CHARACTERISTICS OF SIZE-FRACTIONIZED PHYTOPLANKTON AND THEIR RESPONSE TO ENVIRONMENTAL FACTORS IN TYPICAL LAKES OF SOUTHEASTERN HUBEI PROVINCE, CHINA

XIE, Y.-Z.[#] – LIU, M.-Q.[#] – LIU, Z.-X. – HOU, J.-J.[*] – LIU, X.-X. – ZHOU, F. – CHEN, Q. – SUN, L.-L.

Hubei Key Laboratory of Edible Wild Plants Conservation and Utilization, Hubei Normal University, Huangshi 435002, China

Hubei Engineering Research Center of Typical Wild Vegetable Breeding and Comprehensive Utilization Technology, Hubei Normal University, Huangshi 435002, China

National Demonstration Center for Experimental Biology Education, Hubei Normal University, Huangshi 435002, China

[#]The two first authors made equal contributions to this paper

**Corresponding author*
e-mail: jjhou@hbnu.edu.cn; phone: +86-71-4651-1613

(Received 15th Mar 2020; accepted 9th Jul 2020)

Abstract. We studied the size-fractionized phytoplankton community structure characteristics and their response to environmental factors by investigating the photosynthetic pigment concentration and composition; composition and abundance of algal phyla levels; and environmental factors in typical lakes of Hubei Southeastern, China for four seasons. High-performance liquid chromatography (HLPC) results show that the total chlorophyll concentration was 36,418.62 mg/m^3, and the contribution rates of microplankton, nanoplankton, and picoplankton to the total biomass were 13.43%, 49.08%, and 37.49%, respectively. Fucoxanthin, alloxanthin, zeaxanthin, and chlorophyll b were the main photosynthetic pigments. However, the spatial and temporal distribution had significant differences in the four typical lakes. Chemical taxonomy (CHEMTAX) calculation indicates that the dominant species were *Diatoms* and *Cryptophytes* in spring, *Euglenophytes* in summer, *Euglenophytes* and *Cyanobacteria* in autumn, and *Euglenophytes* and *Cryptophytes* in winter. The *Chrysophyte* and *Dinoflagellates* have the lowest proportion for all seasons. The redundancy analysis (RDA) demonstrates that the key environmental factors for the succession were Total Nitrogen (TN) and Total Phosphorus (TP). The application of the HPLC-CHEMTAX method has provided the first analysis of the community structure of size-fractionized phytoplankton in typical lakes of Hubei Southeast, China, and environmental factors affect the succession of size-fractionized phytoplankton over time. This study provides theoretical bases that the comprehensive research on different size phytoplankton in freshwater.
Keywords: *HPLC, community structure, photosynthetic pigment, chemical taxonomy, redundancy analysis*

Introduction

East Lake (Wuchang District, Wuhan City, Hubei Province, China) is a typical semi-enclosed lake in the middle and lower reaches of the Yangtze River (Yun et al., 2015), and it is the second largest urban lake in China. The catchment area is 190 km^2, and the lake water area is 34.59 km^2. The average water depth is 2.2 m, with the highest value reaching 6 m. However, Wuhan is a typical industrial city, the domestic, industrial, and agricultural waste water discharge has significantly increased with its rapid industrialization and urbanization, which has resulted in the deterioration of the water

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

quality of the East Lake. The trend has changed from phosphorus limitation to nitrogen restriction, and eutrophication is intensifying. Cihu Lake (Huangshi City, Hubei Province, China) is the largest lake in Huangshi with a water area of approximately 1.0×10^7 m^2, catchment area of 6.28×10^7 m^2, and average water depth of 1.75 m (Yan, et al., 2015). The effect of its geographical location (i.e., proximity to a city, low water level, and limited purification capacity) and absence of a reasonable treatment have resulted in a large amount of industrial wastewater discharge. Water pollution is serious concerning the lake's eutrophic state. Qingshan Lake (Huangshigang District, Huangshi City, Hubei Province, China), which is located in the northern part of the Cihu Lake, typical urban lake, has a catchment area of 6.2 km^2, water area of 0. 52 km^2, and depth of 16.83 m (Li et al., 2013). It consists of four sub-lakes. Qinggang Lake (Huangshigang District, Huangshi City, Hubei Province, China) is located on the west bank of the Yangtze River and east of Qingshan Lake. Compared with Qingshan Lake, Qinggang Lake, Cihu and East Lake have more domestic sewage discharge, their smaller water areas, poorer self-recovery ability, and serious water pollution are often observed to be in a nutritious state.

Phytoplankton participates in the material cycle and energy flow as the main contributors of primary productivity; they also play an important role in the freshwater ecological system (Callieri, 2008). Phytoplankton consists of microplankton (20-200 μm), nanophytoplankton (2-20 μm), picophytoplankton (0.2-2 μm), and ultraphytoplankton (< 5 μm) (Robineau et al., 1999); each type has different contributions to the primary productivity and biomass. Nishibe et al. (2015) determined that the primary production was low in winter and composed mostly of small phytoplankton (< 10 μm), whereas large phytoplankton (> 10 μm) became the major producers in spring with high production. Current studies on the phytoplankton size structure mainly focus on marine ecosystems (Wang et al., 2014; Le et al., 2014; Joan et al., 2015), which are rarely reported in freshwater waters. Therefore, the study of freshwater bodies has important theoretical and practical significance, especially with lakes as the research object. However, fresh water has a special nature because of its ecological environment, including the complexity and variability of biological factors in freshwater bodies, organisms, and the environment, which determine the study complexity in freshwater bodies.

Therefore, the current study clarifies the compositions of phytoplankton community and their relationship with environmental factors in four urban lakes (Cihu Lake, East Lake, Qingshan Lake and Qinggang Lake) with different eutrophication levels in Huangshi City or Wuhan City, Hubei Province, China by using high-performance liquid chromatography (HPLC)-chemical taxonomy (CHEMTAX) and redundancy analysis (RDA).

Materials and methods

Study area and sampling strategy

Cihu Lake (30012' N, 11503' E) is located in the center of Huangshi City, Hubei Province, China. Three sampling points (*Fig. 1A*) are set up according to the morphological characteristics, geographical location, and pollution degree of Cihu Lake. East Lake (30033' N, 114023' E) is located in Wuchang District, Wuhan City, Hubei Province, China, which composed of Guozhen Lake, Fruit Lake, Tangling Lake, Tuan Lake, Hou Lake, Xiaotan Lake, Luoyan and other small sub-lakes. Guozheng

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

Lake is the main lake district of East Lake, where three sampling points are also set up (*Fig. 1B*). Qingshan Lake is located in the northern part of Cihu Lake, which consists of four sub-lakes: fish pond, attached lake 1, main lake district, and attached lake 2, we selected representative main lake district located in Qingshan Lake Park for sampling, three sampling points were set up (*Fig. 1C*). Three sampling points are also set up in Qinggang Lake, which is located east of Qingshan Lake and southwest of the Tiger's Head (*Fig. 1D*). The GPS latitude and longitude of each monitoring point are shown below (*Table 1*). The sampling times were March 2016, August 2015, November 2015, and January 2016 for spring, summer, autumn, and winter, respectively. Sampling once per season, a total of four lakes were sampled, each lake set up three sampling points. At each sampling point, 900 mL of surface water is collected with plexiglass sampler at a depth of 0.5 m.

Determination of photosynthetic pigment contents of the size-fractionized phytoplankton

A 900 mL water sample was collected at each sampling point and divided into three equal parts. The first water sample was directly filtered with a 0.7 μm GF/F filter membrane to obtain phytoplankton above 0.7 microns. The second water sample was first filtered with a 20 μm sieve, and then the filtrate was collected and filtered with a 0.7 μm GF/F filter membrane for store to obtain phytoplankton of 0.7 to 20 microns. The third water sample was first filtered with a pore size of 5 μm, and then the filtrate was collected and filtered with a 0.7 μm GF/F filter membrane for store to obtain phytoplankton of 0.7 to 5 microns.

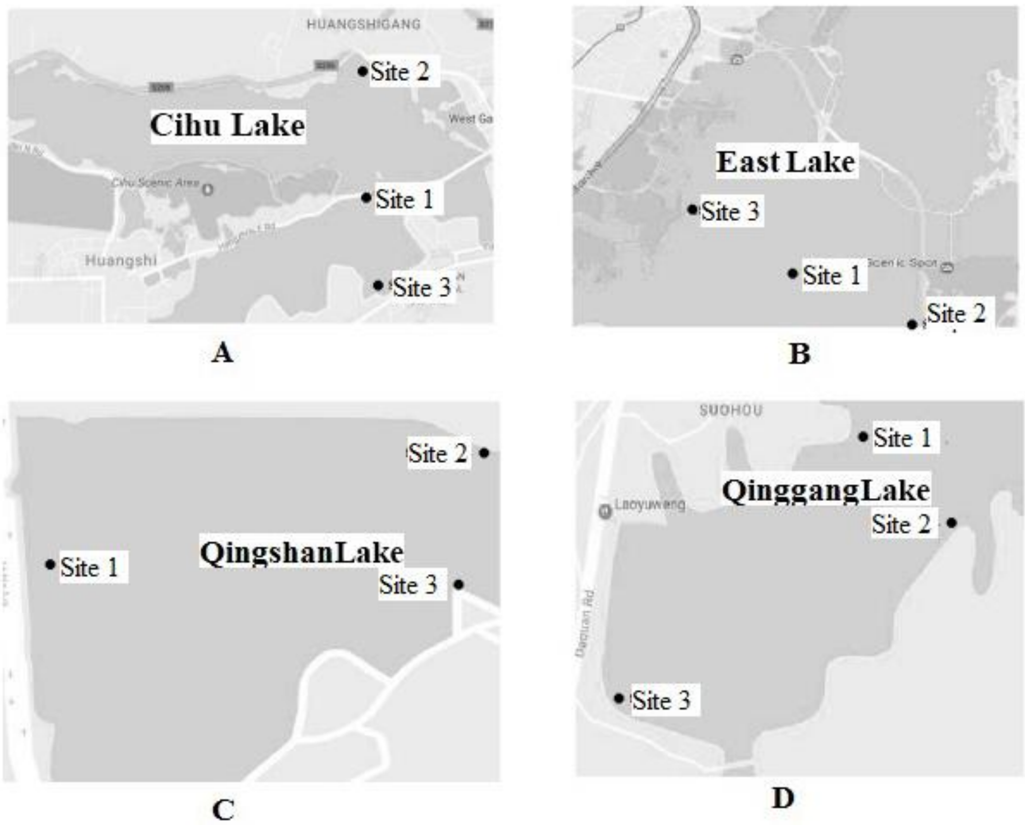


***Figure 1.** Location of the sampling sites in the study area*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

Table 1. *The GPS latitude and longitude of each monitoring point*

Lakes	Monitoring points	longitude	latitude
Cihu Lake	Site 1	E115°3’40.8”	N30°12’39.08”
Cihu Lake	Site 2	E115°3’36.7”	N30°13’23.8”
Cihu Lake	Site 3	E115°3’35.6”	N30°11’40.3”
East Lake	Site 1	E114°23’35.7”	N30°32’44.3”
East Lake	Site 2	E114°23’53.4”	N30°32’59.8”
East Lake	Site 3	E114°22’20.3”	N30°34’19.8”
Qingshan Lake	Site 1	E115°3’251”	N30°14’12.3”
Qingshan Lake	Site 2	E115°3’18.1”	N30°13’52.9”
Qingshan Lake	Site 3	E115°3’16.6”	N30°2’0.2”
Qinggang Lake	Site 1	E115°1’59”	N30°14’4”
Qinggang Lake	Site 2	E115°1’34”	N30°14’4”
Qinggang Lake	Site 3	E115°1’33”	N30°14’2”

The membrane with the sample was cut into several 5 mm × 1 cm pieces (not excessively small or large) and placed in a 2 mL centrifuge tube. Approximately 1 mL DMF was added and shock mixed into the - 20 °C refrigerator for 40 min, centrifuged at 4,000 r/min for 5 min, filtered through a 0.22 μm filter, and mixed with an equal volume of 1 M ammonium acetate solution for the HPLC analysis according to the reference method (Liu et al., 2017). The mobile phase consisted of methanol [1 M ammonium acetate; methanol = 80:20 (pH = 7.2)] and a mobile phase B: 100% methanol. The detection wavelength was 440 nm, injection volume was 100 μL, and flow rate was 1 mL/min. The photosynthetic pigment standards include fucoxanthin (Fuco), neoxanthin (Neox), violaxanthin (Viol), zeaxanthin (Zeax), diadinoxanthin (Diad), alloxanthin (Allo), chlorophyll a (Chl a), and chlorophyll b (Chl b), which were prepared according to our patent (Chinese Number: 201410022083.4). Quantitative, gradient elution, and quantitative calculation were performed according to our published method (Liu et al., 2017). The pigment concentrations of phytoplankton in the samples were calculated by the differential method. The pigment concentrations of microphytoplankton (> 20 μm), nanophytoplankton (5-20 μm), and picophytoplankton (0.7-5 μm) were obtained by subtractive method. The characteristics of the size-fractionized phytoplankton community structure were analyzed by utilizing CHEMTAX.

Chemtax analysis of the pigment data of the size-fractionized phytoplankton

The initial pigment ratio can directly affect the Chemtax calculation (Mackey et al., 1996). In studies of different habitats, including an estuary (Lionard et al., 2008), a bay (Madhu et al., 2014), lagoons (Sarmento and Descy, 2008), and freshwater bodies (Guisande et al., 2008), many adjustments were made to the selection of the initial ratio and pigment matrix. The intent was to isolate and incubate the phytoplankton in the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

laboratory to discover the pigment/Chl a ratio, but the ratio for every phylum was complex. The optimal initial pigment ratios in the current study were obtained from literature (Liu et al., 2017). The pigment concentration and initial pigment ratio data were inputted utilizing the CHEMTAX software version 1.95. The new pigment ratio data were obtained from the first run. The second CHEMTAX run employed this output pigment ratio as the input pigment ratio. After five to seven repetitions, there is no change in the output of the algae composition in phylum level, which indicates that the final results have been obtained. (Latasa, 2007) reported that multiple operations of the CHEMTAX analysis lowered the dependence on the initial pigment ratio, the transformation of different pigment ratios with continuous runs was always directed towards the true value, which improves the reliability and accuracy of the results. The relative abundance of size-fractionized phytoplankton that contributes to the Chl a biomass was calculated. The spatial and temporal differences were analyzed through the ANOVA statistical analysis.

Analysis of environmental factors

Water sample environmental factors comprised temperature (T), pH, total phosphorus (TP), ammonia nitrogen (NH_4^+-N), total nitrogen (TN) and $KMnO_4$ index, measured according to Water and Wastewater Monitoring and Analysis Methods of ministry of environmental protection of the people's republic of China.

Analysis of relationship between community structure and environmental factors

First, the species variables were analyzed by detrending correspondence analysis (DCA). If the longest length of the gradient was less than or equal to 3.0, then the linear model of principal component analysis (PCA) or redundancy analysis (RDA) was more appropriate. RDA was then applied to analyze the relationship between the environmental factors and size-fractionized phytoplankton community structure. Utilizing the previous selection and Monte Carlo tests, a minimal subset of the environmental factors was adopted for the RDA analysis, which explains a significant ($P < 0.05$) variation within the species data. Only these environmental factors are shown on the biplots. The ordination plots were made by Canoco for the Windows 4.5 software.

Results

Spatial and temporal distribution of the total biomass of size-fractionized phytoplankton in typical lakes of Hubei Southeast, China

The Chl a concentration was analyzed to evaluate the size-fractionized phytoplankton biomass in the typical lakes of Hubei Southeast. The spatial and temporal distributions of the total biomass were then clarified. Results are shown in *Figure 2*. The total biomass of the size-fractionized phytoplankton in the Lake centeris higher than those of other areas. For different seasons, the total biomass of the size-fractionized phytoplankton in the same lake has a large difference, and the highest value was observed in summer. The total biomass of the size-fractionized phytoplankton in the same lake was also different, and the highest total biomass of phytoplankton was that of nanoplankton. The spatial and temporal distributions of the total biomass of size-fractionized phytoplankton in the typical lakes of Hubei Southeast are as presented follows:

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

The contribution of the size-fractionized phytoplankton to the total biomass in Cihu Lake is shown in *Figure 2A*. The ratios of the total biomass in each site to the total biomass in all sites were 40.65%, 29.47%, and 29.89%, and the highest biomass was observed in sampling site 1. The biomass for all phytoplankton was the highest in autumn and smallest in winter. Among the microplankton, nanoplankton, and picoplankton, the highest contribution to the total biomass was that of nanoplankton. The contribution of the size-fractionized phytoplankton to the total biomass in East Lake is shown in *Figure 2B*. The ratios of the total biomass in each site to the total biomass in all sites are 45.05%, 28.77%, and 26.18%, and the highest biomass appeared in sampling site 1. The biomass for all phytoplankton was the highest in summer. The contributions of microplankton, nanoplankton, and picoplankton to the total biomass are relatively close. The contribution of the size-fractionized phytoplankton to the total biomass in QingShan Lake is shown in *Figure 2C*. The ratios of the total biomass in each site to the total biomass in all sites are 50.39%, 28.53%, and 21.08%, and the highest biomass was observed in sampling site 1. The biomass for all phytoplankton was the highest in summer. Among the microplankton, nanoplankton, and picoplankton, the highest contribution to the total biomass was that of nanoplankton. The contribution of the size-fractionized phytoplankton to the total biomass in QingQang Lake is shown in *Figure 2D*. The ratios of the total biomass in each site to the total biomass are 46.10%, 21.99%, and 31.91%, respectively, and the highest biomass was observed in sampling site 1. The biomass for all phytoplankton was the highest in summer. Among the microphytoplankton, nanophytoplankton, and picophytoplankton, the highest contribution to the total biomass was that of nanophytoplankton.

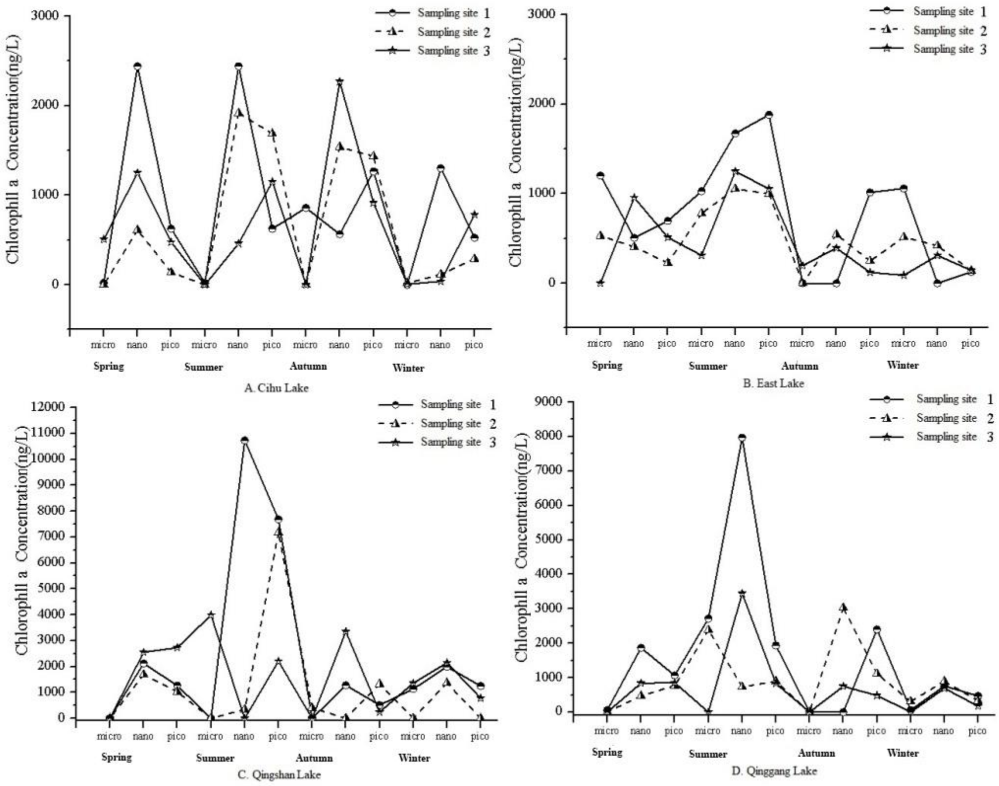


Figure 2. *Biomass of size-fractionized phytoplankton in Cihu, East, Qingshan and Qinggang lakes*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

Temporal and spatial distributions of the photosynthetic pigments of the size-fractionized phytoplankton

The photosynthetic pigment content of the size-fractionized phytoplankton was detected by utilizing HPLC technology in typical lakes of Hubei Southeast, China. The temporal and spatial distributions were then analyzed. The results show that the main biomark pigments in typical lakes of Hubei Southeast, China have eight types: Fuco, Neox, Viol, Allo, Lute, Zeax, Chl a, and Chl b. Among these pigments, Fuco, Allo, and Zeax were the main biomark pigments. The pigment compositions of the size-fractionized phytoplankton were similar, but the concentrations were different. Seven types of photosynthetic pigments from size-fractionized phytoplankton in the time distribution were relatively different, but their spatial distributions were relatively concentrated. The pigment concentrations in Qingshan and Qinggang Lakes were also higher. The spatial and temporal distributions of Fuco are shown in *Figure 3A*. The concentration range of Fuco was approximately 0 μg/m^3 to 2,341.91 μg/m^3, and it was highest in summer. A comparison of the Fuco concentration of the size-fractionized phytoplankton shows that nanophytoplankton was the highest in the four seasons. The spatial and temporal distributions of Neox are shown in *Figure 3B*. The concentration range of Neox was approximately 0 μg/m^3 to 262.99 μg/m^3, and it was also highest in summer. A comparison of the Neox concentration of the size-fractionized phytoplankton shows that nanophytoplankton was the highest in the four seasons. The spatial and temporal distributions of Viol are shown in *Figure 3C*. The concentration range of Viol was approximately 0 μg/m^3 to 1,024.40 μg/m^3, and it was highest in autumn. A comparison of the Viol concentration of the size-fractionized phytoplankton shows that nanophytoplankton was the highest in spring, autumn, and winter, whereas picoplankton was the highest in summer. The spatial and temporal distributions of Allo are shown in *Figure 3D*. The concentration range of Allo was approximately 0 μg/m^3 to 6,231.79 μg/m^3, and it was highest in summer. A comparison of the Allo concentration of the size-fractionized phytoplankton show that microphytoplankton was the highest in summer, whereas nanoplankton was the highest in the other seasons. The spatial and temporal distributions of Zeax are shown in *Figure 3E*. The concentration range of Zeax was approximately 0 μg/m^3 to 1,499.53 μg/m^3, and it was highest in autumn. A comparison of the Zeax concentration of the size-fractionized phytoplankton shows that nanophytoplankton was the highest in autumn, whereas picoplankton was the highest in the other seasons. The spatial and temporal distributions of Lute are shown in *Figure 3F*. The concentration range of Lute was approximately 0 μg/m^3 to 539.03 μg/m^3, and it was highest in summer. A comparison of the Lute concentration of the size-fractionized phytoplankton shows that nanophytoplankton was the highest in the four seasons. The spatial and temporal distributions of Chl b are shown in *Figure 3G*. The concentration range of Chl b was approximately 0 μg/m^3 to 4,504.55 μg/m^3, and it was highest in summer. The spatial and temporal differences among the seven photosynthetic pigments (except for Chl a) were analyzed utilizing the ANOVA statistical analysis. Results show that the photosynthetic pigment concentration had a significant difference in time ($P < 0.05$), but no significant difference in space ($P > 0.05$).

Composition of the size-fractionized phytoplankton community structure

The size-fractionized phytoplankton community structure was identified with CHEMTAX based on the pigment concentration data and initial pigment ratio. The

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

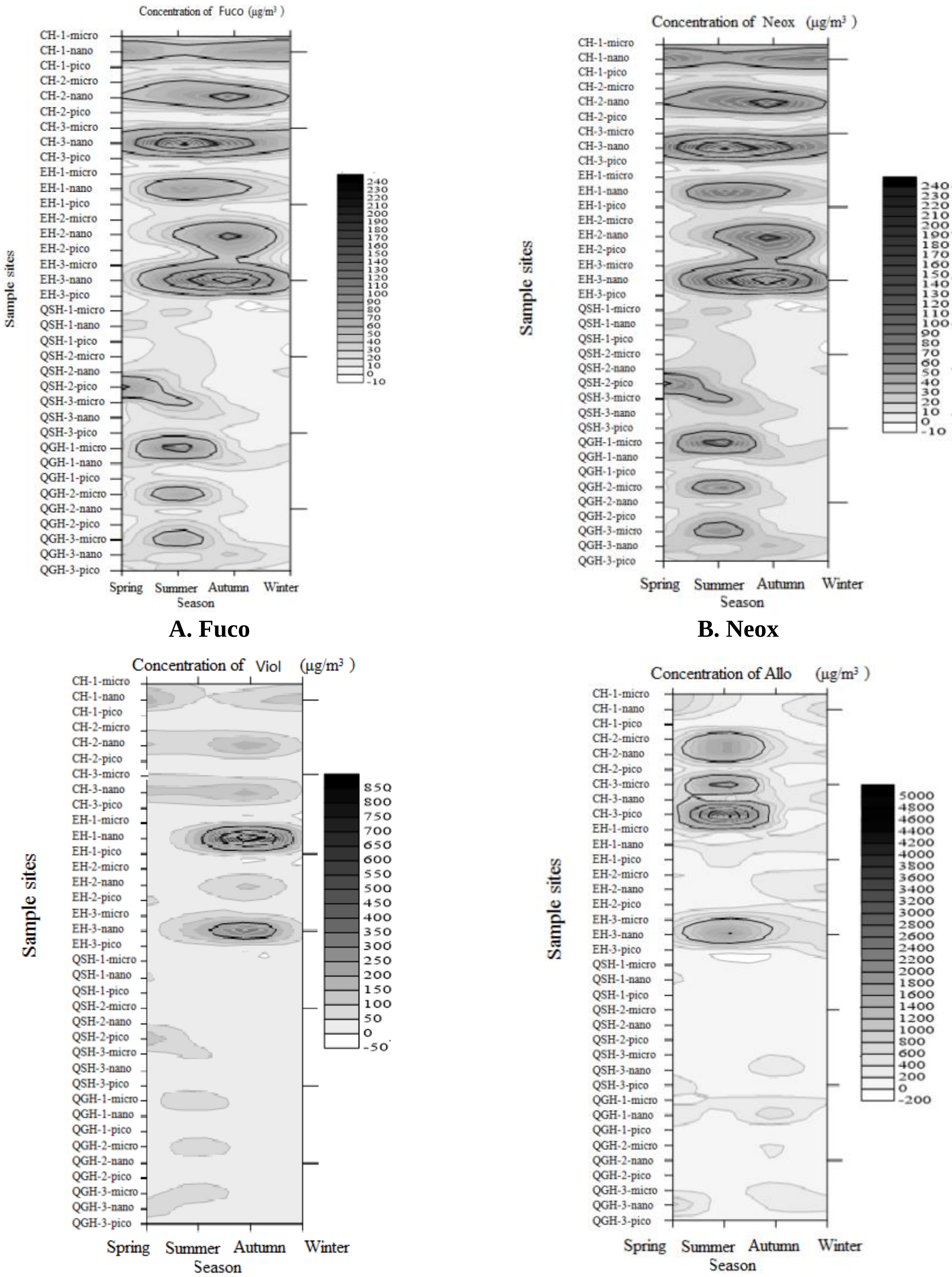
composition of the size-fractionized phytoplankton community structure is shown in *Figure 4*. Seven phytoplankton phyla were identified in the research area: *Cryptophytes*, *Diatoms*, *Chlorophytes*, *Cyanobacteria*, *Chrysophytes*, *Euglenophytes*, and *Dinoflagellates*. The size-fractionized phytoplankton community structure in spring is shown in *Figure 4A*. The results indicate that *Diatoms* dominated the sampling area in the Cihu, East, and Qinggang Lakes, and the highest percentage of *Diatoms* (91.39%) was at sampling site 2 in East Lake. *Cryptophytes* dominated the sampling area in Qingshan Lake, and the highest percentage (94.74%) was at sampling site 1. The dominant species was different for the size-fractionized phytoplankton. *Diatoms* and *Cryptophytes* were the dominant phyla for microphytoplankton, whereas *Diatoms* were the dominant phyla for nanophytoplankton. *Cryptophytes* were the dominant phyla for picophytoplankton. The size-fractionized phytoplankton community structure in summer was shown in *Figure 4B*. The results indicate that *Euglenophytes* and *Cyanobacteria* were the dominant phyla. The community structure largely changed, and the succession occurred between the *Euglenophytes* and *Diatoms* in the Cihu, East, and QingGang Lakes. By contrast, the percentage of cyanobacteria in QingShan Lake was higher than *Euglenophytes*. The dominant phyla did not have a clear difference for the size-fractionized phytoplankton. *Euglenophytes* were the dominant species for microphytoplankton, nanophytoplankton, and picophytoplankton. The size-fractionized phytoplankton community structure in autumn was shown in *Figure 4C*. The results indicate that *Euglenophytes* and *Cyanobacteria* were the dominant phyla, and the variation range of the community structure was small in all sampling areas. The dominant phyla were different for the size-fractionized phytoplankton. *Euglenophytes* were the dominant phyla for microphytoplankton, and the percentage was the highest at sampling site 1 in Cihu Lake. Cyanobacteria were the dominant species for nanophytoplankton. The size-fractionized phytoplankton community structure in winter is shown in *Figure 4D*. The results indicate that *Euglenophytes* were the dominant phyla in the Cihu and East Lakes, and *Cryptophytes* were the dominant phyla in the Qingshan and Qinggang Lakes. The dominant species did not have an evident difference for the size-fractionized phytoplankton, and the dominant phyla were *Euglenophytes* and *Cyanobacteria*. Thus, the dominant species were *Diatoms*, *Duglenophytes*, *Cryptophytes*, and *Cyanobacteria* all year round. The percentage of dominant species in summer was more than 50%, which may cause an outbreak of water bloom. The spatial and temporal differences were analyzed by utilizing the ANOVA statistical analysis. The results indicate that the community structure had a significant difference in time ($P < 0.05$), but no significant difference in space ($P > 0.05$).

Relationship between the size-fractionized phytoplankton community structure and environmental factors

The environmental variables were first analyzed with DCA. The longest gradient length was less than three, which was suitable for the RDA analysis (*Fig. 5*). Utilizing the forward selection and Monte Carlo tests, the environmental factors showed significant ($P < 0.05$) variation within the species data in all seasons, except for autumn. The environmental factors did not significantly affect the size-fractionized phytoplankton community structure in autumn. Therefore, the relationship between the ultraphytoplankton community structure and environmental factors was clarified by RDA in spring, summer, and winter. The RDA results for spring, summer, and winter are presented as follows. The dominant phylum in spring (*Fig. 5A*) was *Diatoms*. The

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

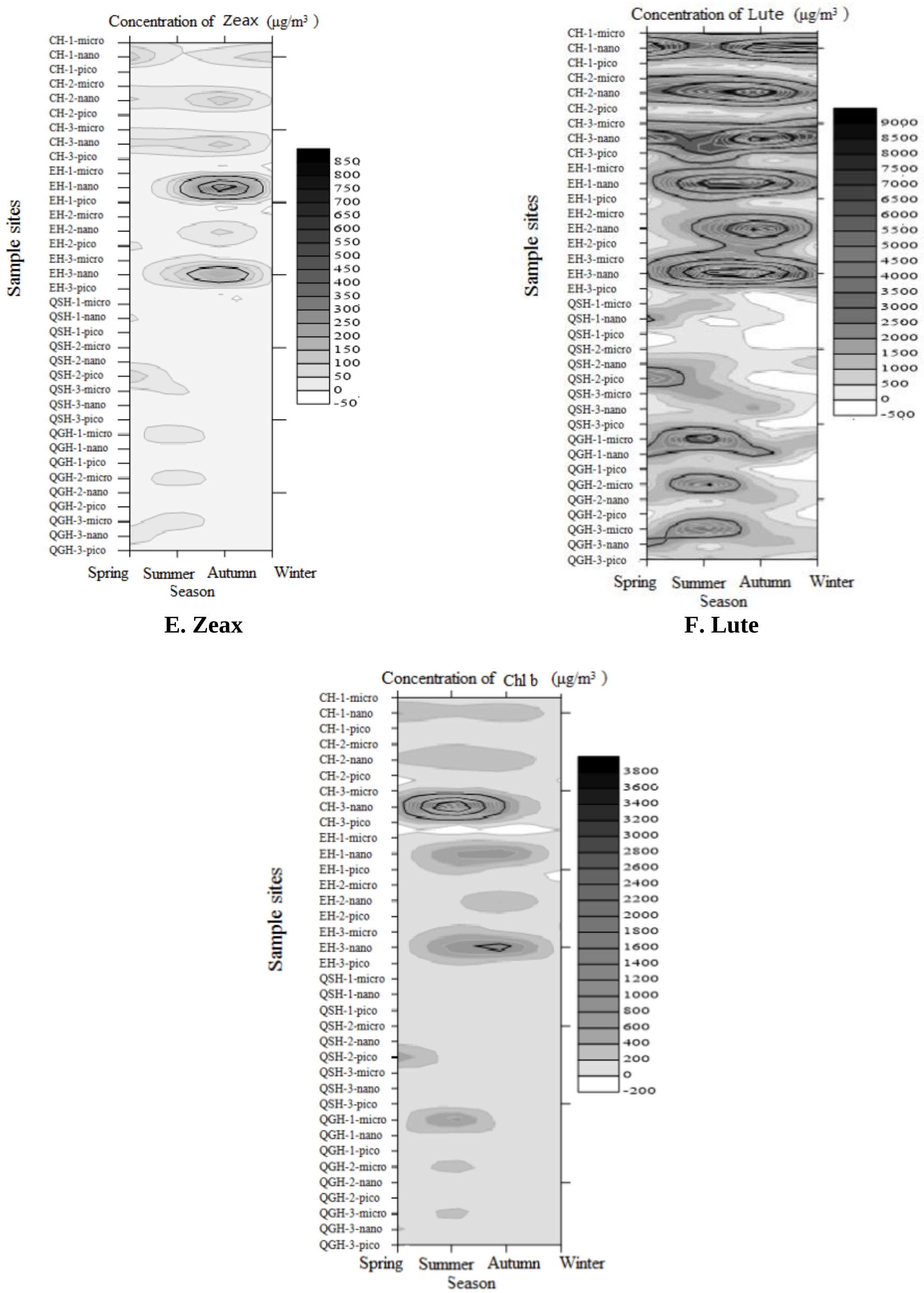
Diatoms were positively correlated with pH and temperature, whereas they were negatively correlated with TP, TN, and NH_4^+-N. The ordination biplot indicates that TN was the most significant environmental factor. The dominant phylum in summer (*Fig. 5B*) was *Euglenophytes*, which were positively correlated with TP, TN, and pH, whereas they were negatively correlated with temperature. The ordination biplot indicates that TP was the most significant environmental factor. The dominant phylum in winter (*Fig. 5C*) was *Euglenophytes*. *Euglenophytes* were positively correlated with NH_4^+-N, whereas they were negatively correlated with TP and TN. The ordination biplot indicates that TP was the most significant environmental factor.



A. Fuco **B. Neox**

C. Viol **D. Allo**

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905



E. Zeax **F. Lute**

G. Chl b

Figure 3. *Temporal and spatial distribution of photosynthetic pigments. CH: Cihu Lake; EH: East Lake; QSH: Qingshan Lake; QGH: Qinggang Lake*

Discussion

The size-fractionized phytoplankton in four seasons was analyzed. The dominant phytoplankton in the Qingshan, Qinggang, and Cihu Lakes was nanophytoplankton. However, the percentage of microphytoplankton, nanophytoplankton, and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

picophytoplankton in East Lake was relatively balanced. Seasonal differences were observed in the phytoplankton composition; microphytoplankton was the dominant phyla in spring and winter, whereas nanophytoplankton was the dominant phyla in summer and autumn. A significant grade structure succession occurred, which was similar to the findings (Wollschläger et al., 2015). The community structure of the size-fractionized phytoplankton in the typical lakes of Hubei Province, China has a significant difference among the seasons, but the composition of the size-fractionized phytoplankton in the same season was highly similar. Nanophytoplankton and picophytoplankton were also the dominant phyla, and these results were similar to marine ecosystem (Huang, 2018).

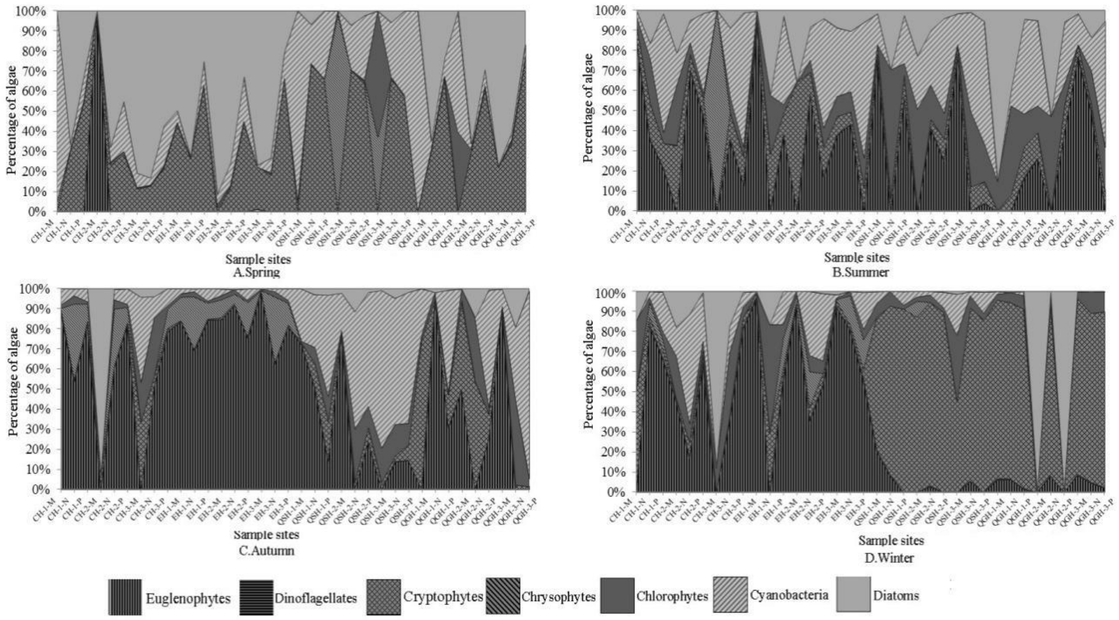
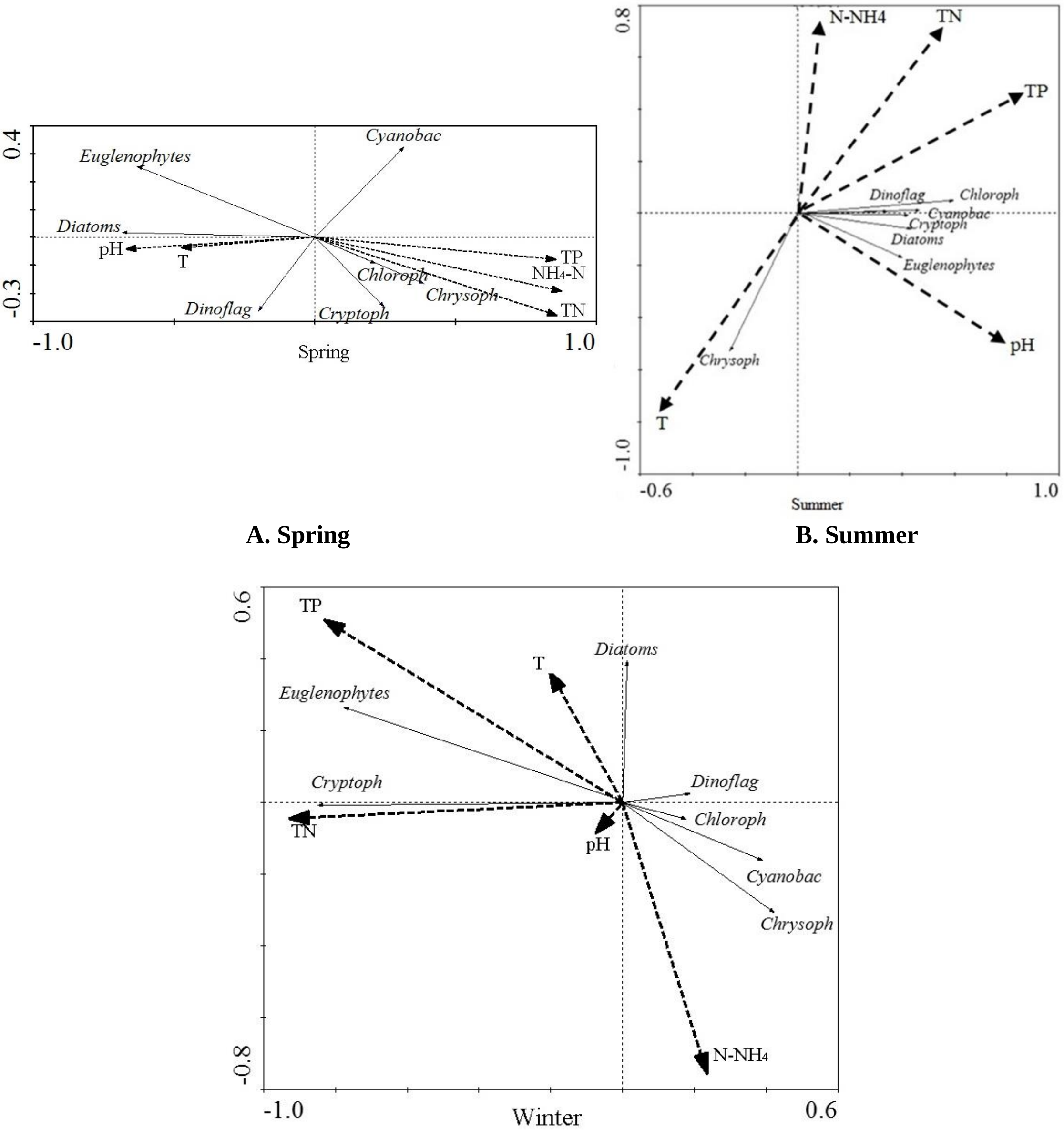


Figure 4. *The size-fractionized phytoplankton community structure. CH: Cihu Lake; EH: East Lake; QSH: Qingshan Lake; QGH: Qinggang Lake*

The community structure of size-fractionized phytoplankton was highly similar in the Cihu and East Lakes, and a small difference was observed among the seasons. The dominant phylum was *Euglenophytes.* Sun (2014) stated that *Euglenophytes* were mostly grown in water with pH 6.5 to 8.5. In our study, the pH and temperature were the optimal conditions for *Euglenophytes* growth. However, *Euglenophytes* have a strong ability of movement and migration, so they could occupy the surface and subsurface waters during the day for photosynthesis (Willén, 1992). Given this competitive relationship, other algae types could hardly reach the upper water. The surface water in this study was collected from the Cihu and East Lakes, which may be one of the reasons why the dominant phylum was *Euglenophytes*. The community structure of the size-fractionized phytoplankton in the Qingshan and Qinggang Lakes were highly similar, and a significant difference among the seasons was observed. *Cryptophytes* was the dominant phylum in spring and winter, whereas *Euglenophytes* and cyanobacteria were the dominant phyla in summer and autumn. This phenomenon is mainly related to their geographical environment. *Cryptophytes* creased to large number in the Qingshan and Qinggang Lakes in spring and winter, which may be due to the following reasons. On the one hand, *Cryptophytes* have high tolerance to low light (Marshall and Laybourn-Parry, 2002). The water in the study areas (Qingshan and Qinggang Lakes) was turbidier, which limited the light and growth of other phytoplankton, and *Cryptophytes* adapted to low light growth occupy an advantageous position. (Lewitus et al., 1991). On the other hand, the reproductive cycle was short at approximately 0.8 d to 3 d (Liu et al., 2012), this factor also provided the conditions for the *Cryptophytes* to become the dominant phylum. The dominant phylum in summer and autumn was cyanobacteria. Previous reports (Zhang et al., 2009; Li et al., 2010) states that the increasing temperature and eutrophication status are blue-green algae outbreak conditions. Results shown that the eutrophication of the Qinggang and Qingshan Lakes was more serious than the other two lakes.



A. Spring **B. Summer**

C. Winter

Figure 5. *Ordination biplot of environmental variables and the size-fractionized phytoplankton assemblages obtained by RDA for different seasons*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

The size-fractionized phytoplankton succession was affected by the environmental factors and nutrients. Previous reports on lakes and reservoirs states that oligotrophic systems have < 0.2 mg/L TN and < 0.01 mg/L TP, whereas eutrophic systems have TN > 0.5 mg/L and TP > 0.02 mg/L (Peng et al., 2013). The lowest values of TN and TP during the sampling period were 1.31 and 0.06 mg/L, respectively, which indicates that the typical lakes of Hubei Southeast, China fall into the category of eutrophicated water bodies. We then had to clarify the relationship between the environmental factors and ultraphytoplankton. Our findings shown that the relationship between environmental factors and size-fractionized phytoplankton had no clear significance ($P > 0.05$) in autumn, which indicates that the environmental factors did not affect the size-fractionized phytoplankton community structure in this season. By contrast, the relationship between the environmental factors and ultraphytoplankton was significant ($P < 0.05$) in the other three seasons, and the most significant environmental factors were TP and TN. The most limiting nutrient for algae growth in the freshwater ecosystem was generally phosphorus (Zhang et al., 2016). Feng et al. (2016) determined that phosphorus was the most significant factor that affects algal bloom in eutrophic conditions. Nitrogen also played an important role in the size-fractionized temporal dynamics in our study. This result was consistent with previous report (Barroso et al., 2016) that TN was the main driver of the phytoplankton structure.

Conclusions

We applied the HPLC-CHEMTAX method successfully and identified eight biomarker pigments and seven ultraphytoplankton (groups or classes) in the typical lakes of Hubei Southeast, China for the first time. The dominant phytoplankton types were *Diatoms* and *Euglenophytes*. The concentration of photosynthetic pigment had a significant difference in time ($P < 0.05$), but no significant difference in space ($P > 0.05$).

During the study period, the typical lake of Hubei Southeast, China was found to belong to the category of eutrophicated water bodies. Nanophytoplankton biomass was higher than microphytoplankton and picophytoplankton biomass, the community structure had a significant difference in time ($P < 0.05$) and was higher in summer than the other three seasons, but no significant difference in space ($P > 0.05$). Action should be taken to improve water quality to reduce the risk of harmful algal blooms.

The relationship between the environmental factors and ultraphytoplankton had a significant difference ($P < 0.05$) in the other three seasons (except autumn), and the most significant environmental factors were TP and TN, affecting size-fractionized phytoplankton succession in spring, summer and winter.

In future research, we can combine molecular biology techniques such as qPCR, high-throughput sequencing, metagenomics, single-cell sequencing with HPLC-CHEMTAX technology to study size-fractionized phytoplankton and obtain more complete information on community structure. Moreover, the biodiversity of specific groups of size-fractionized phytoplankton in freshwater lakes are requied to be further studied.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (41171045), the Research Fund for Science and Technology Innovation Team of Hubei Normal University (T201504) and the Central Government Guided Local Science and Technology Development Project of Hubei Province (No.2017ZYYD008).

REFERENCES

[1] Barroso, H. D., Becker, H., Melo, V. M. M. (2016): Influence of river discharge on phytoplankton structure and nutrient concentrations in four tropical semiarid estuaries. – Brazilian Journal of Oceanography 64(1): 37-48.

[2] Callieri, C. (2008): Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. – Freshwater Reviews 1(1): 1-28.

[3] Cui, D. Y., Wang, J. T., Tan, L. J. (2016): Response of phytoplankton community structure and size-fractionated chlorophylla in an upwelling simulation experiment in the western south China sea. – Journal of Ocean University of China 15(5): 835-840.

[4] Feng, W., Zhu, Y., Wu, F., Meng, W., Giesy, J. P., He, Z., Fan, M. (2016): Characterization of phosphorus forms in lake macrophytes and algae by solution 31P nuclear magnetic resonance spectroscopy. – Environmental Science and Pollution Research 23(8): 7288-7297.

[5] Guisande, C., Marciales, L. J., Hernández, E., Aranguren, N., Mogollón, M., Rueda-Delgado, G. (2008): Testing of the CHEMTAX program in contrasting Neotropical lakes, lagoons, and swamps. – Limnology and Oceanography: Methods 6(12): 643-652.

[6] Hong, H. S., Ruan, W. Q., Huang, B. Q., et al. (1997): The Taiwan Strait primary productivity and its regulation mechanism. – Journal of Marine Chinese, Beijing: Ocean Press 12-15 (in Chinese).

[7] Huang, D. L. (2018): Distribution and Influencing Factors of Phytoplankton Grain Size Structure in the Coastal Waters of Guangxi Beibu Gulf. – Guangxi University, Nanning (in Chinese).

[8] Joan, S., Font-Muñoz, et al. (2015): Estimation of phytoplankton size structure in coastal waters using simultaneous laser diffraction and fluorescence measurements. – Journal of Plankton Research 37(4): 740-751.

[9] Latasa, M. (2007): Improving estimations of phytoplankton class abundances using CHEMTAX. – Marine Ecology Progress Series 329(Jan): 13-21.

[10] Le, F. F., Hao, Q., Jin, H. Y., et al. (2014): 2012 in Chukchi Sea and phytoplankton in the sea area adjacent to the existing volume and primary productivity of grain structure study. – Journal of ocean 36(10): 103-115 (in Chinese).

[11] Lewitus, A., Caron, D., Miller, K. (1991): Effect of light and glycerol on the organization of the photosynthetic apparatus in the facultative heterotroph Pyrenomonas salina (Cryptophyceae). – Journal of Phycology 27: 578-587.

[12] Li, J., Ji, F. F., Hua, J. H. (2013): Community structure of phytoplankton and water quality assessment in summer in lake Qingshan. – Natural Sciences Journal of Harbin Normal University 29(5): 61-65 (in Chinese).

[13] Li, Z., Guo, J. S., Fang, F., et al. (2010): The relationship between seasonal variation of cyanobacteria in Three Gorges backwater area of Xiaojiang River and the main environmental factors. – Environmental Science 31(2): 301-309 (in Chinese).

[14] Lionard, M., Muylaert, K., Tackx, M., Vyverman, W. (2008): Evaluation of the performance of HPLC-CHEMTAX analysis for determining phytoplankton biomass and composition in a turbid estuary (Schelde Belgium). – Estuarine, Coastal and Shelf Science 76(4): 809-817.

[15] Liu, X. X., Lu, X. H., Chen, Y. W. (2012): Spatiotemporal dynamics of crypto biomass in northern taihu lake. – Lake Science (1): 144-150 (in Chinese).

[16] Liu, X. X., Li, J. Y., Bi, Y. H., Hou, J. J., Li, Y. T., He, Y. Y. (2017): Characterization of ultraphytoplankton pigments and functional community structure in Xiangxi Bay, China, using HPLC-CHEMTAX. – Journal of Freshwater Ecology 32(1): 103-118.

[17] Mackey, M. D., Mackey, D. J., Higgins, H. W., Wright, S. W. (1996): CHEMTAX-a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. – Marine Ecology Progress Series 144: 265-283.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905

[18] Madhu, N. V., Ullas, N., Ashiwini, R., Meenu, P., Rehitha, T. V., Lallu, K. R. (2014): Characterization of phytoplankton pigments and functional community structure in the Gulf of Mannar and the Palk Bay using HPLC-CHEMTAX analysis. – Continental Shelf Research 80: 79-90.

[19] Marshall, W., Laybourn-Parry, J. (2002): The balance between photosynthesis and grazing in antarctic mixotrophic crytophytes during summer. – Journal of Freshwater Ecology 47(11): 2060-2070.

[20] Nishibe, Y., Takahashi, K., Shiozaki, T., Kakehi, S., Saito, H., Furuya, K. (2015): Size-fractionated primary production in the Kuroshio Extension and adjacent regions in spring. – Journal of Oceanography 71(1): 27-40.

[21] Peng, C. R., Zhang, L., Zheng, Y. Z., Li, D. H. (2013): Seasonal succession of phytoplankton in response to the variation of environmental factors in the Gaolan River, Three Gorges Reservoir, China. – Chinese Journal of Oceanology and Limnology 31(4): 737-749 (in Chinese).

[22] Robineau, B., Legendre, L., Michel, C., Budeus, G., Kattner, G., Schneider, W., Pesant, S. (1999): Ultraphytoplankton abundances and Chlorophyll a concentrations in ice-covered waters of northern seas. – Journal of Plankton Research 21(4): 735-755.

[23] Sarmento, H., Descy, J. P. (2008): Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes. – Journal of Applied Phycology 20(6): 1001-1011.

[24] Solorzano, G. G., Martinez, M. G., Vázquez, A. L., Garfías, M. B., Zuñiga, R. E., Conforti, V. (2011): Trachelomonas (Euglenophyta) from a eutrophic reservoir in Central Mexico. – Journal of Environmental Biology 32(4): 463-471.

[25] Sun, F. Z. (2014): The prevention and cure of the algae bloom in aquaculture. – Guide to the Fishing for Wealth 21: 53-53 (in Chinese).

[26] Wang, G. F, Zhou, W., Lin, G. F., et al. (2014): Validation and evaluation of the biological optical inversion model of the phytoplankton size structure in the northern South China sea. – Journal of Laser Biology 23(6): 502-515.

[27] Willén, E. (1992): Planktonic green algae in an acidification gradient of nutrient-poor lakes. – Archiv für Protistenkunde 141(1-2): 47-64.

[28] Wollschläger, J., Wiltshire, K. H., Petersen, W., Metfies, K. (2015): Analysis of phytoplankton distribution and community structure in the German Bight with respect to the different size classes. – Journal of Sea Research 99: 83-96.

[29] Yan, Z., Liu, T., Sun, J. X., Zhou, S., Zhou, Y. J., Xiao, W. S. (2015): Accumulation of heavy metals in nanoplankton in two lakes in mining area - take Daye Lake and Cihu Lake in Hubei as examples. – Safety and Environmental Engineering 22(3): 28-34 (in Chinese).

[30] Yun, X. Y., Yang, Y. Y., Liu, M. X., Wang, J. (2015): Concentrations and risk assessment of polychlorinated biphenyls and polybrominated diphenyl ethers in surface sediments from the East Lake, China. – Ecotoxicology 24(1): 172-180.

[31] Zhang, M., Cai, Q. H., Wang, L., et al. (2009): A preliminary study on the process of elimination in Xiangxi Bay of Three Gorges reservoir water algae monitoring Wahson. – Wetland Science 7(3): 230-236.

[32] Zhang, J. J., Zhang, Q., Qin, M. M., Hong, Y. (2016): Selection and characterization of eight freshwater green algae strains for synchronous water purification and lipid production. – Frontiers of Environmental Science & Engineering 10(3): 548-558.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1804_58915905