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COMPARATIVE EFFECTS OF THE QUALITY AND QUANTITY OF LIGHT AND TEMPERATURE ON THE GROWTH OF *PLANKTOTHRIX AGARDHII* AND *P. RUBESCENS*[1]

L. Oberhaus[2]

INRA-UMR CARRTEL, Équipe de Microbiologie Aquatique, 74203 Thonon Cedex, France
Ecole Nationale des Ponts et Chaussées, Centre d'Enseignement et de Recherche Eau, Ville, Environnement (Cereve), 77455 Marne-la-Vallée Cedex 2, France

J. F. Briand[2]

INRA-UMR CARRTEL, Équipe de Microbiologie Aquatique, 74203 Thonon Cedex, France
MFS, Equipe ''Chimie des Produits Naturels Marins,'' Faculté des Sciences et Techniques, Université du Sud Toulon-Var, BP 20132, 83957 La Garde Cedex, France

C. Leboulanger

INRA-UMR CARRTEL, Équipe de Microbiologie Aquatique, 74203 Thonon Cedex, France
IRD UR 167, c/o UMR CNRS 5119, CC093, Université Montpellier II, 34095 Montpellier Cedex 5, France

S. Jacquet

INRA-UMR CARRTEL, Équipe de Microbiologie Aquatique, 74203 Thonon Cedex, France

and J. F. Humbert[3]

INRA-UMR CARRTEL, Équipe de Microbiologie Aquatique, 74203 Thonon Cedex, France
Institut, Pasteur-URA CNRS 2172, Unités des Cyanobactéries, 75015 Paris Cedex, France

The effects of temperature, light intensity, and quality on the growth of the cyanobacteria *Planktothrix agardhii* (Gomont) Anag. et Komárek and *P. rubescens* (Gomont) Anag. et Komárek were assessed in batch cultures. The relative competitiveness of the green-pigmented *P. agardhii* and the red-pigmented *P. rubescens* was evaluated in separate and mixed cultures, under different light intensities and qualities (green, red, and white), and at two different temperatures, chosen as representative of the natural conditions favoring the respective blooms of each species. In monocultures, the *P. rubescens* strain appeared to be particularly well adapted to low intensities of green light and displayed a strong photoinhibition under high irradiance levels. The *P. agardhii* strain appeared less specialized with regard to light quality and also less sensitive to photoinhibition at higher irradiances. In competition experiments, temperature (15°C vs. 25°C) was the most important parameter in determining relative fitness of the species and competitive success. At 15°C, *P. rubescens* appeared to be much more competitive than *P. agardhii*, while *P. agardhii* was more competitive at 25°C. Under low irradiance, however, the pigmentation of these strains was of primary importance in determining the outcomes of the competition experiments. On the basis of our experimental results and on field observations, we propose that the successful growth leading to the proliferation of these two differently pigmented strains may largely depend on the combined conditions of light and temperature. The two strains, being genetically close relatives, could therefore be considered as two ecotypes that are adapted to different light and temperature environments. Competition experiments showed that the combination of these parameters largely controls the success of one strain in comparison to the other.

Key index words: **competition; cyanobacteria; growth; light; *Planktothrix agardhii; Planktothrix rubescens;* temperature**

***Abbreviations:* AP, allophycocyanin; DIC, dissolved inorganic carbon; OD, optical density; PAR, photosynthetic active radiation; PC, phycocyanin; PE, phycoerythrin; TCC, Thonon Culture Collection**

Competition among phytoplankton species for environmental resources remains poorly understood, especially among closely related species.

[1]Received 11 December 2006. Accepted 26 June 2007.
[2]These authors contributed equally to this work.
[3]Author for correspondence: e-mail humbert@thonon.inra.fr or humbert@pasteur.fr.

Stomp et al. (2004) suggested, through experiments and mathematical modeling, that the coexistence of red and blue-green pigmented strains of *Synechococcus* could be explained by an adaptive divergence in pigment composition, allowing the use of different light spectra (560–570 nm for the red; 620–630 nm for the blue green) for photosynthesis by these two strains of picocyanobacteria. These authors proposed, therefore, that this adaptive divergence results from a natural selection process driven by the competition for access to light, and that differences in photosynthetic characteristics promote a form of niche differentiation, thus contributing to the explanation of Hutchinson's paradox of the plankton.

As observed for the marine picocyanobacteria *Synechococcus*, two strains with contrasting pigmentation can also be identified in the freshwater and filamentous *Planktothrix* genus. *Planktothrix rubescens* is a red, phycoerythrin-rich, filamentous cyanobacterium generally occurring in deeper, stratified mesotrophic lakes in temperate areas (Jacquet et al. 2005). This species is morphologically very difficult to distinguish from *P. agardhii*, except that the latter is green and phycocyanin rich. Using a genetic approach, it has been demonstrated that these two species are probably conspecific (Humbert and LeBerre 2001), and that they indeed may constitute two ecotypes of the same species adapted to different environmental conditions. *Planktothrix agardhii* is in fact often collected in eutrophic water bodies characterized by lower depths and higher water temperatures than those where *P. rubescens* occurs (Mur and Schreurs 1995, Briand et al. 2002, Walsby and Schanz 2002).

The red and blue-green pigmented *Planktothrix* strains are generally not sympatric but have been observed to coexist in some rare cases (Davis et al. 2003). *Planktothrix agardhii* is widely distributed in temperate areas, whereas *P. rubescens* occurs in a restricted number of lakes, most often located in mountainous areas or in northern latitudes. Thus, *P. rubescens* appears to be a very efficient species in occupying ecosystems characterized by low light and temperature conditions, while *P. agardhii* seems to be a species that typically grows in the first few meters of the water column or in shallow waters, where irradiance and temperature are greater.

With the goal of testing the ability of the two strains to occupy different niches, we evaluated their relative fitness (expressed as growth rate) in variable environments defined by light quality and quantity, and temperature. In a first experimental protocol, the growth of each strain was estimated in monocultures exposed to different light and temperature conditions, while in the second one, mixed cultures of the two *Planktothrix* strains were used to evaluate their relative competitive capacities.

MATERIALS AND METHODS

Culture conditions. Clonal, nonaxenic strains of *P. rubescens* (TCC 29-1, Thonon Culture Collection ref 29-1) and *P. agardhii* (TCC 83-2) were used, after isolation from two French subalpine lakes, Lake Bourget (45°44′ N, 5°52′ E) and Lake Nantua (46°10′ N, 5°35′ E), respectively. A preculture of each strain was grown under continuous conditions in a 2 L turbidostat (Feuillade and Feuillade 1979) supplied with Z medium (Zehnder in Staub 1961; composition: $NaNO_3$, 0.467 g · L^{-1}; $MgSO_4$, $7H_2O$ 0.025 g · L^{-1}; K_2HPO_4, 0.031 g · L^{-1}; $Ca(NO_3)_2$, $4H_2O$, 0.059 g · L^{-1}; Na_2CO_3, 0.021 g · L^{-1}; FeEDTA; and a micronutrient solution containing trace amounts of 14 essential metals) at 20°C. Precultures were grown under 20 μmol photons · m^{-2} · s^{-1} of white light, provided by daylight fluorescent tubes (OSRAM Lumilux® ''de luxe''; OSRAM, München, Germany), on a 16:8h light:dark (L:D) cycle. Inocula of these exponentially growing precultures were then used to prepare batch cultures in autoclaved 250 mL Pyrex Erlenmeyer flasks containing 100 mL of Z medium. All experimental light conditions were also provided by OSRAM Lumilux® ''de luxe'' daylight fluorescent tubes on a 16:8 L:D cycle. Experimental cultures were illuminated by fluorescent tubes placed on two sides, in a V formation. Light intensity gradients were obtained by placing Erlenmeyer flasks at variable distances from the light source. Light intensity was measured in the center of Erlenmeyer flasks filled with 100 mL of culture medium, using a spherical microquantum sensor (US-SQS; LI-COR, Lincoln, Nebraska, USA) and a LI-1400 data logger (LI-COR). Culture flasks were returned to the exact placements corresponding to measured light intensities after each growth measurement, and lamps were kept stationary. Experiments were performed in temperature-controlled growth chambers, and temperature was monitored regularly. Variations in light quality were created using different filters placed around the lamps supporting the fluorescent tubes: Leefilters® no. 121 (Lee Filters, Hampshire, UK) produced ''green light'' (absorbing 88% of light in the blue range, 32% of that in the green, and 80% of light in the red = 12% blue light + 68% green light + 20% red light), and no. 022 produced ''red light'' (absorbing 100% of light in the blue range, 90% of that in the green, and 10% of that in the red = 0% blue light + 10% green light + 90% red light), while ''white light'' resulted from an absence of filters (spectra available on the Leefilters® Web site, http://www.leefilters.com/).

Experimental cultures were briefly agitated by hand on a daily basis to assure homogenization inside the flask and to avoid significant sedimentation, clumping, or flotation of the cyanobacteria. Cell density was estimated by determining the optical density (OD) at 750 nm for the two strains. OD was measured using a Perkin-Elmer® Lambda 2 Spectrophotometer (Perkin-Elmer Life and Analytical Sciences, Waltham, MA, USA). Batch cultures were started at low cell concentrations ($OD_{750} \sim 0.06$) to avoid any self-shading effects on early growth. Culture sampling was performed approximately every 3 d under sterile conditions for 21 d, which corresponded to the duration of the exponential growth phase at 15°C (Briand et al. 2005).

Light absorption spectra for *P. rubescens* and *P. agardhii* were measured for diluted in vivo cultures, using a Perkin-Elmer Lambda 2 Spectrophotometer. Centered absorbance spectra showed peaks for *P. agardhii* at ~430, 625–630, and 680 nm, and at ~430, ~525–575, and 680 nm for *P. rubescens* (Fig. 1). These values correspond to absorption of light in the blue, red-orange, and red spectra by *P. agardhii*, due to the presence of chl *a* and large amounts of phycocyanin, and to absorption of light in the blue, green-yellow, and red spectra by *P. rubescens*, due to the principal presence of chl *a* and phycoerythrin. These two strains are not known to show chromatic acclimation.

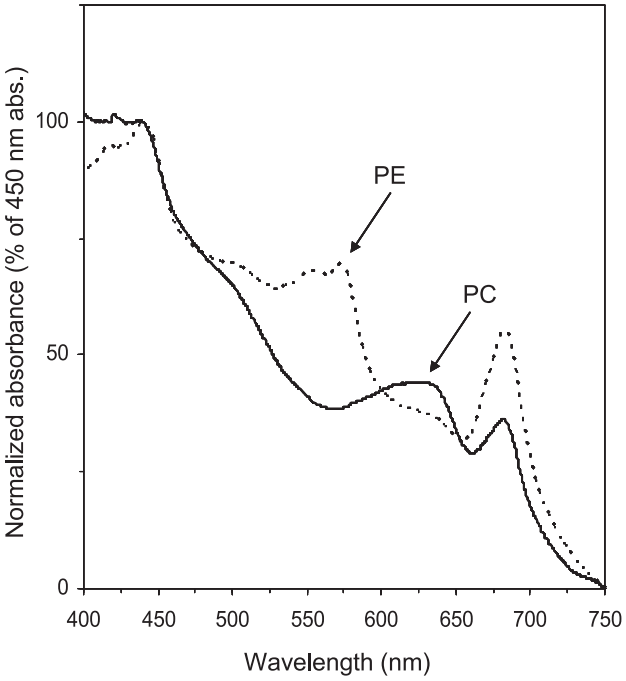

FIG. 1. Light absorption spectra (centered absorbance data) for *Planktothrix rubescens* (broken line) and *P. agardhii* (solid line). PE, phycoerythrin; PC, phycocyanin.

Growth characterization under different light conditions. For the variable light experiments using monocultures, one culture of each strain was grown at 12–14 light intensities in white, green, and red light at 15°C and 25°C. A range of light intensities from 3 to ~300 μmol photons · m^{-2} · s^{-1} was used for most experiments, with the exceptions of *P. rubescens* under white light at 15°C (I_{max} = 197 μmol photons · m^{-2} · s^{-1}), *P. agardhii* under red light at 15°C (I_{max} = 234 μmol photons · m^{-2} · s^{-1}), and both strains under green light at 25°C (I_{max} = 246 and 245 μmol photons · m^{-2} · s^{-1}, respectively). pH levels were measured at the end of our batch culture experiments conducted at 25°C in white light (mean = 8.9, n = 10, SD = 0.47). These values were slightly lower than those of turbidostat cultures from which all batch cultures originated (mean = 10, n = 2, SD = 0.42), meaning that no carbon limitation occurred during the experiments.

A maximum growth rate at low cell densities μ_{log} (d^{-1}) was obtained for each strain, grown at each experimental light intensity, and for all light type–temperature combinations using a solution for the classic logistic growth model (Verhulst 1838, 1845):

$$N_t = \frac{KN_0}{N_0 + (K - N_0)\,e^{-\mu_{log} t}} \quad (1)$$

where N_t is the biomass at time t, measured as OD; N_0 is the biomass of the inoculate; and K is the carrying capacity of the culture environment (i.e., the maximum attainable biomass K in the given environment). The adjustment to measured OD data was carried out using the least-squares method, over the duration of each experiment (i.e., ~21 d). Resulting values for μ_{log} were plotted against light intensity (Fig. 2). An exponential equation including a correction for photoinhibition was used to plot the theoretical growth (μ_{theo}) curve as a function of light intensity (I). It is based on the equation of photosynthesis versus irradiance relationship

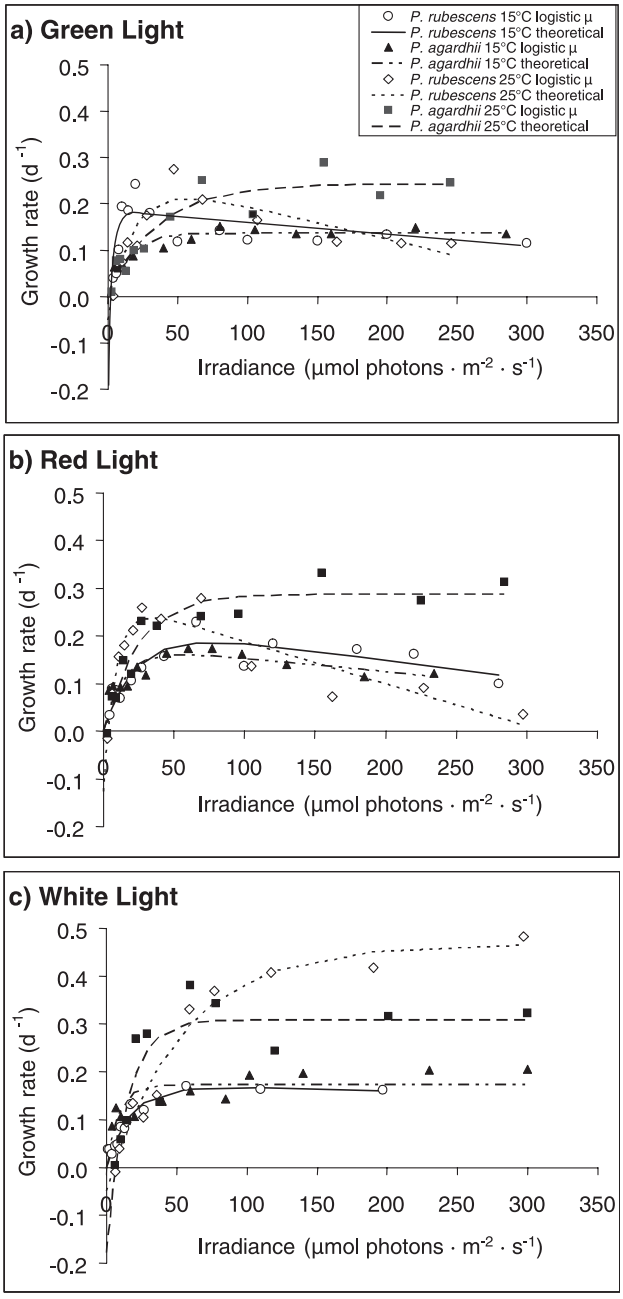

FIG. 2. Growth rate versus irradiance curves for *Planktothrix rubescens* and *P. agardhii* in green (a), red (b), and white (c) light at 15°C and 25°C. Points represent overall experimental growth rates obtained from the logistic model (parameter μ_{log}, eq. 1), whereas lines represent modeled values for μ_{theo} (eq. 2) adjusted to all points shown, for each light intensity (I).

used by Webb et al. (1974) and modified by Bright and Walsby (2000), for which we replaced photosynthesis rates by growth rates:

$$\mu_{theo} = \mu_{max}\left[1 - e^{(-\alpha I/\mu_{max})}\right] + R + \beta I \quad (2)$$

where μ_{max} is the maximum growth rate, α is the initial slope of the light-limited portion of the μ versus I curve, I is the light intensity, R is the estimated value for growth in the dark (i.e., should be negative due to the respiratory consumption of organic carbon and subsequent biomass decrease), and β

is a parameter describing photoinhibition. The least-squares method was once again used, in this case to fit this model to μ_{log} values. A range of parameter values (μ_{max}, α, R, and β) was obtained for each experiment using the jackknife method (Efron and Tibshirani 1993, Dagnelie 1998, Palm 2002). Standard error was calculated for parameter estimates using the formula:

$$\sigma^*_{\theta^*_J} = \sqrt{\frac{n-1}{n}\sum_{i=1}^{n}(\theta^*_{(-i)} - \theta^*_J)^2} \quad (3)$$

with

$$\theta^*_J = \frac{1}{n}\sum_{i=1}^{n}\theta^*_{(-i)} \quad (4)$$

where $\theta^*_{(-i)}$ is the estimated parameter value obtained by elimination of the observation i. All model adjustments were performed by the Solver function found in Excel® software (Microsoft, Redmond, WA, USA).

Competition between P. rubescens *and* P. agardhii. For mixed culture experiments, inocula from the two turbidostats were mixed at equal trichome densities (OD_{750} = 0.06) and in equal volumes in each sterilized 250 mL Erlenmeyer flask, for a total culture volume of 100 mL. Experiments were performed in triplicate at 15°C and 25°C and in white, green, and red light. Three different light intensities (10, 50, and 120 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) were tested. Relative abundance of *P. rubescens* and *P. agardhii* filaments was evaluated at experiment onset and then at day 15 and ~day 30, and filaments were fixed with formaldehyde (1.5% of total volume). Experiments were stopped ~day 30 to prevent nutrient-limitation effects. Filament measurements and counting were performed for 1 mL of well-mixed sample using the technique of Utermöhl inverted microscope (Axiovert 200; Carl Zeiss, Oberkochen, Germany) technique (Uthermöhl 1958). Strains were identified based on their visible differences in diameter and color. Biovolumes were calculated from filament length measurements and the previously measured average diameters of each strain (considering *P. rubescens* trichomes as cylindrically shaped, with a mean width of 6 μm, and *P. agardhii* trichomes as having a mean width of 4 μm).

RESULTS

Monoculture experiments. For the duration of experiments, growth was kept exponential for most of the time. By using growth rates calculated from the logistic model instead of the exponential growth model, effects of medium limitation (including nutrient and dissolved inorganic carbon [DIC] limitation, self-shading, etc.) did not affect comparisons between experimental conditions. The resulting μ versus I curves for both strains from the six light quality–temperature combinations are shown in Fig. 2. Growth rates were higher at 25°C than at 15°C (Sign test on μ_{max} values, $P < 0.05$; Fig. 3c), with the only exception being *P. rubescens* in green light. OD_{750} at the end of monoculture experiments ranged from 0.05 to 1.26 and 0.03 to 0.83, respectively, at 25°C and 15°C. In addition, photoinhibition was more frequently (Fisher exact probability test, $P < 0.05$) observed in *P. rubescens* ($\beta < 0$ in five of six experiments) than for *P. agardhii* ($\beta < 0$ in one of six experiments; Figs. 2 and 3b).

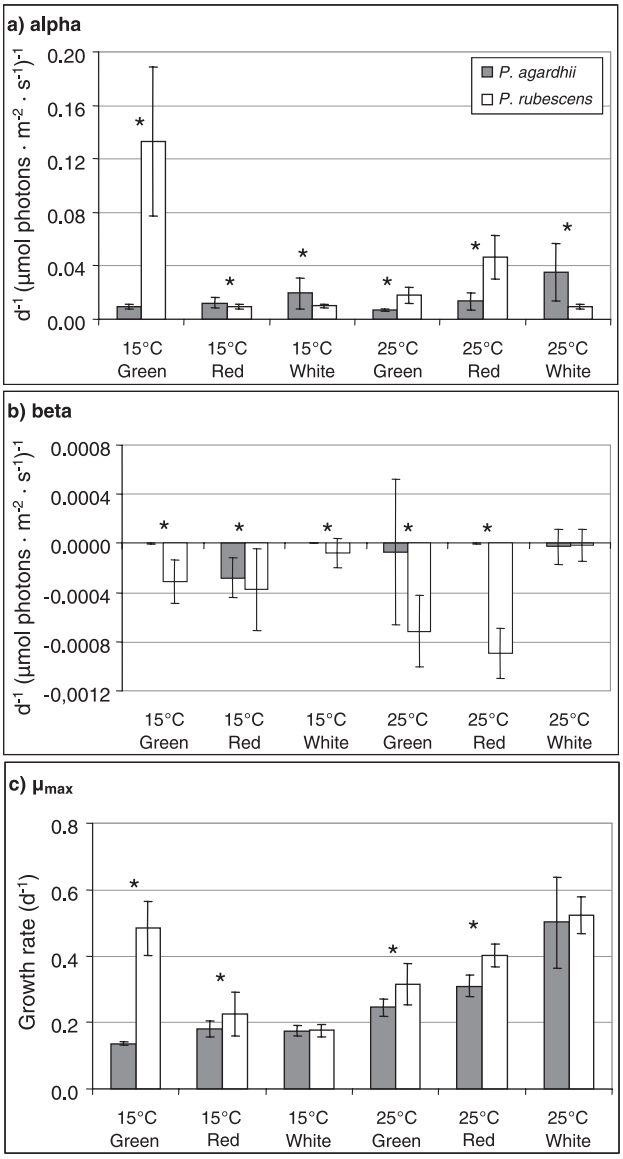

FIG. 3. Estimated parameter values for alpha (a), beta (b), μ_{max} (c), and corresponding standard errors obtained using the jackknife (eqs. 3 and 4). Significant differences (Mann–Whitney, $P < 0.01$) between parameters for *Planktothrix agardhii* and for *P. rubescens* are evidenced by * in the figure.

Differences in parameter estimates obtained for the two strains were significant (Mann–Whitney, $P < 0.01$) for most experimental conditions tested (Fig. 3). The most pronounced difference observed between the two strains was the higher growth performance of the red-pigmented *P. rubescens* at very low intensities of green light at 15°C (Fig. 2a). This phenomenon is reflected by initial slope values (α; Fig. 3a), for which *P. rubescens* showed the highest value of all experiments (0.133 [d^{-1}][μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$]$^{-1}$), including that of *P. agardhii* for the same conditions (0.0095 [d^{-1}][μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$]$^{-1}$). In green light, *P. rubescens* also showed the greatest value for μ_{max} (0.485 $\cdot$ d^{-1})

of all experiments performed at 15°C (Fig. 3c). This value was most closely comparable to μ_{max} values for *P. agardhii* ($0.501 \cdot d^{-1}$) and *P. rubescens* ($0.523 \cdot d^{-1}$) grown under white light at 25°C.

In red light, there were notable differences between growth efficiencies of the two strains at 25°C. *P. rubescens* showed a higher value for α at 25°C ($0.046\ [d^{-1}][\mu mol\ photons \cdot m^{-2} \cdot s^{-1}]^{-1}$) than *P. agardhii* ($0.013\ [d^{-1}][\mu mol\ photons \cdot m^{-2} \cdot s^{-1}]^{-1}$; Fig. 3a). Maximum growth rates (μ_{max}) between the two strains also varied under these experimental conditions, that of *P. rubescens* ($0.402\ d^{-1}$) being greater than that of *P. agardhii* ($0.309\ d^{-1}$; Fig 3c). These conditions also provided the greatest difference in photoinhibition between the two strains, as shown by β values (-8.95×10^{-4} for *P. rubescens* and -1.67×10^{-7} for *P. agardhii*; Fig. 3b).

The greatest observed growth rates of both strains occurred in white light at 25°C, where values for μ_{max} more than doubled from those observed at 15°C (Figs. 2c and 3c). Little photoinhibition was observed for this light type (Fig. 3b). The three cases in which differences between parameter estimates for the two strains were not statistically significant (Mann–Whitney *U*-test) corresponded to cultures grown in white light. This was the case for estimates of β for cultures grown at 25°C, and also that of μ_{max} for cultures grown at 15°C and 25°C (Fig. 3, b and c).

Mixed-culture competition experiments. In terms of total biomass, greater biovolumes were often observed at 50 or 120 rather than at 10 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (data not shown) in mixed cultures. In comparing average relative abundances of *P. rubescens* and *P. agardhii* observed initially and at the end of competition experiments for the three light intensities tested (10, 50, and 120 μmol photons $\cdot m^{-2} \cdot s^{-1}$), it appears that at 15°C, *P. rubescens* dominated at the end of seven of the nine experimental conditions tested, while at 25°C, *P. agardhii* dominated at the end of seven of the nine experiments (Fig. 4). However, a few exceptions to this temperature-based trend were observed. The first occurred in low-level green light at 25°C (Fig. 4d). At 10 μmol photons $\cdot m^{-2} \cdot s^{-1}$, replicates showed that *P. agardhii* filaments comprised 34, 67, and 87% of the total biovolume, and 10, 13, and 83% at 50 μmol photons $\cdot m^{-2} \cdot s^{-1}$. The second exception occurred in low red light at 15°C (again 10 and 50 μmol photons $\cdot m^{-2} \cdot s^{-1}$), where the proportions of *P. agardhii* and *P. rubescens* remained relatively stable over time, in comparison with other results at this temperature (Fig. 4b).

Several examples of complete or near-complete dominance at days 29–32 were observed. *P. rubescens* at 15°C and 10 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of green light (Fig. 4a) was 99.98% of the total biovolume in the three replicates. Another involved *P. agardhii* at 25°C and 120 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of red light (Fig. 4e), which dominated at an average of 99.6 ± 0.8%. Strong dominance was also shown by *P. agardhii* at 25°C, at 50 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of red light (99.1 ± 1.5%) and at 120 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of green light (97.9 ± 2.7%; Fig. 4, e and d).

DISCUSSION

These experiments were designed to examine the selective advantage that the different pigments of the strains *P. rubescens* (chl *a*; allophycocyanin [AP], phycocyanin [PC], phycoerythrin [PE]) and *P. agardhii* (chl *a*, AP, and PC) may confer on them, allowing them to successfully inhabit their respective niches and proliferate in water bodies they occupy. Monoculture experiments first confirmed that the two species shared certain ecophysiological characteristics, such as relatively low growth rates and tolerance of low light and low temperature. Previous studies have shown the capabilities of *P. agardhii* and *P. rubescens* to grow effectively at low light intensities (Zimmermann 1969, Van Liere and Mur 1978, Mur and Schreurs 1995, Bright and Walsby 2000), but few have examined differences in growth over a range of light and temperature combinations. However, despite their shared capacities of growth at low irradiances, *P. rubescens* and *P. agardhii* occupy different ecological niches as shown by their respective classification into two different functional groups: R (inhabits metalimnia of mesotrophic stratified lakes; low-light tolerance; sensitive to instability) and S1 (inhabits turbid mixed layers; tolerance of highly light-deficient conditions; sensitivity to flushing; Reynolds et al. 2002). Our results revealed that the red *P. rubescens* performed particularly well in low quantities of green light at 15°C (Figs. 2a and 3a), which corresponds well with temperature and light-quantity conditions present at its habitual metalimnetic position (Walsby and Schanz 2002, Davis et al. 2003, Jacquet et al. 2005).

Monoculture experiments also showed that photoinhibition occurred more frequently for *P. rubescens* than for *P. agardhii* under most conditions tested. In green light, *P. rubescens* was strongly photoinhibited at high light intensities, probably due to the fact that this species is particularly well adapted to the use of low quantities of light and thus is less able to persist in certain high irradiance levels. In red light, growth of *P. agardhii* was only slightly photoinhibited, which is in agreement with the fact that this species can inhabit the first few meters of lakes and ponds, where it can be temporally exposed to high irradiance levels (Briand et al. 2002). Under white light, growth rates of both strains were not greatly reduced at high intensities, perhaps since the amount of light received at each wavelength (i.e., green, red, etc.) was small compared with the amount that the cultures received in filtered-light experiments. For example, for a given light intensity, the quantity of photons at red

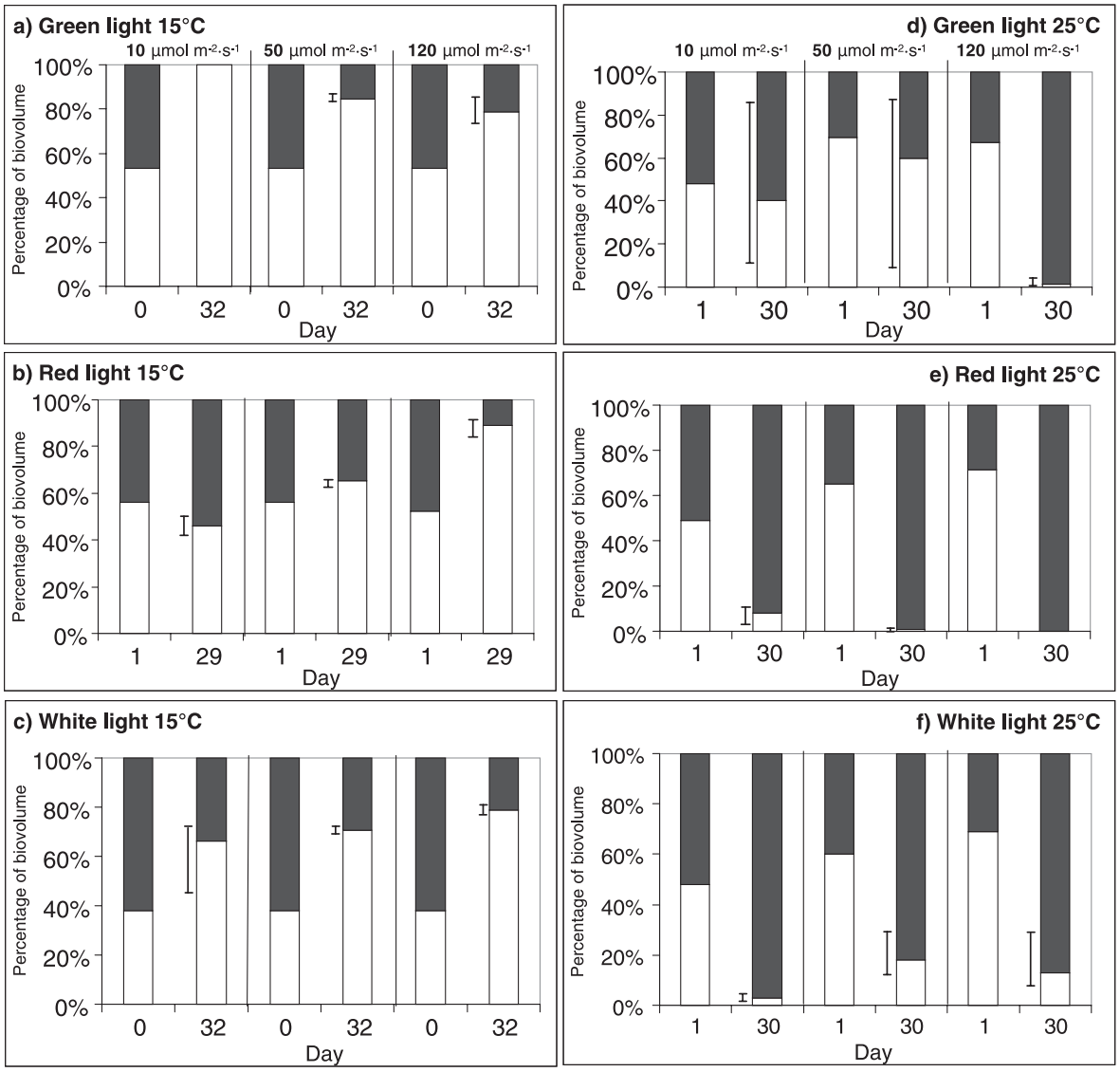

FIG. 4. Relative abundances of *Planktothrix rubescens* (white) and *P. agardhii* (black), beginning and end of experiment, in mixed-culture competition experiments. Percentages are based on microscope-determined biovolumes ($mm^3 \cdot mL^{-1}$) averaged for three experimental replicates. Minimum and maximum relative abundances obtained at the end of the experiment for the three experimental replicates are indicated by bars.

wavelengths is greater in red light than in white light, where photons are distributed among the different wavelengths. Considering that the pigments in the photosynthetic antennae absorb at specific wavelengths (primarily red wavelengths for *P. agardhii*; green wavelengths for *P. rubescens*), white light may thus be less damaging than filtered red and green light. A lack of growth photoinhibition at high white-light intensities (200 µmol photons $\cdot m^{-2} \cdot s^{-1}$) was also observed for *P. rubescens* by Bright and Walsby (2000).

Some otherwise surprising results observed in monoculture experiments might be explained by the fact that the filters used did not allow a complete selection of the desired wavelengths. This possibility could explain, for example, why *P. agardhii* outperformed *P. rubescens* at higher intensities of green light, in fact composed of 20% red light, although this outcome was not expected. Finally, monoculture experiments suggested that *P. agardhii* appeared less light-spectrum specialized than *P. rubescens,* which displays a more niche-specific pigment composition (Utkilen et al. 1985, Feuillade 1994). Indeed, the α and μ_{max} parameters (Fig. 3) varied greatly for *P. rubescens* in regard to the light quality, which was not as much the case for *P. agardhii.*

In the mixed-culture competition experiments, temperature clearly appeared to be the key parameter in determining the performance of one strain as compared to the other, *P. rubescens* generally dominating at 15°C, and *P. agardhii* at 25°C. The

exceptions to this trend occurred in low intensities of light qualities thought to favor each strain (i.e., green light for *P. rubescens* and red light for *P. agardhii*). In the case of low-intensity red light at 15°C, replicate responses were similar, showing relatively stable abundances over time. While in low-intensity green light at 25°C, the observed apparent stability of average proportions over time was in fact due to large variation among replicates. Perhaps the greater quantity of red light transmitted by the green filters (20%) may have played a role. However, it is possible that an exception to the temperature trend occurred in these conditions, given the dominance of *P. rubescens* in low-intensity green light at 15°C. These exceptions may represent environmental conditions that allow coexistence of the two strains.

From competition results, we can conclude that in general, temperature, which influences many cellular processes related to growth, may be the most important factor in assessing dominance between these two strains, whereas light quality and quantity, whose influence depends more on photosynthetic pigments, might jointly be slightly less important.

In monocultures, if the greatest interstrain differences in values for each parameter are examined, these occur in green light at 15°C for α and μ_{max}, and in red light at 25°C for β. These conditions (low green light, higher red light) also correspond to two of the four observed examples of complete or near-complete dominance by one strain in competition experiments. In our study, the mixed-culture approach used seemed to better highlight the effects of small though important differences in growth rates between the two strains as well as the conditions under which these differences occur. For example, whereas no significant difference between strains was observed for μ_{max} values from monocultures under white light, these same conditions produced dominance of one strain over the other in mixed cultures. The outcome of such conditions would have been difficult to predict based solely on the monoculture results, and this points out the subtlety and complexity of competition mechanisms between strains. Competition experiments of longer length may have produced different final outcomes (Huisman et al. 1999, Stomp et al. 2004), but to avoid the effects of nutrient limitation in our batch cultures, we chose to limit our experiments to 30 d. This length allowed us to test the effects of temperature and light during the mostly exponential phase of growth.

The experimental results in general coincide with field observations in which the combined effects of light and temperature have also been reported. In Lake Bourget, a deep (z_{max} = 142 m) lake located near the French Alps, *P. rubescens* proliferates frequently (Oberhaus et al. 2003, Jacquet et al. 2005). As with *P. rubescens* in Lake Zürich (Micheletti et al. 1998, Walsby and Schanz 2002) and Blelham Tarn (Davis et al. 2003), the stratified population typically undergoes an important biomass increase in the summer metalimnion (z_{meta} = 12–15 m in Lake Bourget), whose temperature often lies near 15°C. Field measurements of spectral profiles from Lake Bourget (data not shown) showed that as in other inland water bodies poor in yellow substances (Zimmermann 1969, Kirk 1994), green light predominated at this depth. Thus, the low-intensity green light and cooler temperature that seemed to favor *P. rubescens* in the laboratory correspond to the conditions in the late spring and summer metalimnion and help explain its important summer biomass increase. The strain of *P. rubescens* that proliferates in Lake Bourget showed comparable growth rates at temperatures of 15, 20, and 25°C at 30 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of white light, and greatly decreased growth rates at l0, 30, and 35°C (unpublished data). The combination of the lower temperature, although not optimal for growth, and very low intensities of a light quality resulting in an abundance of phycoerythrin seem to result in favorable growth for *P. rubescens* in this combination of otherwise suboptimal conditions. Many algae are unable to grow under such conditions, allowing *P. rubescens* metalimnetic summer biomass increases with little competition. Zimmermann (1969) noted that in Lake Lucerne the population maximum of *P. rubescens* stratified at depths where the intensity of green light was at 1%–9% of that occurring at the surface and where temperature ranged from 10°C to 15°C. He concluded that this combination was responsible for the formation of *P. rubescens* biomass maxima at metalimnetic depths.

In the case of *P. agardhii*, conditions favoring its growth in our experiments also correspond to those of the habitats in which it often occurs. Its increased growth performance at 25°C is suggestive of the warmer summer epilimnion temperature or possibly that of shallower water bodies, such as those in which it proliferates in the Netherlands (Mur and Schreurs 1995), Germany (Wiedner et al. 2002), or in France near Paris (Briand et al. 2002). *Planktothrix agardhii* was less easily photoinhibited than *P. rubescens*, especially in red light at 25°C, making it better suited to growth nearer the surface, and it also exhibited versatility, growing well at low intensities of white light. We assume that low-light tolerance may also be useful in shallow lakes, where despite heavy self-shading, it may grow and proliferate throughout a well-mixed water column. In addition, its tolerance of lower temperatures may allow it to survive throughout the year, including during winter months (Briand et al. 2002, Wiedner et al. 2002).

Because of the coexistence of *P. rubescens* and *P. agardhii*, the case of Blelham Tarn, English Lake District (Davis et al. 2003), is relevant to our study. The changes in the vertical distributions of

P. rubescens and *P. agardhii* filaments in Blelham Tarn were related to vertical profiles of temperature and light attenuation and to continuous records of surface irradiance. Stomp et al. (2007) provide an interesting hypothesis for the coexistence of these two strains, based on turbidity. They observed that red and green picocyanobacteria often coexisted in intermediately turbid waters, whereas red strains dominated in clear lakes and oceans, and green strains in turbid lakes. Davis and Walsby (2002) noted that the potential growth of *P. agardhii* and *P. rubescens*, individually determined in white light, highlighted two factors that may vary between these two organisms and affect their growth in addition to their pigmentation: cell size and gas vesicles. Thus, in rare cases of coexistence of these two strains, mutually favorable spectral and temperature conditions, or perhaps factors other than light climate and temperature, could be determinant.

Based on our experimental results and on field observations, we might conclude that the successful growth of these two strains depends on the combined conditions of light and temperature. Due to its high concentrations of PE, allowing the capture of low quantities of green light, and to its ability to grow at low temperatures, *P. rubescens* seems very efficient in occupying the metalimnic layer of deep lakes in late spring and summer, or the entire water column in winter. In contrast, *P. agardhii* is more efficient in growing in the upper water column, due to its ability to absorb sufficient energy from the entire PAR spectrum in a very turbid and relatively low-irradiance environment, and also due to its resistance to photoinhibition in higher irradiance levels. This cyanobacterium is one of the most frequently observed in water blooms in temperate zones during the summer. However, *P. agardhii* is also able to survive in winter and form perennial blooms in shallow eutrophic lakes in temperate areas (Briand et al. 2002).

As reported with comparable experiments on two closely related marine picocyanobacteria of the genus *Synechococcus*, also principally differentiated by their pigment composition (Stomp et al. 2004), differences in photosynthetic characteristics may offer subtle opportunities for niche differentiation in the same water layer. In addition, this pigment differentiation within *Synechococcus* and *Planktothrix* genera could allow the occupation of a larger range of niches within an ecosystem (i.e., metalimnic vs. epilimnic layers in deep lakes) or a larger range of ecosystems (i.e., shallow vs. deep lakes). But our study showed that these two *Planktothrix* strains may also be differentiated by their responses to temperature. The mechanisms regulating the combined influence of light and temperature or other factors on competitive success remain relatively unclear. With this perspective in mind, current work on the sequencing of the genomes of these two *Planktothrix* strains should help us to identify specific metabolic pathways involved in their adaptations to environmental conditions.

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