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Original Research

A Kinetics Model for Predicting *Microcystis* Growth Based on the Synergistic Effect of Nitrogen and Phosphorus on the Growth of *Microcystis densa* (Cyanobacteria)

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Abstract

Microcystis blooms occur frequently in subtropical lakes and reservoirs and cause serious ecological and economic losses. A novel *Microcystis* bloom caused by *Microcystis densa* has broken out in many reservoirs in South China. In order to control *M. densa* bloom, it is important to predict the growth of *M. densa*, which is influenced by the synergistic uptake and assimilation of nitrogen and phosphorus. Therefore, it is necessary to develop a model of the response of *Microcystis* growth to nitrogen and phosphorus concentrations. Our study focused on both nitrogen and phosphorus, and studied their synergetic effect on *M. densa* growth. The growth kinetics, including the maximum specific growth rates and the half-saturation constant of *M. densa* for both nitrogen and phosphorus, were also determined. A modified model with Monod kinetics was developed to explain the synergistic effects of nitrogen and phosphorus on *M. densa* growth. The specific growth rate of *M. densa* can be predicted by nitrogen and phosphorus concentrations. This modified model was verified by the actual measures of the Lianhe Reservoir and Liuxihe Reservoir. It could relatively accurately predict the growth level of *M. densa* and helped for the daily monitoring of reservoir management for *M. densa* growth.

Keywords: kinetics model, *Microcystis densa*, nitrogen, phosphorus, growth

Introduction

Cyanobacteria *Microcystis* blooms are common in many lakes and reservoirs where they usually cause negative consequences for environment and human health [1, 2]. Usually, these *Microcystis* blooms are cuased by *Microcystis aeruginosa*, which produces toxins that poison aquatic life [3]. However, A new *Microcystis* bloom caused by *Microcystis densa* has emerged in some subtropical reservoirs in southern China [4]. *M. densa* are common in rivers, lakes and reservoirs [5], but there is no or rarely reports of its bloom before 2012. However, a *M. densa* bloom has occurred every summer in Lianhe Reservoir since 2012, causing serious damage to the ecology.

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The mechanisms of *Microcystis* outbreaks have been studied, and it is suggested that their proliferation is the effect of multiple factors, such as the decrease in the ingestion rates of zooplankton, the weakening of the net flows of estuaries, and suitable temperatures and nutrients [6]. Among these, increasing nutrient inputs are widely considered an important factor for high densities of *Microcystis* [7]. Bioavailable nutrients concentrations, including nitrogen (N) and phosphorus (P), can affect the physiological conditions and the growth of *Microcystis*. *M. densa* belongs to *Microcystis* (Cyanobacteria) and it's growth is also affected by the concentrations of N and P. Therefore, in order to timely detect fast growth of *M. densa* and take controlling measures for preventing bloom, establishing a *M. densa* growth model based on N and P concentrations in water column is necessary.

N is the crucial nutrient for *Microcystis* in temperate and subtropical lakes and reservoirs and affects photosynthesis and the biosynthesis of macromolecules, such as proteins, nucleic acids, and chlorophyll [8]. Nitrate (NO_3^-) is the dominant form of dissolved inorganic nitrogen in most lakes and reservoirs and can be taken up by *Microcystis* for development [9, 10]. Although cells using NO_3^- must induce the release of nitrate reductase and expend extra energy, NO_3^- can be more readily accumulated within cells than other forms of nitrogen [11]. Comparing to NO_3^-, ammonium (NH_4^+) can be transported directly in algal cells without additional energy, but NH_4^+ concentration in natural water body is not very high, and excess cellular NH_4^+ is toxic to algal cells [12]. Therefore, NO_3^- is still considered the primary preferred nitrogen source for phytoplankton in the aquatic ecosystem.

P is also the essential nutrient that is required for the growth of *Microcystis* and their DNA, RNA, and energy transfer. Bioavailable phosphorus correlates with the abundance and growth rates of phytoplankton [13]. However, P in many freshwater ecosystems is often found in a very low concentration, particularly in the surface layer [14, 15]. Therefore, P is considered a key component in for *Microcystis* development [16]. Many studies have suggested that some cyanobacteria including *Microcystis,* can still bloom when the P concentrations is near or below detection limits [17]. Many studies also showed that P can be absorbed quickly into algal cells through alkaline phosphatase (AP) in the condition of phosphorus limitation. But the mechanism of enzyme production is closely related to the phosphorus concentration and algal species [18, 19]. Therefore, it is necessary to understand the effect of P on *Microcystis* growth at different concentrations, especially under very low concentrations of phosphorus.

The integrated function of nutrients on the growth rates and competing ability of phytoplankton has been the focus of increasing attention. Several studies reported that the stoichiometry of N:P can successfully account for shifts in environmental changes and cellular conditions [20, 21]. N and P can influence both the metabolism of algal cells and the production of organic compounds, which addresses the variation of cell growth rates and physiology and even affects the ability of phytoplankton to survive [22-24]. Therefore, the growth of *Microcystis* in natural water body is affected by N and P together. It should be considered simultaneously the effects of N and P on the growth of *M. densa*.

To understand the synergistic promoting mechanism of N and P the growth rate of *M. densa*, the affinity of *M. densa* for N and P was used to evaluate the ability of uptake and assimilation for nutrients, determining their growth rates and the ability to compete in the phytoplankton community. The affinity coefficients, including the maximum specific growth rate (μ_{max}) and half-saturation constant (K) of *M. densa* for N and P, are the parameters which indicate the response of phytoplankton growth to nutrients. The μ_{max} indicates that the ratio of cells increased from the previous day. The higher the μ_{max} is, the faster the algal cells grow. The K indicates the ability of algal cells to get nutrients in oligotrophic lakes or reservoirs. The lower the K is, the lower the concentrations of nutrients that the algal cells can obtain. Therefore, a high μ_{max} and low K could be beneficial to the competition of phytoplankton on nutrients. Most research has focused attention on the effects of a single nutrient (N or P) rather than integrated N and P on the growth of *M. densa*. However, it is impossible to have a single nutrient in lakes and reservoirs, N and P usually worked together. Therefore, the integrated action of N and P on the growth of *M. densa* is particularly important. When the integrated values of μ_{max} and K of *M. densa* for N and P were known, the biomass of *M. densa* could be estimated by measuring the nutrient concentrations.

In order to build a model which can predict *M. densa* growth, the objective of our work was to (1) quantitatively evaluate the mechanistic active growth model describing the response of *M. densa* growth to both N and P, (2) provide the simplest model to describe *M. densa* growth performance based on the different concentrations of N and P, (3) contribute to the understanding of the nutrient mechanisms of *M. densa* blooms in subtropical reservoirs, and (4) help to predict the growth of *M. densa*.

Materials and Methods

Algal Strain and Culture Condition

The Chinese strain of *Microcystis densa* (No. MD-1) was isolated from the water column of Lianhe Reservoir (23°17′57.2″N, 113°55′8.8″E) in Guangdong Province during a *M. densa* bloom in July 2017 and was maintained in the School of Environmental Science and Engineering, Sun-Yansen University, China.

Prior to the experiment, the culture of *M. densa* was re-inoculated 4 times during the exponential phase

in BG-11 medium for Blue Green Algae [25]. $NaNO_3$ was used to be N source in the media and the concentration was reduced gradually from 1.5 to 0.7, and 0.1, then to 0.05 g l^{-1}. K_2HPO_4 was P source and the concentration was from 40 to 10, and 1, then to 0.1 mg l^{-1}. The cultures were maintained at 28±1ºC in a light dark cycle of 12:12 with irradiation of 100 µmol photons m^{-2} s^{-1}. The transfer of these cultures for continuous maintenance was conducted aseptically.

The antibiotics, including penicillin G and streptomycin sulfate, were used to prevent bacterial contamination 48nh before the next inoculation [26]. The check for bacterial contamination using 4',6-diamidino-2-phenylindole (DAPI) (Sigma) staining at regular intervals was made by microscopic inspection.

The Growths of *M. densa* under N and P Substrates with Different Concentrations

The initial cell densities of *M. densa* in the media of both N and P substrates were ~ 0.1 × 10^6 cells ml^{-1}. BG-11 medium was used for the culture of *M. densa*. For the experiment under N subtrates, $NaNO_3$ as N substrates was added into the culture with the final concentrations of 0, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 2, 4, 8, 20 and 40 mg N l^{-1}, while the P concentration was added according to BG-11 medium. For the experiment under P substrates, K_2HPO_4 as P substrates was added to the culture of *M. densa* with final concentrations of 0, 0.001, 0.005, 0.01, 0.02, 0.04, 0.08, 0.1, 0.5, 1, 10, and 20 mg P l^{-1}, while the N concentration was added according to BG-11 medium. These treatments were all performed in triplicate, and were maintained at 28±1ºC in a light dark cycle of 12:12 with an irradiation of 100 µmol photons m^{-2} s^{-1}.

The samples for the cell counts were obtained daily and were fixed in a 2 % acid Lugol's solution. The cell density was measured with a Sedgwick-Rafter counting chamber under a fluorescence microscope CKX41 (Olympus, Japan) with 200-400 × magnification. The phytoplankton species were identified as suggested by Hu and Wei [5]. The specific growth rates (μ, d^{-1}) of *M. densa* meant relative growth rate and were calculated according to the equation:

$$\mu = \frac{ln\,N_2 - ln\,N_1}{t_2 - t_1} \quad (1)$$

...where N_2 and N_1 were the cell densities of *M. densa* at days t_2 and t_1, respectively. In this study, for example, when the cell density of *M. densa* at day 1 was N_1 and the cell density at day 2 was N_2, the μ (d^{-1}) was calculated: $\mu = (\ln N_2 - \ln N_1)/(2-1) = \ln (N_2/N_1)$.

Model Development

The model was quantitatively evaluated to describe the specific growth rates of *M. densa* responding to different N and P concentrations. Based on the Monod equation [27], the specific growth rates of *M. densa* (μ, d^{-1}) against the N and P concentrations were calculated as below, respectively:

$$\mu_N = \mu_{maxN} \frac{C_N}{K_N + C_N} \quad (2)$$

$$\mu_P = \mu_{maxP} \frac{C_P}{K_P + C_P} \quad (3)$$

...where μ_N, and μ_P are the specific growth rate of *M. densa* (d^{-1}) under N, and P substrates, respectively; μ_{maxN}, and μ_{maxP} are the maximum specific growth rates (d^{-1}) of *M. densa* for N and P, respectively; C_N, and C_P are the concentrations of N (mg l^{-1}), and P (mg l^{-1}) in the treatments, respectively. In addition, K_N and K_P are the half-saturation constants for the N (mg l^{-1}) and P (mg l^{-1}) substrates, respectively.

The specific growth rate of *M. densa* (μ_A) associated with the synergistic effects of N and P can be expressed in the form:

$$\mu_A = \left(\mu_{maxN} \frac{C_N}{K_N + C_N}\right)\left(\mu_{maxP} \frac{C_P}{K_P + C_P}\right) \quad (4)$$

...where μ_A is the specific growth rate of *M. densa* responding to both N and P substrates. Once the concentrations of nitrogen (C_N) and phosphorus (C_P) in the water column are known, the specific growth rate of *M. densa* cells (μ_A) can also be calculated to determine the growth status of *M. densa*. Combined with the results of field investigation, we believe that if μ_A increases continuously for 2 ~ 3 days and the value is higher than 0.1 d^{-1}, it is highly likely that *M. densa* cells have reached the exponential growth stage. At this time, it is necessary to take measures to prevent the further proliferation of *M. densa*.

Q_N (pg N $cell^{-1}$) and Q_P (pg P $cell^{-1}$) were the minimum cell quota for N and P, respectively. They derived from the number of N or P starved cells grown in the media with limited N or P concentrations, which can be expressed as below, respectively:

$$Q_N = \frac{C_N}{N_{fN} - N_{iN}} \quad (5)$$

$$Q_P = \frac{C_P}{N_{fP} - N_{ip}} \quad (6)$$

...where N_{fN} and N_{iN} are the cell numbers at the stationary and initial phases of growth in N substrate, respectively, while N_{fP} and N_{iN} were those in P substrate, respectively.

Model Verification

Lianhe Reservoir (23°17′57.2″N, 113°55′8.8″E), which is located in Guangdong Province, South China, is a typical canyon-shaped reservoir in the subtropical

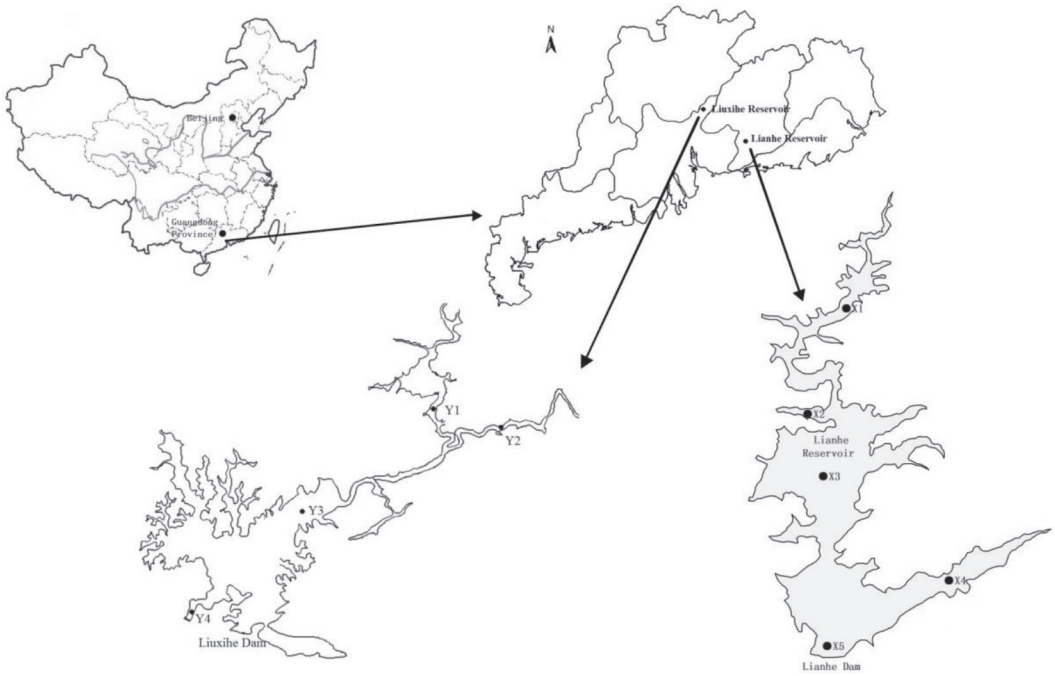

Fig. 1. Illustration of Lianhe Reservoir and Liuxihe Reservoir showing the location of sampling stations from 2014 to 2018.

region (Fig. 1). The dam was built at the southwest part of the study area in the 1970s and supports multiple purposes including drinking water supply, irrigation, hydroelectric power generation, and flood control. The hills surrounding the reservoir are covered with pine and spruce. There are also some villages and farmlands surrounding the reservoir. There are no industrial or urban pollution sources near the reservoir. Since the summer of 2012, a *M. densa* bloom has covered the reservoir and causes a highly publicized drinking water crisis.

Liuxihe Reservoir (23°45′50″N, 113°46′52″E), located in Guangdong Province, South China, is a large canyon-type reservoir on the north side of the tropic of cancer (Fig. 1). It was built in 1958, with a dam located in its southwestern. The inflow water of the reservoir mainly comes from two upstream tributaries. Natural forests are planted around the reservoir with little human intervention. The reservoir is an important drinking water source for downstream residents. However, *M. densa* bloom has occasionally occurred in the reservoir over the last decade.

Sampling and Analysis

Sampling was taken at 0.5 m below the water surface from X1 to X5 in June, July, and August 2015~2017 in Lianhe Reservoir and from Y1 to Y4 in June, July, August and September 2014~2018 in Liuxihe Reservoir (Fig. 1). The water temperature (WT), pH and dissolved oxygen (DO) were measured *in situ* using a multi-parameter water quality meter EXO2 (YSI, USA). The samples for TN and TP concentration analysis were collected in pre-sterilized 500 ml polyethylene bottles in triplicate. They were transported to the laboratory as soon as possible and stored at 4ºC. After being filtered through 0.45 μm cellulose acetate membranes (Whatman CA), TN and TP were carried out using standard combined persulfate digestion methods for water quality [28].

Water samples for determining the *M. densa* species and its cell density were also collected at 0.5 m below the surface from X1 to X5 in June, July, and August 2015~2017 in Lianhe Reservoir and from Y1 to Y4 in June, July, August and September 2014~2018 in Liuxihe Reservoir, respectively. Samples were collected in triplicate in 1 l polyethylene bottles and were preserved with acidic Lugol's iodine solution (2 % final concentration) *in situ* for later enumeration using a sedimentation technique. *M. densa* was identified, as suggested by Hu and Wei [5]. The cell density was measured with a Sedgwick-Rafter counting chamber under a fluorescence microscope (Olympus, Japan) at 200-400 × magnification.

Data Analysis

A one-way ANOVA with a Tukey test was performed to compare the differences among the treatments of each test parameter. A P-value<0.05 was regarded as significant and <0.01 as highly significant. Prior to analysis, the data were tested for the normality and homogeneity of variation. A Log 10 or square-root transformation of the data was performed prior to any statistical test when necessary. Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., USA).

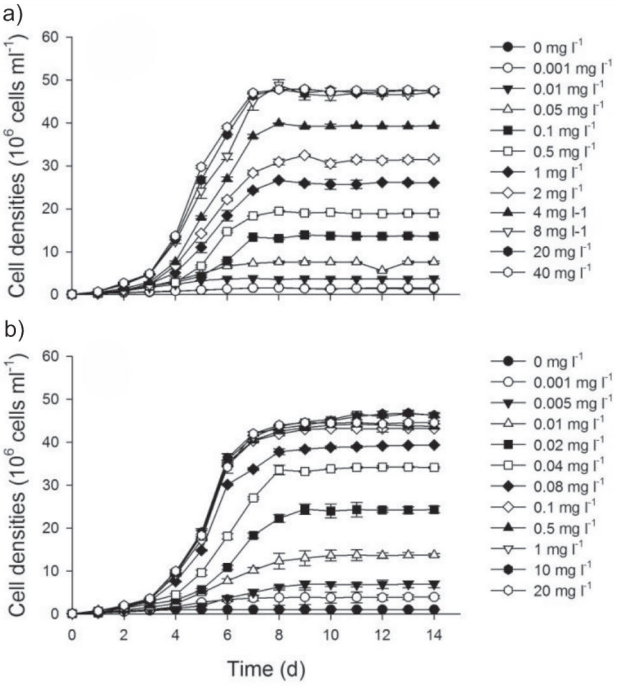

Fig. 2. The growth of *M. densa* during treatments with different concentrations of a) N and b) P. The values are the mean±S.D. (n = 3).

Results

The Growth of *M. densa* under N and P Substrates with Different Concentrations

The *M. densa* cells in treatments grew well, except for those treatments that had N concentrations of 0 and 0.001 mg l^{-1}, and their maximum cell densities appeared on Day 8~9 (Fig. 2a). The cells in the treatments of 0.01~40 mg l^{-1} were in the exponential growth stage from Day 3 and transitioned to the stationary phase after Day 9. The maximum cell densities in the treatments of 8, 20, and 40 mg l^{-1} (47.8±0.3~48.8±1.2 × 10^6 cells ml^{-1}) were almost the same (Fig. 2a). The maximum cell densities in the concentrations of 0 and 0.001 mg l^{-1} were significantly lower than those in other treatments (P<0.05), and neither of them reached the exponential growth stage.

M. densa cells responding to different P concentrations increased rapidly and achieved the exponential growth stage at Day 3, except the treatment of 0 mg l^{-1} (Fig. 2b). As the P concentrations increased, the maximum cell densities in each treatment gradually increased. When the P concentration exceeded 1 mg l^{-1}, there was little difference in the growth curves between these treatments (Fig. 2b). The maximum cell density (46.8±0.7×10^6 cells ml^{-1}) was found at Day 14. The cells reached the stationary phase after Day 9.

Equations of specific growth rate curves fit well with the Monod equation with an R^2 of 0.70 and 0.89 for N and P substrates, respectively (Fig. 3a; Fig. 3b). The K for N substrate (0.15±0.02 mg l^{-1}) was significantly higher than that for P substrate (0.03±0.00 mg l^{-1}) (P<0.05) (Table 1). The maximum growth rates (μ_{max}) for N and P substrates were 0.53±0.03 and 0.60±0.03 d^{-1}, respectively (Table 1). *M. densa* had an approachable affinity to P substrate comparing to N substrate. The mean value of Q_N (186.5 fg N $cell^{-1}$) was significantly higher than the value of Q_p (71.7 fg P $cell^{-1}$) (P<0.05) (Table 1).

Model Evaluation and Parameters

The modified model of the specific rates of growth of *M. densa* responding to the N under different P concentrations was shown, and all curves conformed to the Monod equation (Fig. 4). At the same concentration of P, when N concentrations <1 mg l^{-1}, $\ln(\mu_A)$ was positively proportional to the N concentrations, while N concentrations ≥1 mg l^{-1}, the value of the $\ln(\mu_A)$ reached a plateau. This indicated that adding the N concentration would only promote rapid reproduction of *Microcystis* in situations of lower N concentrations. Therefore, N concentration was a critical factor affecting the growth of *M.densa*.

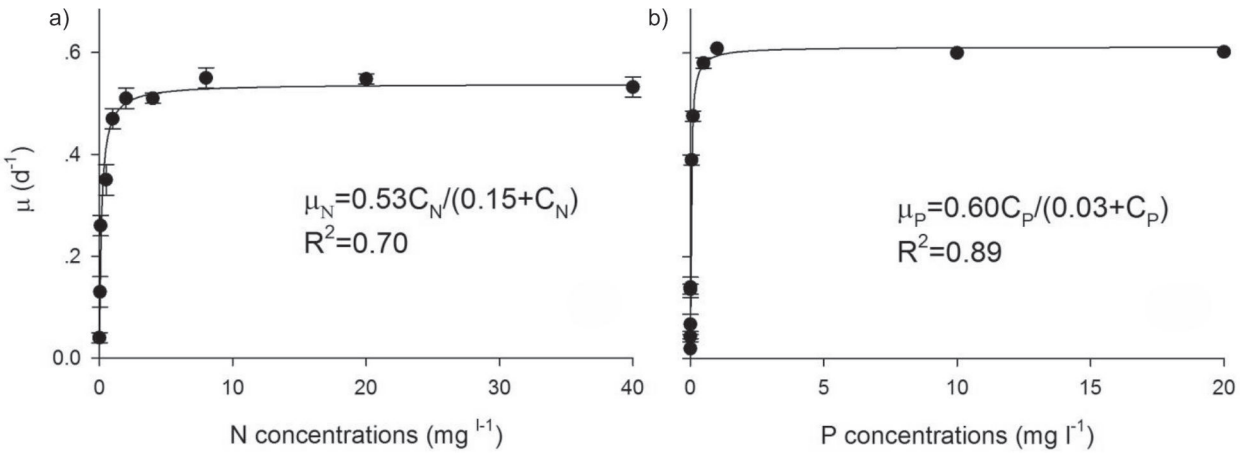

Fig. 3. The specific growth rates of *M. densa* (μ) under a) N and b) P substrates. The values are the mean±S.D. (n = 3).

Table 1. The calculated cell-specific parameters (the maximum specific growth rate, μ_{max}; the half-saturation constant for the substrate, K; the coefficient of correlation, R^2; and the minimum cell quota, Q) for the growth kinetics of N and P substrates by *M. densa*. The values are the mean±S.D. (n = 3).

Substrate	μ_{max} (d^{-1})	K (mg l^{-1})	R^2	Q (fg $cell^{-1}$)(mean±S.E.)
N (NO_3^-)	0.53±0.03	0.15±0.02	0.70	186.5±277.3
P (PO_4^{3-})	0.60±0.03	0.03±0.00	0.89	71.7±142.8

The model can also be calculated under other P concentrations (Table 2). With increasing P concentrations, the values of $\mu_{max\text{-}A}$ in the model became increasingly high. The lowest $\mu_{max\text{-}A}$ value was 0.01 d^{-1} when the P concentration was 0.001 mg l^{-1} and the highest was 0.31 d^{-1} when the P concentration was over 0.7 mg l^{-1} (P<0.05). However, when the P concentration was higher than 0.7 mg l^{-1}, both the $\mu_{max\text{-}A}$ (0.31 d^{-1}) and K (0.15 mg l^{-1}) maintained at constant values. Therefore, the P concentration, like N concentration, was also a critical factor for controlling the growth of *M. densa*.

Model Verification

WT, pH and DO concentrations were similar in Lianhe Reservoir and Liuxihe Reservoir, with mean WT of 33.2 and 32.9ºC, mean pH of 8.1 and 8.2, mean DO concentration of 8.7 and 8.2 mg l^{-1} in Lianhe Reservoir and Liuxihe Reservoir, respectively (Table 3). NO_3^--N and PO_4^{3-}-P concentrations in Lianhe Reservoir were higher than those in Liuxihe Reservoir (Table 3). The variation range of NO_3^--N in Lianhe Reservoir was from 0.10 to 1.19 mg l^{-1} and the mean value was 0.59 mg l^{-1}, while the variation range in Liuxihe Reservoir was 0.13~1.21 mg l^{-1} and the mean value was 0.51 mg l^{-1}. The variations of PO_4^{3-}-P were not significant in these two reservoirs (p<0.05), with the concentration of 0.049 mg l^{-1} in Lianhe Reservoir and of 0.013 mg l^{-1} in Liuxihe Reservoir, respectively (Table 3).

Accordingly, the measured data from the Lianhe Reservoir and other documents [29-32] were used to verify the relationship between the specific growth rates of *M. densa* (μ_A) and the concentrations of NO_3^--N and PO_4^{3-}-P (Fig. 5a). The modified model ($\mu_{max\text{-}A}$ = 0.20 d^{-1} and K_N = 0.15 mg l^{-1}) was similar to the curve based on actual measures ($\mu_{max\text{-}A}$ = 0.19 d^{-1} and K_N = 0.12 mg l^{-1}) when the PO_4^{3-}-P concentration was 0.05 mg l^{-1}, which showed that our modified model was consistent with real cases.

The measured data in Liuxihe Reservoir were also used to verify the accuracy of the model (Fig. 5b). The PO_4^{3-}-P concentration in Liuxihe Reservoir maintained at approximately 0.01 mg l^{-1} all the year around. Therefore, based on the PO_4^{3-}-P concentration, the equation with $\mu_A = 0.08C_N/(0.15+C_N)$ was chosen to used in predict the specific growth rate of *M. densa* (Table 2). According to the actual measurement results, the fitted equation with $\mu_A = 0.08C_N/(0.16+C_N)$ is in good agreement with the modified model we calculated (Fig. 5b). It indicated that the model could accurately predict the growth status of *M. densa* according to the concentrations of NO_3^--N and PO_4^{3-}-P in the water column.

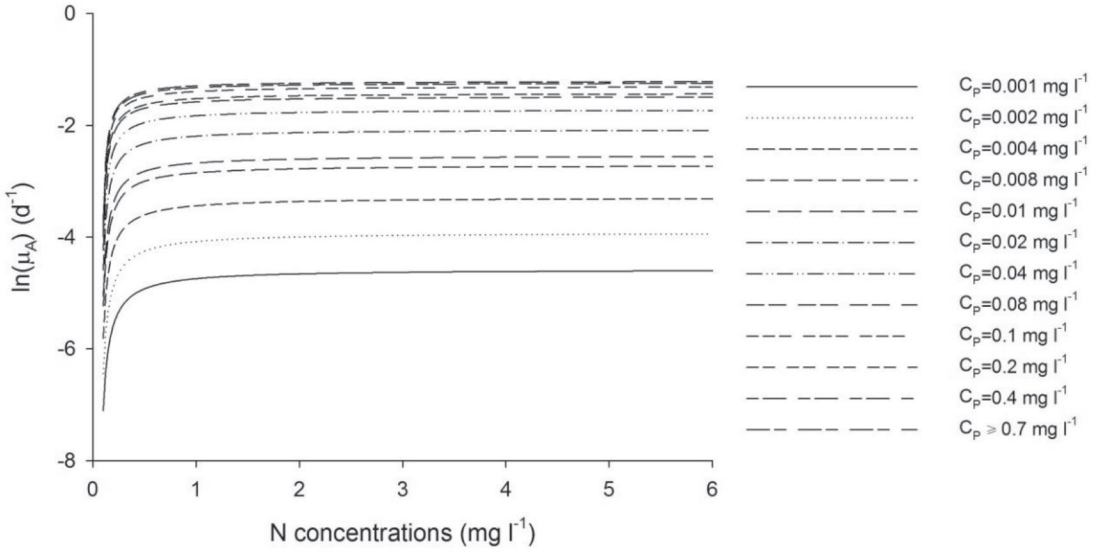

Fig. 4. The models of the specific growth rates of *M. densa* (μ_A) responding to N concentrations based on different P concentrations.

Table 2. The equations and correlation parameters, and the minimum ratios of N:P while the μ_A cells transition into the stable growth period, which corresponds to the calibrated models under different concentrations of the P substrate (P concentrations, C_p = 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mg l^{-1}).

C_p (mg l^{-1})	Equations	$\mu_{max\text{-}A}$ (d^{-1})	μ_p (mg l^{-1})	N:P
0.001	$\mu_A = 0.01C_N/(0.15+C_N)$	0.01	0.02	2214.3
0.002	$\mu_A = 0.02C_N/(0.15+C_N)$	0.02	0.04	1107.1
0.003	$\mu_A = 0.03C_N/(0.15+C_N)$	0.03	0.05	738.1
0.004	$\mu_A = 0.04C_N/(0.15+C_N)$	0.04	0.07	553.6
0.005	$\mu_A = 0.05C_N/(0.15+C_N)$	0.05	0.09	442.9
0.006	$\mu_A = 0.05C_N/(0.15+C_N)$	0.05	0.10	369.0
0.007	$\mu_A = 0.06C_N/(0.15+C_N)$	0.06	0.11	316.3
0.008	$\mu_A = 0.07C_N/(0.15+C_N)$	0.07	0.13	276.8
0.009	$\mu_A = 0.07C_N/(0.15+C_N)$	0.07	0.14	246.0
0.01	$\mu_A = 0.08C_N/(0.15+C_N)$	0.08	0.15	221.4
0.02	$\mu_A = 0.13C_N/(0.15+C_N)$	0.13	0.24	110.7
0.03	$\mu_A = 0.16C_N/(0.15+C_N)$	0.16	0.30	73.8
0.04	$\mu_A = 0.18C_N/(0.15+C_N)$	0.18	0.34	55.4
0.05	$\mu_A = 0.20C_N/(0.15+C_N)$	0.20	0.38	44.3
0.06	$\mu_A = 0.21C_N/(0.15+C_N)$	0.21	0.40	36.9
0.07	$\mu_A = 0.22C_N/(0.15+C_N)$	0.22	0.42	31.6
0.08	$\mu_A = 0.23C_N/(0.15+C_N)$	0.23	0.44	27.7
0.09	$\mu_A = 0.24C_N/(0.15+C_N)$	0.24	0.45	24.6
0.1	$\mu_A = 0.24C_N/(0.15+C_N)$	0.24	0.46	22.1
0.2	$\mu_A = 0.28C_N/(0.15+C_N)$	0.28	0.52	11.1
0.3	$\mu_A = 0.29C_N/(0.15+C_N)$	0.29	0.55	7.4
0.4	$\mu_A = 0.30C_N/(0.15+C_N)$	0.30	0.56	5.5
0.5	$\mu_A = 0.30C_N/(0.15+C_N)$	0.30	0.57	4.4
0.6	$\mu_A = 0.30C_N/(0.15+C_N)$	0.30	0.57	3.7
0.7	$\mu_A = 0.30C_N/(0.15+C_N)$	0.30	0.58	3.2
0.8	$\mu_A = 0.31C_N/(0.15+C_N)$	0.31	0.58	2.8
0.9	$\mu_A = 0.31C_N/(0.15+C_N)$	0.31	0.58	2.5
1.0	$\mu_A = 0.31C_N/(0.15+C_N)$	0.31	0.58	2.2

Discussion

Effects of N and P on the Growth of *M. densa*

N concentrations in subtropical and tropical reservoirs varied greatly from 0.1~4 mg l^{-1} [33, 34]. Algae species chose suitable N concentration for their growth. For *M. densa*, it could live well in a wide range of N concentrations which was from 0.05 to 40 mg N l^{-1} (Fig. 2a). It meant that *M. densa* could survive in most waters. When the N concentration could not satisty the growth of other algae, *M. densa* could still survive and continue to growth, which might be one of the reasons why it bloomed in Lianhe Reservoir. In order to demonstrate the superiority of *M. densa* in population competition, more experiments are needed.

There was no significant difference in the time of *M. densa* reaching the exponential growth stage and the stationary phase under different N concentrations. It indicated that N concentration did not affect the growth cycle of *M. densa* cells. The specific growth rate and the maximum cell density of *M. densa* increased

Table 3. Summary statistics of available average data from Lianhe Reservoir in June, July and August 2015~2017 and Liuxihe Reservoir in June, July, August and September 2014~2018.

	Lianhe Reservoir			Liuxihe Reservoir		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
WT (ºC)	31.1	34.5	33.2	31.7	34.3	32.9
pH	7.8	8.7	8.1	7.6	8.9	8.2
DO	8.0	9.7	8.7	7.8	9.5	8.2
NO_3^--N (mg l^{-1})	0.10	1.20	0.59	0.13	1.52	0.71
PO_4^{3-}-P (mg l^{-1})	0.043	0.057	0.049	0.011	0.015	0.013

with the increasing of N concentration. However, when N concentration was higher than 8 mg l^{-1}, the cell growth curves tended to be consistent. These results suggested that the concentration of approximately 8 mg l^{-1} was the maximum threshold of *M. densa* for N demand. This concentration was significantly higher than the N concentration in most lakes and reservoirs [35, 36], which suggested that *M. densa* could grow rapidly in most waters and N concentrations in natural waters would not restrict the growth of *M. densa*.

The Redfield ratio has long been regarded as the optimum allocation of nitrogen and phosphorus for algal metabolism [37]. However, the ratio of N:P was more than 16:1 in the lakes and reservoirs where *Microcystis* blooms occurred, such as the Lianhe Reservoir, Liuxihe Reservoir, Chopim Reservoir, and Salto Grande Reservoir [38, 39]. The low concentration of P in ambient environment affects the intracellular P storage. Therefore, *Microcystis* possesses a variety of mechanisms to enhance P uptake and storage in response to P deficiency or starvation. In this study, P concentration at a very low level (0.001~0.01 mg l^{-1}) could also promote algal cells to enter the exponential growth stage (Fig. 2b). It indicated that the cell density of *M. densa* seemed to be unaffected by low P concentrations. The reason for these might be that the K of *M. densa* for P was 0.03±0.00 mg l^{-1}, which was significantly lower than that for N (0.15±0.02 mg l^{-1}) (Table 1) (P<0.05). This indicated that the *M. densa* cells had a high affinity with P and could still take up P while the concentration was very low, even near the detection limit. We suggest that *M. densa* cells, which tolerate low P for a long time, have adapted to their surroundings. *M. densa* can grow faster than other species who can not adapt to low P environments, which may be another reason for the bloom. Surely, this viewpoint also needed more experiments to confirm.

Even the P concentration was at low level (0.001~0.01 mg l^{-1}), *M. densa* still continued to proliferate (Fig. 2b). When the P concentration exceeded 0.7 mg l^{-1}, the values of $\mu_{max\text{-}A}$ (0.31 d^{-1}) and μ_P (0.58 d^{-1}) were invariant, which suggested that the P pool in the *M. densa* cells was saturated and no longer required external P (Table 2). This showed that *M. densa* cells had a high P storage capacity and maintained a high P uptake rate. Species such as some of *Microcystis* are considered to be better suited for a low P environment as a survival strategy for environmental adaptation. They have the high ability to scavenge for available P to allow them to out-compete other species and be the dominant species in the phytoplankton community. Other studies also mentioned that *Microcystis* could

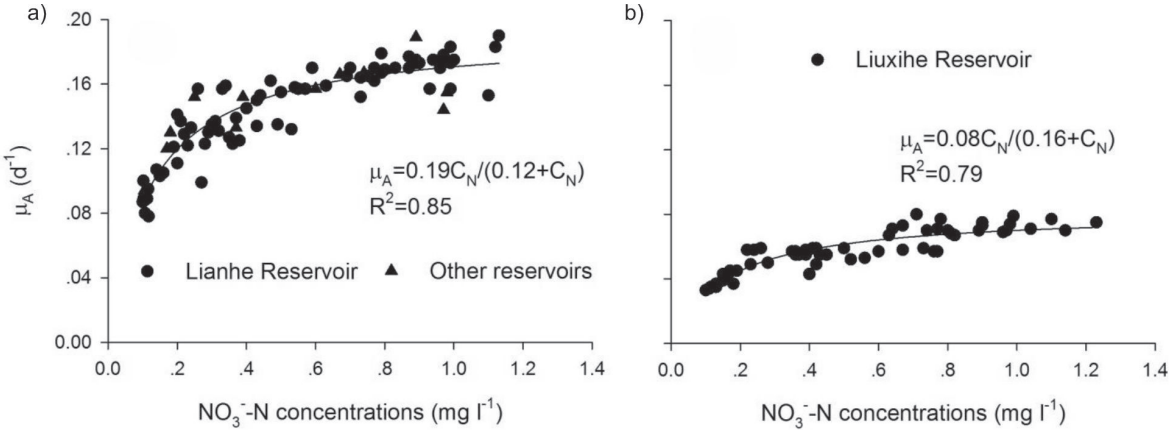

Fig. 5. The relationship of the specific growth rates of *M. densa* (μ_A) in response to the NO_3^--N concentrations (C_N) when a) the PO_4^{3-}-P concentration was 0.05 mg l^{-1} based on the actual measures of Lianhe Reservoir (solid circles) and other studies (solid triangles) and when b) the PO_4^{3-}-P concentration was 0.01 mg l^{-1} based on the actual measures of Liuxihe Reservoir (solid circles).

consistently accumulate the newly P [40] and had a high efficiency for P utilization to cause an algal bloom [18].

Comparing with Other Common *Microcystis* Species

Microcystis aeruginosa are a common *Microcystis* species in many eutrophic lakes and reservoirs [41]. We took *M. aeruginosa* as a typical example and compared the growth characteristics between *M. densa* and *M. aeruginosa*. Many studies showed that *M. aeruginosa* had the competitive advantage only under high N concentration and low N concentration would hinder the growth of *M. aeruginosa* [41, 42]. However, *M. densa* cells could reached the exponential growth stage when N concentration was from 0.05 to 40 mg l^{-1} (Fig. 2). It is generally believed that if N concentration was higher than 0.2 mg l^{-1} and P concentration was higher than 0.02 mg l^{-1}, the waters was in an eutrophication state. Therefore, *M. densa* could multiply rapidly in both oligotrophic and eutrophic waters, which indicated that *M. densa* had a wider survival scope comparing with *M. aeruginosa*. N concentration was not the primary environmental factor that restricted the growth of *M. densa*, but it was a constrain for *M. aeruginosa*.

In this study, the specific growth rates of *M. densa* were from 0.01~0.31 d^{-1} (Table 2). But the average growth rate of *M. aeruginosa* was approximately 0.37 d^{-1} [43], and sometimes this value even reached 0.64 d^{-1} [44]. Obviously, the specific growth rate of *M. densa* was lower than that of *M. aeruginosa*, which meant that it took longer for *M. densa* cells to transition to the stationary phase. Meanwhile, our study showed that the lower ratios of N:P, the higher the specific growth rate of *M. densa* (Table 2). But some studies showed that N:P ratio did not influence the growth rate of *M. aeruginosa* [45], which was different from the situation of *M. densa*. Therefore, the growth of *M. densa* seemed to be more influenced by the ambient nutrient concentration and lower N:P ratio would favor the growth of *M.densa*.

Environmental Implications

M. densa is a novel bloom species observed in recent years, taking up and assimilating nitrogen and phosphorus from ambient environment for rapid growth. It was been indicated that the frequent occurrence of *Microcystis* blooms was closely related to increasing N or P inputs in fresh waters [16, 46]. Many studies focused on a single nutrient as the substrate and their monotonic relationship with *Microcystis*. These studies surely helped understanding the uptake and assimilation of *Microcystis* for N and P [17-19, 47]. However, N and P coexisted in water column and could be simultaneously taken up by *M. densa* cells for growth and metabolism. Our study focused on the growth response of *M. densa* to both N and P as substrates and evaluated the growth model combined with N and P. It is hoped that our results could contribute to understanding the growth of *M. densa* in utilizing both N and P and the prediction of the cell densities of *M. densa* based on N and P concentrations.

Assuming the P concentration, a modified model provided a prediction of the cell densities of *M. densa* using the least number of input parameters. A crucial implication of a modified model was that the concentrations of N and P both controlled the growth rates of *M. densa*. For a water column with different nutrient levels, such as oligotrophic, mesotrophic, or eutrophic lakes and reservoirs, calculating the specific growth rate might be accomplished using a different μ_{max} and K of *M. densa*. The equation proposed by Monod [27] was a promising method to describe the growth kinetics of *M. densa* and determine the parameters. As long as the N and P concentrations in the water column were determined, the specific growth rate and growth status of *M. densa* could be predicted.

The annual variations of N and P concentrations in lakes or reservoirs are a dynamic process. The concentrations of N and P are usually low in reservoirs when they are first formed. Then, the concentrations increase steadily year by year from oligotrophic to mesotrophic and even eutrophic. The Lianhe Reservoir, built in the 1970s, had no previous algal bloom until 2012. According past data of N and P concentrations, we believed that the growth trajectory of *M. densa* in the past several decades could be deduced. Moreover, the water column where *M. densa* are suited to grow in the waters with the same physical and chemical environment characteristics, such as higher water temperature, lower disturbance, and enough dissolved oxygen content. Therefore, this modified model is hoped to apply to all subtropical reservoirs which suffer *M. densa* bloom. Of course, this model needs to be validated in more reservoirs and further refined. We still believed that this model is conducive to the reservoir management office to find growth status of *M. densa* in time and take early measures to prevent the further outbreak of *M. densa*.

Conclusions

A modified model with Monod kinetics was built to describe the synergetic respond of *M. densa* growth to different concentrations of N and P. The specific growth rate of *M. densa* can be calculated according to N and P concentrations in water column. This model was verified by the actual measures of the two typical subtropical reservoirs and could relatively accurately predict the growth level of *M. densa*. Moreover, N and P are equally important for the growth of *M. densa*. Therefore, our study helped for the daily monitoring of reservoir management for *M. densa* growth and understanding the synergetic effect of N and P to *M. densa* growth.

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Conflict of Interest

The authors declare no conflict of interest.

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