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Metabolism and resources of spherical colonies of *Nostoc zetterstedtii*

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Abstract

Constraints imposed by the spherical form and gelatinous matrix of centimeter-thick colonies of the cyanobacterium *Nostoc zetterstedtii* on its functional properties were tested by examining the scaling of its composition, light absorption, photosynthesis, and respiration to individual size. In three summer experiments with colonies collected from the bottom of oligotrophic lakes of low inorganic carbon concentrations (dissolved inorganic C, DIC), metabolism and pigment density of colonies were scaled to their surface area as most algal filaments were confined to a 2-mm-thick outer shell. *Nostoc* absorbed 96% of incident light from the surface to the center because of high areal pigment density, but absorbed photons were used with low quantum efficiency (11–38 mmol O_2 mol^{-1} photon) and photosynthesis was low relative to dark respiration (2.0–5.4). Therefore, *N. zetterstedtii* is threatened by reduced light availability and only extended to lake depths receiving about 12% of surface irradiance, whereas mosses, characeans, and angiosperms with thin photosynthetic tissues grew deeper (3.1–7.5% of surface irradiance). *Nostoc* ameliorated the restrictions of low lake DIC and long diffusion paths by active transport that could extract most external DIC, accumulate DIC in the colony 150-fold above external concentrations, and retain respiratory CO_2. The energy cost of solute transport and gel formation in *Nostoc* colonies and extensive self shading restrict their potential growth, whereas colony formation should prevent grazing and increase longevity and nutrient recirculation. *Nostoc zetterstedtii* has become one of rarest freshwater macroalgae because of widespread lake eutrophication reducing water transparency and increasing competition from taller and faster-growing stands of filamentous algae and higher plants.

If you pay a visit to nutrient-poor soft-water lakes in Sweden you may be lucky to find the remarkable, centimeter-large spherical colonies of the cyanobacterium *Nostoc zetterstedtii*. The species is known from about 50 Swedish lakes, 2 lakes in Denmark and Germany and 1 lake in Finland and Spain, whereas it is absent from other parts of the world (Bengtsson 1995; Vestergaard 1998; Mollenhauer et al. 1999). Throughout its restricted distribution range, *N. zetterstedtii* has declined in occurrence along with increasing humification of the lakes and enhanced shading from phytoplankton and benthic plants following eutrophication and today it is red-listed as one of the rarest photosynthetic species whatsoever (Bengtsson 1986; Mollenhauer et al. 1999). The more common cousin, *Nostoc pruniforme*, has also declined in abundance along with eutrophication of its main growth sites in oligotrophic and mesotrophic hard-water lakes (Mollenhauer et al. 1999; Sand-Jensen et al. 2008).

Whereas *N. pruniforme* resembles a prune and has a smooth colony surface, *N. zetterstedtii* has a granulated surface like a blackberry. Both species form a relatively firm gelatinous colony in the 2-mm-thick outer shell where most of the *Nostoc* filaments are embedded, although the center of large colonies contains few filaments and is more aqueous (Raun et al. 2009). Thus, functionally, *Nostoc* colonies may behave more like hollow than solid spheres. We hypothesize, accordingly, that pigment density, photosynthesis, and respiration should be closely scaled to the surface area of the colonies and that metabolic rates per unit volume or weight, consequently, should decline with colony size.

In terms of uptake of light, inorganic carbon, and nutrients, the colony form of *Nostoc* appears to be very unsuitable because the surface area (A) for exchange with the outer environment is very small relative to the volume (V) supported by the exchange (Nielsen and Sand-Jensen 1990; Markager and Sand-Jensen 1994). For a spherical colony 3 cm in diameter, the $A:V$ ratio is only 2 cm^2 cm^{-3} and although it is 5.7 cm^2 cm^{-3} for a hollow sphere with all activity confined to a 2-mm-thick outer shell, the $A:V$ ratio is still some 100-fold lower than the 400–4000 cm^2 cm^{-3} for filamentous algae and single-layered moss leaves with cell thicknesses of 0.01–0.10 mm.

Spherical form, large dimension, and dark green color of *Nostoc* colonies should lead to high self shading within the colony and to low light-use efficiency of photosynthesis (Agustí et al. 1994). For example, the upper hemisphere of the spherical colonies should completely shade the lower hemisphere and filaments in the outer shell should mutually shade each other and filaments located deeper into the colony. Accordingly, we hypothesize that light-use efficiency of *Nostoc* colonies should be lower than that of species with thin photosynthetic tissues. As a consequence, *Nostoc* colonies should not be able to attain very low light compensation points, defined as the irradiance at which photosynthesis balances respiration, because there is no reason to expect special capabilities of *Nostoc* to reduce respiration relative to photosynthesis. On the contrary, there are sustained costs of producing the conspicuous colony gel, which does not contribute to photosynthesis.

Nostoc zetterstedtii is mainly distributed in soft-water lakes of low alkalinity and low dissolved inorganic carbon (DIC: $CO_2 + HCO_3^- + CO_3^{2-}$, Vestergaard 1998). The diffusive supply of inorganic carbon from outside is further constrained by the large size and low $A:V$ ratio of the colonies and by the high density of filaments in the outer shell of the colony without direct contact with lake water.

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Table 1. Environmental conditions and lower depth limits of submerged plants in seven Danish lakes known to house *Nostoc zetterstedtii* (Lake Hampen and Lake Ulstrup) or *Nostoc pruniforme* (the remaining lakes). Environmental variables are means of bimonthly summer measurements from May to October in 2004–2007. Plant depth limits were determined by scuba diving and percentage of surface irradiance (% SI) at the depth limit was estimated assuming 10% surface reflection and 15% subsurface irradiance at the Secchi depth.

Variable	Hampen	Ulstrup	Almind	Slaaen	Knud	Ravn	Esrum
DIC (mmol L^{-1})	0.15		0.60	1.16	1.94	2.12	2.47
pH	7.5		7.6	7.9	8.5	8.2	8.7
Total P (mg L^{-1})	0.047	0.04	0.011	0.011	0.027	0.029	0.15
Total N (mg L^{-1})	0.159	1.0	0.27	0.21	2.2	4.61	0.45
Chlorophyll *a* (μg L^{-1})	4.7	10.0	4.0	2.0	12	7.5	6.3
Secchi depth (m)	4.8	5.2	5.5	6.0	3.9	3.7	4.3
Plant depth limit (m)	8.5	7.1	6.3	8.7	6.1	4.1	7.0
% SI at limit	3.1	7.5	10.2	5.7	4.6	11.1	4.1

Moreover, the diffusion path is long from the water to the site of photosynthesis. Concentrations of free CO_2 may be somewhat elevated on top of the sediment surface, where *Nostoc* colonies are located, because of microbial release and groundwater seepage of CO_2 from the sediments. However, to ameliorate potential carbon limitation of photosynthesis, we anticipate that *Nostoc* must have very active carbon uptake to extract DIC from the lake water and attain reasonably high quotients of CO_2 to O_2 at the site of Rubisco activity within the cells. *Nostoc* may also be able to retain inorganic carbon from nighttime respiration for later use during daytime photosynthesis. Such efficient extraction and accumulation of carbon are known for unicellular cyanobacteria (Kaplan et al. 1980; Sültemeyer et al. 1998).

Despite the unsuitable form and size of large *Nostoc* colonies for efficient resource use, they include several species in nutrient-poor lakes (*N. pruniforme* and *N. zetterstedtii*, Vestergaard 1998), ephemeral calcareous ponds (*Nostoc commune*), and rice paddies (*Nostoc sphaeroides*, Li and Gao 2004). Accordingly, there must be advantages associated with the form and size of the colonies such as longevity (Dodds and Castenholz 1988), low grazing (Moore 1978), and N_2 fixation. Because of the supposedly persistent nature and low losses, we hypothesize that *Nostoc* colonies should be able to live and grow at low irradiances in nutrient-poor lakes despite their unfavorable size and form for use of light, DIC, and other essential solutes. We do not anticipate, however, that colonies of *N. pruniforme* and *N. zetterstedtii* can grow as deep in lakes as characeans, filamentous algae, and mosses because of less efficient use of light, but we do expect that they should come close because the lower depth boundary is a combined physiological–ecological boundary set by the net balance between growth and losses by grazing, senescence, and physical removal (Sand-Jensen and Madsen 1991; Markager and Sand-Jensen 1994).

Our overall objective was to determine the metabolic features in relation to light, DIC, pigment content, and dry weight density of *N. zetterstedtii* of different colony sizes. The first specific objective was to test if photosynthesis and respiration scaled to the surface area of colonies. The second specific objective was to test if light-use efficiency was lower and light compensation point was higher for thick *Nostoc* colonies than for thin photosynthetic tissues of macroalgae, mosses, and angiosperm leaves. The third specific objective was to test if *Nostoc* indeed uses inorganic carbon very efficiently. The final objective was to test whether the experimentally derived light compensation point of *Nostoc* colonies for 24-h day–night periods accords with light availability at their lower depth boundary in lakes and examine if species with thin photosynthetic tissues penetrate deeper to lower irradiances.

Methods

Study sites and depth distribution—The presence of *N. zetterstedtii* and *N. pruniforme* has been examined over several years by the Danish counties in 88 Danish lakes with submerged vegetation. Water chemistry and Secchi depth are known from these lakes on the basis of measurements every 14 d from May to November as described by Vestergaard (1998). *Nostoc zetterstedtii* has been reported from two lakes and *N. pruniforme* from six lakes, all of which are relatively deep, oligotrophic to mesotrophic lakes with transparent water, low phytoplankton biomass, and fine growth conditions for submerged vegetation (Table 1).

The classical Danish *N. zetterstedtii* locality, Lake Hampen, was used for sampling colonies for experiments. The lake used to have a low DIC concentration (ca. 0.15 mmol L^{-1}; Sand-Jensen and Søndergaard 1981; Vestergaard 1998) that has increased somewhat recently. The other sampling locality is the DIC-poor oligotrophic Swedish Lake Värsjö (ca. 0.15 mmol L^{-1}). All known Danish localities for *N. pruniforme* have a medium to high DIC (0.6–2.4 mmol L^{-1}).

The depth distribution of *N. zetterstedtii*, *N. pruniforme*, and submerged species of charophytes, filamentous green algae, mosses, and vascular plants was determined by scuba diving along 10–20 transects during midsummer in two Danish lakes in mid-Jutland, Lake Hampen, and Lake Ulstrup in 2004. Mean values of Secchi transparency on the basis of bimonthly measurements from May to November

were used to determine the percentage of subsurface light at the lower depth boundary assuming that 10% of incident irradiance is lost by surface reflection and 15% of subsurface irradiance reaches the Secchi depth. Although 10% surface reflection is a standard mean value, the mean percentage of surface irradiance in the Secchi depth ranges from 8% to 20% among lakes and 15% was selected as an appropriate mean value (Sand-Jensen and Søndergaard 1981). Surface irradiance is known from continuous measurements of the photon flux density (400–700 nm, photosynthetically active radiation) at nearby meteorological stations, allowing estimation of photon flux density at the depth boundary of *Nostoc* and other submerged plants.

Photosynthesis vs. light—Nostoc colonies for photosynthesis–light experiments were collected in September 2005 (series 1) and June 2006 (series 2) in Lake Hampen and in June 2008 in Lake Värsjö (series 3). Experiments were conducted at different times and sites and with numerous replicates (a total of 47 colonies) to make sure that our results were reproducible and generally valid. Colonies were kept in the laboratory at 15°C in dim light in aquaria with plenty of water of the same DIC concentration and a chemical composition of major cations, anions, and dissolved nutrients as in sampling lakes (pure groundwater diluted to 0.15 mmol L^{-1} DIC) and used for experiments within a few days. Additional experiments were made with colonies from Lake Värsjö in June–July 2008.

Photosynthesis at low and high irradiance (30 and 200 μmol m^{-2} s^{-1}) and dark respiration were determined as changes in dissolved oxygen under stirred conditions for individual colonies placed in closed glass bottles mounted on a rotating wheel in an incubator set at 15.0°C. The high irradiance was sufficient to saturate photosynthesis of *N. sphaeroides* (Li and Gao 2004) and presumably also of *N. zetterstedtii*. Each series consisted of 15–16 colonies ranging in size from about 0.5 to 10 g fresh weight. Because of large colony volumes relatively long incubation periods are needed to establish new oxygen gradients across the colony surface following changes in irradiance, and thereby ensure constant oxygen exchange rates between the colony and the water for most of the incubation time. New steady-state gradients are established more rapidly at high metabolic rates at high light than at low metabolic rates at low light and in darkness and incubation time was adjusted accordingly. Also, we performed the experiments in the order: darkness, low light, and high light to reduce the time needed to establish steady-state release of oxygen. Measurements of oxygen gradients across the colony surface by oxygen microelectrodes show that 2 h are required to attain steady state after changes from darkness to low light and 25 min after changes from low to high light (A. L. Raun unpubl.), which is a short time relative to the applied incubation periods. Finally, we adjusted incubation time, colony volume, and water volume relative to each other such that sufficient changes of dissolved oxygen (>20% air saturation) took place during the incubation to permit precise calculation of metabolic rates, but not too excessive changes happened (<50%) for confounding effects of oxygen accumulation or depletion to appear.

Dissolved oxygen concentrations were measured directly in incubation bottles with colonies and in water-filled blanks after incubations lasting 2–4 h at high light, 12–18 h at low light, and 8–12 h in the dark using a Clark-type microelectrode (OX500, Unisense) in a temperature-constant water bath at 15.0°C. The microelectrode was connected to a picoamperometer (PA2000, Unisense) and the signal was logged on a computer through an ADC16 converter (Picolog). The microelectrode was calibrated in air-saturated water and in oxygen-free water bubbled with N_2 gas.

Photosynthesis at low irradiance (NP_{Low}) and dark respiration (R) were used to calculate light-use efficiency (α) and light compensation point (I_C) according to the linear increase of photosynthesis with irradiance of the slope, α up to the low irradiance, I_{Low} of 30 μmol m^{-2} s^{-1}:

$$\alpha = 2(NP_{Low} + R)I_{Low}^{-1} \quad (1)$$

where R is given as positive values. We multiplied by a factor of 2 to permit comparisons with values for flat tissues, where respiration and photosynthetic variables are referred to the one-sided area. This is also the proper calculation of light-use efficiency of *Nostoc* colonies, which are illuminated from one side during incubations such that half of the spherical colony is in the shade. Only two points were used to define the line and calculate α and I_C but this is sufficient because of the linearity at low irradiances. We deliberately attempted to attain very accurate metabolic rates in the dark and in low light by making long incubations to attain steady state and combined this by using excessive replication to ensure highly reliable determinations of α and I_C and of the influence of colony size.

Colonies are large and dark green and their cyanobacteria experience extensive self shading. Light attenuation through the colonies was measured on six specimens ranging in diameter from 1.5 to 3.0 cm. Colonies were cut in half and each colony hemisphere was placed on top of a Li-Cor quantum sensor illuminated by a halogen spot. Measurements were compared with reference measurements at the same incident irradiance, but no shading of the sensor. To test the influence of self shading on photosynthetic performance, eight colonies from Lake Värsjö were cut into 1–2-mm pieces to minimize self shading and similar numbers of intact colonies incubated for comparison as already described. Because relatively large pieces were used, the great majority of *Nostoc* filaments will be left intact and we regard the results as a minimum estimate of the influence of self shading because some harm will be induced and some self shading will remain even in the 1–2-mm pieces.

Colony size was expressed as mean diameter, fresh weight, and volume, enabling calculation of the percentage of water and the specific mass density. Surface area was calculated from mean diameter assuming spherical shape. Calculations of surface area from colony volume yielded virtually the same results. We did not correct for the granulated outer surface of the colonies in the calculations, but this underestimation will be of the same relative magnitude in all determinations.

Concentrations of chlorophyll *a* (Chl *a*) in the colonies were measured on subsamples of homogenized freeze-dried material by extraction in ethanol, measurements of absorbance in a spectrophotometer, and calculations of chlorophyll concentrations according to the formula in Wintermans and DeMots (1965). Organic carbon was measured on other subsamples using a Carbo Erla CHN analyzer.

DIC extraction, internal pools, and dark refixation—DIC extraction capacity was measured on *Nostoc* colonies freshly collected from lake sediments and placed outdoors in two large aquaria during June–July exposed to dim light (20% of solar irradiance) and holding stagnant water of the same DIC concentration (0.15 mmol L^{-1}) as in Lake Värsjö.

To determine the biological DIC extraction capacity from the internal DIC pool, individual colonies were incubated at high irradiance (200 μmol m^{-2} s^{-1}) and 15°C over 18–20 h in closed bottles filled with water containing about 0.15 mmol L^{-1} DIC and traces of oxygen. After the extended incubation period, changes in oxygen, pH, and DIC were measured and HCO_3^- and free CO_2 were calculated from DIC, pH, temperature, and ionic strength according to Mackereth et al. (1978). We then calculated the biological extraction capacity from the DIC pool of the colony by setting the sum of biological DIC extraction capacity from the colony (unknown) and from the external water (measured) equal to the production of oxygen (measured), all in moles. Other studies showed that a molar quotient of oxygen to DIC of 1.0 was applicable. We removed oxygen from the water before light incubations to prevent bubble formation and too-high oxygen concentrations during incubation. However, because this experiment intended to measure the DIC extraction capacity and, thus, the ability of *Nostoc* to generate very low DIC, high oxygen, and high pH in the water, these bottle effects were intentional.

DIC pools within *Nostoc* colonies were also measured chemically by a destructive technique before and after extended light and dark incubations by transferring colonies to bottles filled with 0.1 N HCl, then stoppered and left for 48 h to convert all inorganic carbon to free CO_2 and reach equilibrium with the acidic medium before measuring the CO_2 concentration. If *Nostoc* can extract all DIC from the colonies during extended photosynthesis in the light, the biological DIC extraction capacity will be equal to the internal DIC pool measured chemically. Because *N. zetterstedtii* lives in lakes of low pH and DIC, the colonies have no precipitates of carbonates that would be dissolved by the acid treatment. During extended incubations in closed bottles, changes in DIC and oxygen were measured in the water, changes in internal DIC were measured on similarly incubated sacrificed colonies, and internal oxygen was assumed to equal that in the external water. The latter assumption will only lead to small errors because measurements of internal oxygen with microelectrodes inserted into the colonies revealed that they at most deviate twofold from external concentrations (A. L. Raun unpubl.) even during very intense photosynthesis. This will at most result in 3% error in oxygen exchange rates for 2 cm^3 colonies incubated in 70 cm^3 of water, which is the typical ratio between colony volume and water volume in the experiments.

Changes in internal DIC pools and external DIC and oxygen pools were measured in a final experiment during extended light and dark incubations at three initial DIC concentrations (0.99 mmol L^{-1}, 0.29 mmol L^{-1}, and 0.10 mmol L^{-1}) prepared by diluting pure alkaline groundwater with distilled water. This experiment permitted us to determine the molar balance between total exchange (sum of colony and water) of DIC and oxygen in light and darkness, determine the sensitivity of photosynthesis to falling external DIC concentrations, test whether DIC uptake from the water declines with lower concentrations and thereby puts a greater toll on the internal DIC pool to sustain photosynthesis, and, finally, test whether internal DIC pools reduced in the light are reestablished in the dark.

Concentrations of DIC were measured on minute (25–100 μL) samples injected into an infrared gas analyzer (ADC225 MK3, Analytical Development) as described previously (Vermaat and Sand-Jensen 1987).

Results

Composition of colonies with size—Fresh weight increased in proportion to colony volume with a slope not significantly different from 1.0 ($p < 0.05$; *t*-test, df = 15). Colonies were slightly heavier than water, having a mean specific density of 1.056 ± 0.0082 g cm^{-3} (±SE) and a mean dry weight of 5.5% ± 0.5% of the fresh weight. The mean proportion of N_2-fixing heterocyst relative to normal cells in the *Nostoc* filaments (0.065 ± 0.0074) was independent of colony size.

Because most *Nostoc* filaments were located in the outer shell of the colonies, Chl *a* normalized to colony surface area also remained constant with colony size, averaging 20.4 ± 0.65 μg cm^{-2} in series 1 ($p > 0.05$; *t*-test, df = 15). Light was strongly absorbed (96.3% ± 0.39%) from the illuminated surface to the center of the colonies.

Photosynthesis–light relationships and respiration with colony size—When adjusted for experimental series and type of metabolic rate at high light, low light, and in darkness, metabolic rates per colony surface area did not change systematically or significantly with colony size ($p = 0.49$; ANCOVA; Fig. 1). Averages of photosynthesis at high light varied little among the three experimental series (6.6 to 9.2 μg O_2 cm^{-2} h^{-1}), whereas photosynthesis at low light varied relatively more (0.1 to 2.6 μg cm^{-2} h^{-1}) and so did respiration (1.3 to 4.6 μg cm^{-2} h^{-1}; Table 2). Both photosynthesis at low light and respiration were significantly higher in the first than in the other experiments (Table 2). Photosynthesis at high light was twofold higher than respiration in series 1 and fivefold higher in series 2 and 3.

Light compensation point and light-use efficiency were both independent of colony size when adjusted for differences among series ($p = 0.24$; ANCOVA; Fig. 2).

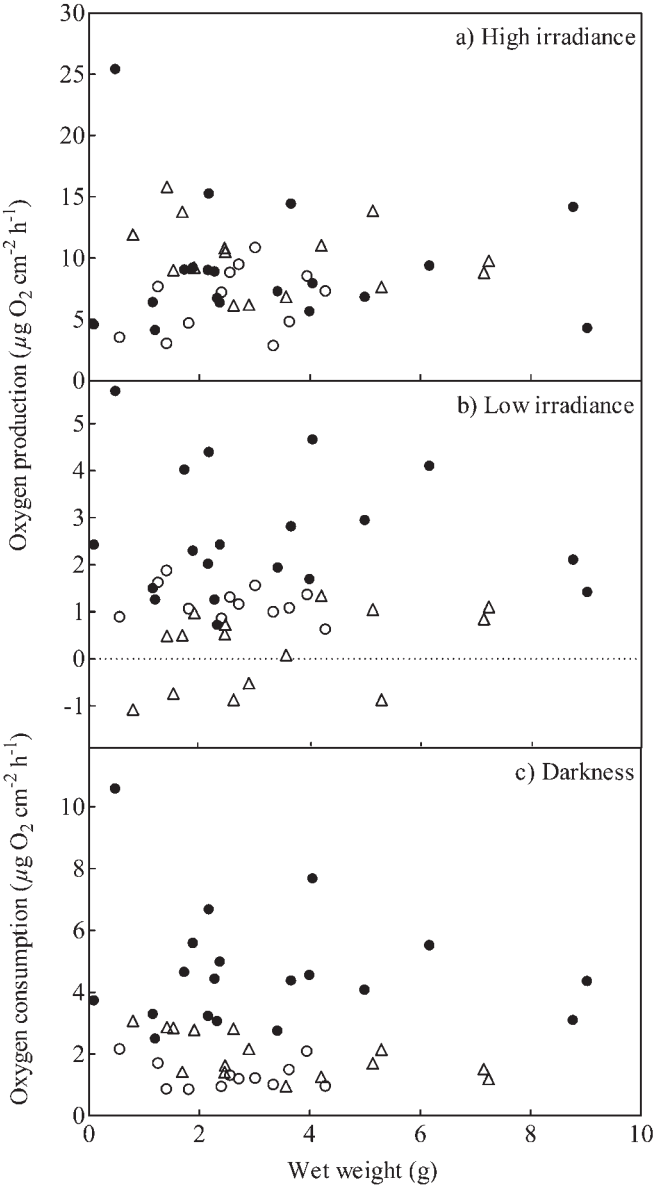

Fig. 1. (a) Photosynthesis of *Nostoc zetterstedtii* at high light (200 μmol m^{-2} s^{-1}), (b) low light (30 μmol m^{-2} s^{-1}), and (c) respiration in the dark in relation to colony size in three experimental series. Series 1: Colonies from Lake Hampen, September 2005 (closed circles); series 2: Lake Hampen, June 2006 (open triangles); series 3: Lake Värsjö, June 2008 (open circles). All metabolic rates are expressed per colony surface area. When adjusted for type of metabolic rate and series, metabolic rates did not change significantly with colony size (p = 0.49; ANCOVA).

Mean light compensation resembled each other in experiment 1 (19.6 μmol m^{-2} s^{-1}) and experiment 3 (15.1) from June in Lake Hampen and Lake Värsjö, whereas some values were markedly higher in series 2 from Lake Hampen in September, resulting in greater variability and a higher mean value (Table 2). Mean light-use efficiency was significantly higher in series 1 (37.4 mmol O_2 mol^{-1} photon) than in series 2 and 3 (10.6 and 12.8). As almost all incoming light is absorbed by the colonies, light-use efficiency is practically equal to quantum efficiency on the basis of absorbed photons.

Low efficiency of *Nostoc* colonies in the use of incoming light at high irradiance was evident from the low mean assimilation number (±SE) measured in series 1 (0.52 ± 0.09 mg O_2 mg Chl a h^{-1}), which can be used for comparison with the photosynthetic efficiency of free-living microalgae traditionally expressed on a chlorophyll basis. Photosynthesis and light-use efficiency are in part constrained by self shading within the colonies. When self shading was reduced by cutting the colonies into 1–2-mm pieces, photosynthesis at high and low light, photosynthesis relative to respiration, and photosynthetic efficiency all increased highly significantly ($p < 0.01$; t-test, df = 6; Table 3).

DIC extraction, refixation, and internal accumulation—*Nostoc* colonies used DIC very efficiently and drove pH up to high levels (10.97–11.07) during 18–20 h of light incubation in closed bottles where only traces of free CO_2 (0.28–0.95 nmol L^{-1}) and HCO_3^- (13–35 μmol L^{-1}) remained (Table 4). Oxygen accumulated in the bottles during extended incubations and the quotient of O_2 to HCO_3^- reached high levels (18–29), reflecting high DIC extraction capacity and low photorespiration.

During light incubations at different external DIC, total oxygen release matched total DIC use from the water and the colony matrix with a molar quotient not significantly different from 1.0 (legend to Fig. 3). As anticipated, DIC use from the water decreased with falling DIC concentrations in the water, whereas DIC use from the colony matrix increased (Fig. 3). Because of the alternative DIC supply from the colony matrix, photosynthesis measured as either oxygen release or DIC uptake declined only moderately despite very low external DIC in the water and most DIC uptake occurring from the internal pool (Table 5). In the dark, molar uptake of oxygen was also equivalent to combined molar release of DIC to the water and the colony matrix and here more DIC accumulated within those colonies that had been deprived of internal DIC during the preceding light incubation in low-DIC water (Fig. 3). There is considerable variability among colonies in these experiments, because internal DIC pools vary among colonies and changes in their pool size during light or darkness is calculated from mean values of other colonies (similarly treated) sacrificed before incubations minus individual pool size in individual colonies sacrificed after incubations.

During extended light incubations in low-DIC water in a subsequent experiment, use of internal DIC amounted to 16.3–24.5 μmol g^{-1} colony over 20 h in two independent collections from outdoor aquaria, if we assume a molar quotient of 1.0 in the exchange of DIC and oxygen (Table 6). These biologically derived estimates of DIC accumulation accorded with chemical determinations of the internal DIC pools measured initially (19.0–23.7 μmol g^{-1} colony, Table 6), implying the *Nostoc* is capable of extracting all DIC from the colony if needed during extended light incubations. During subsequent extended dark incubations of the colonies in low-DIC water, substantial oxygen was consumed from the water to sustain

Table 2. Photosynthesis at high (NP_{High}) and low light (NP_{Low}) and dark respiration (R) per entire colony surface area of *Nostoc zetterstedtii* in three experimental series (*see* Fig. 1). Also shown are $NP_{High}:R$, light compensation point (I_C, μmol m^{-2} s^{-1}), and light-use efficiency (α, mmol O_2 mol^{-1} photons). Mean ± SE of 15–16 measurements in each series. Significant differences are shown by different letters ($p < 0.05$; Kruskal–Wallis followed by Dunn's multiple comparison test).

Series	NP_{high} (μg cm^{-2} h^{-1})	NP_{Low} (μg cm^{-2} h^{-1})	R (μg cm^{-2} h^{-1})	$NP_{High}:R$	I_C (μmol m^{-2} s^{-1})	α (mmol O_2 mol^{-1} photon)
1	9.19±1.3ab	2.60±0.4a	4.68±0.5a	2.03±0.2a	19.6±0.6a	38.4±4.2a
2	9.10±1.3a	0.06±0.2b	2.02±0.2b	5.25±0.8b	28.5±3.1a	10.8±1.2b
3	6.55±0.7b	1.19±0.1b	1.31±0.1b	5.35±0.6b	15.1±0.7b	12.8±0.8b

respiration in the colonies but either no release or a small uptake of DIC took place, implying that internal accumulation of DIC from respiration amounted to 8.6–13.2 μmol g^{-1} over 20 h (Table 6).

Depth distribution and minimum light requirements—*Nostoc zetterstedtii* is abundant during summer in shallow water in Lake Hampen and it extends to a depth limit of 5.0 m, receiving about 12.5% of surface irradiance (SI, Table 7). Certain mosses (*Fontinalis antipyretica*), characeans, and angiosperms extend deeper to 8.5 m, with only 3.1% of SI. Because angiosperms reach up into the water they receive a greater percentage of surface irradiance than creeping mosses and characeans. In Lake Ulstrup, *N. zetterstedtii* grows to a depth limit of 5.6 m with about 11.7% of SI and *N. pruniforme* reaches 6.2 m with 9.4% SI (Table 7). Here, moss species (*Fontinalis antipyretica*) and filamentous green algae reach 7.0 m with 7.8% SI and *Potamogeton pectinatus* penetrates slightly deeper to 7.1 m.

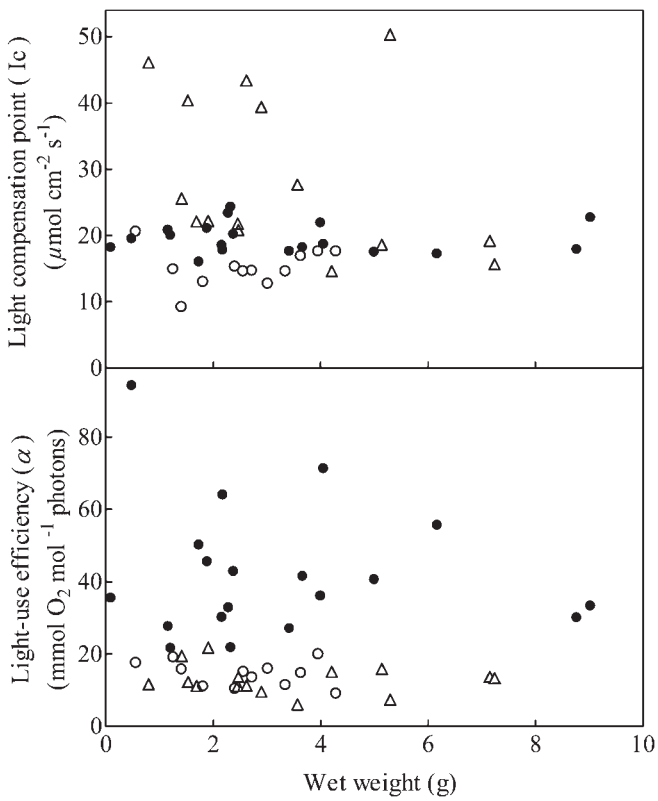

Fig. 2. Light compensation point (I_C) and light-use efficiency (α) of *Nostoc zetterstedtii* in relation to colony size in three experimental series (*see* legend to Fig. 1). When adjusted for influence of series, there were no significant changes with colony size ($p = 0.24$; ANCOVA).

Discussion

Scaling of colony metabolism—Overall, photosynthesis and respiration increased in proportion to surface area of *N. zetterstedtii* colonies, resulting in constant rates per surface area across different colony sizes (Fig. 1). Scaling to surface area is anticipated when we consider that algal filaments are mostly confined to the outer 2-mm-thick shell of the colony while the center is an aqueous gel with few filaments (Raun et al. 2009). Chl *a* is also scaled to surface area such that photosynthesis and respiration relative to chlorophyll are independent of colony size. The almost constant chlorophyll density at the colony surface and the low chlorophyll concentration in the colony center also suggest that self shading is independent of colony size once they exceed about 5 mm in diameter.

Photosynthesis, respiration, and Chl *a* also increased in proportion to surface area of *N. pruniforme* colonies differing from 7 to 17 mm in diameter such that metabolic rates per chlorophyll as anticipated remained constant independent of colony size (Raun et al. 2009). In contrast, photosynthesis per surface area doubled and photosynthesis per Chl *a* increased fourfold from 2.1-mm to 7.1-mm colonies of *N. sphaeroides*, whereas photosynthesis per chlorophyll and light-use efficiencies were extremely low

Table 3. Photosyntheses at high (NP_{High}, 200 μmol m^{-2} s^{-1}) and low light (NP_{Low}, 30 μmol m^{-2} s^{-1}) and dark respiration (R) of intact colonies of *Nostoc zetterstedtii* and colonies cut into 1–2-mm pieces. Light compensation point (I_C) and light-use efficiency (α) at low irradiance are also shown. Photosynthesis and respiration are expressed per entire colony surface area. Significant differences were found between intact and cut colonies for NP_{High}, NP_{Low}, $NP_{High}:R$, and α ($p < 0.01$; t-test). Mean ± SE of eight replicates.

Variable	Unit	Intact colonies	Cut colonies
NP_{high}	μg cm^{-2} h^{-1}	6.8±0.92	23.8±0.95
NP_{Low}	μg cm^{-2} h^{-1}	1.23±0.081	2.77±0.42
R	μg cm^{-2} h^{-1}	1.18±0.16	1.59±0.13
$NP_{High}:R$	Dimensionless	5.8±0.81	16.6±1.2
I_C	μmol m^{-2} s^{-1}	14.1±1.2	11.1±1.7
α	mmol O_2 mol^{-1} photon	14.8±1.4	26.2±2.3

Table 4. Final pH, DIC, HCO_3^-, free CO_2, and O_2 in the water after 18–20 h of *Nostoc* photosynthesis at high irradiance in closed bottles in two independent collections (A and B) and two consecutive experiments (1 and 2) for each collection separated by 8 h of darkness. Mean initial DIC was 149–162 μmol L^{-1}, initial pH was 8.26–8.45, and initial O_2 was 5–10 μmol L^{-1} in the two experiments. Mean ± SE of four replicates for each collection and experiment.

Collection experiment	pH	DIC (μmol L^{-1})	HCO_3^- (μmol L^{-1})	CO_2 (μmol L^{-1})	O_2 (μmol L^{-1})	O_2 : HCO_3^-
A1	10.94±0.040	174±30	35	0.95	644±49	18
A2	11.07±0.045	77±19	13	0.28	373±29	29
B1	11.06±0.050	144±31	24	0.54	690±93	29
B2	11.06±0.070	100±58	17	0.38	363±6	21

(Gao and Ai 2004). These results for *N. sphaeroides* are unexpected and difficult to explain.

Light-use efficiency and metabolic balance—Incident light was used with low efficiency by *N. zetterstedtii* colonies as 11–38 mmol O_2 mol^{-1} photon was produced as an average in the three series. A similarly low efficiency of 27 mmol O_2 mol^{-1} photon was calculated for the large, spherical, and thick-walled colonies of the Mediterranean macroalga *Codium bursa* (Geertz-Hansen et al. 1994). Because the colonies absorb all incident photons, quantum efficiency is equal to the reported light-use efficiency. In large compilations of measurements on many species with thin photosynthetic tissues, most quantum efficiencies are markedly higher, at 70–120 mmol O_2 mol^{-1} photon for microalgae, 37–79 for macroalgae and submerged plants, and close to 100 for CO_2-enriched terrestrial C-3 plants (Björkman and Demmig 1987; Frost-Christensen and Sand-Jensen 1992).

One constraint on light-use efficiency measured as oxygen production in *N. zetterstedtii* and other colonies such as *C. bursa* is light attenuation by colored substances, dead algae, and other particles within the colony. As there is no mechanism to get rid of senescent or dead algae from the colony their competition with photosynthetic pigments for photons will reduce quantum efficiency. This attenuation does not exist for free-living organisms and is one possible reason for the lower light-use efficiency of *Nostoc* and *Codium*. Light attenuation by nonphotosynthetic pigments and structural substances in living *Nostoc* filaments is a second constraint that, however, also influences light-use efficiency of free-living organisms. A third constraint, specific for *Nostoc* and other cyanobacteria, derives from use of photons for adenosine triphosphate (ATP) production by cyclic photophosphorylation in N_2-fixing heterocysts. Cyclic photophosphorylation uses photons for ATP production without concurrent oxygen production and has been suggested as the explanation for the lower quantum efficiency of terrestrial C-4 plants (avg. 69 mmol O_2 mol^{-1} photon) compared with C-3 plants (avg. 106; Björkman and Demmig 1987). A role of cyclic photophosporylation in internal carbon accumulation has previously been proposed for the unicellular cyanobacterium, *Anacystis* (Ogawa et al. 1985) and the green alga, *Clamydomonas* (Sültemeyer et al. 1988) and may also apply for *Nostoc*, considering the high-energy expenditures for active ion transport needed because of long diffusion paths of solutes and dense packing of filaments. High capacity for concentrating DIC within the colony requires energy for ion pumps that must be linked to photosynthetic electron transport and ATP generation.

Nostoc zetterstedtii has a relatively low photosynthesis at high irradiance relative to both chlorophyll (about 0.5 μg O_2 μg^{-1} chlorophyll h^{-1}) and respiration (2.0–5.4). Again it shares these properties with the balloon-like colony *C. bursa* (0.085 μg O_2 μg^{-1} chlorophyll h^{-1} and $NP:R$ 5.6; Geertz-Hansen et al. 1994), whereas free-living unicells have higher rates of photosynthesis per chlorophyll, typically between 2 and 20 (Harris 1978) and $NP:R$ quotients from 5 to 20 (Geider and Osborne 1992). High self shading within *Nostoc* colonies is an important constraint because photosynthesis at high irradiance increased fourfold and $NP:R$ increased threefold when self shading was reduced by cutting the colonies into 1–2-mm large pieces (Table 3). Compared with free-living unicells there are also additional respiratory costs in producing and maintaining the colony matrix and running the necessary energy-demanding ion pumps to ameliorate the depletion of solutes around densely packed algal filaments.

DIC extraction and concentrating capacity—Common understanding of the CO_2-concentrating mechanism in cyanobacteria dictates that it depends on membrane-bound inorganic carbon transport systems that may use both HCO_3^- and CO_2 and a microenvironment within the cell where accumulated DIC can elevate CO_2 at the site of Rubisco (reviewed by Badger and Price 1992, 2003). The microenvironment is the discrete structure, carboxysome, where Rubisco and carbonic anhydrase are packed closely together.

DIC extraction capacity is very high in *N. zetterstedtii* and it probably makes use of the common mechanisms observed for several cyanobacteria. *Nostoc* is able to drive pH above 11 in a DIC-poor medium where HCO_3^- is only about 20 μmol L^{-1}, CO_2 about 0.5 nmol L^{-1}, and the quotient of O_2 to HCO_3^- is about 20 (Table 4), much like the values for unicells adapted to low CO_2 (Badger and Price 1992). Considering the high pH and only traces of CO_2 remaining in the water in closed bottles during long light incubations, there is little doubt that HCO_3^- is used actively by *Nostoc*.

DIC concentrating capacity of *Nostoc* is very high, as internal concentrations averaged over the entire colony volume reached about 20 mmol L^{-1}, which was 150-fold

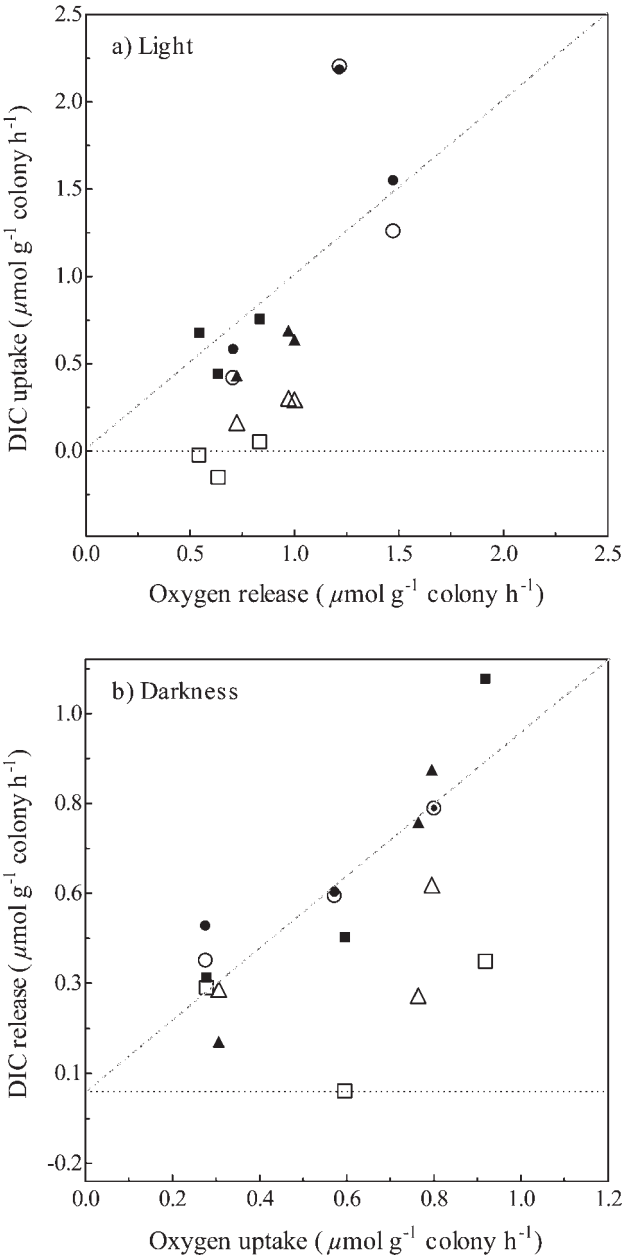

Fig. 3. DIC exchange of *Nostoc zetterstedtii* with the water (open symbols) and with both the water and the colony (filled symbols) as a function of oxygen exchange with the water during (a) 18 h of light incubations and (b) 18 h of dark incubation. Colonies were incubated at three DIC levels containing initially 0.99 mmol L^{-1} (circles), 0.29 mmol L^{-1} (triangles), and 0.10 mmol L^{-1} (squares). Mean (±SD) molar exchange rates of oxygen relative to DIC (water + colony) were 1.19 ± 0.13 in the light and 0.96 ± 0.081 in the dark and not significantly different from 1.0 ($p > 0.05$, *t*-test, df = 6).

higher than in the water. This internal DIC pool means that photosynthesis is little affected by external DIC concentrations (Table 5) because the internal pool alone can support maximum photosynthesis for 11 h in 1-cm-diameter colonies and for 23 h in 2-cm-diameter colonies with no uptake from the water. We would suspect that DIC concentrations are higher within cells than in the colony gel, resulting in a higher accumulation quotient between the cell and the water. High CO_2 accumulation quotients of 500–1000 are not unprecedented among cyanobacteria (Kaplan et al. 1980; Badger and Price 2003).

There is additional and independent evidence for the very efficient carbon concentrating mechanism in *N. zetterstedtii*. Measurements of $\delta^{13}C$ in several submerged macrophytes from Lake Värsjö (A. Winkel unpubl.) showed markedly less negative values for *N. zetterstedtii* (−12.7‰ ± 0.2‰), in support of active carbon concentrating mechanisms (Finlay 2004), compared with typical passive users of CO_2 from the water (−32.7‰ for *Fontinalis antipyretica*, −33.0 for *Juncus bulbosus*, and −30.7 for *Myriophyllum alterniflorum*) and potential active users of HCO_3^- among angiosperms (−25.5 for *Potamogeton berchtoldii* and −24.7 for *Potamogeton alpinus*). Markedly less negative $\delta^{13}C$ values have also been observed for *N. pruniforme* compared with benthic algae in a California stream (Finlay 2004), confirming the existence of very active DIC-concentrating mechanisms of colonial *Nostoc* species.

Colony growth calls for particularly efficient DIC-concentrating mechanisms because cells are densely packed in a stagnant gel, where photosynthetic substrates (e.g., HCO_3^- and CO_2) and products (e.g., O_2 and OH^-) have up to 2-mm-long paths of molecular diffusion from and to the external water. Thus, during intense photosynthesis cells are embedded in a gel having high O_2, high pH, and low CO_2. Microelectrode measurements have so far confirmed the formation of two times oxygen supersaturation and pH values of 10.2 in *Nostoc* colonies placed in air-saturated water of pH 8.0 after a few hours of intense photosynthesis (A. L. Raun unpubl.). Thus, without active carbon-concentrating mechanisms, *Nostoc* colonies would face extensive photorespiration because of unfavorably low $CO_2 : O_2$ quotients and this is obviously not the case because photoinhibition is not observed at high irradiances and photosynthesis is maintained across a wide range of external DIC concentrations (Raun et al. 2009) as long as internal DIC pools are available (Table 5).

Light compensation point, depth distribution, and survival—Light compensation points averaged 15.1 and 19.6 μmol m^{-2} s^{-1} in series 1 and 3 (Table 2) and exceeded mean values of the deep-growing characean *Nitella translucens* (5.3 μmol m^{-2} s^{-1}), the moss *F. antipyretica* (5.9), and the angiosperm *Elodea canadensis* (6.6) in long-term growth experiments in continuous light at 7°C (Sand-Jensen and Madsen 1991). Even though we accept that light compensation points are lower at 7°C than at 15°C in the *Nostoc* experiments because of lower respiratory maintenance costs, temperature correction applying a standard Q_{10} value of 2.0 would only raise mean light compensation points of the three species to 9–11 μmol m^{-2} s^{-1}, which is still appreciably lower than for *N. zetterstedtii*. This comparison supports our initial hypothesis that inefficient light use in *Nostoc* colonies combined with higher respiratory costs to produce and maintain the colony and perform a more costly uptake of solutes should lead to higher minimum light requirements than for characeans,

Table 5. Photosynthetic release of oxygen of *Nostoc zetterstedtii* over 18 h of extended high irradiance at three different initial and resulting final levels of DIC in the water (DIC_w) and within the colonies (DIC_I). Respiratory oxygen uptake over the subsequent 18 h of dark incubation is also shown. Mean ± SE of six replicates. Significant differences between final internal DIC pools and photosynthetic and respiratory rates are shown by different letters ($p < 0.05$, one-way ANOVA followed by Tukey tests).

Initial DIC_W (μmol L^{-1})	Final DIC_W (μmol L^{-1})	Final DIC_I (μmol L^{-1})	Photosynthesis (μg O_2 cm^{-2} h^{-1})	Respiration (μg O_2 cm^{-2} h^{-1})
0.990	0.338	17.2±1.1a	13.3±0.57a	5.8±0.45a
0.294	0.124	13.8±0.61b	10.6±0.53b	6.0±0.65a
0.103	0.109	7.4±0.45c	7.5±0.86c	5.7±0.65a

mosses, and angiosperms with thin photosynthetic tissues. Distribution of submerged plants in two lakes supports this conclusion because *N. zetterstedtii* grew to depth limits receiving about 11.7–12.5% of surface irradiance, whereas *F. antipyretica* grew to greater depths receiving 3.1–7.8%, characeans to 3.1–11.7%, and *E. canadensis* to 3.1% in Lake Hampen (Table 7). Light compensation points of 15.1–19.6 μmol m^{-2} s^{-1} for *N. zetterstedtii* corresponds to mean daily light compensation points of 1.3–1.7 mol m^{-2} d^{-1}, which is 3.7–4.9% of the mean daily solar irradiance of 35 mol m^{-2} d^{-1} in May–July. About 4.2 mol m^{-2} d^{-1} is received at the depth limit of *N. zetterstedtii* in the two lakes in May–July. This surplus of light compared with the light compensation point is expected because May–July has the highest solar irradiance and an energy surplus is needed to cover negative metabolism in darker months. For the entire year, the mean daily incident irradiance at the depth limit of *N. zetterstedtii* is about 2.2 mol m^{-2} d^{-1} and this is also slightly more than the measured light compensation points. A surplus of light is, however, needed to support net growth and balance unavoidable losses by senescence, mechanical removal, and other processes (Sand-Jensen and Madsen 1991; Markager and Sand-Jensen 1994). This surplus is small, emphasizing that depth limits in the lakes are set by available light.

The measured low photosynthetic rates of *Nostoc* colonies support the notion that growth is slow, that large colonies reaching 5 cm in the studied lakes are some years old (e.g., Dodds and Carstenholtz 1988), and that mortality must be similarly low for the population to persist. Large *Nostoc* colonies are probably free of any attack from invertebrate grazers (Moore 1978) and no fish or birds are known to consume the colonies. In the face of low resource use from the surrounding water, efficient recycling of nutrients and carbon is possible within the large colony volume because of little diffusive loss to the surrounding water and, thereby, the unusual colony form offers an important advantage (Vaqué et al. 1994). Also, accumulation of organic reserves during favorable seasons may support survival during later unfavorable seasons. In all these respects, *N. zetterstedtii* resembles the balloon-like *C. bursa*, which is regarded to rank among the slowest-growing macroalgae (Nielsen and Sand-Jensen 1990; Geertz-Hansen et al. 1994).

In summary, we expect that N_2 fixation, DIC concentration mechanisms, and the long-lived, persistent nature of *Nostoc* colonies are the main reasons why they can coexist with macroalgae and plants in predominantly resource-poor environments despite inefficient uptake of light and dissolved nutrients caused by the large size and spherical form of the colonies. If, on the other hand, these pristine environments are subjected to cultural eutrophication *Nostoc* colonies cannot take advantage of the richer environment but decline in abundance and eventually become extinct because of greater shading from phytoplankton and benthic filamentous macroalgae and rooted plants that form denser and taller stands, while *Nostoc* is left to succumb in the shadow at the sediment surface. Fortunately, strong phosphorus pollution control in Sweden means that although *N. zetterstedti* is very restricted in its distribution, there is no immediate risk of its global extinction.

Table 6. DIC consumed from internal DIC pools of *Nostoc* colonies over 20 h in the light, DIC accumulated in internal DIC pools over 20 h in the dark, and DIC initially present in the colonies before light incubations in the morning measured on sacrificed colonies in acid. Incubations were made at low external DIC (ca. 0.08 mmol L^{-1}). Values were calculated in μmol C g^{-1} colony fresh weight assuming a molar exchange ratio of oxygen to DIC of 1.0. Mean ± SE of four replicates from two independent collections, A and B.

Collection	DIC consumed in light	DIC accumulated in darkness	Internal DIC before light
A	24.5±5.6	8.6±2.4	23.7±1.7
B	16.3±2.9	13.2±5.0	19.0±3.2

Table 7. Lower depth limits of *Nostoc zetterstedtii*, other macroalgae, mosses, and angiosperms, and percentage surface irradiance (% SI) reaching the depth limit in summer 2006 in Lake Hampen and summer 2004 in Lake Ulstrup. Depth limits were determined by scuba diving.

	Lake Hampen		Lake Ulstrup	
Species	Depth limit (m)	% SI	Depth limit (m)	% SI
Nostoc zetterstedtii	5.0	12.5	5.6	11.7
Nostoc pruniforme			6.2	9.4
Elodea Canadensis	8.5	3.1	4.5	
Ranunculus species			5.5	
Potamogeton species	8.5	3.1	7.1	7.5
Characean species	8.5	3.1	5.6	11.7
Moss species	8.5	3.1	7.0	7.8
Filamentous green algae			7.0	7.8

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