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Marine ciliate community in relation to eutrophication of coastal waters in the Yellow Sea*

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Abstract We assessed the potential of marine ciliate community as an indicator to coastal water quality using water samples collected from four stations in the Yellow Sea in the summer 2000. The four stations were characterized by different levels of pollution. The ciliate communities consisted primarily of tintinnids and aloricate ciliates that were <30 μm. A total of 78 species were classified: 55 species at Station 2, 51 species each at Stations 1 and 4, and 47 species at Station 3. The mean number of species at each site was 29.2±2.0 (Station 1), 28.5±2.9 (Station 2), 27.8±1.7 (Station 3), and 24.5±2.3 (Station 4). The abundance was highly variable: 19 331±11 187 ind./L at Station 1, 7 960±5 639 ind./L at Station 2, 29 015±12 999 ind./L at Station 3, and 8 190±4 658 ind./L at Station 4. Our results suggest that neither the simple chemical analysis (e.g. chemical oxygen demand, dissolved inorganic nitrogen, and phosphate) nor the eutrophication/pollution index adequately described the water quality at the four stations. The same was true of the number of species and their abundance, both of which had no correlation with the chemical indices. In contrast, Margalef's diversity index values (3.12 at Station 2, 2.89 at Station 1, and 2.64 at Stations 3 and 4) generally discriminated the water quality status of the four stations. The difference in water quality among the stations was strongly supported by the pattern of species richness (i.e. the total number of species) of ciliates at each station. Our evaluation was consistent with the results of long-term water quality monitoring at the four stations. With increasing eutrophication, we observed also a compositional and functional shift in the ciliate assemblages from algivorous oligotrich/choreotrich to nonselective-omnivorous gymnostomatids to bacterivorous-detrivorous scuticociliatids. Thus, ciliates may be used to indicate the coastal water quality status of a given site.

Keyword: biomonitoring; chemical evaluation; ciliates; community structure; eutrophication; marine pollution

1 INTRODUCTION

Ciliates are an important component of aquatic ecosystems and are characterized by a short generation time (hours to days) and delicate membrane. Thus, ciliates are more responsive to environmental changes than other large eukaryotic organisms. For example, the community structure of ciliates is known to be affected by pollution (Cairns et al., 1972; Beaver et al., 1982; Parker, 1983; Pratt et al., 1992; Decamp et al., 1999; Madoni, 2005). Individual populations and assemblages of ciliates have been extensively evaluated as bioindicators of water quality under various types of stress (e.g. Bark et al., 1985; Dale, 1991; Kalavati et al., 1997; Xu et al., 2005). However, most studies have addressed the relationships between ciliate communities and freshwater environments. The response of ciliates to marine pollution is not well documented (Curds, 1982; Revelante et al., 1985; Lynn et al., 1992). In particular, the effect of environmental quality on marine ciliate community structure is not known.

Our objective was to assess the ciliate community response to marine eutrophication. We measured a range of chemical indices and documented the relationships between ciliate community structure

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(species composition, abundance, and diversity) and environmental conditions in the coastal waters of the Yellow Sea. In addition, we assessed the potential for using marine ciliate communities as indicators of chemical and total environmental quality.

2 MATERIAL AND METHOD

2.1 Study areas, sample collection and environmental factors

The Yellow Sea is a semi-enclosed marginal sea surrounded by China and the Korean Peninsula. The coastal water masses in the Yellow Sea have been continuously stressed by various types of pollutants. We selected four stations that had different levels of pollution in the coastal waters of Incheon, Korea to assess the response of ciliate communities to marine pollution (Fig.1). Station 1 was located at the mouth of Man-Suk Port. This site was moderately polluted by inputs from domestic sewage and a sewage treatment plant (Kim et al., 2004). Station 2 near Pal-Mi Island was the cleanest site, and was located at furthest from coast (Son et al., 2003). Station 3 was located outside Shihwa Lake, an artificial lake that has experienced environmental deterioration since 1994. Station 3 was heavily polluted by organics, nutrients, and heavy metals from industrial discharge (Park et al., 1997). Station 4 was located in Incheon Harbor, the second largest port in Korea. The water at this site was semi-enclosed and polluted mainly by discharge of domestic and industrial sewage as well as oil pollutants (Cho et al., 2009).

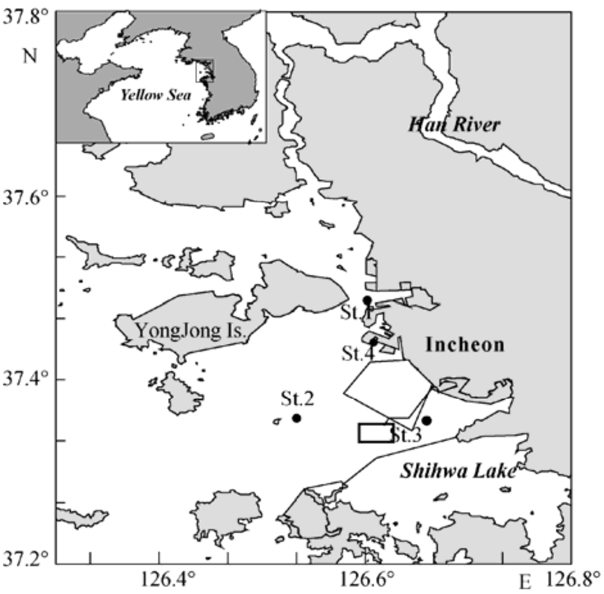

Fig.1 Map showing the sampling stations (St. 1–4) in the coastal region of Korea (eastern Yellow Sea, inset) during summer 2000

We collected a total of 24 samples using a 10 L bucket from the surface water (~1 m) at the four stations during six cruises that were conducted on June 14, 16, 20, 23, 28, and July 4, 2000. A 1-L subsample of each was immediately preserved with Lugol's iodine (1% final concentration). After transported to the laboratory, the water samples were settled for 48 h, and then concentrated by siphoning off the supernatant to give a final volume of 50 ml. The supernatant was transferred to a polycarbonate bottle and stored at 4°C in the dark until processed. Temperature, salinity, pH, and dissolved oxygen (DO) were measured in-situ using a CTD meter (Sea-Bird). Additional water samples were collected for the measure of chemical oxygen demand (COD), dissolved inorganic nitrogen (DIN) and phosphate (DIP), suspended solids (SS), and chlorophyll *a* (Chl *a*) concentrations. These environmental factors were measured following the methods of Parsons et al. (1984) and MOMAF (1998).

The seawater eutrophication level was evaluated using the eutrophication index (E) suggested by Zou et al. (1983): $E = \mathrm{COD}\times\mathrm{DIN}\times\mathrm{DIP}\times10^6/4\,500$, in which the concentrations are expressed in mg/L. The water mass is regarded as eutrophic when the value of $E > 1$, and the higher the value the more eutrophic the water.

The chemical pollution level was assessed with the chemical evaluation index (Pe), suggested by Shen et al. (1995). We followed the modification of Xu et al. (2000), who used DO, COD, SS, DIN, and DIP to evaluate marine water quality. The Pe values were calculated using the formula: $Pe = Pa/5$, where $Pa = \sum Pi$, Pi = Cd/Co (for the variable DO, Pi = Co/Cd); Cd, the concentration of the tested chemical variable; and Co, the upper limit of the concentration of the chemical variable stipulated in Grade II of the standards for surface water of Korean marine environmental conservation. The evaluation classes of marine environmental quality status are proposed as follows (Xu et al., 2000):

< 1 conformable or basically conformable to the Grade II standard for surface water;

1–2 slightly polluted;

2–3 polluted;

3–4 heavily polluted;

> 4 extremely polluted.

2.2 Ciliate enumeration and identification and diversity measurements

We estimated the number of species (S) and the abundance (N) of ciliates that were ≥ 15 μm in each sample using a microscope. The ciliates were counted using a 1-ml grid and each sample was counted twice at a magnification of 200× or 400×. To avoid omitting rare species, two additional subsamples were scanned at a magnification of 100×. We used a partial protargol impregnation (Wilbert, 1975) to identify certain dominant species, which were defined as those whose abundance contributed to more than 10% of the total ciliate abundance in each sample. Prior to protargol staining, we replaced Lugol's fixative with the Bouin's fluid, as suggested in Montagnes et al. (1993). The higher taxonomy of ciliates followed the system of Corliss (1979), which is based primarily on the ciliary structures of ciliates and is thus convenient for studies such as ours.

Margalef's, Shannon's and Simpson's indices were calculated as follows:

Margalef's index: $d = (S-1)/\ln N$, where S=number of species in one sample, and N = total number of individuals per liter.

Simpson's index (D) following Pielou's (1969) modification: $D = 1-\sum n_i (n_i-1)/N (N-1)$, where n_i = the number of individuals in the i^{th} species and N = the total number of individuals.

Shannon's index: $H' = -\sum P_i \ln P_i$, where $P_i = n_i/N$, the proportional abundance of the i^{th} species.

2.3 Statistical analyses

We included eight environmental variables (temperature, salinity, DO, COD, DIN, DIP, SS, and Chl *a*) and five indices of the ciliate community structure (species number, abundance, and Margalef's, Shannon's, and Simpson's indices) in a principal components analysis (PCA) to examine the similarity of environmental conditions in 24 samples, where the relative positions of the samples in hypospace reflects their similarity.

Spearman's correlation analysis was used to identify the relationships among ciliate community parameters (species number, abundance, Margalef's, Shannon's, and Simpson's indices) and the main environmental variables (COD, DIN, DIP, DO, SS, Chl *a*, *E*, and *Pe*) in the 24 samples. In addition, we evaluated the relationships between the abundance of dominant species and the environmental factors.

The data were checked for normality prior to analysis. Non-normal data were transformed using $\log(x+1)$. All statistical analyses were performed in SAS (1983).

3 RESULT

3.1 Environmental factors

There were minor differences in water temperature, salinity, and pH values among the four stations during the summer cruises (Fig.2). Station 2 had the best water quality, with the lowest concentrations of COD, DIN, and DIP. Stations 1 and 4 had relatively high concentrations of COD, DIN, and DIP but low concentrations of DO. Station 3 had the highest COD concentration, suggesting that the water was heavily polluted by organic matter. The DIN and DIP concentrations at Station 3 were very low in summer, while the concentrations of chlorophyll *a* and DO were the highest.

All four stations were highly eutrophic (E: 1.8 to 78.4) (Fig.2). Stations 4 (E=30±24.5) and 1 (E=23.5±17.6) were extremely eutrophic, followed by Stations 3 (E=7.7±5.7) and 2 (E=4.3±2.1). The *Pe* values followed the same order as the eutrophication index values: Station 2<Station 3<Station 1<Station 4 (Fig.2). The chemical evaluation indicated that Station 2 was slightly polluted (Pe=1.6±0.2) whereas Stations 3 (2±0.5), 1 (2.7±0.5), and 4 (2.8±0.6) were polluted.

3.2 Ciliate community parameters

The ciliate communities at the four stations were dominated by tintinnids and small aloricate ciliates of no more than 30 µm in length (Fig.2). We observed a total of 78 ciliate species, including 30 tintinnid species that accounted for ~38.5% of the total species richness, 28 species of Oligotrichina (~35.9%), 14 species of Gymnostomatea, 4 species of Scuticociliatida, and one species each of Suctoria and Hypotrichida (Table 1). Among these, 55 species were observed in the samples from Station 2, 51 from Stations 1 and 4, and 47 species from Station 3. The mean species numbers in each sample were 29.2±2.0 at Station 1, 28.5±2.9 at Station 2, 27.8±1.7 at Station 3, and 24.5±2.3 at Station 4 (Fig.2).

The abundance varied significantly throughout time. The mean number of individuals was 19 331±11 187 ind./L at Station 1, 7 960±5 639 ind./L at Station 2, 29 015±12 999 ind./L at Station 3, and 8 190±4 658 ind./L at Station 4 (Fig.2). The mean contribution of loricate tintinnids to total ciliate abundance was 55% at Station 4, 52% at Station 1, 35% at Station 3, and 30% at Station 2. Among the stations, the higher abundance generally concurred with the higher number of species. The lowest abundance (3 828 ind./L), observed at Station 4, was associated with the lowest number of species (21) observed in this study. The very high abundances observed at Stations 1 (40 150 ind./L on July 4 and 23 140 ind./L on June 23) and 2 (17 562 ind./L on

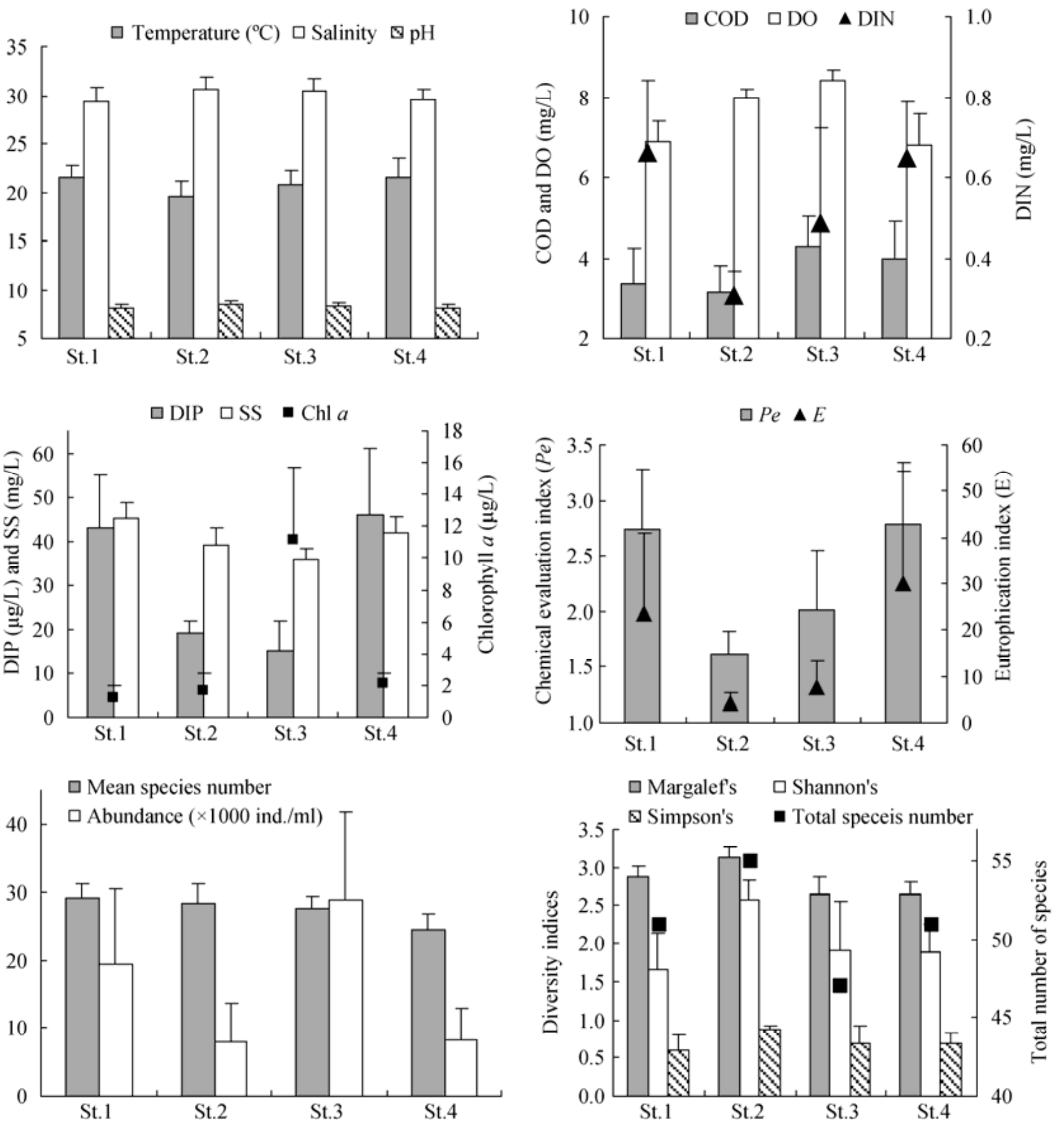

Fig.2 Variations in the environmental factors (temperature, salinity, pH, COD, DO, DIN, DIP, SS, chlorophyll *a*, and eutrophication and chemical evaluation indices) and ciliate community parameters (mean species number, abundance, total species number, and Margalef's, Shannon's and Simpson's indices) at the four stations (St. 1−4)

June 23 and 12 235 ind./L on July 4) also corresponded with the highest number of species (31, 32, 32, 34, respectively) in a sample. However, the highest abundance (Station 3: 49 449 ind./L) observed in this study was associated with the lowest number of species (25) and the highest COD concentration. At Station 3 the lowest abundance (13 304 ind./L on June 23) corresponded with the highest chlorophyll *a* concentration (17.73 μg/L) in the study, suggesting that the bloom of *Gymnodium spirale*, *Gymnodium* sp., and *Ceratium furca* depressed the ciliate population.

The Margalef's diversity index revealed the following order: Station 2 (3.12)>Station 1 (2.89)>Stations 3 and 4 (both 2.64) (Fig.2). Shannon's and Simpson's indices yielded a similar pattern: Station 2 (2.58, 0.87, respectively)> Station 3 (1.91, 0.70) ~Station 4 (1.89, 0.70)>Station 1 (1.65, 0.61). Shannon's and Simpson's index values were strongly affected by the dominant species. For example, the occurrence of *Leprotintinnus simplex*, which accounted for 80% of the total abundance on June 20 and 78% on June 23 at Station 1, yielded very low index values. Likewise, the index values decreased sharply when *L. simplex* contributed to ~ 72% of total abundance and *Myrionecta rubra* (=*Mesodinium rubrum*) to 75% at Station 3.

Among the 12 dominant species, *L. simplex* and *Mesodinium pupula* were dominant at all stations (Tables 1 and 2). At Station 1, *L. simplex* accounted for 55%–80% of the total abundance between 14–23 June. However, by July 4 there was a shift in the

Table 1 Ciliate taxa found at the four stations in Inchon coastal waters in summer 2000

Taxa	Occurrence			
	Station 1	Station 2	Station 3	Station 4
Tintinnina (species number)	19	22	18	17
Tintinnidium semiciliatum	+	+	+	+
Leprotintinnus simplex	+*	+*	+*	+*
L. neriticus	+	+	+	+
L. nordqvisti	+	–	–	–
Tintinnopsis baltica	+	+*	+*	+*
T. tubulosa	+	+	+	+
T. diversicervica	–	+	–	–
T. plagiostoma	–	+	–	–
T. mortensenii	+	+	+*	+
T. beroidea	–	+	–	–
T. radix	+	+	+	+
T. nucula	+	+	–	+
T. schotti	+	+	–	–
T. karajacensis	+	+	+	-
T. lohmani	+	+	+	+
T. japonica	+	–	+	+
T. brevicollis	+	–	+	+
T. cylindrica	–	–	+	–
T. rotundata	–	–	–	+
T. aperta	–	–	–	+
Stenosemella steini	+	+	+	+
Codonellopsis lusitanica	+	+	+	+
Coxiella ampla	–	+	–	–
C. annulata	+	+	–	–
Favella ehrenbergii	+	+	+*	+
F. arizona	–	–	+	–
Undella hyalinella	–	-	+	–
Proplectella ovata	–	+	–	+
Dictyocysta californiensis	–	+	–	–
Eutintinnus tubulosus	+	+	+	+
Oligotrichina (species number)	23	22	20	20
Strombidium compressum	+*	+*	+*	+*
S. minor	–	+	–	–
S. lagenula	+	+	+	+
S. sulcatum	+	+	+	+
Strombidium sp.1 ((15–20) μm×(12–15) μm)	+*	+	+	+
Strombidium sp.2	+	+	+	+
Strombidium sp.3	+	+	+	+
Strombidium sp.4	+	+	+	+
Strombidium sp.5 ((25-30) μm×(20-25) μm)	–	+*	–	–
Strombidium sp.6	+	+	–	–
Strombidium sp.7	–	+	–	–
Strombidium sp.8	–	+	–	+
Strombidium sp.9	+	+	+	+
Strombidium sp.10	+	–	+	+
Strombidium sp.11	+	–	–	–
Halteria sp.1	+	+	–	+
Halteria sp.2	+	+	+	+
Tontonia caudatum	+	+	+	+
Tontonia sp.	+	–	+	–
Strobilidium sp.1	+	+	+	+

To be continued

Table 1 continued

Taxa	Occurrence			
	Station 1	Station 2	Station 3	Station 4
Strobilidium sp.2	+	+	+	+
Strobilidium sp.3	+	–	–	–
Strobilidium sp.4	–	–	+	+
Meseres sp.	+	+	+	–
Strombidinopsis cheshiri	+	+	+	+
S. minima	+	+	+	+
S. sphaira	+	+	+*	+
Strombidinopsis sp.	+	–	+	+
Gymnostomata (species number)	6	9	9	10
Urotricha sp.	–	+	–	+
Prorodon sp.	+	+	+	+
Balanion comatum	+	+	+	+
Litonotus sp.	–	–	–	+
Lacrymaria sp.	+	–	–	+
Myrionecta rubra	+	+	+*	+*
Mesodinium pupula	+*	+*	+*	+*
M. velox	–	+	+	-
M. pulex	–	-	+	–
Mesodinium sp.	–	+	–	+
Cyclotrichium sphaericum	+	+	+	–
Monodinium sp.	–	–	+	+
Didinium sp.	–	+	+	–
Quasillagilis constanziensis	–	–	–	+
Suctoria (species number)	0	0	0	1
Acineta infundibuliformis	–	–	–	+
Scuticociliatida (species number)	3	2	0	2
Uronema marinum	+*	+	–	+
Cyclidium sp.	+	+	–	–
gen. sp.1	+	–	–	–
gen. sp.2	–	–	–	+
Hypotrichida (species number)	0	0	0	1
Euplotes rariseta	–	–	–	+

+: present; −; absent; *: dominant species

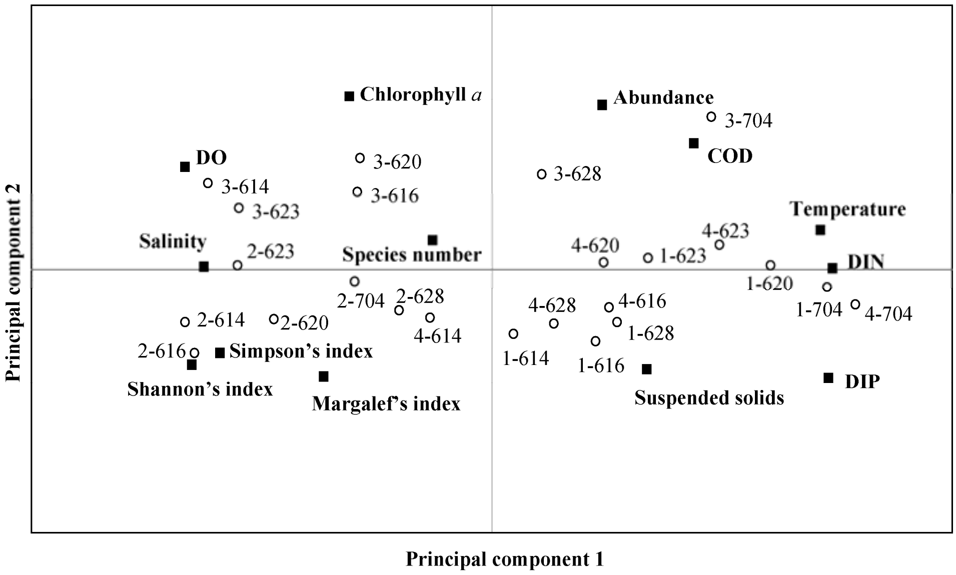

Fig.3 Principal component ordination of four stations incorporating 24 samples (e.g. 1-614, of which 1 indicates Station 1 and 614 indicates the sampling date (June 14)) based on physicochemical and biological data

dominant species (*Uronema marinum*), coincident with the highest DIN concentration (0.998 mg/L) and the second highest DIP concentration (0.065 mg/L). Station 2 was dominated by four species that contributed to between 12%–41% of the total abundance. Station 3 was dominated by ten species (range: 10%–75% of the total abundance). At Station 3 the abundance of the non-toxic, red tide species *M. rubra* decreased significantly during the bloom of *Prorocentrum triestinum* on June 16, and was undetectable during the bloom of *Gymnodium spirale*, *Gymnodium* sp., and *Ceratium furca* on June 23. However, levels gradually recovered with the decrease in abundance of other algae species by June 28. The abundance of *M. rubra* peaked at 37 093 ind./L on July 4, coincident with the bloom of some dinoflagellate species, causing water discoloration. Station 4 was characterized by six dominant species (range: 10%–74% of total abundance). *U. marinum* occurred in very low numbers at Station 4 (24 versus 11 150ind./L at Station 1) on July 4, which overlapped with the lowest DO concentration (5.4 mg/L), and the highest DIP (0.074 mg/L), *E* (78.4), and *Pe* (3.8) values, as well as the second highest COD (5.2 mg/L) and DIN concentrations (0.998 mg/L).

3.3 Relationships between ciliate community parameters and environmental factors

The distribution of each station/sample plotted by PCA is shown in Fig.3. The first 2 factors accounted for 58% of the total variability of the environmental indices while the first 3 factors explained 73% of the total variability. The plots of the stations/samples revealed a distinct geographic pattern. Almost all samples from Stations 1 and 4 except 4-614 were separated clearly on PCA axis I (PC I), which was characterized by high concentrations of DIN and DIP. The samples from Station 3 were strongly associated with PC II, which was characterized by high concentrations of chlorophyll *a*, DO, and COD and high abundance. Conversely, the samples from Station 2 appeared to associate with PC III, which was characterized by high species number and diversity index values (Fig.3).

Spearman's correlation analyses suggested that the number of species in each sample had no correlation with any of the environmental factors. Furthermore, the abundance was only weakly correlated with chlorophyll *a* (Table 2). In contrast, Margalef's index was very significantly negatively correlated with COD, which was also true of the correlation between Shannon and Simpson's indices and DIN, *E*, and *Pe* at the four stations. There was a weak correlation between Margalef's index and DIN, *E*, and *Pe* (Table 2). However, when the samples from Station 3 (the only site where a red tide occurred) were excluded from the analyses, there was no correlation between COD and the community parameters, while Margalef's index was negatively correlated with DIN (P=0.003 3), DIP (P=0.006 2), *E* (P=0.000 6), and *Pe* (P=0.004 0). Similar correlations were obtained for Shannon and Simpson's indices.

The dominant species, *Tintinnopsis baltica* and *Favella ehrenbergii*, were very significantly negatively correlated with DIP and *E*. *T. baltica* was also very significantly positively correlated with DO and chlorophyll a (Table 3). Likewise, *Strombidium* sp.5, which was found only at Station 2, the cleanest site, was very significantly negatively correlated with DIN, *E*, and *Pe*. *Strombidinopsis sphaira* was significantly correlated with all variables except COD and DIN, suggesting that this species may be used as an indicator of clean water with low suspended

Table 2 Spearman's correlation coefficients (r, upper line) and probability values (*p*, lower line) between ciliate community parameters and the primary chemical and biological variables measured at Stations 1–4

Items*	Species number (S)	Abundance (N)	Margalef's diversity (d)	Shannon's diversity (H')	Simpson's diversity (D)
Chemical oxygen demand (COD)	-0.223 0	0.307 8	**-0.539 5**	-0.504 0	-0.443 1
	0.295 0	0.143 4	**0.006 5**	0.012 0	0.030 1
Dissolved inorganic nitrogen (DIN)	-0.130 9	0.272 7	-0.503 1	**-0.609 9**	**-0.556 1**
	0.542 0	0.197 4	0.012 2	**0.001 6**	**0.004 8**
Dissolved inorganic phosphate (DIP)	-0.193 6	-0.161 4	-0.158 9	-0.438 1	-0.468 2
	0.364 8	0.451 3	0.458 4	0.032 3	0.021 0
Chlorophyll *a* (Chl *a*)	0.094 9	0.428 0	-0.025 7	-0.106 7	-0.045 1
	0.659 1	0.036 9	0.905 1	0.619 8	0.834 3
Eutrophication index (E)	-0.253 3	0.061 8	-0.444 1	**-0.672 6**	**-0.661 2**
	0.232 5	0.774 4	0.029 7	**0.000 3**	**0.000 4**
Chemical evaluation index (Pe)	-0.145 8	0.172 2	-0.448 4	**-0.632 3**	**-0.615 9**
	0.496 5	0.421 0	0.028 0	**0.000 9**	**0.001 4**

* Dissolved oxygen and suspended solids were excluded as there was no correlation. Bold indicates very significant correlations (P<0.01)

Table 3 Spearman's correlation coefficients (*r*, first line) and probability values (*p*, second line) between environmental variables and the abundance of dominant species. Bold indicates very significant correlations (*P*<0.01)

Items	COD	DIN	DIP	DO	SS	Chl *a*	*E*	*Pe*
Leprotintinnus simplex	0.049 6	0.190 0	0.314 1	-0.302 2	0.411 6	0.105 3	0.359 6	0.325 3
	0.817 9	0.373 8	0.135 0	0.151 3	0.045 7	0.624 5	0.084 3	0.120 9
Tintinnopsis baltica	-0.239 1	-0.423 7	**-0.693 9**	**0.600 7**	-0.142 4	**0.721 3**	**-0.631 1**	-0.491 1
	0.260 6	0.039 1	**0.000 2**	**0.001 9**	0.507 0	**<0.000 1**	**0.000 9**	0.014 8
Tintinnopsis mortensenii	0.305 2	0.276 2	0.325 5	-0.332 3	0.011 3	0.159 7	0.347 8	0.255 4
	0.147 0	0.191 4	0.120 7	0.112 6	0.958 2	0.456 0	0.095 9	0.228 4
Favella ehrenbergii	-0.333 0	-0.463 3	**-0.567 0**	0.368 8	-0.375 7	0.308 2	**-0.603 2**	0.493 0
	0.111 9	0.022 6	**0.003 9**	0.076 2	0.070 4	0.142 9	**0.001 8**	0.014 4
Strombidium compressum	0.093 2	-0.027 4	-0.333 3	0.375 3	-0.496 7	0.478 8	-0.260 6	-0.152 0
	0.665 0	0.898 9	0.111 5	0.070 8	0.013 5	0.017 9	0.218 8	0.478 2
Strombidium sp.1	-0.300 5	0.004 0	-0.269 9	0.160 6	-0.170 1	-0.075 1	-0.314 0	-0.092 9
	0.153 6	0.985 1	0.202 2	0.496 0	0.426 8	0.727 4	0.135 1	0.665 9
Strombidium sp.5	-0.353 7	**-0.635 1**	-0.401 4	0.304 6	-0.231 6	-0.227 9	-0.589 7	**-0.630 3**
	0.090 0	**0.000 9**	0.051 9	0.147 8	0.276 3	0.284 2	0.002 4	**0.001 0**
Strombidinopsis sphaira	0.036 9	-0.362 3	**-0.546 5**	0.478 8	**-0.525 1**	**0.608 0**	-0.497 7	-0.437 3
	0.864 2	0.081 9	**0.005 7**	0.017 9	**0.008 4**	**0.001 6**	0.013 3	0.032 6
Mesodinium pupula	0.242 5	0.245 3	-0.146 2	0.201 9	**-0.582 1**	0.284 5	-0.006 5	0.100 9
	0.253 6	0.248 0	0.495 6	0.344 2	**0.002 8**	0.177 9	0.975 9	0.639 0
Myrionecta rubra	0.240 1	0.205 5	0.082 5	-0.046 5	0.019 8	-0.057 0	0.155 9	0.149 3
	0.258 4	0.335 4	0.701 5	0.829 2	0.926 9	0.791 2	0.467 1	0.486 2
Balanion comatum	-0.012 9	-0.183 5	-0.453 1	0.332 1	-0.411 9	0.395 4	-0.365 9	-0.245 4
	0.952 4	0.390 8	0.026 2	0.112 9	0.045 5	0.055 8	0.078 7	0.247 8
Uronema marinum	0.058 6	**0.598 5**	**0.667 4**	**-0.768 5**	0.253 0	-0.484 6	**0.668 0**	**0.684 0**
	0.785 8	**0.002 0**	**<0.000 1**	**<0.000 1**	0.232 9	0.016 4	**0.000 4**	**0.000 2**

solids and high chlorophyll *a*. *Myrionecta rubra* had no correlation with any chemical variables. *U. marinum* was rare at Station 2, predominant at the highly eutrophic Station 1, but present at distinctly lower levels at the slightly more eutrophic Station 4. *U. marinum* was the only species that was significantly positively correlated with DIN, DIP, *E*, and *Pe*, but negatively correlated with DO and chlorophyll a (Table 3).

4 DISCUSSION

4.1 Chemical evaluation of marine water quality

Our data suggest that all four sampling stations were highly eutrophic, with eutrophication index values ~79 times greater than those obtained from the Bohai Sea in August 2002 (Wang et al., 2009). The chemical assessment indicated that the water quality of the four stations could be ranked in the order of Station 2>Station 3>Station 1>Station 4 during the summer of 2000. This is consistent with the chemical evaluation conducted in winter-spring 2000 (Xu et al., 2000). However, the eutrophication index values were markedly lower at Station 4 and slightly lower at Stations 2 and 3 during the summer when compared with the winter. This was largely due to the decrease in nutrient availability. For example, the DIN concentrations were significantly lower during the summer (0.485 mg/L) than during the winter-spring (1.122 mg/L, Xu et al., 2000, 2002).

Nonetheless, neither the eutrophication nor the chemical evaluation index was able to adequately describe the pollution status of the four stations. The deficiency of this simple chemical evaluation was well illustrated at Station 3 where the nutrient/eutrophication level was comparatively low, resulting in seemingly better water quality than at Stations 1 and 4 (Fig.2). However, Station 3 was severely impacted by other pollutants including heavy metals and organic contamination discharged from Shihwa Lake (Park et al., 1997). This was the only station that experienced frequent red tides (Choi et al., 1997; present study). Li et al. (2004) noted that the nonylphenol (NP) concentrations in Shihwa Lake (near Station 3) and its in-flowing creeks were among the highest in the world, reaching the threshold level for sublethal effects on a variety of organisms (USEPA, 1997). This explains the highest COD concentrations that were detected at Station 3 (Fig.1, 2).

Likewise, the simple chemical evaluation was of limited use in evaluating the water quality at the highly eutrophic Station 4. Cho et al. (2009) noted that the area surrounding Station 4 contains a large metallurgic center and an oil-refinery. Thus, a large proportion of the pollutants from these two sources are transported into a relatively small area of the marine environment. The ciliate population at Station

4 was clearly depressed by contamination from these and other sources. Both the abundance and the number of species were distinctly lower at Station 4 than at Station 1, even though the chlorophyll *a* concentrations and nutrient levels were similarly high at both locations (Fig.2).

4.2 Biological evaluation using ciliate communities

Neither the species number nor abundance was closely correlated with the individual chemical variables. However, Margalef's index, which combines the two parameters, generally distinguished the water quality status of the four stations as: Station 2 > Station 1 > Station 3 ~ Station 4. The evaluation for Stations 1–3 is consistent with Xu et al. (2002), who used a modified PFU (polyurethane foam unit) method to discriminate their pollution status. The species richness (i.e. the total number of species) measure indicated that the water quality was slightly better at Station 4 than at Station 3. In summer, the sites were ranked according to species richness in the order: Station 2 (55) > Station 1 ~ Station 4 (51) > Station 3 (47). The pattern was similar in the winter-spring: Station 2 (58)> Station 1 (44) > Station 4 (43) > Station 3 (34) (Xu et al., 2000; and original data for that of Station 4). There are at least two possible explanations for the higher total number of species at most stations in the summer: 1) the water quality was improved due to the depletion of nutrients and/or 2) the increase in abundance was associated with a greater likelihood of detecting rare species. The overall health of the sites, based on both summer and winter-spring water sampling may be ranked in the order: Station 2 > Station 1 > Station 4 ≥ Station 3. This is consistent with the results of an integrated environmental assessment of the four stations (Park et al., 1997; Son et al., 2003; Kim et al., 2004; Cho et al., 2009).

In comparison with other techniques for marine biomonitoring (e.g. the modified PFU method mentioned above), the advantages of using Margalef's index of marine planktonic ciliates include sampling simplicity, low cost, reliable environmental estimation, and relaxed taxonomic standards (Xu et al., 2000, 2002). These factors are important considerations for the biological assessment of marine open waters. It is worthy of note that Margalef's index is sensitive to rare species, which are significant for their contribution to the stability of community structure because disturbed or polluted natural systems are often restored by species that were previously rare (Patrick, 1988). In contrast, both Shannon's and Simpson's indices are highly influenced by the dominant species, whose occurrence may be influenced by environmental factors unrelated to pollution. A sharp increase in the abundance of a few species will strongly decrease the index values (Mugurran, 1988). Given this, Karydis et al. (1996) suggested that both Shannon's and Simpson's indices were not good estimators of eutrophication, despite the voluminous literature, including our study, which indicate the correlation between the two.

4.3 Ciliate community composition related to eutrophication

Our results suggest that the nutrient profile was one of the main factors structuring the ciliate communities in these coastal waters. The decrease in species richness and species diversity at some sites was reflective of an increase in pollution. We observed a relative increase in tintinnid contribution to the total ciliate abundance coincident with an increase in the level of eutrophication, consistent with Revelante et al. (1985). The often predominant algivorous tintinnids, *T. baltica* and *F. ehrenbergii*, which are easy to identify by their distinctive shell structure, may be used as indicators of low levels of nutrients/eutrophication. In contrast, the bactivorous-detrivorous *U. marinum* is an indicator of eutrophication/organic-rich and oxygen-poor environments. This ciliate was predominant at the second most eutrophic Station 1, but infrequent at the slightly more eutrophic Station 4. This further strengthens our hypothesis that Station 4 was heavily stressed by other pollutants (e.g. oil). Moreover, consistent with the trophic response of freshwater ciliates (Beaver, 1982), we observed a compositional shift from the marine Oligotrichida (=oligotrich and choreotrich) to Gymnostomata to the small-bodied Scuticociliatida with an increase in eutrophication. Likewise, we observed a functional shift from marine algivores to nonselective omnivores to bacterivores-detritivores with the increase in eutrophication.

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