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Planktonic protist communities in a semi-enclosed mariculture pond: structural variation and correlation with environmental conditions

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In order to evaluate the environmental status within a mariculture pond, temporal variations of physico-chemical factors, protist community structure and interactions between biota and environmental conditions were investigated during a complete cycle in semi-enclosed shrimp-farming waters near Qingdao, north China. Results revealed that: (1) a total of 54 protist taxa with ten dominant species was present, comprising 4 chlorophyceans, 2 chrysophyceans, 5 cryptophyceans, 10 dinoflagellates, 3 euglenophyceans, 10 diatoms, 18 ciliates and 2 sarcodines; (2) a single peak of protist abundance occurred in October, mainly due to the chlorophyceans, diatoms and chrysophyceans, while the bimodal peaks of biomass in July and October were mainly due to the ciliates, dinoflagellates and diatoms; (3) the succession of protist communities significantly correlated with the changes of nutrients, salinity and temperature, especially phosphate, either alone or in combination with NO_3; (4) species diversity and evenness indices were found to be relatively independent of physico-chemical factors, whereas species richness and the ratio of biomass to abundance were strongly correlated with water temperature and abundances of bacteria. It was concluded that planktonic protists are potentially useful bioindicators of water quality in a semi-enclosed mariculture system.

Keywords: aquaculture, environmental stress, microbial ecology, shrimp-farming, temporal variations

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INTRODUCTION

Planktonic protists are the main components of the microplankton community and play an important role in the functioning of mirobial food webs, especially in terms of energy flow and element cycling in aquatic ecosystems (Finlay & Esteban, 1998). Autotrophic protists are responsible for the bulk of primary production in most aquatic habitats; protozoan grazers transfer the production of algae and of the bacteria that grow on algal exudates to higher trophic levels in the food chain. Structural changes of protist communities may significantly affect other components of the aquatic food web, and thus may influence the distribution and abundance of both lower and higher organisms (Finlay & Esteban, 1998). Some protists can tolerate extreme environmental conditions and inhabit biotopes that are unfavourable to most metazoans (Patterson *et al.*, 1989). Furthermore, with their rapid growth and delicate external membranes, protists may react more quickly to environmental change than most other eukaryotic organisms and thus serve as bioindicators of water pollution (Shen *et al.*, 1990).

Semi-enclosed mariculture waters are typically characterized by their small size, poor exchange of water with the sea, heavy disturbance from the introduction of cultured animals, and high nutrient and/or contaminant inputs either from mariculture sources or of autochthonous origin. This commonly results in eutrophic or hypertrophic environments that are subject to recurrent eutrophication events. Furthermore, environmental conditions (e.g. water temperature, salinity, pH, nutrients, etc.) are often highly variable on short spatial and/or temporal scales resulting in significant changes in the abundance, biomass, diversity and community structure of microorganisms (Nuccio *et al.*, 2003). Although there have been a number of reports of protist community dynamics in mariculture waters and in semi-enclosed marine habitats such as lagoons (Pitta *et al.*, 1998; Gilabert, 2001; Cytryn *et al.*, 2003), such studies have yet to be carried out in semi-enclosed mariculture waters.

A six-month baseline survey of planktonic protists was carried out in a semi-enclosed shrimp-farming pond near Qingdao, China. The farming of shrimp was responsible for great variations of environmental factors in the pond thus offering the opportunity for us to analyse the relationships

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between planktonic protists and a range of physico-chemical and biological parameters. It should be noted, however, that because of the constraints of the methods used not all protist groups could be investigated so no data are available for the picoplanktonic forms (0.2–2 μm), for example. The aims of this study were to document the taxonomic composition and the temporal variation of planktonic protists in a semi-enclosed mariculture pond, to monitor the community structure of the planktonic protists and investigate relationships with a range of environmental factors.

MATERIALS AND METHODS

Study site

The shrimp-farming pond is located on the Laoshan Bay coast near Qingdao, China (Figure 1). It is a shallow marine pond, maximum depth about 1.2 m, with a mud–sandy bottom and covers an area of about 800 m^2. It is connected to the sea via a long and narrow canal that may be closed by means of a sluice gate. The shrimp juveniles were introduced on 15 June 2002 and fed with artificial granular foodstuff after two weeks. During the period of study (May to October), the water depth in the pond increased by 1.1 to 1.2 m about every two weeks due to seawater influx.

Sampling

Fifteen samples (referred to as 22 May etc) were collected every ten days from May to October 2002. All water samples were collected at a depth of 0.5 m. For quantitative studies and for the identification of ciliate, 1000 ml water samples were fixed with Bouin's solution to a final concentration of 10%. For the identification of autotrophs, 1000 ml of water was concentrated to 50 ml by filtering through a 20 μm-mesh plankton net *in situ* (Shen *et al.*, 1990).

For the measurement of concentrations of dissolved inorganic nitrogen (DIN, sum of NO_3-N, NO_2-N and NH_3-N) and soluble reactive phosphate (SRP), 1000 ml seawater was collected and analysed following standard methods (APHA, 1989). For the measurement of chlorophyll-*a* (chl *a*), a further 500 ml water sample was filtered through Whatman 25 mm GF/F filters by gentle vacuum filtration; following extraction of the filter paper in 90% DMF (N, N-dimethyl foramide) for 24 hours at 4°C, the concentration of chl *a* in the supernatant was determined using a spectrophotometer (UV-1601, Shimadzu) (Talling & Driver, 1961). For enumeration of bacteria, glutaraldehyde was added to 100 ml seawater to give a final concentration of 1.25%.

Water temperature, salinity and pH were recorded with appropriate sensors (WTW) at a depth of 0.5 m, and turbidity was measured *in situ* using a turbidimeter (Hach 2100P, Hach).

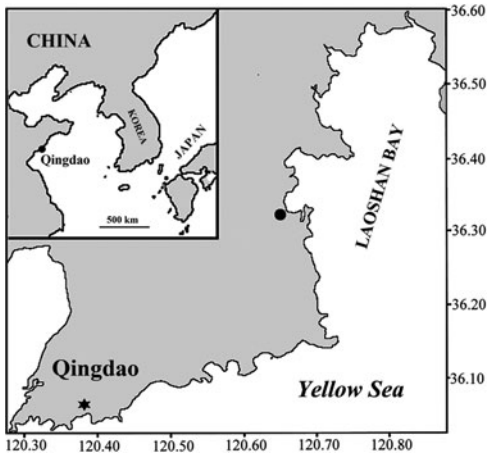

Fig. 1. Map showing the location of the semi-enclosed shrimp-farming pond on the Laoshan Bay coast.

Identification and enumeration

For identification of ciliates, the quantitative protargol staining (QPS) method (Montagnes & Taylor, 1994) and the identification guides of Song *et al.* (2003) were used. Identification of other protists was performed following Steidinger & Tangen (1997).

For protist enumeration (except for ciliates), 800 ml of Bouin's-fixed sample was settled for 48 hours resulting in 30 ml of concentrated sediment. A 0.1 ml sub-sample of this sediment was placed in a Perspex counting chamber and enumerated under a light microscope at a magnification 400× (Shen *et al.*, 1990). For ciliate enumeration, 200 ml of Bouin's-fixed sample was directly used for enumeration of ciliated protozoa by the QPS method (Montagnes & Taylor, 1994). All counts were repeated three times, and three subsamples from each sample yielded a SE of <8% of the mean values of counts.

Biovolume estimates were determined for nanoplankton (2–20 μm) from microscopical measurement of cell dimensions and assuming spherical or ellipsoidal shape. Microplankton (20–200 μm) biovolumes were determined from measurements of their linear dimensions and using volume equations of appropriate geometric shape (Winberg, 1971). Phototrophic and heterotrophic nanoplankton was converted to carbon biomass using a conversion factor of 183 fg C μm^{-3} (Dennett *et al.*, 1999). Diatom biovolumes were converted to carbon values using the modified Strathmann equation (Smayda, 1978). For other microplankton, conversion factors were 140 fg C μm^{-3} for dinoflagellates and non-loricate ciliates, and 53 fg C μm^{-3} for tintinnid ciliates (Putt & Stoecker, 1989; Stoecker *et al.*, 1994).

Bacteria in seawater were counted by epifluorescence microscopy. Cells were stained by adding the DNA specific fluorochrome, 4′6-diamidino-2-phenylindol (DAPI; Sigma) to a final concentration of 0.12 mg ml^{-1} and collected on a black nucleopore filter (0.2 μm pore size) supported by a 0.8 μm backing filter. At least 1000 bacteria were counted in each sample. In all cases, examinations were made at 1000× magnification using UV light excitation (Sherr *et al.*, 1987).

Data analysis

Species diversity (H'), evenness (J) and species richness (d) of samples were calculated as follows:

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

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

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

where H' = observed diversity index; Pi = proportion of the total count arising from the i^{th} species; S = total number of species; and N = total number of individuals.

The community structures of samples were analysed using the PRIMER (Plymouth Routines in Multivariate Ecological Research) package (Clarke & Warwick, 1994). A Bray–Curtis similarity coefficient matrix was calculated on root-transformed data, and the separate clusters were identified by hierarchical clustering (CLUSTER) and multidimensional scaling (MDS) ordination. Differences between groups of community samples were tested by the PRIMER program ANOSIM. The contribution of each species to the average Bray–Curtis dissimilarity between groups of samples and to similarity within a group was examined by the program SIMPER analysis.

The multivariate biota-environment (BIOENV) procedure (Clarke & Warwick, 1994) was used to explore the potential relationships between the abiotic features of the water and the similarity patterns among biological samples. BIOENV functions within the PRIMER program and allows either a full search of all abiotic variable combinations or of specific subsets, e.g. all combinations containing certain variables or containing a fixed number of variables. Chl *a* was omitted from the environmental matrix due to its collinearity with temperature. Data for NO_3-N, NO_2-N NH_3-N and SRP were standardized by logarithmic transformation before analysis. Analysis of univariate analysis was carried out using the software SPSS (version 11).

RESULTS AND DISCUSSION

Environmental variables

The values for 10 environmental variables in each of 15 samples are shown in Table 1. Water temperature ranged from 19.5°C to 31°C levelling off steadily from May to June, increasing slowly and then dropping sharply after peaking in late August.

Salinity averaged around 28.7 psu, maintaining high levels from May to the middle of July, but dropping sharply to the lowest level (8.1 psu) in end of July (22 July sample) due to the heavy rainfall, and reverting to its original levels from the end of July to the beginning of September.

The pH values remained relatively stable ranging from 7.24 to 8.05 while turbidities exhibited an increasing trend and peaked at the end of September.

Concentrations of chl *a* were characterized by double peaks, the first of which (60.39 $\mu g\,l^{-1}$) occurred at the end of June and the second in mid-September (77.58 $\mu g\,l^{-1}$).

The turbidity averaged around 8.38 ntu, maintaining low levels from May until mid-September and increasing to a maximum value of 20.3 ntu on 24 September.

The average value of DIN over the whole sampling period was 5.5 $mg\,l^{-1}$. There was an initial decline in DIN between May and mid-June followed by an increasing trend after the introduction of shrimp juveniles. NH_3-N (mean 3.10 $mg\,l^{-1}$) represented 56% of total DIN and exhibited an increasing trend, whereas NO_3-N (mean 0.89 $mg\,l^{-1}$) and NO_2-N (mean 1.31 $mg\,l^{-1}$) levelled off steadily reaching maximum values of only 1.8 and 2.4 $mg\,l^{-1}$ respectively.

The concentrations of SRP ranged from 0.01 to 6.24 $mg\,l^{-1}$, and were much higher in the period after the introduction of shrimp juveniles (13 June) than in the period before.

Densities of bacteria (mean 4.03×10^6 ml^{-1}) ranged from 1.89×10^6 to 7.25×10^6 ml^{-1} (Table 1), the lowest value being on 22 May (i.e. before the shrimp juveniles were introduced) and the highest on 5 August.

Taxonomic composition and taxa distribution

A total of 54 protists were identified during the six-month survey comprising 4 chlorophyceans, 2 chrysophyceans, 5 cryptophyceans, 10 dinoflagellates, 3 euglenophyceans, 10 diatoms, 18 ciliates and 2 sarcodines (Table 2). Ciliates, dinoflagellates and diatoms were the most common forms, accounting for 32%, 19% and 19% respectively of the species recorded. The other 6 groups were represented by comparatively few species (Table 2; Figures 2 & 3a).

The species number of protists in the 15 samples varied considerably with respect to the shrimp-farming cycle. The

Table 1. Environmental variables in mariculture pond water samples between May and October 2002.

	T (°C)	S (psu)	pH	Tur (NTU)	NH_3-N ($mg\,l^{-1}$)	NO_2-N ($mg\,l^{-1}$)	NO_3-N ($mg\,l^{-1}$)	SRP ($mg\,l^{-1}$)	chl a ($\mu g\,l^{-1}$)	bacteria (10^5 ml^{-1})
22 May	24.00	26.80	7.97	3.40	1.30	4.40	0.70	0.01	9.51	18.87
3 June	26.80	32.50	7.67	5.38	2.50	1.10	1.50	0.07	7.30	47.76
13 June	24.80	32.30	7.53	4.97	0.70	1.10	0.90	0.08	5.88	56.61
26 June	25.50	31.50	7.54	7.39	1.20	0.80	0.70	1.16	60.39	30.39
8 July	27.70	34.30	7.74	5.00	2.00	1.00	1.10	6.48	4.68	39.58
18 July	27.30	32.70	7.79	5.72	2.50	0.90	0.60	4.34	8.31	24.55
26 July	27.70	8.30	7.87	5.01	3.60	0.90	0.60	4.80	10.67	26.89
5 August	27.70	21.70	8.05	9.64	3.50	1.30	1.20	5.92	19.69	72.48
15 August	29.10	25.10	7.58	8.06	1.80	0.90	1.00	1.86	6.83	33.07
25 August	31.00	28.50	7.87	6.22	2.60	0.80	1.20	5.10	10.35	27.72
4 September	27.70	31.30	7.84	9.65	5.50	1.30	1.20	5.72	42.87	42.08
14 September	21.00	29.20	7.59	9.05	1.60	1.10	0.90	4.56	77.58	38.74
24 September	23.00	32.50	7.75	20.30	3.90	1.80	1.20	6.24	69.96	53.77
4 October	23.00	32.30	8.01	17.50	5.90	2.40	1.10	5.60	35.53	42.08
14 October	19.50	32.10	7.95	11.30	4.30	2.10	1.30	5.20	52.68	47.26

chl *a*, chlorophyll-*a*; NTU, nephelometric turbidity units; S, salinity; SRP, soluble reactive phosphate; T, temperature; Tur, turbidity.

Table 2. List of the protist species found in 15 samples, including body size, mean abundances and biomass.

Species	Body size	Abundances	Biomass
Chrysophyceans			
Chromulina sp.	4–5 × 4–5	++++	+++
Ochromonas sp.	10–20 × 10–15	++	+
Euglenophyceans			
Euglena sp.1	20–30 × 4–6	+	+
Euglena sp.2	30–40 × 4–6	++	++
Eutreptia sp.	20–35 × 4–6	+	+
Cryptophyceans			
Hillea fusiformis	5–8 × 5–7	++	++
Hillea marina	2–4 × 2–4	++	+
Chroomonas marina	18–22 × 8–10	+	+
Hemiselmis sp.	10-15 × 5–10	+	+
Teleaulax acuta	12–15 × 7–10	++	++
Dinoflagellates			
Prorocentrum minimum	15–20 × 10–15	++	++
Prorocentrum rostratum	18–22 × 8–10	++	++
Prorocentrum sp.1	18–25 × 7–9	+	+
Prorocentrum sp.2	18–25 × 7–9	+	+
Gymnodium sp.	5–7 × 5–7	++	+
Gyrodinium spirale	30–35 × 15–20	++	+++
Peridinium sp.1	10–14 × 8–12	+	++
Peridinium sp.2	10–14 × 8–12	++	+
Peridinium sp.3	8–10 × 5–7	++	++
Peridinium sp.4	30–35 × 25–30	++	++++
Chlorophyceans			
Dictyocha sp.	2–3 × 2–3	+	+
Mamiella sp.	1–2 × 1–2	++ + +	++
Pseudoscourfieldia marina	3–4 × 5–6	++	+
Chlorella sp.	5–7 × 5–7	+	+
Diatoms			
Minidiscus sp.	20–25 × 20–25	+	+
Navicula sp.1	40–45 × 5–10	+	++
Navicula sp.2	40–45 × 7–8	+	+
Thalassiosira sp.	5–6 × 5–6	+++	+
Chaetoceros sp.1	6–9 × 5–7	++	+
Chaetoceros sp.2	6–8 × 5–6	++	+
Manguinea sp.	30–40 × 4–5	+	+
Ephenera sp.	10–16 × 4–5	++++	+++
Gyrosigma sp.	40–45 × 4–5	+	++
Achnanthes sp.	30–40 × 15–20	+	++
Ciliates			
*Condylostoma*sp.	90–120 × 70–90	+	++
Dysteria sp.	45–55 × 25–35	+	++
Uronema marinum	30–45 × 16–22	+	++
Euplotes vannus	90–140 × 60–80	+	++
Euplotes minuta	50–70 × 40–55	+	++++
Podophrya sp.	35–45 × 35–45	+	+
Tintinnopsis beroidea	18–22 × 10–15	+	+++
Tintinnopsis lohmanni	60–110 × 35–55	+	++
Strombidium acutum	30–55 × 30–45	+	++
Strobilidium neptuni	35–60 × 40–50	+	++
Mesodinium pupula	30–55 × 30–45	+	+++
Didinium sp.	70–90 × 50–70	+	+++
Urotricha venatrix	80–120 × 40–80	+	+++
Rimostrombidium orientale	25–35 × 20–30	+	++
Askenasia stellaris	30–45 × 25–30	+	+
Balanion comatum	5–15 × 10–25	+	+
Holophrya gargamella	70–90 × 50–70	+	++
Gymnozoum sp.	35–70 × 25–40	+	+++
Sarcodines			
Amoeba sp.	35–45 × 35–45	+	++
Thecamoeba sp.	35–45 × 35–45	+	+

Body size (μm): length × width; abundance (ind ml^{-1}): + = 0–50, ++ = 50–500, ++ + = 500–5000, ++ + + = over 5000; biomass (μg l^{-1}): + = 0–10; ++ = 10–100; ++ + = 100–1000; ++ + + = over 1000.

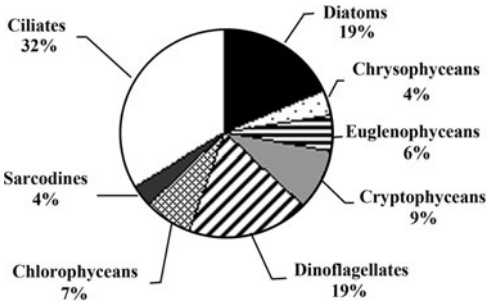

Fig. 2. Composition of planktonic protist communities; the percentage of the total number of species recorded throughout the period of sampling is shown for each group.

temporal variation of species number showed a bimodal distribution during the six-month period with peaks in August and October. The maximum values were 21 species in August and 20 species in October. Ciliates, dinoflagellates and diatoms were primarily responsible for the two peaks. The lowest species number (6 species) was found in the 26 June sample, the first sample after the introduction of shrimp juveniles (Figure 3a).

Protist species diversity is generally lower in the semi-enclosed biotopes than in more open-water sites.

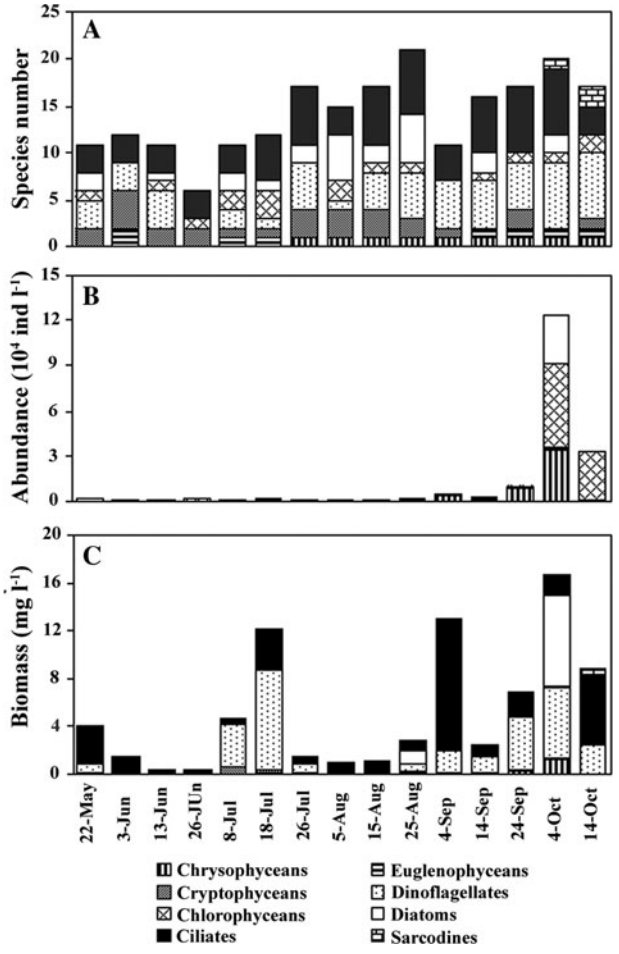

Fig. 3. Temporal variations of species richness (A), abundance (B) and biomass (C) of protists obtained.

Investigations of temperate coastal, inshore and estuarine sites have revealed over 30 ciliate taxa in the Jiaozhou Bay of Qingdao, China (Gong *et al.*, 2005), compared with the 18 taxa in the shrimp-farming biotope. Diversities of dinoflagellates, diatoms, chlorophyceans, cryptophyceans and chrysophyceans in the pond were also low, but their abundances were very high, so they are therefore likely to make a significant contribution to microplankton dynamics (Gilabert, 2001; Nuccio *et al.*, 2003).

Abundance and temporal variation

The temporal variation of the protist abundance had unimodal distribution (Figure 3b). The abundances maintained relatively low values (mean 1.35×10^3 ind ml^{-1}) from May to September, followed by a peak in October when maximum cell densities reached 1.23×10^5 ind ml^{-1}. Chlorophyceans (e.g. *Mamiella* sp.), chrysophyceans (e.g. *Chromulina* sp.) and diatoms (e.g. *Ephenera* sp.) were primarily responsible for the October peak reaching abundances of 5.44×10^4 ind ml^{-1}, 3.48×10^4 ind ml^{-1} and 3.24×10^5 ind ml^{-1} respectively. Of the total protist abundance for the six-month period, chlorophyceans accounted for 47.71%, chrysophyceans 25.43%, and diatoms 19.45% compared to dinoflagellates (3.13%), cryptophyceans (2.93%), ciliates (0.98%), euglenophyceans (0.35%) and sarcodines (0.02%) (Figure 4).

There were 10 dominant species, the individual abundance of which exceeded 30% of the total at some point during sampling period: *Chromulina* sp., *Hillea fusiformis*, *Hillea marina*, *Teleaulax acuta*, *Peridinium* sp.2, *Peridinium* sp.4, *Mamiella* sp., *Thalassiosira* sp.1, *Ephenera* sp., and *Pseudoscourfieldia marina*. The abundance of five of these (*Hillea marina*, *Hillea fusiformis*, *Pseudoscourfieldia marina*, *Teleaulax acuta* and *Peridinium* sp.4) had one high peak and at least one other smaller peak whereas the other five (*Chromulina* sp., *Peridinium* sp.2, *Mamiella* sp., *Thalassiosira* sp.1 and *Ephenera* sp.) occurred in significant abundances on only one occasion (Figure 5). These ten dominant species showed a clear succession from May to October (Figure 5).

At least one previous investigation has demonstrated the numerical dominance of flagellates and cryptophyceans, with relatively low numbers of other protist groups such as diatoms, in a marine lagoon (Nuccio *et al.*, 2003). Moreover, the studies on other semi-enclosed marine waters, for example various Mediterranean lagoons, the Varano Lagoon near the Adriatic Sea, and the Center-Western Sardinia lagoon, have also revealed that protist abundances show distinct peaks in abundances of a limited number of species in the summer and occasionally in the winter, mainly flagellates (e.g. chlorophyceans, cryptophyceans and euglenophyceans) and diatoms (Gilabert, 2001). There are, however, no previous studies of protist communities in semi-enclosed shrimp-farming ponds with which to compare our data.

In the present study, the autotrophic groups (cryptophyceans, chlorophyceans and other small flagellates) were the most abundant mainly due to their ability to bloom rapidly. Similar findings have previously been reported for marine lagoons (Gilabert, 2001; Nuccio *et al.*, 2003).

Compared with lower latitude fjords, the protist abundance in the semi-enclosed pond is very high. Ciliate abundance in our sampling pond, for example, ranged between 1.6 to 7.8×10^5 ind l^{-1}, whereas in Ellis Fjord, maximum

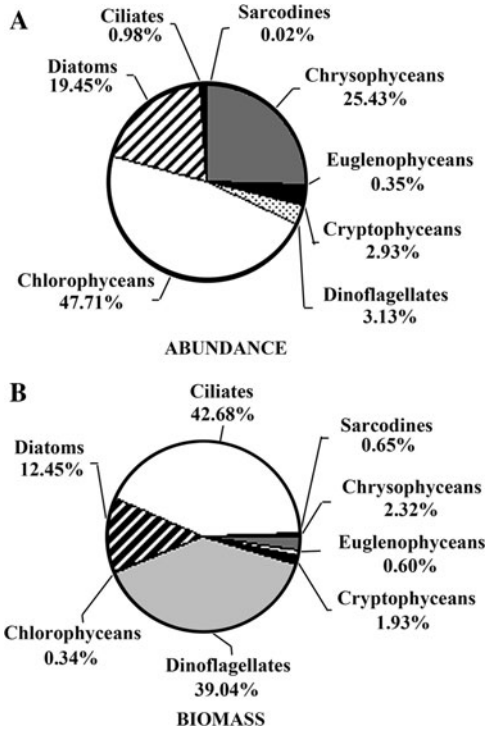

Fig. 4. Proportions of cumulative abundances (A) and biomasses (B) of protists throughout the period of sampling.

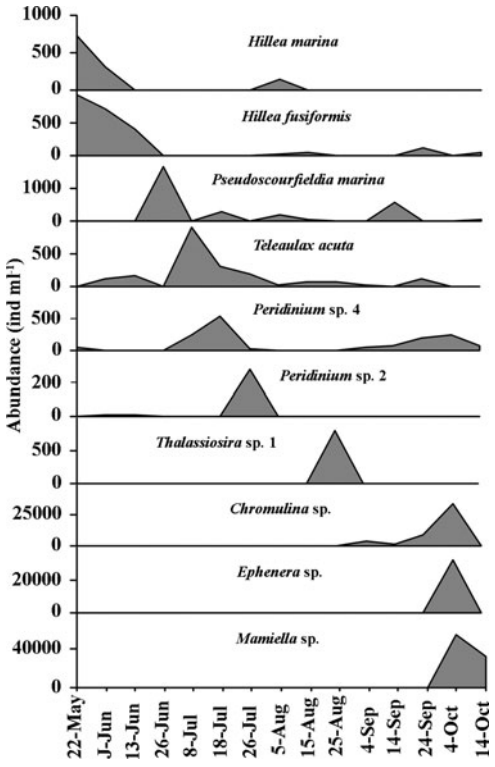

Fig. 5. Abundance (ind ml^{-1}) and temporal succession of the 10 dominant protist species.

abundance only reached 2.2×10^2 ind l^{-1} (Grey *et al.*, 1997). Compared with coastal waters around the Antarctic, the ciliate abundances in the mariculture pond were also high. In Admiralty Bay, for example, ciliate numbers remained little more than $3-4 \times 10^3$ ind l^{-1} (Brandini, 1993).

Biomass and temporal variation

The temporal variation of biomass exhibited a clear bimodal distribution with one peak in July and another in October, but only the latter corresponded to the abundance peak (Figure 3c). The maximum values were 12.14 mg l^{-1} in July and 16.74 mg l^{-1} in October. Dinoflagellates (e.g. *Peridinium* sp.4) and ciliates (e.g. *Urotricha venatrix*) were responsible for the July peak when their biomasses reached 8.43 and 3.37 mg l^{-1} respectively, while diatoms, dinoflagellates and ciliates were the major contributors to the October peak with biomass values of 7.52, 5.96 and 1.79 mg l^{-1} respectively. Ciliates, dinoflagellates and diatoms accounted for 42.68%, 39.04% and 12.45% respectively of the total protist biomass compared to chrysophyceans (2.23%), cryptophyceans (1.93%), sarcodines (0.65%), euglenophyceans (0.60%) and chlorophyceans (0.35%) (Figure 4).

Temporal patterns of community structure

Although autotrophic and heterotrophic species appeared in almost all samples, the patterns of protist communities in 15 samples exhibited a clear temporal succession in relative species composition, abundance and biomass (Figure 6). In terms of the relative abundances, the patterns of protist communities might be distinguished as five structural types each of which dominated at different times during the period of study: (1) cryptophyceans (maximum 96.18%) dominated the protist communities from May to June; (2) dinoflagellates (maximum 56.73%) in June and July; (3) diatoms (maximum 71.44%) in August; (4) chrysophyceans (maximum 83.05%) in September; and (5) chlorophyceans (maximum 96.54%) in October (Figure 6b). The temporal variation of relative biomass showed an alternate functional change of protist communities: the heterotrophic protists, mainly represented by the ciliates, gave rise to four peaks, one each in June, August, September and October, followed by three peaks of autotrophic forms in July, August and October, respectively (Figure 6c).

The variation of protist community structure showed high frequency oscillations with rapidly increasing and decreasing blooms. The lowest densities occurred from May to August and highest ones during September and October 2002. Despite the variability in the protist abundance and biomass over short time scales, a recurrent pattern was apparent. During the sampling period, the autotrophic protists were always the dominant group, mainly cryptophyceans during May and June, dinoflagellates from July to August, chrysophyceans and chlorophyceans during September and October 2002, and diatoms in August and October. The protist community structure appeared to be more diverse from early July to the middle of September with varying contributions of ciliates, dinoflagellates, diatoms, cryptophyceans and chlorophyceans. To some extent, these findings are consistent with previous findings, e.g. the elevated number of cryptophyceans when temperatures are lower, the high abundances of certain flagellate groups such as the chlorophyceans

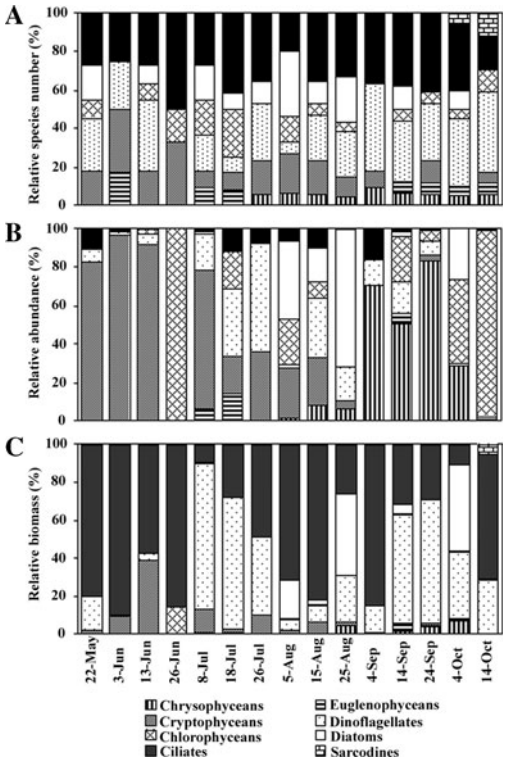

Fig. 6. Temporal succession of protist communities during the period of study.

and cryptophyceans associated with poor abundances of diatoms, and the co-dominance of diatoms and dinoflagellates in the summer period (Dennett *et al.*, 2001; Nuccio *et al.*, 2003). Pitta *et al.* (1998) also found evidence that seasonal factors were more important than the effects of fish-farming in influencing planktonic protist communities, although this study was carried out in an open, rather than a semi-enclosed, mariculture system.

A dendrogram of the 15 samples was plotted using group-average clustering from Bray–Curtis similarities on square root transformed abundances (Figure 7a). The cluster analysis resulted in the 15 samples falling into 2 groups at a 25% similarity level ($P < 0.001$): group I was composed of the first eight samples (May to beginning of August), and group II the last seven samples (from August to October). The MDS ordination shows a temporal distribution of samples in agreement with the dendrogram with 2 groups appearing at separated locations on the plot (Figure 7b).

Analysis of similarities (ANOSIM) revealed that the two groups are significantly different at the 85.86% dissimilarity level ($P < 0.001$). Similarity percentage (SIMPER) analysis showed that the cryptophyceans *Teleaulax acuta* and *Hillea fusiformis* and chlorophycean *Pseudoscourfieldia marina* dominated group I, while the chrysophycean *Chromulina* sp. and the dinoflagellates *Gyrodinium spirale* and *Prorocentrum rostratum* dominated group II. Furthermore, ANOSIM indicated that group I was clustered into three subgroups (dissimilarity 74.15%; Ia, Ib and Ic) and group II into two subgroups (dissimilarity 73.81%; IIa and IIb) at $P < 0.01$ level (Figure 7). SIMPER analysis revealed that: subgroup Ia was characterized by the cryptophyceans *Hillea fusiformis* and *H. marina*; subgroup Ib by the chlorophycean *Pseudoscourfieldia marina* and the cryptophycean *Teleaulax acuta*; subgroup Ic,

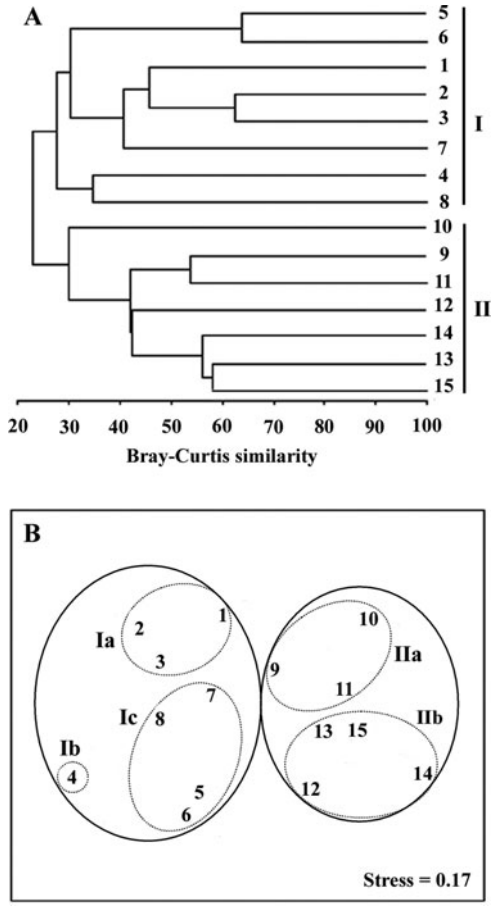

Fig. 7. Cluster analysis (A) and MDS ordination (B) of protist communities of 15 samples. 1, 22 May; 2, 2 June; 3, 13 June; 4, 26 June; 5, 8 July; 6, 18 July; 7, 26 July; 8, 5 August; 9, 15 August; 10, 25 August; 11, 4 September; 12, 14 September; 13, 24 September; 14, 4 October; 15, 14 October; I, group I; II, group II; Ia, Ib, Ic, subgroups in group I; IIa, IIb, subgroups in group II.

the cryptophycean *Teleaulax acuta* and the dinoflagellate *Peridinium* sp.4; subgroup IIa, the dinoflagellate *Peridinium* sp.3 and the cryptophycean *Teleaulax acuta*; and subgroup IIb, the chrysophycean *Chromulina* sp., the chlorophycean *Mamiella* sp. and the dinoflagellate *Prorocentrum rostratum*.

Interaction between biota and environmental variables

The correlations (Spearman, SPSS) between the various environmental variables and abundance, biomass, biomass/abundance ratios (B/A), species diversity, species evenness and species richness in 15 protist samples are shown in Table 3. The protist abundance exhibited significant positive correlations with turbidity, chl *a*, DIN, DIN + SRP, NH_3-N and NO_2-N, and a significant negative correlation with water temperature. For protist biomass, significant positive correlations were found with DIN, DIN + SRP, NH_3-N, NO_2-N and pH. By contrast, the biomass/abundance ratio (B/A) was positively correlated with water temperature, but was negatively correlated with chl *a* and the concentration of bacteria. Both diversity (H') and evenness (J) indices of protist species showed significant positive correlations with water temperature and were negatively correlated with salinity, while the species richness values only exhibited significant negative correlation with salinity (Table 4).

Table 4 summarizes the correlations between abundance of dominant species and environmental variables. The chlorophycean *Pseudoscourfieldia marina* was negatively correlated with DIN ($r = -0.681$, $P < 0.01$); *Teleaulax acuta* was positively correlated with water temperature ($r = 0.521$, $P < 0.05$), but was negatively correlated with NO_2-N and chl *a*; *Mamiella* sp. and *Chromulina* sp. were positively correlated with turbidity ($r = 0.684$, 0.858, $P < 0.01$), and also with nutrients such as DIN and NH_3-N (Table 4); there was a strong positive correlation between *Peridinium* sp.4 and salinity ($r = 0.541$, $P < 0.05$) and total nutrients ($r = 0.603$, $P < 0.05$); a significant positive correlation between *Ephenera* sp. and pH was also noted ($r = 0.612$, $P < 0.05$).

For all 15 samples collected during the six-month period, the top 6 correlations between biota and environmental variables, established by BIOENV analysis, are dominated by nutrients, salinity and water temperature (Table 5). The highest correlation occurred with the combination of NO_3-N and SRP. It was also found that the nutrient SRP is the only one variable that was in each of the top six correlations with both abundance and biomass (Table 5).

Species diversity, evenness and richness indices are commonly employed in community investigations and are amenable to simple statistical analysis (Ismael & Dorgham, 2003).

Table 3. Correlations between environmental variables and species diversity (H'), species evenness (J), species richness (d), abundance, biomass and biomass/abundance ratio (B/A) of the protist community.

	Abundance	Biomass	B/A	H′	J	d
T	−0.608**	−0.236	0.653*	0.546*	0.543*	0.246
S	0.252	0.372	−0.039	−0.555*	−0.528*	−0.511*
pH	0.411	0.500*	0.023	0.250	0.170	-0.002
Tur	0.614**	0.421	−0.439	−0.093	−0.175	0.077
NH_3-N	0.529*	0.686**	−0.027	−0.007	−0.118	0.045
NO_2-N	0.560*	0.441*	−0.326	−0.254	−0.229	−0.220
NO_3-N	0.300	0.175	−0.235	−0.194	−0.199	0.044
DIN	0.626**	0.694**	−0.075	−0.111	−0.174	−0.158
SRP	0.357	0.475*	0.064	−0.096	−0.175	−0.009
DIN + SRP	0.625**	0.761**	0.007	−0.050	−0.154	−0.084
Chl *a*	0.686**	0.239	−0.504*	−0.239	−0.293	−0.046
Bacteria	0.027	−0.152	−0.465*	−0.345	−0.295	0.072

*$P < 0.05$; **$P < 0.01$; DIN, dissolved inorganic nitrogen; see Table 1 for other abbreviations.

Table 4. Correlations between abundance of 10 dominant protists and environmental variables (see Table 1 for abbreviations).

	T (°C)	S (psu)	pH	Tur (NTU)	NH_3-N (mg l^{-1})	NO_2-N (mg l^{-1})	NO_3-N (mg l^{-1})	DIN (mg l^{-1})	SRP (mg l^{-1})	DIN + SRP (mg l^{-1})	Chl *a* (μg l^{-1})
Chromulina sp.	−0.352	−0.126	0.286	0.858**	0.671**	0.517*	0.303	0.581*	0.535*	0.708**	0.632*
Hillea fusiformis	−0.283	−0.084	−0.100	−0.240	−0.289	0.364	0.155	0.039	−0.491	−0.408	−0.177
Hillea. marina	−0.014	−0.201	0.335	−0.319	−0.170	0.390	0.191	0.216	−0.368	−0.222	−0.207
Teleaulax acuta	0.521*	0.366	−0.323	−0.472	−0.126	−0.522*	−0.149	−0.348	0.099	−0.180	−0.629*
Peridinium sp.2	0.086	−0.049	−0.239	−0.488	−0.121	−0.175	−0.098	−0.175	−0.396	−0.380	−0.352
Peridinium sp.4	−0.426	0.541*	0.282	0.291	0.425	0.375	−0.112	0.396	0.461	0.603*	0.199
Mamiella sp.	−0.619*	0.297	0.381	0.684**	0.629*	0.590*	0.372	0.629*	0.406	0.623*	0.439
Pseudoscourfieldia marina	−0.018	−0.060	−0.368	0.039	−0.485	−0.485	−0.285	−0.681**	−0.140	−0.401	0.127
Thalassiosira sp.1	0.437	−0.186	0.155	−0.060	0.062	−0.406	0.219	−0.062	0.062	<0.001	−0.062
Ephenera sp.	0.055	−0.354	0.612*	0.243	0.480	0.225	−0.061	0.398	0.322	0.452	0.125

*$P < 0.05$; ** $P < 0.01$.

Table 5. Summary of results from biota-environment (BIOENV) analysis, with the top 6 correlations corresponding to different variables.

Rank	Abundance – environment		Biomass – environment	
	r	Variables	*r*	Variables
1	0.252	NO_3-N, SRP	0.279	NO_3-N, SRP
2	0.247	SRP	0.261	SRP
3	0.239	S, NH_3-N, NO_3-N, SRP	0.230	NH_3-N, NO_2-N, NO_3-N, SRP
4	0.230	T, NO_3-N, SRP	0.213	S, NO_3-N, SRP
5	0.225	NO_2-N, NO_3-N, SRP	0.193	S, NH_3-N, NO_3-N, SRP
6	0.224	T, NH_3-N, NO_3-N, SRP	0.183	S, NO_2-N, NO_3-N, SRP

r, Spearman correlation coefficient; for abbreviations see Table 1.

In our case, however, univariate correlation analysis showed that these indices only correlated at a high level of significance with water temperature and salinity, but failed to show significant correlations with nutrients. All three indices sharply increased in the end of June and exhibited a peak in the middle of August. This might have been due to low salinity and high water temperature during this period.

Univariate correlation analysis demonstrated that there were significant correlations between the protists (abundance and biomass) and environmental variables, such as water temperature, turbidity, chl *a* and nutrients. The chrysophycean *Chromulina* sp. and the chlorophycean *Mamiella* sp. showed significant correlations with nutrients, which might be the reason that these two species dominated the communities and gave rise to the October peaks of protist abundance. Furthermore, their strong correlation with turbidity suggests that *Chromulina* sp. and *Mamiella* sp. were largely responsible for the elevated levels of turbidity in the water during this period. The cryptophycean *Teleaulax acuta* bloomed in the warmest months (from May to August) and was positively correlated with water temperature, while the chlorophycean *Mamiella* sp., which dominated the protist communities in the autumn when the water was cooler, was negatively correlated with water temperature.

The biomass/abundance (B/A) ratio of the community, i.e. the mean protist body-size, showed a strong positive correlation with water temperature and negative correlations with chl *a* and the concentration of bacteria. That is to say, the higher the water temperature, the more large-sized species were present, and the higher the abundance of phototrophic microorganisms or bacteria, the more small-sized forms dominate. This is consistent with the use of abundance/biomass comparison (ABC) plots to determine levels of disturbance (Warwick, 1986). This method, which is usually used in benthic macrofauna studies, might thus also be suitable for biomonitoring levels of organic pollution using planktonic protist communities.

Multivariate analyses were more sensitive than univariate ones for detecting changes in community structure. Cluster analysis revealed the difference of protist communities between summer (group I) and autumn (group II). Furthermore, MDS ordination analysis clearly showed the succession sequence of 15 communities due to the disturbance from shrimp-farming activities: subgroup Ia, which was composed of the first three samples, represented the community structure before the introduction of shrimp juveniles;

subgroup Ib, which comprised only the first sample after shrimp-farming started, was significantly different in terms of community structure, almost certainly due to disturbance caused by shrimp-farming; subgroup Ic (8 July, 18 July, 26 July and 5 August), IIa (15 August, 25 August and 4 September) and IIb (14 September, 24 September, 4 October and 14 October) exhibited variations in the structural patterns of protist communities during the shrimp-farming period.

The BIOENV analysis demonstrated that NO_3-N and SRP were the most important factors influencing the structure of the planktonic protist community, based on all 15 samples. Moreover, nutrients were always among the top combinations of variables along with salinity and water temperature, suggesting that the succession of protist communities is significantly related to these parameters in the semi-enclosed shrimp-farming waters. It should be noted, however, that the present study was restricted to the nano- and microplanktonic protists. Other methods, such as denaturing gradient gel electophoresis (DGGE) and real-time PCR which have previously been used for analysing prokaryote communities in mariculture ponds (Cytryn *et al.*, 2003), might usefully be employed in order to expand the range of the protist data to include, for example, the picoplanktonic forms.

In conclusion, the results of this study demonstrate that planktonic protists are abundant and diverse in the semi-enclosed mariculture pond near Qingdao and that they are correlated with various environmental parameters including nutrients such as nitrogen (NO_3-N) and phosphate (SRP), both individually and in combination. This suggests that planktonic protists might be useful bioindicators of water quality in such systems. However, further studies are needed on a range of semi-enclosed mariculture ponds and over extended time periods in order to verify this conclusion.

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