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RESEARCH ARTICLE

# Use the predictive models to explore the key factors affecting phytoplankton succession in Lake Erhai, China

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**Abstract** Increasing algae in Lake Erhai has resulted in frequent blooms that have not only led to water ecosystem degeneration but also seriously influenced the quality of the water supply and caused extensive damage to the local people, as the lake is a water resource for Dali City. Exploring the key factors affecting phytoplankton succession and developing predictive models with easily detectable parameters for phytoplankton have been proven to be practical ways to improve water quality. To this end, a systematic survey focused on phytoplankton succession was conducted over 2 years in Lake Erhai. The data from the first study year were used to develop predictive models, and the data from the second year were used for model verification. The seasonal succession of phytoplankton in Lake Erhai was obvious. The dominant groups were Cyanobacteria in the summer, Chlorophyta in the autumn and Bacillariophyta in the winter. The developments and verification of predictive models indicated that compared to phytoplankton biomass, phytoplankton density is more effective for estimating phytoplankton variation in Lake Erhai. CCA (canonical correlation analysis) indicated that TN (total nitrogen), TP (total phosphorus), DO (dissolved oxygen), SD (Secchi depth), Cond (conductivity), T (water temperature), and ORP (oxidation reduction potential) had significant influences ($p < 0.05$) on the phytoplankton community. The CCA of the dominant species found that *Microcystis* was significantly influenced by T. The dominant Chlorophyta, *Psephonema aenigmaticum* and *Mougeotia*, were significantly influenced by TN. All results indicated that TN and T were the two key factors driving phytoplankton succession in Lake Erhai.

**Keywords** Water bloom · Phytoplankton succession · Predicted models · Lake Erhai

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## Introduction

Increasing and frequent algal blooms are worldwide environmental concerns that have attracted much attention (Paerl and Huisman 2008). These blooms can cause irreparable damage to aquatic ecosystems and aquatic organisms (Hallett et al. 2016; Landsberg 2002). Algal blooms have also seriously damaged the economic value of water. Moreover, toxic metabolites produced by algae (Smith et al. 2008) could lead to second pollution to both drinking water and aquatic products. Previous studies had illuminated many algae treatment methods, such as biological control (Gumbo et al. 2008; Ma et al. 2012; Xie 1996), physical control (Liu et al. 2013a; Rajasekhar et al. 2012), and chemical control (Ibelings et al. 2016). Many of these measures had beneficial effects and value for improving water quality. However, the abundance of algae could be determined in advance with predictive models of the dynamic relationships between phytoplankton communities and environmental factors over time, and these

models could also help significantly reduce and mitigate the associated damage.

The composition and quantity of phytoplankton are strongly affected by environmental factors. Phytoplankton communities react directly to nutrient levels and are effective indicators for assessing ecological water quality (Katsiapi et al. 2016). Numerous field and laboratory studies have addressed how changes in environmental factors, including nutrients (Glibert et al. 2005; Glibert and Burkholder 2006; Heisler et al. 2008), temperature (Paerl and Huisman 2008, 2009), pH, and other factors (Jiang et al. 2014) have impacted phytoplankton. The results of these studies have not always been consistent. For example, (Xu et al. 2015) found that nitrogen had negligible effect on phytoplankton growth at some sites in Lake Taihu, but another study that was also conducted in Lake Taihu indicated that nitrogen was the primary limiting nutrient for phytoplankton (Xu et al. 2010). Even, the same factors could have different effects in different predictive models (Persaud et al. 2015). Many of the previous studies have focused on the same types of lakes. In these lakes, the nutrient levels were relatively high, and the Cyanobacteria dominance was clear and stable. Predictive models for Cyanobacteria biomass have adequately represented phytoplankton abundance (Beaulieu et al. 2014). However, in other lakes, the composition and dominant groups of phytoplankton alternated, and Cyanobacteria variations could not represent the whole phytoplankton community. Chlorophyta was also one of the main groups that had a high risk of forming blooms. High proportions of diatoms have also been recorded and have seriously influenced the water quality (Birk et al. 2012; Pandey et al. 2017; Zelazna-Wieczorek and Nowicka-Krawczyk 2015). Models for predicting the densities and biomass of Chlorophyta or Bacillariophyta are also important to understand the variations in the phytoplankton community. Predictive models can greatly assist with algae abundance estimations once the models are verified to be reliable and applicable.

As a water resource for Dali City, the phytoplankton variations in Lake Erhai were significantly influence the water supply and human health. However, few studies have conducted to explore the seasonal variation of phytoplankton community in Lake Erhai. In the present study, a systematic survey of the whole area of Lake Erhai was implemented for 2 years from May 2014 to April 2016. Phytoplankton and various physicochemical parameters were monitored monthly during the study period. The data from the first study year were used to develop predictive models; therefore, the seasonal variations of the phytoplankton community and the environmental factors were characterized from May 2014 to April 2015. The data from the second year were applied to verify the reliability and practicability of the models.

## Materials and methods

### Study area

Lake Erhai (25° 36′ − 25° 58′ N, 100° 05′ − 100° 18′ E) is a plateau lake located in Yunnan Province, China, with an area of approximately 250 $km^2$ (Davis et al. 2009). The lake has faced serious threats of intensive eutrophication caused by increasing nutrient inputs and the over-use of water resources (Yu et al. 2014). From 1957 to 2013, the phytoplankton density in Erhai Lake rose by approximately one hundred times, from $22.4 \times 10^4$ to $2043.3 \times 10^4$ cells/L. Since the water bloom dominated by *Anabaena* first broke out in 1996, it has occurred every year in most bays of Lake Erhai. In 1998, 2003, 2006, 2009, and 2013, the bloom covered the entire lake.

### Sampling and analyzing

The distribution of 18 sample sites across whole Lake Erhai was showed in Fig. 1. Every water sample was a mixture connected from three layers, 0–0.5 m of surface water, the middle and the bottom of 0–0.5 m over sediment (Niu et al. 2011). Values of scchi depth (SD), water depth (WD), Water temperature (T), pH, dissolved oxygen (DO), conductivity (Cond), and oxidation-reduction potential (ORP) were measured in situ (Tao et al. 2012).

Chemical parameters, including total nitrogen (TN), ammonia nitrogen ($NH_4$-N), nitrate nitrogen ($NO_3$-N), total phosphorus (TP), dissolved total phosphorus(TDP), phosphate phosphorus ($PO_4$-P), and Chlorophyll a (Chl-a) were measured in laboratory for each sample.

One liter of water sample for phytoplankton identification was fixed in situ with acetic Lugol's solution (Parsons et al. 1984). Then each sample was concentrated to 50 ml after sedimentation for 48 h in the laboratory. 0.1 ml concentrated sample was counted using an Olympus microscope (BX50, Olympus, Tokyo, Japan) under magnification of 400 times after complete mixing. Phytoplankton species were identified with reference to the methods detailed by (Hu and Wei 2006) and (John et al. 2002).

### Statistical analyses

The data on the physicochemical parameters are shown as the mean value of the 18 sites. All graphs were made by the Origin 8.0 software. CCA (canonical correlation analysis)

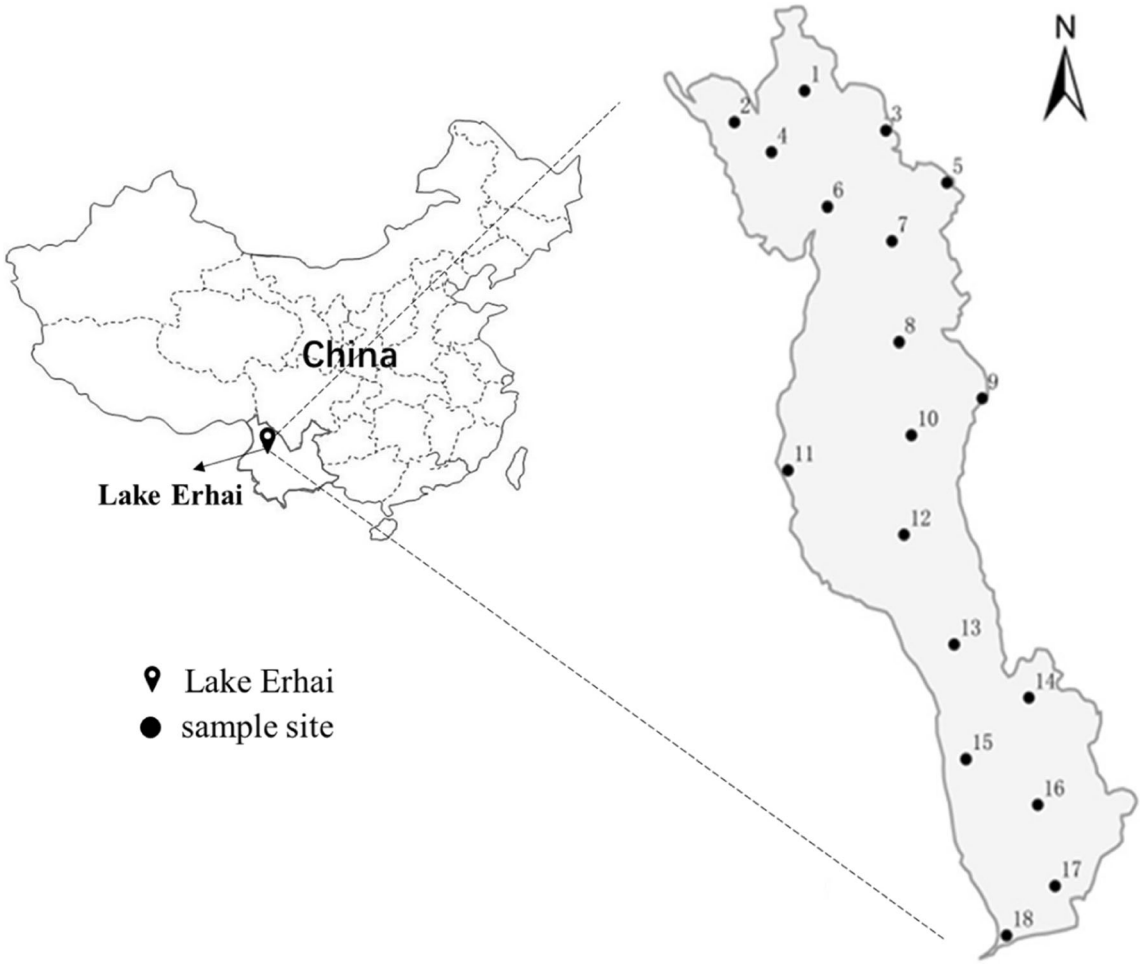


**Fig. 1** The distribution of 18 sample sites in Lake Erhai

was performed by the Canoco 5.0 software to explore the relationship between the phytoplankton composition and environmental factors. Forward selection was chosen to determine the impact factors that were significant ($p < 0.05$) to the phytoplankton community changes. The dominant taxa were defined as those with densities higher than 50% of the total phytoplankton (Downing et al. 2001; Persaud et al. 2015; Yang et al. 2017).

General linear models (GLMs) were used to predict the phytoplankton and Cyanobacteria biomass, and the models were implemented using the mgcv package of (R Core Team 2014; Wood 2004). The density and nutrient data (μg/L) were log-transformed to reduce deviations. The adjusted R squared ($r^2_{adj}$) and AIC (Akaike information criterion) values were used to select the best models. The optimal modeling theory was defined by two aspects (Dong et al. 2012): (i) the influence of every predictor in the model is significant and (ii) the $r^2_{adj}$ based on the last conditions after it was adjusted is maximal and the AIC is minimal. Every factor was taken into account at first, and the factors that were not significant according to their $p$ values ($p < 0.05$) were then eliminated.

## Results

### Water quality in Lake Erhai

The monthly variations of the physical and chemical variables during our study of Lake Erhai are presented in Fig. 2. The TN concentration peaked in October and reached 1.048 mg $L^{-1}$ (Fig. 2a). The highest TP content was 0.038 mg $L^{-1}$ and occurred in November (Fig. 2b). The $NH_4$-N concentration fluctuated from 0.01 to 0.09 mg $L^{-1}$, and the $PO_4$-P concentration ranged from 0.001 to 0.01 mg $L^{-1}$. The T changed from 11.76 to 24 °C (Fig. 2d). The DO concentration varied from 5.40 mg $L^{-1}$ (June) to 8.66 mg $L^{-1}$ (March) (Fig. 2f). The Cond and ORP demonstrated opposing seasonal variations, and both peaked in April (312.22 ms $cm^{-1}$, 283.68 mv) then

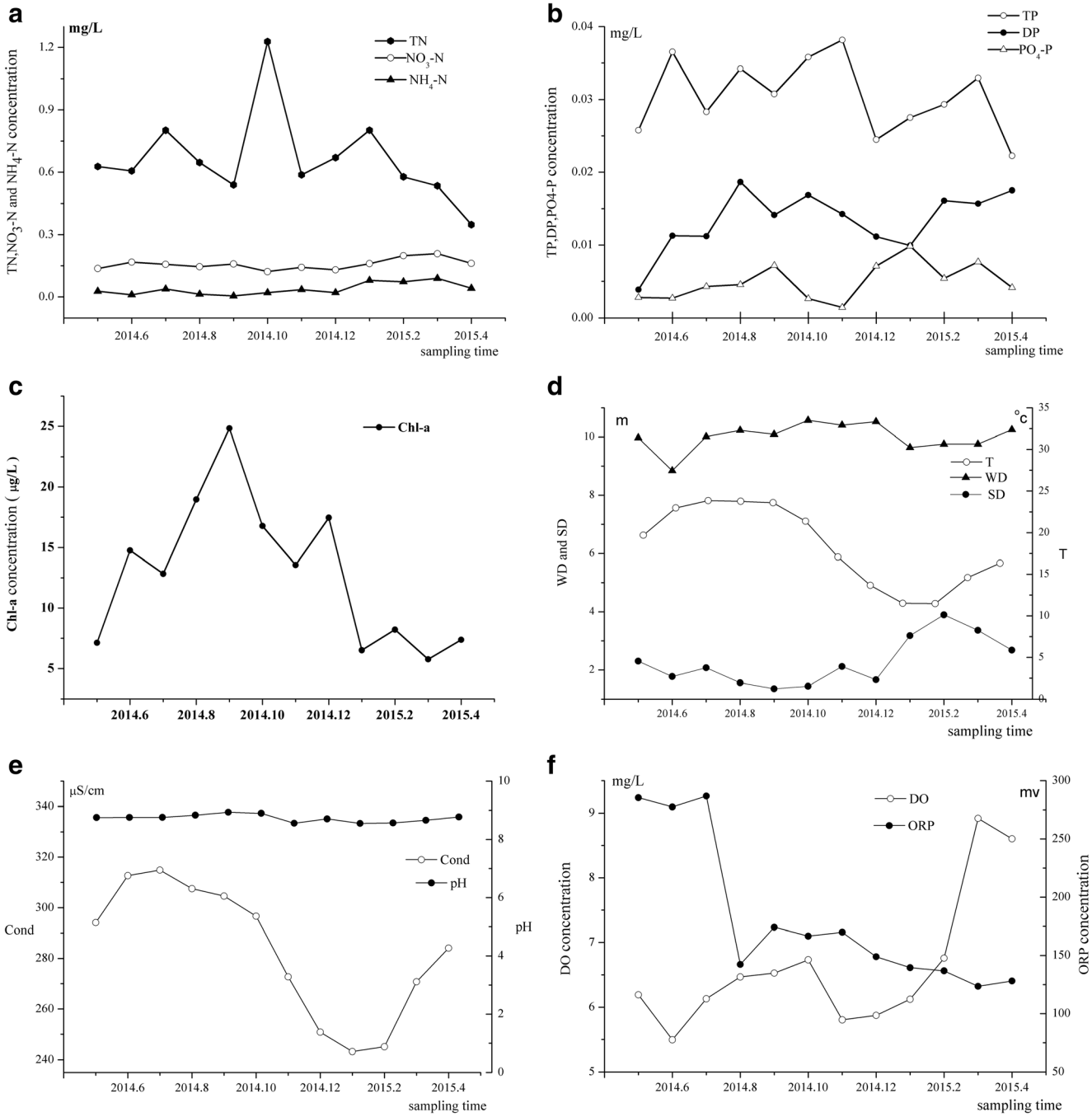


**Fig. 2** Environmental parameters from May 2014 to April 2015 in Lake Erhai. **a** Nitrogen content including TN, $NH_4$-N, $NO_3$-N. **b** Phosphorus content including TP, TDP, and $PO_4$-P. **c** Chl-a content. **d** WD, SD, and T. **e** pH, Cond. (**f**) DO and ORP

experienced persistent declines until October. The highest SD was 3.81 m and occurred in February 2015. The pH had a relatively small range from 8.5 to 8.93.

## Dynamics of phytoplankton

In Lake Erhai, a total of 74 phytoplankton species belonging to 43 genera and 7 phyla were recognized, including 16 genera of Chlorophyta, 17 genera of Bacillariophyta and 6 genera of Cyanophyta. The other genera belonged to Pyrrophyta, Chrysophyta, Cryptophyta, and Euglenophyta. Bacillariophyta had the largest percentage of genera and accounted for 39.5% of the total genera. Chlorophyta had the next largest percentage of genera with 37.2%, while Cyanophyta had only 14% of the genera. Chlorophyta, Bacillariophyta, and Cyanophyta occurred throughout the lake over the whole year. Chrysophyta and Cryptophyta only appeared at a few sample sites in one or 2 months.

The total phytoplankton cell density increased beginning in May 2014 and peaked in October 2014 when it reached $3.3 \times 10^7$ cells/L. Then, the cell density decreased and remained low until April. In other eutrophic lakes, Cyanophyta was found to be preponderant in the phytoplankton community across the whole year and contributed more than 90% to the phytoplankton abundance. However, in Lake Erhai, the dominant groups changed. The seasonal succession of phytoplankton density was obvious, and the pattern is shown in Fig. 3. From June to September, Cyanophyta was the dominant group, and its proportion reached the maximum of 92.2% in August. From October to January, Chlorophyta was the dominant group, and the largest contribution occurred in November when it accounted for 85.5% of the algae community. Bacillariophyta dominated the algae community in the winter.

The changes in the phytoplankton biomass were inconsistent with those in the phytoplankton density. The largest algal biomass was 4.42 mg/L and occurred in December. The smallest algal biomass (1.13 mg/L) occurred in February. The seasonal variation of the biomass of different algae is shown in Fig. 4. With respect to biomass, Cyanophyta did not have obvious advantages over the other groups and was only dominant in August and September. From October to January, Chlorophyta was the dominant algae. In the winter, Bacillariophyta was the dominant algae.

There were six dominant taxa of phytoplankton in Lake Erhai (Fig. 5). *Aphanizomenon* was the dominant species in May 2014. *Microcystis* were the most common Cyanobacteria in the summer. *Psephonema aenigmaticum* and *Mougeotia* were the dominant taxa in the autumn. In the winter, *Fragilaria construens* and *Melosira* showed absolute advantages over the other taxa. Although the algal density in December was lower than that in October, the biomass of the dominant species in December, *Mougeotia*, was much higher than the biomass of *Psephonema aenigmaticum* in October. This resulted in a higher phytoplankton biomass in December than in October. In addition, the biomass of *Ceratium hirundinella,* which was found at very low densities at many sample sites, significantly influenced the total phytoplankton biomass because of its large volume. One *Ceratium hirundinella* is one thousand times larger than *Microcystis* and *Psephonema aenigmaticum*. However, *Ceratium hirundinella* was less frequent or abundant during the blooming season than at other times. The presence of *Ceratium hirundinella* maintained the high phytoplankton biomass in Lake Erhai even in the winter and spring. The seasonal species variations and the algae cell sizes resulted in the different tendencies between biomass and density in our study.

## Predicting models for phytoplankton

### *Predicting models for phytoplankton density*

Increasingly frequent occurrences of water blooms resulting from increasing algae abundance have made it urgent to develop predictive models in Lake Erhai. In other eutrophic lakes, the phytoplankton composition was relatively simple, and Cyanobacteria was the dominate species, so many researches focused on cyanobacteria, and models only developed for biomass (Kosten et al. 2012; Persaud et al. 2015). In Lake Erhai, Cyanobacteria, Chlorophyta, and Bacillariophyta were all important phytoplankton groups. The seasonal variations of phytoplankton density and biomass were different. Therefore, in

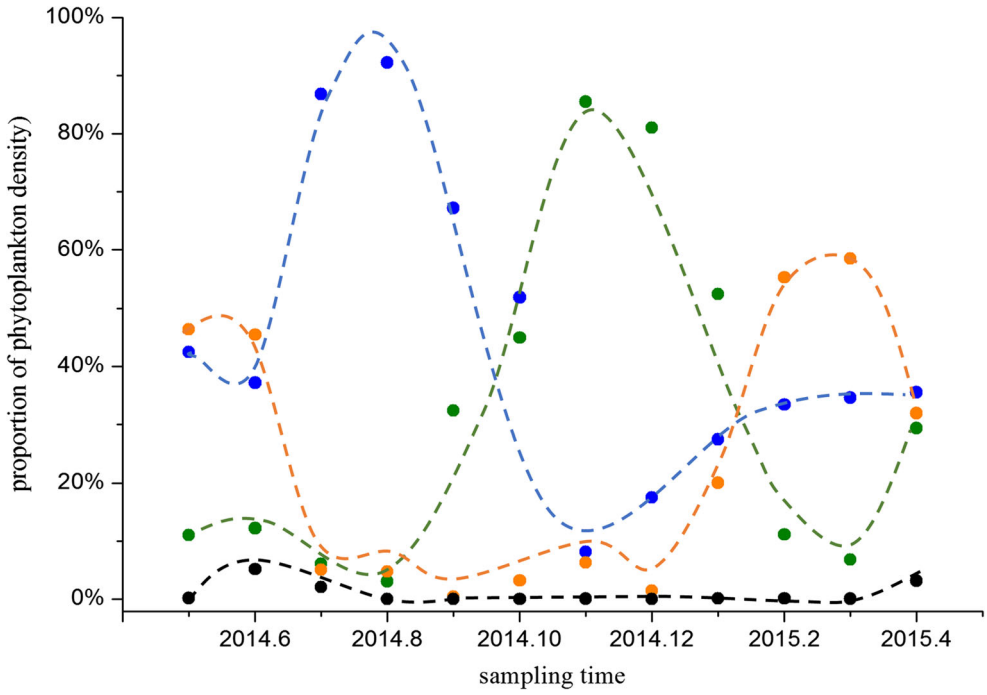


**Fig. 3** Composition density of the phytoplankton community in Lake Erhai from May 2014 to April 2015. Blue dash lines indicate Cyanophyta; green dash lines, Chlorophyta; orange dash lines, Bacillariophyta; and black dash lines, others

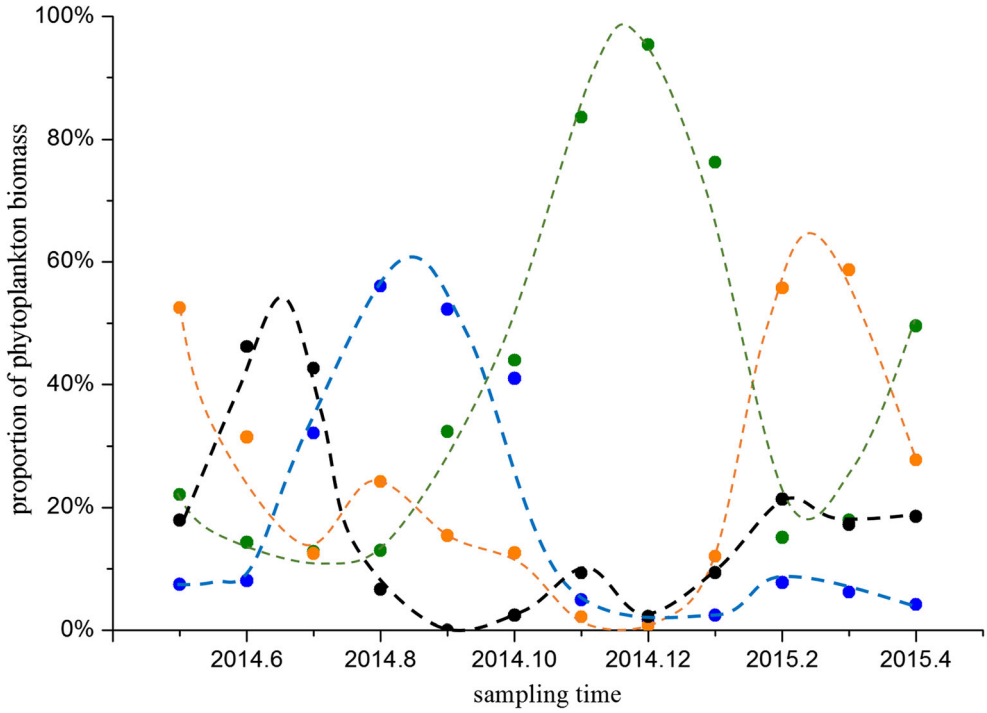


**Fig. 4** Biomass composition of the phytoplankton community in Lake Erhai from May 2014 to April 2015. Blue dash lines indicate Cyanophyta; green dash lines, Chlorophyta; orange dash lines, Bacillariophyta; and black dash lines, others

our research, the density and biomass of these three groups of phytoplankton were studied and analyzed, and predictive models were also developed for these two aspects of the taxa. The fitted models for phytoplankton density are shown in Table 1. TN, SD, and T were the three significant predictors of phytoplankton density ($r^2_{adj}$ = 0.51, AIC = 185.9). The variance of the Cyanobacteria density was explained by SD and T ($r^2_{adj}$ = 0.429, AIC = 461.2), Chlorophyta was predicted by TN, T, and DO ($r^2_{adj}$ = 0.51, AIC = 438.02), while Bacillariophyta was predicted by SD and T ($r^2_{adj}$ = 0.277, AIC = 459.25).

*Predicting models for phytoplankton biomass*

Predictive models for algal biomass were developed, and the results are shown in Table 2. Phytoplankton biomass was predicted by TN and SD ($r^2_{adj}$ = 0.186, AIC = 772.02). The Cyanobacteria biomass was relatively well predicted by TN, T, DP, DO, and Cond ($r^2_{adj}$ = 0.57, AIC = 240.39). The Chlorophyta biomass was negatively influenced by $NO_3$, SD, and Cond ($r^2_{adj}$ = 0.407, AIC = 561.71). The predictors that included $NO_3$, T, DO, and Cond accounted for 0.285 of the variation of the Bacillariophyta biomass.

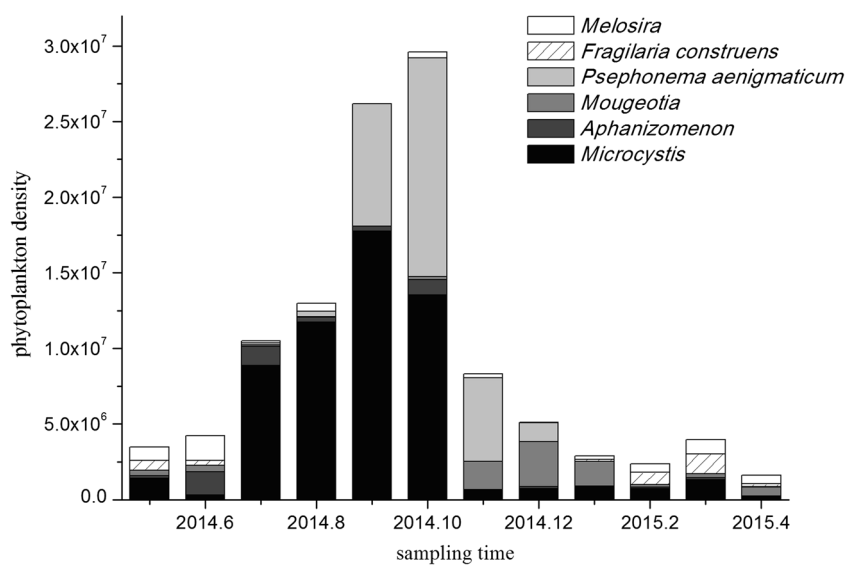


**Fig. 5** The seasonal variation of the dominant species in Lake Erhai

**Table 1** Predictive models for phytoplankton density in Lake Erhai

| Models | $r^2_{adj}$ | AIC |
|---|---|---|
| log-phytoplankton density = 4.08 + 0.85 logTN*** − 0.15 SD*** + 0.037 T*** | 0.51 | 185.9 |
| log-Cyanobacteria density = 4.63–0.18 SD* + 0.10 T*** | 0.429 | 461.2 |
| log-Chlorophyta density = − 2.06 + 1.80 logTN*** + 0.13 T*** + 0.12 DO*** | 0.51 | 438.02 |
| log-Bacillariophyta density = 8.63–0.62 SD*** − 0.067 T*** | 0.277 | 459.25 |

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

## Discussion

### The influence of environmental factors on phytoplankton succession

The relationships between the phytoplankton and environmental factors are shown in the CCA plot (Fig. 6). Seven environmental factors, TN, TP, DO, SD, Cond, T, and ORP (significant influence, $p < 0.05$) significantly affected the phytoplankton community. The eigenvalues of SPE AX1 and SPE AX2 together explained 49.89% of the variation of the algae species in Lake Erhai. The first axis was correlated with T, DO, Cond, pH, and TP. The second axis was correlated with TN and SD. Cyanophyta had positive relationships with T, Cond, ORP, and pH but had negative relationships with DO. Chlorophyta was positively correlated with TN and DO. Bacillariophyta preferred high SD and TP and low T.

In our study, Cyanophyta had a weak relationship with TN; this was because one of the main Cyanobacteria species, *Aphanizomenon*, can fix nitrogen and can grow in waters with low nitrogen concentrations (Bradburn et al. 2012). For the dominant species *Microcystis*, T had more important influence (Carey et al. 2012; Rigosi 2014) when the T in summer was the optimum temperature for *Microcystis* growth (Paerl and Paul 2012; Reynolds and Reynolds 2006). As Cyanophyta have phycobiliproteins, it can make Cyanophyta harvest light with wide wavelengths range and have better low-light utilization efficiency (Liu et al. 2013b; Oliver et al. 2012; Singh et al. 2009). These characteristics supported the normal growth of Cyanobacteria in water with low SD. Furthermore, Cyanobacteria blooms may even increase local water temperatures through the intense absorption of light. Some studies have indicated that the temperatures of surface blooms can be at least 1.5 °C above those of ambient waters. This positive feedback provides additional competitive dominance of Cyanobacteria over other phytoplankton. In Lake Erhai, the water temperatures in the winter were optimal for diatom growth. The nutrient requirements of diatoms are lower than those of blue-green algae, which resulted in the prevalence of diatoms over other groups in the winter.

Studies have indicated that seeking out the reason for frequent outbreaks of water blooms in lakes, identifying the dominant species, and exploring the key factors driving dominant species succession are extremely important (Hudnell and Dortch 2008). *Aphanizomenon, Microcystis*, *Psephonema aenigmaticum*, and *Mougeotia* were the dominant species in the summer and the autumn when the risk of water bloom outbreak was high in Lake Erhai. Therefore, the correlations between the densities of these four species and the environmental factors were analyzed (Fig. 7). The analysis was conducted using only the data on the dominant species densities and the physicochemical variables from July to December. The eigenvalues of SPE AX1 and SPE AX2 together explained 56.8% of the variation in the dominant species in Lake Erhai. The results indicated that T was the most important environmental factor that explained 39.3% of the variation and had the greatest positive influence on *Microcystis*. Another main Cyanobacteria species, *Aphanizomenon*, was positively related to ORP, TN, and SD. The other two species were both positively related to TN concentrations. In early summer, as the water temperature was slowly rising, *Anabaena* and *Aphanizomenon* were rapidly increasing due to their high tolerance to low nitrogen and nitrogen fixation abilities (O'Neil et al. 2012). When the temperature was continuously rising and reached the peak in the summer, *Microcystis* was dominant and out-competed all other species (JÖHnk et al. 2008; Reynolds and Reynolds 2006). When the TN concentration was increasing and the water temperature was falling in the autumn and early winter, *Psephonema aenigmaticum* and *Mougeotia* successively began

**Table 2** Predictive models for phytoplankton biomass in Lake Erhai

| Models | $r^2_{adj}$ | AIC |
|---|---|---|
| Phytoplankton biomass = − 0.824 + 1.592 logTN* − 0.556 SD*** | 0.186 | 772.02 |
| Cyanobacteria biomass = − 2.69 + 1.27 logTN*** + 0.169 T*** + 0.406 DP** + 0.0954 DO** − 0.016 Cond*** | 0.57 | 240.39 |
| Chlorophyta biomass = 16.292–0.470 $\log NO_3$** − 1.032 SD*** − 0.0422 Cond*** | 0.407 | 561.71 |
| Bacillariophyta biomass = − 7.01 + 0.480 $\log NO_3$*** − 0.153 T*** + 0.070 DO* + 0.0312 Cond*** | 0.285 | 300.75 |

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

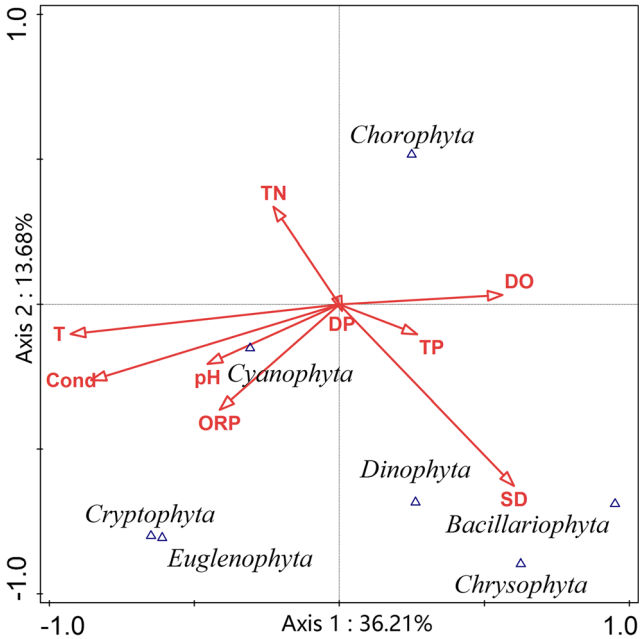


**Fig. 6** Phytoplankton species-environmental CCA biplot (phytoplankton groups are represented by the "△" symbols). The red arrows represent each environmental variation (significant $p < 0.05$)

to prevail over the other species. In the winter, Bacillariophyta began to dominate in both number and variety. From this research, we can conclude that high T was the main reason for the dominance of *Microcystis* in summer. The dominance of *Psephonema aenigmaticum* in the autumn resulted from the rising TN concentrations. This result indicates that rising T and high TN concentrations are the two main indicators of water bloom risks in Lake Erhai.

## The influence of environmental factors on predictive models

The influences of the environmental factors on the phytoplankton community were explored, and the results were nearly consistent with the model predictions. We found that the density models did not have the same predictors as the biomass models. The total phytoplankton density models ($r^2 = 0.51$) were stronger than the phytoplankton biomass models ($r^2 = 0.186$). There were several reasons for the differences between the biomass and density models. First, the phytoplankton biomass was obviously influenced by the biomass of *Ceratium hirundinella*, which has a large cell size and occurs at lower frequencies in the lake. In addition, the dominant species such as *Microcystis*, *Aphanizomenon*, and *Mougeotia* are filamentous or colony-forming species; even if the cells are small, they could have many cells in one filament or colony. The models that predicted both Cyanobacteria and Chlorophyta density and biomass were stronger and more reliable than the Bacillariophyta models. The TN was still the key factor for Chlorophyta in both the density and biomass models. In our study, the Bacillariophyta models did not perform well, which may be a result of the poor abundance of diatoms. Compared to those of the other nutrients in the models, the nitrogen concentration was much more important than the phosphorus concentrations in Lake Erhai, which was an important factor in previous studies in other lakes (Elser et al. 2007; Paerl 2008).

## Verification of the predicted models

Predictive models for phytoplankton in Lake Erhai were developed. The total phytoplankton density, Cyanobacteria density, and Chlorophyta density models outperformed the biomass models. However, the reliability and applicability of the models have not been determined. In previous studies, these models have usually been used to indirectly explain the relationship between the phytoplankton community, water blooms, and environmental variations (Kosten et al. 2012). Other research has modeled the Cyanobacteria across a broad range of lakes (Beaulieu et al. 2014). However, few of these models have been verified. Considering the convenience and accuracy, all models in this study were verified for applicability and practicability. The data that were measured from May 2015 to April 2016 were used for verification. The results are shown in Fig. 8.

The verification comparison diagram (Fig. 8) indicated different results and showed that models for density were better than those for biomass. Total phytoplankton density, Cyanobacteria density, and Chlorophyta density had almost the same variation tendencies between the measured and predicted values. Approximately 90.7% of the predicted total phytoplankton values fell with the 90% confidence intervals of the measured

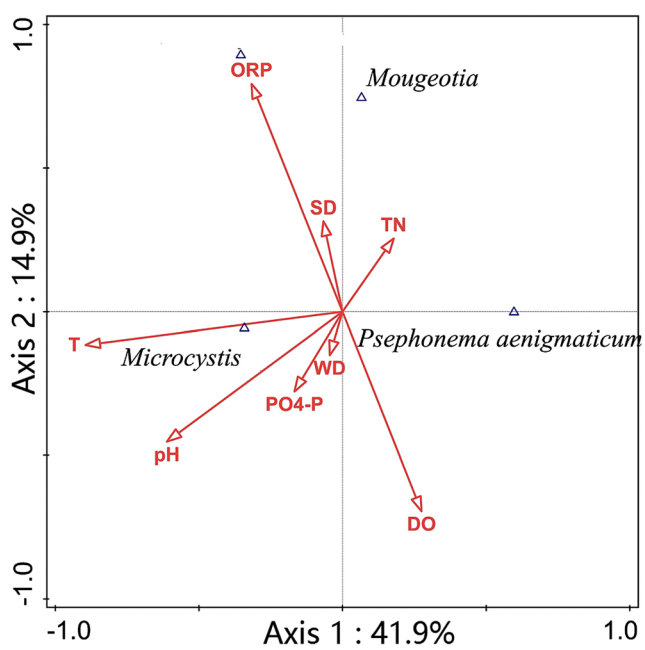


**Fig. 7** Dominant species-environmental CCA biplot (phytoplankton groups are represented by the "△" symbols). The red arrows represent each environmental variation (significant $p < 0.05$)

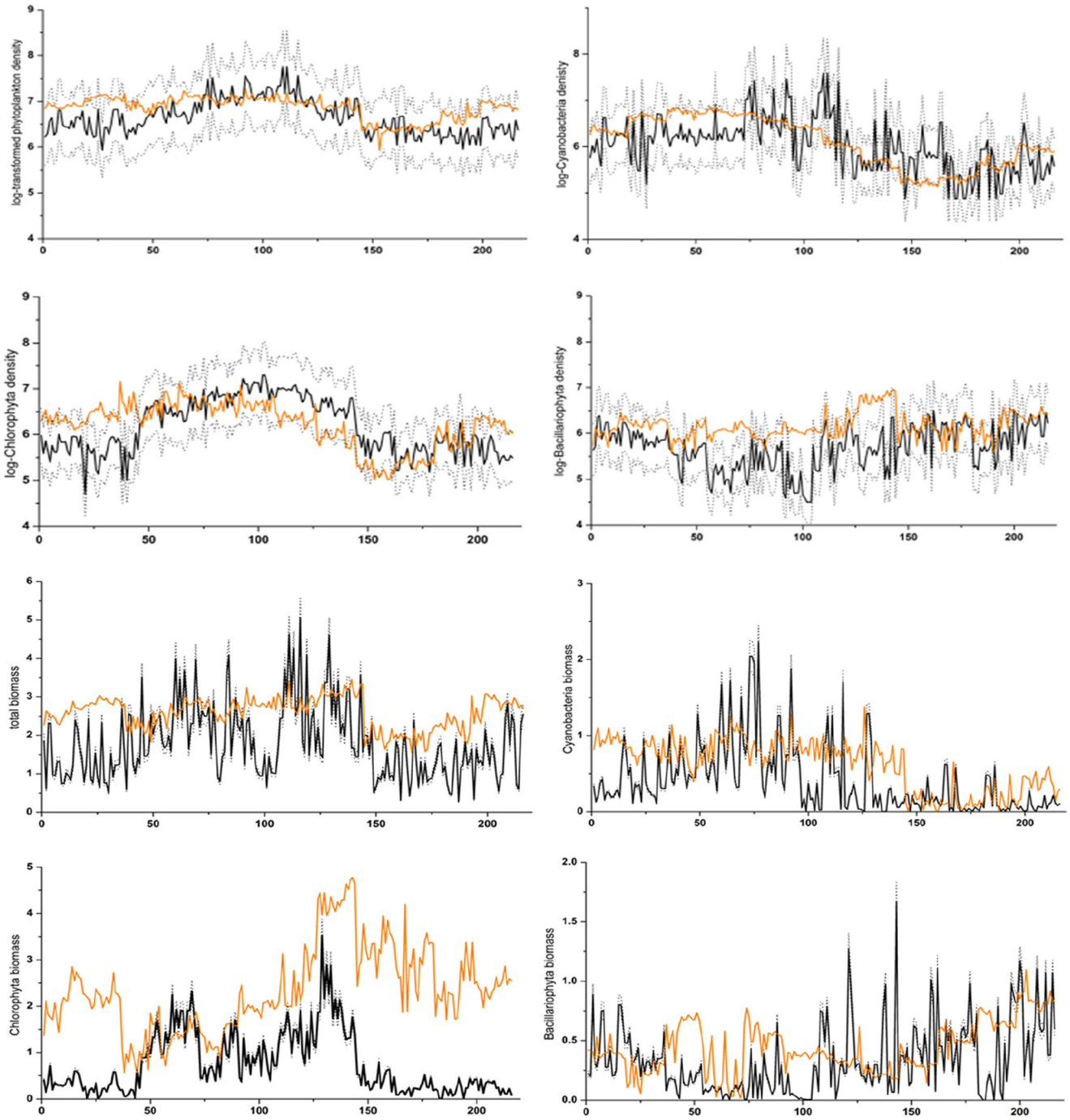


**Fig. 8** The verification between measured and predicted phytoplankton. All density data was log-transformed. Sample sites number was the sites from May 2015 to April 2016; every month had 18 samples and totaled 216 sites, numbered consecutively. Solid black line indicates observed values; dash lines, 90% confidence limits; and orange lines, predictive values

values. In addition, the proportion of Cyanobacteria density was 84.7%. The measured and predicted values for biomass were not suitable, and the variations were widely inconsistent because the phytoplankton community in Lake Erhai was not stable. Both the predicted and observed Cyanobacteria densities had the same tendencies. However, the main species in Lake Erhai differed during the two study years. In the first year, *Microcystis* was the dominant species throughout the summer, and it contributed much more to the Cyanobacteria density and biomass. *Aphanizomenon* also exhibited a large proportion in May. However, for 2016, *Aphanizomenon*, *Microcystis*, and *Anabaena* were all main species, and even *Pseudanabaena* had a high density at some sites at times. These differences resulted in the irregular changes in Cyanobacteria biomass. The other algae

exhibited the same problems, which is why the verifications of the density and biomass models had different results. From the developments and verification of predictive models, we can conclude that compared to algae biomass, algae density is more effective for estimating phytoplankton variation in Lake Erhai.

## Conclusion

In our study, the seasonal variations of the phytoplankton community and environmental factors were investigated over 2 years from May 2014 to April 2016 in Lake Erhai. The relationships between phytoplankton and environmental factors were explored. There is obviously seasonal phytoplankton succession in Lake Erhai. In the summer, the dominant algae were Cyanobacteria. Chlorophyta was the dominant group in autumn and Bacillariophyta was dominated in winter. The developments and verification of predictive models indicated that phytoplankton density is more effective for estimating phytoplankton variation in Lake Erhai. The CCA results showed that seven environmental factors, TN, TP, DO, SD, Cond, T, and ORP, had significant influences ($p < 0.05$) on the changes in phytoplankton. T was the key factor for Microcystis dominance in the summer, and TN concentration was the main factor resulting in the dominance of *Psephonema aenigmaticum* and *Mougeotia* in autumn. Our study suggested that T and TN were the key factors for high risk of algal bloom in Lake Erhai.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflicts of interest.

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