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Periphytic ciliate colonization: annual cycle and responses to environmental conditions

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ABSTRACT: Glass slides were used as artificial substrates for collecting periphytic ciliates from scallop-farming waters of Jiaozhou Bay near Qingdao (China) over a period of 1 yr. A total of 37 ciliate species, about half of which belong to the orders Hypotrichida and Cyrtophorida, were identified using living observation and silver impregnation methods. Peaks of ciliate abundance and biomass occurred in November, mainly due to the suctorian *Corynophrya lyngbyi*, while sessile peritrichs (especially *Pseudovorticella sinensis*, *Zoothamnium duplicatum* and *Z. plumula*) dominated the ciliate communities during August. Vagile ciliates had low abundance and biomass despite accounting for a large proportion of the species richness. Almost no typical periphytic ciliates were detected in the winter months (from January to March). Twelve dominant species showed clear succession over the year and were found to correlate with a variety of environmental variables. Univariate and multivariate analyses were performed in order to explore the relationship between ciliate community and environmental conditions (temperature, salinity, pH, dissolved inorganic nitrogen, soluble reactive phosphate, dissolved oxygen, chlorophyll *a*, turbidity). 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 related to nutrients. Multivariate analyses revealed that temperature, nutrients and salinity may best explain the changes in community structure of ciliates colonizing the glass slides.

KEY WORDS: Periphytic ciliate · Temporal variations · Environmental stress · Marine biofilm · Microbial ecology · Scallop farming · Jiaozhou Bay

INTRODUCTION

Ciliates are important components of the aquatic ecosystem and play a crucial role in the functioning of microbial food webs (Finlay et al. 1979, 1988, Azam et al. 1983, Pratt & Crains 1985, Sherr & Sherr 1987, Caron & Goldmann 1990). Several ciliates inhabit environments that are unfavorable to most metazoans and some can tolerate what would be extreme environmental conditions to macrofauna (Fenchel 1969, Patterson et al. 1989). Furthermore, with their rapid growth and delicate external membranes, ciliates may react more quickly to environmental changes than most other eukaryotic organisms and can thus serve as bioindicators of water pollution (Cairns et al. 1972, Dale 1991, Foissner et al. 1992, Pratt & Balczon 1992, Al-Rashid & Sleigh 1995, Coppellotti 1998).

Glass slides may be used as artificial substrates that allow microorganisms to form a periphyton or biofilm, in which periphytic ciliates are usually in high abundance and richness (Cairns & Yongue 1968, Foissner et al. 1992). Compared with sampling periphytic ciliates from natural substrates such as stones (Foissner et al. 1992) and macrophytes (Baldock et al. 1983), collection using glass slides seems to be non-destructive, since most species can be observed, enumerated and even identified *in vivo* by observation of the whole slide under an inverted or a stereomicroscope. In addition, the species richness of ciliate communities colonizing glass slides is almost as high as those on natural

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substrates exposed to the same environmental conditions (Agamaliev 1974, Foissner et al. 1992). Differences between fauna have been detected using glass slides in different conditions (Song & Chen 1999, Strüder-Kypke 1999, Strüder-Kypke & Schönborn 1999, Primc-Habdija et al. 2001, Weitere et al. 2003). Moreover, recent studies have demonstrated that periphytic ciliates are strongly related to effluent quality in wastewater treatment processes (Fried et al. 2000, Martín-Cereceda et al. 2001).

Biomonitoring using ciliated protozoa is widely accepted and has many advantages: (1) they are easy to sample compared with other biota such as fish; (2) the generation times are short and they are protected from the environment by only a delicate membrane, so the potential response time to pollution events is fast; (3) periphytic species in particular are relatively immobile, and therefore good for local stress studies; (4) the increasing availability of easily used taxonomic references, and (5) artificial substrates allowing colonization can be standardized for temporal and spatial comparisons (Mohr 1952, Lee 1986, Foissner 1987, Clarke & Warwick 1994). In the interest of using periphytic ciliates as indicators and further tracking the effects of pollution and recovery of the biotic component, it is necessary to have an adequate knowledge of the specific community structures, the relationship with environmental conditions as well as suitable indices. Such studies on marine environment, however, have rarely been carried out (Persoone 1968, Agamaliev 1974, Coppellotti & Matarazzo 2000).

Between October 2000 and September 2001, a 1 yr baseline survey of periphytic ciliates colonizing glass slides was carried out in a scallop-farming area of Jiaozhou Bay, where the most severe form of pollution is the overload of nutrients, especially inorganic nitrogen and phosphorus (Ma et al. 1997). The farming of scallops was responsible for introducing great variations of environmental factors in the study area; thus, it offered an interesting opportunity for a biota–environment analysis. The aims of this study were: (1) to document the taxonomic composition and the temporal pattern of periphytic ciliates colonizing glass slides in the scallop-farming waters; (2) to monitor the population dynamics of the periphytic ciliate communities and their responses to environmental factors and (3) to explore the possibility of using periphytic ciliate community in assessment of marine water quality.

MATERIALS AND METHODS

Study site. Jiaozhou Bay is a semi-closed bay with an area of about 400 km^2 and on average a depth of 7 m. The north of the bay receives inflows from several small rivers and the south is connected with the Yellow Sea. The sampling site was located at the centre of a scallop-farming area (about 16 km^2) with a depth of 10 m (Fig. 1).

Sampling. Twenty-two samples (referred to as Oct-I, Oct-II, etc.) were collected during a 12 mo period from October 2000 to September 2001. Glass slides (2.6 × 7.6 cm) were clipped to a PVC frame, and were immersed in the water at a depth of about 1 m below the surface. The slides were exposed as back-to-back pairs; thus, they could be split and observed directly without cleaning. Slides were placed vertically in the frames, each frame holding 20 sheets of slides. The samples were collected every 15 d. In January and April 2001 samples were lost; therefore, at these times the sampling could only be performed irregularly.

According to Wilbert (1969), there are no significant differences between ciliate communities colonizing slides within the same frame. Thus, for every sampling date 5 replicate slides were randomly selected and then evaluated. The slides were transferred into jars containing water from the sampling site, stored in a cooling box and transported to the laboratory within 1 h for identification and counting.

Water temperature (T), salinity (S), pH, dissolved oxygen concentration (DO) were recorded *in situ* with appropriate sensors (WTW) at the depth of 1 m; turbidity was measured by a turbidimeter (Hach 2100P, Hach). One l of seawater was collected for laboratory analysis of dissolved inorganic nitrogen concentrations (DIN, sum of NO_3-N, NO_2-N and NH_3-N) and soluble reactive phosphate (SRP) followed standard methods (APHA 1989). A further 500 ml water sample was filtered

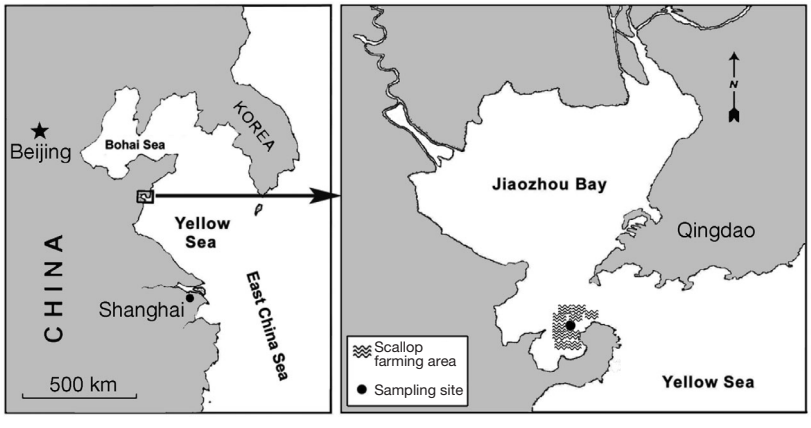

Fig. 1. Map of the study area

through Whatman 25 mm GF/F filters by gentle vacuum filtration; after the extraction of the filter paper in 90% acetone for 24 h at 4°C, the concentration of chlorophyll *a* (chl *a*) in the supernatant was determined using a spectrophotometer (UV-1601, Shimadzu) (Talling & Driver 1961, Jeffrey & Humphrey 1975).

Identification and enumeration of ciliates. Species were first examined at 45-fold magnification using a stereomicroscope to observe the behavior and movement of the cells. They were then transferred using a micropipette to a clean glass slide and placed under a microscope (BH-2 Olympus) at 100- to 1250-fold magnification to reveal cell size and other morphological characters in detail (Foissner et al. 1999). Usually over 30 individuals of each morphotype were picked out with micropipette and then identified to species level using protargol (Wilbert 1975) and Chatton-Lwoff silver nitrate method (Song & Wilbert 1995). Species identifications were made following reference to keys and guides such as Kahl (1931) and Carey (1992). The taxonomic scheme is according to Corliss (1979). Detailed morphological descriptions of most species isolated during the study have been published elsewhere (Gong et al. 2001, Hu et al. 2002, Hu et al. 2003, Ji et al. 2003, Gong & Song 2004a,b, Hu et al. 2004, Lin et al. 2004,). The designation of species as being sessile, vagile or planktonic was made according to their mobility and the ecological niches they occupy. This approach has been used in previous studies including those by Foissner et al. (1992, 1999) and Coppellotti & Matarazzo (2000).

The enumeration and measurement of ciliates *in vivo* was carried out under an inverted microscope as soon as possible after sampling (generally within 1 to 2 h) in order to prevent significant changes in species number and composition. Using bright field illumination, 5 fields of view per slide were randomly chosen for counting. The ciliate concentrations were calculated from all 5 replicate slides to determine average cell density (ind. cm^{-2}).

Biovolume estimates based on 3-dimensional measurements and approximations of shape to standard geometrical configurations (Winberg 1971) were made for most ciliate species fixed with 2% (v/v) formalin. The volumes of individual ciliates were converted to biomass using a conversion factor of 0.14 pg C μm^{-3} (Putt & Stoecker 1989).

Data analysis of samples. Species diversity (H') (Shannon & Weaver 1963), evenness (J) (Pielou 1969) and species richness (d) (Margalef 1968) of samples (apart from samples Jan I to March II due to low number of species present) were calculated as follows:

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

where H' = observed diversity index; P_i = proportion of the total count arising from the ith species; S = total number of species; $J = H'/\ln S$ and $d = (S-1)/\ln N$, where N = total number of individuals.

The community structures of samples were analyzed using the PRIMER package (Plymouth Routines in Multivariate Ecological Research, Clark & Warwick 1994). A Bray-Curtis similarity coefficient matrix was calculated on root transformed data and separate clusters were identified by hierarchical clustering (CLUSTER) and on multidimensional scaling plots (MDS). Differences between species compositions were tested by the PRIMER program ANOSIM.

The multivariate biota–environment (BIOENV) procedure (Clarke & Ainsworth 1993) was used to explore the potential relationships between the abiotic features of 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 normalised by logarithmic transformation before analysis.

RESULTS

Environmental conditions

The results of the physico-chemical analyses of the water samples are shown in Table 1. The water temperature was significantly lower in winter (from January to March); salinity showed little variation (around 30 psu) throughout the year apart from a sharp decrease to 20 psu in late July; pH values ranged from 6.9 to 7.9; turbidities were much lower in the period from April to July, indicating much clearer waters with Sechii depths of about 1.5 to 3.3 m; concentrations of dissolved oxygen generally exceeded 7.0 mg l^{-1} except that 2 lower values (3.8 and 4.3 mg l^{-1}) were recorded in 2 samples in August; concentrations of chl *a* were much higher in the period from April to August (4.25 to 6.75 µg l^{-1}) than that of other periods (0.08 to 3.23 µg l^{-1}). The average value of DIN over the whole year was 0.335 mg l^{-1}; NO_3-N (mean 0.107 mg l^{-1}) represented 68% of the DIN in winter (mean 0.157 mg l^{-1}), whereas NH_3-N (mean 0.522 mg l^{-1}) became the main component (89%) of DIN (mean 0.584 mg l^{-1}) in summer with extremely high concentrations in July and early August. The concentration of SRP ranged from 0.006 to 0.345 mg l^{-1} (mean 0.099 mg l^{-1}) and showed no clear trend throughout the year, although there was a minor peak in early August that coincided with one for NO_2-N.

Table 1. Environmental factors of sampling water between October 2000 and September 2001. Chl *a*: chlorophyll *a*; DO: dissolved oxygen concentration; NTU: nephelometric turbidity units; S: salinity; SRP: soluble reactive phosphate; T: temperature; Tur: turbidity

	T (°C)	S (psu)	pH	Tur (NTU)	NO_2-N (mg l^{-1})	NH_3-N (mg l^{-1})	NO_3-N (mg l^{-1})	SRP (mg l^{-1})	DO (mg l^{-1})	Chl *a* (µg l^{-1})
Oct-I	23.0	31.0	7.9	2.10	0.052	0.023	0.037	0.345	12.2	2.03
Oct-II	20.0	31.5	8.2	3.20	0.055	0.014	0.046	0.114	13.8	1.89
Nov-I	17.0	30.0	8.1	2.46	0.063	0.035	0.058	0.095	15.0	1.00
Nov-II	10.0	29.0	7.5	7.82	0.069	0.063	0.062	0.076	16.0	0.80
Dec-I	11.0	27.0	7.1	2.66	0.056	0.067	0.081	0.076	17.6	0.33
Dec-II	8.0	28.5	7.4	5.91	0.015	0.065	0.030	0.102	8.5	0.08
Jan	6.0	31.0	7.7	4.69	0.007	0.074	0.095	0.300	11.2	0.44
Feb-I	3.5	30.0	7.6	4.44	0.001	0.019	0.111	0.006	12.1	0.82
Feb-II	3.5	28.0	7.7	3.15	0.005	0.050	0.134	0.050	11.8	1.68
Mar-I	4.0	28.0	7.7	4.21	0.002	0.063	0.107	0.008	10.5	1.59
Mar-II	5.0	28.0	7.8	1.47	0.003	0.280	0.086	0.310	9.4	2.00
Apr	8.0	29.0	7.7	0.85	0.015	0.230	0.064	0.060	12.5	2.03
May-I	11.0	30.0	7.6	1.56	0.007	0.560	0.017	0.060	10.6	6.76
May-II	17.0	29.0	7.2	1.11	0.006	0.420	0.021	0.010	7.5	4.25
Jun-I	19.0	30.5	7.4	1.47	0.006	0.100	0.014	0.060	8.6	5.34
Jun-II	16.0	30.0	7.5	2.29	0.006	0.380	0.025	0.040	6.7	5.43
Jul-I	15.0	29.0	7.4	2.31	0.018	1.170	0.023	0.020	6.5	5.10
Jul-II	12.0	30.0	6.9	2.40	0.009	0.850	0.010	0.010	8.5	5.27
Aug-I	14.0	20.0	7.1	4.52	0.080	0.630	0.018	0.110	3.8	6.41
Aug-II	13.0	30.0	7.2	3.13	0.002	0.041	0.075	0.025	4.3	4.96
Sep-I	9.0	30.0	7.4	3.19	0.036	0.352	0.021	0.105	6.8	3.24
Sep-II	9.0	29.5	7.4	5.09	0.046	0.089	0.036	0.271	6.5	2.04

Taxonomic composition and annual cycle of abundance and biomass

A total of 37 ciliate species representing 10 orders and 30 genera were found during the 1 yr survey (Table 2). Hypotrichida and Cyrtophorida were the 2 orders that represented most species, accounting for 36 and 23% respectively of the species recorded; each of the other 8 orders had a comparatively low numbers of species (Table 2, Fig. 2).

The temporal variation of abundance clearly exhibited a bimodal distribution during the course of the year, with 2 peaks, 1 in autumn and 1 in summer (Fig. 3). The maximum cell densities were 1245.6 ind. cm^{-2} (SD = 242.1) in November 2000 and 806.5 ind. cm^{-2} in August 2001. The sessile suctorian *Corynophrya lyngbyi* was responsible for the autumn peak when it had an extremely high abundance (1173.2 ind. cm^{-2}, SD = 98.5). Two peritrichous species, *Pseudovorticella sinensis* (437.5 ind. cm^{-2}, SD = 117.8) and *Zoothamnium duplicatum* (366.2 ind. cm^{-2}, SD = 87.4) gave rise to the summer peak. The sessile ciliates accounted for 83% of the total abundance for the whole year, while the vagile and planktonic ciliates accounted for 16 and 1% respectively (Fig. 4).

Biomass variation did not follow the bimodal pattern as abundance. A single peak for biomass (128 µg C cm^{-2}) occurred in November 2000, corresponding to the abundance peak (Fig. 3). The peak abundance in summer 2001 that was due to the 2 peritrich species did not, however, result in a distinct peak for biomass. This is mainly due to the smaller biovolume of *Pseudovorticella sinensis* and *Zoothamnium duplicatum* relative to *Corynophrya lyngbyi* (50 to 70 vs. 110 to 130 µm in cell length). The sessile ciliates accounted for 89% of the total biomass for the whole year, the vagile and planktonic ciliates accounted for only 9 and 2%, respectively (Fig. 4).

The species number of ciliates in the samples varied significantly with respect to seasons. The lowest species numbers were observed in the winter months (from January to March 2001), when there was usually 1 planktonic ciliate *Uronema marinum*; species numbers were relatively higher in spring, summer and late autumn despite of minor fluctuations, with 2 peaks in December 2000 (11 species) and July 2001 (14 species) (Fig. 3). The variation in species numbers was mainly due to the vagile ciliates, the cumulative total of which accounted for 73% during the period of sampling (Fig. 4).

There were 12 species the individual abundances of which exceeded 30% of the total at some point during the year. These were: *Orthodonella hamatus*, *Holosticha heterofoissneri*, *Hartmannula angustipilosa*, *Trochilia sigmoides*, *Pseudokeronopsis qingdaoensis*, *Acineta tuberosa*, *Amphileptus litonotiformis*, *Coryno-*

Table 2. List of the species of ciliates recorded in 22 samples, including biohabit (Se: sessile; V: vagile; P: planktonic), body size (length × width in µm), and degree of average abundance (+ = 0–10 ind. cm^{-2}; ++ = 10–100 ind. cm^{-2}; +++ = 100 – 400 ind. cm^{-2}; ++++ = over 400 ind. cm^{-2})

Species	Biohabit	Body size	Abundance
ORDER: Haptorida			
Chaenea teres (Dujardin, 1841)	P	100–400 × 12–40	+
Lacrymaria marinum Kahl, 1933	V	200–300 × 20–40	+
ORDER: Prostomatida			
Holophrya oblonga Maupas, 1883	V	300–500 × 30–50	+
Placus salinus Dietz, 1964	V	40–45 × 20–40	+
ORDER: Pleurostomatida			
Amphileptus litonotiformis Song, 1991	V	120–220 × 50–80	+
Litonotus paracygnus Song, 1994	V	150–250 × 30–60	+
ORDER: Cyrtophorida			
Aegyriana oliva Deroux, 1974	V	80–100 × 60–70	+
Brooklynella sinensis Gong & Song, 2005	V	40–50 × 20–30	+
Chlamydonella pseudochilodon Deroux, 1976	V	30–75 × 20–50	+
Dysteria derouxi Gong & Song, 2004	V	100 × 40	+
Hartmannula angustipilosa, Deroux & Dragesco, 1968	V	40–80 × 20–50	+
Hartmannula derouxi Gong & Song, 2004	V	60–120 × 30–70	+
Hypocoma acinetarum, Collin, 1907	V	30–50 × 15–25	+
Trochilia sigmoides Dujardin, 1841	V	20–30 × 10–18	+
Trochilioides recta (Kahl, 1928)	V	40–60 × 20–30	+
ORDER: Nassulida			
Orthodonella gutta (Cohn, 1866) Kahl, 1931	V	140–200 × 60–100	++
ORDER: Suctorida			
Acineta tuberosa, Ehrenberg, 1834	Se	180–200 × 40–50	++
Corynophrya lyngbyi (Ehrenberg, 1833)	Se	110–130 × 80–90	++++
ORDER: Peritrichida			
Pseudovorticella sinensis Ji, Song & Al-Rasheid, 2003	Se	50–60 × 35–45	++++
Zoothamnium duplicatum Kahl, 1933	Se	70 × 40	+++
Zoothamnium plumula, Kahl, 1933	Se	50–70 × 30–40	+++
ORDER: Scuticociliatida			
Pleuronema coronatum Kent, 1881	P	50–70 × 30–40	+
Uronema marinum Dujardin, 1841	P	30–40 × 25–28	+
ORDER: Hypotrichida			
Aspidisca leptaspis Fresenius, 1865	V	60–80 × 40–50	+
Aspidisca steini (Buddenbrock, 1920)	V	20–35 × 15–27	+
Diophrys scutum (Dujardin, 1841)	P	140–200 × 70–100	++
Euplotes rariseta Curds et al. ,1974	V	30–40 × 20–25	+
Euplotes vannus, (Müller, 1786)	V	90–140 × 60–80	+
Holosticha bradburyae Gong et al., 2001	V	150–320 × 25–75	+
Holosticha diademata, (Rees, 1883) Kahl, 1932	V	80–90 × 28–50	+
Holosticha heterofoissneri Hu & Song, 2001	V	115–135 × 32–45	+
Oxytricha enigmatica Dragesco & Dragesco-Kernéis, 1986	P	80–100 × 30–40	+
Oxytricha saltans (Cohn, 1866) Kahl, 1932	P	40–80 × 15–30	+
Parabirojimia similis Hu, Song & Warren, 2002	V	140–300 × 30–50	+
Pseudokeronopsis qingdaoensis Hu & Song, 2000	V	130–240 × 50–70	++
Thigmokeronopsis rubra Hu, Warren & Song, 2004	V	140–200 × 40–50	++
ORDER: Oligotrichida			
Eutintinnus inquilinus (Müller, 1776)	P	100–110 × 30–40	+
Strombidium sulcatum, Claparède & Lachmann, 1858	P	30–45 × 30–40	+

phrya lyngbyi, *Pseudovorticella sinensis*, *Thigmokeronopsis rubra*, *Zoothamnium duplicatum* and *Z. plumula*. The first 5 species occurred in more than one season while the last 7 species appeared in significant numbers during only 1 season (Fig. 5).

Temporal patterns of community structure

Cluster analysis based on square root transformed abundances resulted the 22 samples falling into 3 groups at a 12% similarity level (analysis of similarities

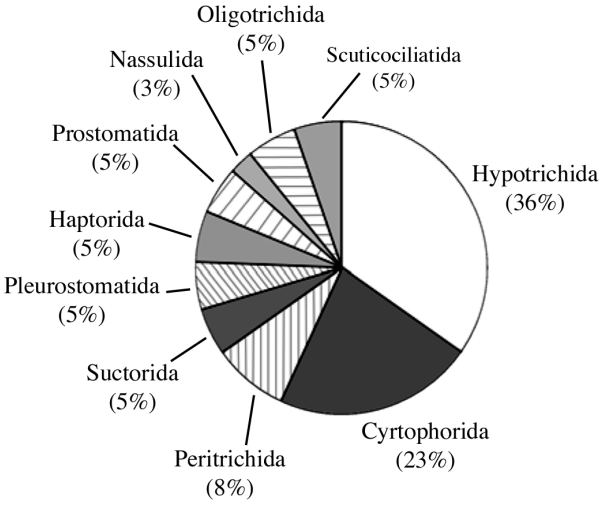

Fig. 2. Composition of periphytic ciliate communities; the percentage of the total number of species recorded throughout the period of sampling is shown for each order

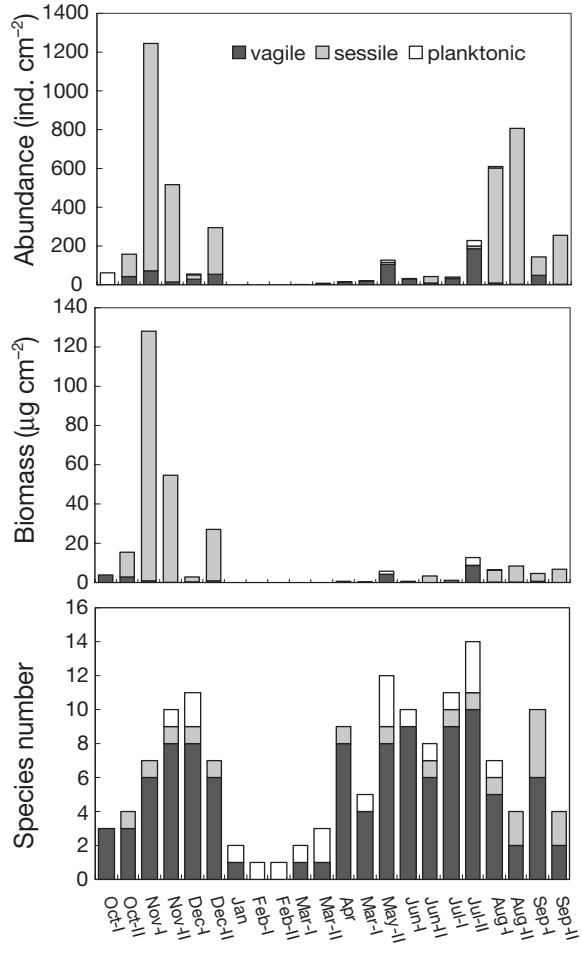

Fig. 3. Temporal variations of abundance, biomass and species number of vagile, sessile and planktonic ciliates in colonized biofilms. Two samples collected in 1 mo with an interval of 15 d were referred to as month-I and month-II

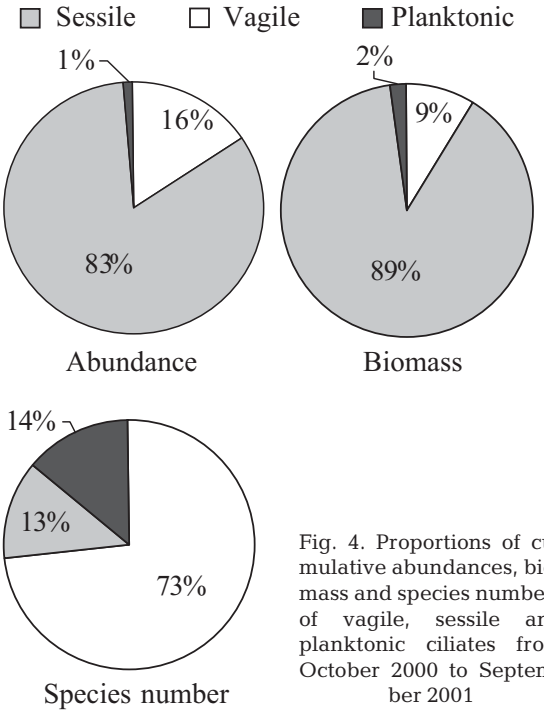

Fig. 4. Proportions of cumulative abundances, biomass and species numbers of vagile, sessile and planktonic ciliates from October 2000 to September 2001

[ANOSIM], $p < 0.001$): group I was composed of the winter month samples (Jan, Feb-I, Feb-II, Mar-I and Mar-II); group II, the 2 August samples (Aug-I and Aug-II); and group III, the rest of the samples (Fig. 6). Furthermore, at 19% similarity level, group III are clustered into 3 subgroups: IIIa, IIIb and IIIc (see Fig. 7). The MDS ordination shows a temporal distribution of samples in agreement with the dendrogram with the 3 groups appearing at separate locations on the plot (Fig. 7).

Linking biota to environmental factors

Table 3 summarizes the correlations between the various environmental parameters and species diversity, species evenness and species richness, excluding the 5 samples collected in winter (Jan, Feb-I, Feb-II, Mar-I, Mar-II) because of the lack of organisms on the slides. All 3 indices show significant positive relationships with water turbidity and NO_2-N, while significant correlations between species richness and nutrients such as NH_3-N, DIN and SRP are also noted.

Correlations between abundance of dominant species and environmental factors are shown in Table 4. Significant positive relationships were found between *Amphileptus litonotiformis* and NO_3-N ($r = 0.52$, $p < 0.05$), and between *Hartmannula angustipilosa* and NH_3-N ($r = 0.57$, $p < 0.05$); the suctorian *Corynophrya lyngbyi* was positively correlated to pH value ($r = 0.49$,

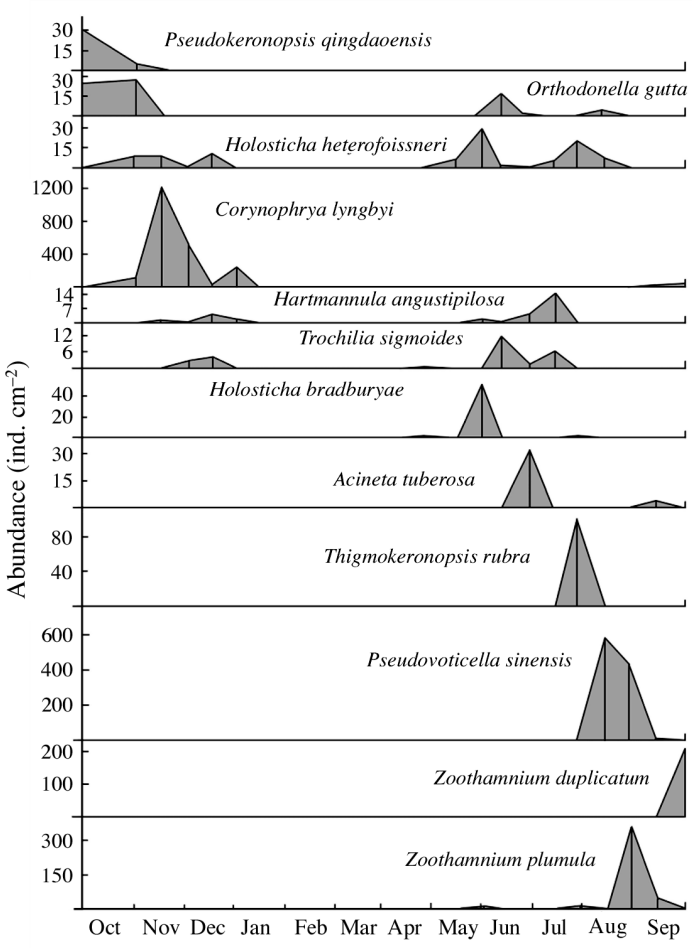

Fig. 5. Abundances (ind. cm^{-2}) and temporal distribution of the 12 dominant ciliate species

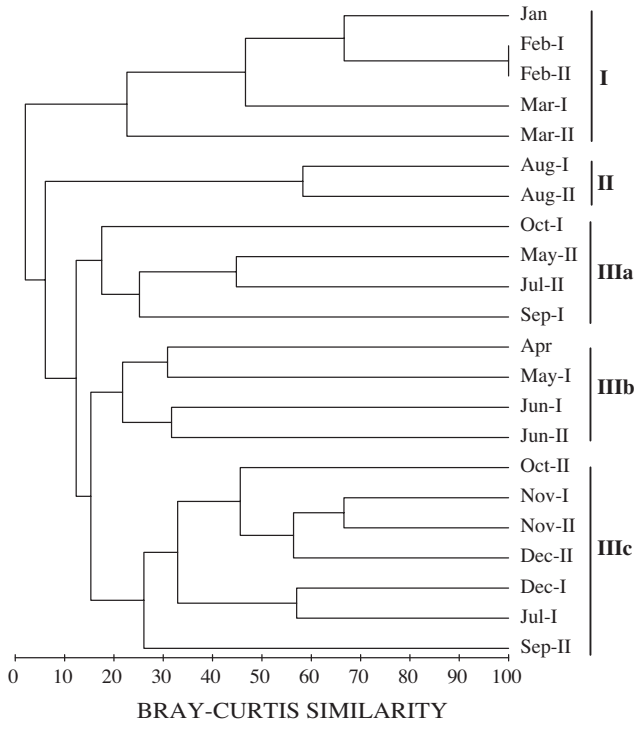

Fig. 6. Dendrogram of 22 samples, using group-average clustering from Bray-Curtis similarities on square root transformed abundances. I = group I; II = group II; IIIa, b, c = subgroup a, b, c in group III (see 'Results' for details). Two samples collected in 1 mo with an interval of 15 d were referred to as month-I and month-II

$p < 0.05$); *Orthodonella hamatus* showed a strong significant positive relationship with water temperature ($r = 0.70$, $p < 0.01$); *Pseudokeronopsis qingdaoensis* correlated with SRP with high level of significance ($r = 0.74$, $p < 0.01$), and also with water temperature ($r = 0.59$, $p < 0.05$); there was a strong negative relationship between *Pseudovorticella sinensis* and salinity ($r = -0.70$, $p < 0.01$) and DO ($r = -0.53$, $p < 0.05$); there was a significant positive correlation between *Zoothamnium duplicatum* and SRP ($r = 0.52$, $p < 0.05$).

For all the 22 samples collected over the year, the top 6 correlations between biota and environmental variables, established by BIOENV analysis, are dominated by temperature and nutrients (Table 5). The highest correlation occurs with the combination of 3 variables: temperature, NO_2-N and NO_3-N. Another BIOENV analysis for 17 samples of biota (with the 5 winter samples excluded) and environmental variables showed a similar result: temperature, nutrients and salinity are all closely correlated to the community structure of periphytic ciliates (Table 5).

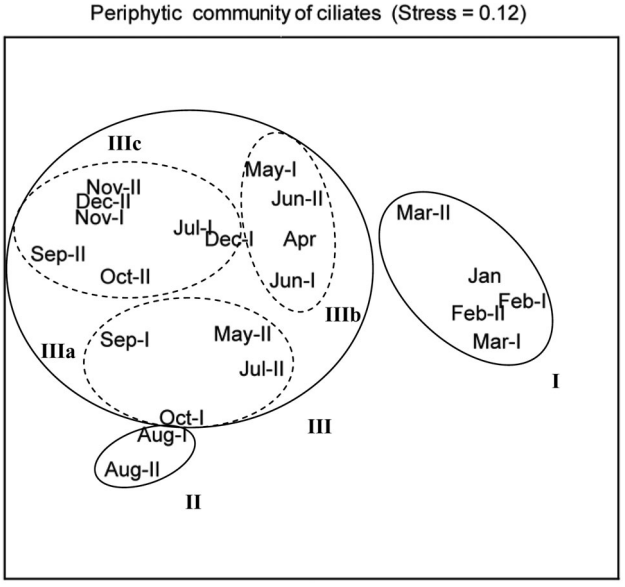

Fig. 7. Multidimensional scaling ordination of 22 samples for abundance data. Groups established from the cluster analysis are indicated (continuous line at 12% similarity level and discontinuous line at 19% similarity level). Stress = 0.12 corresponds to a good ordination with no real prospect of a misleading interpretation

Table 3. Correlation between environmental factors (DIN = dissolved inorganic nitrogen; see Table 1 for other abbreviations) and species diversity (*H'*), species evenness (*J*), species richness (*d*), abundance, biomass and biomass/abundance ratio (B/A) of the ciliate community. *p < 0.05; **p < 0.01

	H'	*J*	*d*	Abundance	Biomass	B/A
T	−0.04	0.13	−0.11	0.01	0.08	0.31
S	0.28	0.46*	0.04	−0.20	0.09	0.35
pH	−0.28	−0.03	−0.34	0.15	0.47	0.56*
Tur	−0.71**	−0.75**	−0.53*	0.34	0.28	0.34
NO_2-N	−0.59*	−0.55*	−0.49*	0.40	0.32	0.29
NH_3-N	0.40	0.18	0.50*	−0.26	−0.32	−0.50*
NO_3-N	0.32	0.22	0.34	0.31	0.32	0.33
DIN	0.35	0.18	0.46	−0.22	−0.27	−0.46
SRP	−0.33	−0.01	−0.53*	−0.04	−0.01	0.04
DIN + SRP	0.27	0.14	0.33	−0.24	−0.3	−0.48*
DO	−0.03	0.04	−0.03	0.03	0.44	0.60*
Chl *a*	0.25	0.18	0.29	−0.20	−0.45	−0.58*

DISCUSSION

Sampling strategy

Biological colonization of a new artificial substrate is a dynamic process, the primary stage of which generally exhibits the following succession: at first, bacteria colonize the slide, followed by diatoms and autotrophic flagellates; the next groups are bacterivorous, vagile species of amoebae and ciliates; then larger species with a broader feeding spectrum and sessile feeders occur (Railkin 1995, Strüder-Kypke 1999). During the primary colonization process, the number of species generally increases and then equilibrates, following the MacArthur-Wilson equilibrium model (MacArthur & Wilson 1967, Franco et al. 1998). Once equilibrium of immigration ends, the early and late interactive phases follow, during which internal factors such as competition and predation pressure become more important (Cairns & Henebry 1982, Railkin 1995).

The time taken for primary colonization to reach equilibrium greatly depends on environmental factors such as water temperature (seasonality) and trophic conditions. Strüder-Kypke (1999) found that the primary colonization in bog lakes reaches its climax after 6 wk during winter months but after only 4 wk in summer. Equilibrium was reached after 1 mo for the mesotrophic White Sea (Railkin 1995), up to 12 wk in oligotrophic lakes (Bamforth 1982), and on average within 2 wk in eutrophic habitats (Wilbert 1969). These findings suggest that, given certain parameters remain constant (e.g. artificial substrate, target habitat and period of exposure), the colonized community is possibly a function of at least 2 environmental aspects, namely seasonality and trophic conditions.

During our 1 yr survey, glass slides were exposed for a fixed period of 15 d, which is sufficient for optimal ciliate colonization in most months of the year (Persoone 1968, Agamaliev 1974). However, few or even no typical periphytic ciliates were found in the winter samples: Jan, Feb-I, Feb-II, Mar-I and Mar-II. The most likely explanation for this is that when the water temperatures were extremely low (3.5 to 6°C), the time to reach equilibrium was probably far longer than 2 wk.

Glass slides proved to be a robust, inexpensive and reliable method for collecting periphyton ciliates. Other forms of artificial substrate that are commonly used to collect protozoa communities for bioassess-

Table 4. Correlation between abundance of dominant ciliates and environmental factors (see Table 1 for abbrevations). *p < 0.05; **p < 0.01

	T (°C)	S (psu)	pH	Tur (NTU)	NO_2-N (mg l^{-1})	NO_3-N (mg l^{-1})	NH_3-N (mg l^{-1})	SRP (mg l^{-1})	DO (mg l^{-1})	Chl *a* (µg l^{-1})
Acineta tuberosa	0.10	0.11	−0.03	−0.11	−0.25	−0.18	0.07	−0.13	−0.21	0.24
Amphileptus litonotiformis	−0.37	−0.04	−0.07	0.37	−0.20	0.52*	−0.14	−0.19	−0.04	−0.30
Corynophrya lyngbyi	0.05	0.10	0.49*	0.28	0.43	0.34	−0.33	−0.01	0.48	−0.48
Hartmannula angustipilosa	0.07	−0.03	−0.11	−0.13	−0.15	−0.04	0.57*	−0.29	−0.12	0.10
Holosticha heterofoissneri	0.20	−0.11	−0.29	−0.33	−0.10	−0.32	0.36	−0.35	−0.02	0.19
Orthodonella hamatus	0.70**	0.33	0.46	−0.22	0.14	−0.14	−0.21	0.40	0.16	−0.14
Pseudokeronopsis qingdaoensis	0.59*	0.23	0.39	−0.14	0.25	−0.03	−0.26	0.74**	0.19	−0.20
Pseudovorticella sinensis	−0.01	−0.7**	−0.34	0.18	0.21	0.00	0.09	−0.06	−0.53*	0.41
Thigmokeronopsis rubra	−0.10	−0.10	−0.42	−0.09	−0.22	−0.30	0.42	−0.22	−0.08	0.22
Trochilia sigmoides	0.22	0.07	−0.13	−0.16	−0.17	−0.17	0.10	−0.20	0.06	0.15
Zoothamnium duplicatum	−0.07	0.11	−0.24	0.00	0.27	0.27	−0.17	−0.19	−0.37	0.20
Zoothamnium plumula	−0.27	0.04	−0.05	0.29	−0.05	−0.05	−0.16	0.52*	−0.20	−0.15

Table 5. Summary of result from biota–environment (BIOENV) analysis, with the top 6 correlations corresponding to different variables (p = Spearman correlation coefficient). See Table 1 for abbreviations

Rank	22 samples		17 samples	
	p	Variables	p	Variables
1	0.588	T, NO_2-N, NO_3-N	0.439	T, S, NO_2-N, NO_3-N, SRP
2	0.581	T, NO_2-N	0.425	T, S, NO_2-N, NO_3-N
3	0.536	T, NO_2-N, SRP, NO_3-N	0.420	T, S, NO_2-N, NH_3-N NO_3-N, SRP
4	0.515	T, NO_2-N, NH_3-N	0.416	S, NO_2-N, SRP, NO_3-N
5	0.511	T, NO_2-N, SRP	0.410	T, S, NO_2-N, NH_3-N
6	0.508	NO_2-N, NO_3-N	0.409	T, S, NO_2-N, NH_3-N, SRP

ment include polyurethane foam units (PFU) (Pratt & Kepner 1992, Xu et al. 2002). PFUs are particularly suited for investigating species numbers over an expanding time scale (e.g. 1, 2, 4, 8, 16 d). Such a strategy is, however, not so well suited for studies that involve long time-scales (e.g. 1 yr) or large number of sites because it is very demanding in terms of labour and time. In addition, squeezing PFUs may result in the failure to recover certain types of ciliates, e.g. sessile and highly thigmotactic species.

Taxonomic composition

In the present study, 37 species representing 30 genera and 10 orders of ciliates were detected. This result is similar to the previous report by Persoone (1968), who, using the same sampling method, found 30 ciliate species (belonging to 21 genera and 9 orders) in a polluted harbour at Ostend, Belgium, also over a period of 1 yr. Comparing the taxonomic compositions of the 2 communities, 5 species (*Trochilioides recta*, *Acineta tuberosa*, *Corynophrya lyngbyi*, *Uronema marinum* and *Euplotes vannus*) and 14 genera (accounting for 66.7% of genera recorded in the Ostend study) were found at both locations. Comparison at the order level indicates even higher similarity between the 2 faunas: 8 out of the 9 ciliate orders in Ostend were also present in the Qingdao samples. Over half the species in the Ostend samples were from the orders Hypotrichida (30%) and Cyrtophorida (23.3%). The same 2 orders accounted for similar proportions of the species composition in the present study (36 and 23%, respectively; see Fig. 2).

A large number (130) of periphytic ciliates were found on a combination of submerged objects and glass slides in the Caspian Sea (Agamaliev 1974). Species in orders Hypotrichida (36.2%) and Peritrichida (18.5%) accounted for over half of the total. However, it should be noted that the higher species richness of periphytic ciliates in the Caspian Sea compared to the present study was almost certainly due to the larger number of samples (500) and the wider range of locations sampled.

Coppellotti & Matarazzo (2000) investigated ciliate colonization on glass slides in the Lagoon of Venice and found 45 species representing 34 genera, 12 (40%) of which were also found at Qingdao. Like the ciliate faunas of Qingdao, Ostend and the Caspian Sea, the Hypotrichida represented the largest proportion of species (33%) in the Venice study, the second largest being the Peritrichida (17.8%). Cyrtophorida accounted for only 2%. In addition, 5 species of karyorelictids, namely *Trachelocerca lacrymariae*, *T. multinucleata*, *Tracheloraphis gracilis*, *Remanella multinucleate* and *Geleia swedmarki*, which are usually considered to be benthic species (Fenchel 1969), were also included on the species list. The depth at which the artificial substrate was submerged might explain this finding since most samples of the Lagoon of Venice were recovered from just 60 cm above the bottom (Coppellotti & Matarazzo 2000).

Considering the clear links between the ecological niches of protists and their morphology (Fenchel 1986, 1987), Franco et al. (1998) classified various taxonomic orders of ciliates into feeding categories, based on 3 parameters: the structure and function of the oral apparatus; the way the ciliate collects its food; and the size of the captured food particles. The taxonomic order itself, however, circumscribes certain aspects of the morphology of any given ciliate and hence, to a certain extent, provides a clue to its ecology. In our study, the predominance of the dorsoventrally flattened hypotrich species is almost certainly due to this adaptation of protozoa that crawl on surfaces (Fenchel 1987). The bilaterally flattened cyrtophorids were the second largest group in both the Ostend and Qingdao surveys, but only a minor component of the ciliate communities in the Venice Lagoon and Caspian Sea areas (Agamaliev 1974, Coppellotti & Matarazzo 2000). This is in contrast to the situation in freshwater habitats, where the peritrichs usually dominate the periphytic communities (Shen et al. 1990, Song & Chen 1999).

Univariate and multivariate analyses

Univariate correlation analysis for community and environmental factors was carried out on data sets omitting the 5 winter samples. Species diversity, evenness and richness indices are commonly employed in community studies and are amenable to simple statisti-

cal analysis (Magurran 1991, Ismael & Dorgham 2003). In our case, however, diversity and evenness generally failed to show significant relationships with environmental factors whereas species richness did. A similar finding was demonstrated in a diatom community study which also involved the use of artificial substrates (Vaultonburg & Pederson 1994).

All 3 indices sharply decreased in the Aug-I sample when *Pseudovorticella sinensis* dominated the community. This may have been due to the low salinity and DO, both of which showed highly significant negative correlations with the abundances of other peritrich species such as *Zoothamnium duplicatum*.

The ratio of biomass to abundance (B/A) of the community, i.e. the mean body-size of species in a sample, showed strong negative correlations with nutrients. That is to say, the higher nutrients load, the more small-sized species were present. This is consistent with the use of abundance/biomass comparison (ABC) plots to determine levels of disturbance (Warwick 1986). This method, which is usually for benthic macrofauna studies, might thus also be suitable for biomonitoring using periphytic ciliate communities.

Multivariate analyses were more sensitive than univariate ones for detecting changes in community structure. For example, the 2 samples in August (Group II) comprised a distinct cluster at a similarity level of 12% (Fig. 6) that corresponded to the decrease in salinity and the increase in concentrations of NO_3-N, NO_2-N and SRP. The separation of other groups basically reflects seasonal effects, but with few exceptions (e.g. Jul-I was grouped together with autumn samples in subgroup IIIc). This indicates other physico-chemical variables also play roles in the differentiation of community structures.

The subsequent BIOENV confirms that temperature is the most important factor influencing the structure of the periphytic ciliate community, based either on the entire year's samples or on those with the winter data omitted. Likewise, nutrients were always among the top combinations of variables in both cases whereas salinity was only occasionally an important factor, particularly when the data from the winter samples were omitted from the analysis. Since many ciliates are consumers of bacteria and algae in microbial loops, nutrients may affect the growth and structure of attached bacteria and diatoms and further indirectly affect the communities of periphytic ciliate on glass slides.

Multivariate analysis was also employed but failed to reveal any relationships between periphytic ciliate community on glass slides and environment with pollution of heavy metals (Coppellotti & Matarazzo 2000). They classified ciliates into 3 groups (i.e. suctoria, peritrichs and vagile ciliates) rather than investigating each species individually. This significantly reduced the dimensions of the biota matrix and inevitably weakened the sensitivity of the multivariate analysis.

In summary, our studies demonstrate that variations of periphytic ciliate communities were not only seasonal but were also highly correlated to the concentrations of dissolved nutrients in the water and hence, to some extent, show potential for the assessment of water quality. Further studies, e.g. site-by-site comparisons of community responses to specific environmental stress such as heavy metals or organic pollutants, are needed to further explore the possibility of using periphytic ciliates in marine water biomonitoring.

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