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Short research note

Eco-physiological responses of nitrogen-fixing cyanobacteria to light

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Abstract The eco-physiological responses of three nitrogen-fixing cyanobacteria (N-fixing cyanobacteria), *Aphanizomenon gracile*, *Anabaena minderi*, and *Ana. torques-reginae*, to light were assessed under nutrient saturation. The N-fixing cyanobacteria were isolated into monocultures from a natural bloom in a shallow colored lake and their growth irradiance parameters and pigment composition were assessed. The different ecological traits related to light use (μ_{max}, α, I_k) suggest that these N-fixing cyanobacteria are well adapted to low light conditions at sufficient nutrients, yet interspecific differences were observed. *Aphanizomenon gracile* and *Anabaena minderi* had high relative growth rates at low irradiances (ca. 70% of those in high light), low half saturation constant for light-limited growth ($I_k <$ 9.09 μmol photon m^{-2} s^{-1}) and high efficiency ($\alpha < 0.11$ day^{-1} μmol $photon^{-1}$ m^2 s). Conversely, *Ana. torques-reginae* showed poorer light competitiveness: low relative growth rates at low irradiances (ca. 40% of those in high light), low α (0.009 day^{-1} μmol $photon^{-1}$ m^2 s) and higher I_k (35.5 μmol photon m^{-2} s^{-1}). Final densities in *Aphanizomenon gracile* and *Anabaena minderi* reached bloom densities at irradiances above 30 μmol photon m^{-2} s^{-1} with different hierarchy depending on irradiance, whereas *Ana. torques-reginae* never achieved bloom densities. All species had very low densities at irradiances ≤17 μmol photon m^{-2} s^{-1}, thus no N-fixing blooms would be expected at these irradiances. Also, under prolonged darkness and at lowest irradiance (0 and 3 μmol photon m^{-2} s^{-1}) akinetes were degraded, suggesting that in ecosystems with permanently dark sediments, the prevalence of N-fixing cyanobacteria should not be favored. All species displayed peaks of phycocyanin, but no phycoeritrin, probably due to the prevailing red light in the ecosystem from which they were isolated.

Keywords Nitrogen fixing cyanobacteria · Light · Growth-irradiance · Eco-physiological traits · Akinetes

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Light levels that phytoplankton receive may oscillate between darkness in the aphotic zone to irradiances higher than 1,500 μmol photon m^{-2} s^{-1} in the surface.

Growth rate responses to these different light levels are among major traits that determine ecological success of phytoplankton species (Litchman & Klausmeier, 2008). The species or groups capable of growing at lower light intensities can competitively displace species/groups with higher light requirements (Huisman & Weissing, 1994). In addition to the amount of light, the quality of the available light in the medium also affects the eco-physiological responses of phytoplankton; this may favor the development of species with accessory pigments.

Individual species and taxonomic groups differ in major parameters of light-dependent growth and these differences often define contrasting ecological niches. For example, cyanobacteria are known to be good light competitors associated with low light environments, while green algae generally require higher irradiances (Richardson et al., 1983; Reynolds, 1984). In order to increase our ability to generalize across taxonomic groups, we need to assess light-related traits in a greater number of species from diverse environments. Moreover, within a single major taxonomic group, individual species likely differ in their responses to light. This interspecific variation is still poorly characterized because of the limited number of species tested.

Nitrogen-fixing (N-fixing) cyanobacteria (Nostocales) recurrently bloom in water bodies worldwide. Their massive and persistent growth is responsible for significant losses of diversity and ecosystem functioning in aquatic ecosystems (Huisman et al., 2005). N-fixing cyanobacteria possess various ecological traits that favor their success in aquatic ecosystems, including accessory pigments (phycobiliproteins), nitrogen fixation, buoyancy regulation, resting stages (akinetes), toxin synthesis, and grazer resistance. Light availability (quantity and quality) may impact these traits at the physiological level and ultimately affect ecological niches and success of N-fixers. There is a need to better understand how environmental factors, such as light, shape the eco-physiological responses of N-fixing cyanobacteria.

Here we assessed the eco-physiological responses to light of three N-fixing cyanobacteria isolated from a natural bloom. The species were taxonomically identified and their light-dependent growth parameters, densities, and absorption spectra were determined.

We isolated three species of N-fixing cyanobacteria from a natural bloom in the Otamendi RAMSAR floodplain wetland, Argentina (34°10′ to 34°17′S; 58°48′ to 58°53′W), in early austral autumn 2006. These species were grown into monocultures in sterile WC medium (Guillard, 1975); cycloheximide was used to prevent the development of eukaryotic algae (final concentration of 75 mg l^{-1}). The taxonomic identification was based on the morphological characters: the heterocyte and akinete pattern distribution and the relative position of the heterocytes with respect to akinetes, following Komárek & Zapomělová (2007, 2008). The three N-fixing cyanobacteria (Nostocales) were identified as *Aphanizomenon gracile* (Lemmermann) Elenkin, *Anabaena minderi* Huber-Pestalozzi, and *Anabaena torques-reginae* Komárek.

Growth irradiance curves ($\mu - I$) were obtained for each of these N-fixing species. Cultures were grown in 250-ml flasks, with 200 ml of full strength WC medium (Guillard, 1975) ensuring oversaturation of essential elements ($N = 14$ mg l^{-1}, $P = 3.1$ mg l^{-1}), in batch culture. They were exposed to 12 different irradiances of cool white fluorescent light, from limiting to saturating: 0–350 μmol photon m^{-2} s^{-1}. In order to attain the 12 irradiances, we used different positions within an environmental chamber, and layers of neutral density screen (from 1 to 5) for the lowest irradiances. Light was measured with a Biospherical QSL Quantum Scalar Laboratory sensor (Biospherical Inc., USA) in distilled water at the start of the experiment and directly in the cultures at the end of the experiment. Flasks were kept at a constant temperature of 20°C with a photoperiod of 16:8 h of light and dark. Each flask was swirled daily.

Before starting the growth irradiance ($\mu - I$) experiment, monocultures were preconditioned to each experimental irradiance for 5 days to allow cultures to acclimate (from August 13th to 18th, 2006). This period started with the inoculation of ca. 200 filaments ml^{-1} of *Aphanizomenon gracile* and *Anabaena minderi*, and of ca. 100 filaments ml^{-1} of *Ana. torques-reginae* because densities within this monoculture were very low. At the end of the precondition period, algae were quantified and dilutions were performed to avoid effects of self-shading and of nutrient limitation due to high biomasses. Dilutions were made in *Aphanizomenon gracile* and *Anabaena torques-reginae* at the highest irradiances (200, 250, and 350 μmol photon m^{-2} s^{-1}) and in *Ana. minderi* at 100, 200, 250, and 350 μmol photon

m^{-2} s^{-1}, using WC medium. The $\mu - I$ experiment lasted 4 days, from August 19th to 23rd, 2006. By the end of the experiment each flask was exposed to 9 days at a given irradiance (including the precondition period and the $\mu - I$ experiment).

Each day during the $\mu - I$ experiment 20 ml were removed from each culture and fixed with a 2% Lugol solution for enumeration. If densities were low, cultures were sedimented in graduated cylinders of 25 ml for 48 h (Litchman, 2000). Phytoplankton counts were performed using a Palmer–Maloney counting chamber with a Nikon Eclipse 80i microscope (magnification 250×). The counting unit was the individual (filament); at least three transects were counted for dense samples and the whole chamber for diluted samples. Heterocytes and akinetes were also quantified. Cultures were not axenic, nevertheless, regular microscopic observations revealed that heterotrophic bacteria were below 1% of the total density.

At the end of the $\mu - I$ experiment the absorption spectra was measured for each monoculture at each irradiance, as described in Litchman et al. (2002), to assess pigment composition.

The growth rate at each irradiance was calculated using the slope of the natural logarithm of the density plotted vs. time. The linear part of this curve, corresponding to the exponential growth, was used, including at least 3–4 points.

The growth rate data were fitted to a Monod equation in which the growth rate saturates at high irradiance (Eq. 1)

$$\mu(I) = \mu_{\max}\frac{I}{I + I_k} \quad (1)$$

where μ = growth rate (day^{-1}), μ_{max} = maximum growth rate (day^{-1}), I = irradiance (μmol photon m^{-2} s^{-1}) and I_k = half saturation constant (μmol photon m^{-2} s^{-1}). The initial slope of the growth irradiance curve, growth efficiency α (day^{-1}μmol $photon^{-1}$ m^2 s), was calculated as $\alpha = \mu_{max}/I_k$. Substituting $I_k = \mu_{max}/\alpha$ in Eq. 1 gives Eq. 2 (Schwaderer et al., in prep.)

$$\mu(I) = \frac{\mu_{\max} I}{I + \frac{\mu_{\max}}{\alpha}} \quad (2)$$

This growth irradiance curve was fitted using Marquardt–Levenburg minimization, Origin 7.5 program. Final average irradiance values were used for fitting the curve; these irradiances were lower than the ones initially assayed (from 0 to 38% lower) due to attenuation by the biomass (Table 1). Light attenuation increased more at higher irradiances (Table 1).

The species identified as *Aphanizomenon gracile*, *Anabaena minderi* and *Anabaena torques-reginae* (Nostocaceae) are filamentous, isopolar, planktonic, solitary, with gas vesicles, and capable of fixing nitrogen. Filaments in *Anabaena minderi* and *Aphanizomenon gracile* are straight, whereas in *Anabaena torques-reginae* are coiled; filament morphology did not vary with the light availiability. Akinetes in *Aphanizomenon gracile* are cylindrical and remote from the heterocytes, but in *Anabaena minderi* they are ovoid and adjacent to the heterocytes and in *Ana. torques-reginae* sphaerical and adjacent to heterocytes.

Aphanizomenon gracile had the highest μ_{max} and duplication rate, closely followed by *Anabaena minderi*, but *Ana. torques-reginae* had about threefold lower μ_{max} and duplication rate (Table 2). All species saturated their growth at highest irradiances with no photoinhibition (Fig. 1). The parameterized maximum growth rate (μ_{max}) matched well the maximum values empirically observed (μ) (Fig. 1). In *Aphanizomenon gracile* and *Anabaena minderi* the estimated growth rates at low irradiances (17 μmol photon m^{-2} s^{-1}) were about 70% of those in high irradiance (200 μmol photon m^{-2} s^{-1}): 0.65 vs. 0.96 day^{-1} in *Aphanizomenon gracile* and 0.48 vs. 0.68 day^{-1} in *Anabaena minderi*. In *Ana. torques-reginae*, however, the growth rate at low light was 40% less than at high light (0.10 vs. 0.27 day^{-1}). The initial slope of the $\mu - I$ curve (α) was the highest in *Aphanizomenon gracile*, closely followed by *Anabaena minderi* and much lower in *Ana. torques-reginae* (Table 2). In all species, the half saturation

Table 1 Initial and final average irradiances (μmol photon m^{-2} s^{-1})

Initial	0	3	5	10	15	25	40	60	100	200	250	350
Final	0	3	4	8	13	17	31	42	80	137	199	218

Table 2 Parameters obtained from the Monod model: μ_{max}: maximum growth rate (day^{-1}), α: initial slope of the growth irradiance curve (day^{-1} µmol photon^{-1} m^2 s) and I_k: half saturation constant for light limited growth (µmol photon m^{-2} s^{-1})

	μ_{max}	α	I_k	R^2	χ^2	Dupl.
Aph. gracile	1.00 (0.10)	0.11 (0)	9.09	0.78	0.04	1.45
Ana. minderi	0.71 (0.06)	0.09 (0.04)	7.88	0.88	0.009	1.03
Ana. torques-reginae	0.32 (0.06)	0.009 (0.004)	35.55	0.78	0.003	0.46

Values of R^2 and χ^2 are provided. The duplication rate (Dupl. = day^{-1}) was calculated from the ln (2), where 0.69 day^{-1} corresponds to one duplication. Values in *parentheses* represent standard deviations

constant for light limited growth (I_k) was below 40 µmol photon m^{-2} s^{-1} (Table 2), indicating that these N-fixing cyanobacteria are well adapted to low light conditions. Nevertheless, interspecific differences existed: I_k was very low in *Anabaena minderi* and *Aphanizomenon gracile*, but four times higher in *Anabaena torques-reginae* (Table 2). In all irradiances, the species light absorption spectra showed peaks of phycocyanin (PC, $\lambda = 620$ nm), but no peaks of phycoerythrin ($\lambda = 560$ nm). The pigment composition (the presence or absence of different accessory pigments) was similar at different irradiances.

Final densities were very low at irradiances ≤17 µmol photon m^{-2} s^{-1} but at irradiances ≥30 µmol photon m^{-2} s^{-1} they increased proportionally with the irradiance (Fig. 2). In *Aphanizomenon gracile* and *Anabaena minderi* densities at irradiances ≥40 µmol photon m^{-2} s^{-1} (≥1 × 10^4 ind ml^{-1}) were similar to those encountered in natural blooms. Above 80 µmol photon m^{-2} s^{-1} *Aphanizomenon gracile* was the species with the highest density, whereas between 30 and 80 µmol photon m^{-2} s^{-1} *Anabaena minderi* was the densest species (Fig. 2). Densities in *Ana. torques-reginae* were always the lowest compared to other monocultures and never reached bloom densities (Fig. 2).

Akinetes were seldom observed in *A. minderi*, but in *Aphanizomenon gracile* and *Anabaena torques-reginae* akinete densities were high. At irradiances ≥5 µmol photon m^{-2} s^{-1} akinetes occurred without any consistent pattern related to light availability. In the dark and at lowest irradiance (0 and 3 µmol photon m^{-2} s^{-1}), however, akinete densities decreased during the experiment and the integrity of some akinetes was lost. Heterocyte dynamics in all species did not show any pattern associated with light availability.

The different ecological traits related to light use (μ_{max}, α, I_k) highlight that these N-fixing cyanobacteria are well adapted to low light conditions. This coincides with the low light requirements of Cyanobacteria as a group. We have also studied light traits of a green alga culture (*Ankistrodesmus* sp., data not shown) grown under the same experimental conditions as the N fixers. N fixers had at least three times lower half saturation (I_k) values and about 50% higher relative growth rates at low irradiance than the green alga, showing that N fixers are better low light competitors. Nevertheless, at high irradiances, all N fixers had much lower maximum growth rates than the green alga. Thus, under nutrient sufficiency, N-fixers should outcompete green alga at low irradiances, whereas the opposite could occur at high irradiances. Schwaderer et al. (in prep.) found similar results in a meta-analysis of growth irradiance parameters of 115 algal species from all algal taxonomic groups; differences in light physiological traits between groups defined different ecological responses to light availability.

As a group, the N fixers showed low light adaptation, however, *Aphanizomenon gracile* and *Anabaena minderi* showed better light competitiveness than *Ana. torques-reginae*. *Aphanizomenon gracile* and *Anabaena minderi* had low half saturation (I_k), showed high efficiency (α) and high relative growth rates at low irradiances. Similar results were observed by McCausland et al. (2005) for the blooming *Anabaena circinalis* culture strain ACMB 13. Conversely, *Ana. torques-reginae* had at least three times higher I_k, two and a half times lower μ_{max}, one order of magnitude lower α and almost 50% less relative growth rates at low irradiances.

The different light levels tested affected the N fixers' growth rates, their blooming capabilities and viability of akinetes, but did not affect their filament morphology, nor did it trigger chromatic adaptation. Densities of all N-fixing cyanobacteria below 17 µmol photon m^{-2} s^{-1} were extremely low, thus no blooms would be expected at these irradiances.

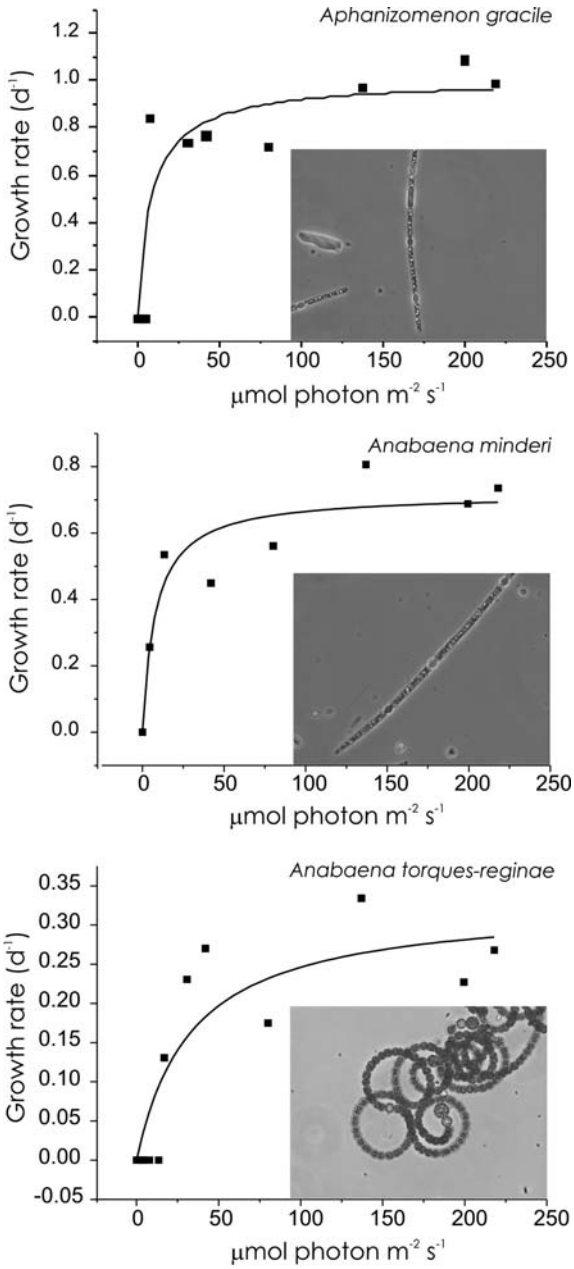

Fig. 1 Growth-irradiance curves ($\mu - I$) of the assayed species. Every *dot* represents the growth rates at each irradiance and the *solid line* represents the fit of the Monod model

Densities of *Aphanizomenon gracil*e and *Anabaena minderi* at irradiances between 40 and 200 µmol photon m^{-2} s^{-1} were similar to blooms in nature, with changes in the hierarchy of the dominant species within a bloom: *Aphanizomenon gracile* would dominate at high irradiances and *Anabaena minderi* at lower irradiances. The low densities of *Ana. torques-reginae* at all irradiances suggest that this may not be an important bloom-forming species. This, in conjunction with its lower light competitiveness may account for its rareness, as *Ana. torques-reginae* has only been found in few sites in the world (Komárek & Zapomělová, 2007).

The akinetes, resting structures that differentiate from vegetative cells, are easily detached from the filament and sink to the sediments, where they remain dormant. The ease with which they are generated might be related to the sensitivity of a given species to environmental variables. The low frequency of akinetes in *Anabaena minderi* may suggest a low persistence in the ecosystem, or that the environmental conditions did not favor the development of resting structures. In the other two species which produced akinetes, their viability decreased under prolonged darkness, suggesting that in ecosystems with permanently dark sediments the prevalence of N-fixing cyanobacteria should not be favored.

All three N-fixing cyanobacteria species had phycocyanin but no phycoeritrin, because in the RAMSAR Otamendi wetland the prevailing wavelengths are red, as a consequence of the high humic content of its waters (Rodríguez, 2008). Chromatic adaptation was not observed during the experiment, probably because light quality was always the same (white light).

Recently, Zapomělová (2008) reported that the 16S rRNA gene sequences of two *Anabaena*'s coiled morphospecies (*Anabaena reniformis* Lemmermann and *Aphanizomenon aphanizomenoides* (Forti) Horecká et Komárek) with spherical akinetes positioned adjacent to the heterocyte, cluster separately from most planktonic *Anabaena* species. The author proposes to relocate all species with such morphological characteristics into a new genus *Sphaerospermum* genus *novum*, including: *Anabaena reniformis*, *Aphanizomenon aphanizomenoides*, *Anabaena torques-reginae*, *Anabaena eucompacta*, *Anabaena oumiana*, and *Aphanizomenon capricorni*. Even though the 16S rRNA gene sequences of *Ana. torques-reginae* still needs to be analyzed to confirm that it belongs to this cluster, its different ecophysiological responses to light and their poor blooming capabilities reported here support Zapomělová's (2008) suggestion. Like in *Anabaena torques-reginae*, the N-fixing cyanobacteria *A. reniformis* and

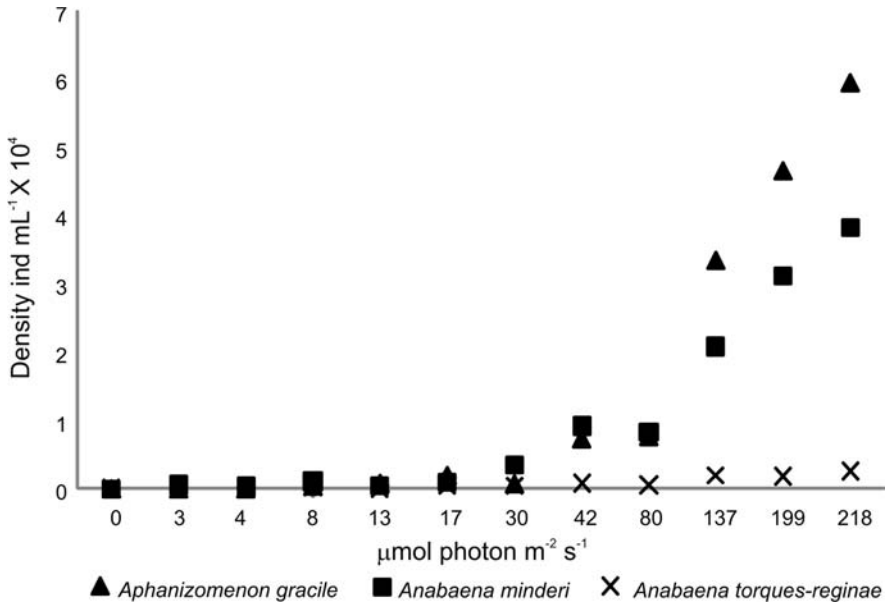

Fig. 2 Species densities at the end of the experiment in day ml^{-1} at each irradiance

Aphanizomenon aphanizomenoides have only been reported a few times from isolated localities worldwide (Cronberg & Annadotter, 2006; Komárek & Zapomělová, 2007). It is possible that this genetic heterogeneity also reflects different ecological success among planktonic N-fixing cyanobacteria.

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