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RESEARCH PAPER

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Growth characteristics and growth modeling of *Microcystis aeruginosa* and *Planktothrix agardhii* under iron limitation

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Abstract Although iron is a key nutrient for algal growth just as are nitrogen and phosphorus in aquatic systems, the effects of iron on algal growth are not well understood. The growth characteristics of two species of cyanobacteria, *Microcystis aeruginosa* and *Planktothrix agardhii*, in iron-limited continuous cultures were investigated. The relationships between dissolved iron concentration, cell quota of iron, and population growth rate were determined applying two equations, Monod's and Droop's equations. Both species produced hydroxamate-type siderophores, but neither species produced catechol-type siderophores. The cell quota of nitrogen for both *M. aeruginosa* and *P. agardhii* decreased with decreasing cell quota of iron. The cell quota of phosphorus for *M. aeruginosa* decreased with decreasing cell quota of iron, whereas those for *P. agardhii* did not decrease. Iron uptake rate was measured in iron-limited batch cultures under different degrees of iron starvation. The results of the iron uptake experiments suggest that iron uptake rates are independent of the cell quota of iron for *M. aeruginosa* and highly dependent on the cell quota for *P. agardhii*. A kinetic model under iron limitation was developed based on the growth characteristics determined in our study, and this model predicted accurately the algal population growth and iron consumption. The model simulation suggested that *M. aeruginosa* is a superior competitor under iron limitation. The differences in growth characteristics between the species would be important determinants of the dominance of these algal species.

Key words Iron · Growth rate · Uptake rate · Model simulation · Cyanobacteria

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Introduction

In Lake Kasumigaura, a eutrophic lake in Japan, *Microcystis* blooms had been observed every summer until 1986, but they disappeared in 1987, and thereafter *Planktothrix* spp. were instead dominant for a long time. *Planktothrix* spp. became uncommon from 2001 onward (CGER 2004). The change of the dominant species of cyanobacteria in Lake Kasumigaura is very difficult to explain reasonably. Takamura et al. (1992) challenged explaining this change from the point of view of nitrogen:phosphorus ratio (N:P ratio). Before 1986, the N:P ratio was less than 10, but it exceeded 20 after 1987. The optimal N:P ratios for the growth of *Microcystis* spp. and *Planktothrix agardhii* are 4.1 (Rhee and Gotham 1980) and 12.0 (Zevenboom 1980), respectively. Takamura et al. (1992) suggested that the shift from *Microcystis* spp. to *Planktothrix* spp. was related to the change of limiting nutrient from nitrogen to phosphorus in Lake Kasumigaura at that time. However, *Microcystis* spp. did not become dominant even though the N:P ratio decreased to less than 10 after 1993 (CGER 2004). Therefore, the observed change of dominant species cannot be explained by only the N:P ratio after 1993.

It has also been suggested that iron may limit the growth of bloom-forming cyanobacteria in Lake Kasumigaura (Yagi et al. 1987; Aizaki and Aoyama 1995; Imai et al. 1999; Nagai et al. 2004). Iron is an essential micronutrient for algal growth, and, in particular, cyanobacteria have a higher cellular iron requirement than other algae (Brand 1991). The average concentrations of dissolved iron in the deepest part of Takahama-iri Bay in Lake Kasumigaura were 1229 nM for 1980–1986, 673 nM for 1987–2000, and 374 nM for 2001–2002 (CGER 2004). Moreover, the chemical speciation of iron as well as its concentration is important, because bioavailability of iron for phytoplankton is changed significantly by iron complexation with dissolved organic matter. Inorganic iron (Fe′, the sum of free hydrated and hydrolyzed ferric iron species) is available for uptake, whereas organically complexed iron is thought to be unavailable (Hudson and Morel 1993). Our previous

study (Nagai et al. 2006) provided the evidence that the growth of *Microcystis aeruginosa* and *P. agardhii* in water from Lake Kasumigaura was inhibited by iron limitation as a consequence of iron complexation with dissolved organic matter.

Fujimoto et al. (1997) investigated the growth characteristics of *M. aeruginosa* and *Phormidium tenue* (a filamentous cyanobacterium similar to *Planktothrix* spp.) under nitrogen and phosphorus limitations. Moreover, they proposed a competition model between *M. aeruginosa* and *P. tenue* under a gradient of N:P ratio (Fujimoto et al. 1999). However, no studies have evaluated quantitatively the iron requirements and uptake ability for freshwater cyanobacterial species.

In this article, we investigate the growth characteristics under iron limitation for *M. aeruginosa* and *P. agardhii*. Siderophores (Greek: siderous = iron, phorus = bearer) are low molecular weight (400–1200 Da) iron-binding compounds (siderophore + Fe^{3+} = a ferrisiderophore complex) that facilitate the transport of ferric ions into cells during periods of iron deficiency (Wilhelm 1995). The presence of two types of siderophores (hydroxamate-type and catechol-type) has been reported, and therefore we measured the production of the two types of siderophores. Moreover, we propose a kinetic model including iron bioavailability for algal population growth in non-steady-state iron-limited continuous cultures to predict the effect of iron limitation on the dominance of particular algal species.

Methods

Algal growth kinetics

Two well-established equations describe algal growth characteristics under steady nutrient limitation. Monod's equation (Monod 1949) expresses the relationship between algal population growth rate (μ) and dissolved nutrient concentration (S):

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S} \quad (1)$$

where μ_{max} is the maximal growth rate and K_s is the half-saturation constant for growth. Droop's equation (Droop 1973) expresses the relationship between growth rate (μ) and cell quota (Q):

$$\mu = \mu'_{max} \cdot \left(1 - \frac{Q_{min}}{Q}\right) \quad (2)$$

where μ'_{max} is the apparent maximal growth rate that would occur if Q were infinite, and Q_{min} is the minimal cell quota.

Compared to the foregoing well-established growth rate relationships, nutrient uptake kinetics are less well understood. Nutrient uptake rate (p) is usually expressed by the Michaelis–Menten equation:

$$p = \frac{p_{max} \cdot S}{K_p + S} \quad (3)$$

where p_{max} is the maximal uptake rate and K_p is the half-saturation constant for uptake. The maximal uptake rate (p_{max}) has been observed to vary with the previous degree of starvation of the algae, hence with their cell quota (Morel 1987). Metal speciation is an important factor in the uptake of metals by algae, and therefore S in Eq. 3 should not be total metal concentration but bioavailable metal concentration.

Medium and culture conditions

Axenic unialgal cultures of *Microcystis aeruginosa* NIES-44 and *Planktothrix agardhii* NIES-204 obtained from the NIES (National Institute for Environmental Studies, Japan) Microbial Culture Collection were used for culture experiments (Kasai et al. 2004). The two strains were isolated from Lake Kasumigaura. *Microcystis aeruginosa* NIES-44 has already lost the ability to aggregate; *P. agardhii* NIES-204 can be suspended well in liquid culture without attaching to the wall of the culture tubes. Stock cultures were maintained at 25°C and under a photon flux of 10 μE m^{-2} s^{-1} continuous light in a modified CB medium of the following composition: 6.35×10^{-4} M $Ca(NO_3)_2 \cdot 4H_2O$, 2.30×10^{-4} M K_2HPO_4, 8.11×10^{-5} M $MgSO_4 \cdot 7H_2O$, 6.13×10^{-4} M bicine [*N*,*N*-bis(2-hydroxyethyl)glycine], 1.00×10^{-5} M $Na_2EDTA \cdot 2H_2O$, 1.00×10^{-6} M $FeCl_3 \cdot 6H_2O$, 5.46×10^{-7} M $MnCl_2 \cdot 4H_2O$, 4.84×10^{-7} M $ZnCl_2$, 5.04×10^{-8} M $CoCl_2 \cdot 6H_2O$, and 3.10×10^{-8} M $Na_2MoO_4 \cdot 2H_2O$, in which the pH was adjusted to 9.0. Milli-Q water (resistance 18.3 MΩ; Millipore, USA) was used to prepare the culture medium. The MINEQL+ computer program (Schecher and McAvoy 1992) was used to calculate the concentration of inorganic iron (Fe′, the sum of free hydrated and hydrolyzed ferric iron species) in the medium. Stability constants of the metal–bicine complexes (NIST 2004) were used in the calculation. The concentration of Fe′ was calculated to be 0.79% of the total concentration of dissolved iron within a range of 1000 nM or less.

Iron-limited continuous culture

The algae were grown in non-steady-state continuous cultures under iron limitation. The medium used in the growth experiments was the modified CB medium described above. Initial concentration of iron in the culture vessels was 1000 nM. Iron concentration in the inflowing medium was reduced to 200 nM to achieve an initial phase of iron-unlimited growth followed by iron-limited growth. Culture experiments were performed in a single set of borosilicate glass vessels with several input ports. Cultures were aerated with filter (pore size, 0.2 μm) sterilized air. A control volume of 1 l culture was maintained via overflow, with medium inflow at a dilution rate (the rate of medium inflow divided by the control volume) of 0.15 day^{-1}. To avoid growth inhibition by strong light during a lag time of algal growth, light intensity for the first 3 days was set at a photon flux of 10 μE m^{-2} s^{-1}. It was then raised to 20 μE m^{-2} s^{-1} of continuous

light for the rest of experimental time. Temperature was maintained at 25°C, and cultures were mixed with a magnetic stirrer. *Microcystis aeruginosa* and *P. agardhii* were inoculated into the medium to give initial densities of 6800 cells ml^{-1} and 1710 filaments ml^{-1}, respectively.

Every 2 or 3 days, overflowed culture medium was sampled and algal cell counting was undertaken using a microscope (BX-51; Olympus, Tokyo, Japan). Optical density of the overflowed sample was also measured, as absorbance at a wavelength of 750 nm with a spectrophotometer (UV-2200; Shimadzu, Kyoto, Japan) using a 10-mm pathlength glass cell. Part of the overflow sample was filtered through acid-washed (by 0.1 M HCl and then Milli-Q water) glass fiber filters (GF/F; Whatman, Brentford, UK). The filters were used to determine chlorophyll-*a* (Chl-*a*) concentration by spectrophotometry after extraction with 100% methanol (Marker et al. 1980). The filtrates were used for chemical analyses. Dissolved iron concentration (D-Fe) was measured by cathodic stripping voltammetry after 3 h UV-irradiation (Nagai et al. 2004). Iron analysis was conducted in duplicate. After tenfold concentration (by freeze drying) of the filtrates, hydroxamate-type and catechol-type siderophores were quantified by the Csaky test modified by Gillam et al. (1981) and the test of Rioux et al. (1983), respectively. The concentrations of nitrate and phosphate were measured with an autoanalyzer (TRAACS 800; Bran + Luebbe, Tokyo, Japan). The culture vessels and all glassware were cleaned before use by soaking in 3 M HCl for 3 days, followed by rinsing with Milli-Q water. Acid-washed (by 1 M HCl and then Milli-Q water) pharmed tube (Saint-Gobain K.K.) was used for sending culture medium. The iron concentrations in inflowing medium were measured at the beginning and end of the culture experiment to confirm the effect of absorption and desorption of iron on the vessel wall. The iron concentrations were not different (from 171 nM to 170 nM), and the effect was thought to be negligible.

During the growth experiments, growth rate (μ, d^{-1}) on day t was calculated as

$$\mu = \frac{\ln A_t - \ln A_{t'}}{t - t'} + D \tag{4}$$

where A_t is the optical density on day t, $A_{t'}$ is the optical density on the previous sampling day (t′), and D is the dilution rate (d^{-1}). For each sample, the volume of overflowed culture medium was measured and used to calculate D. Total iron concentration (T-Fe) on day t was calculated as

$$\text{T-Fe} = \left[\int_{t'}^{t} \{S_0 + (S_{ini} - S_0)e^{-D \cdot t}\} dx\right] / [t - t'] \tag{5}$$

where S_0 is the iron concentration in the inflowing medium and S_{ini} is the total concentration of iron on day zero. The values of S_0 and S_{ini} were the measured iron concentration of inflowing medium and culture medium just before inoculation, respectively. The cell quota of iron (Q-Fe) on day t was calculated as

$$\text{Q-Fe} = \frac{\text{T-Fe} - \text{D-Fe}}{N_t} \tag{6}$$

where N_t is the number of algae on day t (cells or filaments l^{-1}). Values of N_t were calculated using the following equations derived from the linear regression equations between the optical density and counted number of algae obtained from the continuous culture experiment:

$$N_t(\textit{Microcystis}) = A_t \times 6.80 \times 10^9 \tag{7}$$
$$N_t(\textit{Planktothrix}) = A_t \times 2.95 \times 10^8 \tag{8}$$

Cell quota of nitrogen and phosphorus were calculated in the same way as Eq. 6.

Measurement of iron uptake rates

Experiment 1: Measurement of iron uptake rates in the iron-limited batch cultures.

Iron uptake was quantified as iron disappearance from the medium (Gress et al. 2004). Iron-reduced medium (150 ml, Fe 200 nM) was distributed to a single set of eight sterile 300-ml glass flasks. Cells were inoculated and grown under the same conditions as the continuous cultures. Flasks were shaken with a reciprocal shaker at 60 rpm. When algal growth reached stationary phase (almost all initial iron was incorporated into algal cells), iron was added to each of the eight cultures with different levels of iron concentration (200–6000 nM) from a filter-sterilized stock solution of $FeCl_3 \cdot 6H_2O$. Culture samples (25 ml) were taken immediately after iron addition, and again after 3, 6, and 9 h. Part of each sample was used to measure the optical density, and the rest was filtered through glass fiber filters (GF/F) for iron analysis. Iron concentrations in the filtrates were determined by inductively coupled plasma-atomic emission spectrometry (ICAP-750; Nippon Jarrell-Ash, Tokyo, Japan) after acidification by HNO_3. The time courses of iron concentrations were fitted by quadratic regression, and the slope at time zero was used to estimate uptake rate. The blank experiment was conducted by iron addition (2000 nM) to culture medium that was not inoculated with algal cells. The iron concentrations at the blank experiment were 1943 nM (0 h), 1980 nM (3 h), 1909 nM (6 h), and 1930 nM (9 h).

Experiment 2: Investigation of relationship between iron uptake rate and previous degree of cell starvation.

Cells were grown in iron-limited batch cultures as for experiment 1. When algal growth reached the stationary phase, iron was added to the cultures with nine levels of iron concentration (0–6000 nM). After 2 days of incubation, a further iron addition was performed to each culture with 2000 nM iron, and then samples were taken every 3 h. Uptake rates were estimated from the time courses of iron concentrations, as described in experiment 1.

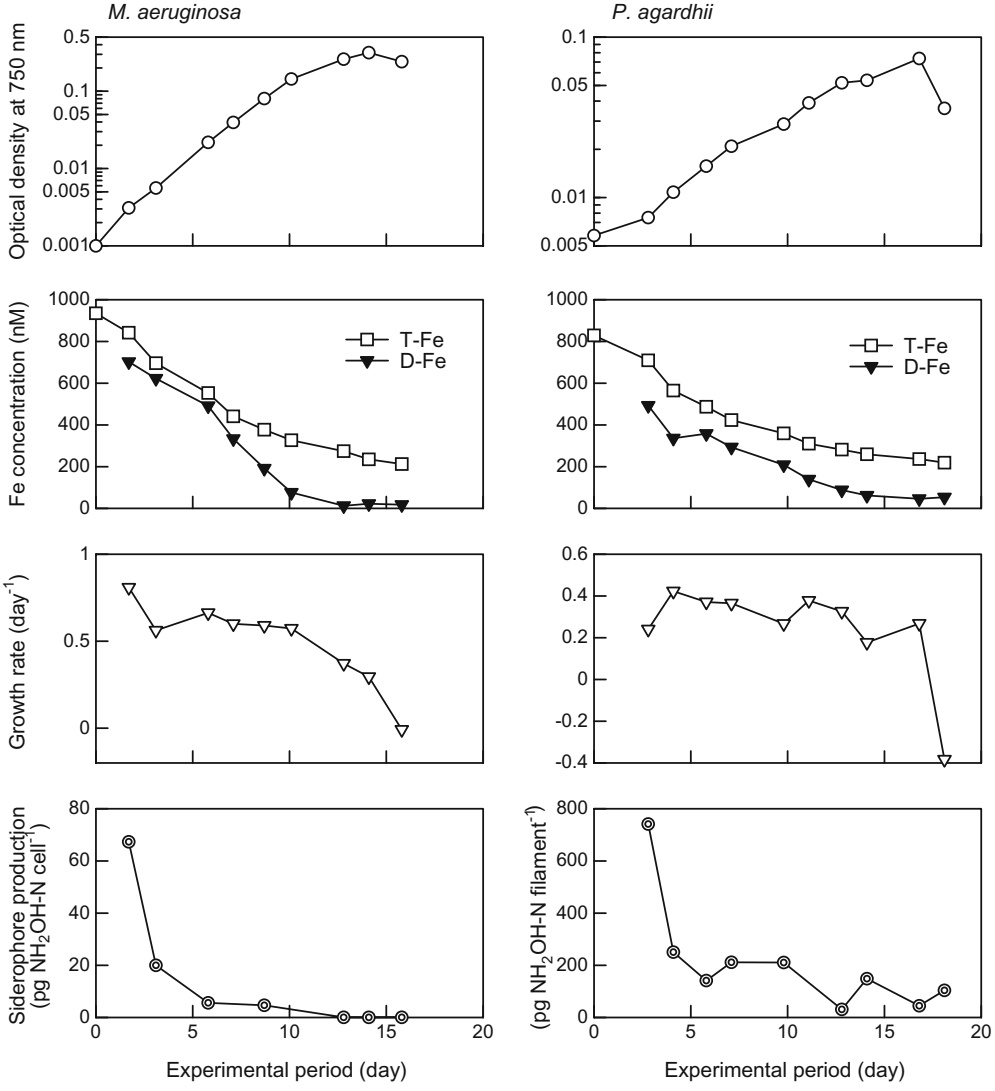

Fig. 1. Time courses of optical density, total iron concentration (T-Fe), dissolved iron concentration (D-Fe), growth rate, and production of hydroxamate-type siderophores in a single set of iron-limited continuous cultures

Results

Iron-limited continuous cultures

During growth of the iron-limited continuous cultures, the algal densities of both *M. aeruginosa* and *P. agardhii* increased exponentially with time (Fig. 1). The negative values of growth rate were found on day 16 for *M. aeruginosa* and on day 18 for *P. agardhii*, without apparent stationary phases. The growth experiments were terminated on these days. Both species produced hydroxamate-type siderophores, but neither species produced catechol-type siderophores. Large amounts of hydroxamate-type siderophores were produced during the early stages of the cultures (Fig. 1).

The values obtained for μ and D-Fe were fitted to Monod's equation using least-squares regression as $\mu = \mu_{max} \cdot \text{D-Fe}/(K_s + \text{D-Fe})$ (Fig. 2). The regressed equations yielded μ_{max} of 0.675 day^{-1} and K_s of 17.1 nM for *M. aeruginosa*, and μ_{max} of 0.419 day^{-1} and K_s of 39.2 nM for *P. agardhii*. These K_s values are represented as K_s(D-Fe), as they are constants in the relationship between total dissolved iron and μ. The constants in the relationship between inorganic iron and μ was defined as $K_s(Fe')$, and it was estimated from $[Fe'] = 0.0079 \times [\text{D-Fe}]$ that $K_s(Fe')$ values were 135 pM and 310 pM for *M. aeruginosa* and *P. agardhii*, respectively. Moreover, the values obtained for μ and Q-Fe were fitted to Droop's equation using least-squares regression as $\mu = \mu'_{max}(1 - \text{Q-Fe}_{min}/\text{Q-Fe})$ (Fig. 2). The regressed equations yielded μ'_{max} of 0.719 day^{-1} and Q-Fe_{min} of 0.0617 fmol $cell^{-1}$ for *M. aeruginosa*, and μ'_{max} of 0.430 day^{-1} and Q-Fe_{min} of 4.08 fmol $filament^{-1}$ for *P. agardhii*.

Cell quota of nitrogen and phosphorus, and cellular Chl-*a* content

Nitrogen cell quota decreased with decreasing Q-Fe for both *M. aeruginosa* and *P. agardhii* (Fig. 3). Phosphorus cell

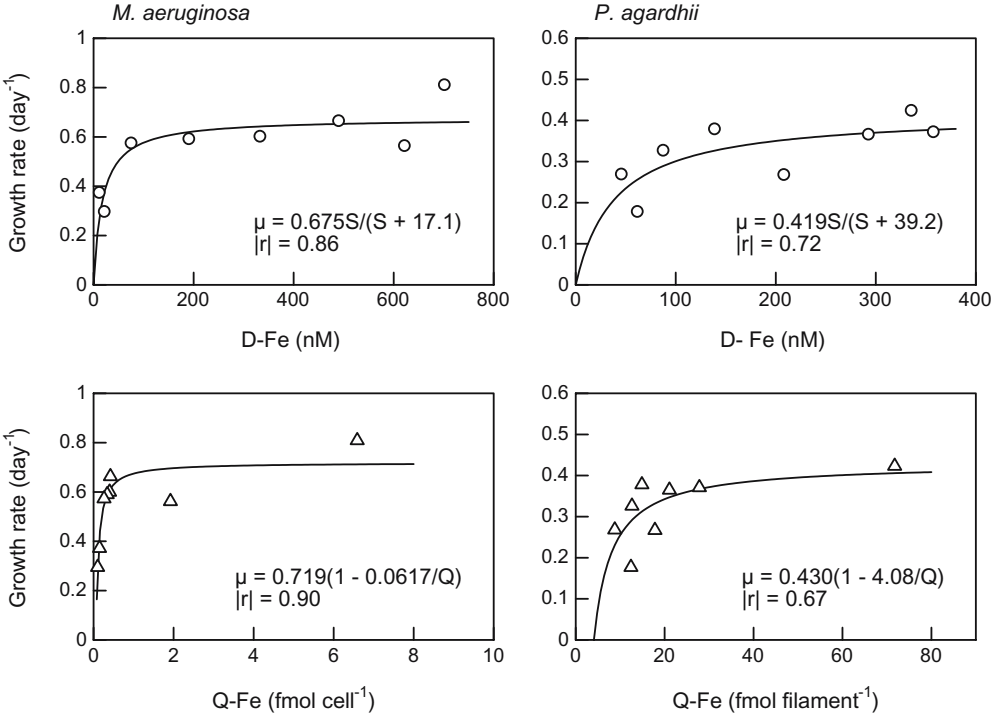

Fig. 2. Relationships between growth rate and dissolved iron concentration fitted to Monod's equation (*upper graphs*) and between growth rate and cell quota of iron fitted to Droop's equation (*lower graphs*) in iron-limited continuous cultures. Lag time in growth was observed for *Planktothrix agardhii*, and the data of first sample in the culture were not plotted

quota also decreased with decreasing Q-Fe for *M. aeruginosa*, but varied without a clear relationship to the Q-Fe for *P. agardhii* (Fig. 3). The cellular N:P ratio ranged from 3.2 to 8.7 for *M. aeruginosa* and from 2.1 to 14.9 for *P. agardhii* (Fig. 3). The cellular N:P ratio for *P. agardhii* clearly decreased with decreasing Q-Fe, but the ratio for *M. aeruginosa* varied without a clear relationship to the Q-Fe.

Cellular Chl-*a* content ranged from 0.3 to 0.7 for *M. aeruginosa*, and from 10.3 to 16.7 for *P. agardhii* (Fig. 3). The cellular Chl-*a* content decreased at the iron-limited condition for *M. aeruginosa* but did not decrease significantly for *P. agardhii*.

Iron uptake rates

There was a clear difference in the iron uptake characteristics between *M. aeruginosa* and *P. agardhii*. In experiment 1 (estimation of uptake rates by iron-starved cells), *p*s (uptake rates) of *M. aeruginosa* were very low and lacked any clear relationship to the iron addition, but those of *P. agardhii* clearly increased with increasing iron addition (Fig. 4). The results of experiment 1 suggest that iron uptake by starved cells of *M. aeruginosa* does not start immediately after iron is added, but for *P. agardhii* it does.

In experiment 2 (investigation on relationship between iron uptake rates and previous degrees of cell starvation) the *p* of *M. aeruginosa* increased as the concentration of initially added iron (added 2 days before measurement of uptake rates) increased (Fig. 4). However, the *p* of *P. agardhii* decreased as it increased (Fig. 4). Algal densities increased by a factor of 1.5–1.8 times and 1.0–1.2 times for *M. aeruginosa* and *P. agardhii*, respectively, in the 2 days between the initial addition of iron and the first measurement of iron uptake rate. The iron uptake of *M. aeruginosa* was relatively slow and was followed by rapid cell division. On the other hand, the iron uptake of *P. agardhii* was fast despite their low growth rate.

Discussion

Growth characteristics under iron limitation

This study is the first investigation to report on growth kinetics of freshwater algae under iron limitation using the equations developed by Monod and Droop. The values of μ and D-Fe were well represented by Monod's equation for both *M. aeruginosa* and *P. agardhii*. The values of μ_{max} for *M. aeruginosa* were higher than those for *P. agardhii*, and the values of K_s for *M. aeruginosa* were lower than those for *P. agardhii*. This result suggests that *M. aeruginosa* would be the superior competitor under iron limitation. The values of μ and Q-Fe were also well represented by Droop's equation for both species in non-steady-state continuous cultures. Therefore, Droop's equation provides a useful model for predicting algal growth and iron consumption under iron limitation in non-steady-state conditions.

It have been demonstrated that many cyanobacterial species are capable of producing siderophores (Wilhelm 1995). Although production of hydroxamate-type sidero-

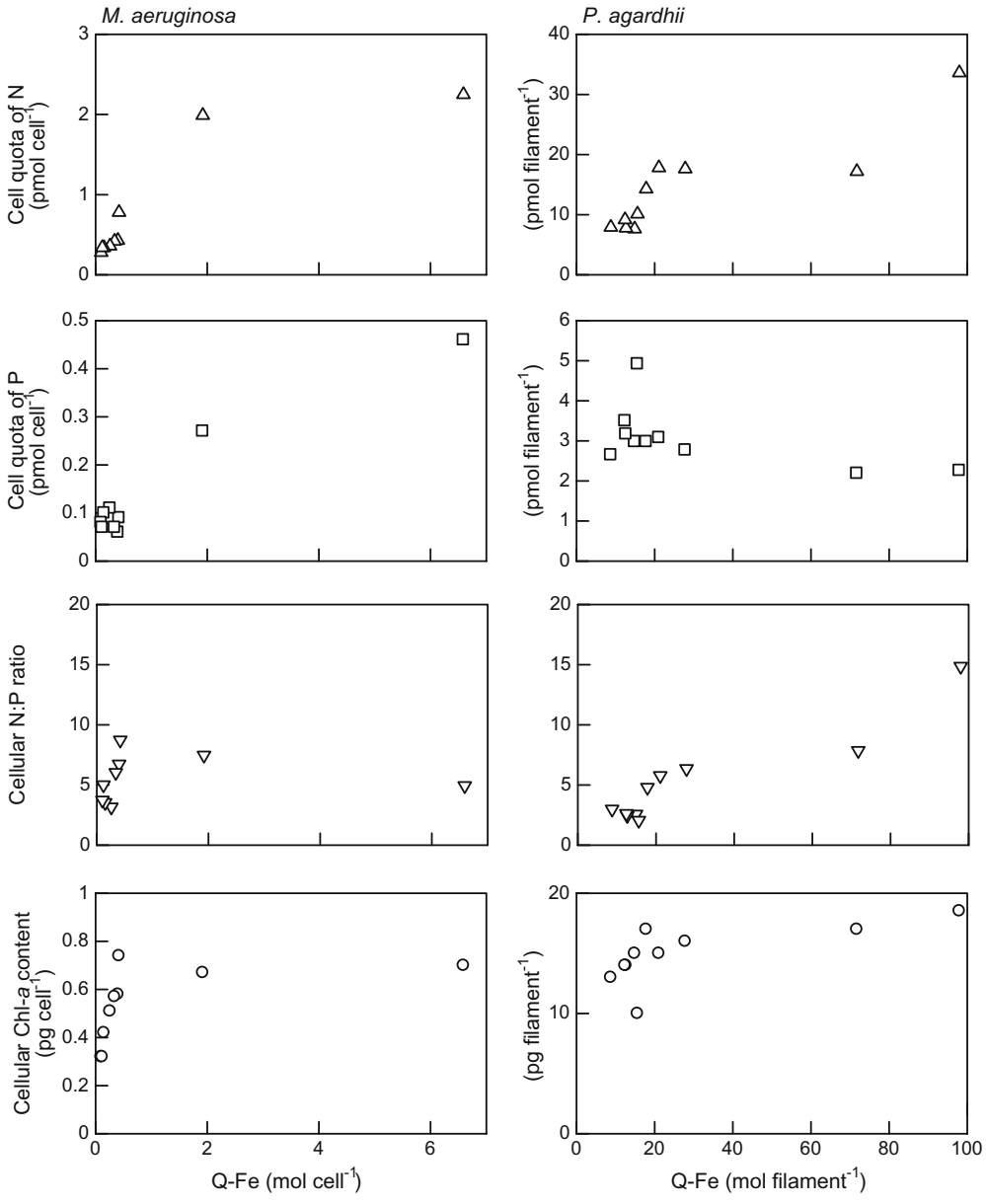

Fig. 3. Cell quota of nitrogen and phosphorus and cellular nitrogen : phosphorus ratio, and cellular chlorophyll *a* (Chl-*a*) content as a function of cell quota of iron (*Q-Fe*) in iron-limited continuous cultures

phores by *M. aeruginosa* under iron limitation was reported by Imai et al. (1999), there was no study testing the ability of production of catechol-type siderophores for *M. aeruginosa*. The effect of siderophores produced by *M. aeruginosa* appears to be small, because no recovery of μ at low iron concentration was observed (Fig. 2); this is consistent with observations reported by McKnight et al. (1979) and by Imai et al. (1999). McKnight et al. (1979) showed that *M. aeruginosa* produces weakly binding hydroxamate-type ligands, and Imai et al. (1999) showed that there was no substantial recovery of μ related to production of hydroxamate-type siderophore for *M. aeruginosa*. Brown and Trick (1992) reported production of both hydroxamate-type and catechol-type siderophores and increase of growth rate under iron limitation for *Oscillatoria* (*Planktothrix*) *tenuis*. A recovery at low iron concentration was not observed in this study, probably the result of the lack of production of catechol-type siderophores. We used the same methods as they did for detection of siderophores, and their culture experiment was conducted using axenic batch culture and BG-11 medium (pH 7.4) at 25°C under a photon flux of $70\,\mu E\,m^{-1}\,s^{-1}$. The ability of siderophore production may differ among species even within the same genus.

Nitrate uptake and metabolism require energy to reduce nitrate to ammonium; this redox reaction is catalyzed by Fe-containing enzymes, nitrate and nitrite reductases. Therefore, nitrate uptake is restricted by iron limitation (Milligan and Harrison 2000). It has been reported that cell quotas of nitrogen and phosphate decrease with keeping a constant cellular N : P ratio under iron limitation (Greene et al. 1991; Takeda 1998). On the other hand, Timmermans et al. (2004) examined silicate, nitrate, and phosphate consumption ratios under iron limitation and reported that phosphate consumption per cell remained fairly constant in

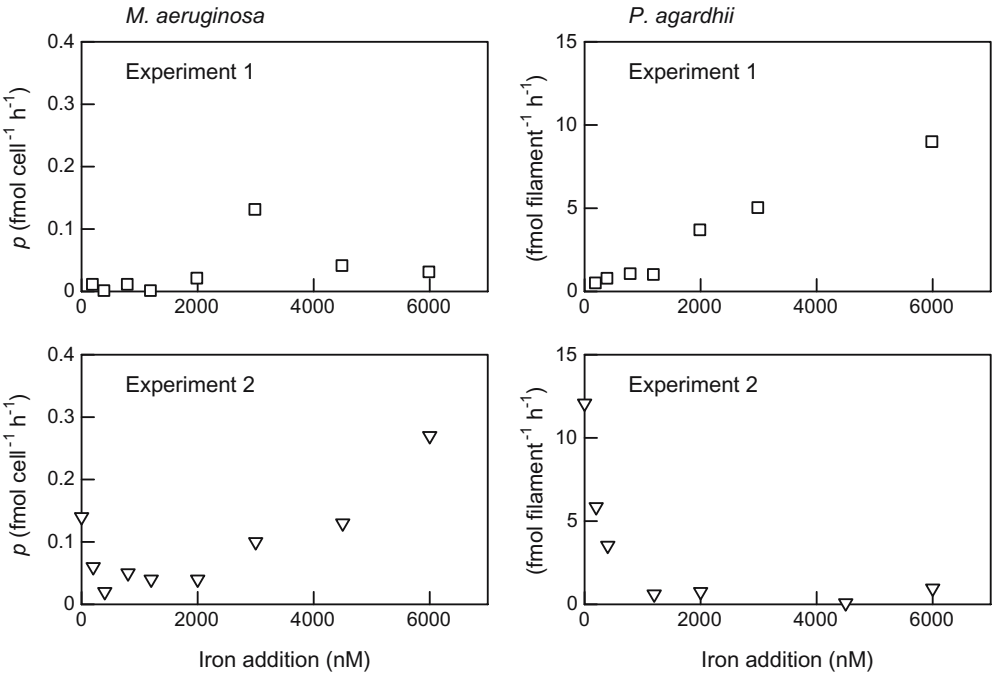

Fig. 4. Iron uptake rates (p) measured in a single set of batch cultures. *Experiment 1:* Iron uptake rates measured just after iron addition (200–6000 nM) to iron-starved cells. *Experiment 2:* Iron uptake rates measured just after iron addition (2000 nM) to cells undergoing different degrees of iron starvation prepared by 2 days incubation after initial addition of iron (0–6000 nM) to iron-starved cells

response to changing iron concentration for three of four tested species of diatom. Cell quotas of nitrogen for both *M. aeruginosa* and *P. agardhii* decreased with decreasing Q-Fe in our study, and these findings are consistent with the results of previous work. Cell quota of phosphorus for *M. aeruginosa* decreased with decreasing Q-Fe, while those for *P. agardhii* did not. These results suggest that the response of phosphate uptake to iron limitation differs between species and may depend on the strategies of individual species to prevent iron deficiency. *Microcystis aeruginosa* may employ a strategy of reducing all material requirements to prevent iron deficiency. This concept is consistent with our finding that the decline of cellular content of Chl-*a* for *M. aeruginosa* under iron limitation was greater than that for *P. agardhii*.

The results of our iron uptake experiments suggest that the iron uptake rate of *M. aeruginosa* does not depend on cell quota, because their iron uptake was relatively slow and was followed by rapid cell division and decreasing cell quota. However, the results also suggest that the iron uptake rate of *P. agardhii* greatly depends on cell quota, as their iron uptake proceeded quickly despite their low growth rate. As a result, *P. agardhii* is likely to become sated with iron immediately after the addition of a large amount of iron.

Simulation model of algal growth under iron limitation

We have developed a kinetic model of population growth for a single algal species in continuous culture under iron limitation by modifying the model proposed by Grover (1991), which is based on Droop's equation. The state variables are S (dissolved iron concentration, mol l^{-1}), Q (cell quota of iron, mol $cell^{-1}$ or mol $filament^{-1}$), and N (algal density, cells l^{-1} or filaments l^{-1}). The kinetic model is represented by the following equations:

$$\frac{dS}{dt} = D(S_0 - S) - N \cdot p \tag{9}$$

$$\frac{dQ}{dt} = p - \mu'_{max}(Q - Q_{min}) \tag{10}$$

$$\frac{1}{N} \cdot \frac{dN}{dt} = \mu'_{max}\left(1 - \frac{Q_{min}}{Q}\right) - D \tag{11}$$

where D is dilution rate (h^{-1}), S_0 is iron concentration in the inflowing medium (mol l^{-1}), and p is iron uptake rate (mol $cell^{-1}$ h^{-1}).

Morel (1987) proposed that the relationship between p_{max} and Q can be expressed as follows:

$$p_{max} = p^{hi}{}_{max} - (p^{hi}{}_{max} - p^{lo}{}_{max}) \cdot \frac{Q - Q_{min}}{Q_{max} - Q_{min}} \tag{12}$$

where $p^{hi}{}_{max}$, $p^{lo}{}_{max}$, Q_{max}, and Q_{min} are the bounds on the ranges of p_{max} and Q. The bounds $p^{hi}{}_{max}$ and Q_{min} correspond to the physiological state of zero growth ($\mu = 0$), and the bounds $p^{lo}{}_{max}$ and Q_{max} correspond to the physiological state of maximal growth ($\mu = \mu_{max}$). The p of *M. aeruginosa* is assumed to be independent of Q. Therefore, it is thought that $p_{max} = p^{hi}{}_{max} = p^{lo}{}_{max}$ and p_{max} may not be variable. The p of *P. agardhii* is assumed to be dependent on Q as well as the extracellular concentration of inorganic iron. Moreover, $p^{lo}{}_{max}$ is almost zero and represents a special case of Eq. 12. Therefore, p_{max} for *P. agardhii* may be variable and be expressed as follows:

$$p_{max} = p^{hi}{}_{max} \cdot \left(1 - \frac{Q - Q_{min}}{Q_{max} - Q_{min}}\right) \tag{13}$$

Thus, p is calculated as follows:

Table 1. Estimated parameters for growth model of *M. aeruginosa* and *P. agardhii*

Parameter	*Microcystis aeruginosa*	*Plantktothrix agardhii*
μ'_{max} (h^{-1})	0.0300	0.0179
Q_{min} (fmol $cell^{-1}$ or fmol $filament^{-1}$)	0.0617	4.08
p_{max} (fmol $cell^{-1}$ h^{-1} or fmol $filament^{-1}$ h^{-1})	0.272	12.1
K_p (nmol l^{-1})	60.0	160
Q_{max} (fmol $filament^{-1}$)	–	159

$$p(M.aeruginosa) = \frac{p_{max} \cdot S'}{K_p + S'} \quad (14)$$

$$p(P.agardhii) = \frac{p_{max} \cdot S'}{K_p + S'} \cdot \left(1 - \frac{Q - Q_{min}}{Q_{max} - Q_{min}}\right) \quad (15)$$

where S′ is inorganic iron concentration (mol l^{-1}) calculated as 0.0079 × S. The effect of siderophore production was taken to be small, and thus not considered in this model.

The kinetic constants μ'_{max} (maximal growth rate), Q_{min} (minimal cell quota), p_{max} (maximal uptake rate), K_p (half-saturation constant for iron uptake), and Q_{max} (maximal cell quota) defined in this study are shown in Table 1. The values of μ'_{max} and Q_{min} were determined based on the experiments using iron-limited continuous cultures (see Fig. 2). The value of p_{max} was determined from the iron uptake experiments. In experiment 1, saturation of iron uptake was not observed (see Fig. 4), and thus fitting of the data to the Michaelis–Menten equation, $p = p_{max} \cdot$ D-Fe/(K_p + D-Fe), was not possible. Therefore, the values of p_{max} were defined as the maximal values of iron uptake rate determined in the iron uptake experiments. To determine values for K_p, simulated growth curves derived from a range of provisional K_p values were compared with results of the continuous culture experiments. Then, the final values of K_p were defined as those that produced a simulated growth curve which corresponded best to the results of the continuous culture experiments. The Q_{max} value for *P. agardhii* was calculated from the equation $\mu_{max} = \mu'_{max}(Q_{max} - Q_{min})/Q_{max}$ (Morel 1987).

A Runge–Kutta algorithm with 0.1-h step size was used for dynamic simulations of algal growth in iron-limited continuous cultures. Initial values of N and D were the same as those in the continuous culture experiments, and measured values of initial S, S_0, and Q were used for calculation. Predicted algal growth was compared with the results of the iron-limited continuous culture experiments (Fig. 5). The model accurately predicted the observed algal growth and D-Fe except for those during the lag time found in the early days of the culture of *P. agardhii*. This result suggests that our model and the parameters such as cell quota and uptake rate estimated in our study are appropriate. Because our model does not take into account the algal death phase, declines of algal density from day 16 for *M. aeruginosa*, and from day 18 for *P. agardhii*, were not predicted.

Recently, the FeL model of iron acquisition, including reduction of organically complexed iron(III) to iron(II) induced by membrane-bound reductases, was proposed

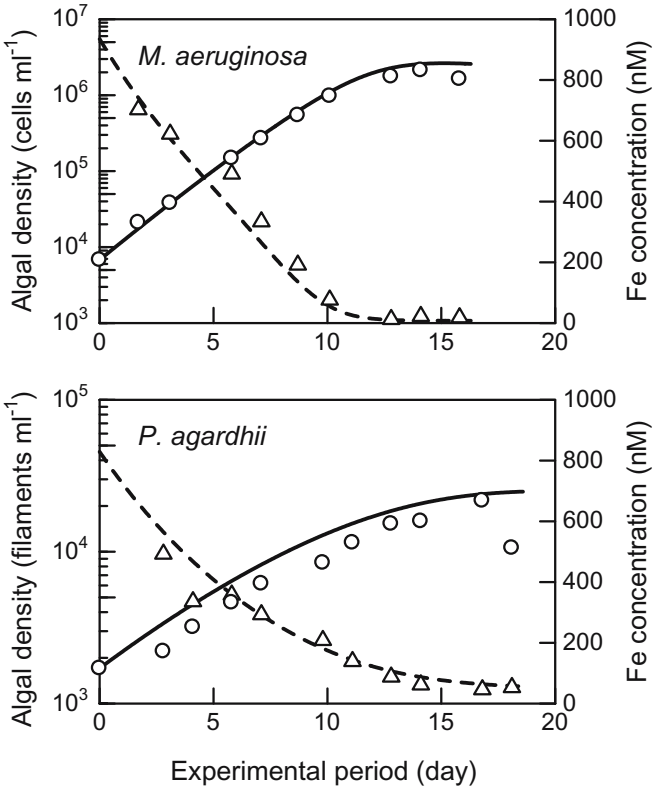

Fig. 5. Simulation of algal growth under iron-limited continuous cultures. *Circles* and *triangles* show measured algal density and dissolved iron concentration, respectively. *Solid lines* show predicted trajectories for algal density by the simulation model, and *dashed lines* show those for dissolved iron concentration

(Shaked et al. 2005; Salmon et al. 2006). However, the growth of *M. aeruginosa* and *P. agardhii* was predicted accurately by considering that only Fe′ is determining Fe uptake in our study. Our growth model was validated using algal growth in an artificial culture medium. Therefore, the validation of the model in natural lake waters should be conducted in a further study.

Effect of iron limitation in aquatic systems

Interspecific competition between *Microcystis aeruginosa* and *Planktothrix agardhii* under iron limitation was simulated using the model developed in this study. The competition model consists of one equation expressing variation of S and equations for each species expressing variations of Q and N. The value of D used was 0.1 day^{-1}, and iron concentration was set to 50 nM, which is an approximate average concentration of dissolved iron in the center of Lake Kasumigaura (Nagai et al. 2006). The model simulation predicted *M. aeruginosa* to be an overwhelmingly superior competitor under iron limitation (Fig. 6). This result is consistent with our interpretation of the differences in K_s and μ_{max} values for the two algal species. Although iron limitation may be the reason that *Planktothrix* spp. no longer grows in Lake Kasumigaura, the possibility of the occur-

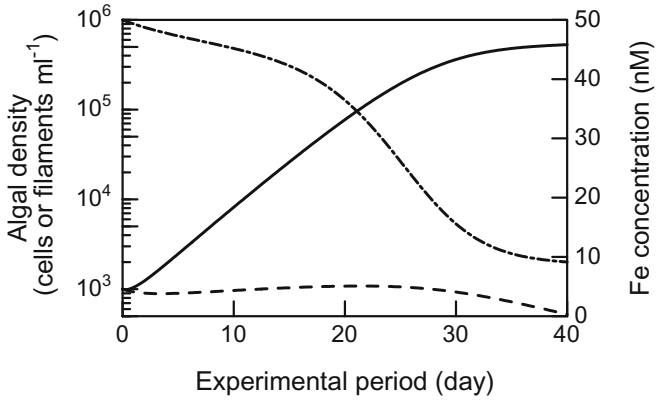

Fig. 6. Simulation of interspecific competition between *Microcystis aeruginosa* and *Planktothrix agardhii* in an iron-limited continuous culture. The initial value of S is 50 nM, the initial values of Q are each Q_{min}, and the initial values of N are 1000 cells or filaments ml^{-1}; S_0 is 50 nM and D is 0.1 day^{-1}. *Solid line* and *dashed line* show predicted trajectories for algal density of *M. aeruginosa* and *P. agardhii*, respectively. The *one-point chain line* shows the predicted trajectory for dissolved iron concentration

rence of *Microcystis* blooming (roughly defined as cell density of $\geq 10^5$ cells ml^{-1}) in Lake Kasumigaura was indicated (Fig. 6). Therefore, the inability of *Microcystis* spp. to grow in Lake Kasumigaura may be caused by other factors, in addition to iron limitation.

The optimum cellular N:P ratio has been known to be species specific (Rhee and Gotham 1980). These optimum N:P ratios were estimated under iron-replete conditions. However, cellular N:P ratio was found to change in response to iron limitation for both *M. aeruginosa* and *P. agardhii* in this study. Our study also showed the cellular N:P ratio for *P. agardhii* decreased to a greater extent than that for *M. aeruginosa* under severe iron limitation. Therefore, the effect of N:P ratio on the mechanism of species dominance needs reconsideration. There are multiple limiting nutrients (such as nitrogen, phosphorus, and iron) in natural aquatic systems, and we therefore need to consider the interrelationships between nitrogen and iron, and between phosphorus and iron, as well as the individual effects of these nutrients on algal growth.

In this study, we have investigated the details of growth characteristics and developed kinetic models of population growth and iron consumption under iron limitation for two species of cyanobacteria, *M. aeruginosa* and *P. agardhii*. We have shown that growth characteristics under iron limitation are markedly different between *M. aeruginosa* and *P. agardhii*. Moreover, our model was able to describe iron competition between *M. aeruginosa* and *P. agardhii* quantitatively. Thus, the differences in growth characteristics will be important determinants of the dominance of these algal species in natural aquatic systems.

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