Photosynthetic physiology and growth as a function of colony size in the cyanobacterium *Nostoc sphaeroides*

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Algal size can affect the rate of metabolism and of growth. Different sized colonies of *Nostoc sphaeroides* were used with the aim of determining the effects of colony size on photosynthetic physiology and growth. Small colonies showed higher maximum photosynthetic rates per unit chlorophyll, higher light saturation point, and higher photosynthetic efficiency ($a$) than large colonies. Furthermore, small colonies had a higher affinity for DIC and higher DIC-saturated photosynthetic rates. In addition, small colonies showed higher photosynthetic rates from 5 – 45°C than large colonies. There was a greater decrease in $Fv/Fm$ after exposure to high irradiance and less recovery in darkness for large colonies than for small colonies. Relative growth rate decreased with increasing colony size. Small colonies had less chl $a$ and mass per unit surface area. The results indicate that small colonies can harvest light and acquire DIC more efficiently and have higher maximum photosynthetic rates and growth rates than large colonies.

**Key words**: colony size, DIC, growth rate, light, *Nostoc sphaeroides*, photoinhibition, photosynthesis, temperature

**Introduction**

*Nostoc sphaeroides* is an edible blue-green alga (cyanobacterium) found in water-filled paddies in some mountain areas of Hubei province in middle China (Li, 2000). *N. sphaeroides* reproduces by forming hormogonia or small colonies on the surface of large colonies (Li, 2000). From late autumn to early spring, local residents fill paddies with water, encouraging the growth of *N. sphaeroides*. Local residents also called it ‘Tian-xian-mi’ (the rice that comes from heavenly immortals) or ‘Ge-xian-mi’ according to some legends. *N. sphaeroides* has been used as food and a herb in China for more than 1600 years. Recent studies showed that *N. sphaeroides* has the potential to be a healthy food (Huang, 1997; Liu, 2000).

*Nostoc sphaeroides* can fix atmospheric nitrogen and may release the bound nitrogen and, like other *Nostoc* species (Dodds *et al*., 1995), can increase nitrogen input to rice paddies. Desiccated *N. sphaeroides* can fully or partly recover metabolism upon rehydration; the time required for recovery is correlated with the length of the storage period. *N. sphaeroides* can also tolerate salt-stress (Li, 2000; Li & Gao, 2003). These properties make *N. sphaeroides* a good biofertilizer for rice paddies.

The relationship between algal cell size and metabolic rate has been discussed extensively (Banse, 1976; Taquchi, 1976; Finkel & Irwin, 2000; Finkel, 2001; Gillooly *et al*., 2001; Raven & Kübler, 2002). Small algae usually have a thinner diffusion boundary layer, a smaller ‘package effect’ and higher surface to volume ratios (Raven & Kübler, 2002). They consequently have higher metabolic rates with higher capacity of solutes influx/efflux and less self-shading. Small algae also have higher specific growth rates (Sunda & Huntsman, 1997; Raven, 1999). However, these studies were mainly restricted to unicellular algae or very small colony-forming planktonic algae (up to several millimeters).

Benthic colony-forming algae can be larger than planktonic algae (Raven & Kübler, 2002). In larger benthic colonies, diffusion boundary layers (DBL), which increase with increasing colony radius (Ploug *et al*., 1999a,b), are thicker and may more severely decrease the nutrient uptake rate and retard the diffusion of CO$_2$ and O$_2$. In addition, more pigments occur per unit surface area in large colonies, so that the ‘package effect’ may be more severe in them than in the largest unicellular algae (Raven & Kübler, 2002). Steep gradients of O$_2$ concentration, photosynthetic rate and light were found in colonies of *N. parmelioides* (Dodds, 1989b). Photosynthesis- irradiance characteristics...
for cells at different depths in an algal colony may be varied. In some benthic algal communities, affinity for light \(x\) was lower while light saturation point \(I_s\) was higher for surface cells than for the interior cells (Dodds et al., 1999). For whole algal communities, light saturated photosynthetic rate and \(x\) (both expressed on a chlorophyll basis) decreased with increasing biomass and colony thickness (Enriquez et al. 1996; Dodds et al., 1999).

*Nostoc sphaeroides* can grow to several centimeters in diameter. For different sized colonies, there are different physical and physical-chemical properties. Besides light, dissolved inorganic carbon and temperature are key environmental factors. Their effects on the physiology and growth of *N. sphaeroides* may also vary with colony size, but have rarely been investigated. The aim of this study was to describe how photosynthetic and growth responses to light, dissolved inorganic carbon and temperature in colonies of *N. sphaeroides* changed with colony size. The results may provide useful data for the mass cultivation of *N. sphaeroides*.

### Materials and methods

*Nostoc sphaeroides* was obtained from the FACHB-collections of the Institute of Hydrobiology, Chinese Academy of Sciences. Small colonies, about 0.03 – 0.04 mm in diameter, developed from induced hormogonia were used as inocula. The initial chlorophyll \(a\) concentration was about 2.8 \(\mu g\) per litre of culture medium. Specimens were cultivated in BG110 medium (Stanier et al., 1971) in a CO₂ chamber (E7, Conviron, Canada) at 25°C, with constant light (approximately 96 \(\mu mol\) m\(^{-2}\) s\(^{-1}\) from cool-white fluorescent tubes), and aerated. *N. sphaeroides*, with a regular spherical form, were used in the following experiments.

Photosynthetic oxygen evolution of different sized colonies of *N. sphaeroides* was measured at 25°C in a Clark-type oxygen electrode chamber (Rank Brothers, England), using a halogen light source, and irradiances were plotted from different sized colonies of *N. sphaeroides*. Photosynthetic irradiance response (P-E) evolution was measured for at least 5 min at each irradiance. Photosynthetic irradiance response \(P-E\) curves were followed in darkness at the same temperature.

Photosynthetic oxygen evolution of different sized colonies of *N. sphaeroides* was measured at 25°C in a Clark-type oxygen electrode chamber (Rank Brothers, England), using a halogen light source, and irradiances up to 950 \(\mu mol\) m\(^{-2}\) s\(^{-1}\). Irradiance was measured with a quantum sensor (LI-185B, Li-Cor Instruments). Oxygen evolution was measured for at least 5 min at each irradiance. Photosynthetic irradiance response \(P-E\) curves were plotted from:

\[
P = \frac{P_m}{1 + \tanh \left( \frac{\gamma}{P_m} \right) + R_d}
\]

where \(P_m\) is maximal photosynthetic rate, \(P_m\) is dark respiration rate, \(R_d\) is a nonlinear curve fitting of the data to Jassby’s formula using Microcal Origin (Version 5.0, Microcal Software Inc.). \(R_d\) and \(x\) were obtained from linear regressions of the irradiance-limited part of the P-E curves at the onset of the saturated photosynthesis \(E_a\) was calculated as the point where the maximal photosynthetic rate intercepts the initial slope \(x\) of P-E curve \(E_a = \left( \frac{P_m}{1 + \tanh \left( \frac{\gamma}{P_m} \right) + R_d} \right) / \gamma\). The light compensation point was calculated as: \(E_a = - R_d / \gamma\).

Photosynthetic oxygen evolution as a function of dissolved inorganic carbon (DIC) was measured at 25°C and 650 \(\mu mol\) m\(^{-2}\) s\(^{-1}\). DIC-free medium was obtained by aerating acidified medium (by adding HCl to the medium, \(pH < 5\)) with CO₂-free N₂ for about 1 h (Gao, 1999). 

\[
E_a = \frac{P_m + R_d}{\gamma}
\]

\(E_a\) was obtained from the FACHB-N. sphaeroides culture. The dark respiration rate \(E_a\) was measured as the point where the maximal photosynthetic rate intercepts the initial slope \(x\) of P-E curve.

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 sized colonies were not significant. Photosynthetic responses to DIC concentrations in different sized colonies of \textit{N. sphaeroides} are shown in Fig. 2. \(V_{\text{max}}\) decreased significantly with increasing colony size (ANOVA, \(p < 0.001\)). \(K_{0.5}\) (DIC) increased significantly (ANOVA, \(p < 0.01\)) with increased colony sizes (Table 2), suggesting that large colonies had a lower affinity for DIC.

Photosynthetic rates of small colonies of \textit{N. sphaeroides} at all temperatures were higher than those of large ones (Fig. 3). \textit{N. sphaeroides} of different sizes all reached their highest photosynthetic activity at 35°C, and died at 50°C. The larger the colonies, the smaller the changes of photosynthetic activity at 35°C to those at 5°C were 8.7, 5.6 and 3.6 for colonies with diameters of 0.08, 0.2 and 0.6 cm, respectively.

Obvious photoinhibition occurred in \textit{N. sphaeroides} under 1300 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) (Fig. 4). \(F_v/F_m\) values for \textit{N. sphaeroides} decreased significantly (ANOVA, \(p < 0.001\)) after exposure to 1300 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) for only 15 min. The effects of high irradiance were more marked in large colonies, which also showed slower and less complete recovery in darkness than small colonies. Recovery of \(F_v/F_m\) could be divided into a quick and a slow phase. The quick phase lasted for 90 and 120 min and the rate of recovery during this phase was 0.38% \(\text{min}^{-1}\) and 0.22% \(\text{min}^{-1}\) for colonies of 0.08 and 0.20 cm, respectively. The recovery of \(F_v/F_m\) was nearly complete after 4 h in darkness for colonies of 0.08 cm but, in larger colonies (0.2 cm), recovery had reached only 78% of initial values after 20 h in darkness.

The relative growth rate of colonies of \textit{N. sphaeroides} decreased significantly with increasing colony diameters (Fig. 5; ANOVA, \(p < 0.001\)). Colonies 0.08 cm in diameter had an RGR 2.3 times greater than that of colonies 0.6 cm in diameter.

The chlorophyll \(a\) content (on both an area and a dry weight basis) of different sized colonies of \textit{N. sphaeroides} increased significantly (ANOVA, \(p < 0.001\)) with increased colony diameter (Table 3), whereas the surface area to volume ratio decreased significantly (ANOVA, \(p < 0.05\)) Water content in colonies with diameter of 0.15 cm was

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**Table 1. Parameters of photosynthesis-irradiance curves for Nostoc sphaeroides colonies of different sizes**

<table>
<thead>
<tr>
<th>Colony size ((\Phi)) (cm)</th>
<th>(P_m) (chl) ((\mu\text{mol O}_2\ \text{mg}\ chl\ \text{a} \text{h}^{-1}))</th>
<th>(\alpha) (chl)</th>
<th>(E_h)</th>
<th>(R_d)</th>
<th>(E_c)</th>
<th>(P_m) (dw) ((\mu\text{mol O}_2\ \text{mg}\ \text{dw} \text{h}^{-1}))</th>
<th>(\alpha) (dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>374.1 ± 16.0c</td>
<td>5.4 ± 0.1c</td>
<td>73.0 ± 3.0</td>
<td>21.3 ± 6.6</td>
<td>4.0 ± 1.3</td>
<td>2101 ± 89c</td>
<td>30 ± 1abc</td>
</tr>
<tr>
<td>0.20</td>
<td>243.2 ± 22.1b</td>
<td>5.0 ± 0.3bc</td>
<td>56.6 ± 6.3</td>
<td>35.7 ± 8.5</td>
<td>7.1 ± 1.2</td>
<td>1737 ± 158b</td>
<td>35 ± 2abc</td>
</tr>
<tr>
<td>0.30</td>
<td>189.4 ± 19.3ac</td>
<td>3.5 ± 0.8abc</td>
<td>61.1 ± 9.9</td>
<td>20.3 ± 1.7</td>
<td>6.0 ± 1.5</td>
<td>1287 ± 131c</td>
<td>24 ± 5abc</td>
</tr>
<tr>
<td>0.67</td>
<td>147.3 ± 9.5bc</td>
<td>3.4 ± 0.4bc</td>
<td>51.4 ± 7.8</td>
<td>23.8 ± 8.8</td>
<td>7.3 ± 3.5</td>
<td>1189 ± 76c</td>
<td>27 ± 3abc</td>
</tr>
</tbody>
</table>

Within each column, values with different superscript letters are significantly different at \(p = 0.05\) (Tukey’s test). Values are means ± SD (\(n = 6\)) derived from P-E curves of Fig.1. Units: \(P_m\) (\(\mu\text{mol O}_2\ \text{mg}^{-1}\ \text{chl} \text{a} \text{h}^{-1}\)) or (\(\mu\text{mol O}_2\ \text{g}^{-1}\ \text{dw} \text{h}^{-1}\)), \(\alpha\) (\(\mu\text{mol O}_2\ \text{mg}^{-1}\ \text{chl} \text{a} \text{h}^{-1}\)) (\(\mu\text{mol m}^{-2}\text{s}^{-1}\)) or (\(\mu\text{mol O}_2\ \text{g}^{-1}\ \text{dw} \text{h}^{-1}\)) (\(\mu\text{mol m}^{-2}\text{s}^{-1}\)) \(-1\), \(E_h\) and \(E_c\) (\(\mu\text{mol m}^{-2}\text{s}^{-1}\)), \(R_d\) (\(\mu\text{mol O}_2\ \text{mg}^{-1}\ \text{chl} \text{a} \text{h}^{-1}\)).
significantly lower than that in larger colonies (\(p < 0.05\), Tukey’s test).

**Discussion**

The changes of \(P_m\) and \(z\) with colony size (Table 1) in this study are consistent with the results of Enrı´quez et al. (1996) and Dodds et al. (1999). Both of these papers reported a negative relationship between both \(P_m\) and \(z\) (on the basis of carbon/chlorophyll) with assemblage thickness/biomass (mg chl m\(^{-2}\)) in periphyton communities. Lower chl \(a\) contents per unit surface area in small colonies (Table 3) avoid packaging and self-shading among chl \(a\) molecules. This means that individual chl \(a\) molecules in small colonies have more chance of being excited by light energy at a given irradiance, and this can lead to a higher \(z\) (Taquchi, 1976; Enrı´quez et al., 1994; Rodrigues et al., 2000).

In this study, P-E measurements were carried out in BG110 medium without additional inorganic carbon. The DIC concentration was only 189 \(\mu\)M, which was unlikely to have been saturating for photosynthesis, since it was lower than the \(K_{0.5} \text{ (DIC)}\) for N. sphaeroides colonies with diameters of 0.23 (196 \(\mu\)M) and 0.60 cm (260 \(\mu\)M) and only slightly higher than that for 0.08 cm colonies (Table 2). Consequently, the \(P_m\) values obtained (Table 1) were for light saturation, which are determined by carbon procurement (Taquchi, 1976; Henley, 1993; Lambers et al., 1998).

Cyanobacteria can take up both CO\(_2\) and HCO\(_3^-\) as their photosynthetic carbon source and have a CO\(_2\) concentration mechanism (CCM; Salon et al., 1996; Süttemeyer et al., 1998). The active transport of inorganic carbon into the cell cytosol is regarded as an important component of a functional CCM, and this may lead to a higher affinity for inorganic carbon (Süttemeyer et al., 1998).
Photosynthesis and growth of different sized Nostoc colonies

Table 3. Chlorophyll \(a\), water content and surface area/volume ratio (s/v) for Nostoc sphaeroides colonies of different sizes

<table>
<thead>
<tr>
<th>Colony size ((\Phi) cm)</th>
<th>chl (a) ((\mu g \text{ cm}^{-2}))</th>
<th>chl (a) [mg g(^{-1})] (dw)</th>
<th>Water content (%)</th>
<th>s/v (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67</td>
<td>13.9 ± 3.4(^a)</td>
<td>8.1 ± 1.7(^b)</td>
<td>6500(^b)</td>
<td>8.96(^c)</td>
</tr>
<tr>
<td>0.45</td>
<td>11.4 ± 1.2(^a)</td>
<td>8.2 ± 0.9(^b)</td>
<td>7160(^b)</td>
<td>13.3(^b)</td>
</tr>
<tr>
<td>0.30</td>
<td>6.0 ± 0.3(^b)</td>
<td>6.8 ± 0.3(^b)</td>
<td>7630(^b)</td>
<td>20.0(^bc)</td>
</tr>
<tr>
<td>0.23</td>
<td>5.8 ± 0.4(^b)</td>
<td>7.1 ± 0.5(^b)</td>
<td>7800(^b)</td>
<td>26.7(^b)</td>
</tr>
<tr>
<td>0.15</td>
<td>5.0 ± 0.4(^b)</td>
<td>5.6 ± 0.4(^b)</td>
<td>2610(^b)</td>
<td>40.0(^a)</td>
</tr>
</tbody>
</table>

Within each column, values with different superscript letters are significantly different at \(p = 0.05\) (Tukey’s test). Chl \(a\) contents are means ± SD (\(n = 3\)); water content and s/v are means of two measurements.

So anything that influences the diffusion and transport of DIC or enzymatic assimilation of DIC will also affect \(P_m\) and affinity for DIC. The higher surface to volume ratios of small colonies (Table 3) allow more cells to come into direct contact with the culture medium. Thinner sheaths, thinner diffusion boundary layers (DBL), and less slime result in fewer barriers for diffusion of DIC, \(O_2\) and other nutrients in small colonies (Chang, 1980; Bebout et al., 1993; Ploug et al., 1999b). Consequently, DIC and other nutrients can be supplied to surface and inner cells in small colonies more effectively under high light conditions. As a result of the greater resistance to \(O_2\) diffusion, especially in high light environments, DIC and other nutrients can be supplied to surface and inner cells in small colonies more effectively under high light conditions, and this may result in a higher \(P_m\) and higher affinity for DIC. In addition, the efflux of \(O_2\) can be retarded by the sheath, slime and DBL (Prufert-Bebout et al., 1993; Ploug et al., 1999a; Potts, 2000), so that higher \(O_2\) partial pressure may exist around the surface and interior cells in large colonies as a result of the greater resistance to \(O_2\) diffusion, especially in high light environments. Higher \(O_2\) concentrations compete with CO\(_2\) for Rubisco and lower both \(P_m\) and the affinity for DIC in large colonies.

There was no evidence of photoinhibition at high irradiances (up to 950 \(\mumol\ m^{-2}\ s^{-1}\)) in the P-E measurements (Fig. 1) but substantial inhibition of \(Fv/Fm\) was observed in \(N. sphaeroides\) during longer exposures to 1300 \(\mumol\ m^{-2}\ s^{-1}\) (Fig. 4). Rodrigues et al. (2000) also reported that \(Laminaria\ abyssalis\) and \(Laminaria\ digitata\) did not show photoinhibition at 1600 \(\mumol\ m^{-2}\ s^{-1}\) in P-E measurements, but significant inhibition of photosynthetic \(O_2\) evolution and \(Fv/Fm\) was observed after exposure to 1000 \(\mumol\ m^{-2}\ s^{-1}\) for 15 min. Absence of photoinhibition in P-E measurements does not mean that photoinhibition does not occur (Henley, 1993; Hill, 1996): irradiances which are supersaturating for surface cells may be below saturation for deeper-layer cells, so that stimulation of deeper-layer cells may compensate for inhibition of surface cells (Hill, 1996; Dodds et al., 1999); photoinhibition is time-dependent, and the short exposures used for P-E measurements may be too short to observe photoinhibition; \(Fv/\)

\(Fm\) is a more sensitive indicator of photoinhibition than \(P_m\) (Henley, 1993).

Both the increase in thermal dissipation of excess excitation energy and damage to \(PSII\) are responsible for the decrease of \(Fv/Fm\) under high light (Demmig-Adams & Adams, 1992). Photoinhibition induced by increase of thermal dissipation is regarded as a photoprotective reaction and usually decreases rapidly in the dark, but photoinhibition caused by photodamage requires more time for recovery because it involves the synthesis of new proteins. In large colonies, more severe photoinhibition and a less complete recovery indicated that more damage had been done to \(PSII\) of large colonies in high light. In addition, the higher \(P_m\) in small colonies of \(N. sphaeroides\) can dissipate more light energy and thus prevent the occurrence of photoinhibition (Henley, 1993; Lambers et al., 1998). Less photoinhibition in small colonies has important ecological meanings; it can protect them against the damage induced by high irradiance and maintain high photosynthesis under midday sun in the field.

Some species of \(Nostoc\) are able to endure extreme environmental temperatures (Dodds et al., 1995). One strain of \(N. muscorum\) can survive at 60 ± 5°C in hot springs (Greenwood & Steenbergen, 1976). Many species of \(Nostoc\) are the predominant biota in cold polar environments such as ice shelves, glaciers and ice-capped lakes (Vicent, 2000). This study shows that \(N. sphaeroides\) maintained net oxygen evolution from 5–45°C, but died at 50°C (Fig. 3). \(N. sphaeroides\) grows in the water-filled paddies in winter and is harvested in spring. During its growth, the environmental temperature is low and may be near or below 0°C, so that active photosynthesis at low temperatures is essential to achieve an increase in biomass. Light and the supply of \(CO_2\) are two key factors that influence the slope of the temperature-response curve. In the experiments, photosynthesis was measured at saturating irradiances in BG110 medium, so that DIC supply was limiting. Thus the inefficient supply of \(CO_2\) in large colonies as
discussed above is partly responsible for the low value for the initial slope.

Small colonies exhibited higher growth rates than large colonies, and similar trends were observed in marine phytoplankton (Sunda & Huntsman, 1997). *N. sphaeroides* was cultured at 96 μmol m⁻² s⁻¹, which was above $E_k$, so that the diffusion of DIC and O₂ may play an important role in the growth of *N. sphaeroides*, as suggested for other *Nostoc* species (Dodds, 1989a). But colony size had no effect on the growth of *N. pruniforme* in the field (Dodds & Castenholz, 1987) and light seemed to be the most important factor. These measurements of the growth of *N. pruniforme* by Dodds & Castenholz (1987) were conducted at low temperatures (about 4°C) and low irradiances (mean values 0.11–0.31 J m⁻² d⁻¹), and the growth rates ranged from 0.692–3.05 mg (g Fw)⁻¹ d⁻¹, compared with 148–343 mg (g Fw)⁻¹ d⁻¹ for *N. sphaeroides* in our experiments.

In addition to its effects on the physiological responses and growth rate, colony size affects the storage of resources. Large colonies can contain more mucus, which are mainly composed of extrapoly saccharides (EPS). EPS include arabinose, galacturonic acid, ribose, xylose and glucose, and can be consumed as an energy source when there is no exogenous energy. Continued inorganic nitrogen uptake and protein synthesis in the dark, at the expense of mucus as a carbon source, was found in *Phaeocystis* colonies (Lancelot et al., 1986). The storage of water is another important role of EPS (Potts, 2000) and water content was higher in larger colonies (Table 3). Furthermore, the sheath of cyanobacteria has been found to contain metal elements (Tease & Walker, 1987). We also found that the loss of water was slow in larger colonies (unpublished data). This suggests that large colonies can exist longer in some stress conditions.

Our results suggest that several parameters of the photosynthetic responses to light, inorganic carbon and temperature, as well as growth rate are functions of colony size in *N. sphaeroides*. Small colonies have higher affinities for light and DIC, and consequently higher photosynthetic rates and relative growth rates than large colonies. Small colonies have the advantage in utilizing limited environmental resources and restricting damage by high light. If we want more productivity in mass cultivation of *N. sphaeroides*, small colonies are preferred.

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### References


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