Cycling of dissolved oxygen concentration within colonies of *Nostoc pruniforme* measured with a fluorescence quenching microprobe

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Summary

1. Colonies of *Nostoc pruniforme* collected from a benthic lake population had dissolved oxygen (O_2) concentration changes from anoxia in the dark to more than 10^3 µmol L^{-1} in the light, four times saturation level at 20 C. Regardless of colony size (≤ 4 cm) similar patterns of increased or decreased O_2 concentration occurred during repeated light-dark cycles.

2. Most metabolic activity occurred within two hours of incubation during either light or dark periods. For the remainder of 12 to 48 hours in constant conditions, rate of change in O_2 was minimal. Apparently internal supplies of nutrients and photosynthates were quickly exhausted, and slow rates of diffusion across the colony border limited external supplies, and thus both photosynthesis and respiration.

Introduction

Nostoc is a globally distributed genus of a freshwater and terrestrial cyanobacterium, most commonly recognized in colonial form (Dodds, Gudder and Mollenhauer, 1995; Scherer and Zhong, 1991). In the present study we selected *N. pruniforme* as the best species description although Geitler (1932) had misgivings of the 'outmoded classification system' that persists with several similar or identical forms of the genus. *N. sphaeroides* may be synonymous although Geitler describes its habitat as terrestrial on wet rocks or damp soil. The genus *Nostoc* is polyphyletic (Beltrami, 2008) thus an updated phylogeny awaits a more systematic assessment of species descriptions.

Colonies of the filamentous and diazotrophic cyanobacterium *Nostoc pruniforme* form from individual trichomes. Cell division forms aseriate masses that develop into spheres, as illustrated for *N. punctiforme var. populorum* (Geitler 1932). Subsequent cell division increases colony diameter. The larges colony found, 22 cm, may be the upper size limit likely due to colony death and decomposition (Dodds and Castenholz, 1988). Within each spherical colony a collection of

filaments is embedded in a gelatinous glycan (polysaccharide) matrix, varying in color from blue-green, bright green, yellow, brown, black or bright green. The glycan has a density slightly less than water, ~0.968 (Garcia-Pichel, Wade and Farmer, 2002) thus the colonies are easily moved by gentle water currents, and occasionally float. Gas vesicles are not formed in *N. pruniforme* and are only formed in *N. caeruleum* (Mollenhauer et al., 1999). Buoyancy can be adjusted with changes in the volume of the glycan matrix secreted by denser cells, as well as by gas bubbles we observed trapped within the colonies.

Vegetative cells are 3.5 – 4.5 μm in diameter**,** subspherical to spherical, and can differentiate into terminal or intercalary heterocysts, as reviewed in detail (Adams and Duggan, 1999). Likely vegetative cells can also form akinetes (spores) under stress such as nutrient limitation, as observed in *N. punctiforme* (Meeks et al., 2002), capable of viability after centuries of desiccation (Livingstone and Jaworski, 1980).

The slow growth rate of *N. pruniforme* can be limited by several factors, recently reviewed by Sand-Jensen (2014). The benthic habitat can have low ambient light intensity, especially at high latitudes with short ice-free periods. The presence of phycobilins, especially phycoerythrin staining the glycan matrix, further absorbs light within the colonies. Much photosynthate is consumed in the production of the glycan matrix. Slow diffusion occurs across the static boundary layer on the outside of the colonies, as well as through the interior of the matrix (Sand-Jensen, 2014). Wide fluctuations in both pH and O_2 concentration within the colony must severely limit rates of photosynthesis. Cells within colonies, especially those furthest from the outer boundary, are expected to be severely growth limited by their position as a result of slow diffusion of solutes from the surrounding water.

In an effort to help explain slow growth rates of *N. pruniforme*, we examined several colonies from a natural population with regard to cell distribution within the colonies, and metabolic activity on the basis of O_2 microgradients and changes through time. We were also simply curious about patterns of activity within the colonies.

Methods

Several lakes in New Hampshire and Maine, USA, including remote lakes in Acadia National Park were surveyed by kayak and wading during May – September 2014. In October a large benthic population was reported in Goose Pond, Shapleigh, ME (N 43°33.5' W 70°51.8') where colonies were easily visible. The colonies were resting on and within loose organic sediment at a depth of 1 – 2 m, and were collected with a long-handled net. Colony size varied from 0.5 - 5.0 cm and were identified as *N. pruniforme* based on colony morphology, and dimensions of vegetative cells and heterocysts. Additional colonies were collected within the vicinity of various aquatic plants.

Collected colonies were initially maintained in two 10-gallon tanks near a shaded lab window (18-24 C) with original pond water, sediments (~1 cm thick) and plants found with the colonies (mainly *Myriophyllum tenellum* and *Isoetes* spp.) An original dose of ~10% ASM medium was added. Deionized water was added periodically to offset evaporation.

Experimental setup

Dissolved oxygen (O_2) concentration within colonies was measured initially with a cathodic microprobe (50 μm diameter, Unisense, Aarhus, Denmark) that was replaced with a fiber-optic fluorescence quenching microprobe (Presens, Regensburg, Germany). The latter had the advantage of immunity to electrical fields, non-consumption of oxygen, faster response time, and superior signal/noise ratio. In addition it was much more robust, sheathed in a steel tube (outside diameter 140 μm). The microprobe was mounted in a motorized micromanipulator with controlling software (Unisense) to adjust depth within the colony for experiments at a fixed depth, and to automate micro-profiling with steps of ~100 μm for transect studies. The initial position of the microprobe tip was adjusted manually to be on the surface of the colony.

For each experiment, a single *N. pruniforme* colony was immersed in well water free of organisms and placed on a pad of agar at the base of a plastic centrifuge tube. The tube was immersed in a temperature-controlled water bath from 4 - 33 C.

Light intensity from a white LED lamp varied from 50 – 350 μmol photons m^{-2} s^{-1} and light-dark periodicity was controlled with a programmable timing switch. Light-dark cycles varied in length from 12-12 to 24-24 hours. The entire apparatus was wrapped in two layers of opaque material (a shroud) to eliminate external ambient light. Incident light intensity at the upper surface of colonies was estimated with a quantum sensor (Li-Cor, Lincoln NE, USA).

Temperature and O_2 were sampled at five minute intervals and recorded with a Microx 4 datalogger (Presens). The absolute changes in O_2 were derived from the difference in O_2 between 5-minute intervals.

Results

Physical description

Colony colors ranged from translucent light green to dark olive green to black, regardless of identical collection location and time. At collection, microhabitats varied from the surface of organic sediment to several centimeters within it (Figure 1A, B), or resting within benthic foliage of *Myriophyllum tenellum* and other plants. Colonies were only slightly denser than the water medium at 20 C as they were easily suspended when sediments were agitated by small water currents. Occasionally colonies floated. Unlike terrestrial *Nostoc commune* that is desiccation tolerant because of extracellular polysaccharides (Tamaru et al. 2015), *N. pruniforme* colonies rehydrate poorly and partially, apparently due to different polysaccharides (Fig. 1C).

The colony cover or 'pellicle' has a skin-like texture that can be peeled off in strips. Some colonies included clusters of trichomes growing directly on the outside of the pellicle, liberated from the same or another colony. Algal epiphytes including pennate diatoms were present. Snails and gastrotrichs on the pellicle appear to be grazing on the epiphytic algae. Most of the trichomes in the colony are concentrated in a 1 mm thick green 'shell' at the colony periphery (Figure 1D), while the watery gel-filled interior contains a web of intersecting gel threads containing sparse trichomes (Figure 1E). Also present in the colony interior are starch-like inclusions (~10 μm) and an abundance of rod-shaped, apparently heterotrophic bacteria (Figure

1F), in contrast to hollow colonies of *N. pruniforme* that distinguish it from *N. sphaeroides* (Deng et al., 2008; Whitton, 2011).

Light absorbance within colonies mirrors the filament concentration, occurring mostly within the outer green shell (Figure 1I). As light passes through the colony the green shell absorbs ~92 - 94% of incoming light while the interior gel absorbs < 4% in colonies with diameters of 1.5 to 2.2 mm. A small fraction (6 – 8%) is transmitted out of the colony.

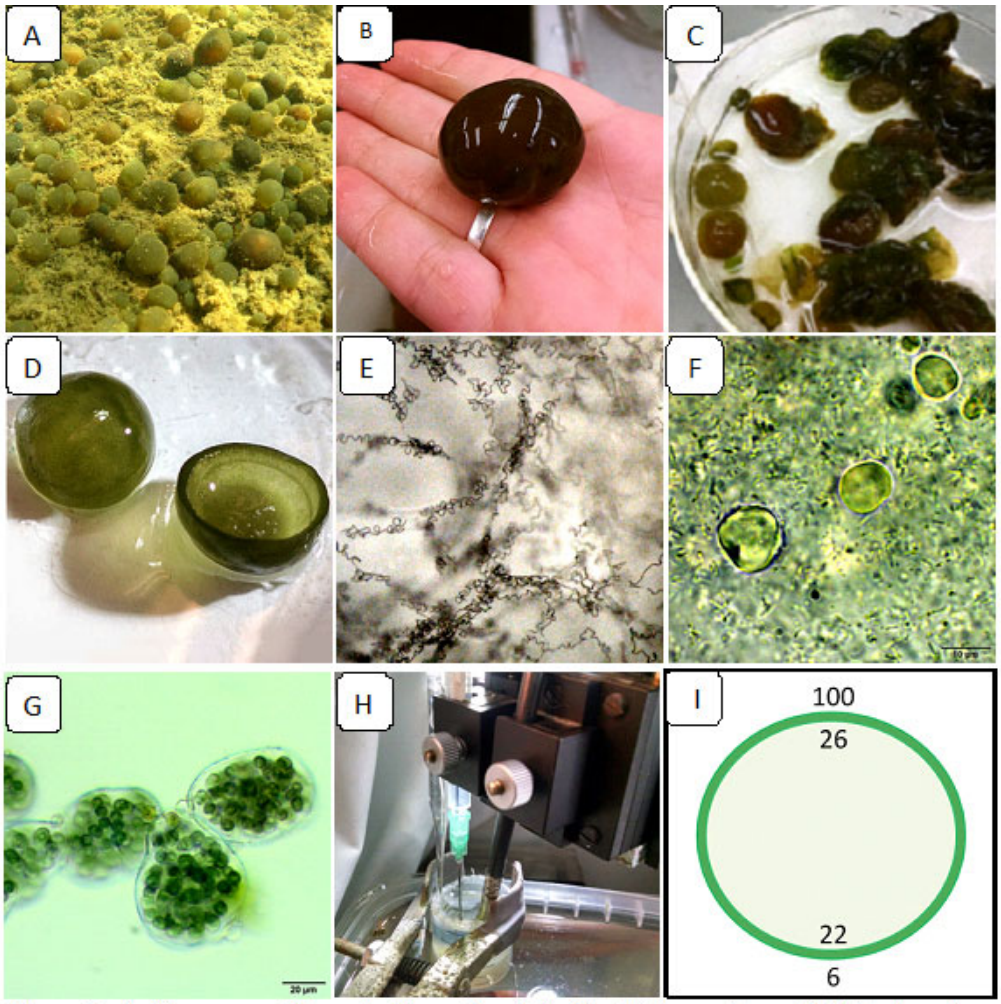

Figure 1. A. *Nostoc* pruniforme in Goose Pond; B. Single 3 cm colony; C. Rehydrated colonies after two weeks of dehydration; D. Dissected colony with internal gel threads removed; E. Gel threads with sparse trichomes; F. Starch-like inclusions and abundant rod-shaped heterotrophic bacteria; G. Aseriate stage of colony formation connected by heterocysts; H. Experimental apparatus including fiber-optic O_2 microprobe, temperature probe, micromanipulator holding probes; vessel and clamp holding colony; and water bath. I. Map of relative light intensity above, within and beneath colony (2 cm diameter).

Changes in [O_2]at discrete depths within Nostoc colonies

Incubation in light, regardless of intensity or depth in the colonies, was usually characterized by an immediate and rapid increase in [O_2], interpreted as net photosynthesis (P_n) typically lasting 30 - 60 minutes, then a decrease during the next hour to low or negligible rates for the remainder of the 24 hour period. A similar pattern occurred at the onset of dark periods as respiration consumed O_2 within an hour, then essentially stopped (Figure 2). Similar patterns occurred on 12-12 hour light-dark cycles with a rise and fall of activity triggered by the onset of each period (light or dark) and persisted for at least five days (Figure 3). Another colony incubated for the same time period had reduced activity each of the five days (Figure 4). A lag with low and declining rates of activity occurred in one colony during the first hour of each light and dark period, followed by a rapid rise of activity for the next hour (Figure 5).

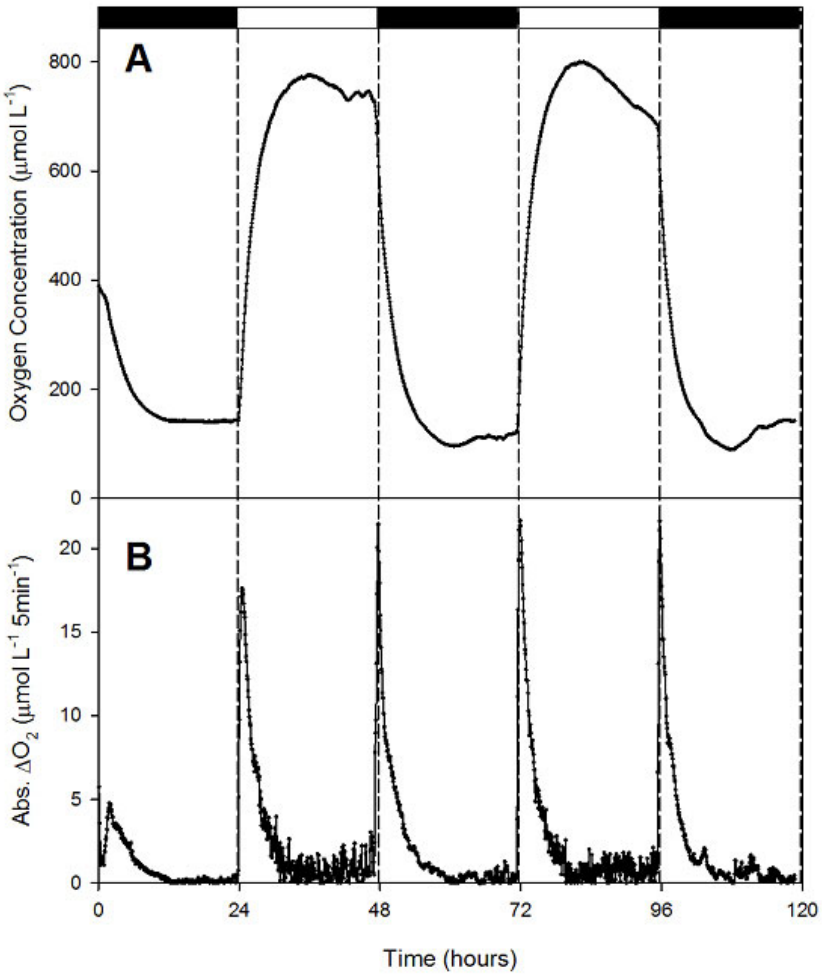

Figure 2. A. [O2] within *Nostoc pruniforme* colony (1cm diameter) measured at five minute intervals at a depth of 2 mm with a 24-24 hour light-dark cycle, 20 C, 250 µmol photons m^{-2} s^{-1}. B. Absolute value of first derivative, rate of change of [O_2].

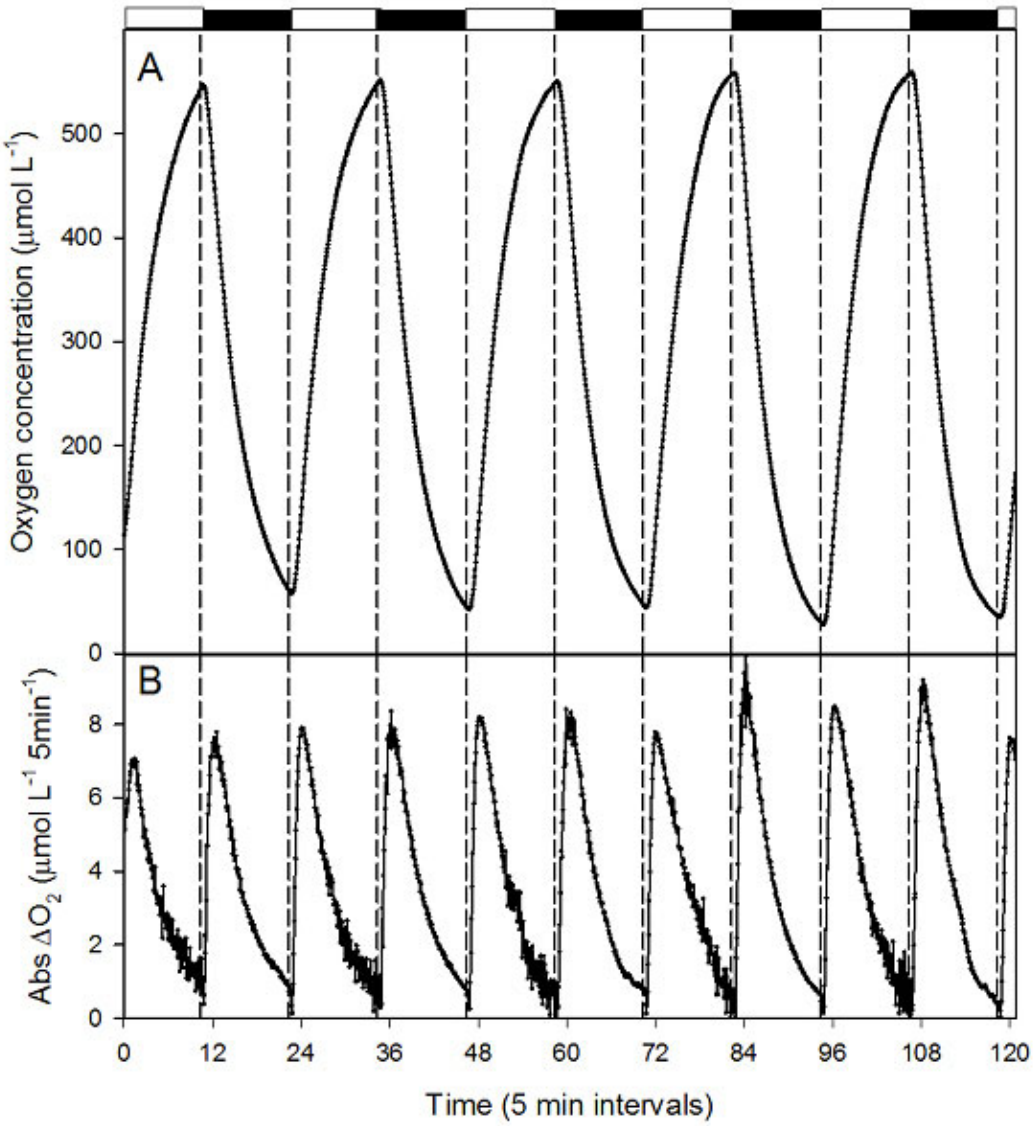

Figure 3. A. [O2] at a depth of 7 mm within *Nostoc pruniforme* colony incubated in 12-12 light-dark cycles at 95 µmol photons m^{-2} s^{-1} for five days. B. Absolute rate of change in O_2 per five minutes. A lag in oxygen concentration change is visible similar to previous experiments with this colony. Noise occurs during the latter part of the light, but not in the dark periods.

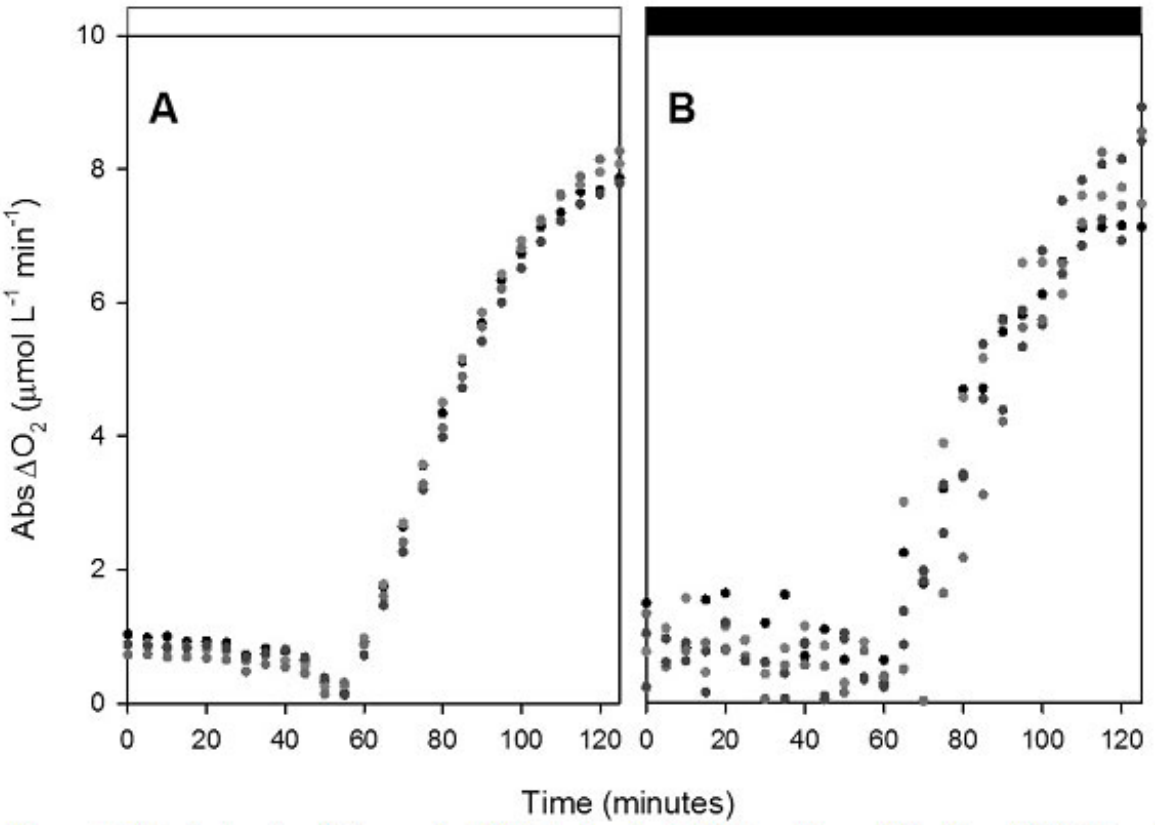

Figure 4. Absolute rate of change in [O_2] during the initial portions of the five A) light and B) dark periods (125 minutes) from Figure 3, emphasizing the declining rate or lag in activity for the first hour and a subsequent rapid increase to maximum at two hours.

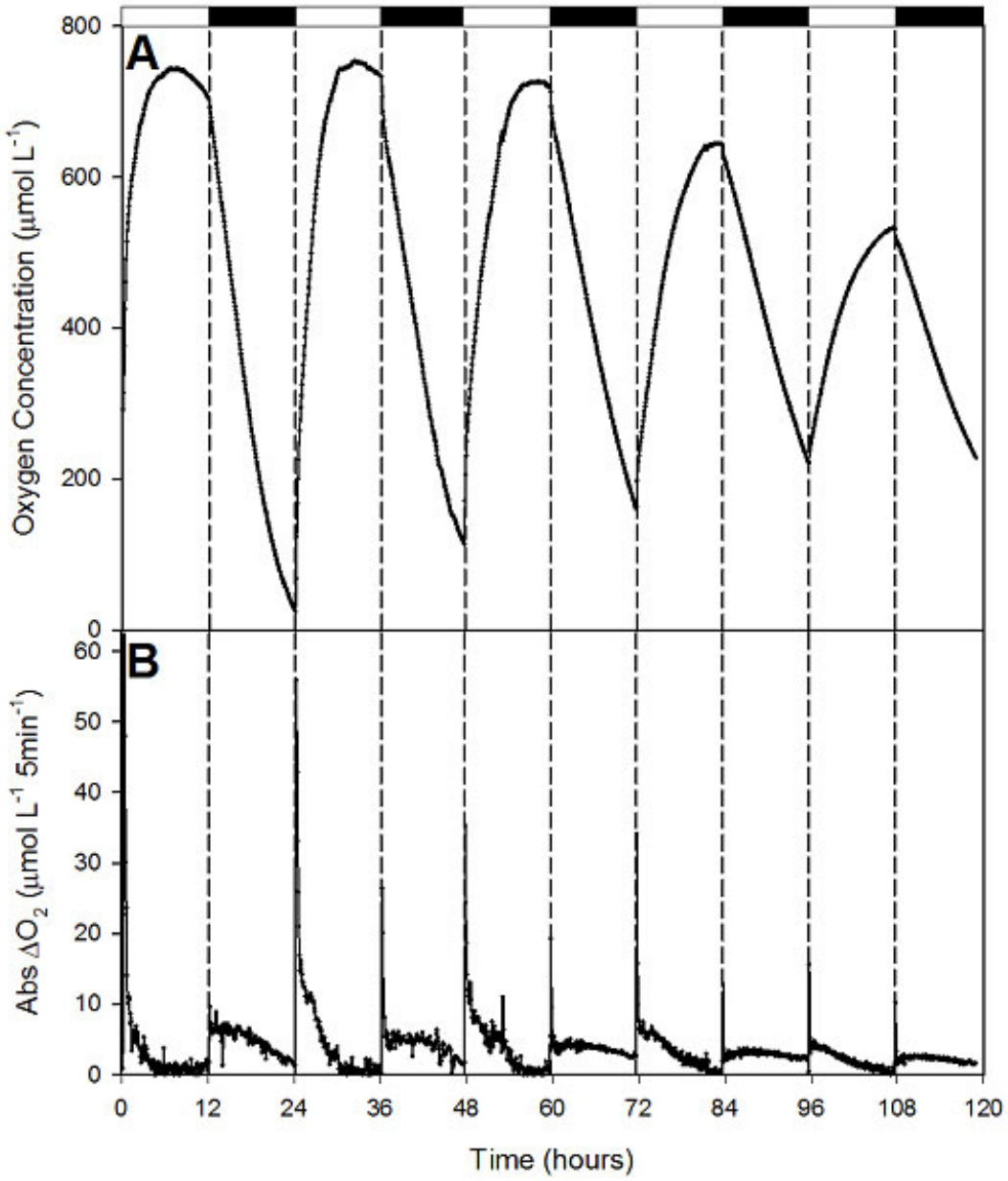

Figure 5. Declining activity during five day incubation at 20 C and light intensity 250 µmol photons m^{-2} s^{-1}.

Rates of change in DO during light-dark cycles.

Colonies under similar conditions had variable levels of activity, independent of size. Where several consecutive light-dark cycles were observed, some colonies repeatedly produced similar rates of P_n and R (Figure 3). Others increased or decreased in activity in subsequent cycles

(Figures 6, 8). An example is change in activity based on areas under the curves decreases during three cycles (Figure 6A), and R decreases more rapidly than P_n (Figures 6B, C).

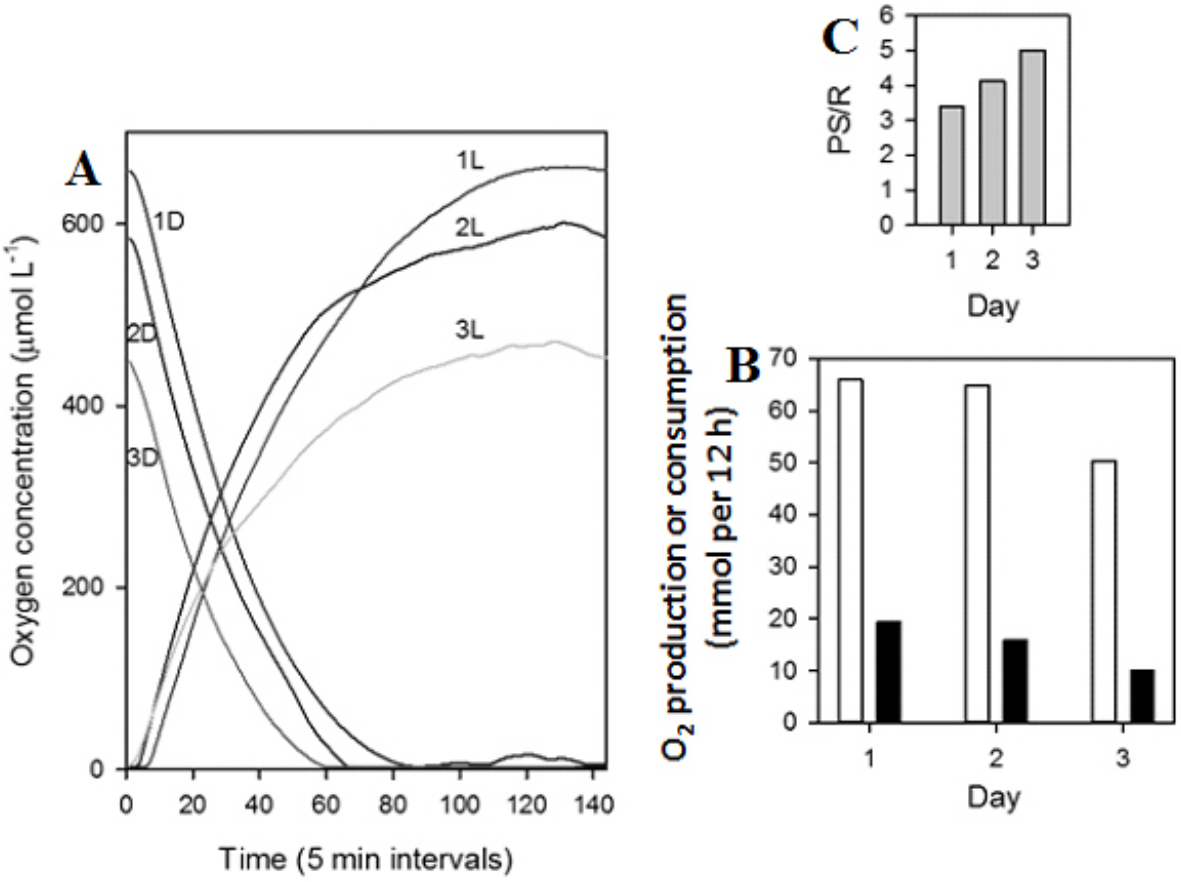

Figure 6. A. Comparison of [O_2] during three consecutive light (L) and dark (D) cycles at 7µm depth in a 2 cm diameter colony. Both P_n and R activity declined with each cycle. B. Area under the curves (white bars – light period; black bars – dark periods). C. Ratio of PS (production) to R (consumption), increasing with each cycle.

Although individual colonies varied in size (1 - 4 cm), color (Figure 1A) and their level of activity, the patterns of P_n and R were generally similar. Light intensity in the range 50 - 250 µmol photons m^{-2} s^{-1} increased P_n and R (Figure 7). O_2 was essentially uniform throughout the colony from the green peripheral shell to the center containing a low concentration of trichomes (depths 2000 - 7000 µm). In contrast the rate of P_n was greatest at a depth of 400 - 500 µm, approximately the mid-depth of the green shell; P_n maximum was > 60 µmol O_2 in a 5 minute interval at 250 µmol photos m^{-2} s^{-1} (Figure 5).

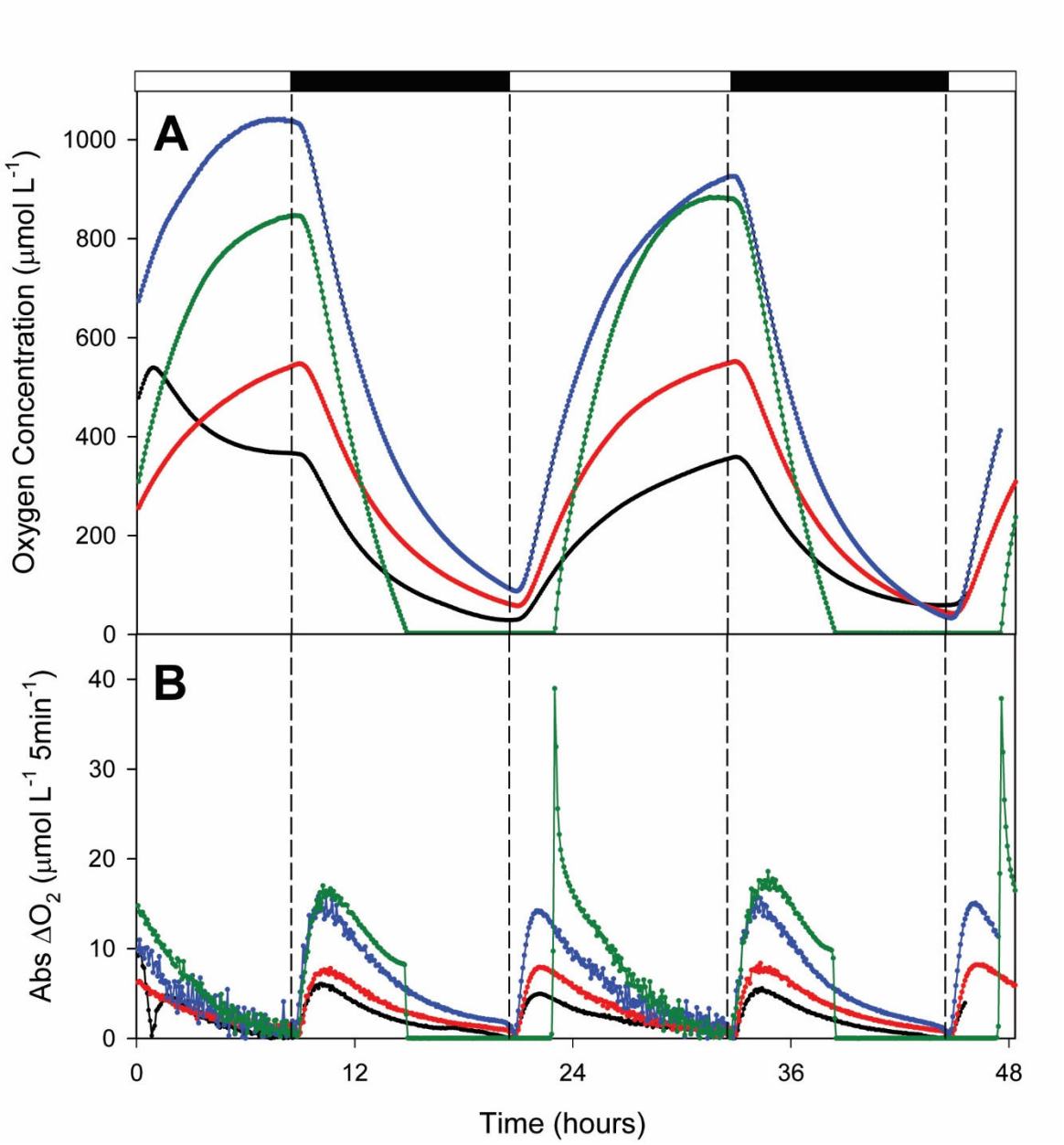

Figure 7. Comparison of O_2 and the delta of various light intensities at 50, 95, 250 and 350 µmol photons m^{-2} s^{-1}. While highest O_2 occurs at 250, highest P_n occurs at 350. Note significant lag in both P_n and R at the highest light intensity, with shorter lags at lower intensities.

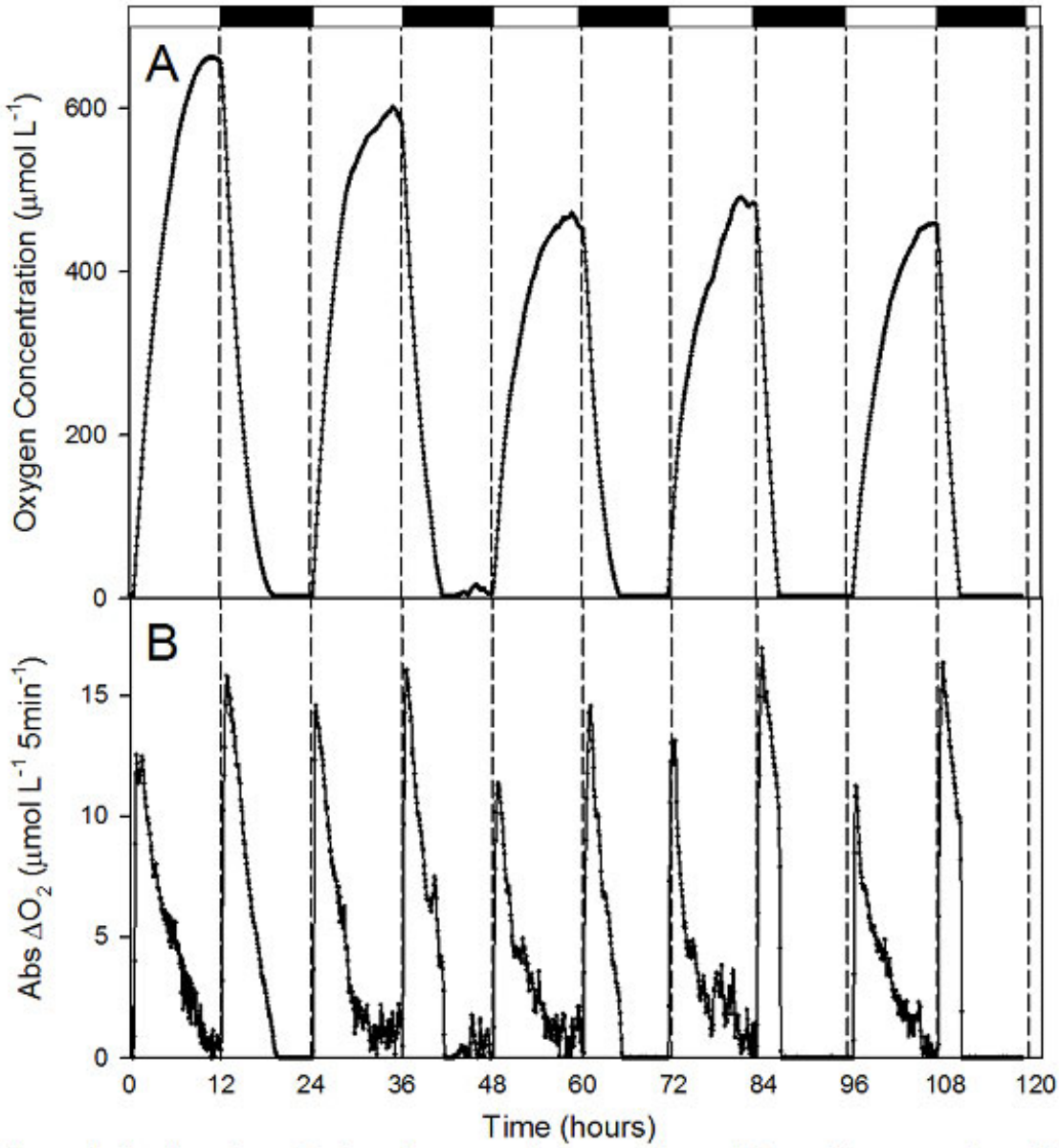

Figure 8. Continuation of colony from previous experiment (Figure 3) measured at ~350 intensity at depth 1 mm on opposite side of colony (lighter in pigment). Lag that occurred on previous side is not apparent in any cycles. [O_2] maximum decreases in first three light periods, and minimum is anoxic within six hours in the dark.

Diffusion of O_2 across colony surface

Simultaneous measurement of internal and external O_2 indicates that diffusion rate across the colony pellicle is low even when large differences develop during both light and dark periods. During a dark period internal O_2 often decreases to a minimum, while external oxygen at the colony border declines from 250 to 200 µmol L^{-1} (Figure 9). During a 250 µmol photons m^{-2} s^{-1} light period, internal O_2 increases from 150 to 465 µmol L^{-1} while external O_2 increases only from 25 - 46 µmol L^{-1}.

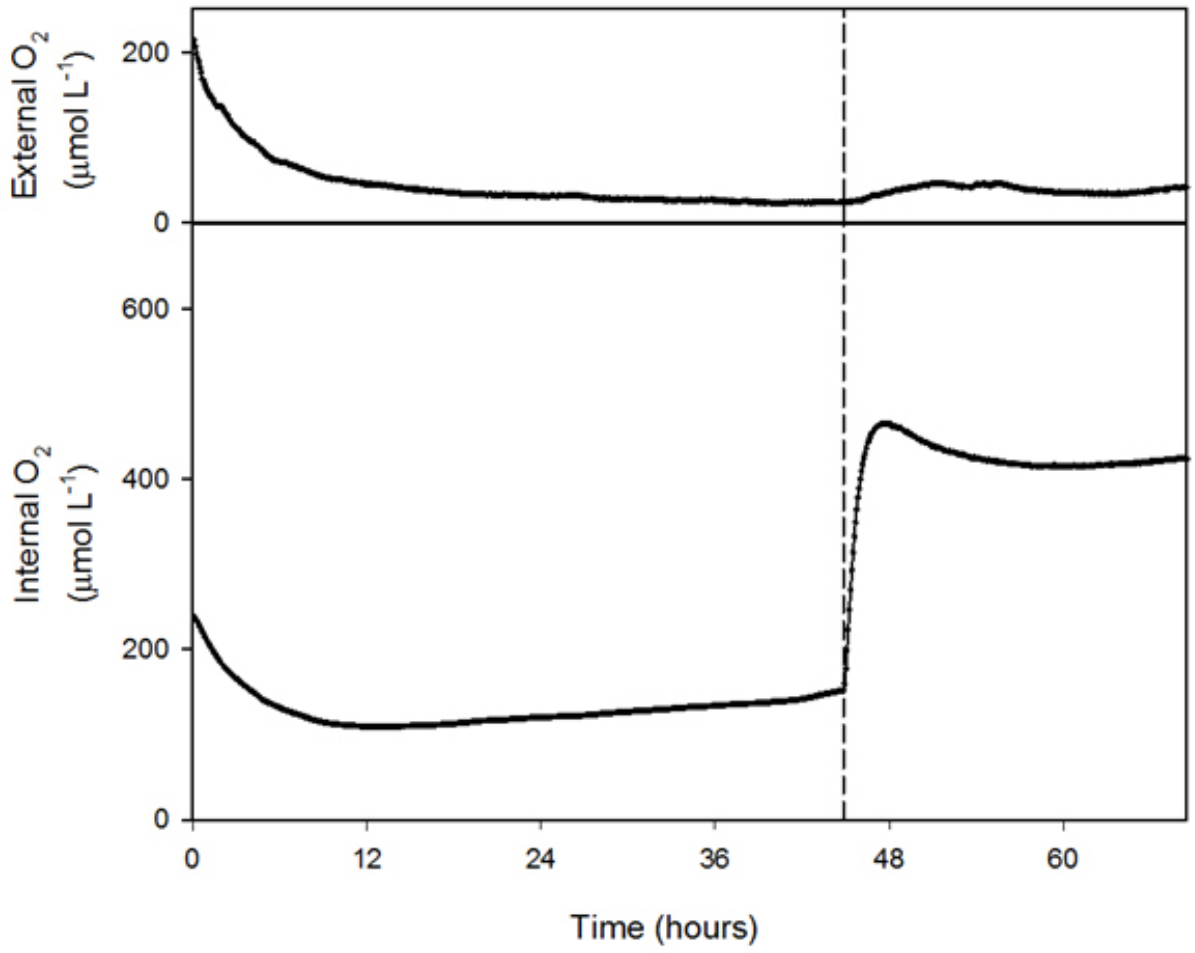

Figure 9. Comparison of internal and external oxygen during transition from dark to light conditions.

Desiccation

After complete desiccation at 20 C and low light intensity for three weeks, *N. pruniforme* colonies struggled to rehydrate during five days (Figure 1C). Colony rehydration failed completely in a subsequent dehydration. Other colonies exposed to sunlight for a single day rehydrated fully. Dehydrated colonies returned to an aquarium at low light intensity gradually faded and disintegrated in two weeks.

Discussion

In New England lakes, *N. pruniforme* colonies are most visible in late summer and disappear beneath siltation in other seasons. Spring sampling beneath the surface of flocculant organic sediment indicates over-winter burial. How the colonies rise above the surface sediment is unclear but may include an increase in buoyancy, perhaps related to increased glycan production.

The concentration and growth rate of *Nostoc* cells depends on the microhabitat surrounding the colony, including light and nutrient (phosphorus) availability and water current, as well as age and size of the colony (Gao and Ai, 2004; Li and Gao, 2004; Dodds and Castenholz, 1988, Dodds, 1989). Smaller colonies grow faster than larger colonies regardless of light or temperature levels (Ibid.). Colonies found in Greenland lakes (Jessica Haney, personal communication) are believed to be relatively old, are pale yellow with few intact filaments and are invaded by other cyanobacteria and insect larvae. O_2 remained at saturation (~251 μmoles L^{-1}) during 12-hour light and dark trials thus the gel matrix remains intact for months or years without trichomes. *N. sphaeroides* colonies differ structurally depending on pollution and soil composition, and can withstand extreme cold temperatures and arid habitats (Deng et al., 2008).

Nostoc parmelioides was observed to be more photosynthetically active at the colony surface relative to at depth within the colony (Dodds, 1989). Three *N. parmelioides* colonies from a stream in Montana, based on a colony profile with a 50 μm increment, had differing patterns of oxygen concentration from colony surface to a colony depth of 300 μm. Each had oxygen gradients and differing concentration of chlorophyll *a*. The variance occurred even though the colonies were suspended in recirculating stream water at constant temperature and illumination (550 μmol photons $m^{2}s^{-1}$) in a laboratory chamber. *Nostoc* trichomes were photosynthetically less active further from the colony surface, with a maximum activity just beneath the surface where there was an optimal balance of light, nutrients (especially PO_4^{-3}, NO_3^{-1} and CO_2) and O_2 (Ibid.). In the present study of *N. pruniforme* photosynthetic rates based on rates of change of O_2 were similar throughout the colonies, and O_2 concentration was uniform along depth gradients. Light was mainly absorbed by the outer 1 mm deep green layer of concentrated trichomes, and the internal light-colored gel zone with relatively sparse trichomes was expected to have lower activity. We suggest that diffusion of internal O_2 is more rapid within the colony than across its border to surrounding water.

Light saturation of P_n

Maximum O_2 production of several colonies incubated at various light intensities (50, 95, 250 and 350 μmol photons m^{-2} s^{-1}) was estimated to be 250 μmol photons m^{-2} s^{-1} at 20 C (Figure 6), with lower production at 350 μmol photons m^{-2} s^{-1}. *N. sphaeroides* has a similar saturation intensity but no inhibition even ≥ 900 μmol photons m^{-2} s^{-1} (Gao and Ai, 2004; Chen et al., 2012). Our low estimate may be the result of photoacclimation (Falkowski and La Roche, 1991) at low light intensity (<10 μmol photons m^{-2} s^{-1}) for several weeks.

Maximum rate of PS and R

Cells are likely oxygen starved after 12 h incubation in dark, and R appeared to be at a minimum based on ΔO_2 min^{-1} during the latter part of the dark period, and the low O_2 often to anoxia. These conditions apparently support, after a brief delay, a rapid rate of O_2 production for 30 minutes during which P_n produces as much as 10^3 µmol O_2. Afterward P_n rate decreases rapidly to near zero for the remainder of the light cycle of 12 - 24 h.

During the post-illumination dark cycle a surge of respiration occurs, similar in duration to the P_n surge, rapidly consumes O_2 to a low level, sometimes to anoxia, then decreases to zero for the remainder of the 12-24 h dark period. The rapid rise and fall of both P_n and R imply exhaustion of internal resources (or extreme internal pH levels) within an hour. Similar post-illumination surges in R occur in the terrestrial lichen endobiont *Nostoc furfuraceum* during the first ten minutes of a dark period, followed by low rates of respiration, were interpreted as a "strong metabolic channeling of recent photosynthate from the phycobiont" (Coxson, Harris and Kershaw, 1982). Typically higher plant respiration measured as CO_2 evolution is rapid and transient when placed in the dark after light exposure. Furthermore P_n is inhibited linearly to 35% as O_2 increases in air from 0 to >20% (Björkman, 1966). Within the glycan matrix of *Nostoc* O_2 exceeded 400% saturation in water, > 10^3 µmol L^{-1} during the current study.

Glyoxylate ($C_2H_1O_3$) produced in cyanobacteria (Bergman, 1980) increases rates of both photosynthesis and N-fixation, rates further enhanced in oxygen starved cells pre-incubated in darkness. Dark R is not affected, and photorespiration may be inhibited. Such effects may help explain the patterns of P_n and R in our experiments (Figures 2, 3, 8).

One hour delay at onset of both light and dark period

With the oxygen microprobe at a depth of 7 µm, one colony repeatedly had low and decreasing rates of P_n at the beginning of light periods, after 12 hours incubation in the dark. Similarly, the colony had the same response after a 12 hour light incubation at the onset of a dark period (Figure 6). We speculate that the delay is metabolic, the result of 'gearing up' for a change in metabolism requiring enzyme production in both light and dark regimes. Possibly, the microprobe is not located at the most active depth of metabolism, and the delay is the result of slow diffusion of O_2 from the site of maximum production and consumption. This seems unlikely given the relatively uniform rates of P_n throughout the colony. A third possibility, that there was a flaw in the light switch control, was eliminated by monitoring with a quantum sensor.

P_n rate at different depths within colony

Dependent on cell and chlorophyll concentrations along a transect through the colonies, P_n rate based on O_2 production diminished with incident light intensity, as expected (Figure 6). Pigments, most likely water-soluble phycobilins that leached into the gel matrix, as well as any iron absorbed (Geitler, 1932) also contributed to light absorbance. Because the filament concentration beneath the peripheral green layer was sparse, the light intensity was relatively constant from the lighted to the shaded side of the colony (Figure 1I). We found little difference

in P_n rate at discrete depths through the inner sparse zone. The high light absorbance of 90% within the first 100 μm of depth in the 'ear-shaped' colonies of *N. parmelioides* (Dodds et al., 1995) as well as the preponderance of chlorophyll in the outer layers of *N. pruniforme* (Raun, Borum and Sand-Jensen, 2009) are similar to our observations. Individual colony profiles (colony surface to base) were usually uniform in O_2 concentration, although the concentration varied with time suggesting either rapid diffusion of oxygen occurred within the colony, or P_n was similar at all depths.

Slow diffusion across the pellicle likely reduces availability of most nutrients, progressively deeper in the colonies. In contrast, the resident internal O_2 and CO_2 are rapidly recycled during light-dark cycles. Active accumulation and efficient use of bicarbonate at the elevated levels of pH (> 8.0) during photosynthesis provides sufficient carbon for at least 22 h at 485 μm photons m^{-2} s^{-1}, afterward becoming unavailable at pH > 10.0 as carbonate (Raun et al., 2009). Chen et al. (2012) found carbon limitation below 0.7 mM DIC at all concentrations of phosphorus tested. In the present study it is likely that DIC within the colonies was recycled during 1 – 2 hours respiration in darkness, then rapidly exhausted in approximately the same time period at light intensities of 50 – 350 μM photons m^{-2} s^{-1}.

N pruniforme colonies may have a uniform yet sparse distribution of trichomes beneath the outer green shell as a result of the specific habitat: Goose Pond, Maine, USA, a relatively oligotrophic, low alkalinity lake. Similar *N. pruniforme* colonies from the eutrophic Lake Esrum, Denmark had an average of 5x more chlorophyll content per wet weight (μg mg^{-1}) in the outer shell than in the 'interior mucus' in colonies up to 6.5 g wet weight (Raun et al., 2009). In contrast, hollow colonies and colonies with concentric rings of filaments have been shown to be produced under different growth conditions, especially light intensity and temperature. In some variants, internal filaments had denser chlorophyll than those near the colony surface (Deng et al., 2008).

Factors affecting colony growth

Phosphorus was likely a limiting factor based on rates of formation of the pre-spherical and young colonies (< 1mm) observed in the aquaria after several weeks and six months respectively. Mineralization of the original lake sediment present in the tanks may have provided necessary macronutrients for growth. According to Chen et al. (2012), formation of young spherical colonies from trichomes of *N. sphaeroides* occurred after eight days at high phosphate concentrations (50 and 250 μM PO_4-P) and increased to 60 – 80% colony formation from trichomes while at lower concentrations (0.5 and 5 μM PO_4-P) fewer than 20% formed. Absolute colony growth rate and chlorophyll *a* concentration were linearly related to increases in phosphorus supply from 0.5 to 250 μM (Ibid.). High concentrations of rod-shaped heterotrophic bacteria were found within the internal matrix (Figure 1F) suggesting a symbiotic relationship that undoubtedly contributes to respiration within the colonies. Heterotrophic consumption of *Nostoc* photosynthate must retard colony growth, and may do so progressively with age and/or size of the colony. Smaller colonies have ~4 times higher rates of P_n g^{-1} than larger ones (2.1 vs. 7.1 mm) at light saturation and twice the relative growth rate (1.5 vs. 5.5 mm) (Gao and Ai 2004).

Even with nutrient limitation, net production of O_2 was 3 to 4 times greater than consumption, measured as area under the first derivative curves in light versus dark. P_n rates were likely limited by high O_2 generated within the colonies, as much as 10^3 µM, 4 times saturation, as well as by high pH levels approaching the pKa (10.35) of bicarbonate dissociation to carbonate that is unavailable to cyanobacteria. Growth of *Nostoc* colonies is sensitive to temperature and can grow in the range 6 – 33 C with maximum growth at 25 C, while 43 C is lethal (Møller, Vangsøe and Sand-Jensen, 2014). *Lack of circadian rhythm*

Although we found sharp maxima in both P_n and R activity in *N. pruniforme*, they consistently occurred at or near the onset of alternating light-dark cycles. Prolonged periods of either light or dark had no change in activity. In contrast, *Synechocystis* sp. PCC 6803 continued to exhibit circadian rhythm in P_n near the onset of four 24-hour periods in continuous light, to which they appeared to be entrained by three preceding days of 12-12 light-dark cycles (Yen et al., 2004). Probably the unicellular *Synechocystis* cultures, incubated in a bottle open to the atmosphere and stirred continuously and provided BG-11 medium were not diffusion limited. If there is potential photosynthetic circadian rhythm in *N. pruiforme* colonies, it may be suppressed by slow diffusion across the pellicle. Length of cycles made little or no difference in patterns of change from 12 to 24 hour periods (Figure 2, 3). This allowed for repetition of multi-day measurements at various depths and light intensities. A lab-cultured strain of *Nostoc* had no circadian rhythm even during a 48 h period of light followed by 48 hours of dark.

Conclusions

Colonies of *Nostoc pruniforme* are highly active for only a brief time, generally 30 – 60 minutes at the onset of both light and dark periods, with O_2 changes as much as 60 µmol L^{-1} in short intervals (5 minutes). Afterward both P_n and R rates decline or cease entirely. We speculate that several metabolic elements are contributing to such a pattern. For example, CO_2 accumulates during the 12 h dark period, fueling P_n in the light period, but is quickly consumed by P_n. Similarly photosynthates accumulate during the light period, and are quickly respired in the dark. O_2 maxima in light probably induces photorespiration, assuming Rubisco is choosing an oxygenase rather than carboxylase pathway (Pierce, Andrews and Lorimer, 1986). As a result of CO_2 and HCO_3^- uptake along with OH^- secretion in light, pH may increase from 4 to 10 in light, limiting P_n above ~9 when most DIC is present mainly as unavailable CO_3^{-2}. Future studies should include microprobes sensitive to CO_2 and pH.

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