New Phytol. (1998), **138**, 579–587

Comparison of the light-limited growth of the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*

BY W. T. (PIM) DE NOBEL[1*], HANS C. P. MATTHIJS[1], ERIC VON ELERT[2] AND LUUC R. MUR[1]

[1] *Laboratorium voor Microbiologie, ARISE, Universiteit van Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands*
[2] *Limnologisches Institut, Universität Konstanz, 78457 Konstanz, Germany*

(*Received 17 June 1997; accepted 10 November 1997*)

SUMMARY

The effect of simultaneous N_2 fixation and light limitation on the growth of two strains of *Anabaena* sp. Bory de St. Vincent and *Aphanizomenon flos-aquae* (L.) Ralfs was investigated using continuous cultures. Under severely light-limited conditions, *Aphanizomenon* showed a broader absorption spectrum (due to the presence of phycoerythrin), a higher maximum efficiency of photosynthesis, a higher steady-state N_2 fixation activity and a higher growth affinity for light than did *Anabaena*. On the other hand, under light saturation, *Anabaena* showed a higher maximum rate of O_2 production and a higher maximum specific growth rate than *Aphanizomenon*. These monoculture results characterize *Anabaena* and *Aphanizomenon*, in relative terms, as a 'sun' and a 'shade' species respectively, and are in accordance with field observations. The difference between the two species in their acclimatory response is discussed in terms of a species-specific alteration of the PSI:PSII stoichiometry. Besides the species-specific modulation of the accessory pigments, such an acclimation would provide a biochemical basis for the observed physiological differences. The monoculture results were used to differentiate the niches of the two species and suggested that *Aphanizomenon* would competitively displace *Anabaena* under N_2-fixing, light-limited conditions. However, when both species were grown together, *Anabaena* became dominant and seemed to be the superior competitor for light. In order to explain this finding, the possible effects of release of allelopathic compounds, or dynamic aspects of light supply, are discussed.

Key words: Competition, cyanobacteria, light limitation, nitrogen fixation, PSI:PSII stoichiometry.

INTRODUCTION

The taxonomically closely related, and morphologically similar, heterocystous cyanobacteria *Anabaena* spp. and *Aphanizomenon* spp. are commonly present in eutrophic shallow freshwater lakes (Mur & Schreurs, 1995; Roijackers & Joosten, 1996). In general, cyanobacterial species capable of N_2 fixation will have a competitive advantage over non-diazotrophs if N_2 is the only N source available for growth. This leaves open the question of what determines the outcome of competitive interaction between different species of diazotrophic cyanobacteria. Whilst this might relate simply to different nitrogenase activities, other biochemical, physiological or ecological factors, either indirectly or not directly related to N_2 fixation, could determine which diazotroph becomes dominant in which environment.

During bloom development, phytoplankton growth in eutrophied systems often becomes N, P or light-limited. Experiments showed that the combination of N_2-fixing and P-limited conditions is disadvantageous for *Aphanizomenon* (Wallström, Johansson & Larsson, 1992; De Nobel *et al.*, 1997*a*,

* To whom correspondence should be addressed.
E-mail: a417pimn@chem.uva.nl

Abbreviations: Chl, chlorophyll *a*; HL, high light; ML, medium light; LL, low light; I_k, light saturation value (μmol photons m^{-2} s^{-1}); K_I, half saturation constant for light-limited growth (μmol photons m^{-2} s^{-1}); OD_{750}, optical density at wavelength 750 nm (cm^{-1}); P/I curve, light saturation curve of photosynthesis; P_{max}, maximum rate of O_2 production (mg O_2 g^{-1} protein h^{-1}); PPFR, photosynthetic photon fluence rate (μmol photon m^{-2} s^{-1}); PSI, photosystem 1; PSII, photosystem 2; q_N, steady-state N_2-fixation activity (μg N mg^{-1} protein h^{-1}); QY, maximum quantum yield for O_2 production (mol O_2 mol^{-1} photons); R_d, rate of respiration in the dark (mg O_2 g^{-1} protein h^{-1}); α, maximum efficiency of photosynthesis (mg O_2 g^{-1} protein h^{-1} μmol^{-1} photons m^2 s); σ_{chl}, chlorophyll-specific optical absorption cross-section (m^2 g^{-1} Chl); σ_{prot}, protein-specific optical absorption cross-section (m^2 g^{-1} protein); μ, specific growth rate (h^{-1}); μ_{max}, maximum specific growth rate (h^{-1}); μ_{max}/K_I, growth affinity for light (m^2 mol^{-1} photons).

b). According to a statistical analysis of field data, the occurrence of *Anabaena* blooms coincides with a relatively high underwater light availability (i.e. a high ratio of euphotic depth and mixing depth), whilst *Aphanizomenon* seems much less light-demanding (Schreurs, 1992). Hence, under N_2-fixing, light-limited conditions, *Aphanizomenon* might have a competitive advantage over *Anabaena*.

This study compares the light-limited growth of *Anabaena* sp. and *Aphanizomenon flos-aquae* under N_2-fixing conditions in continuous culture. The acclimatory responses of light absorption, photosynthetic activity, N_2 fixation activity and growth are examined, in order to ascertain whether *Aphanizomenon* is a 'low-light' or 'shade' species in comparison to *Anabaena*. In addition, a competition experiment under light-limited conditions was carried out in order to test the hypothesis that *Aphanizomenon* is better acclimated than *Anabaena* to N_2-fixing, light-limited conditions.

MATERIALS AND METHODS

Organisms and growth conditions

Anabaena sp. Bory de St Vincent was isolated from a surface bloom in Lake Naardermeer, The Netherlands. *Aphanizomenon flos-aquae* (L.) Ralfs PCC 7905 originated from Lake Brielse Meer, The Netherlands. Both lakes are eutrophic, shallow, freshwater lakes. Hereafter, the two strains are referred to as *Anabaena* and *Aphanizomenon* respectively.

Both species were grown as non-axenic monocultures of single filaments in continuous culture using N-free BG-11 medium (Rippka *et al*., 1979). Continuous cultures were of the diluted-turbidostat type (auxostats), where the dilution rate is adjusted to maintain a constant, low optical density (OD_{750}). Three conditions were used: light-saturated conditions (high light, HL), moderately light-limited conditions (medium light, ML) and severely light-limited conditions (low light, LL). Illumination was provided by circular Philips TLE 32W/33 lamps (three, one and one covered by a neutral density filter respectively), operated in a 12/12 h light/dark cycle. The average photosynthetic photon fluence rate (PPFR) within a culture was calculated according to Van Liere, Loogman & Mur (1978). The OD_{750} values of cultures were kept low (absorption at 750 nm $< 0{\cdot}075$ cm^{-1}) in order to approximate a homogeneously distributed PPFR in monoculture experiments but not during competition experiments. Air-flow through the culture vessel provided mixing and gas exchange. The air was washed twice in 0·05 M H_2SO_4: once before entering the culture to prevent input of air-borne NH_3, and again after leaving the culture to measure NH_3 release. The temperature was maintained at 20 °C ± 1·5 °C and the pH ranged between 6·9 and 7·4.

Steady-state cultures were examined on 2 d separated by at least a week. Unless stated otherwise, samples were taken in triplicate at the middle of the light period. The data represent the average of the two series. Sample measurements were normalized to protein. Protein proved to be a relatively constant fraction of d. wt (*c*. 25 %). A competition experiment with *Anabaena* and *Aphanizomenon* was run in duplicate in chemostats at a dilution rate of 0·004 h^{-1}, after mixing of pre-grown LL continuous cultures.

Protein, chlorophyll, ammonium, culture density and cell numbers

The protein and chlorophyll contents, as well as the concentration of dissolved inorganic N (i.e. NH_4^+), were analysed after centrifugation (4000 ***g***, 4 min). Protein was determined following the Lowry method (Herbert, Phipps & Strange, 1971). Chlorophyll *a* was measured spectrophotometrically in dimethylformamide extracts (Porra, Thompson & Kriedemann, 1989). Dissolved NH_4^+ was measured in the supernatant using salicylate and nitroprusside (Kempers & Kok, 1989). During the competition experiments, the changes in culture density were followed by measurements of OD_{750}, and cell numbers of the two species were monitored microscopically.

Nitrogen fixation

The number of heterocysts, expressed as a percentage of the total cell number, was calculated after counting at least 500 cells. Steady-state N_2-fixation activities (q_N) were calculated by the Droop Cell-Quota model. In a diazotrophically growing steady state, the product of growth rate and total N concentration equals the gross N uptake rate mediated by N_2 fixation (Droop, 1974). Total N was determined after Kjeldahl digestion and subsequent NH_4^+ analysis of samples from the effluent collected over 24 h. The amount of gaseous NH_3 in exhausted air was negligible (< 2 % of total N in the effluent).

Growth/irradiance response

The growth rates in monocultures of *Anabaena* and *Aphanizomenon* were described using Monod kinetics (Monod, 1942):

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

where $\mu_{\max}$ is the maximum specific growth rate, K_I is the half-saturation constant for light-limited growth, and I is the average PPFR (in the culture). The Monod equation was fitted to the data using non-linear regression.

Photosynthesis/irradiance response

Light saturation curves of photosynthesis (P/I curve) were determined from the rates of O_2 exchange in incubation chambers as described by Dubinsky *et al*. (1987). The three fundamental P/I parameters, maximum rate of O_2 production (P_{max}), maximum efficiency of photosynthesis (α) and the rate of respiration in the dark (R_d) were derived by fitting the data to a hyperbolic tangent model (Jassby & Platt, 1976):

$$P(I) = P_{max} \tanh\left(\frac{\alpha I}{P_{max}}\right) - R_d. \quad (2)$$

After 15 min pre-incubation in darkness, respiration was measured for 9 min. Subsequent photosynthesis measurements were made at 12 different PPFRs ranging from 5 to 2000 μmol photons m^{-2} s^{-1}, of which the initial seven were below the light-saturation value ($I_k = P_{max}/\alpha$). Each PPFR was supplied for 3 min and the rates of O_2 exchange were calculated from the linear increase or decrease in O_2 concentration. In order to minimize effects of photo inhibition (Henley, 1993), measurements were completed within 45 min.

Light absorption

Chlorophyll-specific and protein-specific optical absorption cross-sections (σ_{chl} and σ_{prot}) were determined from *in vivo* absorption spectra as described by Kroon *et al*. (1992). The maximum quantum yield for O_2 production (QY) was calculated according to Tilzer (1984) as the ratio between the maximum efficiency of photosynthesis and the optical absorption cross-section ($QY = \alpha/\sigma$).

Chlorophyll fluorescence

Chlorophyll *a* fluorescence measurements were made *in situ* at room temperature using the saturation-pulse method described by Schreiber, Schliwa & Bilger (1986), and the experimental set-up described by Ibelings, Kroon & Mur (1994). A saturating pulse of light (12000 μmol photons m^{-2} s^{-1}, 0·8 s), which induced maximal fluorescence (all PSII reaction centres closed), was administered at 15-min intervals. The fluorescence variables were used to calculate the quantum yield of non-cyclic electron transport as described by Genty, Briantais & Baker (1989). Hereafter, this photochemical efficiency of PSII per absorbed photon is referred to as photon yield (Hofstraat *et al*., 1994).

RESULTS

Growth and nitrogen fixation

Growth/irradiance response. The growth rates of *Anabaena* and *Aphanizomenon* increased with increasing PPFR. *Anabaena* had a higher maximum specific growth rate (μ_{max}) than *Aphanizomenon* (Table 1). However, *Aphanizomenon* had a slightly higher growth affinity for light (μ_{max}/K_I) and thus grows faster than *Anabaena* under severely light-limited conditions (i.e. at PPFRs < 17·7 μmol photons m^{-2} s^{-1} according to the solving of the species' Monod equations).

Table 1. *Maximum specific growth rate (μ_{max}) half saturation constant for light-limited growth (K_I) and growth affinity for light (μ_{max}) for* Anabaena *sp. and* Aphanizomenom flos-aquae *under N_2-fixing conditions*

	μ_{max} (h^{-1})	K_I (μmol photons m^{-2} s^{-1})	μ_{max}/K_I (m^2 mol^{-1} photons)
Anabaena	0·046	95	0·13
Aphanizomenon	0·026	46	0·16

The parameter estimates of μ_{max} and K_I are based on least-squares, nonlinear fits to eqn (1) (see 'Materials and Methods') using the steady-state data from continuous cultures ($r^2 > 0·95$).

Nitrogen fixation. Heterocyst frequencies were identical for the two species and amounted to 5–7 % of total cell number, independent of the conditions of illumination. Acclimation to ML or LL resulted in a decrease of steady-state N_2-fixation activity (q_N) for both species. However, LL hampered q_N of *Anabaena* more than that of *Aphanizomenon* (Table 2).

Photosynthesis

Chlorophyll content. For both species decreased PPFR resulted in a higher chlorophyll *a* (Chl) content, but more so for *Aphanizomenon* than for *Anabaena* (Table 2).

Photosynthesis/irradiance response. The observed changes in the P/I curves (Fig. 1), resulting from the different light conditions (HL, ML & LL), clearly distinguished *Anabaena* from *Aphanizomenon* with respect to their patterns of acclimation to light limitation. The maximum rate of O_2 production (P_{max}) for *Anabaena* increased with decreasing PPFR whilst that for *Aphanizomenon* markedly decreased with decreasing PPFR, in spite of its increased Chl content. For both species the maximum efficiency of photosynthesis (α) increased with decreasing PPFR but, at each condition of illumination α for *Aphanizomenon* was higher than that for *Anabaena*. The rate of respiration in the dark (R_d) did not show a clear pattern, though it tended to decrease with decreasing PPFR and seemed somewhat greater in *Aphanizomenon* than in *Anabaena*. The difference in acclimatory response between both species was reflected in their light saturation values (I_k). For

Table 2. *Steady-state N_2 fixation activity (q_N), chlorophyll* a *content (Chl), maximum rate of O_2 production (P_{max}), maximum efficiency of photosynthesis (α), rate of respiration in the dark (R_d), light saturation value (I_k), chlorophyll-specific optical absorption cross-section (σ_{chl}), protein-specific optical absorption cross-section (σ_{prot}) and maximum quantum yield for O_2 production (QY) for* Anabaena *sp. and* Aphanizomenon flos-aquae *under light-saturating (HL), moderately light-limiting (ML) and severely light-limiting (LL) photosynthetic photon fluence rates (PPFR)*

	Anabaena			*Aphanizomenon*		
	HL	ML	LL	HL	ML	LL
PPFR (μmol photons m^{-2} s^{-1})	190	60	25	160	50	25
q_N (μg N mg^{-1} protein h^{-1})	6·76	5·12	1·40	3·69	3·28	2·56
Chl (mg Chl g^{-1} protein)	23·1	22·4	25·7	20·4	29·5	29·9
P_{max} (mg O_2 g^{-1} protein h^{-1})	375	478	577	341	301	268
α (mg O_2 g^{-1} protein h^{-1} μmol^{-1} photons m^2 s)	1·86	2·51	2·68	2·21	3·36	3·90
R_d (mg O_2 g^{-1} protein h^{-1})	72·4	41·4	44·7	83·0	43·0	82·9
I_k (μmol photons m^{-2} s^{-1})	202	190	215	154	90	69
σ_{chl} (m^2 g^{-1} chlorophyll)	0·77	1·01	1·29	1·13	0·91	1·11
σ_{prot} (m^2 g^{-1} protein)	0·018	0·022	0·033	0·023	0·027	0·034
QY (mol O_2 mol^{-1} photons)	0·09	0·10	0·07	0·08	0·11	0·10

The parameter estimates of P_{max}, α and R_d are based on least-squares, nonlinear fits to eqn (2) (see 'Materials and Methods') using the data from P/I curves ($r^2 > 0·96$).

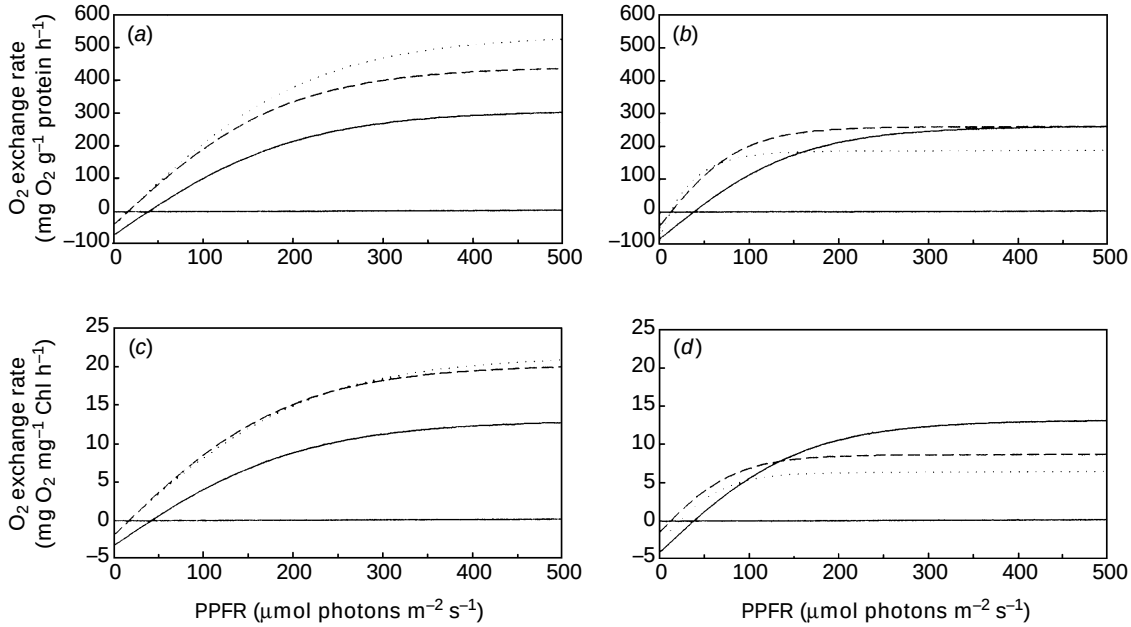

Figure 1. Variation of the rates of O_2 exchange, expressed relative to protein (*a*), (*b*) or chlorophyll (*c*), (*d*), with photosynthetic photon fluence rate for *Anabaena* sp. (*a*), (*c*) and *Aphanizomenon flos-aquae* (*b*), (*d*). P/I curves were determined for steady-state cells originating from cultures that were light-saturated (HL; ——), moderately light-limited (ML; - - -) or severely light-limited (LL; · · ·). Lines are least-squares, non-linear fits to eqn (2) (see 'Materials and Methods') using the data from P/I curves.

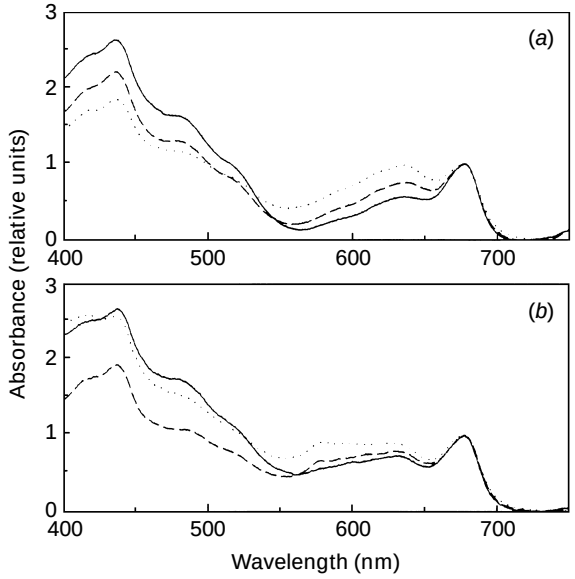

Figure 2. *In vivo* absorption spectra of *Anabaena* sp. (*a*) and *Aphanizomenon flos-aquae* (*b*). Absorption spectra were determined for steady-state cells originating from cultures that were light-saturated (HL; ——), moderately light-limited (ML;---) or severely light-limited (LL; ···). The spectra are normalized to the red absorption peak of chlorophyll *a* (675–680 *nm*).

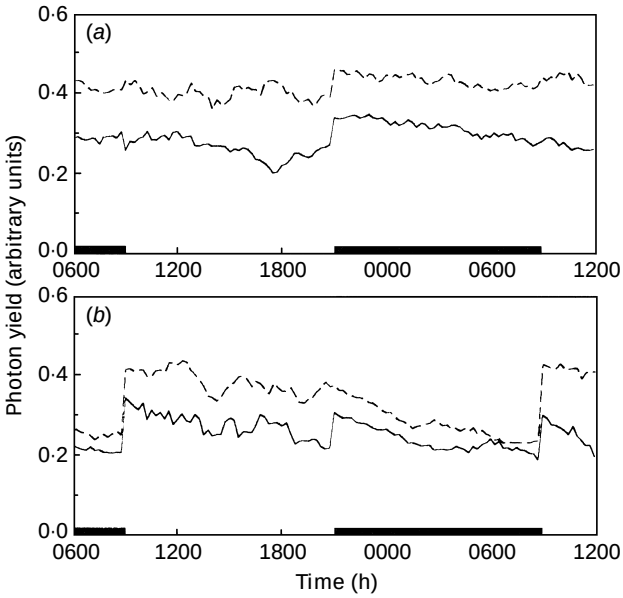

Figure 3. Diel variation of photon yield for *Anabaena* sp. (*a*) and *Aphanizomenon flos-aquae* (*b*). Photon yields are based on *in situ* chlorophyll *a* fluorescence of light-saturated (HL; ——) and moderately light-limited (ML; ---) steady-state cells. The dark periods are indicated by black bars. Note that the time period covers 1·25 diel cycles.

Anabaena, I_k remained more or less constant with decreasing PPFR but, for *Aphanizomenon*, I_k decreased considerably (Table 2).

Light absorption. The normalized absorption spectra of *Anabaena* and *Aphanizomenon* show that absorption by the accessory pigments increased with decreasing PPFR (Fig. 2). For *Anabaena*, absorption increased most strikingly at *c*. 635–640 nm (phycocyanin, PC), indicating an increased PC:Chl ratio. For *Aphanizomenon*, increased absorption at 635–640 nm was accompanied by an even greater increase at *c*. 575–580 nm (phycoerythrin, PE). Hence, the spectra of *Aphanizomenon* reveal a moderate increase in the PC:Chl ratio but a considerably increased PE:PC ratio.

The chlorophyll-specific optical absorption cross-section (σ_{chl}) of *Anabaena* increased with decreasing PPFR whilst σ_{chl} remained more or less constant for *Aphanizomenon* (Table 2). For both species the protein-specific optical absorption cross-section (σ_{prot}) increased with decreasing PPFR but more so for *Anabaena* than for *Aphanizomenon* (Table 2). Based on these values, and those presented for α, the calculated maximum quantum yields for O_2 production ($QY = \alpha/\sigma$) by the two species showed opposite trends (Table 2). Under LL conditions the QY of *Aphanizomenon* increased in comparison to the QY under HL conditions, and eventually became higher than that of *Anabaena*.

Chlorophyll fluorescence. During the light period of both the HL and ML cultures, the photon yields of

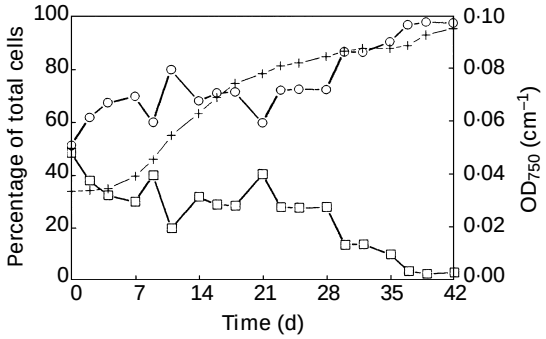

Figure 4. Competition between *Anabaena* sp. (○) and *Aphanizomenon flos-aquae* (□) under N_2-fixing, light-limited conditions. Cells were grown in a chemostat culture at a dilution rate of 0·004 h^{-1}. +, optical density (OD_{750}) of the culture. One of the duplicate experiments is depicted.

Anabaena and *Aphanizomenon* were comparable, although the mean photon yield of HL cells was lower than that of ML cells (0·27 and 0·39 respectively; Fig. 3). The biomass of the LL cultures was too low to allow an accurate measurement of chlorophyll *a* fluorescence (data not shown). Remarkably, the photon yields during the dark period differed for the two species. Although the photon yield of *Anabaena* decreased only slightly during the dark period, that of *Aphanizomenon* showed a pronounced decrease, especially in the ML culture.

Competition

The competition experiment was run in duplicate and the duplicates showed the same result: *Anabaena*

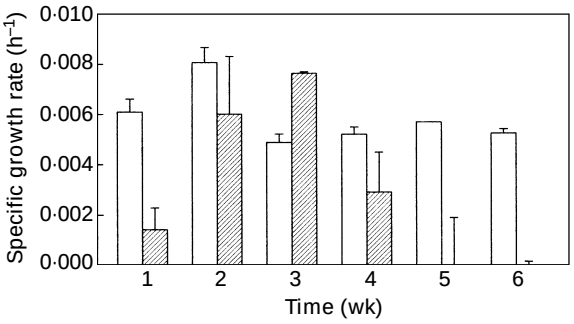

Figure 5. Specific growth rates of *Anabaena* sp. (□) and *Aphanizomenon flos-aquae* (▨) during the competition experiment shown in Figure 4. The specific growth rates were calculated from successive sampling days, and are presented as weekly averages (mean ± SD).

competitively displaced *Aphanizomenon* when the two species were grown together under light-limited conditions (Fig. 4). It should be noted that the turbidity (OD_{750}) increased during the experiment (*c.* 25–20 μmol photons m^{-2} s^{-1}), resulting in a progressive increase in the severity of light limitation, but that the decrease in the average PPFR within the culture was not (yet) enough to become disadvantageous to *Aphanizomenon*. The growth rates of both species are presented in Figure 5. Notwithstanding the qualitative outcome of the experiment, at first both species increased their growth rate. In week 3, *Aphanizomenon* even grew faster than *Anabaena*, whose growth rate had already decreased. *Aphanizomenon* collapsed in week 4, however, whilst the growth rate of *Anabaena* approached the dilution rate. Hence, *Anabaena* eventually prevailed, and seemed to be the superior competitor for light.

DISCUSSION

Light-limited growth of diazotrophs

The growth rate of light-limited cyanobacteria will depend primarily on their capability to capture light and the efficiency with which they use this light energy for growth. Under N_2-fixing conditions, *Anabaena* showed a substantially higher maximum specific growth rate (μ_{max}) than *Aphanizomenon*. The lower μ_{max} of *Aphanizomenon* eventually leads to its competitive displacement under conditions of nutrient and light excess (De Nobel *et al.*, 1997*a*). On the other hand, the slightly higher growth affinity for light of *Aphanizomenon* suggests a potential competitive advantage in case of light-limited conditions.

Growth of diazotrophs relies on a number of processes. The most obvious ones are light absorption, photosynthesis and N_2 fixation. Both species lowered their steady-state N_2 fixation activity (q_N) with decreasing PPFR, but q_N of *Aphanizomenon* was less sensitive to light-limited conditions than was that of *Anabaena*. Interestingly, under P-limited conditions, q_N of *Aphanizomenon* was also less responsive than that of *Anabaena* to changes in the severity of the limitation (De Nobel *et al.*, 1997*a*). It seems that *Aphanizomenon* has a relatively constant but low q_N, whilst *Anabaena* increases its q_N whenever possible (e.g., during the alleviation of P-limited or light-limited conditions).

Photosynthetic activity was determined as O_2 exchange in measurements of P/I curves. The initial slope of the P/I curve equals the maximum efficiency of photosynthesis (α), i.e. the apparent quantum yield of O_2 production per unit of incident irradiance. In general, photosynthetic organisms respond to a decrease in the incident irradiance by increasing α (Henley, 1993). However, the physiological acclimation of the underlying photosynthetic machinery can vary, and organisms have been grouped according to characteristic changes in their P/I curves (Richardson, Beardall & Raven, 1983). Although Wilhelm (1993) rightly advised against the interpretation of P/I curves in terms of changes in the number or size of photosynthetic units (PSU), the observed differences in the acclimatory responses of *Anabaena* and *Aphanizomenon* can easily be addressed that way. Apart from the increased α, the maximum rate of O_2 production (P_{max}) of *Anabaena* also increased (Fig. 1*a*, *c*), corresponding with an increased PSU number (*sensu* Richardson *et al.*, 1983). By contrast, the P_{max} of *Aphanizomenon* was lowered in response to decreased PPFR, whilst α (on a per-protein basis) increased to a greater extent than α in *Anabaena* (Fig. 1*b*, *d*), corresponding with an increased PSU size. However, a more comprehensive approach takes into account the relative occurrence of the two photosystems, PSI and PSII, the accessory pigments and the components of the electron transport system (Wilhelm, 1993), as discussed below. Nevertheless, as a result of these P/I curve responses, it is concluded that the light saturation value (I_k) of *Anabaena* remained relatively constant, whilst it decreased considerably in *Aphanizomenon*. Evidently, *Aphanizomenon* decreases its maximum rate of O_2 production when growing under light-limited conditions, whilst *Anabaena* maintains it at a high level. As a consequence, if the availability of light increases, *Anabaena* can quickly resume non-cyclic electron transport, thus enhancing fixation of CO_2 and, indirectly, of N_2.

The maximum efficiency of photosynthesis, or apparent quantum yield, is determined by the efficiencies of light harvesting and of photochemical conversion of absorbed light quanta, i.e. the product of the absorption cross-section and the maximum quantum yield ($\alpha = \sigma \times QY$; Tilzer, 1984). The two species not only increased their chlorophyll content with decreasing PPFR but also modulated their accessory pigments, resulting in an increased protein-specific optical absorption cross-section (σ_{prot}). Although σ_{prot} became comparable for the two species growing under LL, their patterns of

chromatic adaptation (i.e. acclimation) differed. Despite a less pronounced increase in Chl, *Anabaena* eventually showed higher σ_{chl} values, which are most probably due to its increased phycocyanin-mediated absorption. The more pronounced increase in the Chl content of *Aphanizomenon* coincided with a striking rise in the absorption of light by phycoerythrin, suggesting a capacity for complementary chromatic adaptation (Tandeau de Marsac, 1977). As a consequence of the increased absorption due to phycoerythrin in *Aphanizomenon*, this species seems better equipped than *Anabaena* to absorb green light, normally the only remaining unabsorbed portion of photosynthetically-active radiation that occurs in dense communities or at large depth, i.e. in relative darkness.

Under LL conditions the calculated maximum quantum yield for O_2 production (QY) by *Aphanizomenon* was greater than that by *Anabaena*. In response to decreased PPFR, *Aphanizomenon* increased its α considerably but its σ_{prot} only moderately, whilst the opposite was true for *Anabaena*, which showed a moderate increase of α and a strong increase of σ_{prot}. As a result, the QY of *Aphanizomenon* increased in contrast to that of *Anabaena* (see Table 2 for the calculated values). According to the fluorescence measurements, the photon yield (i.e. the quantum yield of non-cyclic electron transport) increased with decreasing PPFR but was still comparable for both species growing under ML. Unfortunately, the scatter in the data made it impossible to measure the photon yields in the LL cultures.

It is striking that the large increase in the chlorophyll (Chl) content of *Aphanizomenon* is contrasted by a more or less constant chlorophyll-specific optical absorption cross-section (σ_{chl}) and even a decreased maximum rate of O_2 production (P_{max}). This response clearly differs from that of *Anabaena*, in which increased Chl content is accompanied by an increase of both P_{max} and σ_{chl}. This indicates a fairly well-balanced increase of both Chl-containing photosystems together with a greater increase in the accessory pigments, in order to provide enhancement of the non-cyclic electron transport that underpins an increased P_{max}, and an increased σ_{chl} respectively. To explain this species-specific pattern, we postulate that *Aphanizomenon*, in addition to a qualitative change in its accessory pigments, invests its chlorophyll in PSI more than is the case in *Anabaena*.

An increase in the PSI:PSII ratio has been described for a number of growth limitations (Fujita *et al.*, 1994; Grossman *et al.*, 1994), and can be seen as a long-term acclimatory response when maintenance energy needs become relatively important. A relatively high PSI content is also advantageous after differentiation of a vegetative cell into a heterocyst, since PSI activity is the main provider of ATP and reducing power, both of which are needed for N_2 fixation (Wolk, Ernst & Elhai, 1994). Besides the decreased maximum rate of O_2 production and relatively high steady-state N_2 fixation activity in *Aphanizomenon* under LL conditions, other observations support our hypothesis of species-dependent changes in the PSI:PSII stoichiometry in response to light-limited conditions. Firstly, there is the higher maximum quantum yield for O_2 production in *Aphanizomenon*. A relatively high PSI content could increase the yield at PSII due to enhanced consumption of electrons already in the electron transport system, resulting in a higher concentration of open PSII reaction centres (Genty *et al.*, 1989). Secondly, there is the increased rate of respiration in the dark in *Aphanizomenon*. It has been shown that an increase in cyclic electron transport, facilitated by the increase in PSI content, coincides with a concerted change in cyanobacterial respiration (Jeanjean *et al.*, 1993; Hibino *et al.*, 1996). Thirdly, there is in *Aphanizomenon*, a more pronounced decrease in photon yield during the dark period. Increased respiratory electron transport, resulting from an increased PSI:PSII ratio, will still be rate-limited by the terminal cytochrome *c* oxidase (Fujita *et al.*, 1994), leading to an increasingly reduced plastoquinone pool during the dark period. This would cause a substantial lowering of the photon yield.

Competition

Surprisingly, *Aphanizomenon* was competitively displaced by *Anabaena* when growing together under light limitation. One reason for this unexpected result could be that the interactions between the two species were not determined solely by resource competition. If organisms affect each other only indirectly, via uptake of the limiting nutrient or via shading under light-limited conditions, the species able to grow at the lowest nutrient concentration or lowest light condition will competitively displace all other species (Tilman, 1982; Huisman & Weissing, 1994). However, direct interference between species might have influenced growth, and consequently, the outcome of competition. The changes in the specific growth rates of *Anabaena* and *Aphanizomenon* during the competition experiment suggest just such direct interaction. As a consequence of the increase in biomass during the experiments, light limitation became increasingly severe. The growth rate of *Aphanizomenon* surpassed that of *Anabaena* but abruptly ceased. The production of allelopathically-active compounds, whose effects correlate with cell density, could provide a plausible explanation for this pattern (Keating, 1977; Flores & Wolk, 1986; Bagchi, Chauhan & Marwah, 1993). Some strains of *Anabaena* are known to release such growth-inhibitors, and some of these compounds interfere with photosynthetic electron transport and

are presumed to be more inhibitory under light-limited conditions (Von Elert & Jüttner, 1996). However, our strain of *Anabaena* failed to produce zones of growth inhibition in an agar-diffusion assay, following the method described by Von Elert & Jüttner (1997). Nevertheless, allelopathy cannot be excluded, since our strain was not axenic. Associated bacteria might have metabolized extracellular products to a greater extent in the agar-diffusion assay where cells are spatially fixed and non-photoautotrophs will accumulate, than in continuous cultures.

An alternative explanation concerns the light climate in the cultures. In contrast to homogeneously distributed PPFRs in the dilute monoculture experiments, the cells in the dense competition experiment experienced a continuously changing PPFR upon circulation in the vessel. Generally speaking, there is no doubt that a fluctuating PPFR affects the growth of phototrophs (see, for example, Ibelings *et al.* (1994); Schubert *et al.* (1995)). If *Aphanizomenon* is less able to withstand such a dynamic light regime than *Anabaena*, this could explain the outcome of the competition experiment. In this respect, the higher maximum rate of O_2 production in *Anabaena* might be of importance, although the amplitude of the change in PPFR was rather low (at most 25 μmol photon m^{-2} s^{-1}). At this point it is unclear whether dynamic aspects of light supply and use played a role in the competitive growth studied here.

Ecological implications

The physiological evidence presented here might explain the distribution of *Anabaena* and *Aphanizomenon* in eutrophic shallow freshwater lakes, although the extent to which our results can be extrapolated to the genus level and/or to the natural occurrence of these organisms remains to be elucidated (see, for example, Janson, Carpenter & Bergman (1994); Pechar & Masojídek (1995)). However, according to a statistical analysis of field data from 80 Dutch lakes, *Anabaena* blooms coincide with higher underwater availabilities of light to a much greater extent than do *Aphanizomenon* blooms (Schreurs, 1992). This fits well with our monoculture results. For example, the broader absorption spectrum of *Aphanizomenon* (due to the presence of phycoerythrin in this strain), its higher maximum efficiency of photosynthesis, its relative high steady-state N_2 fixation activity and its slightly higher growth affinity for light under severely light-limited conditions, all characterize *Aphanizomenon* as a 'low-light' or 'shade' species (an affinity strategist *sensu* Sommer (1989)). On the other hand, *Anabaena* can be characterized as a 'sun' species, at least in comparison to *Aphanizomenon*, based on, for example, the higher maximum rate of O_2 production in *Anabaena* and its higher maximum specific growth rate (a growth strategist *sensu* Sommer (1989)). Therefore, we expect *Anabaena* to become the dominant N_2-fixing organism upon the development of N-limited conditions in an aquatic system, unless light energy is the growth-limiting factor. In practice a light limitation arises in deep or high-density (i.e. relatively dark) systems. In such systems less quickly developing blooms of *Aphanizomenon* are to be expected. For the same growth-kinetical reasons one should expect a slow replacement of *Anabaena* by *Aphanizomenon* during the development of a bloom of diazotrophs, although our competition experiment shows otherwise. High-density systems and/or dynamic light regimes as present in turbid, shallow lakes might favour *Anabaena* at the cost of *Aphanizomenon*. In general terms, N_2-fixing cyanobacteria can compete for light but, below a certain availability of light, no diazotroph will thrive, due to the higher energetic costs of N_2 fixation (De Nobel *et al.*, 1997*b*). With the degradation of the standing crop and the mineralization of the organic nitrogen pool, non-diazotrophic and less light-demanding species will take over (Zevenboom & Mur, 1980; Reynolds & Bellinger, 1992).

ACKNOWLEDGEMENTS

The authors thank P.C.M. Boers, J. Huisman, B.W. Ibelings and two anonymous referees for their comments on the manuscript. This work was supported by the Institute for Inland Water Management and Waste Water Treatment (RIZA), Lelystad, The Netherlands.

REFERENCES

Bagchi SN, Chauhan VS, Marwah JB. 1993. Effect of antibiotic from *Oscillatoria laete-virens* on growth, photosynthesis, and toxicity of *Microcystis aeruginosa*. *Current Microbiology* **26**: 223–228.

De Nobel WT, Huisman J, Snoep JL, Mur LR. 1997*a*. Competition for phosphorus between the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. *FEMS Microbiology Ecology* **24**: 259–267.

De Nobel WT, Snoep JL, Westerhoff HV, Mur LR. 1997*b*. Interaction of nitrogen fixation and phosphorus limitation in *Aphanizomenon flos-aquae* (Cyanophyceae). *Journal of Phycology* **33**: 794–799.

Droop MR. 1974. The nutrient status of algal cells in continuous culture. *Journal of the Marine Biological Association of the United Kingdom* **54**: 825–855.

Dubinsky Z, Falkowski PG, Post AF, Van Hes UM. 1987. A system for measuring phytoplankton photosynthesis in a defined light field with an oxygen electrode. *Journal of Plankton Research* **9**: 607–612.

Flores E, Wolk CP. 1986. Production, by filamentous, nitrogen-fixing cyanobacteria, of a bacteriocin and of other antibiotics that kill related strains. *Archives of Microbiology* **145**: 215–219.

Fujita Y, Murakami A, Aizawa K, Ohki K. 1994. Short-term and long-term adaptation of the photosynthetic apparatus: Homeostatic properties of thylakoids. In: Bryant DA, ed. *The Molecular Biology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 677–692.

Genty B, Briantais J-M, Baker NR. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**: 87–92.

Grossman AR, Schaefer MR, Chiang GG, Collier JL. 1994. The responses of cyanobacteria to environmental conditions: Light and nutrients. In: Bryant DA, ed. *The Molecular Biology of Cyanobacteria*. Dordrecht: Kluwer Academic Publishers, 641–675.

Henley WJ. 1993. Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *Journal of Phycology* **29**: 729–739.

Herbert D, Phipps PJ, Strange RE. 1971. Chemical analysis of microbial cells. *Methods in Microbiology* **5b**: 209–344.

Hibino T, Lee BH, Rai AK, Ishikawa H, Kojima H, Tawada M, Shimoyama H, Takabe T. 1996. Salt enhances photosystem I content and cyclic electron flow via NAD(P)H dehydrogenase in the halotolerant cyanobacterium *Aphanothece halophytica*. *Australian Journal of Plant Physiology* **23**: 321–330.

Hofstraat JW, Peeters JCH, Snel JFH, Geel C. 1994. Simple determination of photosynthetic efficiency and photoinhibition of *Dunaliella tertiolecta* by saturating pulse fluorescence measurements. *Marine Ecology Progress Series* **103**: 187–196.

Huisman J, Weissing FJ. 1994. Light-limited growth and competition for light in well-mixed aquatic environments: an elementary model. *Ecology* **75**: 507–520.

Ibelings BW, Kroon BMA, Mur LR. 1994. Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes. *New Phytologist* **128**: 407–424.

Janson S, Carpenter EJ, Bergman B. 1994. Fine structure and immunolocalisation of proteins in *Aphanizomenon* sp. from the Baltic Sea. *European Journal of Phycology* **29**: 203–211.

Jassby AD, Platt T. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography* **21**: 540–547.

Jeanjean R, Matthijs HCP, Onana B, Havaux M, Joset F. 1993. Exposure of the cyanobacterium *Synechocystis* PCC6803 to salt stress induces concerted changes in respiration and photosynthesis. *Plant Cell Physiology* **34**: 1073–1079.

Keating KI. 1977. Allelopathic influence on blue-green bloom sequence in a eutrophic lake. *Science* **196**: 885–887.

Kempers AJ, Kok CJ. 1989. Re-examination of the determination of ammonium as the indophenolblue complex using salicylate. *Analytica Chimica Acta* **221**: 147–155.

Kroon BMA, Latassa M, Ibelings B, Mur LR. 1992. The effect of dynamic light regimes on *Chlorella*. I. Pigments and cross sections. *Hydrobiologia* **238**: 71–78.

Monod J. 1942. *Recherches sur la croissance des cultures bactériennes*. Paris: Hermann.

Mur LR, Schreurs H. 1995. Light as a selective factor in the distribution of phytoplankton species. *Water Science and Technology* **32**: 25–34.

Pechar L, Masojídek J. 1995. Colonial forms of the cyanobacterium *Aphanizomenon flos-aquae* represent protection against photosystem II photo-inactivation–fluorescence quenching analysis. *Algological Studies* **77**: 37–43.

Porra RJ, Thompson WA, Kriedemann PE. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* **975**: 384–394.

Reynolds CS, Bellinger EG. 1992. Patterns of abundance and dominance of the phytoplankton of Rostherne Mere, England: evidence from an 18-year data set. *Aquatic Sciences* **54**: 10–36.

Richardson K, Beardall J, Raven JA. 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytologist* **93**: 157–191.

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. 1979. Generic assignment, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111**: 1–61.

Roijackers RMM, Joosten AMT. 1996. The trophic state of shallow lakes in The Netherlands. *Netherlands Journal of Aquatic Ecology* **30**: 219–226.

Schreiber U, Schliwa U, Bilger B. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research* **10**: 51–62.

Schreurs H. 1992. *Cyanobacterial dominance. Relations to eutrophication and lake morphology*. Ph.D. thesis, University of Amsterdam, The Netherlands.

Schubert H, Matthijs HCP, Mur LR, Schiewer U. 1995. Blooming of cyanobacteria in turbulent water with steep light gradients: The effect of intermittent light and dark periods on the oxygen evolution capacity of *Synechocystis* sp. PCC 6803. *FEMS Microbiology Ecology* **18**: 237–245.

Sommer U. 1989. The role of competition for resources in phytoplankton succession. In: Sommer U, ed. *Phytoplankton Ecology. Succession in Plankton Communities*. Berlin: Springer-Verlag, 57–106.

Tandeau de Marsac N. 1977. Occurrence and nature of chromatic adaptation in cyanobacteria. *Journal of Bacteriology* **130**: 82–91.

Tilman D. 1982. *Resource competition and community structure*. Princeton, NJ, USA: Princeton University Press.

Tilzer MM. 1984. The quantum yield as a fundamental parameter controlling vertical photosynthetic profiles of phytoplankton in Lake Constance. *Archiv für Hydrobiologie* **69** (Supplement): 169–198.

Van Liere L, Loogman JG, Mur LR. 1978. Measuring light-irradiance in cultures of phototrophic micro-organisms. *FEMS Microbiology Letters* **3**: 161–164.

Von Elert E, Jüttner F. 1996. Factors influencing the allelopathic activity of the cyanobacterium *Trichormus doliolum*. *Phycologia* **35** (6 Supplement): 68–73.

Von Elert E, Jüttner F. 1997. Phosphorus limitation and not light controls the extracellular release of allelopathic compounds by *Trichormus doliolum* (Cyanobacteria). *Limnology and Oceanography* **42.** (In press.)

Wallström K, Johansson S, Larsson U. 1992. Effects of nutrient enrichment on plankontic blue-green algae in the Baltic Sea. *Acta Phytogeographica Suecica* **78**: 25–31.

Wilhelm C. 1993. Some critical remarks on the suitability of the concept of the photosynthetic unit in photosynthesis research and phytoplankton ecology. *Botanica Acta* **106**: 287–293.

Wolk CP, Ernst A, Elhai J. 1994. Heterocyst metabolism and development. In: Bryant DA, ed. *The Molecular Biology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 769–823.

Zevenboom W, Mur LR. 1980. N_2-fixing cyanobacteria: why they do not become dominant in Dutch, hypertrophic lakes. *Developments in Hydrobiology* **2**: 123–130.