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Annual variation of protozoan communities and its relationship to environmental conditions in a sub-tropic urban wetland ecosystem, southern China

Xinlu Shi[1], Xiangwei Meng[1], Guijie Liu[1], Yong Jiang[2], Songlu Liu[1], Cuicui Hou[1], Qingjuan Meng[1] and Henglong Xu[2]

[1] *Hangzhou Key Laboratory for Animal Adaptation and Evolution, Hangzhou Normal University, Hangzhou 310036, China*
[2] *Laboratory of Microbial Ecology, Department of Marine Ecology, Ocean University of China, Qingdao 266003, China*

Summary

With ease of collection, short life cycles, lack of complex developmental stages, and rapid response to environmental changes, protozoa attract the increasing attention as suitable indicators for bioassessment. In order to reveal the annual variation of protozoan communities and its relation to the environmental conditions in a sub-tropic urban wetland, the protozoan species composition, abundance, diversity, and their correlations with abiotic factors were studied in Xixi wetland, Hangzhou, China. A total of 89 protozoan species comprising 34 ciliates, 13 flagellates, and 42 rhizopods were recorded; 7 of those were the dominant species. The protozoan abundance ranged from 3×10^4 ind. l^{-1} to 19.65×10^4 ind. l^{-1}; ciliates (69.3%) were the primary contributors in terms of relative abundance. The cluster analysis discriminated the protozoan communities into three annual stages: spring, summer-autumn and winter at a 30% similarity level with significant difference. Multivariate correlation analysis showed that temporal variation in protozoan communities was significantly related to the changes of environmental variables, especially water temperature, dissolved oxygen, chemical oxygen demand (COD) and nutrients. Three diversity indices (species richness, diversity and evenness) were significantly correlated with the COD and nutrients. The results demonstrated that the annual variation in protozoan abundance represented a clear seasonal shift in response to environmental changes and thus may be used as a potential indicator for assessing water quality in a sub-tropic urban wetland ecosystem.

Key words: annual variation, protozoan communities, urban wetland, water quality

Introduction

Protozoa are an important ecological group which plays an invaluable role in the functioning of microbial food webs by transferring energy to higher trophic levels in aquatic ecosystems (Cairns et al., 1972; Jiang et al., 2007, 2011; Xu et al., 2009). With ease of collection, short life cycles, lack of complex developmental stages and rapid response to environmental changes, protozoa have proved to be useful bioindicators of water quality in many aquatic ecosystems (Cereceda-Martin et al., 1996; Madoni, 1994; Lee et al., 2004; Jiang and Shen, 2005). Therefore, increasing attention is being focused on protozoa as suitable indicators for bioassessment (Panswad and Chavalparit, 1997; Kchaou et al., 2009; Xu et al., 2005, 2008, 2009).

In recent years, many investigations on the changes of protozoan communities have been carried out in various aquatic ecosystems. Xu et al. (2001) reported the relationship between protozoan community diversity and water quality in the Baiyangdian lake, Xie et al. (2005) demonstrated the relationship of zooplankton community structure and water pollution of the Jinjiang river valley, and Shi et al. (2012) revealed the spatial patterns of protozoan communities for assessing water quality in the Hangzhou section of Jing-Hang Grand Canal in China. However, those investigations were commonly focused on the lakes and rivers, but less – on the sub-tropic urban wetland.

Xixi wetland (30°3'35"N-30°21'28"N, 120°0'26"E -120°9'27"E) is located in the sub-tropic area near Hangzhou city, southern China. It is an urban, farming and cultural wetland in the national wetland park, which has been one of the national 5A-level scenic spots since 2012. The 70% of the area are formed by river, pond, lake overflow, and marshes. It has many environmental functions and plays an important role in controlling flood, regulating runoff, resisting drought, preventing pollution, regulating climate, controlling soil erosion and sedimentation, and beautifying the environment. Up to now, however, human activities have become increasingly frequent in the wetland with the development of economy and society, garbage accumulation, sewage from aquaculture, tourism etc., that cause the destruction of wetland' ecological environment, especially the water environment.

In order to reveal the annual variation of protozoan communities and its relationship to the environmental conditions in a sub-tropic urban wetland, a 1-year research was performed in the protection engineering area of Xixi wetland from May 2012 to April 2013. The aims of this study were: (1) to document the taxonomic composition of protozoan communities; (2) to reveal temporal dynamics in terms of species number, abundance and biodiversity; (3) to determine their relationships to environmental parameters and use as a potential indicator of water quality in sub-tropic urban wetland ecosystems.

Material and methods

Study areas and sampling stations

Xixi wetland is divided into three areas: the eastern ecological protection cultivation area, ecological tourism and leisure area in the central region, and the western ecological landscape closure area; only the central area is open to visitors. Eight sampling stations (Stations 1-8) were selected as the study zones in the central open areas (Fig. 1). A total of 12 sampling events were monthly carried out during the study period of May 2012 through April 2013.

Sampling, identification, and measurements

In order to get quantitative biological samples, a 1,000 ml mixed water sample from the upper-mid-bottom water layers was collected monthly at each station with depths 1–3 m. Samples for counting were preserved *in situ* using Lugol's iodine solution (1.5% final concentration, v/v), precipitated for 48 h, and the final volume was set to 50 ml of the concentrated sediments. A 0.1 ml subsample of this final concentrate was placed in a perspex counting chamber, observed and counted under a light microscope at a magnification of ×400 (Shen et al., 1990). All counts were repeated five times, and three subsamples from each sample yielded a standard error of <8% of the mean values of counts (Xu et al., 2008, 2010).

Identification of protist species was performed according to Shen et al. (1990), Patterson and Hedley (1992) and Foissner et al. (1999). Examination of all live samples was usually completed as soon as possible after sampling (generally within 2-4 h) in order to prevent significant changes in species composition due to predation in the samples, etc. (Shen et al., 1990).

Analysis of environmental factors

Water temperature (T) and dissolved oxygen (DO) were measured using a water quality analyzer (model: HQ30d, HACH), while hydrogen ion

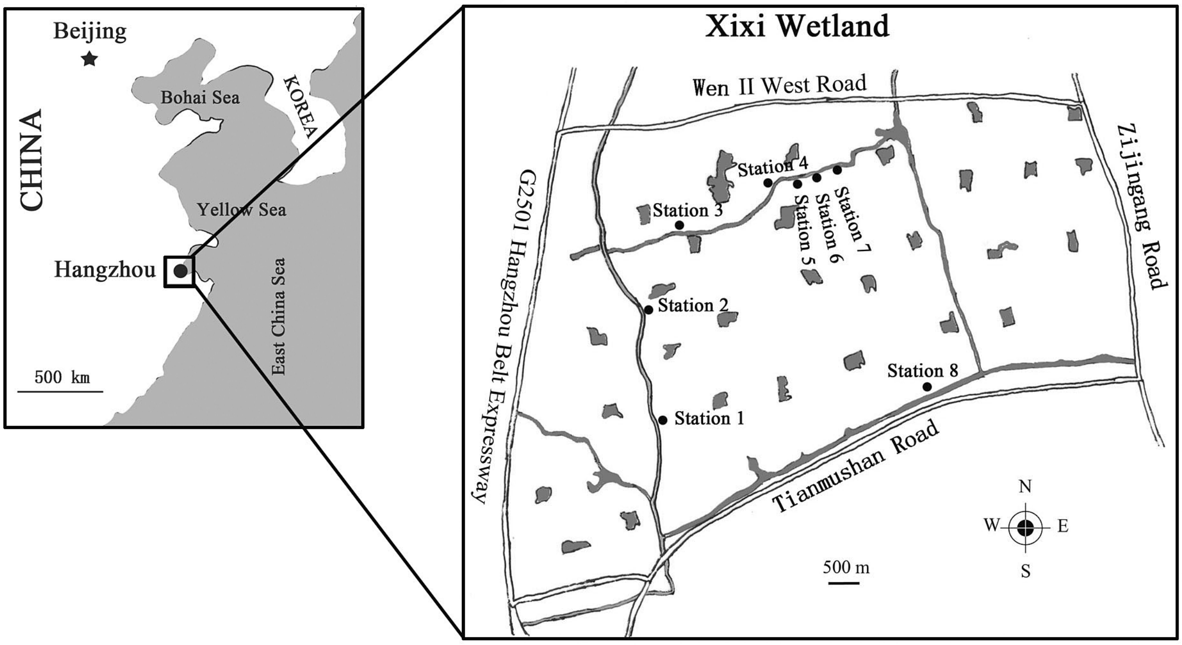

Fig. 1. Map showing the sampling stations of Xixi wetland in Hangzhou, southern China.

concentration (pH) was detected with a pH meter (model: ST20, OHAUS) *in situ*; the determination of chemical oxygen demand (COD), biological oxygen demand (BOD5), total nitrogen (TN), and total phosphorus (TP) followed the standard procedures of American Public Health Association (APHA, 1989).

Data analyses

Species diversity (Shannon-Wiener, *H'*), evenness (Pielou, *J'*) and richness (Margalef, *d*) indices are commonly employed in the community-level investigations and are suitable for simple statistical analyses (Ismael and Dorgham, 2003). The three indices were computed following the equations:

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

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

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

where H' = observed diversity index; P_i = proportion of the i-species in the total count; S = total number of species; and N = total number of individuals.

Multivariate analyses of temporal variations in protozoan community patterns were carried out using the PRIMER v6.0. Bray-Curtis similarity matrices were constructed from log-transformed species abundance data. The clustering analyses of biological samples were conducted using the routine cluster and multidimensional scaling (MDS) ordination (Bray and Curtis, 1957; Anderson et al., 2008; Xu et al., 2011). Differences in samples among sampling stations were tested by the submodule ANOSIM (analysis of similarities) (Clarke and Gorley, 2006). The submodule biota-environment (BIOENV) was used to explore potential relationships between biotic parameters and the abiotic data. The significance of biota-environment correlations was tested using the routine RELATE (Clarke and Gorley, 2006).

Univariate correlation analyses were conducted using the statistical program SPSS v16.0. Data were standardized by logarithmic transformation before analyses (Tan et al., 2010).

Results

Environmental parameters

The monthly mean values of the 1-year long measurements of the environmental variables from eight sampling stations are shown in Table 1.

The change of water temperature (T) was comparatively regular and consistent with the change of season. The study area is located in the

Table 1. Mean values for physicochemical parameters of every month from the eight sampling stations in Xixi Wetland during the study period (25-May-12 to 24-Apr-13).

Date	T (°C)	pH	DO (mg l^{-1})	COD (mg l^{-1})	BOD_5 (mg l^{-1})	TN (mg l^{-1})	TP (mg l^{-1})
25-May-12	24.76±0.82	6.44±0.14	5.99±0.89	16±7.01	6.34±1.42	1.21±0.37	0.067±0.05
26-Jun-12	25.01±0.62	6.33±0.12	4.03±1.33	17.25±10.12	3.58±0.99	1.015±0.52	0.045±0.02
26-Jul-12	31.98±0.93	6.32±0.14	3.24±0.74	19.88±5.54	4.49±1.44	0.824±0.21	0.04±0.02
28-Aug-12	29.83±1.06	7.37±0.23	4.81±1.55	19.38±2.45	7.1±2	0.703±0.16	0.038±0.03
27-Sep-12	25.23±0.64	7.3±0.12	4.16±1.05	16.75±5.31	6.78±1.47	0.966±0.33	0.063±0.03
24-Oct-12	20.95±0.46	7.5±0.28	4.97±1.19	19.88±5.84	5.24±1	0.86±0.31	0.12±0.06
24-Nov-12	13.66±0.67	7.59±0.05	5.61±0.9	13±2.56	3.34±0.64	1.177±0.44	0.066±0.02
26-Dec-12	7.08±1.13	7.66±0.18	7.68±1.37	16.38±7.95	5.9±1.37	1.415±0.34	0.068±0.03
23-Jan-13	7.11±0.77	7.61±0.07	8.2±1.47	12±5.53	5.19±2.3	1.466±0.28	0.047±0.03
27-Feb-13	11.28±0.55	7.06±0.35	8.5±0.54	16±15.19	4.08±1.27	1.493±0.12	0.042±0.01
29-Mar-13	12.85±0.34	6.41±0.18	6.8±1.18	9.13±3.56	4.26±0.73	0.973±0.37	0.034±0.02
24-Apr-13	18.66±0.71	6.36±0.15	6.4±0.6	21.25±14.32	3.71±0.82	0.985±0.25	0.065±0.05

Notes: T – water temperature, DO – dissolved oxygen, COD – chemical oxygen demand, BOD_5 – biological oxygen demand, TN – total nitrogen, TP – total phosphorus.

subtropical climate zone; the temperature was relatively high, ranging from 7.08 to 31.98 °C (annual mean value 19.03 °C), and the wetland water was not frozen throughout the year.

The values of pH ranged from 6.32 to 7.66, averaging 7. Some numerical values showed partial acidulous which was due to silt at the bottom of wetland waters.

The dynamics of DO values and the change of T were basically inverse, DO ranging from 3.24 to 8.5 mg l^{-1} (mean 5.87 mg l^{-1}).

Variation range of COD was from 9 to 21.25 mg l^{-1} (mean 15.18 mg l^{-1}), and the BOD_5 varied from 3.83 to 7.99 mg l^{-1} (mean 5.34 mg l^{-1}).

The concentration of TN peaked two times during the year: the bigger peak from November to February and the minor peak from May to June, with little change during the other months. The concentration of TP was low almost all year round, and only in October TP was high.

Species composition and dominant species

The taxonomic composition of protozoan communities observed during the study period is summarized in Table 2. A total of 89 protozoan species comprising 34 ciliates, 13 flagellates, and 42 rhizopods were identified from 25-May-12 to 24-April-13 in the study area of Xixi wetland.

Dominant species were defined as species with an abundance that exceeded 10% of the total protozoan density in certain months during the study period. There were seven dominant species: *Strobilidium gyrans, Cercomonas crassicauda, Tintinnopsis wangi, Holophrya simplex, Strobilidium velox, Halteria grandinella* and *Difflugia urceolata*. Only one of those species (*Strobilidium gyrans*) peaked during more than one season of the one-year cycle, whereas dominance of the other six species was confined to one season only (Fig. 2).

Annual variation in species number and abundance

The temporal variation in protist species number showed the highest peak in August with 32 species among which ciliates (53.13%) and rhizopods (40.63%) were primary contributors. The lowest values were registered in January and March, only 8 species each month, flagellates (62.5%) being the greatest contributors in January and ciliates (50%) – in March (Fig. 3a and b). Ciliates (42.79%) and rhizopods (40.93%) were primary contributors to the annual variation of species number.

The annual variation in abundance showed a clear seasonal shift (Fig. 3c). The highest abundance value (19.65×10^4 ind. l^{-1}) was recorded in summer (August), when ciliates (77%) were primary contributors. Then, abundance values reduced gradually since autumn (September), the lowest abundance value (3×10^4 ind. l^{-1}) was registered in winter (February), when ciliates and flagellates (35% each) were primary contributors. Finally, abundance values started to rise gradually in spring, from April onward. In the total protozoan abundance, the input of ciliates was 69.28%, rhizopods – 18.89%, flagellates – 11.83%. Ciliates were the primary contributors to the annual variation of protozoan community structure in terms of relative abundance (Fig. 3d).

Table 2. List of protozoan species observed during the study period, their annual abundance, biomass range and occurrence.

Species	Abund.	Biom.	Occurr. (%)
Heliophrya erharsi	++	+	8
Halteria grandinella	++++	++	58
Glaucoma macrostoma	++	+	17
Tintinnidium fluviatile	+++	+	50
Plagiocampa mutabilis	++	+	17
Enchelydium fusidens	++	+	8
Holophrya sulcata	++	+	8
Podophrya fixa	++	+	8
Pleuronema cornatum	++	+	8
Ophryoglena atra	++	+	8
Urotricha agilis	++	+	8
Holophrya simplex	+++	+	67
Podophrya maupasi	++	+	17
Tetrahymena pyriformis	+++	+	25
Trachelius ovum	++	+	8
Strombidium viride	+++	+	33
Colpoda patella	++	+	8
Urotricha farcta	++	+	25
Urotricha furcata	++	+	17
Paramecium aurelia	++	+	17
Pseudoglaucoma musorum	++	+	8
Euplotus terricoda	+++	+	42
Askenasia volvox	++	+	8
Strobilidium velox	+++	++	42
Sathrophilus ovatus	++	+	8
Loxocephalus ellipticus	++	+	8
Tintinnopsis wangi	+++	++	50
Frontonia atra	++	+	8
Urotricha armata	+++	+	17
Didinium balbianii nanum	++	+	25
Trochilia minuta	++	+	8
Strobilidium gyrans	++++	+++	100
Aspidisca costata	++	+	8
Lagynophrya conifera	++	+	8
Phyllomitus undulans	++	+	8
Cercomonas bodo	+++	+	42
Cercomonas crassicauda	+++	+	67
Bodo edax	++	+	8
Cercomonas ovatus	+++	+	25
Mastigella vitrea	++	+	8
Bodo globosus	+++	+	25
Trepomonas steinii	++	+	8
Bodo minimus	+++	+	33
Cercomonas longicauda	++	+	8
Cercomonas radiatus	+++	+	33
Cercomonas agilis	++	+	8
Monosiga ovata	++	+	8
Cyclopyxis arcelloides	++	+	25
Raphidiophrys pallida	++	+	8
Difflugia gramen	++	+	17
Phryganella nidulus	++	+	8
Pelomyxa palustris	++	+	8
Discamoeba guttula	++	+	8
Acanthocystis brevicirrhis	++	+	8
Actinophrys sol	+++	+	25
Astrodisculus radians	++	+	8
Pyxidicula operculata	++	+	25
Diplophrys archer	++	+	8
Pinaciophora fluviatilis	++	+	8
Difflugia avellana	+++	+	58
Clathrulina elegans	++	+	17
Difflugia acuminata	+++	+	33
Euglypha tuberculata	++	+	8
Nebela collaris	++	+	17
Raphidiophrys viridis	++	+	17
Pseudodifflugia gracilis	++	+	17
Difflugia urceolata	+++	++	58

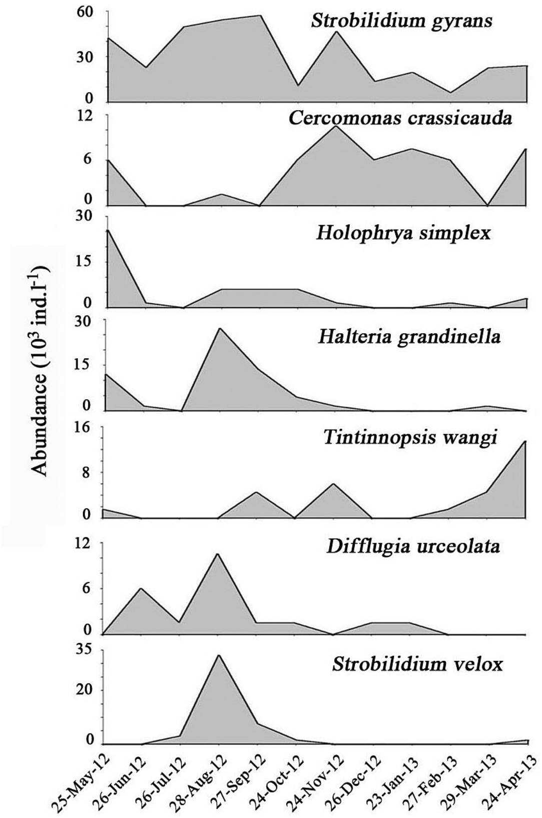

Fig. 2. Annual variations in abundances (ind. l^{-1}) of the seven dominant species in Xixi wetland during the study period of May 2012–April 2013.

Table 2. (Continuation).

Species	Abund.	Biom.	Occurr. (%)
Vannella miroides	++	+	8
Lithocolla globosa	++	+	8
Difflugia globulosa	+++	+	42
Trichamoeba villosa	++	+	8
Hyalodiscus actinophorus	+++	+	42
Cyphoderia ampulla	++	+	8
Striamoeba striata	++	+	8
Cochliopodium bilimbosum	++	+	25
Hedriocystis reticulata	++	+	8
Sphenoderia lenta	++	+	25
Cochliopodium minutum	++	+	8
Hyalosphenia minuta	++	+	25
Trinema enchelys	+++	+	33
Acanthamoeba astronyxis	++	+	8
Euglypha acanthophora	++	+	25
Cucurbitella mespiliformis	++	+	8
Difflugia oblonga	++	+	17
Saccamoeba limna	++	+	8
Lesquereusia epitomium	++	+	8
Euglypha laevis	++	+	8
Centropyxis aerophila	++	+	8
Arcella hemisphaerica	++	+	8

Notes: Abund., abundances (ind. l^{-1}): + = 0–50, ++ = 50–500, +++ = 500–5000, ++++ over 5000; Biom., biomass (μg l^{-1}): + = 0–10, ++ = 10–100, +++ = 100–1000, ++++ over 1000.

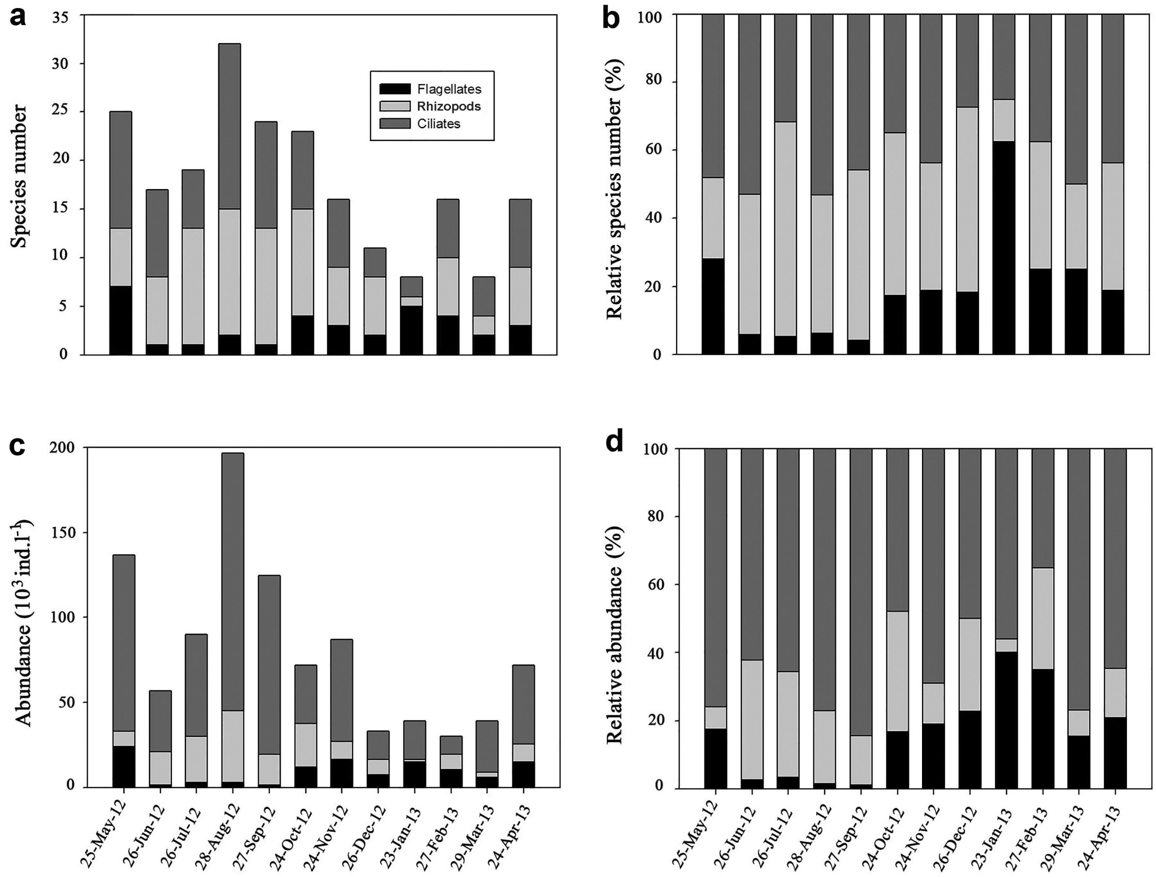

Fig. 3. Species number (a), relative species number (b), abundance (c), and relative abundance (d) of protozoan communities in Xixi wetland during the study period of May 2012–April 2013.

Temporal patterns of protozoan community structure

A dendrogram of the temporal distribution was plotted using the group-average clustering on Bray-Curtis similarities for the protozoan community species-abundance average value of eight sampling stations from every month. The cluster analysis resulted in protozoan communities falling into three groups (I-III) at a 30% similarity level: group I was composed of data from January and December, corresponding to the winter; group II was composed of data from February, April and May, corresponding to the spring; group III including June to November, corresponding to the summer and autumn. Data for March stayed separately. ANOSIM analysis showed a significant difference between the protozoan communities in various seasons (R=0.745, P=0.001). According to the results, the annual variation of protozoan communities in Xixi wetland had an obvious seasonal feature (Fig. 4.).

Annual variation in species biodiversity parameters

The annual variation in species richness and diversity showed an obvious seasonal pattern (Fig. 5); the values were relatively low during the one-year cycle, with maximum in August and May, and minimum in January. Evenness values ranged from 0.75 to 1, with little variation amplitude throughout the year; protozoan communities were evenly distributed.

Linkage between biotic and abiotic data

The results of Spearman correlation analysis showed that, with the exception of pH and TP, biological factors and environmental parameters had a correlation relationship (Table 3). In particular, species numbers highly significantly positively correlated with T (P<0.01), and significantly negatively correlated with TN (P<0.05). Besides, species abundance significantly positively correlated

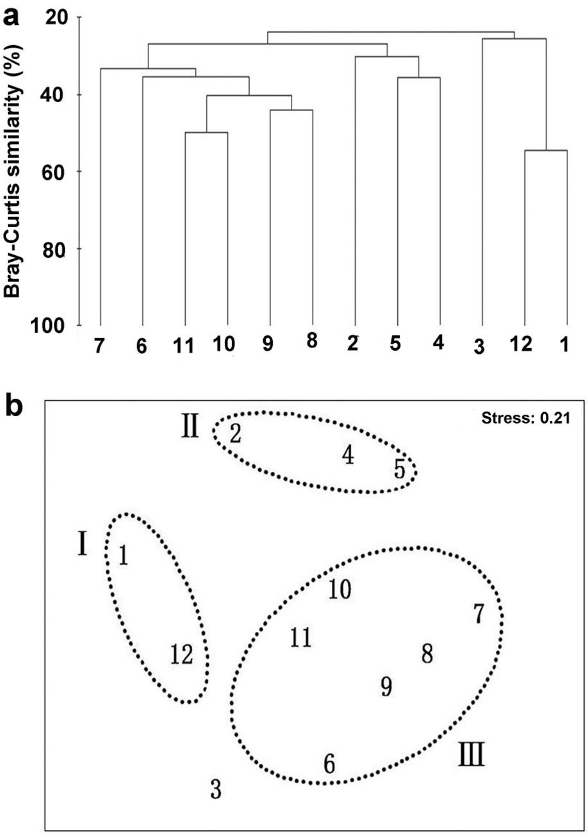

Fig. 4. Multidimensional scaling (MDS) ordination with clustering analysis for protozoan communities in Xixi wetland during the study period of May 2012–Apr 2013. 1=23-Jan-13, 2=27-Feb-13, 3=29-Mar-13, 4=24-Apr-13, 5=25-May-12, 6=26-Jun-12, 7=26-Jul-12, 8=28-Aug-12, 9=27-Sep-12, 10=24-Oct-12, 11=24-Nov-12, 12=26-Dec-12.

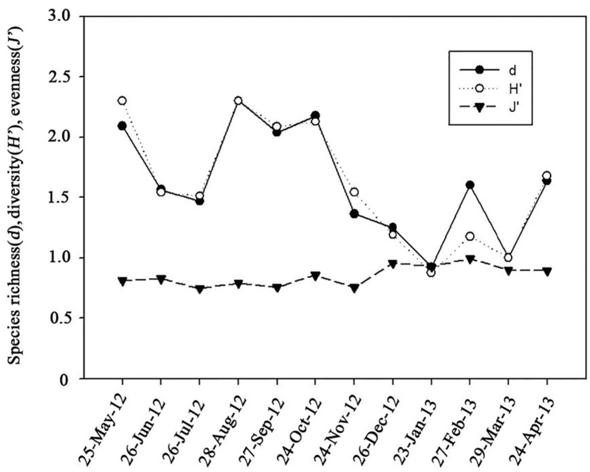

Fig. 5. Temporal variations in species richness (*d*), diversity (*H'*) and evenness (*J'*) of protozoan communities in Xixi wetland during the study period of May 2012–April 2013.

with BOD_5, while species number significantly positively correlated with COD and significantly negatively correlated with DO.

In addition, species richness, diversity and evenness also demonstrated the correlation relationship with water environmental factors: species richness significantly positively correlated with T and COD ($P<0.05$); species diversity highly significantly positively correlated with T ($P<0.01$), significantly positively correlated with COD, significantly negatively correlated with DO and TN; evenness highly significantly negatively correlated with T, highly significantly positively correlated with DO, significantly positively correlated with TN.

Multivariate correlation analysis (BIOENV analysis) demonstrated that temporal variation in protozoan communities significantly correlated with the variation of environmental factors, especially T, pH, DO, COD, TN and TP, either alone or in combinations with one another (Table 4). The highest correlation was between the combination of T, pH, DO, COD and TP (R=0.539; P<0.01). These results confirm that protozoan communities are potential indicators of water quality in wetland systems.

Discussion

Until recently, there has been little understanding of the annual variation in protozoan community pattern in Xixi wetland although many investigations on temporal features of protozoan community patterns and their relationships to changes of environmental conditions were carried out in different aquatic ecosystems (Panswad and Chavalparit, 1997; Kchaou et al., 2009; Xu et al., 2005, 2008, 2009; Sun et al., 2013).

In our study, a total of 89 protozoan species were registered, comprising 34 ciliates, 13 flagellates, and 42 rhizopods; 7 of those were dominant species, of which ciliates (69.28%) were the primary contributors in terms of relative abundance. Our results were also similar to other previously published reports. Shi et al. (2012) reported 88 protozoan species from the Hangzhou section of Jing-Hang Grand Canal during a 1-year cycle from February 2008 to January 2009. Xu et al. (2000) identified 77 protozoan species in Baiyangdian Lake. Xie and Zheng (2000) reported 83 protozoan species

Table 3. Correlation analysis between the abundance (*N*), species number (*S*), species richness (*d*), species diversity (*H'*) and species evenness (*J'*) of protozoan communities and environmental factors.

	N	S	d	H'	J'
T	0.731**	0.792**	0.689*	0.741**	-0.766**
pH	0.019	0.016	0.023	-0.029	0.146
DO	-0.550	-0.597*	-0.495	-0.604*	0.877**
COD	0.383	0.619*	0.663*	0.596*	-0.229
BOD_5	0.635*	0.572	0.542	0.542	-0.175
TN	-0.604*	-0.595*	-0.524	-0.599*	0.673*
TP	-0.009	0.216	0.409	0.428	-0.033

Notes: Abund., abundances (ind. l^{-1}): + = 0–50,++ = 50–500, +++ = 500–5000, ++++ over 5000; Biom., biomass (μg l^{-1}): + = 0–10, ++ = 10–100, +++ = 100–1000, ++++ over 1000.

Table 4. Summary of results from biota-environment (BIOENV) analysis, with the correlations between temporal variation in protozoan communities and different environmental variables.

Rank	Correlation coefficient	Environmental factors
1	0.539	T, pH, DO, COD, TP
2	0.538	T, pH, DO, TP
3	0.537	T, pH, DO, TN, TP
4	0.537	T, DO, COD, TP
5	0.537	T, pH, DO, COD
6	0.536	T, DO, TP
7	0.535	T, DO, COD
8	0.533	T, pH, DO, COD, TN
9	0.532	T, pH, DO, TN
10	0.532	T, DO

in Poyang Lake, and Tan et al. (2010) identified 53 protozoan species during only half a year from May to September 2003 in Songhua River, northeast China.

In the Xixi wetland system, the protozoan abundance ranged from 3×10^4 ind. l^{-1} to 19.65×10^4 ind. l^{-1}, the annual average abundance was 80.875×10^3 ind. l^{-1}. According to relevant reports, the abundances of protozoa in other inland areas of China were, for example, as follows: Dianshan Lake, 3.4×10^3 ind. l^{-1} ~ 5.3×10^3 ind. l^{-1} (Zheng et al., 2009), Yuexiu Lake, 54.9×10^3 ind. l^{-1} ~ 69.1×10^3 ind. l^{-1} (Xu and Zheng, 2000), Baiyangdian Lake, 34.5×10^3 ind. l-1 ~ 37.5×10^3 ind. l^{-1} (Xu et al., 2001). By comparing protozoan abundances in our study and in other fresh waters mentioned above, we can conclude that protozoan abundance was the highest in Xixi wetland.

A number of reports have demonstrated that ciliate assemblages were strongly related to eutrophication status (Beaver and Crisman, 1982, 1989; Jiang et al., 2007). Of the total protozoan abundance in Xixi wetland, ciliates were the primary contributors in the annual variation of protozoan community structure in terms of relative species abundance. This suggests that ciliates played an important role in the eutrophication of the study area which is consistent with the previously reported facts.

Multivariate analyses is a sensitive tool for detecting changes in community structure, and extremely useful for revealing variations of communities on temporal scales (Jiang et al., 2007, 2011; Chen et al., 2008; Xu et al., 2008). In our study, both cluster analyses and MDS ordination demonstrated that temporal patterns of protozoan communities were obviously seasonal. Protozoan communities in Xixi wetland were different in winter, spring, summer and autumn. Moreover, BIOENV analysis and Spearman analysis demonstrated that temporal variation in protozoan communities was significantly related to different environmental variables, especially to T and the concentration of DO, COD, TN and TP. This suggests that the annual variation of protozoan communities is associated with the changes of environmental variables, and that the protists can be used as indicators of water quality in wetland systems (Cereceda-Martin et al., 1996; Madoni, 1994; Lee et al., 2004; Jiang and Shen, 2005).

Species diversity, evenness and richness indices are comprehensive indices for protozoan community survey, and are widely used as the important parameters for evaluating water quality (Xu et al., 2005, 2008; Chen et al., 2008; Kiss et al., 2009; Tan et al., 2010). In general, the higher these indices were, the better the water conditions represented (Ismael and Dorgham, 2003). In our study, the results showed that all three biodiversity parameters were significantly correlated with the chemical factors. Thus, we suggested that these three biodiversity parameters can be used as suitable indicators for assessing water quality in wetland systems (Ismael and Dorgham, 2003; Xu et al., 2005; Jiang et al., 2007).

In summary, the protozoan community structure showed an obvious seasonal feature, and successive annual dynamics of protozoan communities were significantly related to environmental factors. No other study about urban wetland ecosystem was reported yet; our results of survey provide first-hand data for urban wetland research. All three diversity indices (species richness, diversity and evenness) were significantly correlated with the COD and nutrients. The results demonstrated that the annual variation in protozoan abundance represented a clear seasonal shift in response to environmental changes and thus may be used as a potential indicator for assessing water quality in a sub-tropic urban wetland ecosystem.

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References

Anderson M.J., Gorley R.N. and Clarke K.R. 2008. PERMANOVA + for PRIMER guide to software and statistical methods. PRIMER-E Ltd, Plymouth.

APHA (American Public Health Association). 1989. Standard methods for examination of water and wastewater, 17th edn. APHA, Washington DC.

Beaver J.R. and Crisman T.L. 1982. The trophic response of ciliated protozoans in freshwater lakes. Limnol. Oceanogr. 27, 246–253.

Beaver J.R. and Crisman T.L. 1989. Analysis of the community structure of planktonic ciliated protozoa relative to trophic state in Florida lakes. Hydrobiologia. 174, 177–184.

Bray J.R. and Curtis J.T. 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecol. Monogr. 27, 325–349.

Cairns Jr J., Lanza G.R. and Parker B.C. 1972. Pollution related to structural and functional changes in aquatic communities with emphasis on freshwater algal and protozoa. Proc. Acad. Nat. Sci. Phila. 124, 79–127.

Cereceda-Martin M., Serrano S. and Guinea A. 1996. A comparative study of ciliated protozoa communities in activated-sludge plants. FEMS Microbiol. Ecol. 21, 267–276.

Chen Q.H., Xu R.L., Tam N.F.Y., Cheung S.G. and Shin P.K.S. 2008. Use of ciliates (Protozoa: Ciliophora) as bioindicator to assess sediment quality of two constructed mangrove sewage treatment belts in Southern China. Mar. Pollut. Bull. 57, 689–694.

Clarke K.R. and Gorley R.N. 2006. User manual/tutorial. PRIMER-E Ltd., Plymouth.

Foissner W., Berger H. and Schaumberg J. 1999. Identification and ecology of limnetic plankton ciliates. Bavarian State Office for Water Management, Munich.

Ismael A.A. and Dorgham M.M. 2003. Ecological indices as a tool for assessing pollution in El-Dekhaila Harbor (Alexandria, Egypt). Oceanologia. 45, 121–131.

Jiang J. and Shen Y. 2005. Use of the aquatic protozoa to formulate a community biotic index for an urban water system. Sci. Total Environ. 346, 99–111.

Jiang J., Wu S., Shen Y. 2007. Effects of seasonal succession and water pollution on the protozoan community structure in a eutrophic lake. Chemosphere. 66, 523–532.

Jiang Y., Xu H., Al-Rasheid K.A.S., Warren A., Hu X. and Song W. 2010. Planktonic ciliate communities in a semi-enclosed bay of Yellow Sea, northern China: annual cycle. J. Mar. Biol. Assoc. U.K. 91, 97–105.

Jiang Y., Xu H., Hu X., Zhu M., Al-Rasheid K.A.S. and Warren A. 2011. An approach to analyzing spatial patterns of planktonic ciliate communities for monitoring water quality in Jiaozhou Bay, northern China. Mar. Pollut. Bull. 62, 227–235.

Kchaou N., Elloumi J., Drira Z., Hamza A., Ayadi H., Bouain A. and Aleya L. 2009. Distribution of ciliates in relation to environmental factors along the coastline of the Gulf of Gabes, Tunisia. Estuar. Coast. Shelf Sci. 83, 414–424.

Kiss Á.K., Ács É., Kiss K.T. and Török J.K. 2009. Structure and seasonal dynamics of the protozoan community (heterotrophic flagellates, ciliates, amoeboid protozoa) in the plankton of a large river (River Danube, Hungary). Eur. J. Protistol. 45, 121–138.

Lee S., Basu S., Tyler W.C. and Wei W.I. 2004. Ciliate populations as bio-indicators at deer island treatment plant. Adv. Environ. Res. 8, 371–378.

Madoni P. 1994. A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis. Water Res. 28, 67–75.

Panswad T. and Chavalparit O. 1997. Water quality and occurrences of protozoa and metazoa in two constructed wetlands treating different wastewaters in Thailand. Water Sci. Technol. 36, 183–188.

Patterson D.J. and Hedley D. 1992. Free-living freshwater protozoa: a colour guide. Wolfe Publishing, London.

Shen Y., Zhang Z., Gong X., Gu M., Shi Z. and Wei Y. 1990. Modern biomonitoring techniques using freshwater microbiota. China Architecture Building Press, Beijing (in Chinese).

Shi X., Liu X., Liu G., Sun Z. and Xu H. 2012. An approach to analyzing spatial patterns of protozoan communities for assessing water quality in the Hangzhou section of Jing-Hang Grand Canal in China. Environ. Sci. Pollut. Res. 19, 739–47.

Sun Z., Shi X., Xu L., Meng X. and Liu G. 2013. The protozoan community structure and its response to the change of water quality in a typical wetland landscape in summer. Acta Hydrobiol. Sin. 37 (2), 1–10 (in Chinese with English abstract).

Tan X., Shi X., Liu G., Xu H. and Nie P. 2010. An approach to analyzing taxonomic patterns of protozoan communities for monitoring water quality in Songhua River, northeast China. Hydrobiologia. 638, 193–201.

Xie Qi., Li C. and Peng C. 2000. Preliminary studies on community ecology of protozoan in Poyang Lake. Jiangxi Sci. 18 (1), 40–44 (in Chinese with English abstract).

Xie J., Xu Y., Chen Y., Dai C. and Chen C. 2005. The relationship of community structure of zooplankton and the water pollution of the Jinjiang River Valley. Chin. J. Zool. 40 (5), 8–13 (in Chinese with English abstract).

Xu M., Cao H., Xie P., Deng D., Feng W., Xu J. 2005. Use of PFU protozoan community structural and functional characteristics in assessment of water quality in a large, highly polluted freshwater lake in China. J. Environ. Monit. 7, 670–674.

Xu H., Min G.S., Choi J.K., Jung J.H. and Park M.H. 2009. An approach to analyses of periphytic ciliate colonization for monitoring water quality using a modified artificial substrate in Korean coastal waters. Mar. Pollut. Bull. 58, 1278–1285.

Xu H., Song W., Warren A., Al-Rasheid K.A.S., Al-Farraj S.A., Gong J. and Hu X. 2008. Planktonic protist communities in a semienclosed mariculture pond: structural variation and correlation with environmental conditions. J. Mar. Biol. Assoc. U.K. 88, 1353–1362.

Xu H., Warren A., Al-Rasheid KAS, Zhu M. and Song W. 2010. Planktonic protist communities in semi-enclosed mariculture waters: temporal dynamics of functional groups and their responses to environmental conditions. Acta Oceanol. Sin. 29, 106–115.

Xu H., Zhang W., Jiang Y., Min G.S. and Choi J.K. 2011. An approach to identifying potential surrogates of periphytic ciliate communities for monitoring water quality of coastal waters. Ecol. Indic. 11, 1228–1234.

Xu R. and Zheng Y. 2000. Seasonal changes of planktonic ciliate communities in Yuexiu Lake, An Urban Lake of Guangzhou City. J. Lake Sci. 12 (2), 124–131 (in Chinese with English abstract).

Xu M., Zhu J. and Cao H. 2001. The relationship between the protozoan community diversity and the water quality in the Baiyangdian Lake. Acta Ecol. Sin. 21(7), 1114–1120 (in Chinese with English abstract).

Zheng X., Wang L., Gai J., Ji G., Fan Z. and Zhang R. 2009. Dynamic and community structure of zooplankton in Dianshan Lake. Chi. J. Zool. 44 (5), 78–85 (in Chinese with English abstract).

Address for correspondence: Henglong Xu. Laboratory of Microbial Ecology, Department of Ocean University of China, Ocean University of China, Qingdao 266003, China; e-mail: *henglongxu@126.com*