h, and subsequently counted under an Olympus IX 71 inverted microscope (200× or 400×) with photographic measurement system. For each species, a minimum of 20 typical individuals were examined for morphological measurements and species identification according to literatures (Kofoid and Campbell, 1929; Kofoid and Campbell, 1939; Zhang et al., 2012).

2.3. Data analyses

Dominance index (Y) of species in each sample was calculated using the following formula (Xu and Chen, 1989):

\[ Y = \frac{n_i}{N} f_i \]

where \( n_i \) is the number of individuals of species \( i \), \( f_i \) is the frequency of species \( i \) that occurred in a sample and \( N \) is the total number of species.

Species diversity (Shannon-Wiener \( H' \)), evenness (Pielou’s \( J' \)) and richness (Margalef \( D \)) indices were 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:

\[ H' = -\sum_{i=1}^{S} P_i (\ln P_i) \]

\[ J' = H' / \ln S \]

\[ D = (S-1)/\ln N \]

where \( H' \) = observed diversity index; \( P_i \) = proportion of the total count arising from the \( i \)-th species; \( S \) = total number of species; and \( N \) = total number of individuals.

Multivariate analyses of spatial variations in the tintinnid assemblages were analyzed using the PRIMER v6.1 package (Clarke and Gorley, 2006) and the PERMANOVA+ v1.0.6 for PRIMER (Anderson et al., 2008). As most commonly-used similarity coefficient for biological community analysis, Bray-Curtis similarity matrices were computed on four-root transformed abundance data in present study (Xu et al., 2011b). The species distribution pattern of the ciliates among four sampling sites was analyzed using the hierarchical clustering (submodule CLUSTER) on Bray-Curtis similarities from the standardized/transformed species--abundance data (Xu et al., 2011b). The spatial differences of tintinnid communities among the four sampling sites were summarized using the submodule CAP (canonical analysis of principal coordinates) of PERMANOVA+ on Bray-Curtis similarities from the transformed species-abundance data, while the spatial environmental status of the four sampling sites was summarized based on log-transformed/normalized abiotic data (Anderson et al., 2008). Vector overlay of Pearson correlations of dominant species with the two CAP axes is also shown in CAP plots (Anderson et al., 2008). Differences

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**Fig. 1.** Sampling sites in Jiaozhou Bay, near Qingdao, northern China. Site 1 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 2 was fairly heavily polluted area near the mouth of this bay due mainly to intensive mariculture activities and the circulation of inshore waters from site 1. Site 3 was only slightly polluted because it was located relatively distant from the rivers entering the bay. Site 4 was least polluted area for more distance from the river discharges.
between groups of samples/species were tested by the submodule ANOSIM (analysis of similarities) which is a permutation/randomization test and equally effective at testing for assemblage change on biotic Bray-Curtis similarities or environmental change on Euclidean distance matrices (Clarke and Gorley, 2006). The contribution of each species to the average Bray-Curtis similarity among sites, as well as to the similarity within one site, was analyzed using the SIMPER (similarity percentage analysis) program (Clarke and Gorley, 2006). The biota-environment correlation was tested using the routine RELATE. The submodule BIOENV (biota-environment correlation analysis) was used to explore potential relationships between community structure and the abiotic data (Clarke and Gorley, 2006). Univariate correlation analyses were carried out using the statistical program SPSS v16.0 to explore the linear relationships between the individual abundance of dominant species/community structural parameters and environmental variables. Data were log-transformed before analyses (Xu et al., 2008).

3. Results

3.1. Environmental status

Environmental variables at the four sites in Jiaozhou Bay for a 7-year cycle were showed in Fig. 2. All variables showed distinct differences at the four sampling sites except temperature. Temperature ranged between 0.2 and 28.2 °C, while the average values were commonly higher than 14 °C with the exception at St. 3. Salinity ranged between 19.7 and 32.3 with lowest average value at St. 1 and highest at St. 3. Chl a concentration ranged between 0 and 19.84 μg l⁻¹ and the average values were lowest at St. 3 and St. 4 while highest at St. 1. The average concentration values of dissolved inorganic nitrogen (including NH₄-N, NO₂⁻-N and NO₃⁻-N). SRP, and SRSi followed a gradient from maximum to minimum: St. 1 > St. 2 > St. 3 > St. 4, except that the concentrations of SRP were higher at St. 3 than at St. 2.

3.2. Species composition of tintinnid communities

The tintinnid species observed in Jiaozhou Bay during the investigation were presented in Table 1 with the average abundances, occurrence frequency and dominance of each tintinnid species at each of the four sampling sites. A total of 26 tintinnid species belonging to 9 genera in 6 families (Codonellidae, Codonellopsidae, Epiplocylidiidae, Metacylididae, Tintinnidea, and Tintinnidiidae) were recorded. Among the 9 genera recorded, genus Tintinnopsis had the largest number of species (15), followed by genera Codonellopsis, Leprotintinnus, and Tintinnidium (each genus had 2 species), and the remaining genera were represented by one species. Of the 26 species, 10 species that gathered from the top ranked contributors were defined as “dominant” (Table 1).

3.3. Spatial patterns of tintinnid communities

The average species numbers and abundances of tintinnids at each site were shown in Fig. 3a and b. The average species numbers were 25 in all the three sampling sites except St. 2 (Fig. 3a). However, the mean abundances of tintinnids revealed a clear gradient decreasing from St. 1 to St. 4 (Fig. 3b): the mean abundances were higher at heavily polluted sites than at less stressed sites.

Five genera including Codonellopsis, Leprotintinnus, Stenosemella, Tintinnidium, and Tintinnopsis were the primary taxa in record. No obvious spatial difference of relative species composition were revealed among the four sampling sites (Fig. 3c), whereas a clear spatial patterns of relative abundance were presented (Fig. 3d). Compared to the other genera, Tintinnopsis and Stenosemella were the major contributors to the spatial variations in relative abundances. Thus, two structural community types were recognized: (1) those dominated by genus Tintinnopsis (St. 1 and St. 4) and (2) those dominated by genus Tintinnidium and, to a lesser extent, genus Stenosemella (St. 2 and St. 3).

Discrimination among 28 data points from St. 1 to St. 4 averaged from each single year according to Bray-Curtis similarities revealed a spatial pattern in the tintinnid communities (Fig. 4). The first canonical axis (CAP 1) revealed a general gradient from St. 1 to St. 3. It was noticeable that CAP 1 discriminated tintinnid communities sampled at St. 1 (right side of Fig. 4a) from those sampled at St. 3 and St. 4 (left side of Fig. 4a). The second canonical axis (CAP 2) separated samples at St. 4 (upper parts of Fig. 4a) from those sampled at the other sites (lower parts of Fig. 4a). PERMANOVA test revealed significant differences of tintinnid communities among the four sampling sites and between each pair of sites except for paired sites St. 3 and St. 4.
Vector overlay of Pearson correlations of 10 most dominant species (top-5 ranked contributors at each station) with the CAP axes was also shown in Fig. 4b. Although they were the top-5 ranked contributors at each station, vector for one species *Leprotintinnus nordqvisti* pointed towards the sample cloud of St. 4 (upper left part of Fig. 4b); and vectors for five species (*Favella ehrenbergi*, *Tintinnopsis amoyensis*, *Tintinnopsis beroidea*, *Tintinnopsis brasiliensis* and *Tintinnopsis tubulosoides*) pointed towards that of St. 1 (right side of Fig. 4b).

### 3.4. Spatial variations in biodiversity measures

The average species diversity, evenness and richness indices of tintinnids at each site were shown in Fig. 5. All three indices generally represented a clear gradient decreasing from St. 1 to St. 4 except for a decline from St. 3 to St. 4 for species richness, i.e., the mean values were lower at heavily polluted sites than at less stressed sites (Fig. 5).

![Fig. 3. Spatial variations in species number (a), abundance (b, ind l⁻¹), relative species number (c, %) and relative abundance (d, %) of tintinnids at the four sampling sites of Jiaozhou Bay between January 2006 and December 2012.](image-url)
3.5. Linkage between biota and abiota parameters

The relationships among the four sampling sites based on average data for environmental variables and for tintinnid community pattern on Bray-Curtis similarities for species-abundance data of four sampling sites are also shown in Fig. 4. Results of PERMANOVA test for environmental factors were the same as those for tintinnid communities: significant differences were found among the 4 sampling sites and between each pair of sites except for paired sites St. 3 and St. 4. RELATE analysis demonstrated that there was a significant correlation between spatial variation in abundance within the tintinnid communities and changes in environmental variables ($\rho = 0.288$, $P = 0.001$).

For all the sampling sites, the correlations between tintinnid abundances and environmental variables were established by multivariate biota-environment (BIOENV) analysis as shown in Table 2. In the results, the best matching with the tintinnids occurred with the combination of SRSi and NO$_2$-N. It was also notable that the two variables were included in all correlations (Table 2).

Spearman correlations between environmental variables and abundances of the 6 abundant tintinnids were calculated. Of these, 5 species were found to be correlated with environmental variables statistically significant (Table 3). The abundance of *Tintinnopsis amoyensis* was significantly negatively correlated with salinity, but positively correlated with Chl a, SRSi, SRP, NO$_2$-N and NH$_4$-N. The same correlations occurred
Table 2 Summary of results from biota-environment (BIOENV) analysis showing the 10 best matches of environmental variables with spatial variations in tintinnid abundances at four sites in Jiaozhou Bay during a 7-year cycle (2006-2012).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Environmental variables</th>
<th>$\rho$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SRSI, NO$_3$-N</td>
<td>0.335</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>2</td>
<td>SRSI, SRP, NO$_3$-N, NH$_4$-N</td>
<td>0.334</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>3</td>
<td>SRSI, SRP, NO$_3$-N</td>
<td>0.332</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>4</td>
<td>NO$_3$-N</td>
<td>0.328</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>5</td>
<td>SRSI, NO$_3$-N, NH$_4$-N</td>
<td>0.327</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>6</td>
<td>SRSI, SRP, NH$_4$-N</td>
<td>0.325</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>7</td>
<td>NO$_3$-N, NH$_4$-N</td>
<td>0.320</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>8</td>
<td>SRSI</td>
<td>0.318</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>9</td>
<td>SRSI, SRP</td>
<td>0.316</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>10</td>
<td>SRP, NO$_2$-N, NH$_4$-N</td>
<td>0.313</td>
<td>$&lt;0.02$</td>
</tr>
</tbody>
</table>

$\rho$ value: Spearman correlation coefficient; P value: statistical significance level. See Fig. 1 for other abbreviations.

Table 4 Correlations between environmental variables and total abundance (N), species diversity ($H'$), species evenness ($J'$), and richness (D) of tintinnid community at four sites in Jiaozhou Bay during a 7-year cycle (2006-2012).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>$H'$</th>
<th>$J'$</th>
<th>D</th>
</tr>
</thead>
</table>
| T          | 0.103 | 0.349 | 0.406$^*$ | $-0.094$
| S          | $-0.335$ | 0.307 | 0.321 | 0.508$^{**}$
| Chl $a$    | $0.213$ | $-0.153$ | $-0.154$ | $-0.381$
| SRSI       | $0.584^{**}$ | $-0.411^*$ | $-0.397^{**}$ | $-0.726^{**}$
| SRP        | 0.379$^*$ | $-0.130$ | $-0.195$ | $-0.343$
| NO$_2$-N   | 0.664$^{**}$ | $-0.259$ | $-0.316$ | $-0.625^{**}$
| NO$_3$-N   | 0.315 | $-0.384^*$ | $-0.365$ | $-0.539^{**}$
| NH$_4$-N   | 0.478 | $-0.398^*$ | $-0.411^*$ | $-0.612^{**}$

See Fig. 1 for other abbreviations.

$^{**}$ Significant difference at the 0.01.

$^*$ Significant difference at the 0.05.

to *Tintinnopsis berodia* except its correlation with Chl $a$ and SRP. The *Tintinnopsis brasiliensis* was significantly negatively correlated with temperature, but positively correlated to SRSI, NO$_3$-N and NH$_4$-N. Other positive correlation included those between the *Tintinnopsis tubulosoides* and temperature, SRSI and NO$_3$-N, and the *Tintinnopsis tocantinensis* was positively correlated with temperature (Table 4), however, no significant correlation was found between the abundant species *Stenosemella nivalis* and any environmental variable.

PCA ordination with vectors for both structural parameters of tintinnid communities and environmental variables were shown in Fig. 4c. The second canonical axis (PC2) revealed a general gradient from St. 3, St. 2 to St. 1. It was noticeable that PC 1 discriminated tintinnid communities and environmental variables were shown in Fig. 4c. The second canonical axis (PC2) revealed a general gradient from St. 3, St. 2 to St. 1. It was noticeable that PC 1 discriminated species diversity index was negatively correlated to SRSI, SRP, NO$_2$-N, NO$_3$-N, NO$_2$-N, SRP, and SRSI. The community-based ecological parameters (e.g., species richness, diversity, and evenness) of tintinnids also showed a clear correlation to environmental variables. It was noticeable that tintinnid community parameters showed more sensitivity to Chl $a$ and nutrients, which were supposed anthropogenic environmental variables, than temperature and salinity, as reported by Warwick and Clarke (1995, 2001). Compared to abundance of particular species, the community parameters were more reliable. Furthermore, five dominant species (*Tintinnopsis amoyensis*, *T. berodia*, *T. brasiliensis*, *T. tubulosoides* and *Stenosemella nivalis*) were significantly correlated with temperature, salinity, SRSI, and the nutrient. These findings suggest that tintinnids may be used as a bioindicator for discriminating water quality status in marine ecosystems.

In summary, the community structures of the tintinnids represented significant differences among the four sampling sites, and were significantly correlated with environmental variables, especially SRSI and...
nutrients; the community-based parameters were significantly correlated with the nutrients. Based on our data, we suggest that tintinnids might be used as a potential bioindicator for discriminating environmental quality status in marine ecosystems.

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References