Influence of Cultural Conditions on the Cellular Biovolume and Gelatinous Sheath Volume of *Staurastrum arctiscon* (Charophyceae)

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ABSTRACT

This study investigated the influence of cultural conditions on the cellular biovolume (V_C) and gelatinous sheath volume (V_G) of *Staurastrum arctiscon* (Charophyceae) and its growth characteristics during laboratory culturing. A unialgal culture isolated from Lake Biwa was incubated in modified M11 medium containing various concentrations of nitrogen and phosphorous in the form of nitrate and phosphate (0.01 or 0.1 mg P/L). The N/P mass ratio of the medium and incubation temperature ranges were 5 – 100 and 5 – 35°C, respectively. V_C was lower at higher incubation temperature and higher nitrate concentrations, whereas V_G increased with temperature. As V_C and V_G changed, the size of the gelatinous sheath increased relative to the cellular biovolume (V_R) with the water temperature and nitrate concentration. The enhanced growth rate and net production rate with a rising temperature were considered to contribute to the decreased V_C and increased V_G . The positive effect of nitrate concentration on V_G was believed to be attributable to the enhanced net production rate with higher nitrate concentrations.

Keywords: gelatinous sheath, Lake Biwa, N/P ratio, phytoplankton

INTRODUCTION

Phytoplankton and aquatic plants account for most of the primary production in lakes. The phytoplankton population density is not very high in Lake Biwa, Japan, although the primary production by phytoplankton in offshore zones of the lake is believed to account for a significant proportion of the total production because the lake is deep and the euphotic zone reaches depths of 10 – 20 m. Nozaki (2002) reported that the areal net phytoplankton production in the pelagic zone in Lake Biwa was almost equal to or exceeded the areal net production in the littoral zone, where most of the net production was attributed to periphyton. Therefore, the influence of primary production by phytoplankton and their behavior in the field are important for understanding organic pollution in lakes.

Changes in the stratification structure due to climate change (Kumagai, 2008; Sakamoto, 2011) and changes in the nitrogen to phosphorus ratio (N/P ratio) (Tsuda *et al.*, 2006; Hsieh *et al.*, 2010) have been reported in Lake Biwa. To investigate the ecological impact of environmental change in Lake Biwa, Hsieh *et al.* (2010) reported that the phytoplankton community shifted dramatically in response to changes in trophic status driven by phosphorus loadings and physical properties of the water column driven by warming. Furthermore, Ichise *et al.* (2010a) reported that the mean cellular biovolume

tended to decrease in Lake Biwa. In general, smaller organisms show higher specific growth rates (Kagami and Urabe, 2001; Belgrano *et al.*, 2002). Therefore, the predominance of smaller phytoplankton results in higher specific productivity (Yvon-Durocher *et al.*, 2011). In addition to the succession of the phytoplankton community, environmental changes may influence the cellular biovolume of the same phytoplankton species because it is often observed that the same phytoplankton species show diverse body size according to environmental factors in the habitat. However, the mechanism of this cause–effect relationship is unclear at present. Thus, it is necessary to clarify the relationship between environmental factors and the cellular biovolume.

In addition, Ichise *et al.* (2010a) reported that phytoplankton with gelatinous sheaths tended to increase in Lake Biwa. Lange (1976) suggested that the formation and retention of a voluminous sheath provides a microenvironment around the algal cell where essential nutrients, which are present only at submarginal levels in the surrounding water, can become concentrated and made readily available to the cell. In addition, Fogg (1983) reported that extracellular phytoplankton photosynthetic products occasionally accounted for about 25% of the primary production. Thus, the main factors affecting gelatinous sheath production also need to be explored to understand organic pollution due to production within lakes. Light, nutrients, and temperature are important factors that affect photosynthesis (Urabe *et al.*, 1999). Nitrogen and phosphorus in particular are the major nutrients that affect primary production (Smith, 1979; Downing, 1997; Hall *et al.*, 2005). Therefore, this study investigated the influence of water temperature, nitrogen, and phosphorus on the cellular biovolume and gelatinous sheath volume of *Staurastrum arctiscon* (Charophyceae).

The large green algae like *Staurastrum* spp. are considered to be the main primary producers in the food web of Lake Biwa (Kagami and Urabe, 2001; Sekino *et al.*, 2007). *S. arctiscon* is a large green alga with a gelatinous sheath (Fig. 1A) and its population density in Lake Biwa has increased from an average of 0.3 cells/mL in the 1980s to 0.4 cells/mL in the 1990s and to 2.3 cells/mL in the 2000s. Accordingly, *S. arctiscon* was examined in this study.

Fig. 1 - *S. arctiscon* on a slide glass without Chinese ink (A) and with Chinese ink (B).

MATERIALS AND METHODS

Phytoplankton species examined

A unialgal culture of *S. arctiscon*, isolated from Lake Biwa, was cultured in M11 medium (Hagiwara, 1984) containing 16.5 mg N/L of nitrate as a single nitrogen source and 1.78 mg P/L of phosphate as a single phosphorus source, while the calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) concentration was changed from 40 to 30 mg/L. Briefly, 100 mL of modified M11 medium was poured into Erlenmeyer flasks with screw caps (200 mL) and autoclaved at 121°C for 20 min. An aliquot (1 or 2 mL) of *S. arctiscon* preculture was inoculated into each Erlenmeyer flask. The basic culture conditions were incubation for 20 – 26 days at 20°C with photosynthetic active radiation (PAR) at 36.5 $\mu\text{mol}/\text{m}^2/\text{s}$ and a light:dark cycle of 12:12 h in a table top biotron (LH-55-RDS; Nippon Medical & Chemical Instruments, Japan). The pre-cultured *S. arctiscon* was cultured again for 2 weeks in modified M11 medium without nitrogen and phosphorus to establish starvation. Next, the starved cells were used for incubation experiments. All incubation experiments were conducted in triplicate using batch cultures with gentle shaking every 2 or 3 days. The culture conditions were modified to determine the effects of the N/P mass ratio of the medium (5 – 100), incubation temperature (5 – 35°C), and the concentration of phosphorus (0.1 mg P/L or 0.01 mg P/L). PAR was set at 36.5 $\mu\text{mol}/\text{m}^2/\text{s}$ in these cultures. The nitrogen and phosphorus concentrations were adjusted by changing the amounts of sodium nitrate (NaNO_3) and dipotassium hydrogenphosphate (K_2HPO_4), respectively. The population density of *S. arctiscon* was monitored every 2 – 3 days using a plankton counting plate with a stereomicroscope (CX41LF; Olympus Optical, Japan).

Estimation of the specific growth rate

The specific growth rate of *S. arctiscon* was estimated from the slope of the regression line of equation (1) using population density data from the logarithmic growth phase,

$$\ln M = \alpha + \mu t \quad (1)$$

Where, μ is the specific growth rate (per day), M is the population density of *S. arctiscon* (cells/mL), α is an intercept, and t is the incubation period (days). The dependency of the specific growth rate on the nutrient concentration followed the Monod equation (2). The coefficients of the equation were determined using a Lineweaver–Burk plot (3),

$$\mu = \frac{\mu_{\max} C}{K + C} \quad (2)$$

$$\frac{1}{\mu} = \frac{1}{\mu_{\max}} \left(\frac{K}{C} + 1 \right) \quad (3)$$

Where, $\mu_{\max}$ is the maximum specific growth rate (per day), C is the nutrient concentration (mg/L), and K is the half-saturation constant (mg/L).

Volume measurement

Several drops of the culture solution containing *S. arctiscon* and Chinese ink diluted

threefold with distilled water were placed on a slide with a frame and a 1-mm-interval scale. The slide was covered with a cover slip and the phytoplankton were observed using a digital microscope (VH-5000; Keyence, Japan) (Fig. 1B). We measured the lengths of the major and minor axes of the cell and gelatinous sheath, and the radius and height of branched protrusions using a computer program (VH-H1A5; Keyence, Japan). The total volume, cellular biovolume, and gelatinous sheath volume of *S. arctiscon* (6) were calculated using the following equations:

$$V_T, V_E = \frac{\pi}{6} ab^2 \quad (4)$$

$$V_B = r \times r \times \pi \times h \quad (5)$$

$$V_C = V_E + nV_B \quad (6)$$

$$V_G = V_T - V_C \quad (7)$$

Where, V_T is the total volume ($\mu\text{m}^3/\text{cell}$), V_E is the volume of the ellipsoidal part of a cell ($\mu\text{m}^3/\text{cell}$), a is the major axis of an ellipsoid (μm), b is the minor axis of an ellipsoid (μm), V_B is the volume of a branched protrusion ($\mu\text{m}^3/\text{protrusion}$), r is the radius of a branched protrusion (μm), h is the height of a branched protrusion (μm), V_C is the cellular biovolume ($\mu\text{m}^3/\text{cell}$), V_G is the gelatinous sheath volume ($\mu\text{m}^3/\text{cell}$), and n is the number of branched protrusions per cell (30 per cell). The size of the gelatinous sheath relative to the cellular biovolume (V_R) was also determined using equation (8).

$$V_R = \frac{V_G}{V_C} \quad (8)$$

RESULTS

Growth characteristics of *S. arctiscon*

Figure 2 shows the specific growth rate of *S. arctiscon* as a function of water temperature, nitrate concentration, and phosphate concentration. The optimum growth temperature was 25°C. The responses of the specific growth rate to nitrate and phosphate concentrations resembled Monod's equation. The half-saturation constants were estimated to be $K_N = 0.076$ mg N/L for nitrate and $K_P = 0.011$ mg P/L for phosphate. Thus, *S. arctiscon* growth was weaker at high temperatures and with phosphate limitation, but stronger with nitrate limitation when compared with *S. dorsidentiferum* (optimum temperature 30°C, $K_N = 0.091$ mg N/L, $K_P = 0.0075$ mg P/L; Suzuki *et al.*, 2012).

Fig. 2 - Specific growth rate of *S. arctiscon* as a function of water temperature, nitrate concentration, and phosphate concentration. Error bars indicate the standard deviations for triplicate cultures.

Effects of water temperature and nutrients on the cellular biovolume and total volume

Table 1 shows V_G and V_C results. A higher temperature appeared to increase V_G and to decrease V_C in Table 1. However, the effects of phosphate concentration and the N/P mass ratio on V_G and V_C were not clear in culture medium.

The V_R as a function of the N/P mass ratio and water temperature are shown in Fig. 3. Like V_G , V_R seemed to increase with a rise in temperature. However, the effects of the phosphate concentration and N/P mass ratio on V_R produced no clear trend.

Multiple regression analysis was applied to V_G , V_C , and V_R using the water temperature, nitrate concentration, and phosphate concentration as predictor variables (Table 2). The water temperature had positive regression coefficients with V_G and V_R , but a negative regression coefficient with V_C at $p < 0.05$. Rhee and Gotham (1981) observed that V_C of a green algal species *Scenedesmus* sp. decreased with an increase in temperature. The relationship between V_C of *S. arctiscon* and water temperature was the same as that of *Scenedesmus* sp. The nitrate concentration had positive regression coefficients with V_G and V_R ($p < 0.05$), whereas it had no significant regression coefficient with V_C . The phosphorus concentration had no significant regression coefficients with all volumes. Thus, V_C decreased with rising water temperature, while the gelatinous sheath production was enhanced with rising water temperature and nitrate concentration, which resulted in an increase in V_R .

Table 1 - Gelatinous sheath volume (V_G) [$\times 10^3 \mu\text{m}^3/\text{cell}$] and cellular biovolume (V_C) [$\times 10^3 \mu\text{m}^3/\text{cell}$] of *S. arcticus*. N/P is the N/P mass ratio of the culture medium, while St indicates starvation. Ranges indicate the standard deviations for triplicate cultures.

V_G [$\times 10^3 \mu\text{m}^3/\text{cell}$]						
N/P	Low P concentration (0.01 mg P/L)			High P concentration (0.1 mg P/L)		
	15°C	20°C	25°C	15°C	20°C	25°C
St	1190 $\pm$ 281					
5	992 $\pm$ 303	1239 $\pm$ 306	1940 $\pm$ 597	777 $\pm$ 192	1169 $\pm$ 418	1068 $\pm$ 296
10	991 $\pm$ 396	1078 $\pm$ 407	1389 $\pm$ 411	903 $\pm$ 297	1123 $\pm$ 352	1717 $\pm$ 327
30	911 $\pm$ 297	704 $\pm$ 171	1470 $\pm$ 324	924 $\pm$ 311	1087 $\pm$ 307	1263 $\pm$ 240
50	1259 $\pm$ 279	927 $\pm$ 164	2043 $\pm$ 372	1448 $\pm$ 561	1659 $\pm$ 658	1412 $\pm$ 303
100	1238 $\pm$ 270	797 $\pm$ 109	1863 $\pm$ 249	1428 $\pm$ 380	1106 $\pm$ 660	1744 $\pm$ 263

V_C [$\times 10^3 \mu\text{m}^3/\text{cell}$]						
N/P	Low P concentration (0.01 mg P/L)			High P concentration (0.1 mg P/L)		
	15°C	20°C	25°C	15°C	20°C	25°C
St	111 $\pm$ 23					
5	107 $\pm$ 20	89 $\pm$ 15	90 $\pm$ 14	109 $\pm$ 20	94 $\pm$ 17	103 $\pm$ 25
10	116 $\pm$ 19	95 $\pm$ 14	89 $\pm$ 16	110 $\pm$ 21	101 $\pm$ 17	88 $\pm$ 9
30	115 $\pm$ 23	102 $\pm$ 16	95 $\pm$ 8	102 $\pm$ 13	96 $\pm$ 17	88 $\pm$ 19
50	141 $\pm$ 21	104 $\pm$ 13	110 $\pm$ 9	136 $\pm$ 21	114 $\pm$ 19	74 $\pm$ 14
100	118 $\pm$ 27	74 $\pm$ 8	77 $\pm$ 7	124 $\pm$ 17	61 $\pm$ 4	71 $\pm$ 12

Fig. 3 - Size of the gelatinous sheath relative to the cellular biovolume (V_R) as a function of the N/P mass ratio and water temperature. Starvation represents the end of pre-culture, low P concentration was 0.01 mg P/L and high P concentration was 0.1 mg P/L in the culture medium. Error bars indicate the standard deviations for triplicate cultures.

Table 2 - Results of the multiple regression analysis. The predictor variables were water temperature (WT) [°C], nitrate concentration (N) [mg N/L], and phosphate concentration (P) [mg P/L]. Dependent variables were gelatinous sheath volume (V_G), cellular biovolume (V_C), and relative gelatinous sheath volume (V_R) [$\mu\text{m}^3/\text{cell}$].

Dependent variable	V_G	V_C	V_R
Predictor variable			
WT	50.4×10^3 ($p = 0.001$)	-2.91×10^3 ($p = 0.000$)	0.913 ($p = 0.000$)
N	3.86×10^3 ($p = 0.048$)	-0.104×10^3 ($p = 0.268$)	0.061 ($p = 0.002$)
P	-120×10^3 ($p = 0.359$)	-5.26×10^3 ($p = 0.935$)	-13.4 ($p = 0.276$)
Intercept	249×10^3 ($p = 0.36$)	160×10^3 ($p = 0.000$)	-5.225 ($p = 0.046$)
Adjusted R^2	0.37	0.407	0.702

DISCUSSION

The specific growth rate of *S. arctiscon* increased with a rise in water temperature to a range of 15 – 25°C (Fig. 2). Thus, the cell division rate increased with a rise in water temperature. This suggested that a higher water temperature resulted in a lower V_C because it avoided an excessive accumulation of photosynthetic organic carbon in the cell, which would have occurred with a faster cell division rate. Smaller phytoplankton usually have higher specific growth rates (Kagami and Urabe, 2001; Finkel *et al.*, 2010). There may be a general relationship where the cell size of phytoplankton is inversely correlated with its specific growth rate, even in individuals of the same species.

The mass balance of organic carbon in the cell is expressed as follows:

$$P_{gross} - R = M_{syn} + M_{ex} \quad (9)$$

where, P_{gross} is the gross production rate, R is the respiration rate, M_{syn} is the biosynthesis rate, M_{ex} is the extracellular discharge rate, and $P_{gross} - R$ is the net production rate. It is considered that M_{syn} and M_{ex} are positively correlated with the specific growth rate and production rate of the gelatinous sheath, respectively. Thus, V_G increases when the rate of increase of net production with a change in the cultural conditions is higher than that of M_{syn} . Raven and Geider (1988) reported Q_{10} values for photosynthesis, respiration, and nitrate reductase activity of around 2.6, 2.0, and 1.8, respectively. The nitrate reductase activity correlates with the biosynthesis rate, so the Q_{10} values indicate that the increasing rate of net production with a rise in water temperature is higher than that of M_{syn} . Thus, water temperature can increase V_G by enhancing the net production rate more than M_{syn} .

Geider *et al.* (1988) reported that the intracellular N/C ratio of phytoplankton had a positive relationship with the photosynthesis rate in the same light and temperature conditions. In the present study, the intracellular N/C mass ratio increased with an increase in the nitrogen concentration in the culture medium; therefore, the nitrate

concentration was considered to have a positive effect on the gelatinous sheath volume through the enhancement of P_{gross} .

Recently, the annual mean total phosphorus concentration in the northern basin of Lake Biwa fluctuated around 0.08 mg P/L. Contrary to total phosphorus, the annual mean total nitrogen concentration increased from 0.25 mg N/L in 1983 to 0.34 mg N/L in 1996. But it decreased after 2003 and reached 0.24 mg N/L in 2010. As a result, annual mean N/P mass ratio also decreased from 49 in 1996 to 30 in 2010 (Shiga Prefecture, 2011). Therefore, the N/P mass ratio of 30 under low phosphorus concentration was almost equivalent to the current situation in the northern basin of Lake Biwa. If the total nitrogen concentration decreases further, the reduction of V_G will be expected according to the regression model shown in Table 2. Thus, a decrease in nitrogen concentration might contribute to the control of organic pollution caused by gelatinous sheath in Lake Biwa (Ichise *et al.*, 2010b).

CONCLUSIONS

In this study, we investigated the influence of the cultural conditions on the cellular biovolume (V_C), gelatinous sheath volume (V_G), and the relative size of the gelatinous sheath (V_R) of *S. arcuatus* and its growth characteristics in laboratory culture. The results are summarized as follows:

- (1) The optimum growth temperature for *S. arcuatus* was 25°C. The responses of the specific growth rate to nitrate and phosphate concentrations resembled the Monod's equation. The half-saturation constants were estimated to be $K_N = 0.076$ mg N/L for nitrate and $K_P = 0.011$ mg P/L for phosphate.
- (2) Water temperature had positive regression coefficients with V_G and V_R , but a negative regression coefficient with V_C at $p < 0.05$. A higher water temperature resulted in a lower V_C because it avoided the excessive accumulation of photosynthetic organic carbon in the cell, which would occur with a more rapid cell division rate.
- (3) The nitrate concentration had positive regression coefficients with V_G and V_R at $p < 0.05$, but it had no significant regression coefficient with V_C . The intracellular N/C mass ratio increased with a rise in nitrogen concentration in the culture medium, so it was considered that V_G was increased by the high photosynthesis rate because of the high intracellular N/C mass ratio with a high nitrate concentration.
- (4) The phosphorus concentration had no significant regression coefficients with all volumes at $p < 0.05$.

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