# Planktonic ciliate communities in a semi-enclosed bay of Yellow Sea, northern China: annual cycle

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To reveal the annual patterns of planktonic ciliate communities, planktonic ciliate species composition, abundance and biomass, and responses to environmental conditions, were investigated during an annual cycle in Jiaozhou Bay, Qingdao, northern China. A total of 64 species belonging to five orders (Oligotrichida, Haptorida, Cyrtophorida, Hypotrichida and Tintinnida) were identified, 9 of which were dominant. Ciliate communities presented a clear seasonal pattern in terms of both abundance and biomass. A single peak of ciliate abundance and biomass occurred in late August, mainly due to the oligotrichids, tintinnids and haptorids. The 9 dominant species showed a distinct temporal distribution with seasonal successions of ciliate communities. Multivariate analyses revealed that ciliate abundance was significantly correlated with water temperature, dissolved oxygen and nutrients, especially nitrate nitrogen and soluble reactive phosphate (P < 0.05). These findings provided basic data on annual cycle of planktonic ciliate communities in a semi-enclosed bay of Yellow Sea, northern China.

Keywords: annual cycle, Jiaozhou Bay, marine microbial ecology, planktonic ciliate

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### INTRODUCTION

Planktonic ciliates are important components of microplankton communities and play a crucial role in the functioning of microbial food webs (Finlay et al., 1979, 1988; Azam et al., 1983; Pratt & Cairns, 1985; Sherr & Sherr, 1987; Caron & Goldmann, 1990). They have long been considered as important mediators of energy transfer from pico- and nanoplanktonic production to higher trophic levels (Stoecker & McDowell-Cappuzzo, 1990; Gifford, 1991; Legendre & Rassouldezgan, 1995; Sime-Ngando et al., 1995; Elloumi et al., 2006; Xu et al., 2008). With their rapid growth and delicate external membranes, ciliates react more quickly to environmental changes than most other eukaryotic organisms and can serve as bioindicators of water quality (Xu et al., 2002, 2008; Gong et al., 2005). Although the importance of planktonic ciliate ecology is increasingly recognized, data on annual variation in planktonic ciliate communities are scant (Kchaou et al., 2009).

Jiaozhou Bay is a large shallow eutrophic semi-enclosed bay near Qingdao, northern China. It covers an area of about 390 km<sup>2</sup> with an average depth of about 7 m, and is connected to the Yellow Sea via a narrow opening about 2.5 km in width (Shen, 2001). The water mass movement in this bay is dominated by tidal events and the stratification is weak even in summer, especially in the area near the mouth of the bay (Weng *et al.*, 1992; Yang & Wu, 1999), because of limited

Corresponding author: H. Xu Email: henglongxu@126.com freshwater flux and strong tidal turbulence mixing (Liu *et al.*, 2007). For several decades Jiaozhou Bay has been influenced by anthropogenic activities both in and around the bay (e.g. industry, agriculture and aquiculture) and as a consequence it is subject to eutrophication events (Fan & Zhou, 1999; Liu *et al.*, 2004). Furthermore, environmental conditions (e.g. water temperature, salinity, pH and nutrients) are often highly variable on short spatial and/or temporal scales resulting in significant changes in the abundance, biomass, diversity and community structure of microplanktonic organisms (Nuccio *et al.*, 2003; Xu *et al.*, 2008). Although there have been a number of investigations on plankton community dynamics in Jiaozhou Bay (Shen, 2001; Zhang & Wang, 2001; Liu *et al.*, 2005, 2008), annual dynamics of planktonic ciliates have yet to be investigated.

In the present study, a one-year baseline survey was carried out from June 2007 to May 2008 in Jiaozhou Bay, in order to analyse the spatial and temporal dynamics of the ciliate communities. The main objectives were: (1) to document the taxonomic composition of planktonic ciliate communities; (2) to investigate temporal dynamics in terms of planktonic ciliate species number, abundance and biomass; and (3) to determine the relationship between planktonic ciliate communities and environmental parameters.

### MATERIALS AND METHODS

## Sampling strategy

Five sampling sites (A-E) were selected in Jiaozhou Bay near Qingdao, northern China (Figure 1). A total of 24 cruises were

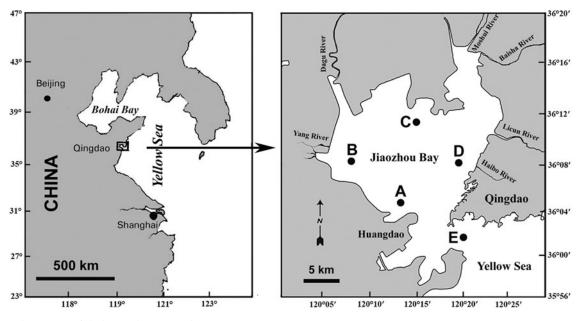


Fig. 1. Sampling stations of planktonic ciliates in Jiaozhou Bay.

carried out biweekly over a one-year period from June 2007 to May 2008. Water samplings (referred to as 10-Jun-07 etc.) were carried out from a depth of about 1 m. Both for quantitative measures and for identification of ciliates, 1000 ml of seawater was fixed with acid Lugol's iodine solution (2% final concentration, volume/volume) (Pitta *et al.*, 2001; Kchaou *et al.*, 2009). Water temperature (T), pH, salinity (S), chlorophyll-*a* (Chl *a*) and dissolved oxygen concentration (DO) were measured *in situ*, using a multi-parameter kit (MS5, HACH). Samples for nutrient analyses were preserved immediately upon collection. Soluble reactive phosphate (SRP), ammonium nitrogen (NH<sub>4</sub>-N), nitrate nitrogen

 $(NO_3-N)$  and nitrite nitrogen  $(NO_2-N)$  were determined using a UV-visible spectrophotometer (DR-5000, HACH) according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, 1992).

### Identification and enumeration

For purposes of identification and of enumeration, 1000 ml Lugol's fixed seawater was settled for 48 hours resulting in 30 ml of concentrated sediment (Utermöhl, 1958). For enumeration of ciliates a 0.1 ml aliquot of each concentrated sample was placed in a Perspex chamber and the ciliates were counted under

Samplings	T (°C)	pН	S (psu)	Chl $a (\mu g l^{-1})$	DO (mg l <sup>-1</sup> )	SRP (mg l <sup>-1</sup> )	$NH_4$ -N (mg l <sup>-1</sup> )	$NO_{3}-N (mg l^{-1})$	$NO_2-N (mg l^{-1})$
10-Jun-07	20.33	7.99	32.17	0.98	7.44	0.27	0.29	0.80	0.04
25-Jun-07	22.92	7.99	31.99	0.75	7.16	0.11	0.19	0.70	0.02
10-Jul-07	24.14	8.17	31.65	1.80	8.89	0.12	0.32	0.70	0.03
24-Jul-07	24.63	8.18	31.16	3.05	7.56	0.12	0.21	0.70	0.13
08-Aug-07	26.24	8.11	30.98	1.22	6.55	0.42	0.30	0.44	0.03
23-Aug-07	27.49	8.60	21.34	4.08	8.95	0.13	0.26	0.82	0.15
06-Sep-07	25.30	8.22	29.07	1.54	6.65	0.29	0.45	0.58	0.09
21-Sep-07	23.57	8.04	20.38	0.10	6.32	0.35	1.19	0.98	0.10
06-Oct-07	22.68	8.20	29.43	0.13	5.83	0.23	0.34	0.28	0.11
22-Oct-07	18.75	8.29	30.37	0.24	7.34	0.12	0.43	0.36	0.08
05-Nov-07	15.83	8.36	30.85	0.29	7.72	0.20	0.21	0.52	0.04
20-Nov-07	12.55	8.34	31.47	0.20	8.12	0.17	0.21	0.02	0.04
05-Dec-07	8.92	8.30	31.23	0.30	8.87	0.10	0.38	0.68	0.04
19-Dec-07	7.50	8.43	32.04	0.46	9.89	0.21	0.26	0.34	0.02
03-Jan-08	5.33	8.44	31.77	0.46	9.70	0.10	0.45	0.38	0.02
17-Jan-08	3.35	8.48	31.90	5.31	8.74	0.27	0.15	0.25	0.02
27-Jan-08	1.40	8.47	30.90	1.87	10.88	0.09	0.28	0.18	0.01
18-Feb-08	2.85	7.79	31.14	3.49	11.93	0.09	0.07	0.10	0.05
04-Mar-08	4.88	8.09	31.23	1.04	11.17	0.13	0.11	0.26	0.01
17-Mar-08	7.12	8.01	30.43	7.99	10.13	0.20	0.22	0.36	0.01
01-Apr-08	8.71	8.06	30.36	0.72	10.26	0.07	0.34	0.30	0.01
15-Apr-08	10.81	7.98	31.08	0.65	9.41	0.09	0.23	0.18	0.01
30-Apr-08	13.27	7.89	31.15	0.37	8.23	0.16	0.35	0.28	0.02
15-May-08	15.18	7.89	31.13	0.25	8.40	0.25	0.29	0.36	0.02

Table 1. Environmental variables in Jiaozhou Bay water samplings between June 2007 and May 2008.

T, water temperature; S, salinity; Chl a, chlorophyll-a; DO, dissolved oxygen concentration; SRP, soluble reactive phosphate.

Table 2. List of the species of	ciliates from Ji	iaozhou Bay	recorded	in 120	samples,	including	body	size,	annual	average	abundance,	biomass
and occurrence.												

Species	Body size*	Abund <sup>1</sup>	Biom <sup>2</sup>	Occur <sup>3</sup>	
Oligotrichida					
Halteria grandinella	$15-20 \times 10-15$	+	+	4	
Leegaardiella sol	$18-20 \times 15-18$	+	+	8	
Metastrombidium sonnifer	$48-52 \times 45-50$	+	+	17	
Omegastrombidium elegans	$30-50 \times 20-30$	+	+	13	
Omegastrombidium foissneri	$110 - 150 \times 50 - 75$	++	++	71	
Omegastrombidium jankowski	$100 - 110 \times 30 - 35$	+	+	25	
Parastrombidium faurei	95-155 × 95-150	++	++++	58	
Pseudotontonia simplicidens	$40-50 \times 18-20$	++	+	38	
Pseudotontonia cornuta <sup>[9]</sup>	$25-30 \times 10-12$	+++	+	83	
Rimostrombidium caudatum	$35-50 \times 25-30$	+	+	13	
Rimostrombidium conicum	$25-39 \times 19-31$	+	+	21	
<i>Rimostrombidium glacicolum</i> <sup>[5]</sup>	$30-45 \times 20-30$	+++	++	75	
<i>Rimostrombidium</i> orientale <sup>[2]</sup>	$25-35 \times 20-30$	+++	++	92	
Rimostrombidium veniliae <sup>[8]</sup>	$5-10 \times 5-6$	+++	+	79	
<i>Rimostrombidium sphaericum</i> <sup>[15]</sup>	$5-15 \times 5-10$	+++	+	71	
Rimostrombidium undinum	$10-20 \times 10-15$	++++	++	50	
Spirostrombidium schizostomum	$45-65 \times 30-45$	+	+	8	
Spirotontonia turbinata <sup>[12]</sup>	$60-100 \times 20-60$	+++	+++	75	
Strombidinopsis acuminatum	$70-124 \times 20-30$	++	++	67	
Strombidinopsis cheshiri	$75 - 160 \times 35 - 85$	+	++	17	
Strombidinopsis elegans	$30-40 \times 30-40$	+	+	17	
Strombidinopsis elongata	$55 - 110 \times 35 - 50$	+	+	13	
Strombidium acutum <sup>[13]</sup>	$30-55 \times 30-45$	+++	++	88	
Strombidium apolatum	$40-75 \times 25-45$	++	+	38	
Strombidium capitatum <sup>[4]</sup>	$40^{-75} \times 25^{-45}$ $45^{-70} \times 40^{-60}$	+++	++	100	
Strombidium compressum	$17-28 \times 10-15$	++	+	67	
Strombidium compressum	$50-75 \times 30-50$	+++	+++	,	
Strombidium globosaneum <sup>[10]</sup>	$30-73 \times 30-30$ $25-35 \times 20-30$	++++	++	75 88	
Strombidium montagnesi	$25-30 \times 15-20$	+++	+	46	
Strombidium paracalkinsi <sup>[14]</sup>	$25-50 \times 15-20$ $25-60 \times 25-50$	++	++	67	
Strombidium rapulum	$25-00 \times 25-50$ $50-130 \times 25-65$	+	+	8	
Strombidium styliferum	$35-80 \times 25-40$	++	++	71	
Strombidium sulcatum <sup>[11]</sup>	$35-50 \times 25-40$ $35-50 \times 30-40$	+++	++	67	
Strombidium succitum Strombidium tintinnodes	$35-50 \times 30-40$ $30-50 \times 20-30$	++	+	29	
Tontonia antarctica <sup>[16]</sup>	$30-30 \times 20-30$ $120-150 \times 30-40$	++	++	58	
Haptorida	120-130 × 30-40	<b>TT</b>	<b>TT</b>	20	
Lacrymaria marina	150 000 × 50 60		1	10	
Mesodinium pupula <sup>[6]</sup>	$150 - 300 \times 50 - 60$	+ ++++	+++++	13 100	
Mesodinium pupulu <sup>2</sup> Mesodinium velox	$30-55 \times 30-45$				
Placus salinus	$15-20 \times 8-10$	+	+	13	
Cyrtophorida	50-70 × 30-50	++	+	4	
/ 1		1	1		
Dysteria cristata	$45-55 \times 25-35$	+	+	4	
Hypotrichida		1	1		
Uronychia setigera Tintingi da	$40-60 \times 20-30$	+	+	4	
Tintinnida				0	
Codonella amphorella	$50-55 \times 20-25$	++	+	8	
Leprotintinnus neriticus	$300-400 \times 160-200$	+	++	17	
Leprotintinnus bottnicus <sup>[3]</sup>	$120 - 140 \times 35 - 50$	++++	+++	63	
Stenosemella nivalis	50-60 × 50-60	++	+	25	
Stenosemella pacifica	$26 - 30 \times 22 - 28$	++	+	25	
Stenosemella steini	50-60 × 40-50	+	+	13	
Tintinnopsis acuminata	$50-60 \times 20-25$	+	+	25	
Tintinnopsis baltica	$52-55 \times 32-35$	++	+	46	
Tintinnopsis beroidea	$65-75 \times 25-35$	+	+	21	
Tintinnopsis brasiliensis	$75 - 80 \times 40 - 50$	++	+	25	
Tintinnopsis bütschlii	$80-90 \times 95-110$	+	+	4	
Tintinnopsis chinglanensis	$80-120 \times 30-40$	+	+	8	
Tintinnopsis lobiancoi	$90-100 \times 30-40$	+	+	4	
Tintinnopsis loricata	$130 - 140 \times 60 - 70$	+	+	8	
Tintinnopsis mucicola	$130 - 150 \times 40 - 45$	+	+	17	
Tintinnopsis nana	$10-15 \times 8-10$	+	+	4	
Tintinnopsis orientalis	$80-90 \times 50-55$	++	+	25	

Continued

Table 2. Continued

Species	Body size*	Abund <sup>1</sup>	Biom <sup>2</sup>	Occur <sup>3</sup>
Tintinnopsis parvula <sup>[1]</sup>	$30-40 \times 20-25$	++++	++	63
Tintinnopsis radix	200-300 × 50-60	+	+	8
Tintinnopsis tocantinens	$120 - 130 \times 25 - 40$	+	+	13
Tintinnopsis tubulosoides	$110 - 120 \times 40 - 50$	++	+	25
Tintinnopsis turgida	$80-110 \times 30-50$	+	+	8
Tintinnopsis urnula	40-50 × 30-40	+	+	8

\*, body size ( $\mu$ m) (length × width); 'abundances (ind. l<sup>-1</sup>) (+ = 0-10, ++ = 10-100, +++=100-400, ++++= over 400); 'biomass ( $\mu$ g l<sup>-1</sup>) (+ = 0-10, ++ = 10-100, +++ = 100-200, ++++ = over 200); <sup>3</sup>occurrence (%); numbers in the superscript square brackets = ranks of the top 16 contributive species to the average Bray-Curtis similarity (90.99%) within 120 samples.

a light microscope at a magnification  $400 \times$ . A total of 0.5 ml concentrated samples were counted and yielded a standard error (SE) of <8% of the mean values of counts. Tintinnids were identified using lorica morphology and species description according to Kofoid & Campbell (1929, 1939), Nie (1934) and Yin (1952). Other ciliates were identified following Song *et al.* (2003). Those individuals whose identity could not be ascertained following examination with Lugol's-fixed specimens were picked out with a micropipette and identified using protargol impregnation after re-fixating with Bouin's solution (Montagnes & Humphrey, 1998). The taxonomic scheme used was mainly according to Lynn (2008).

Biovolumes were determined from measurements of their linear dimensions and using volume equations of appropriate geometric shape (Winberg, 1971). Conversion factors of carbon biomass for non-loricate ciliates were 0.14 pg Cm<sup>-3</sup>, and 0.053 pg Cm<sup>-3</sup> for loricate (tintinnid) ciliates (Putt & Stoecker, 1989; Stoecker *et al.*, 1994).

### Data analyses

Species diversity (*H'*), evenness (*J'*) and richness (*d*) of samplings were calculated as follows the equations  $H' = -\sum_{i=1}^{S} Pi(\ln Pi)$ ,  $J' = H'/\ln(S)$  and  $d = (S-1)/\ln(N)$ , where Pi = proportion of the total count arising from the *i*<sup>th</sup> species; S = total number of species; and N = total number of individuals (Xu *et al.*, 2008).

Multivariate analyses of temporal variations in planktonic ciliate communities were analysed using the PRIMER v6.1 package (Clarke & Gorley, 2006; Kim *et al.*, 2007; Xu *et al.*, 2009) and the PERMANOVA+ for PRIMER (Anderson *et al.*, 2008). Bray–Curtis similarity matrices were computed

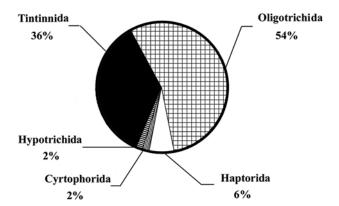


Fig. 2. Taxonomic composition of planktonic ciliate communities and the percentage of cumulative number of species recorded throughout the period of sampling.

on log-transformed data. The clusters of species were assigned by the routine CLUSTER and tested by the ANOSIM (Clarke & Gorley, 2006), while the temporal patterns of communities were summarized using the submodule canonical analysis of principal coordinates (CAP) of PERMANOVA+ on Bray– Curtis similarities. Differences between groups of samples were tested by the submodule PERMANOVA (Anderson *et al.*, 2008). The contribution of each species to the average Bray–Curtis similarity among samples was analysed using the SIMPER program (Clarke & Gorley, 2006). The submodule BIOENV was used to explore the potential relationships between environmental parameters and the biotic data. The

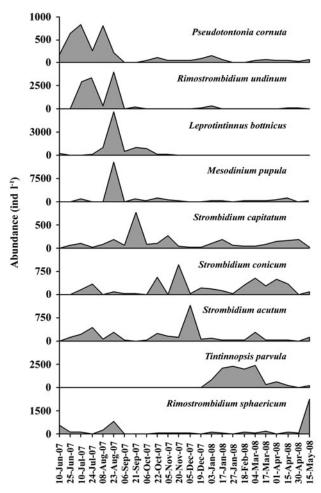


Fig. 3. Annual variations in abundances (ind.  $l^{-1}$ ) of the nine dominant ciliates from June 2007 to May 2008.

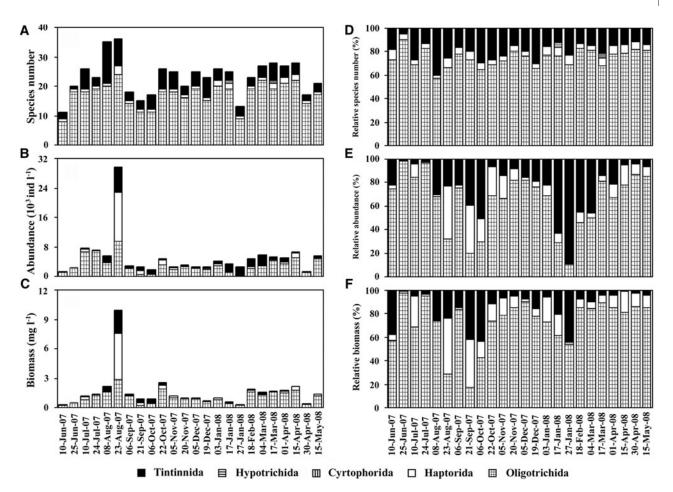


Fig. 4. Annual variations in species number (A), abundance (B), biomass (C), relative species number (D), relative abundance (E) and relative biomass (F) of planktonic ciliates in Jiaozhou Bay, China, from June 2007 to May 2008.

significance of biota-environment correlations was tested using mental test (RELATE analysis) (Clarke & Gorley, 2006).

Univariate analyses of correlations were carried out using the statistical program SPSS v16.0 on log-transformed data (Xu *et al.*, 2008).

### RESULTS

### **Environmental parameters**

The mean values of eleven environmental variables for a total of 24 samplings are summarized in Table 1.

Water temperature followed a clear seasonal pattern, ranging from  $1.40^{\circ}$ C to  $27.49^{\circ}$ C (mean  $14.74^{\circ}$ C).

Salinity was around 30.0 psu and maintained relatively stable levels throughout the year, although with sharp drops in late August (21.34 psu) and again in late September (20.38 psu) due to the heavy rainfall.

The pH values ranged from 7.79 to 8.60, averaging 8.18. Concentrations of Chl *a* peaked three times, in late August (4.08  $\mu$ g l<sup>-1</sup>), in mid-January (5.31  $\mu$ g l<sup>-1</sup>) and in mid-March (7.99  $\mu$ g l<sup>-1</sup>). Values of DO varied inversely with temperature.

Soluble reactive phosphate ranged from 0.07 mg  $l^{-1}$  to 0.42 mg  $l^{-1}$  (mean value of 0.18 mg  $l^{-1}$ ) with a minor peak in early August.

The concentrations of  $NH_4$ -N and  $NO_3$ -N were peaking in late September whereas low concentrations of  $NO_2$ -N were maintained throughout the year apart from a minor increase between July and September.

## Taxonomic composition and annual species distribution

The taxonomic composition of ciliate communities observed during the study period is summarized in Table 2. A total of 64 ciliate species, representing 24 genera and five orders (Oligotrichida, Haptorida, Cyrtophorida, Hypotrichida and Tintinnida), were identified during the one-year survey. Oligotrichids and tintinnids represented the highest numbers of species, accounting for 54% and 36% respectively of the total (Table 2; Figures 2 & 4A). The contribution of the top 16 species to the average Bray–Curtis similarity (90.99%) within samples was summarized using similarity percentage (SIMPER) analysis; the numbers in the square brackets showing the rank (Table 2).

There were nine dominant species, each of which at some time during the sampling period contributed more than 25% of the total planktonic protist abundance (Figure 3). These were: *Pseudotontonia cornuta, Rimostrombidium undinum, Leprotintinnus bottnicus, Mesodinium pupula, Strombidium capitatum, Strombidium conicum, Strombidium acutum,*  Tintinnopsis parvula and Rimostrombidium sphaericum. Five of these species (*Pseudotontonia cornuta*, *Strombidium capitatum*, *Strombidium conicum*, *Strombidium acutum* and *Rimostrombidium sphaericum*) peaked during more than one season of the one-year cycle whereas dominance of the other four (*Rimostrombidium undinum*, *Leprotintinnus bottnicus*, *Mesodinium pupula* and *Tintinnopsis parvula*) was confined to one season only (Figure 3).

## Annual variation in species number, abundance and biomass

The temporal variation in species count showed a unimodal distribution peaking in late August with 36 species (Figure 4A). Tintinnids and oligotrichids were primarily responsible for the peak. The lowest species number (11 species) was found in early June (Figure 4A).

The ciliate abundances also exhibited a unimodal variation with generally low values (mean  $5.03 \times 10^3$  ind.  $l^{-1}$ ) over the one-year period and a distinct peak in August (maximum value  $29.54 \times 10^3$  ind.  $l^{-1}$ ). Haptorids (e.g. *Mesodinium pupula*), oligotrichids (e.g. *Rimostrombidium undinum*) and tintinnids (e.g. *Leprotintinus bottnicus*) were primarily responsible for the peak, reaching abundances of  $13.36 \times 10^3$  ind.  $l^{-1}$ ,  $9.49 \times 10^3$  ind.  $l^{-1}$  and  $6.69 \times 10^3$  ind.  $l^{-1}$  respectively (Figure 4B). Of the total ciliate abundance, oligotrichids accounted for 59.91%, tintinnids 21.81%, haptorids 18.24%, and hypotrichids and cyrtophorids 0.02% each (Figures 2 & 5A).

The variation in biomass showed a similar temporal pattern to that of abundance with one peak (maximum value 9.96 mg  $l^{-1}$ ) in August (Figure 4C). Haptorids (e.g. *Mesodinium pupula*), oligotrichids (e.g. *Spirotontonia*)

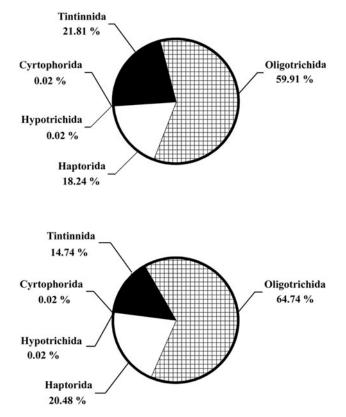


Fig. 5. Proportions of average abundances (A) and biomasses (B) of planktonic ciliates from June 2007 to May 2008.

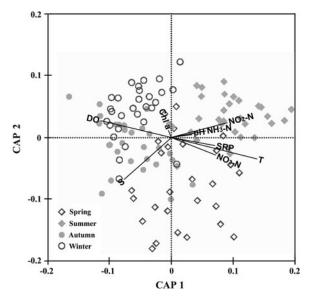
*turbinata*) and tintinnids (e.g. *Leprotintinnus bottnicus*) were the major contributors to the peak when their biomasses reached 4.66 mg l<sup>-1</sup>, 2.90 mg l<sup>-1</sup> and 2.40 mg l<sup>-1</sup> respectively (Figure 4C). Oligotrichids, haptorids and tintinnids accounted for 64.74%, 20.48% and 14.75% respectively of the total ciliate biomass whereas hypotrichids and cyrtophorids each accounted for only 0.02%. These values are fairly consistent with those for relative abundances (Figure 5B).

### Temporal patterns of community structure

Although oligotrichids, haptorids and tintinnids appeared in almost all samples, the ciliate community structure at the 24 sampling points demonstrated a clear temporal succession with respect to species composition, abundance and biomass (Figure 4D, E & F). In terms of the relative abundances, the ciliate communities might be distinguished as four structural types, each of which dominated at different times during the period of study: (1) oligotrichids dominated the ciliate communities from March to July; (2) tintinnids from August to October with a bloom of haptorids in the sampling 23-Aug and 21-Sep; (3) oligotrichids from late October to January; and (4) tintinnids from late January to February (Figure 4E). The relative biomasses showed a similar temporal pattern to that of the relative abundances (Figure 4F).

A seasonal pattern of ciliate communities was discriminated by canonical analysis of CAP on Bray–Curtis similarities from log-transformed species-abundance data of 120 samples from the five sites (Figure 6). The first canonical axis separated the ciliate communities sampled in summer (on the right) from those in autumn and winter (on the left), while the second canonical axis discriminated the samples in spring (lower) from summer and winter (upper) (Figure 6). The PERMANOVA test demonstrated a significant difference between each pair of seasonal groups (P < 0.001).

Vector overlay of environmental variables with the CAP axes is also shown in Figure 6. The sample cloud of spring and summer



**Fig. 6.** Canonical analysis of principal coordinates (CAP) on Bray–Curtis similarities from species-abundance data of 120 samples from five sampling sites in Jiaozhou Bay during the annual cycle from June 2007 to May 2008, and correlations of environmental variables with two CAP axes. See Table 1 for abbreviations.

autumn and winter (left) with salinity, DO and Chl a (Figure 6). The temporal variation in species diversity (H'), evenness (J') and richness (d) indices in 24 ciliate samples during the sampling period is shown in Figure 7. All three community parameters showed a similar temporal variation with peaks corresponding to the season over the one-year cycle and a distinct lowest in January (Figure 7).

## Interaction between biotic data and environmental variables

RELATE analysis revealed that there was a significant correlation between temporal variations in ciliate community structure and changes of environmental variables (R = 0.363; P <0.05). Biota-environment (BIOENV) analysis showed that ciliate communities were significantly related to the variability in the combination of nutrients (NO<sub>3</sub>-N, NO<sub>2</sub>-N and SRP) and water temperature/pH, in terms of both abundance (R =0.462; P < 0.05) and biomass (R = 0.417; P < 0.05).

### DISCUSSION

So far there has been little understanding of the annual patterns of planktonic ciliate communities in Jiaozhou Bay, although a few studies on spatial distribution of large-sized tintinnids have been carried out in this area (Zhang & Wang, 2001; Zhao et al., 2007).

In our study, a total of 64 ciliate species representing 24 genera and 5 orders, were identified during one annual cycle in Jiaozhou Bay. Similarly Kchaou et al. (2009), using the same sampling method, found 56 planktonic ciliate species in eutrophic waters in the Gulf of Gabès, Tunisia, over a period of 1 year. The data for the tintinnid species in Jiaozhou Bay are also consistent with those reported by Zhao et al. (2007)

Annual variations in planktonic ciliate abundance have previously been reported for Jiaozhou Bay (Zhang & Wang, 2001; Zhao et al., 2007). According to these studies the abundances ranged from 0 to  $1.74 \times 10^3$  ind.  $l^{-1}$  and  $0.01 \times 10^3$  ind.  $l^{-1}$ to  $21.30 \times 10^3$  ind.  $l^{-1}$ , which compare closely to those reported here  $(1.34 \times 10^3 \text{ ind. } l^{-1} \text{ to } 29.54 \times 10^3 \text{ ind. } l^{-1})$ . Maximum ciliate abundances in other eutrophic marine

1.2

Species eveness (J'

H

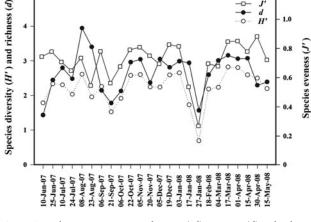


Fig. 7. Annual variations in species diversity (H'), evenness (J') and richness (d) of planktonic ciliate communities from June 2007 to May 2008.

habitats have been reported as follows: Laizhou Bay, China,  $2.38 \times 10^3$  ind. l<sup>-1</sup> (Zhang & Wang, 2000); north-west shelf, Australia,  $1.53 \times 10^3$  ind.  $l^{-1}$  (Moritz *et al.*, 2006); Chesapeake Bay, USA, 22.50  $\times$  10<sup>3</sup> ind. l<sup>-1</sup> (Dolan & Coats, 1990); Gulf of Gabès, Tunisia,  $50.35 \times 10^3$  ind.  $l^{-1}$  (Kchaou et al., 2009). The planktonic ciliate biomass in Jiaozhou Bay also showed a similar pattern to those listed above. This suggests that Jiaozhou Bay is similar to other eutrophic marine habitats in terms of its planktonic ciliate abundance and biomass

In addition, our study revealed that planktonic ciliates exhibited a clear annual/seasonal variation in terms of species composition, abundance and biomass. For example, ciliate species composition demonstrated a seasonal pattern being high in summer and winter, low in spring and autumn whereas abundance and biomass showed similar temporal patterns to each other, each with one distinct peak in August. These findings are consistent with previous reports for periphytic ciliates in Jiaozhou Bay (Gong et al., 2005) and planktonic ciliates in the Gulf of Gabès, Tunisia (Kchaou et al., 2009). Furthermore, CAP demonstrated that the annual variation in the ciliate communities presented a clear seasonal pattern.

Multivariate correlation analysis demonstrated that the temporal variation in planktonic ciliate communities was significantly related to environmental variables, especially the combination of water temperature and nutrients (e.g. NO<sub>3</sub>-N and SRP<sub>3</sub>-N, NO<sub>2</sub>-N and SRP) in terms of both abundance and biomass. Otherwise, nutrients, especially NO<sub>3</sub>-N and SRP, were always among the top combinations of variables. Thus we suggest that the successive annual dynamics of planktonic ciliates are significantly related to eutrophication. This finding was consistent with the reports for detecting the annual changes in ciliate community structure (Gong et al., 2005; Xu et al., 2008).

Species diversity, evenness and richness indices are commonly employed in community-level investigations and are amenable to simple statistical analyses (Ismael & Dorgham, 2003; Gong et al., 2005; Xu et al., 2008). In our case, however, these biological indices generally failed to show significant correlations with environmental parameters, but presented an obvious seasonal pattern.

In summary, the results of this survey demonstrate that: (1) the planktonic ciliate communities in Jiaozhou Bay, northern China, show a significant annual pattern with high diversity in terms of species composition, abundance and biomass; and (2) planktonic ciliate community structure is significantly associated with environmental conditions, in particular nitrates and phosphates. These findings provided basic data on the annual cycle of planktonic ciliate communities in a semi-enclosed bay of Yellow Sea, northern China.

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