Size-dependent growth of *Microcystis* colonies in a shallow, hypertrophic lake: use of the RNA-to-total organic carbon ratio

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Abstract: *Microcystis* was cultured under standard conditions in BG-11 and M-11 media. Using results of an analysis of RNA and total organic carbon (TOC) content, a significant logarithmic relationship between *Microcystis* growth rate and the RNA/TOC ratio was described to measure the growth rate. Colonial *Microcystis* samples collected in a shallow, hypertrophic lake (Lake Taihu, China) during May to November 2012 were divided into six size classes (<75, 75–100, 100–150, 150–300, 300–500, and >500 μm), and the RNA/TOC ratio of each class was analyzed to evaluate differences in growth. The growth rate of colonies in the 150–300 μm size class was highest from May to August, but the growth rate increased along with the increase in colony size from September to November. Our results also indicated that water temperature did not change the relationship between growth rate and colony size, but the growth rate of larger colonies was higher than the growth rate of smaller colonies at conditions of low total nitrogen, low total dissolved phosphorus concentration, and high light intensity. Taken together, these results suggest that large colonial *Microcystis* possess an advantage that is a consequence of this faster growth at lower nutrient concentrations and high light intensities.

Keywords: *Microcystis*; colony; size-dependent; growth rate

Introduction

Large colonial forms of *Microcystis* are considered to be an important contributing factor for algae bloom formation in lakes and reservoirs (Yamamoto et al. 2011). The ecological advantages that contribute to this dominance include fast vertical migration (Kromkamp and Walsby 1990; Nakamura et al. 1993), effective phosphorus uptake (Shen and Song 2007), and protection from growth inhibition factors such as high light intensity (Wu et al. 2011), ultraviolet radiation (Sommaruga et al. 2009), and toxic substances (Wu et al. 2007). *Microcystis* are also protected from predation by zooplankton (Cyr and Curtis 1999; Yang et al. 2009). Most of these advantages promote the growth of *Microcystis* and result in an increase in biomass and the formation of large colonies. However, the self-shading of cells in the colonies continually reduces irradiance of the colony center and inhibits the growth of the internal cells (Yamamoto and Shiah 2010). Therefore, although colony formation has clear benefits, it also hinders the growth of colonial *Microcystis*. However, the relationship between the growth of *Microcystis* colonies and colony size is poorly understood.

Algae growth rate decreases with increasing cell size (Durbin 1977; Geiderlo et al. 1988). There is a significant negative relationship between growth rate and cyanobacterium colony size (Li and Gao 2003). The relative growth rate of *Nostoc sphaeroides* decreases as colony size increases. Gao and Ai (2004) reported similar results. Wilson et al. (2010) measured the growth of individual *Microcystis* colonies of several genotypes and found that growth rate decreases with increasing colony

diameter. However, some researchers have reported that *Microcystis* growth rate is positively correlated with colony size. Yamamoto and Shiah (2010) investigated the growth of colonial *Microcystis aeruginosa* using the frequency of dividing cells (FDC) method and found that large colonies grow at a relatively higher rates compared with small colonies on day 14. Wilson et al. (2006) studied the variation in maximum growth rate for 32 *M. aeruginosa* strains isolated from 12 lakes and found that growth rates are positively correlated with mean colony size. Nielsen (2006) suggested that there is no relationship between colony size and growth rate for colonial cyanobacterium, including *Microcystis*.

In addition to colony size, the growth of colonial *Microcystis* is affected by environmental conditions (e.g., light intensity, water temperature, and nutrition levels). Therefore, contradictory results may be due to differences in culture conditions. Although several experiments have been performed under laboratory culture conditions, the results do not necessarily reflect the natural lake and reservoir environment. The effects of colony size on the growth of colonial *Microcystis* in lakes and reservoirs under varying environmental conditions are still not clear. This is because it is difficult to estimate natural growth rates in the lake and reservoir environment.

The changes in *Microcystis* cell density at a particular location may be used to estimate net growth rate, but these estimates may be affected by spatial transference and predation by zooplankton. The frequently used FDC method (Yamamoto and Tsukada 2009) requires continuous 24-h monitoring. Because of the effect of spatial

transference, samples collected at the same site at different times may appear to be from different locations. Fortunately, the use of cellular RNA content reflects the in situ growth rate of *Microcystis* (Nagai et al. 2011). This method does not require continuous monitoring, but counting cell numbers in irregular colonies is a challenge that should be considered.

In this study, the total organic carbon (TOC) content of algae was used as an indication of *Microcystis* cell biomass. An improved method to estimate the RNA/TOC ratio was used to estimate colony growth rate in situ. The aim of this study was to perform a quantitative analysis of the effects of colony size on colony growth in lakes and reservoirs.

Materials and methods

Organisms and axenic cultures

Organisms: The unicellular *M. aeruginosa* (Kützing) Kützing 1846 strain (FACHB 469) was provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. It was purified using serial dilution and was cultured using long-term axenic cultivation in BG-11 medium (Allen 1968).

Pure cultures: Algae were batch-cultured axenically in triplicate in 1500 mL of sterilized liquid BG-11 and M-11 media (Kameyama et al. 2004) and were exposed to a 12:12 h light: dark cycle. Two media were used to ensure that nutrient composition did not affect the relationship between the RNA/TOC ratio and growth rate. The light

intensity was 60 μmol photons m^{-2} s^{-1} and the temperature was 25°C. Initial cell density was 5×10^4 cells mL^{-1}. Each flask was gently shaken by hand three times daily to prevent the cells from adhering to the inner wall of the flask.

Cell counting and growth rate calculation: Specific growth rates were estimated from daily cell counts. Each sample of cells was counted three times in a hemocytometer (Shanghai Qiujing Glass Instrument Factory, Shanghai, China) under an optical microscope (Olympus CX31, Olympus Corporation) at 400× magnification. When the three sample counts differed by <10%, the mean value was used as the final cell density. If the cell number estimates differed by >10%, additional samples were counted until there were at least samples that met the criterion.

The daily specific growth rate (μ) was estimated as:

$$\mu= \ln(D_t/D_{t-1}) \quad (1)$$

where D_t was the cell density on day t.

RNA and TOC analysis:

Each culture was sampled (20–50 mL volume) in triplicate, and each replicate was filtered through a GF/C filter (Whatman, UK). Each filter was re-suspended in a 50-mL centrifuge tube containing 5 mL 1 mol L^{-1} $HClO_3$, placed in a 90°C water-bath oscillator, shaken at 120 rpm for 15 min, and then centrifuged at 11,550 × g for 15 min. The supernatant was then separated from the pellet. The three samples of supernatant were combined, and RNA content was analyzed using the methods described by Rhee et al. (1978).

Similar volumes of culture samples were filtered through GF/C (Whatman, UK) filters in triplicate, and the filters were freeze dried. The TOC content of the dried filters were analyzed using a TOC-CPN SSM-5500 carbon analyzer (Shimazu, Japan).

Natural sample collection

Samples were collected from Lake Taihu, the third largest freshwater lake in China (2338 km^2, 1.9 m depth). It was selected as a representative shallow, hypertrophic lake. In recent years, massive *Microcystis* blooms have occurred frequently in the lake from May to November (Duan et al. 2009). These algae blooms have covered hundreds of square kilometers.

Sampling was performed once each month in Lake Taihu's Meiliang Bay (Fig. 1), in southeast China, from mid-May to mid-November 2012. The samples were collected from an area with recurring natural *Microcystis* blooms (coordinates: 31° 24' to 31° 28' N; 120° 10' to 120° 12' E). They were collected from a depth of 30 cm below the lake surface, which was an area of high *Microcystis* density. Each 5000-mL plastic bottle containing the algae sample was fixed with formalin [2% (v/v)] immediately after collection. Water temperature was monitored using an electronic thermometer (Mettler SG7, Toledo, OH, USA).

Colony analysis

Microcystis species composition and colony sizes present in the natural sample were analyzed. A 10-mL subsample was shaken thoroughly and then photographed using an Olympus C-5050 digital camera coupled to an Olympus CX31 optical microscope (Olympus America Inc., Center Valley, PA, USA). The photomicrographs

were analyzed using UTHSCSA ImageTool v3.00 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). A minimum of 200 colonies per sample were analyzed to determine: 1) the percent composition of the various sizes of the *Microcystis* colonies; 2) the percent composition of the *Microcystis* species present in the total *Microcystis* biovolume (*M. aeruginosa*, *M. wesenbergii*, *M. flos-aquae*, and other/unidentified *Microcystis* species); and 3) the percent composition of *Microcystis* present in the total plankton biovolume. The *Microcystis* colony biovolumes were calculated using the sphere-volume calculation method.

Sample sieving and analysis

A 200-mL subsample was used for colorimetric total nitrogen (TN) and total phosphorus (TP) analysis after digestion with $K_2S_2O_8$+NaOH (Ebina et al. 1983). Another 2200-mL subsample was filtered through a 64-μm mesh size phytoplankton net, then through a 0.45-μm pore size membrane, and 200 mL of the filtrate was used for colorimetric total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) analysis (similar to the method for the TN and TP analysis). The remaining filtrate was used to remove the alga that might adhere to the sieves.

The remaining 2600 mL formalin-fixed water sample was poured gently through sieves of various pore sizes (six classes: <75 μm, 75–100 μm, 100–150 μm, 150–300 μm, 300–500 μm, and >500 μm). The content of each sieve was re-suspended in 300 mL of the filtrate obtained above. For each size class, six replicates of 10–20 mL of the re-suspended sample were filtered through GF/C (Whatman, UK) filters. Three of

the filters were used for RNA content analysis following the method described previously. The other three filters were freeze dried and were then used for the TOC analysis.

Data analysis

Principal component analysis (PCA) was used to examine the relationships among the environmental factors. The PCA was performed using CANOCO 4.5 software (SCIENTIA Software, Wageningen UR, Wageningen, Netherlands). The differences between environmental factors at different time points were calculated using SPSS 10.0 software (IBM, Armonk, NY, USA).

Results

***Microcystis* growth and variation of cellular RNA and TOC content**

Figure 2 presents the growth curves for *M. aeruginosa* cultured in BG-11 medium and in M-11 medium. The maximum cell density (1700×10^4 cells mL^{-1}) in M-11 medium occurred on day 10, and then the cell density decreased. The cell density in BG-11 medium increased consistently from day 1 to the end of the observation period. The cell density was 3000×10^4 cells mL^{-1} on day 12.

Relationship between growth rate and the RNA/TOC ratio

The results for the relationship between *Microcystis* growth rate and the RNA/TOC ratio (by wt) are presented in Fig. 3. There appeared to be a logarithmic relationship between *Microcystis* growth rate and RNA/TOC for both cultures. This relationship was described using:

$$\mu = 0.625 \times \ln(\text{RNA/TOC}) + 1.056,$$

where μ was the growth rate of *Microcystis* (day^{-1}), and RNA/TOC was the ratio of RNA to TOC, by weight. Lag phase data were excluded from the estimate.

Environmental parameters

The water temperature in Meiliang Bay increased from 19.2ºC to 30.2ºC from May to July 2012 and decreased from 30.2ºC to 10.6ºC from August to November 2012 (Fig. 4). Light intensity remained below 1000 μmol photons m^{-2} s^{-1}.

TDN concentration decreased from 1.2 $mg \cdot L^{-1}$ in May to 0.5 $mg \cdot L^{-1}$ in November (Fig. 5). However, peaks of TN, TP, and TDP appeared in August, with concentrations of 9 $mg \cdot L^{-1}$, 1.2 $mg \cdot L^{-1}$, and 0.15 $mg \cdot L^{-1}$, respectively. The minimum values for TN, TP, and TDP were 1 $mg \cdot L^{-1}$, 0.05 $mg \cdot L^{-1}$, and 0.01 $mg \cdot L^{-1}$, which occurred in September, May, and October, respectively.

The results of the PCA revealed that the environmental factors in Lake Taihu varied during the 7-month study period (Fig. 6). The environmental conditions present during May to August were distinct from the conditions present from September to October, as indicated by the separation of arrows for the respective months in Fig. 6. The differences in environmental factors between the first 4 months (group 1) and the last 3 months (group 2) are presented in Table 1. TN, TDP, and light intensity differed significantly between the two groups. The values for TN and TDP in group 2 were much lower than the group 1 values, but the opposite relationship occurred for light intensity. Colony growth rate increased with increasing colony size when the TN and TDP concentrations were low, and the light intensity was high.

Seasonal variation

Table 2 presents the results for the seasonal percent variation in the composition of various *Microcystis* species comprising the total *Microcystis* biovolume, and the relative abundance of *Microcystis* present in the plankton biomass during the study period. The proportion of *Microcystis* in the plankton biomass was 88.9% in May and was > 93% from June to November. The results for the relative abundance of various *Microcystis* species indicated "other" *Microcystis* were the dominant species in May. The percentage of *M. flos-aquae* and *M. wesenbergii* increased in June, to 29.8% and 29.6%, respectively. *M. wesenbergii* was the dominant species from July to September. *M. aeruginosa* appeared in October (41.1%). *M. flos-aquae* was the dominant species in November (98.3%).

The relative percent abundance of *Microcystis* colonies <75 μm was 44.1% and 34.8% in May and June, respectively (Fig. 7). The abundance of this size class then decreased rapidly, and was <10% from July to October. The relative percent abundance of *Microcystis* colonies >500 μm increased to >40% in July and August. From September to November, each of the largest size classes comprised at least one-third of the *Microcystis* biovolume.

In situ growth rate

The results for in situ growth rates for colonial *Microcystis* of different sizes in different months, calculated using the RNA/TOC method, are presented in Fig. 8. The maximum growth rates of 0.56 day^{-1} and 0.53 day^{-1} occurred in July and August, respectively, for the 150–300 μm colony size. All of the growth rates of colonies <500

μm were <0.0 day^{-1} in November, while the growth rate of colonies >500 μm approached 0.2 day^{-1}.

The overall growth rate increased from May to August, but then decreased from September to November (Fig. 8). The variation in the growth rate patterns for the different size classes could be divided into two types. For type I (May, June, July, and August), the highest growth rate occurred in the 150–300 μm size class. For type II (September, October, and November), the growth rate increased along with the increase in colony size.

The growth rate of colonial *Microcystis* increased with increasing water temperature, but this change was not related to colony size (Fig. 9). The in situ growth rate was greater than zero when the water temperature was >13°C.

Discussion

Assessment of the RNA/TOC method

A significant logarithmic relationship between *Microcystis* growth rate and RNA/TOC ratio was described and was used to estimate the in situ growth rate of colonial *Microcystis* in Lake Taihu. This finding was similar to the relationships described for cellular RNA content and growth rate of other cyanobacteria species (Binder and Liu 1998; Worden and Binder 2003; Lepp and Schmidt 2004). However, this study was different in that TOC content was used to represent *Microcystis* cell biomass. Compared with the use of the cellular RNA method, which was used to analyze in situ growth rate of *Microcystis* in Lake Kasumigaura (Japan; Nagai et al.

2011), this approach was an improvement. Use of this method avoided the error associated with enumeration of natural *Microcystis* cells in irregular colonies and eliminated the inaccuracy caused by variation in *Microcystis* cell size (Nguyen et al. 2012).

The cellular RNA content method was originally developed for assay of water samples from a canal near Lake Kasimigura, which is not affected by wind-induced currents. We improved this method by developing the RNA/TOC method, which we used in Lake Taihu. The lake experiences active wind-induced water movement. However, the relationship between RNA/TOC and growth rate was considered to be intrinsic in this study. Two media with different nutrient concentrations were also used. The results indicated the relationship between RNA/TOC and growth rate was not affected by nutrient concentration. Thus, we considered that it would not be affected by environmental factors, and that the RNA/TOC ratio would reflect the growth characteristics of *Microcystis*. Nonetheless, the data obtained in this study represented only one strain of unicellular *Microcystis*. Its suitability for the measurement of colonial *Microcystis* in the field should be tested further.

There is a close relationship between *Microcystis* biomass and TOC values in laboratory experiments using a single algal strain. This relationship would also occur in the field if *Microcystis* comprised almost 100% of the plankton community. The results presented in Table 2 indicated that the relative abundance of *Microcystis* was >93% from June to November. The minimum value of *Microcystis* abundance was also as high as 88.9% in May. Thus, TOC was an appropriate indicator of

Microcystis biomass and the use of RNA/TOC method was a valid approach.

The growth rate that was used previously to establish the relationship with the cellular RNA content method was based on a calculation that included limiting factors, such as water temperature, light intensity, and nutrient concentration (Nagai et al. 2011). However, the relationship between growth rate and RNA/TOC in the current study was established based on direct measurement under controlled culture conditions. Therefore, the RNA/TOC method should reflect in situ growth rate of *Microcystis* in lakes and reservoirs, at least to some degree.

Effects of environmental factors on the relationship between growth rate and *Microcystis* colony size in Lake Taihu

Maximum growth rates occurred between May and August in the 150–500 μm size class. However, for different colony sizes, growth rate increased with increasing colony size from September to November (Fig. 5). These results suggest that colony forms incur benefits from faster growth, but the largest colonies in Lake Taihu do not always exhibit the fastest growth rates. This difference might be the result of the effects of different environmental factors within the lake.

Colony formation has been considered to be a strategy used by *Microcystis* to reduce the occurrence of photoinhibition under high light intensities (Wu et al. 2011). Thus, larger colonies should experience faster growth rates when high irradiation conditions are present. Shen and Song (2007) reported that the growth of unicellular and small colonial *Microcystis* is inhibited at a P concentration of 0.2 mg L^{-1}, but the growth of the large colonial *Microcystis* is not inhibited when the same conditions are

present. These results indicate that compared with small colonial and unicellular *Microcystis*, large colonial *Microcystis* require lower concentration of P for growth. Our results also suggested the presence of this advantage for the large colonies.

The TN concentration appeared to affect the colony size-dependent growth rate of the *Microcystis* colonies. The mid-sized colonies (150–500 μm) experienced their maximum growth rates while the TN concentration was highest. Growth rate increased with increasing colony size when the TN concentration was lower. This result suggests that large colonial *Microcystis* experience improved growth under lower TN concentrations, which is an observation that has not yet been reported. The TN concentration is also a factor that affects colony size of *Microcystis*. Yang and Kong (2013) reported that lower nitrogen concentrations induce colony formation, because lower nitrogen concentrations exert a positive influence on extra-cellular polysaccharide production. They suggested that an increase in the C: N ratio promotes the incorporation of carbon into polymers (Otero and Vincenzini 2003). Taken together, these observations support the conclusion that lower TN concentration results in colony formation of larger colonies.

Kurger (1978) and Chu (2007) reported that *Microcystis* growth rate increases from about 10 to 30 °C, decreases slightly from 30 to 40 °C, and then slows rapidly when the temperature exceeds 40 °C. The water temperature of Lake Taihu was between 10 and 30 °C, which would not inhibit the growth of colonial *Microcystis*. Thus, the observation that temperature did not alter the relationship between growth rate and colony size (Fig. 9), at least in Lake Taihu, is understandable.

The dynamics of *Microcystis* of different sizes reflects not only the difference attributed to size, but also species-specific differences. Imai et al. (2009) reported that the growth rate of *M. aeruginosa* was significantly higher than the growth rate of *M. wesenbergii* at high temperatures (30 and 35 °C). Zhai et al. (2013) reported that regardless of temperature and nutrient conditions, *M. aeruginosa* is a superior competitor to *M. flos-aquae*. The relationship between environmental factors and size-dependent growth rate may be an indirect rather than a direct relationship. However, the relative abundance of a single *Microcystis* species exceeded 90% during most of the study period. *M. wesenbergii* was the dominant species in July, with a relative abundance as high as 70.2%. The relationship between environmental factors and size-dependent growth rate in June and October was similar to the relationship in the previous and in the following month. Nevertheless, the relative abundances of various *Microcystis* species in both June and October were quite different from the previous and following months. The results of our recent study (Li et al. 2013) suggested that the seasonal succession of *Microcystis* species can be considered to be a colonial morphological change induced by mucilage solubilization. Thus, the relationship between environmental factors and size-dependent growth rate observed in the current study may be credible, and the *Microcystis* species effects may be unimportant.

Relationship between growth rate and colony size under culture and field conditions

The above discussion illustrated that *Microcystis* colonies in the middle size class

(150–500 μm) had the maximum growth rates, except for when the TN and TDP concentrations were low and the light intensity was high. This finding was different from the findings of previous studies that used culture conditions (Table 3). Using culture conditions and the FDC method, Yamamoto and Shiah (2010) measured the growth rates of colonial *Microcystis* through days 14 and 35. They found out that the relationship between growth rate and colony size was negative, and then positive, through the 14th and 35th days of growth, respectively. The nutrient conditions were most likely the only difference between the two time periods. Nutrient availability would be exhausted by the 35th day. Thus, the positive relationship between growth rate and colony size could be explained by the tolerance of large colonies for nutrient limitation. Wilson et al. (2006) reported a positive relationship between growth rate and colony size for *Microcystis* colonies from 35 to 178 μm, which was similar to our results. However, the presence of this relationship was not supported by Wilson et al. (2010).

At present, we cannot explain the reasons for the differences in results between our study and Wilson et al. (2010) and Yamamoto and Shiah (2010) studies. It is possible that the environmental conditions in Lake Taihu were nutrient limited compared with the culture conditions. Under standard culture conditions used, all of the environmental factors were sufficient to support the rapid growth of *Microcystis* colonies. This may also explain why *Microcystis* colony transformed into unicell under standard culture conditions. This phenomenon has been described by Burkert et al. (2001) and Yang et al. (2006). In field conditions, environmental factors (e.g.,

water temperature, light intensity, nutrient concentrations, and hazardous materials) inhibit the growth of colonial *Microcystis*. Under specific environmental conditions, appropriate-sized colonies experience good growth when the benefits are greater than the costs. The optimum size would also vary along with variation in environmental conditions.

In conclusion, the RNA/TOC method based on the significant logarithmic relationship between *Microcystis* growth rate and the RNA/TOC ratio obtained from laboratory experiments reflect in situ growth rate of *Microcystis* in lakes and reservoirs, at least to some degree. The growth rate of colonies in the size class of 150–300 μm was the highest from May to August, and increased along with the increase in colony size from September to November. At low TN and TDP concentrations and high light intensity, the growth rate of larger colonies was faster than the growth rate of smaller colonies. This result suggests that large colonial *Microcystis* possess the advantage of superior growth at lower nutrient concentrations and high light intensity.

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Table 1 Differences in environmental factors between the first 4 months and the last 3 months of the study period. (Group 1 = May to August, group 2 = September to November)

Environmental factors	Groups	Mean	Standard Deviation	P
TN	Group 1	4.62	3.68	**
	Group 2	2.38	1.12	
TDN	Group 1	0.85	0.22	ns
	Group 2	0.48	0.07	
TP	Group 1	0.46	0.53	ns
	Group 2	0.28	0.13	
TDP	Group 1	0.05	0.07	*
	Group 2	0.02	0.00	
Light intensity	Group 1	676	277	**
	Group 2	759	41	
Temperature	Group 1	26.6	5.1	ns
	Group 2	19.3	8.1	

*: P<0.10;
**: P<0.05;
ns: not significant.

Table 2 Seasonal variation in percentages of various *Microcystis* species in the total *Microcystis* biovolume, and in the relative abundance of *Microcystis* in the plankton biomass during the study period.

	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**
Relative abundance of *Microcystis*	**88.9**	**93.5**	**99.4**	**97.2**	**98.9**	**96.7**	**98.3**
M. aeruginosa	0	0	2.2	0	0	41.1	0
M. wesenbergii	0	29.8	37.5	98.4	92.0	0.7	0
M. flos-aquae	2.5	29.6	59.2	1.6	3.2	57.8	98.3
Other *Microcystis*	97.5	40.6	1.1	0	4.7	0.4	1.7

Table 3 Relationship between culture conditions and Microcystis growth rate and colony size.

Researchers	Wilson et al. (2006)	Wilson et al. (2010)	Yamamoto and Shiah (2010)	
Relationship between growth rate and colony size	positive	negative	negative	positive
Method used to estimate growth rate	Logarithmic growth laws	Logarithmic growth laws	FDC	FDC
Initial cell density	10^3 μm^3 mL^{-1}	2 colonies mL^{-1}	2×10^3 cells mL^{-1}	2×10^3 cells mL^{-1}
Light intensity (μmol photons m^{-2} s^{-1})	250	60	100	100
Temperature	25	24	24	24
N concentration (mol mL^{-1})	2	0.5	2.26	2.26
P concentration (mol mL^{-1})	0.18	0.02	0.174	0.174
Duration (day)	9	8	14	35
Colony size (μm)	35-178	100-400	50-400	80-800

Figure captions

Figure 1 Sampling area in Meiliang Bay, Lake Taihu, China.

Figure 2 Growth curves for *M. aeruginosa* grown in BG-11 medium and M-11 medium. Vertical lines represent the maximum and minimum values.

Figure 3 The relationship between *Microcystis* growth rate and the RNA/TOC ratio.

Figure 4 Water temperature and daily maximum light intensity during the field studies.

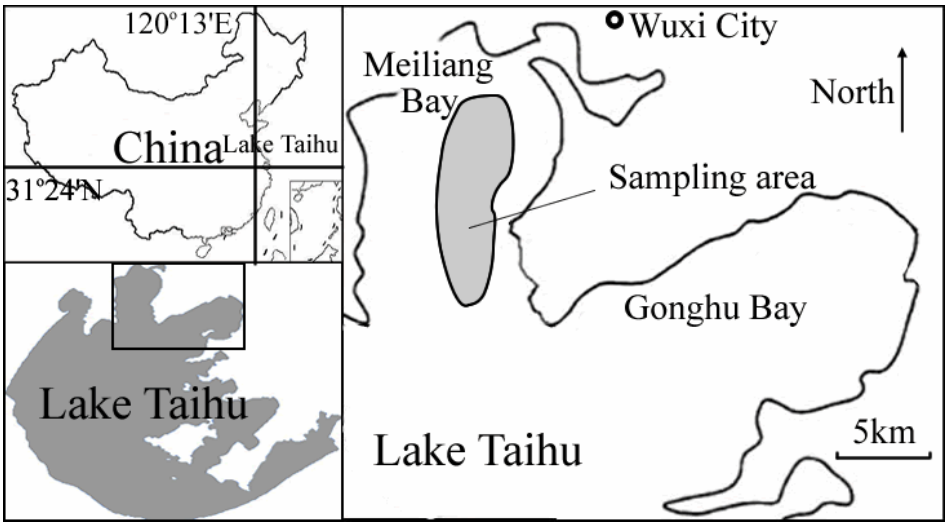
Figure 5 Seasonal variation in nutrient concentration during the field studies. Vertical lines represent the maximum and minimum values.

Figure 6 Principal component analysis (PCA) of environmental factors in Lake Taihu, China.

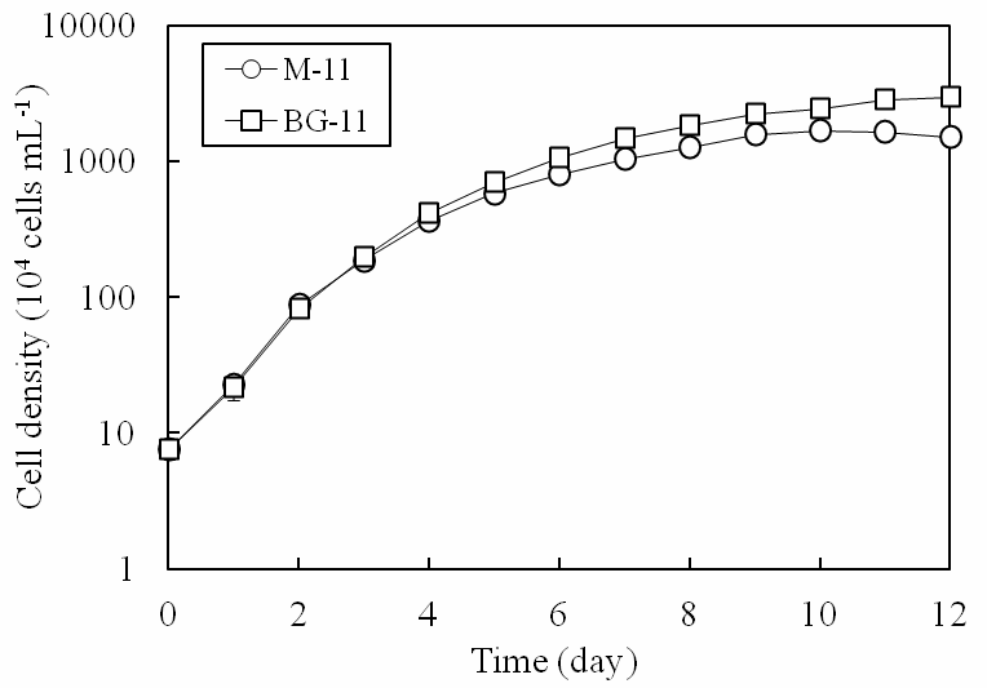
Figure 7 The percentage of different size classes of *Microcystis* during each month.

Figure 8 In situ growth rate of different size classes of colonial *Microcystis* in different months, calculated using the RNA/TOC method. Vertical lines represent the maximum and minimum values.

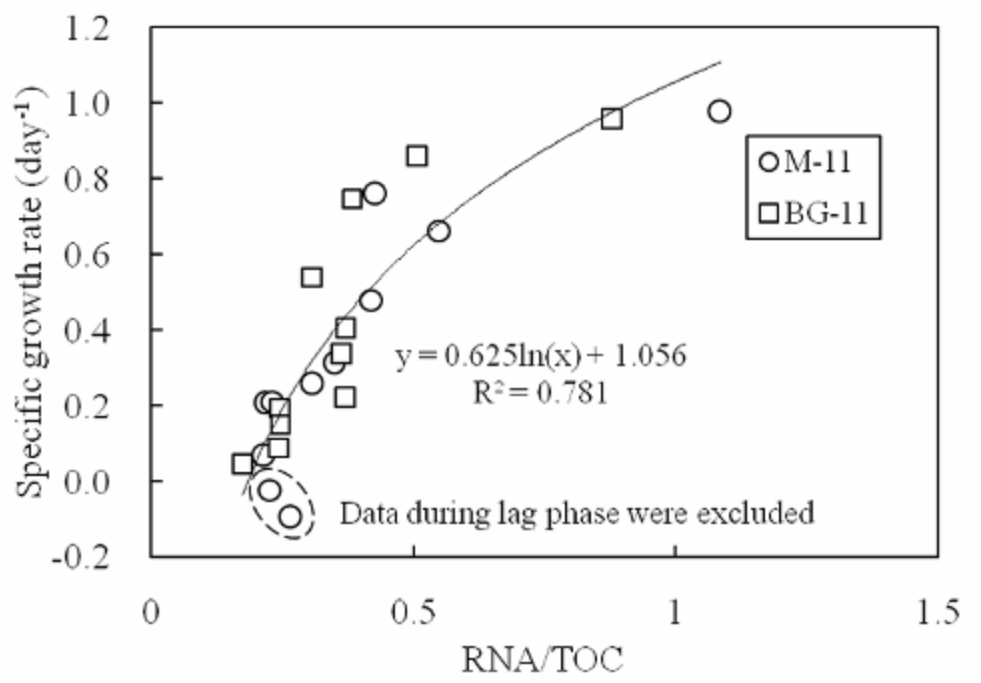
Figure 9 The relationship between growth rate of colonial *Microcystis* and water temperature.


120°13'E
China
Lake Taihu
31°24'N
Lake Taihu
Wuxi City
Meiliang
Bay
North
Sampling area
Gonghu Bay
Lake Taihu
5km

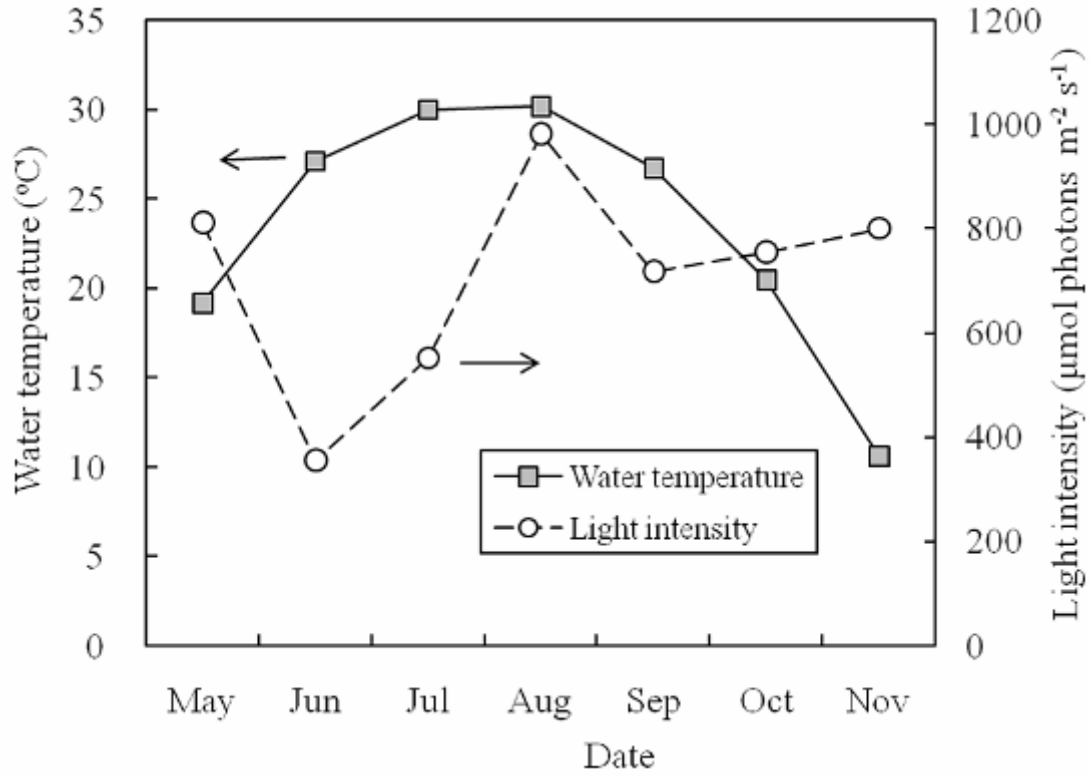
[Fig. 1]


10000
1000
100
10
1
Cell density (10^4 cells mL^{-1})
M-11
BG-11
0
2
4
6
8
10
12
Time (day)

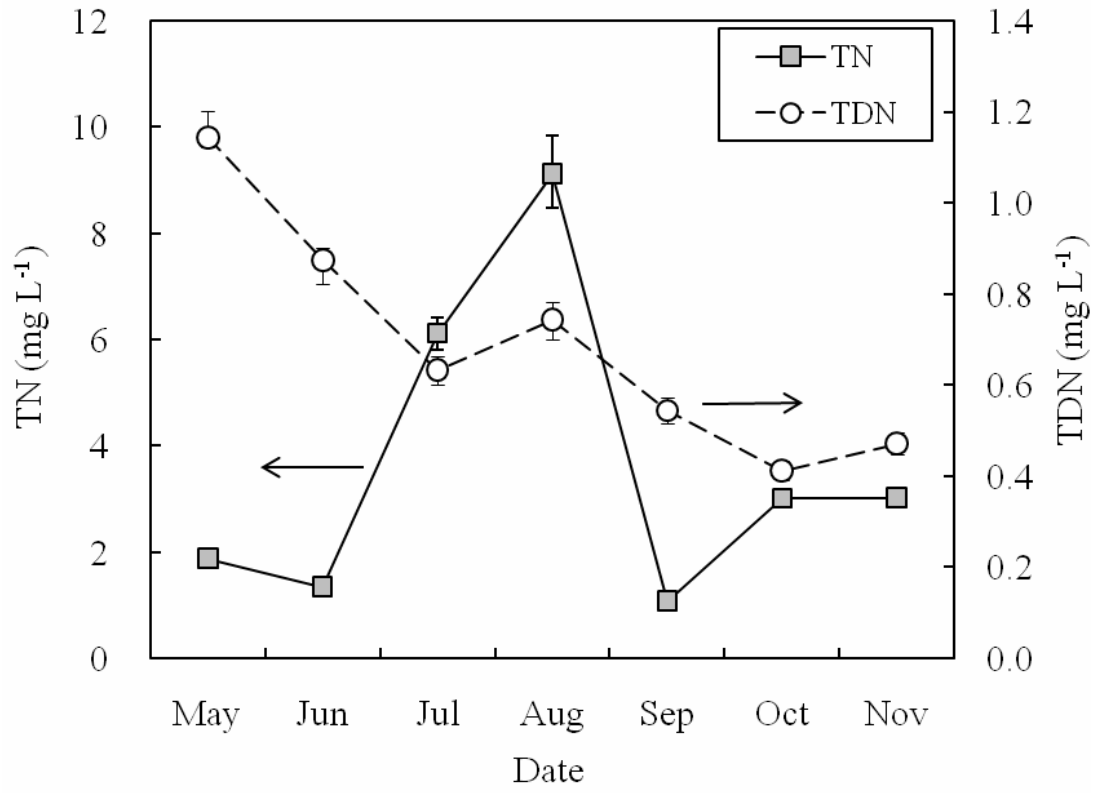
[Fig. 2]

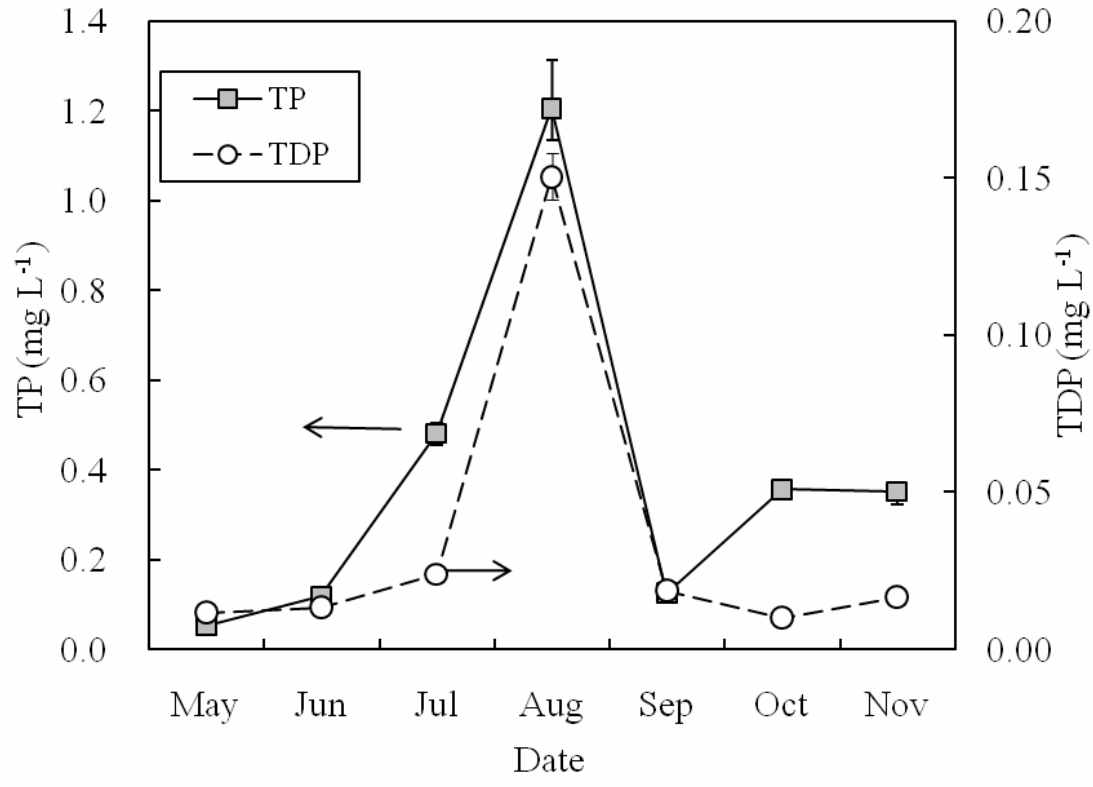

1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Specific growth rate (day-1)
0
0.5
1
1.5
RNA/TOC
M-11
BG-11
y = 0.625ln(x) + 1.056
R² = 0.781
Data during lag phase were excluded

[Fig. 3]

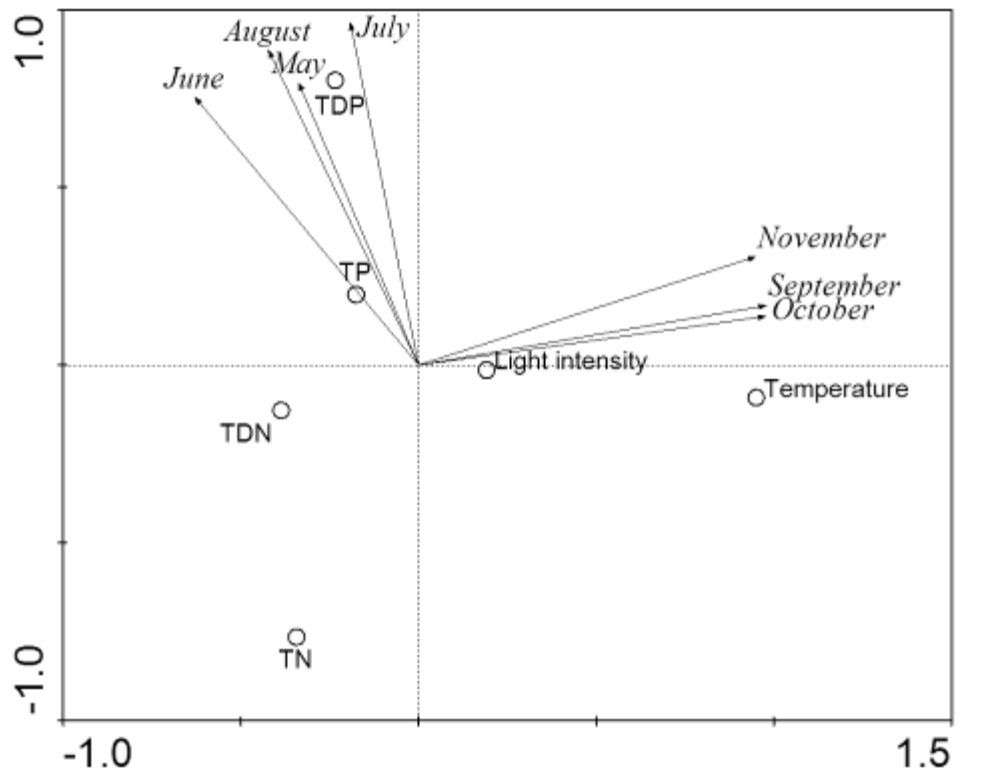

Water temperature (°C)
Light intensity (μmol photons m-2 s-1)
Water temperature
Light intensity
May
Jun
Jul
Aug
Sep
Oct
Nov
Date
0
5
10
15
20
25
30
35
0
200
400
600
800
1000
1200

[Fig. 4]

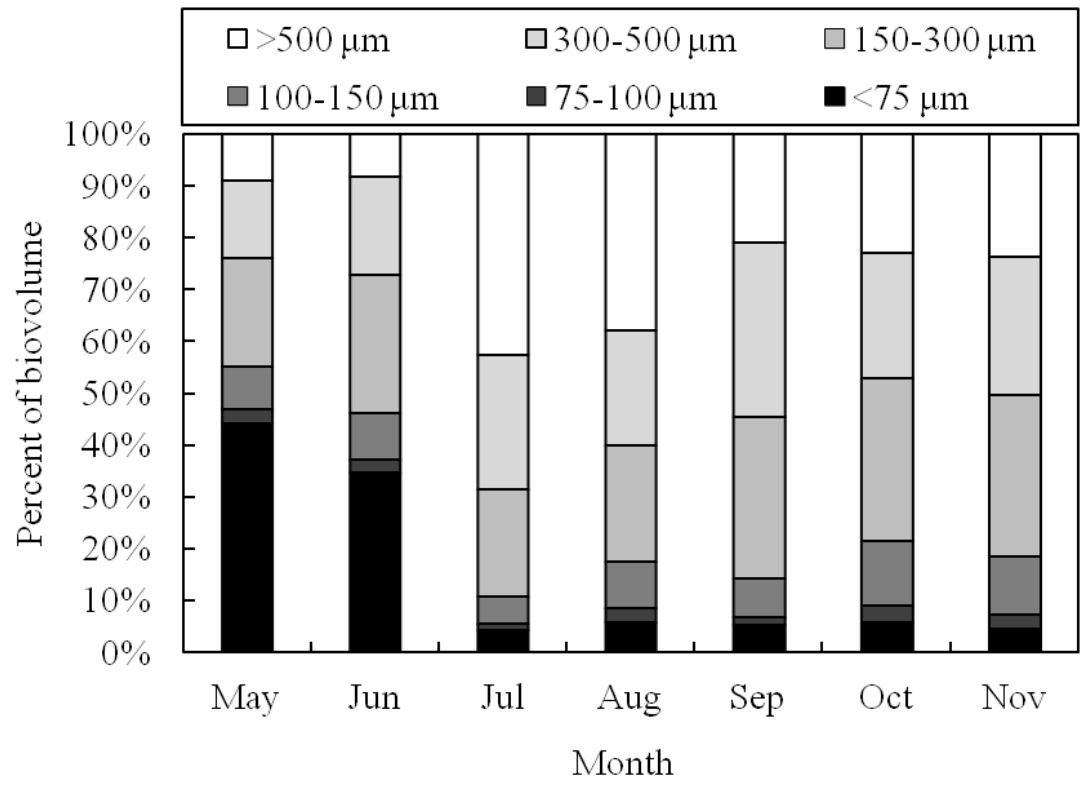

TN
TDN
TN (mg L-1)
TDN (mg L-1)
0
2
4
6
8
10
12
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
May
Jun
Jul
Aug
Sep
Oct
Nov
Date


TP
TDP
TP (mg L-1)
TDP (mg L-1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0.00
0.05
0.10
0.15
0.20
May
Jun
Jul
Aug
Sep
Oct
Nov
Date

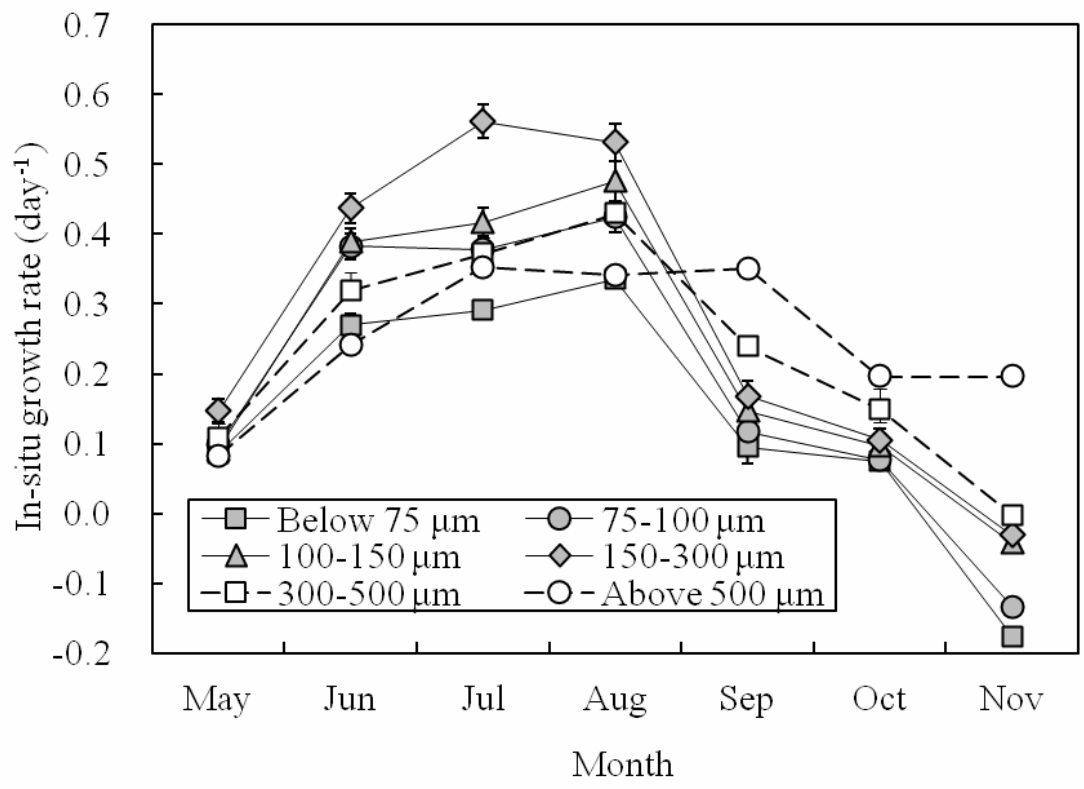
[Fig. 5]


1.0
-1.0
-1.0
1.5
August
July
May
June
TDP
TP
November
September
October
Light intensity
Temperature
TDN
TN

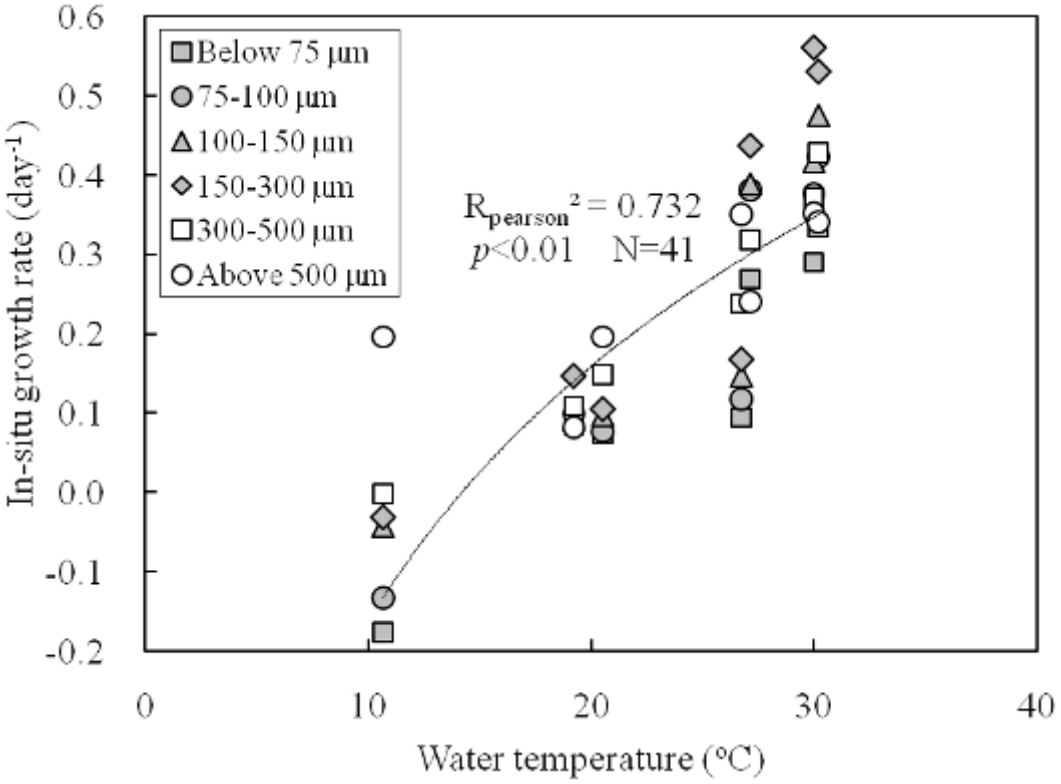
[Fig. 6]


>500 μm
300-500 μm
150-300 μm
100-150 μm
75-100 μm
<75 μm
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent of biovolume
May
Jun
Jul
Aug
Sep
Oct
Nov
Month

[Fig. 7]


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
In-situ growth rate (day-1)
Below 75 µm
75-100 µm
100-150 µm
150-300 µm
300-500 µm
Above 500 µm
May
Jun
Jul
Aug
Sep
Oct
Nov
Month

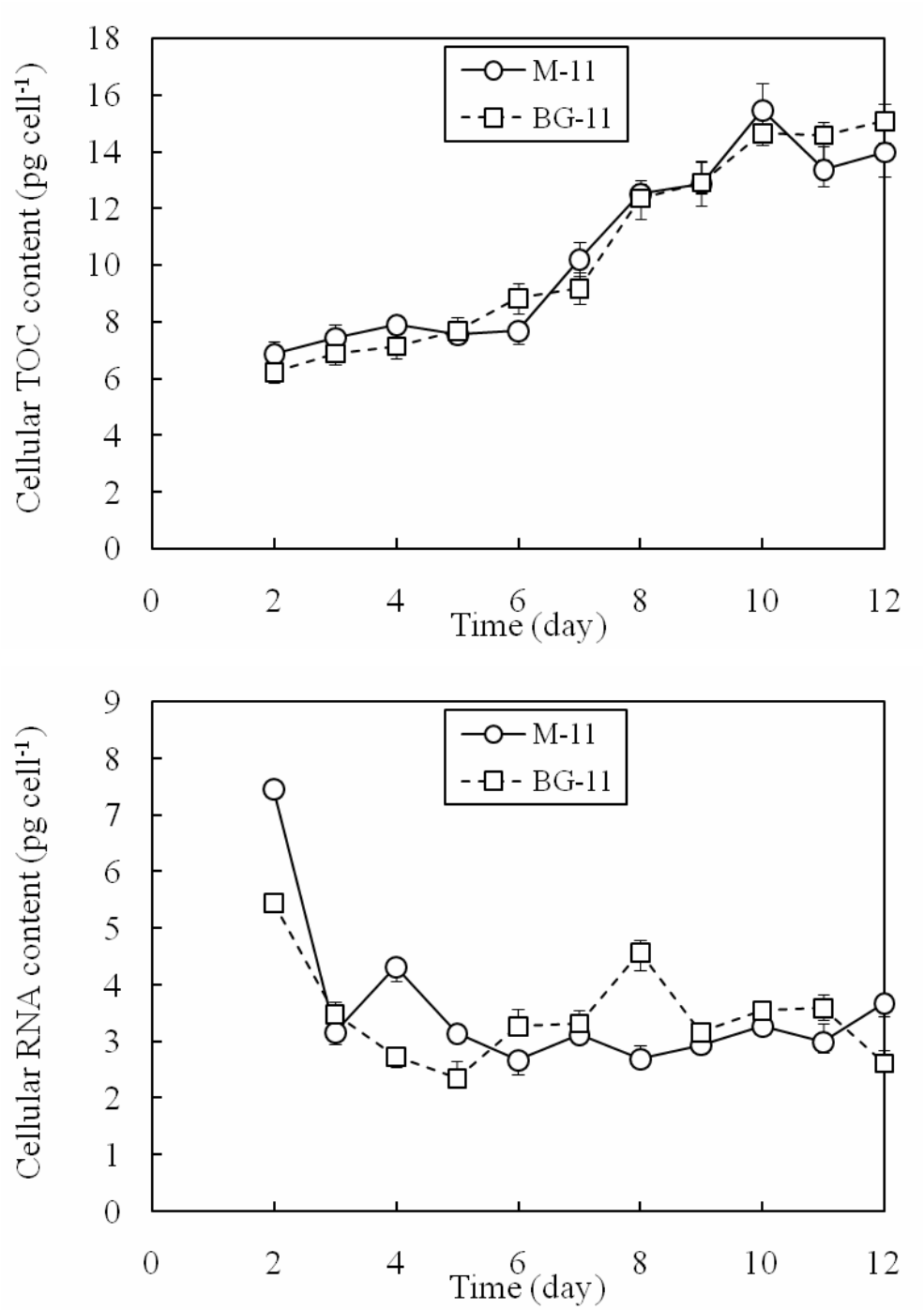
[Fig .8]


Below 75 μm
75-100 μm
100-150 μm
150-300 μm
300-500 μm
Above 500 μm
$R_{pearson}^2 = 0.732$
$p<0.01$ N=41
In-situ growth rate (day^{-1})
Water temperature (°C)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
10
20
30
40

[Fig. 9]

Supplementary materials:

Fig. S1 Cellular TOC and RNA content along with the growth of *Microcystis* under cultured conditions. Vertical lines represent the maximum and minimum value respectively.



[Fig. S1]