INVITED REVIEW

Ecophysiology of gelatinous Nostoc colonies: unprecedented slow growth and survival in resource-poor and harsh environments

Kaj Sand-Jensen*

Freshwater Biological Laboratory, Biological Institute, University of Copenhagen, Universitetsparken 4, 2100 Copenhagen, Denmark

* E-mail ksandjensen@bio.ku.dk

Received: 17 February 2014 Returned for revision: 6 March 2014 Accepted: 1 April 2014

Background

The cyanobacterial genus Nostoc includes several species forming centimetre-large gelatinous colonies in nutrient-poor freshwaters and harsh semi-terrestrial environments with extended drought or freezing. These Nostoc species have filaments with normal photosynthetic cells and N2-fixing heterocysts embedded in an extensive gelatinous matrix of polysaccharides and many other organic substances providing biological and environmental protection. Large colony size imposes constraints on the use of external resources and the gelatinous matrix represents extra costs and reduced growth rates.

Scope

The objective of this review is to evaluate the mechanisms behind the low rates of growth and mortality, protection against environmental hazards and the persistence and longevity of gelatinous Nostoc colonies, and their ability to economize with highly limiting resources.

Conclusions

Simple models predict the decline in uptake of dissolved inorganic carbon (DIC) and a decline in the growth rate of spherical freshwater colonies of N. pruniforme and N. zetterstedtii and sheet-like colonies of N. commune in response to a thicker diffusion boundary layer, lower external DIC concentration and higher organic carbon mass per surface area (CMA) of the colony. Measured growth rates of N. commune and N. pruniforme at high DIC availability comply with general empirical predictions of maximum growth rate (i.e. doubling time 10–14 d) as functions of CMA for marine macroalgae and as functions of tissue thickness for aquatic and terrestrial plants, while extremely low growth rates of N. zetterstedtii (i.e. doubling time 2–3 years) are 10-fold lower than model predictions, either because of very low ambient DIC and/or an extremely costly colony matrix. DIC uptake is limited by diffusion at low concentrations for all species, although they exhibit efficient HCO3 uptake, accumulation of respiratory DIC within the colonies and very low CO2 compensation points. Long light paths and light attenuation by structural substances in large Nostoc colonies cause lower quantum efficiency and assimilation number and higher light compensation points than in unicells and other aquatic macrophytes. Extremely low growth and mortality rates of N. zetterstedtii reflect stress-selected adaptation to nutrient- and DIC-poor temperate lakes, while N. pruniforme exhibits a mixed ruderal- and stress-selected strategy with slow growth and year-long survival prevailing in sub-Arctic lakes and faster growth and shorter longevity in temperate lakes. Nostoc commune and its close relative N. flagelliforme have a mixed stress–disturbance strategy not found among higher plants, with stress selection to limiting water and nutrients and disturbance selection in quiescent dry or frozen stages. Despite profound ecological differences between species, active growth of temperate specimens is mostly restricted to the same temperature range (0–35 °C; maximum at 25 °C). Future studies should aim to unravel the processes behind the extreme persistence and low metabolism of Nostoc species under ambient resource supply on sediment and soil surfaces.

Key words: Gelatinous colonies, cyanobacteria, Nostoc commune, Nostoc flagelliforme, Nostoc pruniforme, Nostoc zetterstedtii, carbon use, carbon concentrating mechanisms, photosynthesis, light use, growth, long-lived, survival, desiccation tolerance, nutrient-poor.

INTRODUCTION

The cyanobacterial genus Nostoc includes many species that are highly diverse with respect to morphology, functional properties, biotic relations and habitat distribution (Dodds et al., 1995). Nostoc species have filaments with normal photosynthetic cells and N2-fixing heterocysts and they periodically form resistant akinetes for survival and short motile filaments (hormogonia) for reproduction (Mollenhauer et al., 1994). Some species are free-living and many species engage in loose or obligate cooperation with land plants and fungi (e.g. lichens; Mollenhauer, 1988; Kaasalainen et al., 2012). A third, fascinating Nostoc type forms large gelatinous colonies of variable shape and structure in rice fields, freshwater lakes, ponds and streams and on alternating wet and dry soils or rock surfaces (Dodds et al., 1995; Gao et al., 2012). The large gelatinous species require special adaptations to obtain sufficient light, nutrients and dissolved inorganic carbon (DIC) (Sand-Jensen et al., 2009b) in water and to survive the extreme variations in temperature, water supply and irradiance on naked soils and rock surfaces (Tamaru et al., 2005; Li et al., 2011; Yu and Liu, 2013). While phototrophs in resource-rich environments have been treated in numerous original papers, reviews and books (Larcher, 2003; Lambers et al., 2008), studies on the adaptations of phototrophs to resource-poor and physically extreme environments are rare. The objective of this review is to evaluate the mechanisms.
behind the extremely low rates of growth and mortality and high persistence, longevity and protection against environmental hazards of gelatinous Nostoc colonies and their ability to economize with highly limiting resources.

The review focuses on four colonial Nostoc species: the sheet-like Nostoc commune, the closely related N. flagelliforme and two species that form spherical colonies, N. pruniforme and N. zetterstedtii. All four species resemble each other by forming centimetre-large gelatinous colonies, but they differ in distribution, habitat preference, growth rate, environmental tolerance and appearance (Fig. 1). Nostoc commune is a globally widespread terrestrial and semi-aquatic species forming inactive crusts when dry, but rapidly changing to 1–5-mm thick sheet-like gelatinous structures when wet (Scherer et al., 1984; Marsh et al., 2006; Novis et al., 2007). Nostoc commune has unprecedented tolerance of environmental extremes (Satoh et al., 2002; Tamari et al., 2005; Sand-Jensen and Jespersen, 2012). It tolerates desiccation during dry seasons and can survive for >100 years on a museum shelf (Cameron, 1962). It tolerates freezing to −60 °C in Antarctic deserts (Davey, 1989; Novis et al., 2007) and to −269 °C in liquid helium in the laboratory (Sand-Jensen and Jespersen, 2012), but rapidly resumes metabolism when rewetted at physiologically suitable temperatures between 0 and 30 °C (Scherer et al., 1984; Taranto et al., 1993; Sand-Jensen and Jespersen, 2012; Möller et al., 2014). Nostoc flagelliforme is also widely distributed in arid and semi-arid areas throughout the world (Gao et al., 2012; Ye et al., 2012). It has a thread-like appearance but resembles N. commune in its genetics, growth habitat, colony composition and extreme tolerance to and rapid recovery from desiccation, freezing and salt stress (Gao and Zou, 2001; Qiu et al., 2004; Huang et al., 2005; Yong-Hong et al., 2005; Liu et al., 2010; Arima et al., 2011). We have observed Nostoc with the typical morphology of both N. commune and N. flagelliforme on the Great Alvar on Öland, Sweden (Sand-Jensen et al., 2010). Nostoc flagelliforme has been classified as a variety of N. commune in arid regions of Spain (Aboal et al., 2010) and recent DNA studies group those two ‘species’ together with N. sphaeroides and N. punctiforme in a common clade (Arima et al., 2011; Gao et al., 2011), which makes species identity dubious. Nonetheless, we maintain the distinction between N. commune and N. flagelliforme here in accordance with most available literature.

Nostoc pruniforme forms a beautiful, dark green, spherical colony with a smooth surface like a plume. It is more common and widely distributed both geographically and ecologically in oligo- and mesotrophic freshwaters in temperate and sub-Arctic regions than its rare freshwater cousin, N. zetterstedtii (Dodds et al., 1995; Raun et al., 2009). Danish alkaline lakes housing N. pruniforme contain >0.7 mM DIC (Sand-Jensen et al., 2009b). In temperate lakes, N. pruniforme appears to grow in diameter from ~0.2 cm in late spring to 2–3 cm in mid-summer and it can rapidly die off, supposedly because of attacks by viruses or bacteria at high summer temperatures (K. Sand-Jensen, unpubl. data). In contrast, in a cold (4 °C), nutrient-poor spring in Oregon, USA, N. pruniforme reached a handball size of 15–17 cm in diameter and weighed 2.6 kg after sustained slow growth for 9–14 years (Dodds and Castenholz, 1987). Very large colonies are also found in cold transparent lakes in Greenland (K. Sand-Jensen, unpubl. data). In addition to their larger size and persistence, they differ from Danish temperate specimens by having a more solid surface and a denser gel. Two additional species, N. calcareous and N. sphaericus (Mollenhauer et al., 1994) resemble N. pruniforme but are not discussed further here.

The fourth species, N. zetterstedtii, is a rare, highly persistent organism whose colonies resemble firm blackberries. It lives in only 50–100 nutrient-poor soft-water lakes in Sweden and in a few other lakes in neighbouring countries (Bengtsson, 1986, 1995; Mollenhauer et al., 1999). Soft-water lakes usually have DIC concentrations of 0.02–0.3 mM and concentrations of total phosphorus <0.1–0.3 μM in open waters (Sand-Jensen et al., 2009b). Among the freshwater phototrophic species studied so far, Nostoc zetterstedtii has unprecedented low growth and mortality rates (Sand-Jensen and Möller, 2011). In oligotrophic Lake Värjsö, Sweden, it takes ~3 years for N. zetterstedtii to double its colony mass and ~30 years to reach the recorded maximum diameter of 7 cm (Sand-Jensen and Möller, 2011). There is no apparent mortality of grazers and pathogenic bacteria and viruses (Sand-Jensen and Möller, 2011). The persistence of N. zetterstedtii is confirmed by its ability to survive for 14 months in the dark at 5 °C with intact colony structure and unaltered fresh mass (K. Sand-Jensen, unpubl. data).

All mentioned Nostoc species must have common structural and functional properties linked to the large size and extensive matrix of the colonies. However, the species differ with respect to metabolism, growth, mortality, tolerance to environmental stress and habitat preference. In this review, I first examine some of the main structural properties of the three main species (N. commune, N. pruniforme and N. zetterstedtii) with supplementary data for N. flagelliforme related to the centimetre-large size and gelatinous matrix of the colonies. Second, I evaluate theoretically the diffusive flux of DIC to the colony surface for a set of environmental constraints on boundary layer thickness and external concentrations. Third, I examine the functional consequences of the large colony size for the use of DIC and light.

![Fig. 1. Images of colonies of rehydrated Nostoc commune (upper left), Nostoc zetterstedtii (upper right, usually more spherical) and N. pruniforme (lower middle).](image-url)
in photosynthesis and growth. Fourth, I compare the tolerance of Nostoc species to environmental stresses related to temperature, freezing and desiccation. Fifth, I evaluate the growth, mortality and longevity of the three Nostoc species and the ecological implications of these properties. The final section points out that costs and benefits of colony formation should be quantified in future studies. For this comparative analysis of the ecophysiology of gelatinous Nostoc colonies, I use published data and include supplementary unpublished data to ensure a comprehensive comparison.

SIZE, SHAPE AND COMPOSITION

Centimetre-thick colonies with photosynthetic filaments embedded in a gelatinous matrix exert large physical constrains on functional properties.

Size and shape

Large size of phototrophs imposes constraints on the use of light and uptake of inorganic carbon and nutrient ions from the surrounding water (Nielsen and Sand-Jensen, 1990; Agusti et al., 1994). For a body of a given shape, surface area (SA ≈ L²) scales to the second power of the linear dimension (L), volume (V ≈ L³) to the third power, and surface area:volume ratio (SA:V ≈ L⁻¹) to the inverse of the linear dimension. These allometric relationships reflect that the supply rates of light and external resources are scaled to the outer surface while the requirements for production and maintenance of the cells and the gelatinous matrix are mainly scaled to the volume.

The spherical shape adopted by N. pruniforme and N. zetterstedtii is a simple shape of low SA:V ratio (3 r⁻¹, where r is the radius of the sphere). Individual filaments of Nostoc embedded in the gelatinous matrix and free-living filaments of cyanobacteria and algae are cylindrical and maintain a high SA:V ratio inversely scaled to the transverse radius of the filament (2 r⁻¹) but independent of filament length. The SA:V ratio of the flat sheet-like structure of Nostoc commune, multicellular algae and leaves of mosses and higher plants is inversely scaled to thickness (Lth) of the structure (SA:V = 2 Lth⁻¹) but is independent of its surface area, volume and total mass as long as tissue thickness remains constant.

The gelatinous colonies of N. commune, N. pruniforme and N. zetterstedtii all have relatively thick tissues (0.1–17 cm) and very low SA:V ratios (0.4–20 cm⁻¹) compared with the thin Nostoc trichomes within the gel (SA:V 4000–8000 cm⁻¹) and the 0.01- to 0.3 cm-thick submerged leaves of mosses and angiosperms (SA:V 70–2000 cm⁻¹) (Table 1). Nostoc commune sheets typically vary in thickness from 0.1 to 0.5 cm when wet and have SA:V ratios of 2–20 cm⁻¹. The smooth spherical colonies of N. pruniforme vary from a diameter of ~0.2 cm and an SA:V ratio of 30 cm⁻¹ to an astonishing maximum diameter of 17 cm and an exceedingly low SA:V ratio of only 0.36 cm⁻¹.

Small colonies of N. zetterstedtii are smooth, have a diameter of ~0.2 cm, just like N. pruniforme, while large colonies, reaching a recorded maximum diameter of 7 cm, have a granulated surface that can be regarded as consisting of small hemispheres (exposed area 2 πr² and planform area πr²) distributed all over a large spherical surface. The granulated surface area has a ~2-fold larger surface area relative to a smooth surface and the SA:V ratio is only 1.7 cm⁻¹ for the largest N. zetterstedtii colonies. The granulated surface could, in theory, double the diffusion-limited uptake of external solutes for N. zetterstedtii by doubling the surface area compared with smooth colonies of N. pruniforme of the same diameter (see section ‘Size effects, diffusive supply and loss of solutes’). The granulated surface could also contribute to the formation of thinner diffusion boundary layers (DBL) around the surfaces of N. zetterstedtii when the granules increase micro-turbulence (Boudreau and Jørgensen, 2001). The micro-topography of the granulated surface can result in thinner DBLs at the protruding tips of the granules and thicker DBLs in the crevices between the granules (Carpenter and Williams, 1993; Jørgensen, 2001) so the diffusion geometry is difficult to interpret. Although diffusive fluxes remain experimentally unexplored, they are, nonetheless, highly relevant considering that N. zetterstedtii usually grows in lakes with lower concentrations of DIC, N and P than N. pruniforme (Sand-Jensen et al., 2009b).

<table>
<thead>
<tr>
<th>Species and phototrophic type</th>
<th>Shape</th>
<th>Diameter/thickness (cm)</th>
<th>SA:V (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. commune</td>
<td>Flat structure</td>
<td>0-1</td>
<td>10 to 20</td>
</tr>
<tr>
<td>N. pruniforme</td>
<td>Sphere</td>
<td>0-5</td>
<td>2 to 4</td>
</tr>
<tr>
<td>N. pruniforme</td>
<td>Sphere</td>
<td>0-2</td>
<td>30</td>
</tr>
<tr>
<td>N. flagelliforme</td>
<td>Sphere, granulated</td>
<td>0-2</td>
<td>30</td>
</tr>
<tr>
<td>N. trichomes</td>
<td>Cylinders</td>
<td>7-0</td>
<td>0.85–1.7</td>
</tr>
<tr>
<td>Submerged leaves</td>
<td>Flat structure</td>
<td>5 × 10⁻⁴</td>
<td>8000</td>
</tr>
<tr>
<td>Single-celled algae or cyanobacteria</td>
<td>Sphere</td>
<td>10 × 10⁻⁴</td>
<td>4000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 × 10⁻³</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 × 10⁻³</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 × 10⁻⁴</td>
<td>30,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 × 10⁻³</td>
<td>3000</td>
</tr>
</tbody>
</table>

Composition of colony matrix

A high proportion of Nostoc colonies is composed of the extracellular matrix, primarily of polysaccharides of high viscosity and molecular weight. The hydrolysed polysaccharides from N. flagelliforme were mainly composed of glucose (43 %), galactose (30 %), xylose (21 %), mannose (6 %) and small amounts of glucuronic and uronic acids (Jia et al., 2007; Pereira et al., 2009). Much of the same main sugars were hydrolysed from hot water extracts of polysaccharides from field samples of N. commune, N. flagelliforme and N. sphaeroides, though small amounts of arabinose appeared in N. flagelliforme and rhamnose and fucose in N. sphaeroides (Huang et al., 1998). The composition of extracellular compared with intracellular polysaccharides was suggested to be quite different in N. commune and N. flagelliforme, perhaps due to a selective mechanism for excretion of polysaccharides (Mehta and Vaidya, 1978). Different studies of N. commune have shown the complex chemistry of the hydrolysed matrix, which includes six to nine monosaccharides, uronic acid, deoxy-sugars, pyruvate, acetate and peptides (Pereira et al., 2009). Scanning electron microscopy has revealed...
a net-like matrix of polysaccharides around *Nostoc* filaments (Cupac and Gantar, 1992), suggesting that their ultrastructure could influence colony shape. The chemical structure, the 3-D network and the presence of special peripheral groups, such as nosturonic acid, are probably essential for the outstanding moisture absorption and moisture retention capacity of *Nostoc* polysaccharides. These properties are highly interesting in biomedical applications involving living cells and tissue (Yu et al., 2010; Li et al., 2011).

When the gelatinous matrix of *N. commune*, *N. flagelliforme* and *N. pruniforme* is hydrated its water content is very high, leading to a soft structure that is easy to cut with a finger nail, as opposed to the very solid structure of *N. zetterstedtii*, which can only be cut, with great force, using a scalpel. Summer colonies of *N. pruniforme* from mesotrophic Lake Esrum with a fresh mass (FM) of 3.5 g have a mass specific density of \(~ \sim 1.01 \) g FM cm\(^{-3}\) while colonies of *N. zetterstedtii* from oligotrophic Lake Värsjö have a specific density of \(~ \sim 1.13 \) g FM cm\(^{-3}\) (Raun et al., 2009; Sand-Jensen et al., 2009b). Both species have gradually decreasing mass specific density for larger colonies, which tend to be hollow and water-filled in the centre of *N. pruniforme* and possess a massive, though less dense, structure without trichomes in *N. zetterstedtii* (Table 2). The carbon mass per surface area [CMA, equivalent to leaf mass per surface area for plants (LMA)] is closely scaled to structural and functional properties of macroagal thalli (Markager and Sand-Jensen, 1994, 1996) and plant leaves (Nielsen and Sand-Jensen, 1989, 1991; Lambers and Poorter, 1992, 2004; Poorter et al., 2012). The CMA increased by a power of \( \sim 0.25 \) relative to colony FM for *N. zetterstedtii* and only 0-05 for *N. pruniforme*, because the first species maintains massive colonies and the second species tends to develop hollow colonies with increasing size (Table 2). Thus, in two summer collections, CMA was \( \sim 8 \)-fold higher for *N. zetterstedtii* than *N. pruniforme* for colonies of 0.1 g FM, while the difference was \( \sim 14 \)-fold for colonies of 3.5 g FM.

The typical differences in CMA values for 3.5 g FM colonies of *N. zetterstedtii* (\( \sim 1400 \) μmol organic C cm\(^{-2}\)) and *N. pruniforme* (\( \sim 100 \) μmol organic C cm\(^{-2}\)) have important implications. Assuming the same net photosynthesis relative to colony surface area of both species and the same proportion of net photosynthesis being incorporated into new biomass, the turnover rate of the colony mass will be 14-fold lower for *N. zetterstedtii* than for *N. pruniforme*. With the same assumption of constant photosynthesis per surface area independent of colony size, biomass turnover and relative growth rate should decline with colony weight in proportion to the increase in CMA; i.e. an 8- to 9-fold decline across the range of colony weights of *N. zetterstedtii* from 0-1 to 4 g FM. We have recorded a decline in the relative growth rate and the surplus of photosynthesis minus respiration per colony mass with increasing colony size in *N. pruniforme*, but did not find the same strong size dependence for *N. zetterstedtii* (Sand-Jensen and Møller, 2011). We propose that allocation to extracellular products and their diffusive loss to the environment may decline with colony size and that these processes are relatively more important for the metabolically less active *N. zetterstedtii*. Reduced loss of exudates with higher colony size could counteract the expected decline in growth rate in larger colonies (see section ‘Size effects, diffusive supply and loss of solutes’).

As emphasized, a very high proportion of the colony volume and fresh mass is made up of the water-rich colony matrix compared with the denser *Nostoc* filaments. Still very high proportions of the carbon content of the colony are bound in the matrix. In *N. commune*, polysaccharides in the matrix constitute > 60% of the colony dry mass (Hill et al., 1997). Among spherical *Nostoc* colonies, filaments are confined to an outer shell, which occupies a falling proportion of the volume with increasing colony size, such that the contribution of the matrix to the total organic content should also increase. If we assume that the organic carbon:chlorophyll a mass quotient in *Nostoc* filaments has a typical range of 30–50 at 15 °C and moderate irradiances (Geider, 1987; his Fig. 13), we estimate that the matrix constitutes 18–45% of the organic carbon mass in the smallest colonies of *N. pruniforme*, while it is 37–62% in large colonies of 3.5 g FM. The proportion of organic carbon in the matrix is much higher in *N. zetterstedtii*, being \( \sim 50–70 \)% in small colonies weighing a few milligrams and \( \sim 90 \)% in large colonies weighing 3-5 g FM. The smaller proportion of living *Nostoc* filaments in the colony mass in *N. zetterstedtii* than in *N. pruniforme* will, as already mentioned, result in less photosynthetic production of new organic material relative to colony mass and therefore much lower growth rates for *N. zetterstedtii* than for *N. pruniforme*.

### Table 2. Organic carbon content per surface area (CMA, μmol C cm\(^{-2}\)) relative to colony fresh mass (FM,g) of three *Nostoc* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Relationship</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. commune</em></td>
<td>CMA = 184 ± 47 (mean ± s.e.)</td>
<td>K. Sand-Jensen, unpubl. data</td>
</tr>
<tr>
<td><em>N. pruniforme</em></td>
<td>Log CMA = 0.0474 log FM + 1.88</td>
<td>C. Møller and K. Sand-Jensen, unpubl. data</td>
</tr>
<tr>
<td><em>N. zetterstedtii</em></td>
<td>Log CMA = 0.26 log FM + 3.13</td>
<td>Sand-Jensen et al. (2009)</td>
</tr>
</tbody>
</table>

**Functional properties of colony matrix**

The gelatinous matrix of *Nostoc* colonies is composed of a complex mixture of polysaccharides (Bertocchi et al., 1990; Yousimura et al., 2012), which is responsible for forming and maintaining the colony shape and protecting the cells against environmental hazards and pathogens (Tamaru et al., 2005; Knowles and Castenholz, 2008). It is fascinating, yet unexplored, how the different colony shapes come about. A variety of disaccharides, including sucrose and non-reducing trehalose, also participate in the protection of cells and matrix (Caiola et al., 1996; Hill et al., 1997; Potts, 1994, 1999, 2000). Poly- and disaccharides protect membranes and macromolecules against freezing, heating, desiccation and salt stress (Tamaru et al., 2005; Sakamoto et al., 2009; Yoshida and Sakamoto, 2009).
The formation and accumulation of trehalose in *N. flagelliforme* and *N. punctiforme* are under strict regulation in response to water limitation (Yong-Hong et al., 2005; Yoshida and Sakamoto, 2009), presumably because trehalose is an essential, though costly, product that diverts organic carbon away from growth processes. The functional importance of the gel is supported by the observation that strains of *N. commune* with no gel and strains where the gel has been physically removed from the filaments become susceptible to injury (Tamara et al., 2005). Addition of exogenous polysaccharides to endolithic *Nostoc* sp. and *Chlorella* sp. increases their tolerance to desiccation (Knowles and Castenholz, 2008), probably by retaining a water layer around the cells and preventing membranes from cracking and macromolecules from undergoing destructive conformational changes. The main polysaccharides observed in the colony gel of *N. commune* have a strong capacity for *in vitro* moisture absorption and water retention, permitting a >10-fold increase in mass when desiccated colonies become rehydrated (Li et al., 2011; Sand-Jensen and Jespersen, 2012).

The gel has a strong adsorption capacity for heavy metal ions that are toxic to cyanobacteria in free ionic form (Bender et al., 1994; De Philippis et al., 2007; Pereira et al., 2009). Non-reducing sugars, UV-screening pigments and water stress proteins also assist in the environmental protection of *Nostoc* colonies (Hill et al., 1997; Potts, 1999). In response to increasing intensity and duration of UV exposure of *N. flagelliforme*, contents of protective syctonemin and mycosporine-like amino acids increase (Büdel et al., 1997; Yu and Liu, 2013). A large number (573) of the genes that were probed (6903) in *N. punctiforme* responded significantly to UVA exposure, 473 genes being up-regulated and only 100 being down-regulated (Soule et al., 2013). As expected, genes encoding antioxidant enzymes and the sunscreen syctonemin were up-regulated, while many genes involved in the synthesis of photosynthetic pigments were down-regulated. Also, *Nostoc* polysaccharides are capable of reducing oxidative damage by scavenging superoxide anions and hydroxyl radicals (Li et al., 2011).

Antibiotic production has been recorded in many *Nostoc* species (de Cano et al., 1986; Bloom and England, 1991; Gromov et al., 1991), including gelatinous colonies (Hameed et al., 2013). When *N. zetterstedtii* was homogenized in water and mixed 1:10 v/v with natural lake water containing bacteria, DNA synthesis stopped immediately (Sand-Jensen et al., 2009a). The polysaccharide nostoflan, isolated from *N. flagelliforme*, has *in vitro* and *in vivo* antiviral effects on a variety of enveloped viruses, including influenza A virus (Kanekyo et al., 2005, 2007, 2008). Symbiotic *Nostoc* in a great variety of lichens either contains toxic microcystins or has the genes for producing them in 12% of 803 lichen specimens collected worldwide (Kaasalainen et al., 2012). Microcystins occurred in 52 different variants in the 803 lichen specimens (Kaasalainen et al., 2012); they are small cyclic peptides and are known to be highly toxic to eukaryotes by inhibiting protein phosphatases (MacKintosh et al., 1990). Microcystins are active at multiple sites with gradually declining effects on nitrogenase activity, respiration, photosynthesis and growth of competing cyanobacteria. Three different classes of peptide compounds (anabaeopeptin, cryptophycin and nostocyclopeptides) could protect the toxin-producing cyanobacteria against bacteria, viruses, fungi, fish and ducks and could be deadly to mice and dogs around paddy fields (Nowruzzi et al., 2012). Their most important ecological effects, however, involve the inhibition of pathogenic viruses and bacteria, potential grazers and competing phototrophs, not mice and dogs.

**SIZE EFFECTS, DIFFUSIVE SUPPLY AND LOSS OF SOLUTES**

As organisms increase in size, diffusion gradually becomes a less efficient means of supplying external dissolved substances.

**Size effects**

Because resource uptake in phototrophic organisms scales to surface area while resource demand scales to volume and mass, a first principle dictates that relative growth rate scales to SA:V and CMA (Nielsen and Sand-Jensen, 1990; Lambers and Poorter, 1992, 2004). Studies on *N. prunifor me* and *N. zetterstedtii* show that chlorophyll content and photosynthetic capacity relative to surface area are approximately independent of colony size because the *Nostoc* trichomes are mainly confined to the 1–2 mm-thick outer periphery of the spherical colony, while its dark central part is mostly devoid of trichomes and photosynthetic activity (Raun et al., 2009; Sand-Jensen et al., 2009b). If *Nostoc* colonies produce hollow spheres with an outer shell of uniform thickness and a water-filled central cavity, CMA would be independent of colony size, just as in the uniformly thick thalli of many macroalgae, plant leaves and the sheet-like structure of *N. commune*. This is also almost the situation for *N. pruniforum* in summer collections in Danish lakes, where CMA increases with colony mass to a power of only ~0.05, corresponding to a 1.3-fold increase in CMA for a 100-fold increase in colony FM (Table 2). Spherical colonies could in this case maintain an almost unaltered growth rate with increasing size provided the extent of diffusive supply of limiting DIC and nutrients remained constant relative to surface area, or that this diffusive flux was not rate-limiting. Because colonies of *N. zetterstedtii* are massive, CMA is high and increases by a power of 0.75 with respect to colony size and thereby should contribute to a low and declining growth rate in larger colonies.

For growth performance, it is therefore crucial how resource fluxes and mass density respond to changes in environmental conditions and colony size and how photosynthates are allocated between new cells, colony matrix and protective organic compounds.

**Resource supply by diffusion and turbulence**

The diffusive flux (*F*_s) of a dissolved substance to a unit surface of a spherical colony is determined by colony size (e.g. radius *r*), thickness of the DBL surrounding the surface (∆), the concentration gradient across the boundary layer and the diffusion coefficient (*D*), according to eqn 1 (Table 3). The flux is highly dependent on size for small colonies, whereas for large colonies (*r ≫ ∆*) the flux gradually approaches the two-dimensional situation for diffusion to a flat plate where the flux relative to surface area is independent of colony radius (eqn 2 in Table 3).
The flux of HCO$_3^-$ was calculated to a unit surface of smooth spherical colonies like those of *N. pruniforme* (eqn 1) of variable size for realistic thicknesses (0.3, 1 and 3 mm) of the DBL in moving and quasi-stagnant water and differences in concentrations of 0-1, 0.5 and 2.0 mm (mm = mmol L$^{-1}$ = μmol cm$^{-3}$) between the external water and the colony surface representative of what would be expected in lakes of low, intermediate and high HCO$_3^-$ concentrations, respectively (Fig. 2). Profiles of dissolved oxygen measured with microelectrodes above the top surface of a *Nostoc parmelioideis* colony as a function of water velocity measured 1 mm above the colony suggested DBL thicknesses of 0.1 mm at 6–10 cm s$^{-1}$, 0.2–0.3 mm at 1 cm s$^{-1}$ and >0.4 mm in quasi-stagnant conditions (Fig. 3 in Dodds et al., 1995). The lower part of the colony resting on the surface has greater DBL thicknesses. *Nostoc zetterstedtii* could be expected to mostly experience concentration differences of 0.1–0.3 mm HCO$_3^-$ and *N. pruniforme* 0.7–2 mm. When *N. commune* (and *N. flagelliforme*) is supplied with rainwater the DIC concentration is very low. However, when water with dissolved HCO$_3^-$ is received from the carbonate-rich soils of the typical growth habitats, the expected DIC range is 0.4–2 mm (Sand-Jensen et al., 2010; Christensen et al., 2013). Because of the granulated surface of *N. zetterstedtii*, the flux is perhaps doubled relative to the smooth surface of *N. pruniforme*, while the flux to the sheet-like colonies of *N. commune* is much lower than the flux to small spherical colonies, but only slightly lower than the values for large colonies of *N. pruniforme*, because they physically approach the two-dimensional diffusion geometry. Only the diffusive flux of HCO$_3^-$ has been calculated, because HCO$_3^-$ constitutes >95% of the DIC pool in alkaline water (>0.5 mm) and ∼85% in soft water (0-1 mm) at air saturation with ∼0.015 mm CO$_2$. All tested *Nostoc* colonies can use HCO$_3^-$ actively (see later).

With an HCO$_3^-$ gradient of 2 mm through a 1-mm-thick DBL, the potential flux is 1440 nmol C cm$^{-2}$ h$^{-1}$ for colonies with a radius of 1 mm (∼5 mg FM) and about half that value (790 nmol C cm$^{-2}$ h$^{-1}$) for colonies with a 10-fold higher radius of 10 mm (∼4.5 g FM) (Fig. 2). The potential flux for the two-dimensional diffusion geometry to a sheet-like structure like that of *N. commune* is 720 nmol C cm$^{-2}$ h$^{-1}$ and is independent of size. Fluxes decline 20-fold when the HCO$_3^-$ gradient is 20-fold smaller (i.e. 0.1 mm across the DBL). In contrast, a thinner DBL of 0.3 mm increases the flux most for large colonies because they are more restricted by diffusion geometry than small colonies. Thus, for a gradient of 2 mm, the calculated flux increases to 3120 nmol C cm$^{-2}$ h$^{-1}$ for a small spherical colony (radius 1 mm), to 2470 nmol C cm$^{-2}$ h$^{-1}$ for a large

### Table 3. Equations 1–5 describe the diffusive flux relative to surface area ($F_s$), volume ($F_v$), shell volume ($F_{sv}$) and entire colony ($F_{col}$) of smooth spherical colonies with a given radius ($r$) of a solute with diffusion coefficient $D$ across a DBL ($δ$) with the concentration gradient $C_m$–$C_o$, where $C_m$ and $C_o$ are concentrations in the bulk medium and at the colony surface, respectively. Equation 6 is for a hollow sphere with an outer shell of thickness $L_s$. Equations 7 and 8 describe the concentration of a solute at the colony surface ($C_r$) produced at a rate of $P_r$ per unit volume of a massive (eqn 7) or hollow colony (eqn 8) and lost by diffusion to stagnant external water of zero concentration.

<table>
<thead>
<tr>
<th>No.</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$F_s = r^{-2} (r + δ) \frac{δ}{δ^{1.1}} D (C_m - C_o) = r^{-1} (1 + r/δ) D (C_m - C_o)$</td>
</tr>
<tr>
<td>2</td>
<td>$F_v \approx δ^{0.5} D (C_m - C_o); (r \gg δ)$</td>
</tr>
<tr>
<td>3</td>
<td>$F_{sv} \approx r^{-1} D C_{sv} (C_o = 0)$</td>
</tr>
<tr>
<td>4</td>
<td>$F_{col} = 4π r D C_{col} (C_o = 0)$</td>
</tr>
<tr>
<td>5</td>
<td>$F_v \approx 3 r^{-2} D C_{sv} (C_o = 0)$</td>
</tr>
<tr>
<td>6</td>
<td>$F_v \approx r^{-1} L_s^{-1} D C_{sv} (C_o = 0)$</td>
</tr>
<tr>
<td>7</td>
<td>$C(r) = \frac{1}{3} P_r D^{-1} r^2; (C_o = 0)$</td>
</tr>
<tr>
<td>8</td>
<td>$C(r) \approx P_r L_s r D^{-1}; (C_o = 0)$</td>
</tr>
</tbody>
</table>

Equations are derived from Nobel (1983) and Denny (1993).
spherical colony (radius 10 mm) and to 2400 nmol C cm\(^{-2}\) h\(^{-1}\) for a sheet-like structure.

What do these fluxes imply in terms of potential growth rate and biomass turnover? The answer is different for *N. pruniforine*, with carbon mass relative to surface area of \(\sim 100 \mu\text{mol C cm}\(^{-2}\) for large colonies (radius 10 mm) compared with \(\sim 1400 \mu\text{mol C cm}\(^{-2}\) for *N. zetterstedtii* of similar size. Assuming an inward flux during 12 daytime hours, a large HCO\(_3\) gradient of 2 mm across a DBL thickness of 1 mm, the potential turnover time of the biomass would be 11 d for *N. pruniforine* and 147 d for *N. zetterstedtii*, not accounting for night-time respiration, while a small gradient of 0.1 mm could only permit a potential turnover time of 210 d for *N. pruniforine* and 2940 d for *N. zetterstedtii* emphasizing the profound influence on carbon turnover of higher CMA for *N. zetterstedtii* compared with *N. pruniforine*. This difference should be enhanced by *N. pruniforine*’s preference for hard-water lakes, ensuring a higher flux, and *N. zetterstedtii*’s predominant existence in soft-water lakes. Thus, typical turnover times of *N. pruniforine* could be of the order of 11 d under suitable conditions in hard-water lakes containing 2 mm DIC for colonies measuring 10 mm in radius as opposed to 2940 d (8 years) for *N. zetterstedtii* of the same size growing in soft-water lakes with only 0.1 mm DIC available. If we account for the possibility of a 2-fold higher inward HCO\(_3\) flux for *N. zetterstedtii* because of the granulated colony surface, the turnover time would drop to 4 years, which is close to the observed values for *N. zetterstedtii* (3 years) in the soft-water Lake Värsjö (Sand-Jensen and Möller, 2011). Also, the turnover time of the biomass of *N. pruniforine* in laboratory experiments at 15–25 °C and in the shallow water of the hard-water Lake Esum (2.5 mm DIC) during early summer was 10–14 d (Møller et al., 2014; C. Möller and K. Sand-Jensen, unpubl. data) and resembled the theoretical estimate of 11 d mentioned above. These calculations suggest that accounting for DIC concentrations, DBL thickness and CMA permits sound predictions of growth and biomass turnover of *Nostoc* colonies.

In addition to DIC, the metabolism and growth of *Nostoc* colonies could be constrained by the supply rate of dissolved inorganic phosphorus (DIP). Nitrogen can be fixed as elementary N\(_2\) and is less likely to be limiting, although growth stimulation by high external concentrations may occur, particularly for terrestrial species with unrestricted access to atmospheric CO\(_2\) (see later). Lakes and ponds with *Nostoc* species mostly have DIP concentrations in the water column ranging from undetectable by standard chemical methods to 0.3 mm (Sand-Jensen et al., 2009b), though higher concentrations can occur at the sediment surface. For a DIP gradient of 0.3 mm across a 1 mm thick DBL, the estimated influx was \(\sim 0.22 \text{nmol P cm}\(^{-2}\)h\(^{-1}\) for small colonies (radius 1 mm) and 0.12 nmol P cm\(^{-2}\) h\(^{-1}\) for large colonies (radius 10 mm). These fluxes should permit a turnover time of the P pool within 6 d for small and 72 d for large colonies of *N. zetterstedtii*, supporting the hypothesis that growth is more likely to be constrained by the supply rate of DIC across gradients of 0.1 mm HCO\(_3\). These calculations agreed with the observation that growth was unaffected by P richness in the sediments of soft-water Lake Värsjö (Sand-Jensen and Möller, 2011). DIC supply is also important in dense epilithic communities growing on inert substrata and covered by relatively thick boundary layers (Sand-Jensen, 1983). Thus, short epilithic algal communities in marine waters have DBL thicknesses similar to those applied for *Nostoc* colonies and they appear to be constrained by the DIC flux to photosynthesis despite high HCO\(_3\) concentration (2 mm) in seawater. Larkum et al. (2003) showed that DBLs of epilithic algal communities covering dead coral surfaces were 0.18–0.59 mm thick under moderate flow (~8 cm s\(^{-1}\)) and >2 mm under quasi-stagnant conditions, and they generated high resistance to DIC influx, limiting primary production. Independent experiments with epilithic algal communities in other coral reef environments appeared to support the conclusion that DIC is a main limiting factor, as elevated nutrient levels had no effect on primary production and growth (Larkum and Koop, 1997; Miller et al., 1999), while large macroalgae in such nutrient-poor waters were stimulated by nutrient amendment (Lapointe et al., 1987).

**Resource supply solely by diffusion**

The flux to *Nostoc* colonies may be controlled entirely by diffusion in a stagnant water layer overlying the sediment. A stagnant surface layer may exist almost permanently in deep water with restricted turbulence and periodically in shallow water during calm weather. The colonies could be buried in a viscous boundary layer a few centimetres thick overlying the sediment (Boudreau and Jørgensen, 2001). When the surrounding water is virtually stagnant and the solute concentration is reduced to zero at the colony surface, the flux per unit surface area is inversely scaled to colony radius, emphasizing the immense advantage of being small (eqn 3 in Table 3). The calculated flux is 720 nmol C cm\(^{-2}\) h\(^{-1}\) for small colonies (radius 1 mm) in water containing 2 mm HCO\(_3\) or half the flux that occurs when DBL is 1 mm thick (eqn 1). For large colonies (radius 10 mm), however, the flux in stagnant water is only 72 nmol C cm\(^{-2}\) h\(^{-1}\), or 11-fold lower than in stirred water with a DBL of 1 mm. The potential biomass turnover time for large colonies as described above would then be 120 d for *N. pruniforine* under stagnant conditions in hard-water lakes and 32 350 d (89 years) for *N. zetterstedtii* in soft-water lakes.
Both values are very high, stressing that the water overlying the sediment is either not entirely stagnant or is enriched with DIC (CO₂ and HCO₃⁻) from sediment decomposition. Maberly’s (1985) measurements 10 cm above the sediments in a shallow bay covered by the moss Fontinalis antipyretica showed elevated CO₂ concentrations relative to surface waters between 0.05 and 0.26 mm on 14 different dates. Concentrations are higher directly on the sediment surface and a near-bed CO₂ concentration of, for example, 0.3 mm CO₂ could permit a 6-fold higher influx of CO₂ than a HCO₃⁻ gradient of 0.1 mm because the diffusion coefficient of CO₂ is approximately twice that of HCO₃⁻ (Denny, 1993). The resulting turnover time could then be 6-3 years for large colonies of N. zetterstedtii if we assume that the granulated surface doubles the influx and 3-2 years if the inward flux continues in both light and darkness. Thus, we cannot exclude that large colonies of N. zetterstedtii could survive in an entirely diffusive environment provided available concentrations of CO₂ and HCO₃⁻ were markedly higher than 0.1 mm. Small colonies a few millimetres in diameter can perform well by diffusive fluxes alone and are likely to be fully embedded in a diffusive boundary above the sediment surface.

Resource supply by diffusion relative to colony volume

The diffusive flux through stagnant water to a smooth, spherical colony follows the well-known formula often used for bacteria (eqn 4 in Table 3; Fenchel et al., 1998) because the colonies are usually so small relative to the thickness of DBL that the supply rate is entirely determined by diffusion. When metabolic activity and the demand for external resources are evenly distributed throughout the colony volume, the flux relative to unit volume (Fv) will be inversely scaled to the second power of radius, emphasizing the likelihood of extreme limitation as the organisms grow in size (eqn 5 in Table 3). The solutions to cope with this rapidly increasing risk of resource limitation with larger size would be to either reduce volume-specific requirements for metabolism (in the case of N. zetterstedtii) or develop a hollow sphere where all activity is restricted to an outer shell of thickness Lsh and volume 4/3π (r² - (r - Lsh)²)) ≈ 4π (r - 0.5Lsh)² Lsh. When r is large relative to Lsh, volume is close to 4π r² Lsh and the flux relative to volume of the outer shell (Fv,r) declines linearly with colony radius (eqn 6 in Table 3).

Thus, a 10-fold increase in colony radius will reduce the flux relative to the surface area and volume of a hollow spherical colony by the same order of magnitude, while the flux relative to the volume of a homogeneous, solid spherical colony declines 100-fold, emphasizing that if large colonies are indeed solid then their central parts must be composed of recalcitrant matrix material and either have no cells or cells of low metabolism. Because N. zetterstedtii colonies with a diameter of 10-20 mm absorb ~96 % of incident irradiance from the surface to the centre (Sand-Jensen et al., 2009b), there is no scope for photosynthesis of Nostoc trichomes in the centre, but there is organic matter present here that could potentially be used by heterotrophic bacteria. The colony matrix is persistent and protected by bactericides (Sand-Jensen et al., 2009a), however, implying that there are no or very few active heterotrophic bacteria living within N. zetterstedtii colonies. The year-long persistence and low respiration rates of the colonies in complete darkness support this implication (see below).

Efflux of solutes

While the large size of gelatinous Nostoc colonies constrains the influx of external resources, it will also limit the efflux of valuable dissolved products derived from metabolism within the colonies. The possible implications of variations in this efflux have been overlooked so far. When the diffusive loss of respiratory CO₂ and inorganic nutrients (e.g. phosphate) from the colony is constrained, they can better be retained and recycled within the colony (see section ‘Size effects, diffusive supply and loss of solutes’). Organic compounds that are produced and to a great extent released from the trichomes to form the extensive colony matrix can also be lost to the surrounding water and this loss can presumably be reduced in large colonies. Perhaps more importantly, antibiotics and toxins produced by Nostoc to reduce the risks of being attacked by harmful bacteria, viruses, mixotrophic algae and animals can be retained within the colony and here reach highly effective concentrations for a limited rate of production. Thus, with increasing colony size a constant production rate of the protective substance per colony volume will lead to increasing internal concentrations, or a constant internal concentration can be attained despite a declining production rate of the protective substance.

The formal analysis shows that a solute produced by a massive spherical organism at a uniform specific rate per unit volume (Pv) and lost by diffusion to stagnant surrounding water with a zero background concentration will have an effective concentration right at the surface of the colony that increases by the second power of the radius (eqn 7 in Table 3). If the concentration is kept constant, the production rate can decline by the second power of the radius. The mathematical formulas have been derived from Denny (1993), who treated an analogue biological example.

Photosynthetic production is constant relative to the surface area of N. pruniforme and N. zetterstedtii (Raun et al., 2009; Sand-Jensen et al., 2009b), in accordance with the fact that photosynthesis is confined to a surface shell of thickness Lsh and an approximate volume of 4π r² Lsh. Because protective substances derive from photosynthetic products, they could have the same scaling properties. Thus, inserting shell volume instead of total colony volume in eqn 7 predicts that the concentration on the colony surface would increase in proportion to colony radius (eqn 8 in Table 3). Alternatively, the concentration would remain constant if the production rate of protective substances declined in proportion to increasing colony radius.

Therefore, the incorporation rate of inorganic carbon into new cells, colony matrix and extracellular products should all be determined as a function of colony size. This would permit direct determination of turnover rates of cells and colony matrix and evaluation of the relative cost of producing and releasing extracellular products. Despite extensive and elaborate work on the biochemistry of Nostoc colonies, this analysis has not been performed as yet.

FUNCTIONAL CONSEQUENCES OF THE USE OF LIGHT AND INORGANIC CARBON

Nostoc concentrates and circulates inorganic carbon efficiently within the large colonies, while the use of light suffers from
extensive self-shading and absorption by non-photosynthetic elements.

Use of light

The large size, long internal light paths and high densities of trichomes within Nostoc colonies imply that light absorbance and internal self-shading are high. Mean light absorbance ranged from 55% in N. commune to 96% in N. zetterstedtii (Table 4). Moreover, because the cells experience high O2 and low CO2 concentrations within the colonies during photosynthesis, maximum photosynthesis relative to cell chlorophyll (i.e. the assimilation number), maximum quantum efficiency of photosynthesis and the ratio of photosynthesis to respiration should be much lower than among free-living unicells. To the extent that photons are absorbed by non-photosynthetic pigments and other coloured substances inside the colonies, maximum quantum efficiency will decline further.

Quantum efficiency was indeed low for N. commune [average 19.1 mmol O2 (mol absorbed photon)−1] and N. zetterstedtii (11.2–39.9 in three series; Table 4). A similarly low efficiency [27 mmol O2 (mol photon)−1] was calculated for the thick-walled, balloon-like green macroalga Codium bursa (Geerth-Hansen et al., 1994) and microbial mats with extensive absorption (40–80%) by structural components (Al-Najjar et al., 2012). In contrast, quantum efficiencies are much higher for free-living microalgae [70–120 mmol O2 (mol photon)−1], macroalgae and submerged plants [37–79 mmol O2 (mol photon)−1] (Frost-Christensen and Sand-Jensen, 1992).

Nostoc zetterstedtii had particularly low light-saturated photosynthesis relative to chlorophyll (average 0.5–0.7 mg O2 mg−1 chlorophyll a h−1) and values were also low for N. commune (average 2.4) and N. prunifome (average 2.1). Likewise, photosynthesis relative to dark respiration (NP:R) was low for N. zetterstedtii (2.0–5.8) and N. commune (average 2.5), but not for N. prunifome (average 10). Again free-living unicells have higher rates of photosynthesis relative to chlorophyll, typically between 2 and 20 (Harris, 1978) and NP:R ratios from 5 to 20 (Harris, 1978; Geider and Osborne, 1992). High self-shading within Nostoc colonies is an important constraint because photosynthesis at high irradiance increased 4-fold and NP:R increased 3-fold when self-shading was reduced by cutting colonies of N. zetterstedtii into 1–2-mm pieces (Sand-Jensen et al., 2009b).

Light attenuation by coloured substances, structural substances and dead or senescent cells within the colonies competes with photosynthetic pigments for photons, and thereby lowers quantum efficiency and increases the light compensation point (where photosynthesis outweighs respiration) compared with free-living unicells (Krause-Jensen and Sand-Jensen, 1998; Al-Najjar et al., 2012). Thus, light compensation points of N. zetterstedtii and N. commune (9.5–19.3 μmol photon m−2 s−1) exceeded those of most unicells (typically 0.8–9 μmol m−2 s−1; Langdon, 1988) and thin thalli and leaves of macroalgae and submerged plants (typically 2–12 μmol m−2 s−1; Sand-Jensen and Madsen, 1991). This comparison supports the hypothesis that inefficient light use in Nostoc colonies combined with higher respiratory costs of producing and maintaining the colony should lead to higher minimum light requirements for survival than for unicells, characeans and plants with thin photosynthetic tissues. Observations of lower light availability at the maximum depth limits of characeans, filamentous algae and mosses (3.1–7.8% of surface light) than of N. prunifome and N. zetterstedtii (9.4–12.5% of surface light) in two Danish lakes support this hypothesis (Table 7 in Sand-Jensen et al., 2009b).

Use of inorganic carbon

The diffusive supply of DIC from outside is constrained by the large size, low SA : V ratio and high density of filaments in the 1–3-mm-thick outer shells of gelatinous Nostoc colonies without direct contact with the surrounding medium. The effective diffusion path involves both the diffusive boundary layer surrounding the colony and the path through the gel to the sites of fixation in the Nostoc filaments, but the internal resistance has not been accounted for.

While increasing the resistance to influx of gas and solutes, the gel will also slow effluxes and contribute to the retention of nighttime respiratory CO2 and facilitate accumulation of internal DIC pools. To ameliorate carbon limitation of photosynthesis and prevent excessive photospiration, Nostoc colonies, like other cyanobacteria (Badger and Price, 1992, 2003), must attain reasonably high quotients of CO2 to O2 at the site of Rubisco activity within the cells. To achieve this, Nostoc colonies must be able to extract high proportions of the DIC pool in the water and have low compensation points of CO2 and HCO3 (Sütemeyer et al., 1998). The ability to accumulate DIC pools within the colony above immediate needs for photosynthesis and to retain respiratory CO2 from nighttime respiration for later use during daytime photosynthesis could assist the carbon supply even further. Such efficient extraction and accumulation of carbon are known for

<table>
<thead>
<tr>
<th>Species</th>
<th>Pmax (mmol O2 cm−2 h−1)</th>
<th>Pmax/dark respiration</th>
<th>Icomp (μmol m−2 s−1)</th>
<th>Quantum efficiency (mmol O2/mol photons)</th>
<th>Absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. commune</td>
<td>353–376</td>
<td>2.4</td>
<td>2.5</td>
<td>9.5</td>
<td>19</td>
</tr>
<tr>
<td>N. prunifome</td>
<td>552–654</td>
<td>2.1</td>
<td>10</td>
<td>5.1</td>
<td>19</td>
</tr>
<tr>
<td>N. zetterstedtii</td>
<td>206–409</td>
<td>0.7</td>
<td>20–5–8</td>
<td>19.3</td>
<td>20</td>
</tr>
</tbody>
</table>

Data are means of measurements in several series.

Pmax, maximum net photosynthesis at light saturation; Icomp, light compensation point.

Sources: N. commune (K. Sand-Jensen, unpubl. data); N. prunifome (Raun et al., 2009); N. zetterstedtii (Sand-Jensen et al., 2009b).
free-living cyanobacteria (Kaplan et al., 1980; Sültemeyer et al., 1998) and phototrophs in lichen photosynthesis (Green et al., 1994), and the potential may also exist for Nostoc trichomes buried within the colony gel.

We found a high mean extraction capacity, of between 74 and 82% of the initial DIC pool in the water (0.3–1.1 mM), during 20 h of photosynthesis in closed bottles with N. commune and N. zetterstedtii where DIC was gradually depleted and pH and O2 increased over time (Table 5). The mean extraction capacity was lower (58%), but probably not fully expressed, in experiments with N. pruniforme*, though it still reflected very active DIC uptake. Efficient DIC use is also known for N. flagelliforme and edible Nostoc Ge-Xian-Me (Gao and Zou, 2001; Qiu et al., 2004; Ye et al., 2012). Efficient active use of external DIC by N. commune and N. zetterstedtii was also reflected in the high final pH in the surrounding water (10.6–11.1) and the low final concentrations of HCO3

\[ \text{N. zetterstedtii} \]

and CO2 (0.3–7 nM) as measures of HCO3

\[ \text{N. pruniforme*} \]

and DIC uptake. Efficient DIC use is also known for free-living cyanobacteria (Kaplan et al., 1980; Badger and Price, 2003).

When I accounted for exchange of DIC during photosynthesis and respiration with both the colony matrix and the external water, I found mean molar exchange quotients of O2 relative to DIC in the light (1.19) and in the dark (0.96) close to 1:0 in experiments with N. zetterstedtii (Fig. 3). These direct measurements confirmed that a high proportion of DIC for photosynthesis in the light was consumed from a DIC pool in the colony, while in the dark a high proportion of respiratory DIC accumulated within the colony (Fig. 3). The measured internal DIC pools in N. zetterstedtii could support maximum photosynthesis for 11 and 23 h in 10- and 20-mm-diameter colonies, respectively, without uptake of external DIC. When N. commune was incubated for 20 h in relatively DIC-rich water containing initially 1.1 mM, it accumulated 16.7–18.7 mol DIC g\(^{-1}\) FM from the support of the immediate requirements for photosynthesis, while it consumed 9–10.2 mol DIC g\(^{-1}\) FM from the colony to support photosynthesis when the initial DIC concentration in the water was only 0.2 mM (K. Sand-Jensen, unpubl. data). Photosynthetic ATP production provides ample energy for active transport of HCO3, and at least N. commune can consume DIC for photosynthesis and at the same time accumulate DIC within the colony in the light when sufficient external DIC is available. Thus, the species does not solely rely on accumulation of respiratory DIC in the dark but can also take up external HCO3 for use in the following light period.

The ability of Nostoc colonies to build up substantial internal DIC pools had been overlooked in the past. This ability means that photosynthesis is less dependent on the immediate external DIC supply. Nonetheless, all three species were limited by the

### Table 5. Extraction capacity of the initial DIC pool (%) of three Nostoc species during photosynthesis for 20 h in closed bottles

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean ± s.e. (%)</th>
<th>No. of measurements</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. commune</td>
<td>72 ± 4</td>
<td>16</td>
<td>K. Sand-Jensen (unpubl. data)</td>
</tr>
<tr>
<td>N. pruniforme*</td>
<td>56 ± 3</td>
<td>5</td>
<td>Raun (2006); Raun et al. (2009)</td>
</tr>
<tr>
<td>N. zetterstedtii</td>
<td>74 ± 9</td>
<td>16</td>
<td>Sand-Jensen et al. (2009)</td>
</tr>
</tbody>
</table>

*Full extraction capacity probably not realized.

### Table 6. Final pH, final DIC, HCO3 and CO2 and final O2/HCO3 ratio of the four Nostoc species after 20 h of photosynthesis in closed bottles. Ranges are shown from several experimental series with many replicates

<table>
<thead>
<tr>
<th>Species</th>
<th>Initial DIC (μmol L(^{-1}))</th>
<th>Final pH</th>
<th>Final DIC (μmol L(^{-1}))</th>
<th>Final HCO3 (μmol L(^{-1}))</th>
<th>Final CO2 (nM L(^{-1}))</th>
<th>O2/HCO3</th>
<th>No. of series</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. commune</td>
<td>106–310</td>
<td>10.68–11.11</td>
<td>12–90</td>
<td>6–34</td>
<td>1–7</td>
<td>9–60</td>
<td>5</td>
</tr>
<tr>
<td>N. pruniforme*</td>
<td>570</td>
<td>10.6</td>
<td>~260</td>
<td>~110</td>
<td>~30</td>
<td>~5</td>
<td>1</td>
</tr>
<tr>
<td>N. zetterstedtii</td>
<td>149–162</td>
<td>10.94–11.06</td>
<td>77–174</td>
<td>13–35</td>
<td>3–10</td>
<td>18–29</td>
<td>4</td>
</tr>
<tr>
<td>N. flagelliforme</td>
<td>3300</td>
<td>10.8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Maximum final pH and minimum final DIC, HCO3 and CO2 probably not attained.

Sources: N. commune (K. Sand-Jensen unpubl. data); N. pruniforme (Raun, 2006; Raun et al., 2009); N. zetterstedtii (Sand-Jensen et al., 2009); N. flagelliforme (Gao and Zou, 2001).
external DIC supply as photosynthetic rates at 0-1 mM DIC externally were \( \approx 20 \% \) of the maximum rate for *N. commune*, 11 \% for small, 13 \% for medium-sized and 25 \% for large colonies of *N. pruniforme* and 60 \% for *N. zetterstedtii* (Figs 4 and 5).

As the internal DIC pool in *N. zetterstedtii* colonies is gradually exhausted during 18 h of light incubation in closed bottles, realized mean rates of O2 production are 1.8-fold lower in water initially containing 0.29 mM compared with water initially containing 0.99 mM DIC (Fig. 3). In *N. pruniforme*, which has higher rates of photosynthesis and DIC requirements per surface area than *N. zetterstedtii* and apparently 2-fold lower internal DIC pools, the dependency of photosynthesis on external DIC was stronger, showing half-saturation constants of 0.78 mM for small colonies (0.1 g FM) and 1.24 mM for larger colonies (2.5 g FM) in 2-h experiments under well-stirred conditions (Fig. 4). Large colonies have higher rates of photosynthesis (164 nmol O2 cm\(^{-2}\) h\(^{-1}\)) at near-zero external DIC than small colonies (55 nmol O2 cm\(^{-2}\) h\(^{-1}\)), probably because of a larger internal DIC pool (being scaled to colony volume) relative to photosynthetic activity (being scaled to colony surface area). The more favourable SA:V ratio in small colonies causes photosynthesis to increase more steeply with increasing external DIC \((\alpha\text{-CO}_2, 319 \text{ nmol O}_2 \text{ cm}^{-2} \text{ h}^{-1} \text{ for a 1-mmol L}^{-1} \text{ increase in external DIC})\) than in large colonies \([\alpha\text{-CO}_2, 197 \text{ nmol O}_2 \text{ cm}^{-2} \text{ h}^{-1} \text{ (mmol DIC L}^{-1})^{-1}]\) (Fig. 4).

The terrestrial and semi-terrestrial *N. commune* and *N. flagelliforme* can use CO2 and HCO\(_3\)– under water and CO2 alone in air. Under water, *N. commune* experiences limitation of photosynthesis at low DIC as rates rise to at least 1 mM. The diffusion coefficient of CO2 in air is \( \approx 2 \times 10^4 \) times higher than that of HCO\(_3\)– in water, and for the same DBL thickness and with complete DIC depletion at the colony surface the CO2 gradient is \( \approx 30-50 \) times lower in air (external CO2 \( \approx 0.02 \text{ mM} \)) than in water containing 0.6–1.0 mM HCO\(_3\)–. Thus, the potential flux would be substantially higher (500- to 700-fold) in air than in water through a diffusive boundary layer of the same thickness overlying the colony surface, but this is followed by the same resistance through the colony to the *Nostoc* filaments in both terrestrial and aquatic habitats. At high irradiance and twice daily

---

**Table 7. DIC consumed from the colony volume of three Nostoc species during extended light periods (~20 h)**

<table>
<thead>
<tr>
<th>Species</th>
<th>DIC consumed ((\mu\text{mol C g}^{-1}) fresh weight)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. commune</em></td>
<td>Mean 95 % CL</td>
<td>Source</td>
</tr>
<tr>
<td></td>
<td>5.8 1.1</td>
<td>K. Sand-Jensen (unpubl. data)</td>
</tr>
<tr>
<td></td>
<td>6.5 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0 1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.2 2.3</td>
<td></td>
</tr>
<tr>
<td><em>N. pruniforme</em></td>
<td>8.67 1.33</td>
<td>Raun (2006), Raun et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>8.57 0.84</td>
<td></td>
</tr>
<tr>
<td><em>N. zetterstedtii</em></td>
<td>16.3 2.9</td>
<td>Sand-Jensen et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>24.5 5.6</td>
<td></td>
</tr>
</tbody>
</table>

---

**Fig. 4.** Mean net photosynthesis (± s.d. of four replicates) of small (0.18 g FM), medium (0.84 g FM) and large (2.71 g FM) spherical colonies of *N. pruniforme* as a function of external DIC concentration (\(\geq 95 \%\) HCO\(_3\)–). The apparent half-saturation constant for small, medium and large colonies was 0.78, 0.78 and 1.24 mM HCO\(_3\)–, the initial linear slope of net photosynthesis at low limiting DIC concentration was 319, 305 and 197 nmol O2 cm\(^{-2}\) h\(^{-1}\) (1 mmol DIC L\(^{-1}\) h\(^{-1}\)) and photosynthesis extrapolated to zero DIC was 55, 72 and 164 nmol O2 cm\(^{-2}\) h\(^{-1}\). From Raun (2006).

**Fig. 5.** Mean DIC uptake during photosynthesis in high light as a function of mean DIC concentration in the water for *N. commune*, *N. pruniforme* and *N. zetterstedtii*. Sources: *N. commune* (K. Sand-Jensen, unpubl. data); *N. pruniforme* (Raun et al., 2009); *N. zetterstedtii* (Sand-Jensen et al., 2009b).
watering, growth of *N. flagelliforme* was doubled in CO₂-enriched air (1500 ppm) compared with atmospheric air (350 ppm at the time; Gao and Yu, 2000). The realized photosynthetic rate of *N. commune* was the same in stirred air-saturated water of 1 mM DIC (0.98 mM HCO₃⁻ + 0.02 mM CO₂) as in atmospheric air with 0.02 mM CO₂ (Sand-Jensen and Jespersen, 2012). Thus, the optimal habitat of *N. commune* could be wet soils where water and inorganic carbon and nutrients are supplied to the lower colony surface, while CO₂ and light are supplied to the upper surface. This is the habitat in 1-cm-deep water-filled depressions in the limestone pavements where we have observed massive growth of *N. commune* (Sand-Jensen et al., 2010).

**TOLERANCE OF ENVIRONMENTAL EXTREMES**

*Freshwater* Nostoc species do not experience extreme temperatures like those experienced by terrestrial species. Active growth of *N. commune*, *N. pruniforme* and *N. zetterstedtii* is, nonetheless, confined to the same temperature range (0–35 °C).

**Temperature and desiccation tolerance**

The terrestrial and semiterrestrial species *N. commune* and *N. flagelliforme* are exceptional in their tolerance of alternating freezing and thawing and of desiccation and rehydration at varying temperatures in their open habitats, located from the Arctic to tropical regions (Li, 1991; Sand-Jensen and Jespersen, 2012). In contrast, the freshwater species *N. pruniforme* and *N. zetterstedtii* are not exposed to freezing and drying or to extreme temperatures in their temperate and sub-Arctic environments. Nonetheless, it appears that active growth of all four species is confined to the same temperature interval between 0 and 35 °C and that the fastest growth takes place around 25 °C (Møller et al., 2014). Both *N. commune* and *N. flagelliforme* are widely distributed species in arid and semiarid bare land throughout the world (Li, 1991; Dodds et al., 1995). *Nostoc commune* survives annual temperature ranges of ~80–60 to 25 °C under Arctic conditions and ~30 to 50 °C under temperate conditions (Davey, 1989; Sand-Jensen and Jespersen, 2012). Surface temperatures in the open habitats of *N. flagelliforme* can reach 78 °C in summer and ~40 °C in winter (Li, 1991). Both species can survive months or years of frost and drought as inactive desiccated crusts, and within minutes to hours can reactivate ion exchange, respiration, photosynthesis and N fixation when water and suitable temperatures again become available (Scherer et al., 1984; Satoh et al., 2002).

Experiments with the temperate species *N. commune* confirmed that photosynthesis and respiration were maintained after 36 h of desiccation of wet or already dry specimens at temperatures from −269 to 70 °C, while temperatures above 70 °C led to death (Sand-Jensen and Jespersen, 2012). During repeated daily cycles of drying for 18 h at −18, 20 and 40 °C and rewetting for 6 h at 20 °C, *N. commune* retained its photosynthetic capacity at −18 and 20 °C but died at 40 °C, presumably because of the greater costs of repair of macromolecules at this higher temperature, which would generate stronger thermal damage than lower temperatures (Sand-Jensen and Jespersen, 2012). In 24-day-long experiments under permanently submerged conditions, *N. commune* also died at 45 °C but survived at 35 °C; growth peaked at 25 °C and declined at the lower temperatures of 15 and 5 °C (Møller et al., 2014). Annual carbon fixation of rehydrated populations of *N. commune* from an Antarctic dry valley was also a strong positive function of increasing temperatures above zero and up to 20 °C, the highest temperature tested (Novis et al., 2007). Thus, there was no obvious sign of temperature adaptation or acclimation across geographical ranges.

*Nostoc flagelliforme*, like other terrestrial *Nostoc* species (Davey, 1989; Dodds et al., 1995; Sand-Jensen and Jespersen, 2012), showed great heat resistance when dry, while it was more susceptible when wet or immersed (Mei and Cheng, 1990). The regular protein pigment structure of photosystem I complexes was destroyed at 70 and 80 °C (Hu et al., 2005). Pretreatment of wet *N. flagelliforme* at 65 °C led to death and temperatures above 45 °C stopped photosynthesis (Mei and Cheng, 1990). The exact temperature effect on survival of *N. commune* and *N. flagelliforme* apparently depends on the duration and frequency of heat exposure and on the length of time available for repair of cellular damage between heat exposures (Sand-Jensen and Jespersen, 2012). Likewise, a longer time was required for maximal photosynthesis and respiration to recover after rehydration of specimens subjected to extended periods of desiccation (Scherer et al., 1984; Potts, 1994; 2000; Qiu and Gao, 1999). The recovery of rehydrated *N. flagelliforme* was light-dependent (Gao et al., 1998) and required potassium (Qiu and Gao, 1999), stressing that a suite of energy-demanding metabolic processes involving expensive protein and lipid synthesis and various cellular repair systems restores cellular structure and catalytic capacity (Angeloni and Potts, 1986; Taranto et al., 1993).

Desiccation is not an issue for freshwater species of *Nostoc* (except when they are washed ashore) and temperature variability is also much lower in freshwater than in terrestrial habitats. In Danish lakes, for example, the annual temperature range is typically 0–25 °C in shallow water and 2–15 °C in deep water (Møller et al., 2014). Nonetheless, the temperature dependence of long-term growth of *N. pruniforme* and *N. zetterstedtii* resembled that of hydrated *N. commune* in that all three species grew fastest at 25 °C and many times slower at 5 °C, while 45 °C led to die-off (Fig. 6 in Møller et al., 2014). So while terrestrial *Nostoc* species possess extraordinary desiccation tolerance there is no major difference in the relationship of active growth with temperature in terrestrial and aquatic specimens from northtemperate localities, though we cannot rule out that different relationships of growth with temperature have evolved in specimens living under Arctic or tropical climates. Because, under temperate conditions, temperature ranges between winter and summer are also profound it would not be a great surprise, however, if these relationships remained constant across geographical ranges. High temperature requirements (≥20 °C) of maximum photosynthesis of *N. commune* from an Antarctic valley with very short frost-free periods support the latter suggestion.

**pH and salt tolerance**

Being photosynthetic organisms capable of using both free CO₂ and HCO₃⁻ at high efficiency, all four *Nostoc* species can push pH in the external water above 10.5 and in some cases even above 11 (Table 6). Exposure of rehydrated *N. commune* to a pH gradient for 36 h also showed that it tolerated pH ranging from 3 to 10, while it died at pH 2 (Sand-Jensen and
Jespersen, 2012). Terrestrial Nostoc species should be able to survive exposure to rainwater of low pH (typically 3.8–4.8) and only very acidic habitats receiving sulphuric acid from the oxidation of metal sulphides are likely to be below pH 3. Therefore, when it comes to direct pH tolerance most terrestrial habitats should be open to colonization by Nostoc. Although N. commune can grow between garden tiles and on open sand soils fed by rainwater, it appears to prefer clay soils and calcareous rocks of high pH, perhaps because available HCO$_3^-$ supports photosynthesis during prolonged illumination. The requirement of HCO$_3^-$ for photosynthesis of aquatic Nostoc is accompanied by a preference for a pH above 7 in the water because of the buffering and alkalinization effect of HCO$_3^-$, thus, at air saturation, freshwaters with pHs of 6.5, 7.5 and 8.5 have HCO$_3^-$ concentrations of 0.05, 0.5 and 5 mM, respectively (Stumm and Morgan, 1981).

Because N. commune and N. flagelliforme often grow in arid habitats with salt accumulation they must be tolerant. Indeed, N. commune retained its photosynthetic capacity upon exposure to alkaline freshwater enriched with NaCl to 20 g kg$^{-1}$, but not 30 g kg$^{-1}$ as in oceanic water (Sand-Jensen and Jespersen, 2012). The closely related N. flagelliforme was also salt-resistant, but photosynthesis, respiration and photosystem II activity declined upon exposure to $>12$ g NaCl kg$^{-1}$ (Ye and Gao, 2004; Yong-Hong et al., 2005). Salt resistance is probably based on the formation of sucrose and trehalose, both of which accumulate under desiccation and exposure to low salt concentrations (Sakamoto et al., 2009) and therefore play a dual role. Tolerance of freezing and desiccation is extraordinary, while salt tolerance is modest. Other cyanobacteria are more salt-tolerant, because of the synthesis of glucosylglycerol in species of moderate tolerance and glycerine betaine and glutamate betaine in species showing high tolerance (Mackay et al., 1984).

ECOLOGICAL ADAPTATIONS AND STRATEGIES

Colonial Nostoc species live in resource-poor environments and succumb in competition with tall macroalgae and plants under richer conditions.

All four colonial Nostoc species discussed here live in resource-poor environments with a limited supply of water and inorganic nutrients on land and a limited supply of nutrients and DIC under water. Phosphorus could be a limiting nutrient for growth but experimental tests are lacking. All species can fix elemental N$_2$, but this is a costly process that requires a rare co-factor (molybdenum). In suspension culture with N. flagelliforme, the variable availability of nitrogen and phosphorus influences the production of new cells and extracellular polysaccharides (Fei et al., 2012).

The terrestrial N. commune and N. flagelliforme grow on nutrient-poor, sparsely vegetated or bare soils and rock surfaces that alternate between being wet and dry. From April to September on the open limestone alvar on Öland, Sweden, measurements of humidity and temperature suggested that populations of N. commune were rehydrated and active at least 26% of the time (Sand-Jensen and Jespersen, 2012). From October to March, populations were probably active during larger parts of the frost-free time.

Maximum recorded growth rates of N. commune floating on water and exposed to atmospheric air under laboratory conditions were 0.050–0.056 d$^{-1}$ at 15–25°C and markedly lower, at 0.020–0.025 d$^{-1}$, at 5 and 35°C (Møller et al., 2014). These growth rates correspond to doubling times of dry mass between 12 and 35 d. Growth rates are intermediate when compared with the extremely low rates (0.0008 d$^{-1}$) of oligotrophic N. zetterstedtii and high rates (0.2–0.4 d$^{-1}$) of nutrient-demanding thin macroalgae (e.g. Cladophora spp. and Enteromorpha spp.; Sand-Jensen and Borum, 1991; Nielsen and Sand-Jensen, 1991). According to Grime’s (1979) classification of plant growth strategies in relation to resource richness and disturbance, the stress strategy (S) of N. zetterstedtii is linked to resource-poor, stable habitats, the ruderal strategy (R) of thin green macroalgae is linked to resource richness and high disturbance, and the competitive strategy (C) of thick macroalgae is linked to resource richness and low disturbance. Resource-poor, highly disturbed habitats do not, in Grime’s concept, support a viable strategy because the growth rate is very low when resources are limiting and disturbance leads to frequent loss of biomass, thus preventing long-term survival. However, N. commune (and N. flagelliforme) can survive disturbance by desiccation, freezing and extreme temperatures with restricted biomass loss in a quiescent stage and possesses a viable strategy in the resource-poor, disturbed terrestrial habitats that Grime considered unoccupied, at least by higher plants. A suite of drought-resistant lichens, mosses and a few flowering plants are capable of surviving in the same resource-poor, disturbed habitats as N. commune and N. flagelliforme (Sand-Jensen et al., 2010) and could be classified as belonging to a mixed S–D strategy, where S represents tolerance of limiting resources and D represents resistance to disturbance. Their biomass survives harsh physical conditions that are detrimental to most other phototrophs. Likewise, in the marine environment, crust-forming coralline red algae are adapted to an extremely low supply of light or nutrients, tolerate high disturbance by wave action and avoid intensive grazing, and thus have a viable S–D strategy with extremely slow growth and biomass turnover (Littler and Littler, 1980; Littler et al., 1986).

The freshwater N. zetterstedtii grows in soft-water, oligotrophic lakes and faces the strongest constraints on nutrients and DIC supply. Nostoc pruniforme grows in mesotrophic, alkaline lakes of higher DIC concentrations, where nutrients remain low from late spring to early autumn due to nutrient uptake by phytoplankton and other benthic phototrophs (Sand-Jensen et al., 2009b). Because both species are perennial, we cannot dismiss the untested possibility that they can take up nutrients in excess during the richer winter season for later use during summer when organic production by photosynthesis is high. This mechanism has been shown for perennial marine macroalgae (Chapman and Cragie, 1977; Lundberg et al., 1989; Lobban and Harrison, 1997). The persistence of Nostoc colonies may therefore offer an opportunity to withdraw external nutrients and produce photosynthates when conditions are favourable and store them within the colony until they are needed for growth and survival. The large size and low SA:V of the colonies can promote internal circulation of valuable substances and reduce their diffuse loss to the environment, as previously discussed (see section ‘Functional consequences of the use of light and inorganic carbon’).

Nostoc zetterstedtii is exceptional among aquatic phototrophic organisms due to its exceedingly low rates of growth, respiration
and mortality as a consequence of the large spherical colony size and high CMA (Sand-Jensen and Møller, 2011). Because direct selection for low growth rate is not a viable evolutionary strategy (Lambers et al., 2008), low growth rate should, as emphasized, be regarded as an indirect consequence of high CMA and investment in a large arsenal of organic compounds as protection against environmental hazards, grazers and pathogens. The maximum growth rate of *N. zetterstedtii* in laboratory experiments was only 0.78 \( \times 10^{-3} \text{ d}^{-1} \), corresponding to a mean doubling time of colony biomass of 2-4 years (Møller et al., 2014). This value resembled the annual mean doubling time of 2.6-3.3 years in field experiments (Sand-Jensen and Møller, 2011).

With such low growth rates, the largest colonies, measuring 7 cm in diameter, are 24-31 years old. We found no senescence and mortality in the examined population of colonies in the field during 13 608 incubation days. This finding implies that the mortality rate was \(<7.3 \times 10^{-5} \text{ d}^{-1} \) and longevity >24 years, in accordance with the high age of the largest colonies. Macerated *N. zetterstedtii* colonies diluted 10-fold in water and added to a sample of lake bacteria immediately stopped all cell division (Sand-Jensen et al., 2009a). Likewise, no grazing by small macroinvertebrates has been observed, and large potential grazers, such as crayfish, fish and swans, are probably unable to consume the large, firm colonies. Recent experiments showed that colonies survived for 14 months in complete darkness at 5 °C with integrity of photosynthesis and fresh mass of the colonies and extremely low daily respiration rates of 0.21 mmol C (mol organic carbon) \(^{-1} \) corresponding to a half-time of the colony carbon biomass of 9 years (K. Sand-Jensen, unpubl. data). I anticipate that *Nostoc* cells can utilize organic compounds from the colony matrix for basal respiration and long-term survival.

The ecological strategy of *N. zetterstedtii* is therefore an extreme example of a stress-selected species living in a very resource-poor environment, growing extremely slowly, being highly persistent thanks to efficient physical and chemical protection against pathogens and grazers, and having efficient recirculation of inorganic carbon (Fig. 3) (Sand-Jensen et al., 2009b). Measured growth rate in dry mass of *N. zetterstedtii* was \(~10\) fold lower than rates predicted from general allometric relationships of growth rate with CMA of aquatic macroalgae (Markager and Sand-Jensen, 1996) and, likewise, of predicted growth rate with the thickness of photosynthetic tissue for a broad range of terrestrial and aquatic plants (Nielsen et al., 1996). This finding suggests that there are extra constraints on the growth of *N. zetterstedtii* linked to limited DIC supply and/ or production of the colony matrix and the protective compounds. The proposed reduced loss of organic solutes from large colonies to the surrounding water has not been tested as yet, though it is plausible given the ability of *N. zetterstedtii* to maintain colony integrity and fresh mass during 14 months in darkness (K. Sand-Jensen, unpubl. data).

*Nostoc pruniforme* grows in oligo-mesotrophic lakes richer in DIC than the main habitats of *N. zetterstedtii*, and its growth, mortality and ecological strategy are also markedly different. Maximum growth rate of *N. pruniforme* in laboratory experiments was 75-fold higher than that of *N. zetterstedtii*, corresponding to a biomass doubling time of 12 d (Møller et al., 2014). Growth rates in a clear-water, DIC-rich lake during early summer resembled these laboratory rates (Møller and Sand-Jensen, unpubl. data 2013). A high DIC (2.0 mM) in the lake water was required to sustain a biomass turnover of 12 d, while incubation in low-DIC water (0.2 mM) reduced net photosynthesis 4-fold and prolonged biomass turnover (Møller et al., 2014). Field studies confirmed that *N. pruniforme* periodically undergoes mass mortality, presumably by attack of microbial pathogens. In terms of the ecological strategy, *N. pruniforme* has species traits belonging to a mixture of ruderal-, stress- and stress-disturbance-selected strategies. The expression of these traits is plastic depending on whether the species lives in cold, oligotrophic freshwaters and forms very large and old colonies or colonies that live for shorter periods in warmer, mesotrophic lakes and reach smaller sizes.

All four *Nostoc* species have one important trait in common. They live directly on the ground surface and are unable to survive in competition with tall, dense terrestrial or aquatic vegetation. This is a main reason why they are primarily confined to resource-poor habitats where tall, dense vegetation cannot develop. The *Nostoc* species therefore face the risk of disappearing when their habitats are subjected to nutrient enrichment. The freshwater *Nostoc* organisms in Scandinavian lakes have been further threatened by large-scale leaching of dissolved humic substances from low-buffered, acidic soils.

### OUTLOOK

Most studies on the ecophysiology of phototrophs have focused on relatively fast-growing species from resource-rich habitats (Larcher, 2003; Lambers et al. 2008). With this review on large gelatinous *Nostoc* colonies I call attention to these competitively inferior species from nutrient-poor habitats. I introduce terrestrial and semiterrestrial *Nostoc* species possessing an unprecedented ability to enter quiescent, desiccated or frozen stages and rapidly resume metabolism when rehydrated. The molecular, biochemical and physiological sequences leading to quiescence and reactivation need to be fully explored and the metabolic costs of frequent desiccation, freezing and rehydration and substantial production of extracellular polysaccharides need quantification in the future. There is strong molecular, biomedical and ecological interest in these processes.

The rare freshwater species *N. zetterstedtii* has unprecedented low rates of metabolism, growth and mortality in its DIC- and nutrient-poor lake habitats. Its ability to take up external DIC and recirculate DIC within the colony is profound. It is challenging to determine whether the large colony size also creates advantages for effective circulation of nutrients within the colony and strong antibiotic effects for relatively low production costs. Future studies should also unravel fluid dynamics and nutrient and DIC conditions in the neglected near-bed habitats of *N. pruniforme* and *N. zetterstedtii* on lake sediments. This effort is needed in order to put the presented diffusion models for uptake and loss of solutes from spherical colonies of different size into proper physical and chemical perspectives in nature.

As free-living organisms and as symbionts in lichens, *Nostoc* species are both pioneers and permanent members of the vegetation of deserts, semideserts, dry grasslands and rock surfaces ranging in geographical distribution from polar to tropical regions. Their N input to these biomes can be of utmost importance (Dodds et al., 1995; Holst et al., 2009; Caputa et al., 2013). In a carefully mapped Low-Arctic tundra landscape, N\(_2\) fixation

---

**OUTLOOK**

Most studies on the ecophysiology of phototrophs have focused on relatively fast-growing species from resource-rich habitats (Larcher, 2003; Lambers et al., 2008). With this review on large gelatinous *Nostoc* colonies I call attention to these competitively inferior species from nutrient-poor habitats. I introduce terrestrial and semiterrestrial *Nostoc* species possessing an unprecedented ability to enter quiescent, desiccated or frozen stages and rapidly resume metabolism when rehydrated. The molecular, biochemical and physiological sequences leading to quiescence and reactivation need to be fully explored and the metabolic costs of frequent desiccation, freezing and rehydration and substantial production of extracellular polysaccharides need quantification in the future. There is strong molecular, biomedical and ecological interest in these processes.

The rare freshwater species *N. zetterstedtii* has unprecedented low rates of metabolism, growth and mortality in its DIC- and nutrient-poor lake habitats. Its ability to take up external DIC and recirculate DIC within the colony is profound. It is challenging to determine whether the large colony size also creates advantages for effective circulation of nutrients within the colony and strong antibiotic effects for relatively low production costs. Future studies should also unravel fluid dynamics and nutrient and DIC conditions in the neglected near-bed habitats of *N. pruniforme* and *N. zetterstedtii* on lake sediments. This effort is needed in order to put the presented diffusion models for uptake and loss of solutes from spherical colonies of different size into proper physical and chemical perspectives in nature.

As free-living organisms and as symbionts in lichens, *Nostoc* species are both pioneers and permanent members of the vegetation of deserts, semideserts, dry grasslands and rock surfaces ranging in geographical distribution from polar to tropical regions. Their N input to these biomes can be of utmost importance (Dodds et al., 1995; Holst et al., 2009; Caputa et al., 2013). In a carefully mapped Low-Arctic tundra landscape, N\(_2\) fixation...
by cyanobacteria (0.68 kg ha\(^{-1}\)) was twice the annual wet deposition of nitrogen (Stewart et al., 2011). It remains unexplored how the high water-absorbing capacity and N\(_2\) fixation of Nostoc can facilitate the colonization of bare or newly exposed mineral surfaces by mosses and higher plants, thereby forming more stable vegetation and more organic soils. With the exposure of new mineral surfaces behind retreating glaciers on a warming Earth, this ecosystem service of Nostoc deserves future attention.

**ACKNOWLEDGEMENTS**

This work was supported by grants from the Willum Kann Rasmussen Foundation to the Centre for Excellence for Lake Restoration Research (CLEAR) and from the Carlsberg Foundation. I thank Mikkel René Andersen, Michael Kühl and Claus Lindskov Møller for help and the referees for constructive suggestions.

**LITERATURE CITED**


Hameed MSA, Hassan SH, Mohammed R, Gamal R. 2013. Isolation and characterization of antimicrobial active compounds from the cyanobacterium


