

Use of Protozoa for Assessing Water Quality in a Humid Subtropical Urban Wetland Ecosystem, Southern China

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Abstract

Multivariate bioassessment based on community data has many advantages to assess environmental quality status in aquatic ecosystems. However, there are few reports on their use for bioassessment in subtropical wetland systems. In this study, the protozoan microfauna and their use in evaluating water quality status was investigated in a humid subtropical urban (XiXi) wetland system in southern China, during the period of June 2013-May 2014. Samples were collected every month at six sampling stations within different pollution/eutrophication levels. A total of 85 protozoan species were recorded, including 67 ciliates, 10 flagellates, 8 sarcodines. A clear variation on spatial scale in protozoan community structures were represented at the six stations. Multivariate approaches revealed that the variations in the community structure were significantly related to the changes of environmental variables, especially nutrients ammonia-N (NH₄-N), nitrate-N (NO₃-N) and total phosphorus (TP), alone or in combination with water temperature (T) and dissolved oxygen (DO) and Four dominant species (*Euglena acus*, *Cucurbitella mespiliformis*, *Codonella acutula* and *Hemiophrys punctata*) were significantly correlated with nutrients. Otherwise, the species richness, diversity and evenness indices represented significant correlations with the nutrients. The results demonstrate that the community-based bioassessment using protozoa may be used as a feasible protocol for determining the water quality status and human disturbance in a humid subtropical urban wetland system.

Keywords: Community-based bioassessment; Protozoan communities; Environment stress; Urban wetland; Humid-subtropical zone

Introduction

Protozoa have many advantages for assessing water quality, such as short life generations and more rapid response to environmental stress than metazoa [1-6]. As an important component of microfauna, protozoa play crucial roles in mediating carbon and energy from bacteria and microalgae to high trophic levels in aquatic microbial food webs, even in extreme environments [7-12]. Thus, special attention has been focused on bioassessment using protozoa as a robust bioindicator for evaluating water quality [13-15]. So far many investigations on such monitoring programs have been carried out based on ecological features at species/population resolutions (e.g. indicator species) or functional parameters (e.g. colonization rate) mainly in freshwater systems (e.g. lake and river) [13]. However, little information on community-based bioassessment using protozoa have been documented in subtropical wetland systems [13,16,17].

Xixi Wetland is the first and a unique national wetland park with integration of domestic urban, farming and cultural wetland in China. It is located in the center area of Hangzhou city, southern China, with an area of 10.08 km². Of the total area, 46% is covered with variety of creeks and ponds [18]. As a humid subtropical urban wetland ecosystem, so far it have intensively been utilized for tourism, recreation, sedimentation and water supply, and thus is of a great conservation value for controlling flood, resisting drought, regulating climate, controlling soil erosion [19]. However, the water quality status of Xixi wetland has been seriously influenced by human activities such as tourist, industrial and agricultural activities [20]. In recent years, a range of ecological issues on water quality monitoring have been studied in freshwater river systems and subtropical canals [5,17]. However, few works on community-based bioassessment using protozoa in Xixi Wetland have been conducted.

In the present study, the protozoan community patterns were studied during a 1-year cycle (June 2013-May 2014) at six sampling stations in Xixi Wetland, southern China. Our aims were focused on: (1) the species composition of protozoa, (2) spatial variations in community structure of protozoa, (3) relationships between the variation in community patterns and changes of environmental variables, and (4) the applicability of bioassessment using protozoa as a robust bioindicator of water quality in freshwater humid subtropical urban wetland ecosystems.

Materials and Methods

Study stations

Six sampling stations (A-F), within a pollution/eutrophication gradient, were selected as study areas (11.5 km²) in the Xixi Wetland. Stations A (30°15'33.16"N, 120°03'26.81"E) and B (30°15'58.53"N, 120°03'17.03"E), near the tourist wharf, receiving organic pollutant

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discharge, were selected as most stressed areas. Station C (30°16'16.59"N, 120°03'19.60"E) and D (30°16'29.99"N, 120°03'59.35"E) were moderately polluted by domestic sewage from hotel and street market. Station E (30°15'58.33"N, 120°4'0.16"E) was located in the outlet of the Xixi wetland and was stressed by pollutions. Station F (30°15'47.02"N, 120°3'57.67"E) was a relatively clean area which was distant from the pollution source (Figure 1).

Data collection

A 1 year baseline with 12 sampling event were conducted from June 2013 to May 2014. At each station, 12 samples were collected (at 8–12 o'clock on day 15) every month. For quantitative and qualitative analyses, a mixed water sample of total 1,000 ml from three layers (upper, mid and bottom) in water column was taken at depths of 1–3 m. The fixation of water samples were carried out by adding Lugol's iodine solution at 2% concentration (volume/volume), and the final volume of 30 ml concentrated sediments were extracted after settlement for 48 h. For quantitative analyses of protozoa, a 0.1 ml subsample of each final concentrated sediment was examined in a Perspex chamber, and the protozoan individuals were counted under a light microscope at a magnification x400. For enumeration, a total of five subsamples (0.5 ml) were checked and yielded a coefficient of variation at <8% level. [21]. For species identification, the published references, such as Shen et al. [13], Patterson and Hedley [22] and Foissner et al. [23], were used. The taxonomic schemes used were according to Levine et al. [24].

Water temperature (T), dissolved oxygen (DO) and pH were measured *in situ* with OHAUS ST20 and HACH HQ30d, while the nutrients ammonium nitrogen (NH₄-N), nitrate nitrogen (NO₃-N), nitrite nitrogen (NO₂-N), total nitrogen (TN) and total phosphorus (TP) and chemical oxygen demand (COD) were measured following the methods [25].

Data analysis

Shannon-Wiener (Species diversity, H'), Pielou (evenness, J') and Margalef (richness, D) indices are 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 P_i =proportion of the total count arising from the i^{th} species; S =total number of species; and N =total number of individuals [6,26].

The values with mean and standard deviation (SD) was applied to describe and to evaluate the variations of the physicochemical variables among sites. All univariate correlation analyses were computed using the software SPSS v16.0. Data were log-transformed before analyses [5].

Multivariate analyses of protozoan communities and their relationships to changes of environmental variables were carried out using the PRIMER v7.0. Protozoan species-abundance data were fourth-root transformed before the Bray-Curtis similarity matrix was constructed while environmental variable data were log-transformed/normalized before the Euclidean distance matrix was computed. The routine Bootstrap Average analysis was used to summarize the spatial patterns of both biotic and abiotic samples based on Bray-Curtis similarity and Euclidean distance matrices, respectively [27-29]. The routine ANOSIM (analysis of similarities) was used to signify the differences in samples among sampling stations; the routine RELATE was utilized to test the significance of biota-environment correlations; and the SIMPER (similarity percentage) analysis was used to summarize the contributions of protozoan species to communities [27,30]. The routine BIOENV (biota-environment) was run for exploring potential matching between biotic and the abiotic data [27].

Results

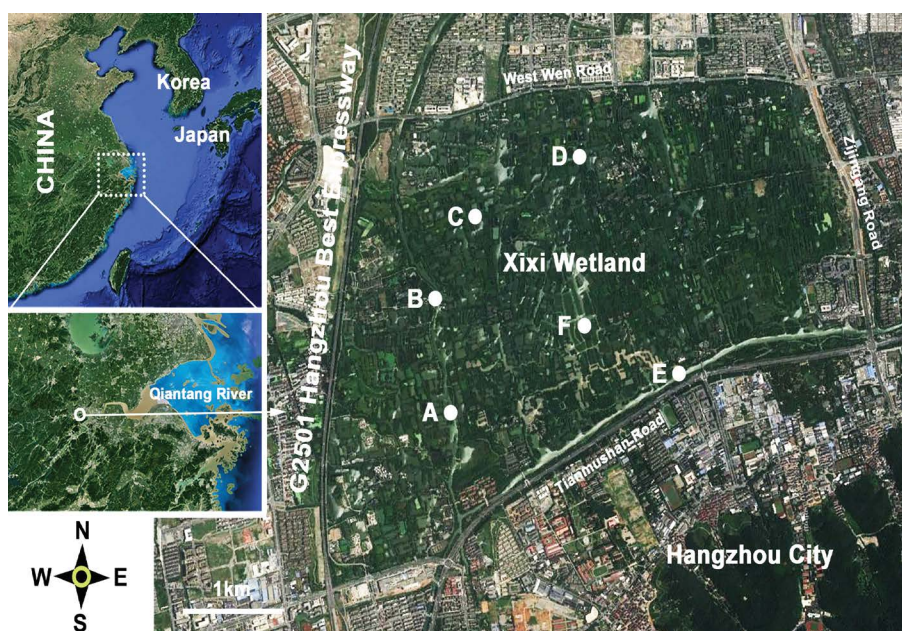


Figure 1: Sampling stations (A–F) in Xixi Wetland, Hangzhou, South China. Stations A/B most stressed in Xixi Wetland near the Tourist wharfs, the pollution being mainly in the form of organic pollutants and nutrients from domestic sewage; stations C/D moderately polluted by domestic sewage from hotel and street market; Station E located in the outlet of the Xixi Wetland was stressed by pollutions. Station F was relatively clean area due to its position in the central of Xixi wetland which was distant from the pollution source.

Environmental variables

The average values for environmental factors at six stations are shown in Figure 2. The average concentrations of DO were ranged from 5.35 mg l⁻¹ (the lowest at station E) to 6.91 mg l⁻¹ (the highest value at station F) (Figure 2b). The pH ranged from 6.93 to 7.29 among the six sampling stations (Figure 2c). The highest values of COD were measured at station A, and the lowest value at station F (Figure 2d). For the nutrients NO₃-N, NO₂-N, TN, TP, high values were found at station A and D with minima at station F, while the high value of NH₄-N occurred at station B and A compared with station C, D, E and F (Figures 2e-2i).

Species composition and distribution

A total of 85 protozoan species, including 67 ciliates, 8 sarcodines and 10 flagellates, were identified in total 72 samples during the study period. The special species distribution among six stations was summarized in Figure 3a. By cluster analysis, the 85 species were resulted in falling into seven groups (I-VII) at a 50% similarity level. For example, group I included 25 common species with high occurrence, while the other six groups (II-VII) composed of taxa with low occurrence: six common species (e.g. *Cucurbitella mespiliformis*, *Euglena oxyuris*, *Euglena acus*, *Euglena caudata*, *Halteria grandinella* and *Tintinnopsis wangi*) in group I appeared over all six stations, eleven (78.6%) species (e.g. *Nassula aurea* and *Hemiohrys meleagris*) of group III occurred independently at station A, four (23.5%) (e.g. *Colpoda*

patella, *Arcella hemisphaerica* and *Lagynophrya retractilis*) of group II at station B, all four forms (e.g. *Euglypha tuberculata* and *Astylozoon faurei*) of group VI at station C and all six species (e.g. *Tetrahymena priformis*, *Trachelophyllum pusillum* and *Colpidium colpoda*) of group VII at station D (Figure 3).

Spatial variations in protozoan community pattern

The spatial variations in species number and abundance of protozoan communities among six stations are summarized in Figures 3a and 3b. In terms of species number, the ciliates were the primarily contributors to the species compositions over all stations (Figure 4a), while the flagellates account for a dominant position at stations F on abundance (Figure 4b). The high and low species numbers were found at stations A/B and D/F (Figure 4a), but the high and low abundances were at station A/C/F and D, respectively (Figure 4b). Noted that the spatial pattern of the communities were consistent with the spatial variation in the water quality from stations A/B to F.

Compared with the relative species composition, a spatial pattern in relative abundance was shown clearly in Figures 3c and 3d. According to the spatial variation in relative abundance, two types of community structure may be identified. For example, stations A, B, C, D and E dominated by ciliates and station F was those dominated by flagellates (Figure 4d).

Relationship between biotic and abiotic variables

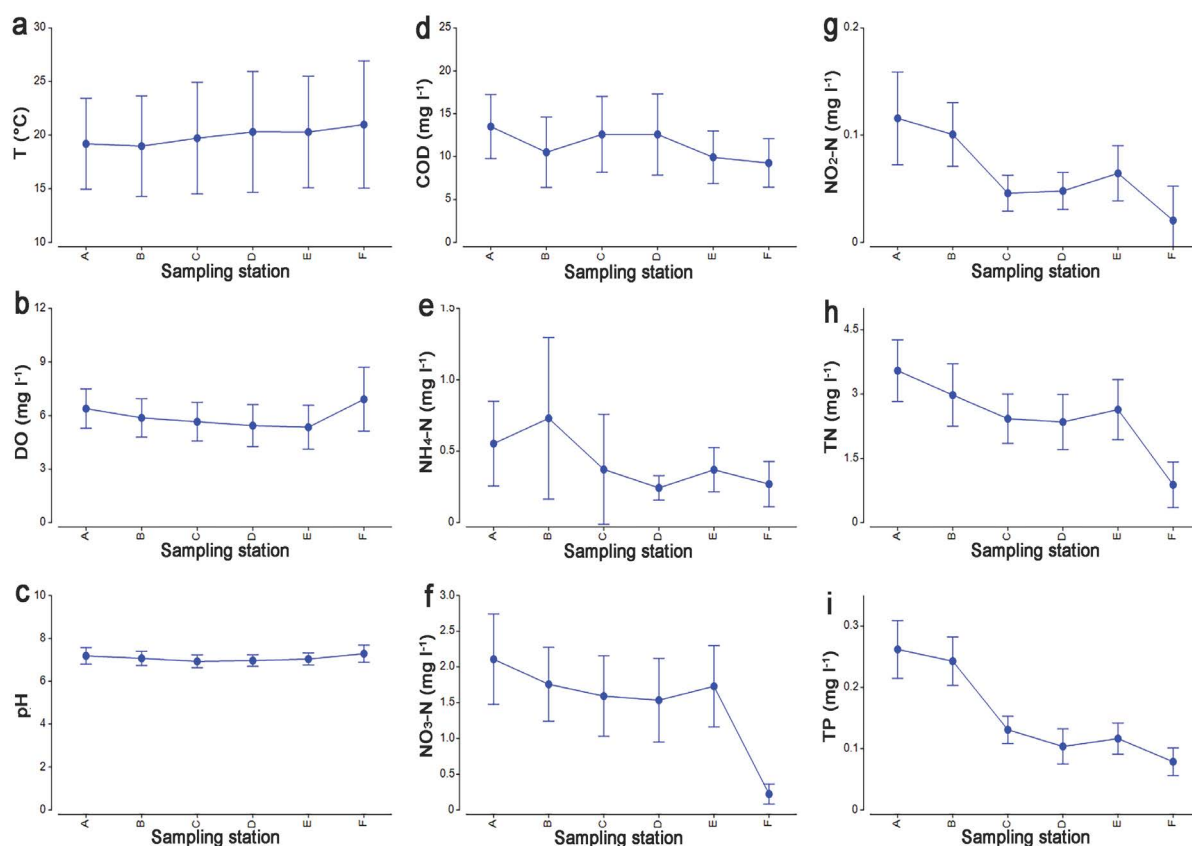


Figure 2: Spatial variations in water temperature (T, a), dissolved oxygen (DO, b), pH (c), chemical oxygen demand (COD, d), ammonium nitrogen (NH₄-N, e), nitrate nitrogen (NO₃-N, f), nitrite nitrogen (NO₂-N, g), total nitrogen (TN, h) and total phosphorus (YP, i) among six sampling stations in Xixi Wetland, southern China, during the study period.



Figure 3: A dendrogram of the species distribution using group-average clustering on Bray-Curtis similarities for fourth-root transformed species abundance data of each species within the protozoan communities from six sampling stations in Xixi Wetland, southern China, during the study period. Abundance (ind l⁻¹): +++>1,000; ++++100–999; ++10–99; +0–9.

The relationships between the spatial patterns of the protozoan communities and spatial variation in environmental conditions among the sampling stations were shown in Figure 5. ANOSIM tests indicated

significant differences between the pairs of A/E, B/F, D/F and E/F ($P < 0.05$). RELATE analysis demonstrated a significant correlation between spatial variations of protozoan communities and changes in environmental

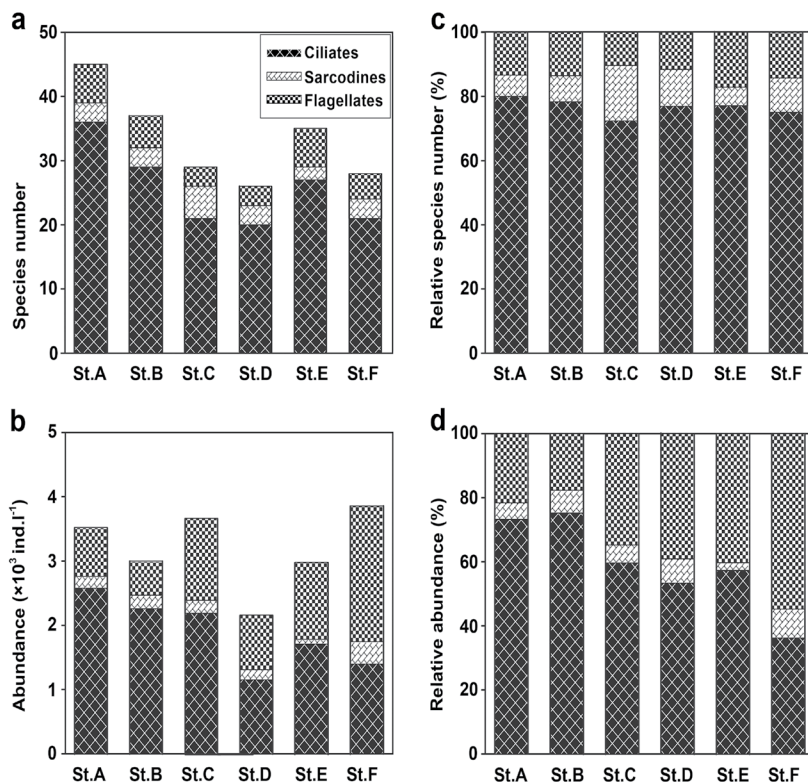


Figure 4: Spatial variations in species number (a), abundance (b), relative species number (c), relative abundance (d) of protozoan communities from six sampling stations (A–F) in Xixi Wetland, southern China, during study period.

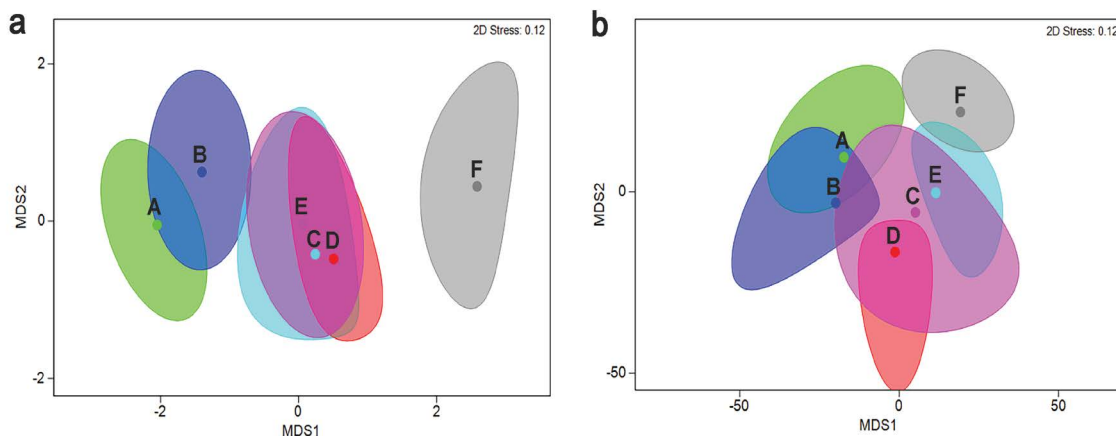


Figure 5: Multidimensional scaling (MDS) ordinations based on bootstrap average analysis for spatial patterns of environmental conditions (a) and protozoan communities (b) among six sampling stations in Xixi Wetland, southern China, during the period.

variables ($R=0.625$; $P=0.003$). The BIOENV analysis showed that the changes in combination of T, DO, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, TP could explain the spatial variation in protozoan communities best ($\rho=0.704$, $P<0.05$) (Table 1).

The values of species number, richness, evenness and diversity represented a significant or a strong significant correlation with nutrients ($\text{NO}_2\text{-N}$, TP or TN or $\text{NH}_4\text{-N}$) (Table 2 and Figures 2 and 6). The evenness and diversity represented a significant negative correlation with T (Table 3). The four species (*Euglena acus*, *Cucurbitella mespiliformis*, *Codonella acutula* and *Hemiohryis punctata*) from 9

dominant protozoan species were significantly related to nutrients $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, TN or TP or significantly negative correlated with T (Table 3).

Discussion

Up to now, a number of ecological studies demonstrated that multivariate analyses are more powerful for discriminating patterns of communities on spatial and temporal scales, in particular for summarizing relationships between community patterns and

environmental changes [31,32,34]. In our study, the statistical multivariate tools demonstrated a significant correlation between

Rank	Environmental variables	ρ value
1	T, DO, NH ₄ -N, NO ₃ -N, TP	0.704
2	DO, NH ₄ -N, NO ₃ -N, TP	0.696
3	DO, pH, NH ₄ -N, NO ₃ -N, TP	0.696
4	DO, NH ₄ -N, NO ₃ -N, NO ₂ -N, TP	0.696
5	DO, NH ₄ -N, NO ₃ -N	0.689
6	T, DO, NO ₂ -N, TP	0.689
7	T, DO, pH, NH ₄ -N, NO ₃ -N	0.689
8	T, DO, pH, NO ₂ -N, TP	0.689
9	T, DO, NH ₄ -N, NO ₃ -N, NO ₂ -N	0.689
10	DO, pH, NH ₄ -N, NO ₃ -N	0.682

T: Water Temperature; DO: Dissolved Oxygen; COD: Chemical Oxygen Demand; NH₃-N: Ammonium Nitrogen; NO₃-N: Nitrate Nitrogen; NO₂-N, Nitrite Nitrogen; TN: Total Nitrogen; TP: Total Phosphorus

Table 1: Best matches of environmental variables with spatial variations in the protozoan abundances at six stations in the Xixi Wetland, southern China, during the study period.

the community patterns and the spatial variations in environmental variables. Otherwise, among 9 dominant protozoan species four forms, *Euglena acus*, *Cucurbitella mespiliformis*, *Codonella acutula* and *Hemiophrys punctata* were identified to be significantly correlated with nutrients. These findings suggest that the community-based bioassessment using protozoa may be used as a feasible protocol for assessing the water quality in the humid subtropical urban wetland systems.

The species number, individual abundance and three biodiversity (species diversity, evenness and richness) indices have widely been utilized as a useful indicator to evaluate water quality in many aquatic environments [33]. For example, the high levels of species richness, and low abundance occur in normal water bodies [4,26,32]. In our study, the species number and the three diversity indices were generally correlated with nutrients. This implies that these ecological parameters of protozoa fauna may be used as useful indicators of water quality in mid-tropical wetland systems.

Parameters	T	DO	pH	COD	NH ₄ -N	NO ₃ -N	NO ₂ -N	TN	TP
S	-0.703	0.175	0.313	0.268	0.741	0.627	0.901*	0.763	0.886*
N	0.034	0.698	0.542	-0.172	0.097	-0.368	-0.081	-0.263	0.088
D	-0.718	0.107	0.265	0.285	0.747	0.673	0.924**	0.801	0.892*
J'	-0.898*	-0.298	-0.238	0.562	0.759	0.877*	0.943**	0.935**	0.878*
H'	-0.870*	-0.066	0.042	0.425	0.826*	0.807	0.995**	0.913*	0.956**

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level. See Table 1 for abbreviations

Table 2: Correlations between average values of the species number (S), abundance (N), richness(D), diversity (H') and evenness Pielou's J'), and environmental variables.

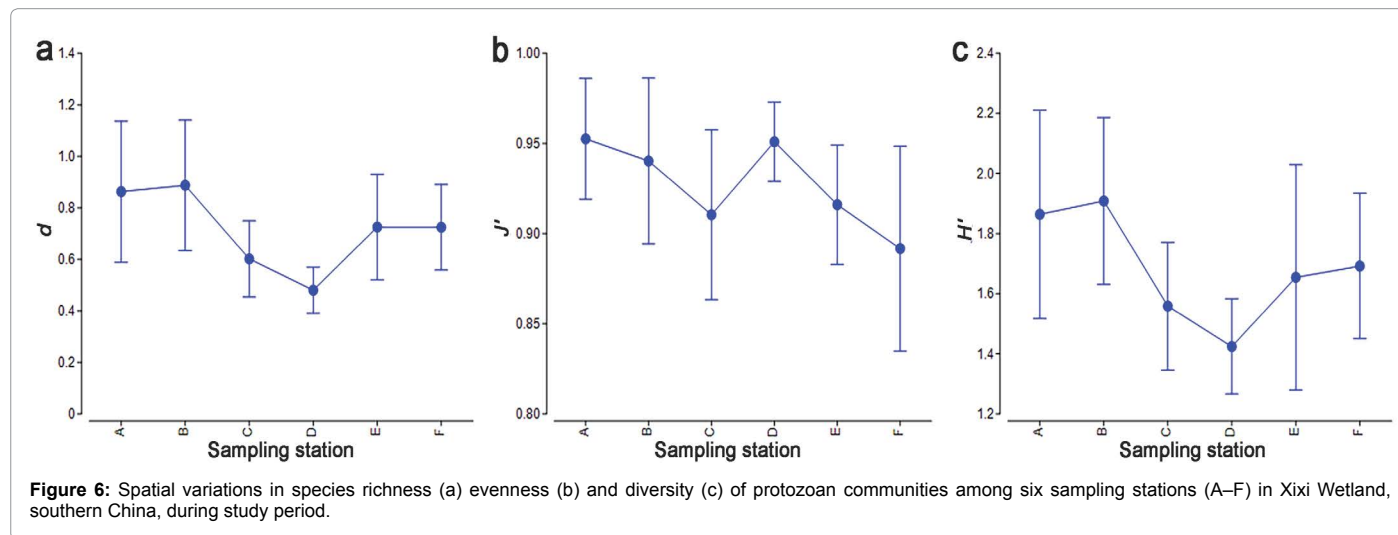


Figure 6: Spatial variations in species richness (a) evenness (b) and diversity (c) of protozoan communities among six sampling stations (A–F) in Xixi Wetland, southern China, during study period.

Species	T	DO	pH	COD	NH ₄ -N	NO ₃ -N	NO ₂ -N	TN	TP
<i>Euglena acus</i>	0.730	0.737	0.648	-0.534	-0.502	-0.948**	-0.706	-0.895*	-0.570
<i>Cucurbitella mespiliformis</i>	0.423	0.756	0.688	-0.593	-0.094	-0.854*	-0.426	-0.744	-0.252
<i>Codonella acutula</i>	-0.868*	-0.224	-0.335	0.176	0.863*	0.522	0.617	0.567	0.703
<i>Hemiophrys punctata</i>	-0.820*	0.001	0.141	0.051	0.952**	0.615	0.893*	0.738	0.913*
<i>Euglena caudata</i>	0.792	0.647	0.678	-0.335	-0.654	-0.742	-0.583	-0.703	-0.552
<i>Euglena oxyuris</i>	0.350	-0.518	-0.620	-0.200	-0.449	-0.162	-0.625	-0.337	-0.635
<i>Tintinnopsis wangi</i>	0.065	-0.664	-0.667	0.068	-0.252	0.291	-0.217	0.127	-0.306
<i>Sphaerophrya soliformis</i>	0.399	0.086	-0.221	-0.026	-0.514	-0.497	-0.693	-0.584	-0.590
<i>Tintinnidium fluviatile</i>	-0.110	0.106	-0.147	0.173	0.048	-0.226	-0.118	-0.213	-0.018

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level. See Table 1 for abbreviations

Table 3: Correlations between four dominant species abundances and environmental variables during the study period.

Connell [34] suggested that with small environmental stress raise, the diversity will be increased because of the decreasing competition, while if the environmental stress increases up to a higher point, the diversity starts to decrease then. Thus, increased eutrophication could lead to either an increase or a decrease in diversity for a certain community. In our study, the three diversity indices of protozoan communities at station F were lower than stations A and B, although latter stations were more polluted than the former.

Conclusion

In summary, the protozoan fauna showed a clear spatial variation among the six sampling stations. Multivariate approaches demonstrated a significant correlation between the spatial patterns of protozoan communities and the changes of environmental variables, especially the combination of nutrients and DO. Four dominant species were found to be significantly related to with nutrients. The species richness, diversity and evenness indices were significantly correlated with the nutrients. The results demonstrated that the community-based bioassessment using protozoa may be used as a feasible protocol for assessing the water quality in humid subtropical urban wetland systems.

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