

*Full Length Research Paper*

# Effects of temperature on recruitment and phytoplankton community composition

Xiao Tan

College of Environment, Hohai University, Nanjing 210098, China. E-mail: [biotan@163.com](mailto:biotan@163.com). Tel: +8613057610908.

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**Effects of temperature on phytoplankton recruitment and variations in phytoplankton community were studied by using hiemal sediment from Taihu Lake and performing a simulation experiment. Sediment samples were cultured in filtered lake water with elevated temperatures. Recruitment patterns and photosynthetic capacity of cyanobacteria, chlorophytes and diatoms were recorded, respectively. Results showed that recruitment of chlorophytes and diatoms was observed above 9°C, but recruitment of cyanobacteria was not evidently detected until 12.5°C. Chlorophytes dominated the phytoplankton community at 12.5 and 16°C, subsequently cyanobacteria established dominance above 19.5°C. In this study, algal cells remained weak photochemical vitality at lower temperatures before recruitment, which reactivated and increased gradually with elevated temperatures.**

**Key words:** Recruitment, temperature threshold, cyanobacteria, blooms, Taihu Lake, phytoplankton community.

## INTRODUCTION

In winter, some species of phytoplankton are capable of dormancy on lake sediment after autumnal sedimentation (Tsuji-mura et al., 2000; Brunberg and Blomqvist, 2002). These benthic portions are able to renew growth and return to the pelagic phase with increased temperature in spring (Ståhl-Delbanco and Hansson, 2002; Karlsson-Elfgren and Brunberg, 2004; Verspagen et al., 2005). Especially, in some eutrophic lakes, recruitment is a key process in cyanobacteria life cycle and blooms formation (Oliver and Ganf, 2000; Kong and Gao, 2005). Various versions of migration traps had been designed to study cyanobacteria recruitment in lakes (Hansson et al., 1994; Brunberg and Blomqvist, 2003; Cao et al., 2005). In labs, some simulation experiments were performed to investigate influences of environmental factors on cyanobacteria recruitment (Ståhl-Delbanco and Hansson, 2002; Li et al., 2004; Tao et al., 2005). According to these field studies and simulation experiment, temperature had been confirmed to play an important role in driving cyanobacteria recruitment (Latour et al., 2004a; Li et al., 2004; Tao et al., 2005). However, previous studies mainly focused on the recruitment of some species of cyanobacteria and did not adequately analyze the variations in phytoplankton community composition

synchronously. Furthermore, cyanobacteria undergo a series of biomass accumulation and population competitive processes with other algae from recruitment to dominance establishment before blooms formation (Cao et al., 2005).

These processes remained to be further understood. Variable chlorophyll *a* fluorescence yield has become an important tool for studying phytoplankton photosynthesis (Schreiber, 1994; Oliver and Whittington, 1998), because it is sensitive to photon flux density and is reliable as a parameter to offer insight into the immediate past light history of phytoplankton (Zhang et al., 2008). The ratio of maximum variable fluorescence to the maximum yield ( $F_v/F_m$ ) has been used to estimate changes in the proportion of functional reaction centers and as an indicator of the photosynthetic capacity of phytoplankton (Falkowski and Kolber, 1995). Moreover, PHYTO-PAM fluorometer allows a separate measurement of the fluorescence signal of each algal group in mixed phytoplankton populations. Accordingly, effects of temperature on photosynthetic capacity of different algal groups could be detected synchronously, which is an important physiological index to analyze recruitment and competitive processes (Latour et al., 2004a). In this

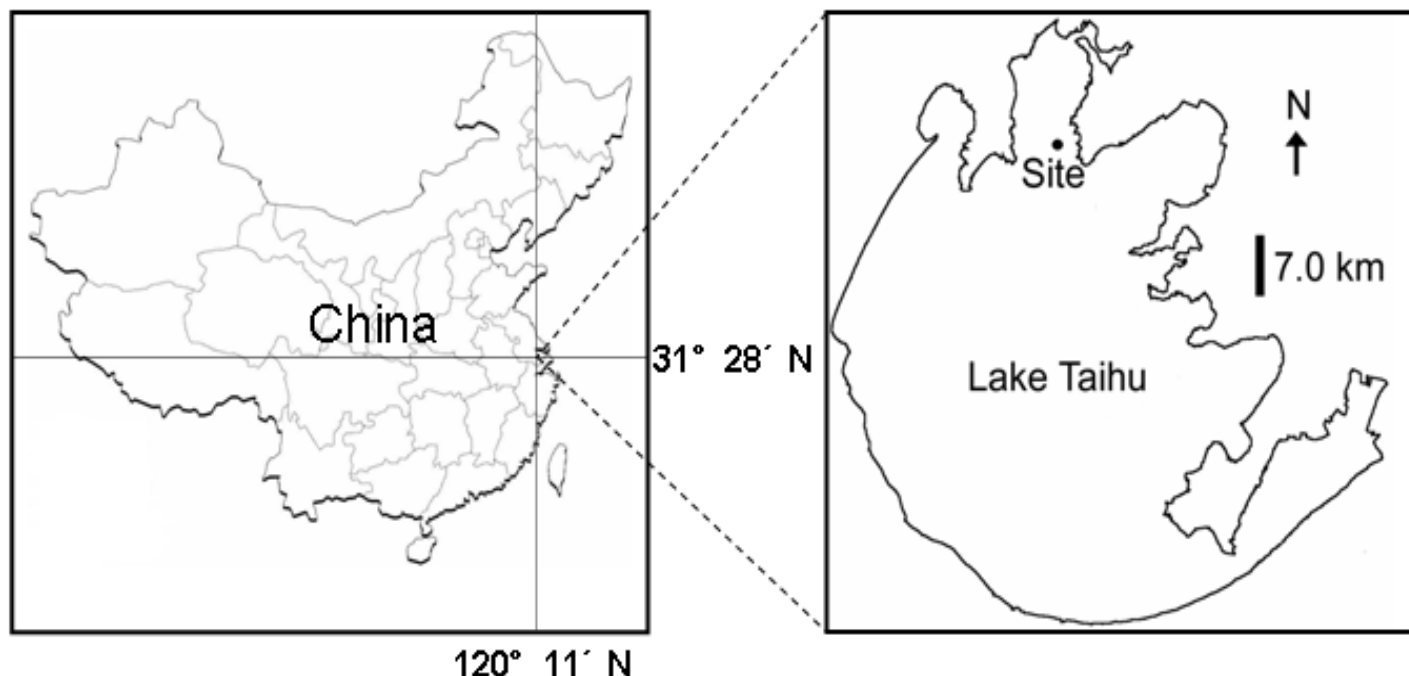


Figure 1. Location of the sampling site.

paper, a simulation experiment was performed by using hiemal sediment samples from Taihu Lake, which were static cultured in light incubator, so as to study the temperature threshold for algae recruitment and effects of temperature changes on algal community composition. Additionally, variations of algal fluorescence were also analyzed to investigate responses of different algal groups to temperature changes.

## MATERIALS AND METHODS

### Lake and sampling site description

Taihu Lake is a large eutrophic lake in China (with an area of 2, 338 km<sup>2</sup> and the annual average water depth of 1.9 m and maximum of 2.6 m) (Hu et al., 2006). Major cyanobacteria blooms composed of *Microcystis* spp. had appeared annually for decades in this lake (Chen et al., 2003). Meiliang Bay lies in the northern part of Taihu Lake, where serious blooms frequently occurred in summer (Chen et al., 2003; Tan et al., 2009). In the present study, the sampling site (31°28'46''N, 120°11'34''E) is located between Meiliang Bay and offshore regions (Figure 1).

### Sample collection and treatment

On January 9, 2008, about 300 g of surface sediment (0 to 3 cm) was collected at the sampling site by a columnar sampler (KC-Denmark). During the sampling period, underwater environmental parameters (such as depth, water temperature, density of cyanobacteria cells and chlorophyll a concentration) were real-time recorded by using a multi-parameter water quality sonde (YSI 6600V2, USA). Sediment samples were transferred to

laboratory immediately and were divided into three equal aliquots approximately. Each portion was laid on the bottom of a beaker. Subsequently, sterilized *in situ* lake water (5 L of 0.22 μm filtrate by Whatman GF/C membrane) were gently added. The three beakers were then incubated under an illumination intensity of 40 μmol photons m<sup>-2</sup> s<sup>-1</sup>, provided by cool white fluorescent lamps (36W FSL, China), with a light-dark period of 12/12 h. The incubation temperature increased along eight levels (5.5, 9, 12.5, 16, 19.5, 23, 26.5 and 30°C), with each temperature being maintained for three days.

### Microscopic analysis of phytoplankton

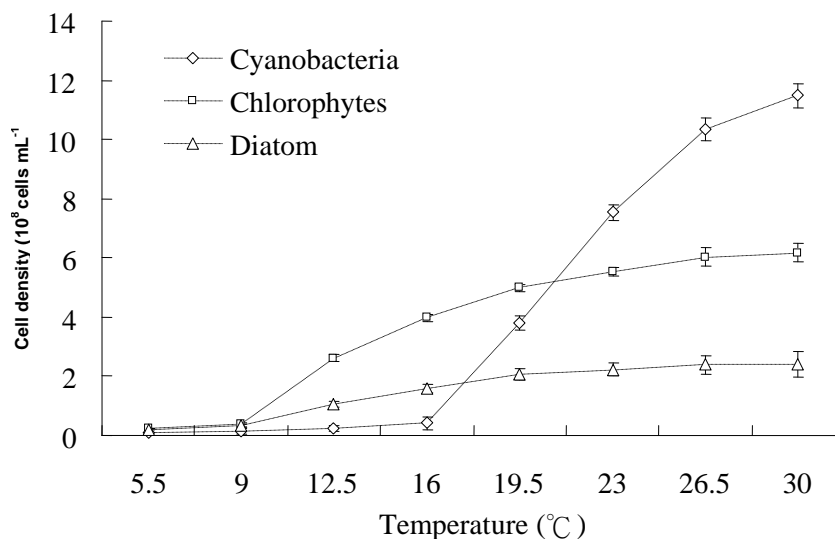
At the beginning and the end of each temperature level, 110 mL of the culture liquid were obtained gently from each beaker by a plastic tube (length=20 cm, diameter=2 cm). 10 ml of the culture liquid were used for algal fluorescence analysis immediately; the residual 100 ml were fixed with Lugol's iodine and sedimented for 48 h prior to microscopic analysis. Phytoplankton cell densities were enumerated by using a haemocytometer. And then, their specific growth rates (SGRs) were calculated according to the following equation:

$$\text{SGR} = \ln(C_t/C_0)/t$$

Where  $C_0$  is the initial cell density at the beginning of each temperature level,  $C_t$  is the cell density at the end and  $t$  is the duration of incubation period under each temperature level in days. Moreover, unicells, dividing cells (two connected cells), and colonies were enumerated. Colonies were grouped into consecutive groups: small colonies (cell number per colony between 2 and with a maximum of 10 cells), middle colonies (cell number per colony was up to 10 and maximum of 100 cells), and large colonies (cell number per colony with more than 100).

**Table 1.** Mean and ranges of real-time recorded environmental parameters at the sampling site.

Environmental parameter	Mean and ranges
Depth (m)	1.95 (1.93 to 1.97)
Water temperature (°C)	5.2 (5.1 to 5.3)
Chlorophyll a ( $\mu\text{g L}^{-1}$ )	0.09 (0.07 to 0.11)
Cyanobacteria cell density (cells $\text{mL}^{-1}$ )	129 (118 to 139)

**Figure 2.** Phytoplankton dynamics during the experiment.

### PHYTO-PAM fluorometer analysis

Algal fluorescence was measured by using a multiwavelength phytoplankton pulse-amplitude-modulated fluorometer (Phyto-PAM Walz, Germany) after dark adaption for 15 min. The Phyto-PAM fluorometer equipped with a special emitter-detector unit (Phyto-ED) for distinguishing cyanobacteria, chlorophytes and diatoms/dinoflagellates by means of four excitation wavelengths (665, 645, 520 and 470 nm). For instance, in chlorophytes chlorophyll fluorescence is much more effectively excited by blue and red light (470, 645 and 665 nm) than by green light (520 nm). In the case of cyanobacteria, almost no chlorophyll fluorescence is excited by blue light (470 nm), while excitation at 645 nm is particular strong due to phycocyanin and allophycocyanin absorption. As for diatoms and dinoflagellates, excitation by blue (470 nm) and green (520 nm) is relatively high resulted from strong absorption by fucoxanthin, chlorophyll c and carotenoids. The fluorescence signals from the four wavelengths excitation were assigned to the three algal groups by using the Phyto-WIN software (version 1.47) and the reference spectra (Zhang et al., 2008). The maximal efficiency of photosystem II photochemistry was determined as  $F_v/F_m$ , which was used for an indicator of the photosynthetic capacity of phytoplankton (Falkowski and Kolber, 1995).  $F_v/F_m$  was calculated by the following equation:

$$F_v/F_m = (F_m - F_0)/F_m$$

Where  $F_0$  is the fluorescence of dark-adapted algal cells stimulated by a weak probe light immediately after 15 min of darkness and  $F_m$

is the maximum fluorescence signal after the closure of all reaction centers by 600 ms pulse of saturating irradiance (Schreiber et al., 2002).

## RESULTS

### Environmental parameters at the sampling site

Sediment samples were collected in a sunny and windless day. Environmental parameters at the sampling site are displayed in Table 1. During the sampling period, water depth of the sampling site fluctuated between 1.93 and 1.97 m. This range was near to the average depth of Taihu Lake. Water temperature was about 5.2°C, which was very close to the initial culture temperature (5.5°C) and was easier for hiemal algae to accommodate. At the sampling site, chlorophyll a concentration and the density of cyanobacteria cells were fairly low in water.

### Phytoplankton dynamics

According to the algae growth curves (Figure 2) and the calculated SGRs, recruitment of chlorophytes and diatoms was observed at 9°C, but the recruitment of

cyanobacteria was not evidently detected until 12.5°C. SGR of cyanobacteria peaked at 19.5°C (about 0.256 D<sup>-1</sup>). As for chlorophytes and diatoms, the maximum of SGRs simultaneously appeared at 12.5°C, reaching to 0.231 D<sup>-1</sup> and 0.175 D<sup>-1</sup>, respectively. Particularly, *Microcystis* spp. (such as *Microcystis aeruginosa*, *Microcystis wesenbergii*, and *Microcystis flos-aquae*) constituted the dominant species of cyanobacteria. After recruitment, pelagic *Microcystis* colonies experienced an enlargement process, but unicells and dividing cells occupied the highest proportion all along (Figure 3).

### Variations in phytoplankton community composition

Phytoplankton community structure at different temperatures was displayed in Figure 4. At the lower temperatures (5.5 and 9°C), cyanobacteria, chlorophytes, diatoms and some species of euglenophyta or chrysophyta were found in the culture media. However, concentration of euglenophyta and chrysophyta cells did not markedly increase with elevated temperatures. Above 12.5°C, phytoplankton community was overwhelmingly composed of cyanobacteria, chlorophytes, and diatoms. Specifically to say, chlorophytes established dominance at 12.5 and 16°C. Subsequently, cyanobacteria maintained dominant position above 19.5°C.

### Algal photosynthetic capacity

The maximal efficiency of photosystem II photochemistry was measured at different temperature levels, and was demonstrated in Figure 5. Algae cells remained weak photochemical vitality before recruitment, their photosynthetic capacity increased gradually after recruitment. As for chlorophytes and diatoms, photosynthetic capacity peaked at 23°C, while that of cyanobacteria reached its peak value at 26.5°C.

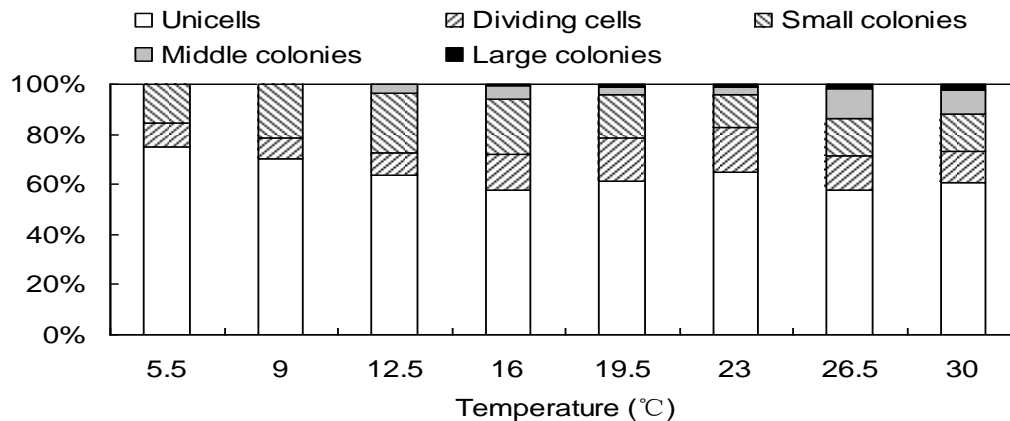
## DISCUSSION

Influences of environmental factors on algae recruitment had been investigated previously (Barbiero and Kann, 1994; Ståhl-Delbanco and Hansson, 2002; Cao et al., 2005; Tao et al., 2005). Based on most of the literature, temperature, light and sediment resuspension were recognized to be the most important driving factors (Tan et al., 2008). In this paper, a simulation experiment was performed by static culture in light incubator, aiming to study effects of temperature on algae recruitment and populations succession. According to the results, we inferred that the temperature threshold for cyanobacteria recruitment would be between 9 and 12.5°C, which was higher than that for chlorophytes and diatoms. This threshold range was in agreement with field studies in

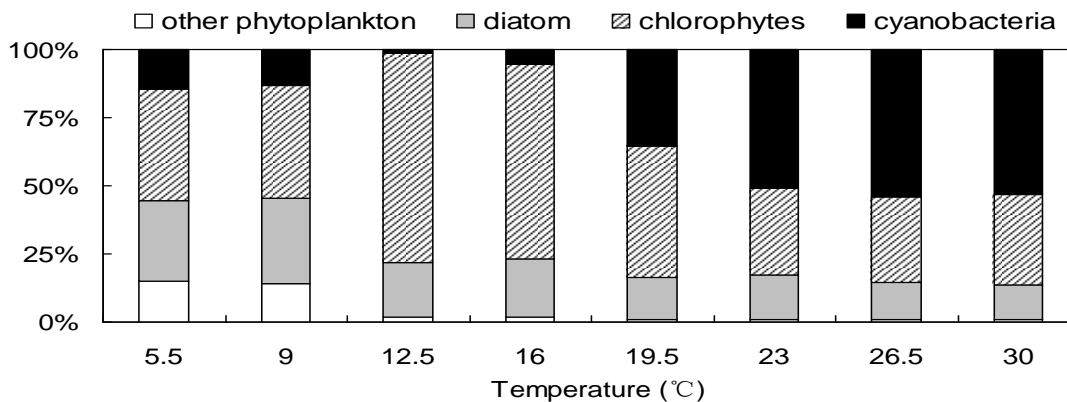
Taihu Lake made by other persons, who reported that benthic cyanobacteria started to grow in March when average temperature was about 10°C (Cao et al., 2005; Zhang et al., 2005). Previous studies indicated the protein synthesis of *Microcystis aeruginosa* accelerated when the temperature rose above 7°C, if below this threshold physiological metabolism of benthic cyanobacteria was bogged down (Càceres and Reynolds, 1984).

It suggested that temperature plays an important role in the recovery of the active form of *M. aeruginosa* in spring (Latour et al., 2004b). Considering facilitated action of resuspension and bioturbation in lakes, migration of overwintering phytoplankton in sediment might be initiated at lower temperature than static incubation (Karlsson-elfgren et al., 2004; Verspagen et al., 2004). In the experiment, sterilized lake water from sampling site was used as culture medium. Therefore, at the beginning of recruitment pelagic algae all originated from sediments. Thereafter, increases in abundances of the pelagic algae could result from two sources: growth of the phytoplankton already present in water and the amounts of recruitment from sediments. These two portions both contributed to the development of phytoplankton in water. Thus, recruitment of phytoplankton played two roles: either as a source of initial pelagic growth or as a supplement to further pelagic development (Cao et al., 2005). In the present study, recruitment of cyanobacteria initiated later than chlorophytes and diatoms, but higher SGR helped cyanobacteria establish and keep dominant position shortly after recruitment. Additionally, colony enlargement phenomena of *Microcystis* were also observed in the absence of zooplankton. Pelagic *Microcystis* mainly existed as unicells or small colonies comprising a couple of cells at lower temperatures and gradually formed large colonies after warming up.

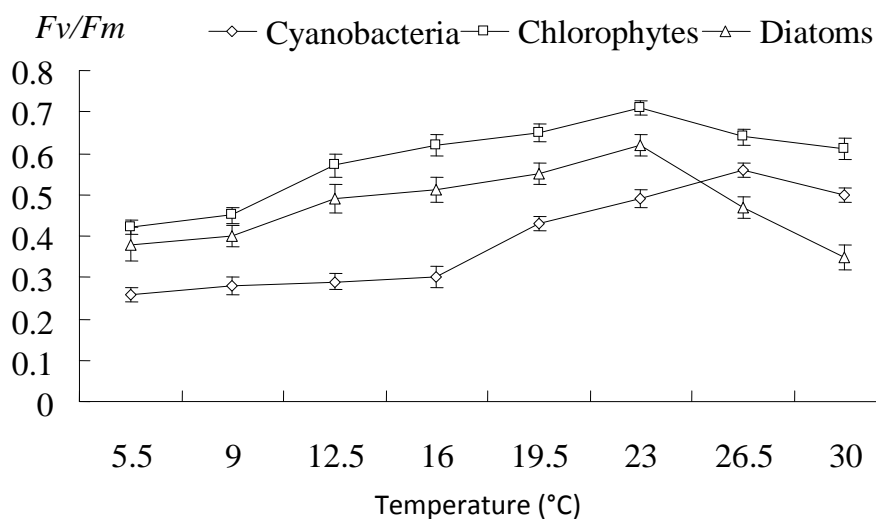
Two mechanisms are involved in the enlarging pattern: one is that colonies are formed when daughter cells of a recently divided cell remain in a regular arrangement during the reproductive process and the other is that formation of colonies is adhesion of already existing single cells (Lüring, 2003). Large colonies consisted of dozens or hundreds of cells, which could be conglomerated together by sheath to form blooms and effectively defend against grazing by zooplankton (Yang et al., 2006). Many *Microcystis* unicells and dividing cells coexisted with colonies after recruitment, the proportion of dividing cells showed a significant correlation to the frequency of dividing cells, which was mainly responsible for algae growth rate and cell viability (Latour et al., 2004b). Thus, rapid proliferation and colony enlargement strategy provided *Microcystis* with an effective competitive power. In contrast, chlorophytes and diatoms, did not maintain high growth rates for occupying ecological space, whose predominance was exceeded by cyanobacteria easily. Previous studies had successfully detected esterase activities of overwintering *M. aeruginosa* as an indicator for cell viability (Latour et al., 2004a).



**Figure 3.** Percentages of *Microcystis* spp. unicells, dividing cells, and colonies. Colonies were grouped into consecutive groups: small colonies (cell number per colony from 3 to 10), middle colonies (cell number per colony between 10 and 100), and large colonies (cell number per colony more than 100).



**Figure 4.** Phytoplankton community composition at different temperature levels.



**Figure 5.** Photosynthetic capacities of cyanobacteria, chlorophytes and diatoms at different temperature levels.

While, esterase activities of different algal groups hardly could be measured separately in mixed samples. In the present study, photosynthetic capacities of cyanobacteria, chlorophytes and diatoms were analyzed by using Phyto-PAM (Walz, Germany) and displayed by  $F_v/F_m$  index, respectively, owing to the absence of dinoflagellates all along. Before recruitment, phytoplankton remained weak photochemical vitality, these dormant algae cells could be referred to as 'physiologically resting cells' (Sicko-goad, 1986). They could be reactivated and increased gradually with elevated temperature (Li et al., 2004). Their maximum of photosynthetic capacity usually occurred at the optimum temperature for physiological metabolism (Blanchard et al., 1996). Interestingly, the maximal efficiency of photosystem II photochemistry did not change with growth rate synchronously. Frequency of algal dividing cells might be under the control of an endogenous component (Latour et al., 2004b), which merits further studies.

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