Algal Response Based on in situ Experiments of Nutrients Enrichment Bioassays in Lake Chaohu (China)

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Abstract: The algal response in relation to nutrient condition changes as well as environmental factors in Lake Chaohu (China) was investigated by in situ experiments of nutrients enrichment bioassays (NEBs) in this study. The results showed that both N and P enrichment could significantly inspire the algal growth especially in July. The highest relative algal growth coefficient compared with control obtained at TN/TP ratio of 10 reached 270% and 163% with respect to Chl-a and algal cell density, respectively. It indicated that algae blooms in Lake Chaohu are prone to occur at the TN/TP ratio around 10:1, which can be an early-warning indicator of algal blooms in Lake Chaohu. Furthermore, besides the nutrient conditions, the algal growth was significantly affected by environmental parameters such as water temperature and dissolved oxygen, which may also act as a trigger of algal blooms. This study suggested an alternative early-warning prediction method for algal blooms and provided basic information for eutrophication management scheme for Lake Chaohu.

1. Introduction

Approximately 90% of the lakes in China are in eutrophic state at the beginning of the 21st century [1]. In recent years, the lake algal blooms occur frequently, and blue algal blooms once overtook Chaohu Lake in Anhui province, China [2,3]. Harmful algal blooms not only lead to the rapid collapse of the aquatic ecosystem because of the release of cyanobacterial toxins, but also threaten aquaculture, water supply, tourism, and even drinking water safety [4]. In China, significant manpower and material and financial resources are put into lake eutrophication control [5,6].

Lake Chaohu, the fifth largest freshwater lake in China, has been subjected to severe eutrophication and cyanobacterial blooms over the past few decades. Survey date [7] found that Lake Chaohu is highly eutrophic and that water quality showed no substantial improvement from 2001 to 2011. For this reason, numerous studies have emphasized the precaution and prevention of algal blooms on Lake Chaohu. In general, algal blooms can be attributed to the excessive loading of N and P, BOD, and other trace elements, appropriate temperature and light, and anthropogenic disturbance [8], of which the excessive enrichment of N and P is the primary reason [9]. Currently, the studies on the correlation of nutrient inputs and algal response are largely based on nutrient enrichment bioassay (NEBs) [10]. The restrictions of nutrients to lakes are examined by observing the changes in algal cell density or Chl-a concentration, which ultimately provides support for eutrophication precaution [11]. NEBs present a more realistic reflection of the nutrient effects on algal specie growth with different phytoplankton communities in different times as the raw lake water is used directly without sterilization, filtration, and addition of algal species [12].
In this study, we aim to determine the key nutrients limiting algal growth using NEBs in Lake Chaohu. The nutrient conditions combining with the environmental physical and chemical indicators will help to predict algal blooms more accurately in Lake Chaohu.

2. Materials and methods

2.1 Sampling site and methods
The study was carried out at Lake Chaohu during the summer season in May–August. It is a left-bank water system in the downstream Yangtze River located between 30° 58´ and 32° 06´ N and 116° 24´ and 118° 00´ E in the Anhui Province of Southeast China, with an average depth of 3 m and an area of 760 km² (Fig. 1). During recent decades, Lake Chaohu has suffered from serious pollution resulting from substantial discharge of urban wastewater and agricultural runoff. It has become one of the most eutrophic lakes in China since the 1980s [7, 13]. The temperature averages 15-16°C throughout the year, 28-30°C in the hottest month (July), and 2-3°C in the coldest month (January) [14]. In general, the meteorological conditions of Lake Chaohu are favorable for the growth of algae.

The sampling site is located in the center of the western half of Chaohu Lake as shown in Fig. 1. As algal blooms are apt to happen in summer season, the experiment was conducted in May, June, July, and August. Surface water (<50 cm) was sampled and mixed in a 500L polyethylene-lined container in the respective four months before the NEBs tests. The characteristics of the sampled water were shown in Table 1.

Table 1. The characteristics of water samples

|       | TP (mg/L) | TDP (mg/L) | PO₄³⁻ (mg/L) | TN (mg/L) | TDN (mg/L) | Chl-a (mg/m³) | Algal cell density (×10⁴ cells/L) |
|-------|-----------|------------|--------------|-----------|------------|---------------|----------------------------------|
| May   | 0.32      | 0.10       | 0.08         | 1.55      | 1.33       | 43.02         | 5073.48                          |
| June  | 0.33      | 0.15       | 0.13         | 1.50      | 1.22       | 33.13         | 3241.72                          |
| July  | 0.17      | 0.09       | 0.06         | 2.00      | 1.48       | 32.46         | 4741.72                          |
| Aug   | 0.18      | 0.08       | 0.05         | 0.52      | 0.42       | 26.66         | 3163.36                          |

*TDP, total dissolved phosphorus; TDN, total dissolved nitrogen; Chl-a, chlorophyll a concentration.

2.2 Nutrient Enrichment Bioassays
The mixed water sample was dispensed into 20L-translucent polyethylene cylindrical containers (with 80% light penetration) and numbered according to the specific program as shown in Table 2. The experimental design included a control (no nutrient addition), phosphorus additions (P treatment) and nitrogen additions (N treatment). The nutrient addition schemes for the NEBs were as Table 2. Potassium dihydrogen phosphate (KH₂PO₄) and potassium nitrate (KNO₃) were used as sources of phosphorus and nitrogen, respectively. For the P treatment, PO₄³⁻-P equivalent to 1, 2, 4, 8, 16 times of initial TP concentration in raw water of Chaohu Lake in the corresponding months (shown in Table 1)
are added, respectively. Meanwhile, the sufficient TN (10mg/L) was supplied in every treatment to simulate the extreme eutrophic state. For the N treatment, NO$_3^-$-N is added in line with the arithmetic or geometric sequence of the initial TN level of Chaohu Lake in the corresponding months and the sufficient TP (5mg/L) was also supplied for each treatment at the same time. All experiments were performed in triplicate.

| Table 2. Nutrient adding methods of NEB test (mg/L) |
|---------------------------------|----------|----------|----------|----------|
|                                | May      | June     | July     | August   |
| Control                        | TN       | TP       | TN       | TP       |
|                                | 1.55     | 0.32     | 1.50     | 0.33     | 2.00     | 0.17     | 0.52     | 0.18     |
| P treatment                    |          |          |          |          |          |          |          |          |
|                                | TN=10mg/L| TN=10mg/L| TN=10mg/L| TN=10mg/L|
|                                | TP       | TN/TP ratio* | TP       | TN/TP ratio | TP       | TN/TP ratio | TP       | TN/TP ratio |
| P1                             | 0.32     | 83.04     | 0.33     | 67.10     | 0.17     | 130.25     | 0.18     | 126.53     |
| P2                             | 0.64     | 41.52     | 0.66     | 33.55     | 0.34     | 65.13     | 0.35     | 63.27      |
| P3                             | 1.28     | 20.76     | 1.32     | 16.77     | 0.68     | 32.56     | 0.70     | 31.63      |
| P4                             | 2.56     | 10.38     | 2.64     | 8.39      | 1.36     | 16.28     | 1.40     | 15.82      |
| P5                             | 5.12     | 5.19      | 5.28     | 4.19      | 2.72     | 8.14      | 2.80     | 7.91       |
| N treatment                    |          |          |          |          |          |          |          |          |
|                                | TN       | TN/TP ratio | TN       | TN/TP ratio | TN       | TN/TP ratio | TN       | TN/TP ratio |
| N1                             | 1.55     | 0.69      | 1.50     | 0.66      | 2.00     | 0.89      | 0.52     | 0.23       |
| N2                             | 2.55     | 1.13      | 2.00     | 0.89      | 4.00     | 1.77      | 1.04     | 0.46       |
| N3                             | 4.55     | 2.02      | 3.00     | 1.33      | 6.00     | 2.66      | 2.08     | 0.92       |
| N4                             | 6.55     | 2.90      | 5.00     | 2.21      | 8.00     | 3.54      | 4.16     | 1.84       |
| N5                             | 8.55     | 3.79      | 9.00     | 3.99      | 10.00    | 4.43      | 8.32     | 3.68       |

*TN/TP ratio is molar ratio.

The NEB tests were carried out once a month during May-August. The various treatment containers were sealed and fastened to a piling pillar on the shore of Lake Chaohu. Supporting by buoyancy, the containers suspend on the water surface to obtain natural light. And the incubation lasted 8 days until the stationary phase of algal growth. During the whole NEB tests, the water samples from each treatment were collected after mixing every day and immediately sent back to the lab for algal and physical and chemical characteristic analysis.

2.3 Algal growth rate
The response of phytoplankton to the treatments was determined by measuring concentration of chlorophyll a (Chl-a) and algal cell density.

The specific growth rate (μ) during the exponential growth (up to the 6-7th day) phase was calculated according to ISO 10253 (1995) [15]:

$$
\mu = \frac{\ln(B_n/B_0)}{t_n-t_0}
$$

Where, $B_0$ the Chl-a or algal cell density at the beginning of the test ($t_0$); $B_n$, the Chl-a or algal cell density after n days ($t_n$).

In order to eliminate the differences caused by the sampled water during difference months, the relative coefficient (RC) of algal growth in comparison to the control is used to compare the impact of additional nutrients on phytoplankton growth [16,17], and the formula is expressed as:
The average specific growth rate based on algal cell density (a) and Chl-a concentration (b) at different N/P treatments.

Yang et al. [7] reported that when the trophic condition was assessed according to the classification proposed by Forsberg and Ryding [18], Lake Chaohu was found to be hypertrophic over 2001 to 2011. The nutrient additions of NEBs were based on the initial concentrations of raw water in Lake Chaohu, which determined that the N and P test levels were high in the NEBs. In the NEBs test, both phosphorus (P treatment) and nitrogen (N treatment) enrichment induced much greater algal growth than the controls indicating strong responses to additions of N and P. Also, it is noticed that the average specific growth rate obtained in July are higher than the ones obtained in the other three months under all the N/P treatments.

The average specific growth rate based on algal cell densities increase with both N and P enrichments as shown in Fig. 2(a). As the TP concentration increased from 0.17 to 2.8 mg/L, the algal growth were sharply activated; but from 2.8 to 5.28 mg/L, the increase of the algal growth rate was not significant in July and August and a decrease was observed in May and June’s treatments. As N additions increasing from 0.52 to 10 mg/L, the average specific growth rate always increased sharply. This indicated that the algal growth can be promoted by the increase of either N or P concentrations in a high eutrophic state, but excess P nutrients may inhibit the growth of algae under certain extra environmental conditions. The Chl-a concentration present nearly the same trend in Fig. 2(b) but presenting a higher growth rate compared with Fig. 2(a). Chl-a concentration and algal cell density can reflect the algal growth process and be used as characterization parameters for algal bloom prediction. The regression analysis reveals that the algal cell density shows positive correlation with Chl-a concentrations and the correlation coefficient ($R^2$) are 0.618, 0.850, 0.899, and 0.831 of May, June, July and August’s treatments, respectively.
3.2 Nutrient Enrichment Bioassay: Effect of TN/TP ratio on algae growth

Algae growth is closely related to the TN/TP ratio. Appropriate TN/TP ratios can provide basic data support for the algal growth status and the control of algal blooms. The TN/TP molar ratios range 0.23–130.25 in this study (Table 2). The RC under different TN/TP ratios are showing in Fig. 4. The highest RC reached 270% in August and 163% in May with respect to Chl-a and algal cell density, respectively. And both of them nearly obtained at TN/TP ratio of 10. When TN/TP ratio is below 10, the RC increased with the increasing TN/TP ratio, while it decreased with the increasing TN/TP ratio above 10.

In this study, as TN/TP ratio is lower than 10 indicating that phosphorus was sufficient, nitrogen addition to the environment with a low TN/TP ratio (<10) is likely to trigger algal blooms in severe eutrophic lakes with high nitrogen and phosphorus concentrations. When TN/TP ratio is above 10, the addition of phosphorus to the high TN/TP ratio environment may result in the quick algal growth. The TN/TP ratio of raw water in Lake Chaohu were 4.8, 4.5, 2.9 (<10) in May, June and August, respectively, while in July, the TN/TP ratio is 11.8 (around 10). As such, nitrogen addition to the Lake Chaohu in May, June and August, and either nitrogen or phosphorous addition in July may result in algal blooms in Lake Chaohu. The TN/TP ratio around 10:1 can be a indicator of forecast and early warning of algal blooms in Lake Chaohu.
3.3 Effect of Environmental factors on the algal growth

Algae growth and algal bloom are affected by numerous factors at the same time. Although the excessive enrichment of N and P is the primary reason of algal blooms, the environmental factors, such as water temperature, light intensity, wind direction, and wind speed may also act as a trigger of algal blooms. Relationship between algal density and water temperature, pH, ORP, DO and electric conductivity (EC) was investigated by Pearson correlation analysis in SPSS 18.0. The results revealed that algal density was strongly related to water temperature and DO, and their correlation coefficients are 0.510 and 0.629, 0.580 and 0.789, 0.508 and 0.792, 0.637 and 0.737 in May, June, July and August, respectively, indicating that algal growth were significantly affected by water temperature and DO (Table 3). However, further studies are needed to elucidate the direct effects of N/P limitations on phytoplankton growth in association with multi-interactive environmental factors.

Table 3. Pearson correlations for environmental variables and algal density.

|        | May       |       |       |        | June      |       |       |
|--------|-----------|-------|-------|--------|-----------|-------|-------|
| pH     | -0.28     | 1     |       | 0.45   | 1         |       |       |
| ORP    | 0.035     | 0.071 | 1     | -0.23  | -0.165    | 1     |       |
| DO     | -0.225**  | 0.030 | -0.465** | 1     | -0.775** | -0.199** | 0.277** | 1 |
| C      | 0.515**   | -0.200** | -0.163 | 0.041  | 1         | 0.144 | 0.354** | 0.012 | -0.090 | 1 |
| Algal density | -0.510** | 0.026 | -0.337** | 0.629** | 0.083     | 0.580** | 0.408** | -0.141 | 0.789** | 0.325** | 1 |

|        | July      |       |       |        | August    |       |       |
|--------|-----------|-------|-------|--------|-----------|-------|-------|
| pH     | 0.130     | 1     | 0.080 | 1      | 1         |       |       |
| ORP    | -0.305**  | -0.261** | 1     | 0.182  | -0.011    | 1     |       |
| DO     | -0.115    | 0.103 | 0.141 | 1      | 0.000     | 0.138 | 0.158** | 1 |
| C      | -0.045    | 0.114 | 0.041 | 0.016  | -0.245**  | 0.557** | 0.008 | 0.089 | 1 |
| Algal density | 0.508** | 0.105 | -0.013 | 0.792** | 0.376**   | 0.637** | 0.548** | -0.137 | 0.737** | 0.341** | 1 |

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level; T, water temperature; EC, electric conductivity.

4. Conclusions

Under different TN/TP ratios and external conditions, there are some different effects on the average specific growth rate, maximum specific growth rate, maximum standing biomass, and RC. As far as Lake Chaohu is concerned, algal blooms are prone to occur at the TN/TP ratio around 10:1; furthermore, nitrogen addition to the Lake Chaohu in May, June and August, and either nitrogen or phosphorus addition in July may result in algal blooms in Lake Chaohu.
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