JOURNAL OF
ECOLOGY AND ENVIRONMENT
http://www.jecoenv.org

Research Paper
J. Ecol. Environ. 38(3): 293-305, 2015

Green algae dominance quickly switches to cyanobacteria dominance after nutrient enrichment in greenhouse with high temperature

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Abstract

In order to understand the mechanisms of conversion between different algal dominance, an experiment was performed in a greenhouse from 22 June to 10 July 2011. The experiment included a treatment group subjected to three instances of nutrient enrichment and a control with no nutrient enrichment. The initial water was dominated by *Ankistrodesmus* of Chlorophyta. The average water temperature at 08:30 h and 14:00 h during the experiment was 31.6°C and 34.6°C, respectively. The results showed that the total nitrogen (TN), total phosphorus (TP), dissolved total nitrogen (DTN), dissolved total phosphorus (DTP), and soluble reactive phosphorus (SRP) concentrations in the treatment were significantly higher than in the control ($P < 0.05$). However, the TN/TP and DTN/DTP in the control was higher than in the treatment ($P < 0.05$). The dominant algae in the control did not change during the experiment, while the dominant algae in the treatment switched to *Planktothrix* of Cyanophyta on day 9. The chlorophyll *a* (Chl-*a*), wet weight of all algae, wet weight of Cyanophyta, and percentage of Cyanophyta in the control were all significantly lower than in the treatment ($P < 0.05$). Amounts of zooplankton, especially rotifers, were present at the end of the experimental period. The density of rotifers between the control and treatment was not significantly different ($P > 0.05$), while the copepod density in the treatment was higher than in the control ($P < 0.05$). We conclude that green algae dominance quickly switches to cyanobacteria dominance after nutrient enrichment in a greenhouse with elevated temperature.

Key words: algal switch, chlorophyta dominance, cyanophyta dominance, high water temperature, nitrogen and phosphorus, zooplankton

INTRODUCTION

Eutrophication mainly caused by nitrogen and phosphorus enrichment has been shown to improve the primary productivity and quicken the propagation of algae (Prepas and Charette 2003, Paerl and Otten 2013). Eutrophication has damaged the function and utility of water bodies, and a large number of management and ecological restoration projects have been conducted to change the widespread eutrophication situation in China (Qin

http://dx.doi.org/10.5141/ecoenv.2015.030

Received 5 February 2015, **Accepted** 27 April 2015

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2007, Qin et al. 2007). One of the most important phenomena resulting from eutrophication is algal blooms, which induce water anoxia, foam, and many other negative impacts (Paerl 1988).

Algal bloom mainly refers to the fast growth and accumulation of phytoplankton (Paerl et al. 2001). There have been many algal blooms in China, such as the blooms in Lake Taihu and Lake Dianchi caused by *Microcystis* spp. cyanobacteria, diatom blooms (Yin et al. 2012), and Euglena blooms (Zhao et al. 1994). Moreover, other large colony-forming cyanobacteria like *Oscillatoria*, *Anabaena*, and *Aphanizomenon* often dominate the plankton in eutrophic lakes (Berger 1975, Schindler 1975, Reynolds 1984, Smith 1986, Trimbee and Prepas 1987).

The formation of algal blooms and especially cyanobacterial blooms are the result of many interacting ecological factors (Paerl 1988, Oliver and Ganf 2000, Chen et al. 2003), e.g., physiological features (Oliver and Ganf 2000), climatic factors (Paerl and Huisman 2008), nutrients (Prepas and Charette 2003, Wang et al. 2010), and selective grazing by zooplankton (Wang et al. 2010). The roles that these factors play during the process are still unclear, and our understanding of the mechanisms is still rudimentary.

However, studying the cyanobacterial bloom process and the influencing factors via field surveys is difficult. On the other hand, it is not easy to replicate the bloom formation process with laboratory experiments using axenic cultured algae. Many algae in laboratory axenic incubations only exist as single cells instead of colonies, which is different from the morphology in the field (Mikheeva and Kruchkova 1980, Lürling and van Donk 1997).

In order to understand the mechanisms of cyanobacterial bloom formation, many *in situ* field investigations were conducted to research the influences of factors such as nutrients and physical characteristics (Jensen et al. 1994, Tang et al. 2006, Ahern et al. 2008). Additionally, some statistical analysis have been performed with long term investigation of the main factors affecting the bloom formation (Jensen et al. 1994, Jacoby et al. 2000), and some physiology-based models have been used to analyze the roles of various factors (Scheffer et al. 1997).

If the algal bloom formation process can be achieved in a controlled environment, the study of this process would be greatly enriched. As cyanobacterial blooms preferentially occur in warm seasons (Paerl and Huisman 2008), a greenhouse can provide the appropriate conditions for the examinations. Our former experiment induced a *Microcystis* bloom formation via nitrogen and phosphorus nutrient enrichment to an algal community with initial green algae dominance (Wang et al. 2010). In order to further investigate the algal bloom formation process, we used a water source with Chlorophyta dominance to study the switch between different algal dominance.

With the widespread eutrophication, many landscape waters also became eutrophic as nutrients concentrations increased and phytoplankton proliferated significantly. Although these waters did not affect people as much as the large lakes did, how these waters will respond to climate change and elevated eutrophication remains unclear. With our survey to many small landscape waters in China, we found the water showed Chlorophyta dominance or both cyanobacteria and Chlorophyta dominance.

Previous studies indicate that increased temperatures can selectively promote cyanobacterial blooms, specifically *Microcystis*, in eutrophic waters (Jöhnk et al. 2008, Jeppesen et al. 2009, Paerl and Huisman 2009, Elliott 2012, De Senerpont Domis et al. 2012). And cyanobacterial blooms are common in eutrophic field. Thus, it seems easy to replicate cyanobacterial bloom formation, especially in eutrophic water with high temperature. Whether *Microcystis* blooms can be generated in eutrophic water with high temperature remains an open question.

Our former experiments performed with different water sources (Wang et al. 2010, 2011) found that the dominant algae after nutrients enrichment changed under different scenarios. To further study the switch from Chlorophyta dominance to other algal dominance, a nutrient enrichment experiment was conducted in a greenhouse with water from a small landscape pond with Chlorophyta dominance. This will provide additional data for the explanation of cyanobacterial bloom formation as well as new methods for future study on the mechanisms of cyanobacterial bloom formation.

MATERIALS AND METHODS

Water source

Water was collected from a ~20 m^2 circular eutrophic pond located at the Fishery Machinery and Instrument Research Institute, Chinese Academy of Fisheries Sciences in Shanghai, China. This pond is located in a subtropical climate, contained abundant phytoplankton, and was stocked with golden carp (*Carassius* sp.) and fancy carp. No surface cyanobacterial blooms have been observed in the pond since its construction at least 10 years ago.

No special feeding was provided for the carp, and the

food web in the pond was mainly comprised of phytoplankton, zooplankton, and carp. The depth of the pond is approximately 40 cm, with a Secchi depth of about 25 cm during the experimental period. The bottom of the pond was pelitic. The pond is in the center of a lawn and is equipped with a fountain and decorative rocks. Tap water is used as a water source for the pond.

At the beginning of the experiment, the chlorophyll *a* content of the pond water was approximately 200 μg/L. The dominant alga was *Ankistrodesmus falcatus var. mirabilis* G. S. West of Chlorophyta (Hu and Wei 2006).

The initial zooplankton was surveyed. The dominant zooplankton was Cyclops of copepodand its larvae (Shen 1979), with a total density of 12.5 ind./L.

Experimental design and monitoring

The experiment was conducted using transparent borosilicate glass jars with a volume of 5.0 L (16.0 cm diameter and 32.0 cm high) in a 182 m^2 greenhouse. The experimental period was during the summer from 22 June to 10 July 2011. A bioassay approach was used that was similar to that of Paerl and Bowles (1987), although our study was not *in situ*.

On the morning of 22 June, the pond water was collected in a large container and gently mixed to ensure an even distribution of phytoplankton. Then, the water was divided amongst the 6 jars. The sunlight was so strong that the glass roof of the greenhouse was covered with light grey cloth. The shading coefficient of the cloth was approximately 80%.

The jars were divided into treatment (+NP) and control groups which each had three replicates. There was no nutrient enrichment in the control, and nutrients were enriched in the treatment group. Nutrients were added thrice during the course of the experiment: at the beginning (day 0), 28 June (day 6), and 4 July (day 12). Enrichment of nutrients on day 0 included organic matter (thick liquid of decomposed *Lemna*) as well as inorganic nitrogen and phosphorus. The enrichment on day 6 and 12 was inorganic nitrogen and phosphorus alone. The content of nutrients added in each enrichment was determined by the net concentration increase, which was calculated according to the volume of water remaining in the jars and the added amount of nutrients.

The thick liquid of organic matter was obtained from the decomposition of *Lemna* in a closed jar that was left in the greenhouse for 2 months. The large particles were filtered out by a 150-mesh sieve before use. The enriched quantity of organic matter was 100 mL liquid for each jar, in which the net increase was as follows: 3.490 mg/L of total nitrogen (TN), 0.788 mg/L of total phosphorus (TP), 2.472 mg/L of dissolved total nitrogen (DTN), dissolved total phosphorus (DTP) 0.137 mg/L, and soluble reactive phosphorus (SRP) 0.113 mg/L. Inorganic nutrients were supplemented as KNO_3 and $K_2HPO_4{\cdot}3H_2O$, and the enrichment was a net increase of 2.1 mg/L of NO_3-N and 0.3 mg/L of PO_4-P at each of the 3 enrichment times.

Our preliminary experiment found that the zooplankton proliferated substantially, although they were filtered away at the beginning. In order to remove as much zooplankton and as little phytoplankton as possible at the beginning, a 125 μm nylon mesh was used to separate the zooplankton according to the technique of Vanni and Temte (1990). The filtration rate for the phytoplankton was lower than 1% (according to a comparison between the chlorophyll *a* concentration before and after the filtration), and hence the affect to phytoplankton content was ignored.

Measured items

The weather during the experiment was recorded. Water temperature; TN, TP, DTN, DTP, and SRP; chlorophyll *a* (Chl-*a*); and the community composition of phytoplankton and zooplankton were also measured.

Weather conditions were recorded daily, and water temperature was measured at 08:30 h and 14:00 h daily. TN, TP, DTN, and DTP concentrations were measured every 6 days. SRP, Chl-*a*, and phytoplankton community composition were measured every 3 days, while the zooplankton community was only determined at the end of the experiment. After the *in situ* water temperature measurement, the water was mixed gently and care was taken not to disturb the algae that had settled at the bottom. Sampling was conducted after the gentle mixing, and nutrient enrichments for the treatment were added as soon as possible. The nutrient concentrations after nutrient enrichments were calculated from the net addition and water volume instead of measurement of sub-samples after enrichment. Sub-samples were collected from the jars at 08:30 - 09:30 h by syphon.

Water temperature was measured using a mercury thermometer held at approximately 5 cm depth. Water for analysis of DTN, DTP, SRP, and Chl-*a* was filtered through GF/C filters (1.2 μm pore size, Whatman, Maidstone, U.K.), which were rinsed with deionized water before use. Measurements of TN, TP, DTN, and DTP were conducted according to the methods of Gross and Boyd (1998), while SRP was determined by molybdenum-antimony-ascorbic

acid colorimetry (Eaton et al. 1995).

Chl-*a* concentration was determined by colorimetry (Lorenzen 1967, Jespersen and Christoffersen 1987), in which water samples were filtered through GF/C filters, and the residue was extracted by 90% hot ethanol. Phytoplankton biomass was expressed as wet weight biomass. For determining phytoplankton density, 50 mL water samples were preserved with 1% Lugols solution and stored in darkness until identification. If the density of phytoplankton was too subtle for accurate counting, the sample was concentrated after settling. For enumeration, two replicate aliquots were placed in 0.1 ml plankton counting chambers that were modified from the Palmer and Maloney design (Palmer and Maloney 1954).

Most cells were observed at 400× magnification via light microscopy (Olympus CX31; Olympus, Japan), while large algal cells were observed at 100× magnification. Cells were mainly identified to the genus level as referenced by morphologies (Chen et al. 2002, Hu and Wei 2006). Algal volumes were calculated based on cell density and cell size measurements. Calculation of the cells volumes was according to their shapes, and the length, height, and diameter measurements were obtained to calculate the volume. The cells with irregular shapes were decomposed to some approximately regular geometry, and then the sum value was calculated as the total volume. At least 30 algal units were measured to obtain the average cell volume for each genera or species. The conversion to wet weight biomass assumed that 1 mm^3 of volume was equivalent to 1 mg of wet weight biomass.

A 112 μm nylon mesh was used to filter all the remaining water for zooplankton sampling at the end of the experiment. The zooplankton samples were preserved with 1% Lugols solution and 4% formalin (final concentration). Identification of zooplankton was according to references (Shen 1979, Zhang and Huang 1991). For the enumeration, two replicate aliquots were placed in 1.0 mL plankton counting chambers under 100× magnification (Zhang and Huang 1991). The identification was mainly to the genus level.

Statistical analyses

Responses of nutrients, Chl-*a*, and phytoplankton biomass (total biomass and the biomass of the main phyla and genera) between the treatment and control were analyzed by two-way ANOVA using repeated measures (nutrient × time). Before comparison, the percentage of total biomass accounted for by the principal phyla was arcsine square-root-transformed, nutrients concentrations were square-root-transformed, and Chl-*a* was log-transformed to increase homogeneity of variances (Underwood 1997). The comparison of zooplankton concentrations was done using a t-test, and the concentrations were log-transformed before comparison. Analyses were performed using SPSS 16.0 (Statistical Product and Service Solutions, IBM, USA). All data are shown as mean ± S.D. The differences are reported as significant if $P < 0.05$.

RESULTS

Phytoplankton community composition and changes

The phytoplankton in the treatment proliferated substantially during the experiment. The total wet weight of the phytoplankton and the wet weight of different phyla of the control are shown in Table 1, and the wet weight of the 5 dominant genera by wet weight are shown in Table 2. The total wet weight of the phytoplankton and the wet weight of different phyla of the treatment are shown in Table 3, and the wet weight of the 5 dominant genera by wet weight are shown in Table 4.

As Table 1 shows, the total wet weight in the control fell during the experiment in comparison with the initial value, and Chlorophyta was dominant for the entire period. The 5 dominant genera were also Chlorophyta, including *Ankistrodesmus*, *Chlorella*, *Gloeotila*, *Scenedesmus*, and *Staurastrum* (Table 2). Table 1 and Table 2 show that there were no substantial changes of the dominant genera in the control during the experiment.

Table 3 shows that the dominant phyla in the treatment during the experiment were Chlorophyta and Cyanophyta. Chlorophyta were dominant during the early stage, while Cyanophyta dominated from day 9 onward. Table 4 shows that the dominant genera in the treatment during the early stage were *Ankistrodesmus*, *Chlorella*, and *Scenedesmus*. On day 9, there is a switch to Cyanophyta genera dominance, including *Planktothrix*, *Chroococcus*, and *Merismopedia*. Table 3 and Table 4 show that there were substantial changes in the dominant genera in the treatment during the experiment, which switches from Chlorophyta dominance to Cyanophyta dominance.

ANOVA results indicated that the total wet weight and the wet weight of Cyanophyta in the treatment were significantly higher than in the control ($P < 0.05$), however, there was no significant difference in the wet weight of Chlorophyta between the control and the treatment ($P > 0.05$) (Table 5).

Table 1. Algal wet weight of total and of different phylum in the control (means ± SD) (mg/L)

Day	Total wet weight	Chlorophyta	Cyanobateria	Bacillariophyta	Pyrroptata	Euglenophyta
0	25.88	23.32	1.62	0.94	-	-
3	10.64 ± 0.99	9.65 ± 0.74	0.09 ± 0.14	0.90 ± 1.25	-	-
6	12.61 ± 1.87	12.01 ± 1.06	0.02 ± 0.02	0.13 ± 0.04	0.05 ± 0.77	0.00 ± 0.00
9	15.07 ± 1.57	14.48 ± 0.83	0.03 ± 0.02	0.20 ± 0.11	0.05 ± 0.7	-
12	13.54 ± 1.10	13.47 ± 1.06	0.02 ± 0.01	0.06 ± 0.05		-
15	10.90 ± 1.94	10.78 ± 1.81	0.01 ± 0.01	0.11 ± 0.11	-	-
18	8.42 ± 3.71	8.35 ± 3.61	0.01 ± 0.01	0.06 ± 0.10	-	-

Table 2. The 5 dominant genera of the control by wet weight (means ± SD) (mg/L)

Dominant genera	0 d	3 d	6 d	9 d	12 d	15 d	18 d
Ankistrodesmus	15.49	8.90 ± 0.45	11.32 ± 0.90	12.58 ± 0.81	11.61 ± 0.92	9.19 ± 1.44	7.23 ± 3.15
Chlorella	3.30	0.29 ± 0.02	0.49 ± 0.16	1.11 ± 0.05	1.17 ± 0.19	0.88 ± 0.40	0.29 ± 0.23
Gloeotila	-	0.22 ± 0.19	-	0.51 ± 0.15	0.52 ± 0.12	0.49 ± 0.28	0.70 ± 0.06
Scenedesmus	2.80	-	0.10 ± 0.05	-	0.07 ± 0.02	-	-
Staurastrum	-	-	0.05 ± 0.07	0.18 ± 0.11	0.07 ± 0.00	0.12 ± 0.14	-
Synedra	-	0.24 ± 0.16	0.08 ± 0.09	0.16 ± 0.14	-	-	-
Asterionella	-	0.62 ± 1. 07	-	-	-	-	-
Cyclotella	-	-	-	-	-	0.08 ± 0.07	-
Stephanodisus	-	-	-	-	-	-	0.06 ± 0.36
Pediastrum	0.70	-	-	-	-	-	-
Coelosphaerium	1.55	-	-	-	-	-	-
Chlamydomonas	-	-	-	-	-	-	0.05 ± 0.10

Table 3. Algal wet weight of the total and of different phylum in the treatment (means ± SD) (mg/L)

Day	Total wet weight	Chlorophyta	Cyanobateria	Bacillariophyta	Euglenophyta	Cryptophyta
0	24.52	22.97	0.36	1.19	-	-
3	10.11 ± 1.71	8.34 ± 1.22	1.31 ± 0.28	0.42 ± 0.27	0.00 ± 0.00	0.05 ± 0.03
6	24.17 ± 0.90	21.34 ± 0.49	2.10 ± 0.41	0.51 ± 0.20	0.00 ± 0.00	0.22 ± 0.12
9	38.36 ± 9.30	4.05 ± 2.37	34.08 ± 7.08	0.00 ± 0.05	-	0.18 ± 0.11
12	74.58 ± 8.81	13.60 ± 1.89	60.82 ± 10.13	0.12 ± 0.12	-	0.03 ± 0.06
15	76.06 ± 2.50	14.24 ± 6.27	61.82 ± 3.93	0.02 ± 0.03	-	-
18	93.99 ± 7.14	22.75 ± 9.71	71.20 ± 6.67	0.05 ± 0.08	-	-

Table 4. The 5 dominant genera of the treatment by wet weight (means ± SD) (mg/L)

Dominant genera	0 d	3 d	6 d	9 d	12 d	15 d	18 d
Ankistrodesmus	15.21	2.83 ± 0.80	1.32 ± 0.13	-	-	-	-
Chlorella	1.97	0.45 ± 0.09	2.11 ± 0.25	1.43 ± 1.06	4.02 ± 0.40	-	-
Scenedesmus	3.29	1.16 ± 0.25	2.45 ± 0.61	1.94 ± 1.40	7.00 ± 1.25	7.14 ± 4.29	8.79 ± 6.09
Planktothrix	-	1.16 ± 0.33	1.60 ± 0.23	32.26 ± 5.46	52.66 ± 12.24	55.04 ± 4.71	61.54 ± 7.80
Chroococcoid	-	-	-	1.22 ± 1.37	5.91 ± 1.46	3.18 ± 1.06	4.20 ± 1.09
Merismopedia	-	-	-	0.58 ± 0.53	2.17 ± 0.78	3.40 ± 0.38	5.43 ± 1.83
Gloeotila	-	3.53 ± 0.30	15.29 ± 0.92	-	-	4.43 ± 1.53	7.62 ± 1.07
Synedra	0.83	-	-	-	-	-	-
Westella	0.45	-	-	-	-	-	-

Composition and density of large zooplankton at the end of experimental period

The composition and density of large zooplankton at the end of the experiment are shown in Table 6. The dominant copepod was Cyclopoida, and the dominant Rotifera was *Monostyla*, *Brachionus*, *Euchlanis*, and *Asplanchna*. As Table 6 shows, cladoceran did not appear during the experiment, while copepods and Rotifera did appear.

T-test analysis results showed that the density of copepods in the treatment was significantly higher than in the control ($P < 0.05$). There were no significant differences between the treatment and the control in comparisons of the density of Rotifera, copepod larva, and the total zooplankton density.

Planktonic Chl-*a* responses to nutrients

Changes in planktonic Chl-*a* are shown in Fig. 1, which shows that the Chl-*a* decreased gradually in the control group and had an upward trend in the treatment group.

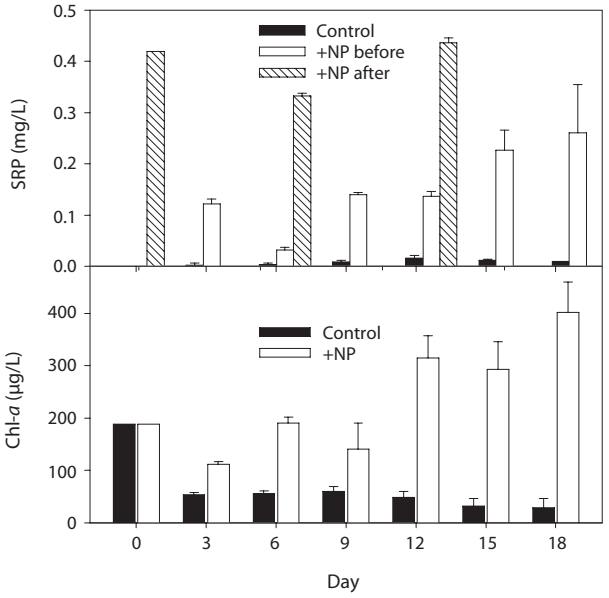

Fig. 1. Changes in SRP and Chl-*a* during the experiment. SRP, soluble reactive phosphorus; Chl-*a*, chlorophyll *a*; +NP before, water sampling before nitrogen and phosphorus addition; +NP after, water sampling after nitrogen and phosphorus addition.

Table 5. Results of the tests of between-subjects effects of repeated-measures ANOVA for comparison of nutrient concentrations and algal biomass

	Treatment versus control	
	F	*P*
TN	691.766	0.000***
TP	609.898	0.000***
DTN	2.419E3	0.000***
DTP	155.486	0.000***
SRP	270.102	0.000***
TN/TP	222.357	0.000***
DTN/DTP	37.303	0.004**
Chl-*a*	126.436	0.000***
Total algal wet weight	1.133E3	0.000***
Wet weight of Cyanophyta	482.726	0.000***
Wet weight of Chlorophyta	2.001	0.230
Percentage of Cyanophyta	890.581	0.000***
Percentage of Chlorophyta	249.674	0.000***

Nutrient (d.f.=1) effects were tested. *, **, and *** indicate significant differences with $P < 0.05$, 0.01, and 0.001, respectively. TN, total nitrogen; TP, total phosphorus; DTN, dissolved total nitrogen; DTP, dissolved total phosphorus; SRP, soluble reactive phosphorus; TN/TP, TN to TP ratio; DTN/DTP, DTN to DTP ratio; Chl-*a*, chlorophyll *a*.

Repeated measures ANOVA results showed that the planktonic Chl-*a* of the control was significantly lower than that of the treatment ($P < 0.05$).

The changes of mean ± S.D. in TN, TP, DTN, DTP, TN/TP, and DTN/DTP for the control and treatment are shown in Fig. 2, and the changes of SRP are shown in Fig. 1. Repeated measures ANOVA results showed that TN, TP, DTN, DTP, and SRP in the treatment group was significantly higher than in the control group ($P < 0.05$), and the TN/TP and DTN/DTP in the control was significantly higher than in the treatment ($P < 0.05$) (Table 5).

Weather conditions and water temperature

The weather during the experimental period was generally sunny or cloudy, except for 4 July (day 12) which was cloudy and rainy. The mean water temperature in the jars at 08:30 h and 14:00 h was 31.6°C and 34.6°C, respectively (Fig. 3).

Table 6. Composition and density of zooplankton at the end of the experiment (means ± SD)

	Copepoda (ind./L)	Copepoda larva (ind./L)	Rotifera (ind./L)	Total zooplankton density (ind./L)
Control	34.3 ± 7.7[a]	0.0 ± 0.0	2941.4 ± 2675.4	2975.7 ± 2667.7
+NP	110.8 ± 48.4[b]	7.8 ± 13.5	2999.5 ± 2639.5	3118.1 ± 2673.4

a and b indicate significant differences with $P < 0.05$. Contol, control group; +NP, treatment group.

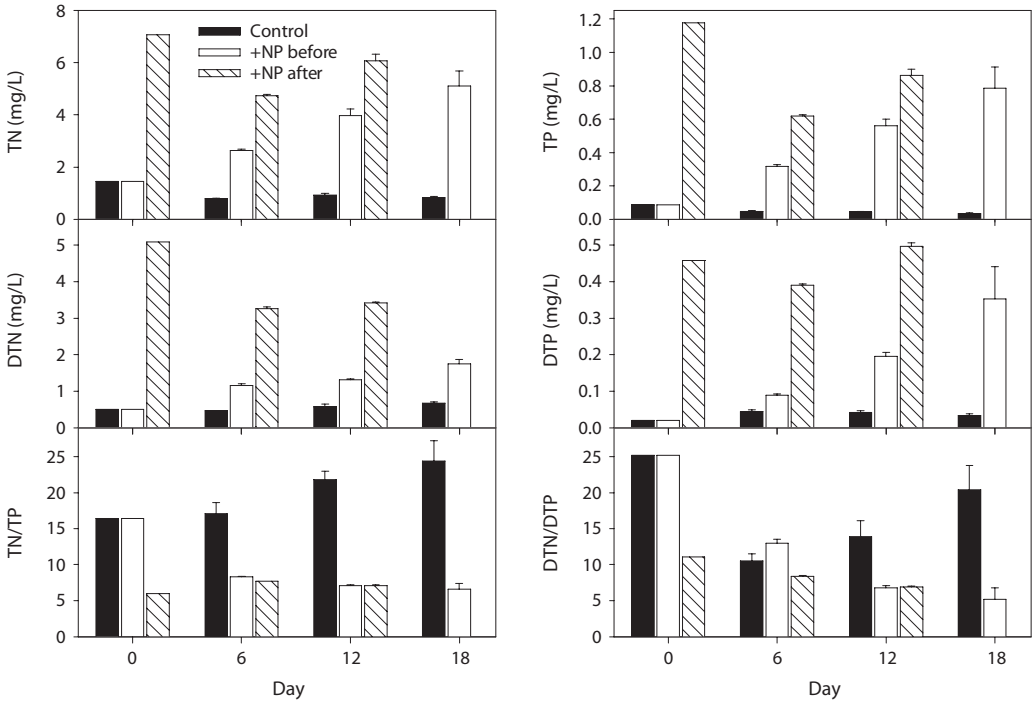

Fig. 2. Changes in TN, TP, DTN, DTP, TN/TP, and DTN/DTP during the experiment. TN, total nitrogen; TP, total phosphorus; DTN, dissolved total nitrogen; DTP, dissolved total phosphorus; SRP, soluble reactive phosphorus; TN/TP, TN to TP ratio; DTN/DTP, DTN to DTP ratio; +NP before, water sampling before nitrogen and phosphorus addition; +NP after, water sampling after nitrogen and phosphorus addition.

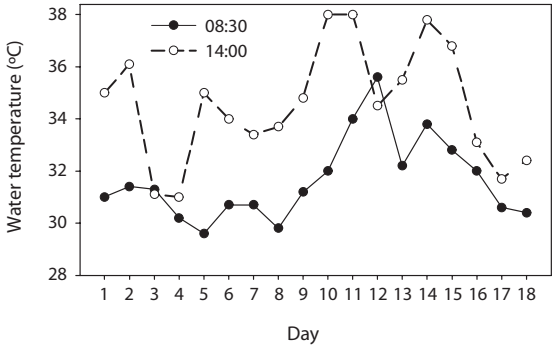

Fig. 3. The daily variations of water temperature at 08:30 h and 14:00 h during the experiment.

DISCUSSION

Resource competition between different phytoplankton is mainly for nutrients, and nutrient concentrations are a key factor affecting phytoplankton community composition (Steinberg and Hartmann 1988). This can be deduced from the succession of phytoplankton to bloom-forming cyanobacteria dominance. Harmful cyanobacterial bloom was inclined to form when the nitrogen and phosphorus nutrient was supplied to satisfy the proliferation need of algae (Richardson 1997, Prepas and Charette 2003, O'Neil et al. 2012). However, it is not necessary for the eutrophic water bodies with high concentrations of phytoplankton that exhibit cyanobacterial blooms (Wang et al. 2004). In this experiment, nutrient enrichment in the treatment induced the switch from Chlorophyta dominance to Cyanobacteria dominance in only 9 days (Table 3 and 4). This shows that nutrient enrichment affected the algal succession markedly. This is similar to the results of our previous experiments (Wang et al. 2010, 2011), in which cyanobacterial blooms were experimentally stimulated outdoors from water bodies initially free of such blooms. Cyanobacterial bloom in natural water usually occurs with eutrophication (Steinberg and Hartmann 1988, Richardson 1997, Prepas and Charette 2003, Qin et al. 2006, O'Neil et al. 2012), which agrees with the natural phenomena in which few cyanobacterial blooms formed in oligotrophic waters.

This experiment illustrated that a *Microcystis* bloom does not necessarily happen in eutrophic water with high temperature and strong solar irradiance, although Chlorophyta dominance switched to Cyanobacteria dominance. However, when TP reached high values in 1996

and 1997, *Microcystis* became less dominant and chlorophytes more dominant in Lake Taihu, China (Chen et al. 2003, Deng et al. 2014). However, the *Microcystis* bloom was extremely severe in the past 20 years in Lake Taihu, China. In shallow lakes and ponds, chlorophytes rather than cyanobacteria are often found to dominate under highly productive conditions (DeNoyelles and O'Brian 1978, Jeppesen et al. 1990, Jensen et al. 1994). This shows that cyanobacteria dominance does not always appear in eutrophic waters.

There are additional *in situ* examples for algal blooms formation after nutrient enrichment. A famous example is the Canadian Experimental Lakes Area (ELA) lake 226, where the lake side receiving carbon, nitrogen, and phosphorus developed eutrophic algal blooms while the side receiving only carbon and nitrogen did not (Schindler 1974). This study showed that phosphorus is very important for algal blooming. A dense *Microcystis* bloom exclusively dominated over the entire enclosure during a summer experiment in 2000 at Donghu Lake, a shallow hypereutrophic lake in Hubei, China (Tang et al. 2006). Hua and Zong (1994) successfully maintained a surface *Microcystis* bloom in enclosures with nutrient enrichment in the Yanghe reservoir of China. Ghadouani et al. (2003) also observed heavy cyanobacterial blooms of mixed-species with *in situ* shallow enclosures enriched with dissolved inorganic nitrogen (DIN) and phosphorus (DIP). However, these experiments were all constructed *in situ* with the influence of sediments and additional factors.

Besides the concentration of nutrients, nitrogen to phosphorus ratios also affect the phytoplankton community composition. It has been discussed in the literature that the TN to TP ratio (TN/TP) can affect the algal composition markedly (Schindler 1977, Liu et al. 2011) and when TN/TP < 29:1 the bloom-forming algae would dominate (Smith 1983). However, some results of research do not agree with the hypothesis. When TN/TP was much larger than 29:1, cyanobacteria dominance has also been observed (Shindler et al. 1980, Harris 1986, McQueen and Lean 1987). Furthermore, cyanobacterial blooms formed in water with relatively low TN/TP values as well. This suggests that low TN/TP values are a consequence of cyanobacterial bloom formation instead of its prerequisite (Xie et al. 2003). In this experiment, the TN/TP and DTN/DTP values in the control were all lower than 29:1, which were larger than those in the treatment, and the dominant algae in the control were Chlorophyta (Table 1 and 2). The TN/TP and DTN/DTP values in the treatment were near 7:1 as it was subjected to nutrient additions of N/P = 7:1, and cyanobacteria dominance formed in the treatment. This indicates that the different TN/TP and DTN/DTP values were not causal factors for the algal difference between the treatment and the control. This result agrees with the opinion of Trimbee and Prepas (1987). Therefore, one of the main causes for the dominant algae switch in this experiment is the concentrations of nutrients.

Besides nutrient concentrations, the feeding from zooplankton was also a causal factor for algal biomass and composition (Geller and Müller 1981, Pace and Cole 2000). The copepod density in the control was significantly lower than in the treatment, while there were no significant difference between the density of Rotifera, copepod larva, and the total zooplankton density (Table 6), suggesting that rotifers were not the main reason for the algal dominance difference between treatments.

Sommer et al. (2001) found that copepods mainly limited the large phytoplankton in a mesotrophic lake. The *Ankistrodesmus* cells of Chlorophyta in this experiment were as long as 70 μm and may have been selectively fed upon by the copepods in the treatment whose density was higher than that in the control. Surveys of the zooplankton community of Lake Taihu (China) during cyanobacterial bloom occurrences found that the dominant population was small cladocerans and that the density of copepods was much smaller (Chen and Qin 1998, Yang et al. 2008). It has been suggested that the small mouthpart of cladocerans is not big enough to feed on cyanobacteria colonies, leading to colonies of cyanobacteria surviving and proliferating to form blooms in Lake Taihu, China. According to the results of zooplankton succession in this experiment, copepods may play an important role in the conversion of the dominant algae. However, the effect of zooplankton selectively feeding on phytoplankton was based on the effect of nitrogen and phosphorus nutrients enrichment.

With global warming and water eutrophication, natural water which is rich in phytoplankton will be inclined to shift to cyanobacteria dominance or severe algal blooms. Research has shown that climate factors including solar radiation time and water temperature affected the formation and time duration of cyanobacteria blooms (Liu et al. 2011, Zhang et al. 2012), and earlier and warmer springs increase cyanobacterial (*Microcystis* spp.) blooms in the subtropical Lake Taihu, China (Deng et al. 2014). With climate changes and elevated eutrophication, many landscape waters would also switch to cyanobacterial dominance or even cyanobacteria blooming.

Different cyanobacteria bloom or dominate. Our previous experiments also provided some examples (Wang et al. 2010, 2011), e.g., a switch from *Scenedesmus* and

Pediastrum (Chlorophyta) dominance to *Microcystis* (cyanobacteria) dominance (Wang et al. 2010) and from *Aphanocapsa delicalissima* (cyanobacteria) dominance to *Microcystis* (Cyanobacteria) dominance (Wang et al. 2011). Moreover, Barica et al. (1980) made different algae species blooms occur in an enclosure experiment via various amounts of nitrogen addition. *Planktothrix rubescens* (Cyanobacteria) dominance appeared in Lake Zürich (Micheletti et al. 1998, Winder 2012). Dominance of filamentous cyanobacteria of the *Oscillatoria* group, such as the genera *Oscillatoria*, *Planktothrix*, and *Lyngbya*, have been frequently observed in shallow lakes (Scheffer et al. 1997).

Filamentous cyanobacteria dominance due to eutrophication is common in the Northern Hemisphere (Ernst et al. 2009). Van den Wyngaert et al. (2011) believed that *Planktothrix rubescens* represented a powerful competitor with prokaryotes, likely due to both its specific physiological (photoheterotrophic) properties and its protection against zooplankton grazing. Furthermore, most filamentous cyanobacteria which can bloom, were nitrogen-fixers, while the non-filamentous cyanobacteria usually cannot fix nitrogen (Oliver and Ganf 2000). To date, that the consensus has been that one of the most important reasons for the nitrogen-fixing filamentous cyanobacteria dominance was the low nitrogen or low nitrogen to phosphorus ratio (Levine and Schindler 1999, Schindler et al. 2008, Vrede et al. 2009, de Tezanos Pinto and Litchman 2010). However, the nitrogen concentration in this experiment was not low, and the nitrogen to phosphorus ratio was not larger than 29:1 in the treatment, which is not high in comparison to reference (Smith 1983). Additionally, we had similar nutrient enrichment levels in several of our previous experiments (Wang et al. 2010, 2011), but they resulted in *Microcystis* dominance instead of filamentous cyanobacteria dominance. Fujimoto et al. (1997) believed that non-nitrogen-fixing cyanobacteria may differ in their responses to N:P ratio and that some are superior competitors at low N:P ratios.

Research on the relationship between nitrogen fixers and carbon has shown that nitrogen fixers are inclined to appear in carbon rich sea water (Moisander et al. 2012). However, we do not know whether the *Planktothrix* in this experiment can fix nitrogen and we did not measure carbon in this experiment. We conjecture that the reasons for the *Microcystis* dominance or filamentous cyanobacteria dominance were dependent on the carbon level in the water. Carbon as well as nitrogen and phosphorus concentration in eutrophic water should be considered in studying the mechanisms of algal bloom formation.

The dominant algae switched from Chlorophyta to Cyanobacteria quickly after nutrient enrichment, although no *Microcystis* bloom formed. This may be related to the high water temperature and strong solar radiation as well as nutrient enrichment. Moreover, cyanobacterial blooms preferentially occur in the hot season (Paerl and Huisman 2008). In this experiment, the mean water temperature in the jars at 08:30 h and 14:00 h was 31.6°C and 34.6°C, respectively. This is higher than the surface water temperature which is approximately 30.0°C at Lake Taihu where severe *Microcystis* blooms occur in summer (Xu et al. 2010).

Field studies and seasonal correlations of the cyanobacteria dominance and water temperature show that cyanobacteria bloom formation preferentially occurs in water with high temperature (Reynolds 1984, Sommer et al. 1986). Foy et al. (1976) concluded that the temperature optima of cultures of *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* fo. *gracile*, *Oscillatoria agardhii*, and *Oscillatoria redekei* were similar to those of other planktonic autotrophs. Robarts and Zohary (1987) reviewed the temperature effects on bloom-forming cyanobacteria, which showed that P_{max} (a maximum photosynthetic rate), R_{est} (specific rates of respiration), and growth rate were temperature-dependent with optima usually at 25°C or greater. This indicated that direct temperature effects were secondary to both indirect temperature effects and nutrients in determining the bloom-forming cyanobacteria in lakes. Wiedner et al. (2007) also concluded that temperature had a major indirect impact on algal population dynamics based on an analysis of the spread of the freshwater cyanobacterium *Cylindrospermopsis raciborskii* in temperate regions.

Cellular processes of many phytoplankton are temperature dependent, and their rates accelerate exponentially with increasing temperature with maximal values occurring between 25 and 40 °C (Reynolds 1984). In this experiment, there were no treatments that varied water temperature to further show the effects of temperature. However, the high water temperature, which is suitable for the fast cellular acceleration, in combination with other factors such as nutrients and light, promotes the green algae dominance quickly switching to cyanobacteria dominance after nutrient enrichment. There must be additional factors affecting the process leading to *Microcystis* blooming. From our experiments with algal dominance switch (Wang et al. 2010, 2011), we conjecture that the dissolved oxygen level of the water body may increase substantially. As the water was taken from its initial environment into containers without sediments, the im-

proved dissolved oxygen level aided Cyanophyta in outcompeting other algae, especially green algae. Additional experiments are needed to explain this phenomenon.

This study provides a quick and simple method for cyanobacteria dominance (or bloom) formation *ex situ*. More experiments are needed to further study the threshold values of the water temperature, solar irradiance, and concentrations of nutrients for a certain algal bloom occurrence or the switch between different algae dominance. Future studies will also need to examine reasons for the dominance of different cyanobacterial genera.

CONCLUSION

In approximately 9 days, initial Chlorophyta dominance was converted to cyanobacteria dominance after nutrient enrichment with elevated water temperature. This provides a simple way for studying the roles of different environmental factors that affect algal bloom formation.

ACKNOWLEDGEMENTS

This study was financially supported by the National Natural Science Foundation of China (No. 41401580), the National "Twelfth Five-Year" Plan for Science & Technology Support Project of China (No. 2012BAD25B01), State Key Laboratory of Lake Science and Environment of China (No. 2010SKL006), and the 948 Program (No. 2013-S7).

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