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Colourful coexistence : a new solution to the plankton paradox

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Chapter 6

The time scale of phenotypic plasticity, and its impact on competition in fluctuating environments

Abstract

Although phenotypic plasticity can be advantageous in fluctuating environments, it may come too late if the environment changes fast. Complementary chromatic adaptation is a colourful form of phenotypic plasticity, where cyanobacteria tune their pigmentation to the prevailing light spectrum. Here, we study the time scale of chromatic adaptation, and its impact on competition among phytoplankton species exposed to fluctuating light colours. We parameterized a resource competition model using monoculture experiments with green and red picocyanobacteria, and the cyanobacterium *Pseudanabaena* that can change its colour within ~7 days by chromatic adaptation. The model predictions were tested in competition experiments, where the incident light colour switched between red and green at different frequencies (slow, intermediate and fast). *Pseudanabaena* (the flexible phenotype) competitively excluded the green and red picocyanobacteria in all competition experiments. Strikingly, the rate of competitive exclusion was much faster when the flexible phenotype had sufficient time to fully adjust its pigmentation. Thus, the flexible phenotype benefited from its phenotypic plasticity if fluctuations in light colour were relatively slow, corresponding to slow mixing processes or infrequent storms in their natural habitat. This shows that the time scale of phenotypic plasticity plays a key role during species interactions in fluctuating environments.

This chapter is based on the paper: Stomp M, MA van Dijk, HMJ van Overzee, M Wortel, C Sigon, M Egas, H Hoogveld, HJ Gons, and J Huisman. The time scale of phenotypic plasticity, and its impact on competition in fluctuating environments. *American Naturalist* (in press).

Introduction

Fluctuations in environmental conditions pose serious challenges to organisms. Many organisms respond to environmental changes by physiological and morphological adaptations. This flexible strategy, known as phenotypic plasticity, may improve their fitness in the new environments (Agrawal 2001). For example, plants increase their leaf area during periods of reduced light (Sultan & Bazzaz 1993), cladocerans develop armoured helmets in the presence of predators (Woltereck 1909; Laforsch & Tollrian 2004), and some green algae aggregate into colonies to reduce their edibility for grazers (Hessen & Van Donk 1993; Lampert *et al.* 1994).

Intuitively, phenotypic plasticity seems a suitable strategy to cope with environmental fluctuations. However, adaptation takes time. If adaptation is too slow, organisms will not be able to keep up with changes in their environment, resulting in a permanent mismatch between the physiology of the organisms and their environmental conditions. Indeed, theory shows that adaptation can even be disadvantageous when it has a strong time delay (Padilla & Adolph 1996; Gabriel 2005). Yet, although many studies have investigated phenotypic plasticity in fluctuating environments (e.g., Chesson *et al.* 2004; Egas *et al.* 2004; Abrams 2006*a*, 2006*b*; Gélinas *et al.* 2007; Van der Stap *et al.* 2007), the time scale of phenotypic adaptation has received surprisingly little attention (Miner *et al.* 2005).

The colourful process of complementary chromatic adaptation is a spectacular form of phenotypic plasticity found in many cyanobacteria. During chromatic adaptation, cyanobacteria change their pigment composition, and thereby their colour (Gaiducov 1902; Tandeau de Marsac 1977; Grossman *et al.* 1993; Kehoe and Gutu 2006). In the presence of green light, they turn their accessory pigments into the complementary colour. That is, they become red. Conversely, in the presence of red light, they turn green. This complementary adaptation optimizes light absorption, and thus favors their photosynthesis and growth. The ecological significance of the spectral tuning of the pigment composition to the ambient light colour is debated for macroalgae (Dring 1981; Ramus 1983). However, theory, competition experiments, and field studies have shown that the underwater light colour is a major selective factor for phototrophic microorganisms (e.g., Montesinos *et al.* 1983; Béjà *et al.* 2001; Rocap *et al.* 2003; Stomp *et al.* 2004). For instance, we showed in a series of competition experiments that red picocyanobacteria win the competition in green light, green picocyanobacteria win the competition in red light, while red and green strains can coexist in the full light spectrum provided by white light (Stomp *et al.* 2004). This matches their field distributions, where red cyanobacteria dominate in clear waters in which green light penetrates the deepest, whereas green cyanobacteria dominate in turbid waters in which red light penetrates the deepest (Pick 1991; Vörös *et al.* 1998; Wood *et al.* 1998; Vila and Abella 2001; Stomp *et al.* 2007*a*, 2007*b*). Coexistence of red and green cyanobacteria is widespread in waters of intermediate turbidity (Stomp *et al.* 2007*a*).

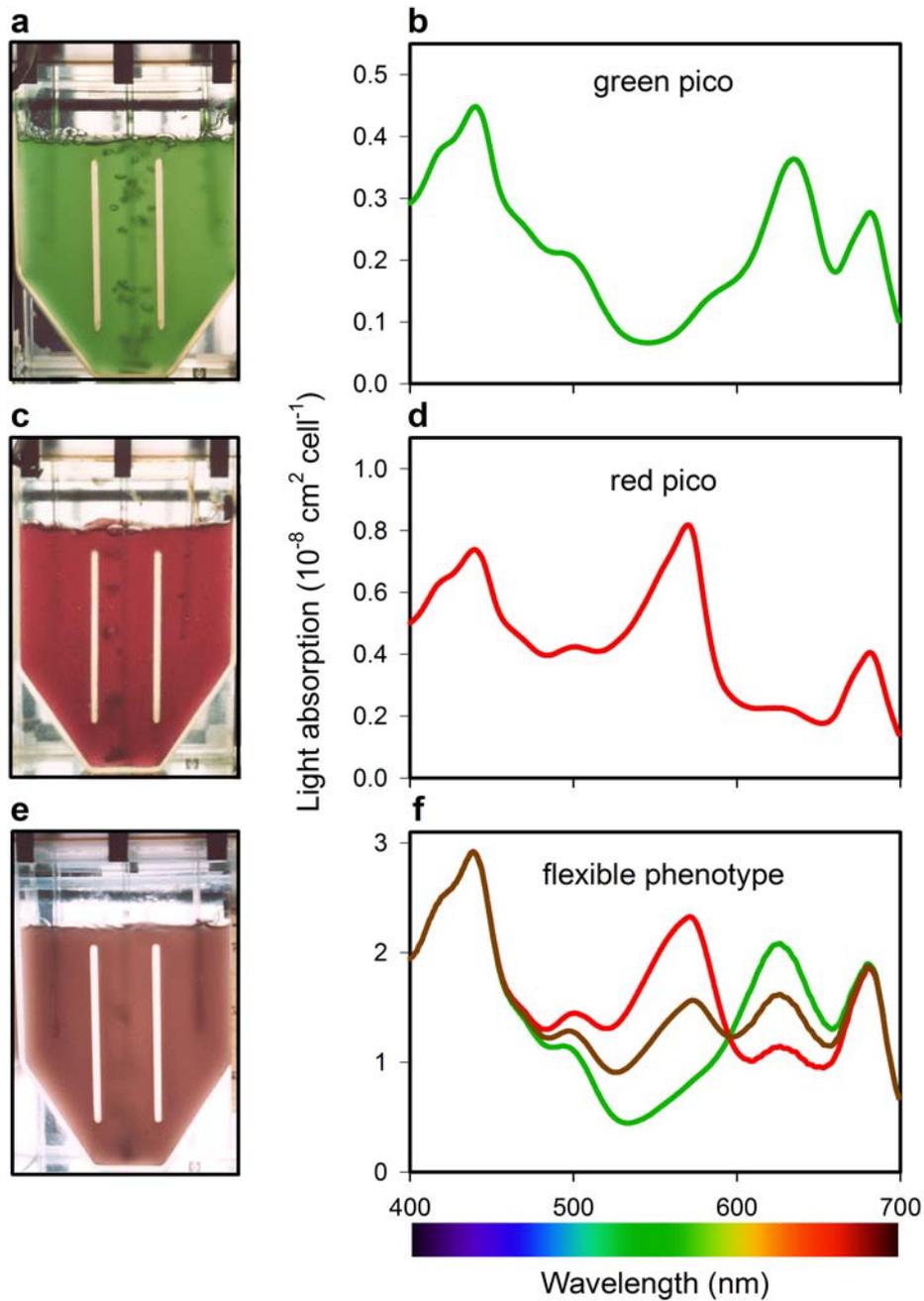


Figure 6.1 Optical characteristics of *Cyanobium* CCY9201 ('green pico'), *Cyanobium* strain CCY9202 ('red pico') and *Pseudanabaena* CCY9509 ('flexible phenotype'). Panels on the left show monocultures of the three strains in light-limited chemostats. Panels on the right show their light absorption spectra. The flexible phenotype can tune its light absorption spectrum to the prevailing light conditions by changing its cellular content of phycoerythrin (absorption peak at ~560 nm) and phycocyanin (absorption peak at ~620 nm).

Here, we study the time scale of phenotypic plasticity in a fluctuating environment, and its impact on interspecific competition. We use three cyanobacteria isolated from the Baltic Sea. One of these species is capable of complementary chromatic adaptation, and can change its colour from red to green (and vice versa) within a week. The other two species are a green and a red picocyanobacterium, both with a fixed pigment composition. We first develop a resource competition model to predict how these species would interact in a fluctuating environment where the light colour switches between red and green. Subsequently, we perform monoculture experiments to estimate the growth parameters and light absorption characteristics of the three species. Finally, the model predictions are tested in a series of competition experiments in which the light colour fluctuates at low, intermediate, and high frequency. The results show how the interplay between the time scale of phenotypic plasticity and the time scale of environmental fluctuations affects the rate of competitive exclusion.

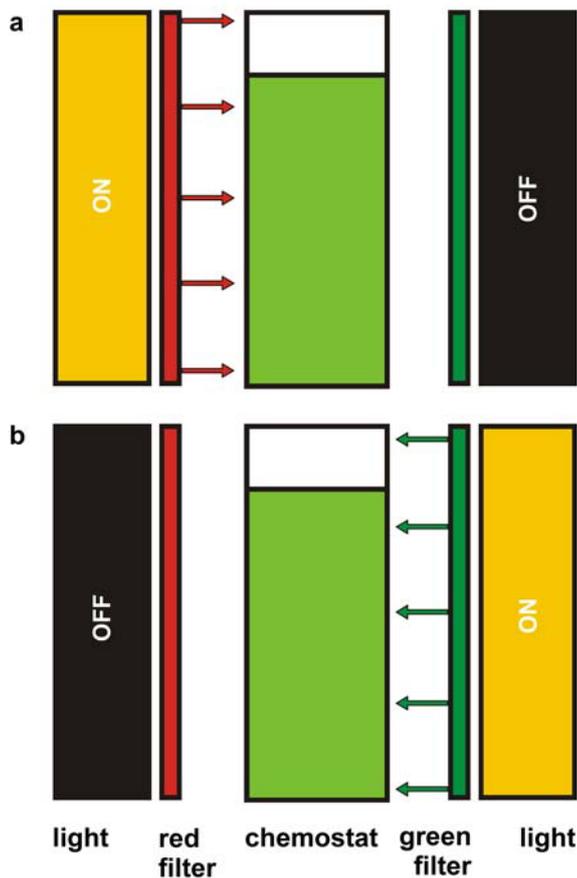


Figure 6.2 Schematic view of the experimental setup. (a) chemostat under red light conditions. (b) chemostat under green light conditions. Fluctuations of incident light color were obtained by time-clock controlled switching between the left and right light sources.

Competition Model

Our theoretical framework on competition for light originates from the competition model developed by Huisman & Weissing (1994, 1995). Their model assumes a direct coupling between changes in phytoplankton population densities and changes in light availability caused by phytoplankton shading. The model treats light as a single resource, and predicts that, in well-mixed waters, the species with the lowest critical light intensity will be the superior competitor for light. The critical light intensity is analogous to the R^* concept (Tilman 1982) in competition for nutrients. It can be measured in monoculture experiments as the light intensity penetrating through the monoculture at steady state. The model has been successfully applied to species with similar light absorption spectra (Huisman *et al.* 1999a; Passarge *et al.* 2006; Agawin *et al.* 2007). Recently, the competition model of Huisman and Weissing was extended to cover the full light spectrum (Stomp *et al.* 2004, 2007a, 2007b). That is, light is treated as a spectrum of resources. This spectral model predicts that species can coexist if they develop different pigments to utilize different parts of the light spectrum (Stomp *et al.* 2004), and will provide the basis for the current study.

We consider a well-mixed water column, in which the phytoplankton species are homogeneously distributed. Nutrients are supplied in excess, so that the species compete for light only. The depth is indicated by z , scaled from zero at the surface to a maximum depth z_m at the bottom of the water column. The water column is illuminated from above, with an incident light spectrum $I_{in}(\lambda)$, where λ is the wavelength. The light spectrum changes with depth, due to selective absorption of different parts of the underwater light spectrum by the phytoplankton community and by the background turbidity caused by water and other non-phytoplankton components. Let $I(\lambda, z)$ denote the light spectrum at depth z . According to Lambert-Beer's law, the change of the underwater light spectrum can be described as (Sathyendranath & Platt 1989; Kirk 1994; Stomp *et al.* 2004):

$$I(\lambda, z) = I_{in}(\lambda) \exp\left(-\sum_{i=1}^n k_i(\lambda)N_i z - K_{bg}(\lambda)z\right) \quad (6.1)$$

where $k_i(\lambda)$ is the specific light absorption spectrum of phytoplankton species i , N_i is the population density of phytoplankton species i , n is the total number of phytoplankton species, and $K_{bg}(\lambda)$ is the background light absorption spectrum. Examples of light absorption spectra of phytoplankton species are illustrated in Figure 6.1. The two peaks at 430 nm (blue light) and 680 nm (red light), shared by all three species, correspond to light absorption by the green pigment chlorophyll *a*. The peak at 560-570 nm corresponds to the red pigment phycoerythrin, which effectively absorbs green light. The peak at 620-630 nm corresponds to the pigment phycocyanin, which absorbs orange-red light. Note from Figure 6.1 that the colour that is least absorbed by a phytoplankton species remains available for other species.

The number of photons absorbed by a single cell of species i (over the PAR range; 400-700 nm) at depth z , $\gamma_i(z)$, depends on the match between the underwater light spectrum and its light absorption spectrum, and can be calculated as (Stomp *et al.* 2004, 2007a):

$$\gamma_i(z) = \int_{400}^{700} I(\lambda, z) k_i(\lambda) d\lambda \quad (6.2)$$

We assume that the specific growth rate of a phytoplankton species is an increasing, saturating function of the number of photons it has absorbed. The specific growth rate thus calculated varies with depth, and is integrated over the entire water column to obtain the total population growth rate (Huisman & Weissing 1994). Accordingly, the population dynamics of species i can be described as (Sathyendranath & Platt 1989; Stomp *et al.* 2007a):

$$\frac{dN_i}{dt} = \left(\frac{1}{z_m} \int_0^{z_m} \frac{p_{\max,i} \gamma_i(z)}{(p_{\max,i} / \varphi_i) + \gamma_i(z)} dz \right) N_i - L_i N_i \quad (6.3)$$

where φ_i is the photosynthetic efficiency (quantum yield) of species i , $p_{\max,i}$ is its maximum specific growth rate, and L_i is its specific loss rate due to factors such as grazing and sinking.

In terms of competition theory, the light spectrum can be interpreted as a spectrum of resources, analogous to the spectrum of seed sizes available for Darwin's finches (Stomp *et al.* 2007a, 2007b). The light absorption spectra of species define which part of the light spectrum they can utilize. Species that are not capable of chromatic adaptation have a fixed light absorption spectrum, whereas species capable of complementary chromatic adaptation have a flexible light absorption spectrum due to variations in phycocyanin and phycoerythrin content (Figure 6.1f). There are different types of complementary chromatic adaptation (Grossman *et al.* 1993; Kehou & Gutu 2006). In our case, we focus on type III, in which the total pool of phycoerythrin (PE) and phycocyanin (PC) remains constant, but the ratio of these two pigments is adjusted to the prevailing underwater light spectrum. Accordingly, we describe the specific light absorption spectrum of the flexible species as:

$$k_i(\lambda) = x_i k_{PE}(\lambda) + (1 - x_i) k_{PC}(\lambda) + k_{other}(\lambda) \quad (6.4)$$

where x_i is the fraction phycoerythrin (i.e., PE/(PE+PC)), $k_{PE}(\lambda)$ is the absorption spectrum of a cell with phycoerythrin only, $k_{PC}(\lambda)$ is the absorption spectrum of a cell with phycocyanin only, and $k_{other}(\lambda)$ is the absorption spectrum due to other pigments in the cell (i.e., chlorophyll a , carotenoids).

We assume that the flexible species changes its phenotype to maximize its specific growth rate under the prevailing environmental conditions (Metz *et al.* 1992; Abrams 1999; Egas *et al.* 2005). In our case, this implies that the species will adjust its phycocyanin and phycoerythrin content to maximize the number of photons it absorbs (Stomp *et al.* 2004):

$$\frac{dx_i}{dt} = \alpha_i \frac{\varphi_i}{z_m} \int_0^{z_m} \frac{\partial \gamma_i(z, x_i)}{\partial x_i} dz \quad (6.5)$$

where α_i measures the rate of chromatic adaptation. Note from this equation that the phycoerythrin fraction (x_i) will change in the direction that yields more light absorption (i.e., a higher γ). The parameter α_i is of central importance in this study, because it determines the time scale of chromatic adaptation.

Numerical simulations of the model were based on a 4th order Runge-Kutta procedure for time integration, and Simpson's rule for depth integration.

Methods

Species

The species chosen for the experiments were the green picocyanobacterium *Cyanobium* strain CCY9201 (formerly known as *Synechococcus* BS4), the red picocyanobacterium *Cyanobium* strain CCY9202 (formerly known as *Synechococcus* BS5), and the filamentous cyanobacterium *Pseudanabaena* strain CCY9509. The latter species can change its colour by complementary chromatic adaptation (type III; Grossman *et al.* 1993; Kehoe & Gutu 2006). In this paper, the three species are referred to as the 'green pico', 'red pico' and 'flexible phenotype', respectively. All three species were isolated from the Baltic Sea (Ernst *et al.* 2003; Haverkamp *et al.* 2008; Acinas *et al.* 2008). The red and green *Cyanobium* strains were also used in earlier competition experiments (Stomp *et al.* 2004).

All three species contain the green pigment chlorophyll *a* with absorption peaks at 430 nm and 680 nm. In addition, the green pico (Figure 6.1a) contains high concentrations of the pigment phycocyanin (PC) which absorbs orange-red light at 620-630 nm (Figure 6.1b). The red pico (Figure 6.1c) contains a little phycocyanin, but its main accessory pigment is phycoerythrin (PE) that absorbs green light at 560-570 nm (Figure 6.1d). The flexible phenotype (Figure 6.1e) can adjust its concentration of PE and PC to the colour of the prevailing light spectrum (Figure 6.1f; Tandeau de Marsac 1977; Grossman *et al.* 1993; Kehoe & Gutu 2006), and thereby changes its own colour from red to green, and vice versa.

Experimental setup

Experiments were performed in chemostats specifically designed to study light-limited growth (Huisman *et al.* 1999a; Stomp *et al.* 2004; Passarge *et al.* 2006). The dilution rate of the chemostats was set at $D = 0.014 \text{ hr}^{-1}$. Flat chemostat vessels were illuminated from the side. Hence, the mixing depth of the chemostats was defined by the width of the vessels, which equaled $z_m = 5 \text{ cm}$. The light source consisted of fluorescent tubes with a white light spectrum (Philips TLD 18W/965). Green light conditions were obtained by a dark green filter (Lee filter #124, Andover, England) placed between the light source and the chemostat vessel, while red

light conditions were obtained by a red filter (Lee filter #26). Fluctuations in incident light colour were generated by time-clock controlled switching between two light sources placed on opposite sides of the chemostats, where one light source used a red filter whereas the other light source used a green filter (Figure 6.2). Monoculture experiments of the three species were carried out under continuous green light, and under continuous red light. Competition experiments, in which all three species were mixed together, were carried out under continuous green light, continuous red light, and fluctuating red/green light conditions. The fluctuations in light colour were characterized by their periodicity (T). We applied the following three fluctuation regimes: $T=0.5$ days (6 hrs red / 6 hrs green), $T=4$ days (2 days red / 2 days green), and $T=28$ days (14 days red / 14 days green).

The incident light intensity (I_{inPAR}) and the light intensity penetrating through the cultures (I_{outPAR}) were measured with a Licor LI-190SA quantum sensor (Lincoln, NE, USA), which integrates the measured number of photons over the entire PAR range (from 400 to 700 nm). In all experiments, we supplied an incident light intensity of $I_{inPAR} = 40 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ to the chemostat vessel. The spectra of the incident light, $I_{in}(\lambda)$, and the light penetration through the vessel, $I_{out}(\lambda)$, were measured by a Licor LI-1800 spectroradiometer (Lincoln, NE, USA). Light absorption spectra of the species were measured by an Aminco DW-2000 double-beam spectrophotometer (SLM Instruments Inc., Urbana, IL, USA), using mineral medium without phytoplankton as a control.

Population densities

Population densities of the species were counted by flow cytometry (Jonker *et al.* 1995; Stomp *et al.* 2004). Because *Pseudanabaena* was much larger than the two picocyanobacteria, direct comparison of the population densities does not provide a good representation of the relative abundances of the different species in the species mixtures. To quantify the contribution of each species in the context of competition for light, we therefore expressed the population densities of the species in terms of their total light absorption. For calculation of the total light absorption by each species, and further details on the experimental conditions and analysis, the interested reader is referred to Appendix C.

Parameter estimation

Model parameters were estimated from the experiments. The preceding paragraphs already specified our measurements of dilution rate (D), mixing depth (z_m), incident light spectrum ($I_{in}(\lambda)$), and the light absorption spectra of the species ($\kappa_i(\lambda)$). Calculation of the background turbidity ($K_{bg}(\lambda)$) is described in Appendix C. We assumed that specific loss rates of the species were dominated by the dilution rate (i.e., $L_i = D$ for all species).

The remaining model parameters were the photosynthetic efficiencies (ϕ) and the maximum specific growth rates (μ_{max}) of all three species, and the rate of chromatic adaptation of the flexible phenotype (α). We estimated the model parameters ϕ and μ_{max} by calibration of the model predictions to time series of population density and light penetration measured in

monoculture experiments. The model calibration was simultaneously applied to monoculture experiments under continuous red light and monoculture experiments under continuous green light, to obtain parameter estimates for φ and p_{max} that applied to both red-light and green-light conditions. For this purpose, the data of population density and light penetration were first log-transformed to homogenize the variances, and subsequently normalized using the total sum of squares as a weighting factor to give equal weight to each of these variables in the calibration procedure. Parameter estimates were obtained by fitting the model predictions to these log-transformed normalized data by minimization of the residual sum of squares, using the Gauss-Marquardt-Levenberg algorithm. The model calibration was performed with the software package PEST (Watermark Numerical Computing, Brisbane, Australia).

Subsequently, the rate of chromatic adaptation of the flexible phenotype (α) was estimated by fitting the model to two separate monoculture experiments. In one experiment, a culture adjusted to green light was exposed to red light for one week, while in the other experiment a culture adjusted to red light was exposed to green light for one week. From these experiments, the parameter α was estimated using the same calibration technique as described above. The species parameters that were estimated from the monoculture experiments were used to predict the population dynamics in the competition experiments.

Rate of competitive exclusion

To examine the dynamics in each competition experiment, we calculated the rate of competitive exclusion (RCE). This rate was defined as (Grover 1988; Passarge *et al.* 2006):

$$RCE = \frac{\Delta \ln(\text{winner} / \text{loser})}{\Delta t} \quad (6.6)$$

where ‘winner’ and ‘loser’ are the population densities of the winning and losing species during the course of competition, measured from the onset of the decline of the loser. Accordingly, RCE can be estimated from the measured population densities by linear regression of $\ln(\text{winner}/\text{loser})$ versus time.

Results

Monoculture experiments

In monoculture, the green pico, red pico and flexible phenotype were all able to grow under both continuous red and continuous green light (Figure 6.3). After inoculation, the population densities increased, absorbed more light, and thus decreased the light intensity penetrating through the vessel. This continued until steady state was reached. The population of the green pico reached steady state within ~8 days when grown in red light, while it took ~21 days to reach steady state in green light (Figure 6.3a, 6.3b). The steady-state population density of the green pico, and its total light absorption, were higher in red light than in green light (Table

6.1). We define the spectrally-integrated light intensity penetrating through a steady-state monoculture as the ‘critical light intensity’ (I_{outPAR}^* ; sensu Huisman & Weissing 1994). The critical light intensity of the green pico was much lower in red light than in green light (Figure 6.3a, 6.3b; Table 6.1), due to its higher population density in red light and its more efficient absorption of red light (Figure 6.1b).

	Green pico	Red pico	Flexible phenotype
Population density ($\times 10^8$ counts mL^{-1})			
Red light	3.59 ± 0.36	1.34 ± 0.09	0.45 ± 0.02
Green light	1.66 ± 0.12	1.31 ± 0.13	0.45 ± 0.04
Light absorption (cm^{-1})			
Red light	0.82 ± 0.07	0.45 ± 0.03	0.55 ± 0.02
Green light	0.22 ± 0.01	0.60 ± 0.05	0.59 ± 0.05
Critical light intensity ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)			
Red light	0.50 ± 0.02	4.69 ± 0.07	0.51 ± 0.07
Green light	7.09 ± 0.15	1.21 ± 0.06	1.18 ± 0.10

Table 6.1 Steady-state traits of the phytoplankton species measured in monoculture experiments. Values are reported as means \pm SD.

The population of the red pico reached steady state within ~ 8 days when grown in green light, while it took ~ 12 days in red light (Figure 6.3c, 6.3d). The steady-state population density of the red pico was similar in green light and red light (Table 6.1). The total light absorption of the red pico was higher in green light than in red light (Table 6.1), because of its more efficient absorption of green light (Figure 6.1d). As a result, the critical light intensity of the red pico was much lower in green light than in red light (Figure 6.3c, 6.3d; Table 6.1).

The population of the flexible phenotype reached steady state within ~ 10 days in both red light and green light (Figure 6.3e, 6.3f). In red light, the flexible phenotype produced mainly phycocyanin pigments, and thereby turned its colour into green (Figure 6.1f). Conversely, in green light the flexible phenotype produced mainly phycoerythrin pigments, turning its colour into red. The steady-state population density of the flexible phenotype, and hence its total light absorption, was quite similar in both red light and green light (Table 6.1). The critical light intensity of the flexible phenotype was lower in red light than in green light (Figure 6.3e, 6.3f; Table 6.1). Under green light, the flexible phenotype had a critical light intensity similar to the red pico, but lower than the green pico. Conversely, under red light, the flexible phenotype had a critical light intensity similar to the green pico, but lower than the red pico.

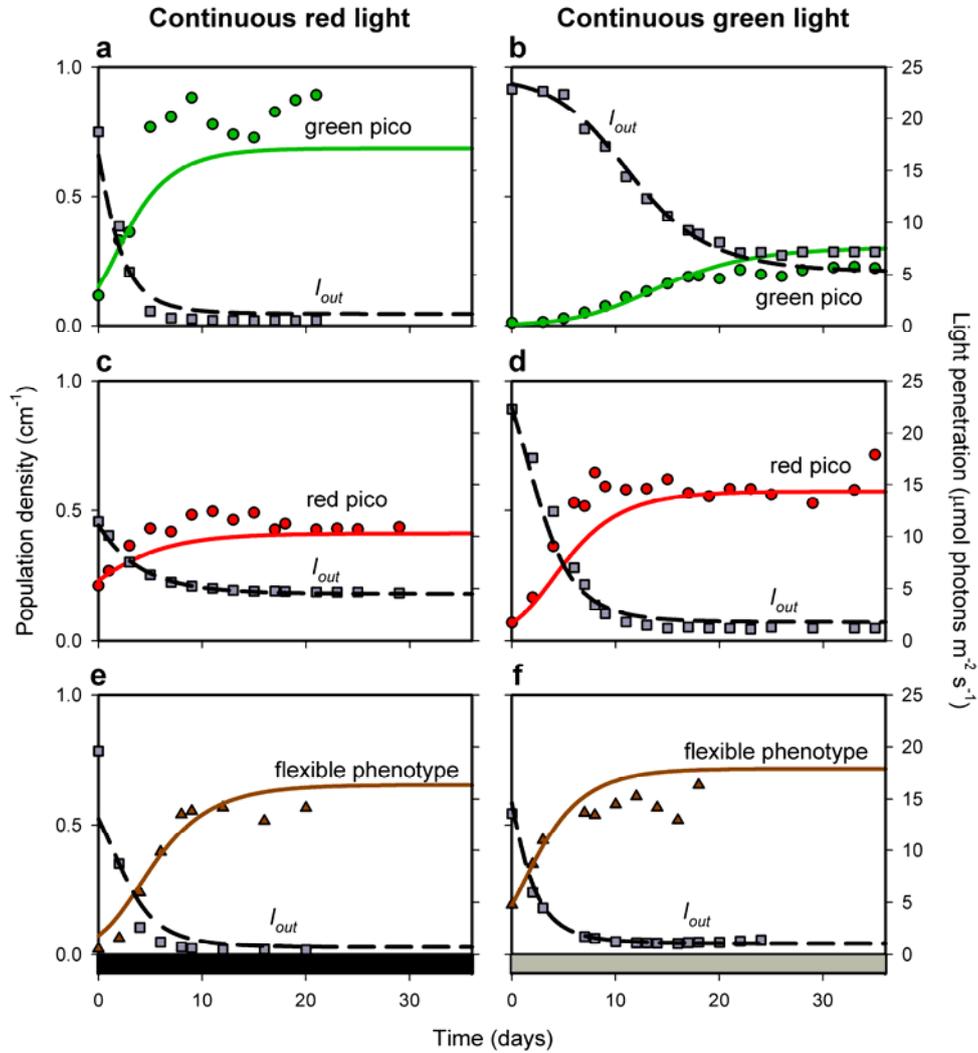


Figure 6.3 Monoculture experiments. (a) and (b), green pico (green circles); (c) and (d), red pico (red circles); (e) and (f), flexible phenotype (brown triangles). The panels on the left-hand side show monocultures grown in continuous red light, while the panels on the right-hand side show monocultures grown in continuous green light. Grey squares indicate the light intensity penetrating through the cultures. Solid lines indicate the population densities predicted by the model. Dashed lines indicate the predicted light penetration. Bars at the bottom of the graphs indicate illumination by red light (black bar) and green light (grey bar). Population densities of the species are expressed by their light absorption. For parameter values, see Table 6.2.

The data from these monoculture experiments were used to calibrate the model parameters of the species (Table 6.2). For this purpose, the model was fitted simultaneously to the time courses of the population densities and light penetration under both red and green light conditions. The calibrated model fitted quite well with the experimental data (Figure 6.3). To

estimate the rate of chromatic adaptation of the flexible phenotype, we simply exchanged the coloured light filters of the two monoculture experiments (Figure 6.3e, 6.3f). When the green light-adapted culture was transferred to red light, it gradually replaced its phycoerythrin by phycocyanin, and thereby turned its colour from red to green in ~ 7 days (Figure 6.4a). Conversely, when the red light-adapted culture was transferred to green light, it gradually replaced its phycocyanin by phycoerythrin, and thereby turned its colour from green to red in 8-9 days (Figure 6.4b). Thus, the rate of chromatic adaptation was rather similar in both directions, although possibly slightly faster from red to green pigmentation than from green to red pigmentation (Table 6.2).

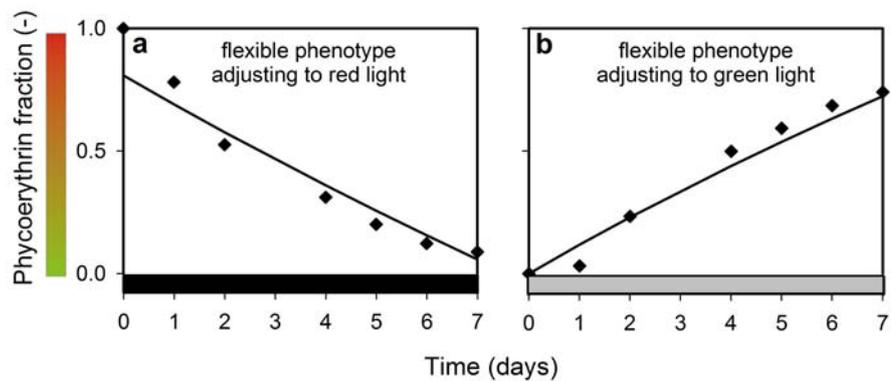


Figure 6.4 Chromatic adaptation of the flexible phenotype. Green-adapted monoculture exposed to red light (left panel). Red-adapted monoculture exposed to green light (right panel). Symbols represent experimental data, lines represent model predictions. Bars at the bottom of the graphs indicate illumination by red light (black bar) and green light (grey bar). The pigment composition of the flexible phenotype is expressed by the phycoerythrin fraction x .

Competition experiments

We performed competition experiments with mixtures of the red pico, green pico and flexible phenotype under continuous red light, continuous green light, and under fast, intermediate, and slow fluctuations in light colour. Under continuous red light, the red pico was rapidly excluded, while the green pico and flexible phenotype coexisted (Figure 6.5a). This is consistent with the critical light intensities for red light measured in monoculture, which were much lower for the flexible phenotype and green pico than for the red pico (Table 6.1). The critical light intensities for red light of the flexible phenotype and green pico were very similar, and their coexistence is probably an example of neutral coexistence. Conversely, under continuous green light, the flexible phenotype rapidly excluded the green pico, while the red pico was displaced more slowly (Figure 6.5b). This is consistent with the critical light intensities for green light, which were much lower for the flexible phenotype and red pico than for the green pico (Table 6.1). Although the critical light intensities for green light of the flexible phenotype and red pico were rather similar, the competition experiment did not reveal neutral coexistence of these two species but showed that the flexible phenotype was a slightly stronger competitor for green light than the red pico.

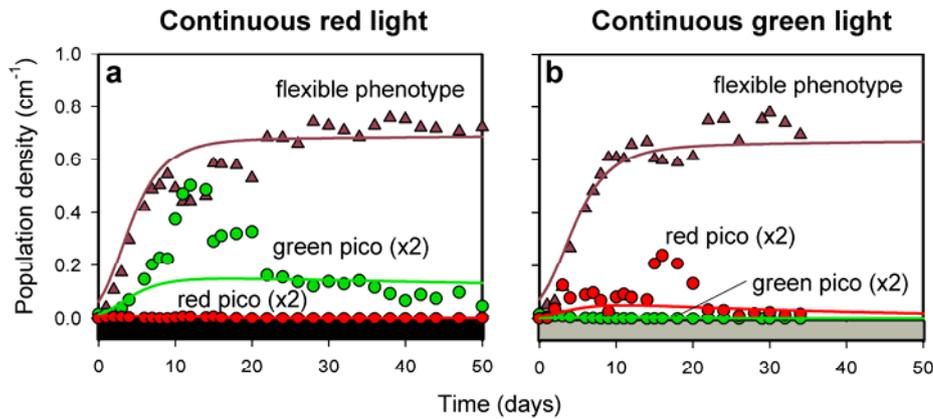


Figure 6.5 Competition experiments between the green pico (green symbols and line), red pico (red symbols and line) and flexible phenotype (brown symbol and line) exposed to continuous red light (a) and continuous green light (b). Symbols represent experimental data, while lines represent model predictions. Population densities are expressed by their light absorption. Bars at the bottom of the graphs indicate illumination by red light (black bars) and green light (grey bars).

In all competition experiments under fluctuating light conditions, the flexible phenotype excluded both the green and red picos (Figure 6.6). When exposed to fast fluctuations in light colour ($T=0.5$ days), the flexible phenotype gradually developed a pigment composition rich in phycoerythrin; fluctuations in pigment composition were not detectable in this experiment (Figure 6.6g). Under intermediate fluctuations in light colour ($T=4$ days), the flexible phenotype changed its pigment composition, but had insufficient time to fully adjust its pigmentation to the prevailing light colour. Hence, the pigment composition of the flexible phenotype fluctuated with relatively small amplitude (Figure 6.6h). Under slow fluctuations in light colour ($T=28$ days), there was sufficient time for the flexible phenotype to fully adjust its pigmentation before the light colour was changed again. In this experiment, the population density, light penetration, and pigment composition of the flexible phenotype fluctuated strongly (Figure 6.6c, 6.6f, 6.6i).

The model predictions, based on the parameters estimated from the monoculture experiments, were in good agreement with the competition experiments (Figure 6.6). However, especially in the fast and intermediate fluctuation regimes, the flexible phenotype had a slightly higher phycoerythrin fraction than predicted by the model (Figure 6.6g, 6.6h). Furthermore, in the slow fluctuation regime, the rate at which the flexible phenotype adjusted its phycoerythrin fraction to green light was consistent with the model predictions, while the adjustment to red light was somewhat more slowly than predicted (Figure 6.6i).

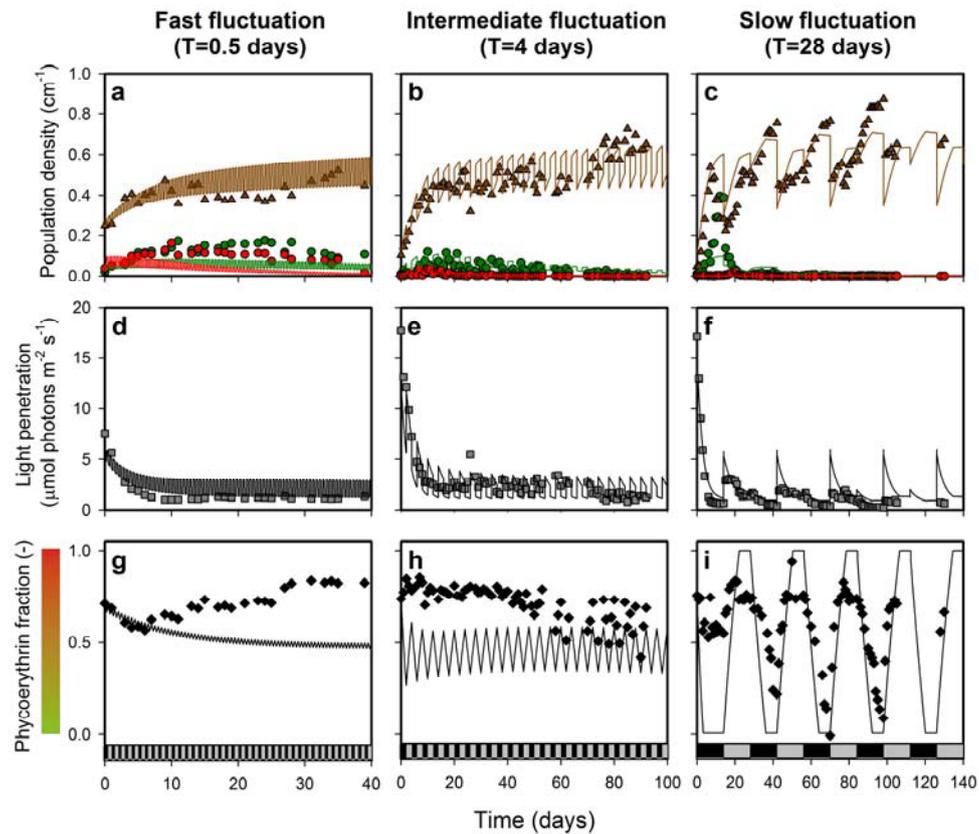


Figure 6.6 Competition experiments between the green pico, red pico and flexible phenotype exposed to fluctuations in light colour. The competition experiments were exposed to fast fluctuations (left panels), intermediate fluctuations (middle panels), and slow fluctuations (right panels). (a-c) Population densities of the green pico (green circles), red pico (red circles) and flexible phenotype (brown triangles). Population densities are expressed by their light absorption. (d-f) Light intensity penetrating through the culture vessel (grey squares). (g-i) Pigment composition of the flexible phenotype (black diamonds) during the competition experiments. Lines represent the model predictions. Bars at the bottom of the graphs indicate illumination by red light (black bars) and green light (grey bars).

At what time scale is chromatic adaptation advantageous?

Summarizing the experimental results, the amplitude of the variation in pigment composition of the flexible phenotype increased with longer periods of environmental fluctuation (Figure 6.7a). The flexible phenotype required ~ 7 days to turn its colour from red to green, while it required 8-9 days to turn its colour from green to red (Figure 6.4). Accordingly, the model predicts that at light fluctuations with a period exceeding ~ 14 days (i.e., 7 days red and 7 days green), the flexible phenotype could fully adjust its pigmentation to red light conditions before the light colour switched to green again (Figure 6.7a). Slightly longer periods of fluctuation ($T > 18$ days; i.e., 9 days red and 9 days green) were required for the flexible phenotype to fully adjust to green light conditions (Figure 6.7a). Note that, at fast fluctuations ($T = 0.5$ days), the flexible phenotype had more phycoerythrin than predicted by the model (Figure 6.7a).

Intuitively, one might think that larger amplitudes in pigmentation of the flexible phenotype would enhance its tuning to red and green light conditions, and thus would promote its light absorption. However, the model predicts that for $T < 14$ days (i.e., the range of periodicities where the amplitude in pigmentation increased with T ; see Figure 6.7a), the time-averaged light absorption by the flexible phenotype remains constant (Figure 6.7b). The explanation is that, with increasing T , the flexible phenotype makes more phycoerythrin to absorb more green light, but as a consequence it will also be more poorly adapted once the red light is switched on again. Thus, the gain in light absorption by a better tuning to green light is offset by a reduced light absorption when the light colour is switched back to red light. As a result, the time-averaged light absorption by the flexible phenotype remains constant over T (for $T < 14$ days). At longer periods ($T > 14$ days), there is sufficient time for the flexible phenotype to fully adjust its pigmentation to the prevailing light colour, and the flexible phenotype starts to benefit from chromatic adaptation (Figure 6.7b). More specifically, the model predicts that, for $T > 14$ days, the flexible phenotype will spend relatively less time changing its pigmentation while it can benefit for longer time spans from its optimal tuning to the prevailing light colour. Hence, for $T > 14$ days, the time-averaged light absorption increases with T , until it approaches an asymptote at which the time spent on chromatic adaptation is negligible (Figure 6.7b). These model predictions were confirmed by the experiments. Although the standard deviations are high, the data show that light absorption by the flexible phenotype was higher at long periods of fluctuation ($T = 28$ days) than at shorter periods ($T = 0.5$ and $T = 4$ days). Also in line with the model predictions, light absorption by the flexible phenotype was higher during red light conditions than during green light conditions.

The rate at which the flexible phenotype competitively excludes the green and red picos is tightly coupled to its efficiency in absorbing red and green light. Since the green pico mainly absorbs red light (Figure 6.1b), the model predicts that its exclusion rate as a function of T will show the same pattern as the average red-light absorption by the flexible phenotype (compare the green lines in Figure 6.7b and Figure 6.7c). Likewise, the exclusion rate of the red pico will show the same pattern as the average green-light absorption by the flexible phenotype (compare the red lines in Figure 6.7b and Figure 6.7c). The competition experiments confirmed that competitive exclusion rates remained independent of the fluctuation period for $T < 14$ days, while the competitive exclusion rate was higher at $T = 28$ days. Also in line with the model predictions, the red pico was excluded faster than the green pico (Figure 6.7c).

What is the advantage of chromatic adaptation? The benefit of phenotypic plasticity can be quantified by comparing the performance of a flexible phenotype with a fixed phenotype that has lost its plasticity. Terauchi *et al.* (2004) discovered a photoreceptor involved in the complementary chromatic adaptation of cyanobacteria. Knock-out mutants lacking the photoreceptor had lost their phenotypic plasticity and displayed a fixed intermediate pigment composition with equal amounts of phycoerythrin and phycocyanin. Accordingly, we calculated the competitive exclusion rates of the red and green picos in case our flexible phenotype would not be able of chromatic adaptation, but would have a fixed intermediate

pigment composition (i.e., a fixed phycoerythrin fraction of 0.5; dashed lines in Figure 6.7c). This shows that when fluctuations are fast ($T < 14$ days), chromatic adaptation does not affect the rate of competitive exclusion. Only when the period of the fluctuations exceeds $T > 14$ days, chromatic adaptation makes a difference (compare solid lines and dashed lines in Figure 6.7c). Thus, chromatic adaptation is advantageous in competition only when there is sufficient time for the flexible phenotype to fully adjust its pigmentation to the prevailing light colour.

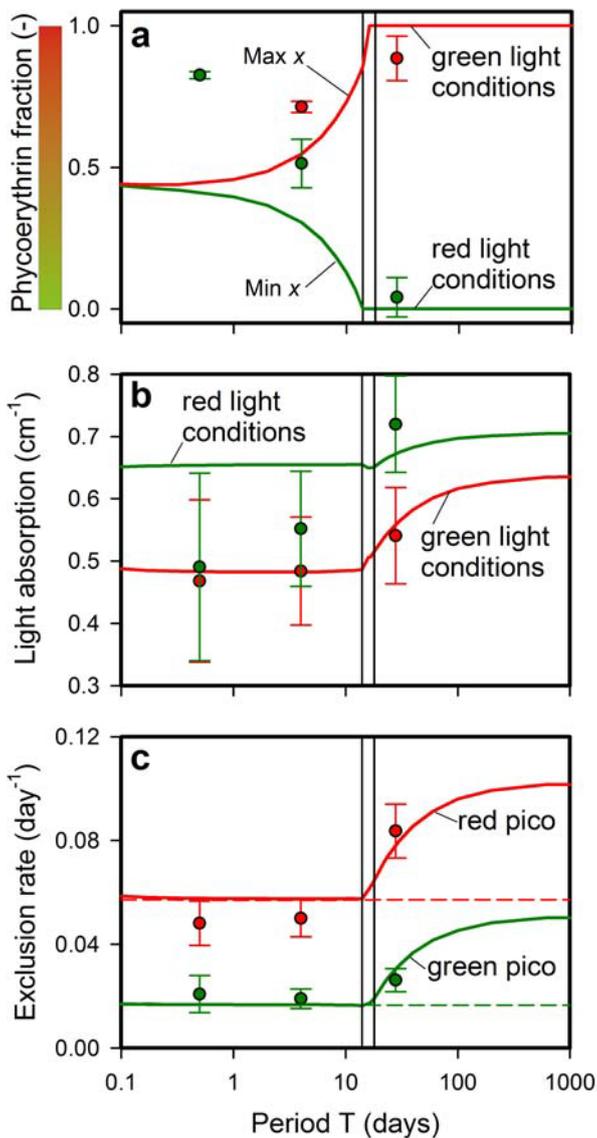


Figure 6.7 Implications of chromatic adaptation at different fluctuation frequencies. (a) Amplitude of the fluctuations in pigment composition of the flexible phenotype, as expressed by the minimum values (green symbols and lines) and maximum values (red symbols and lines) of its phycoerythrin fraction. (b) Time-averaged light absorption by the flexible phenotype during green light conditions (red symbols and line) and red light conditions (green symbols and line). (c) Rates of competitive exclusion of the red pico (red symbols and lines) and green pico (green symbols and lines) in the competition experiments. Lines indicate model predictions, symbols represent the experimental data (\pm SD) from the competition experiments with fast, intermediate and slow fluctuation regimes, respectively. The first vertical line (at $T=14$ days) indicates the periodicity at which the flexible phenotype becomes fully adapted to red light conditions, while the second vertical line (at $T=18$ days) indicates the periodicity at which it becomes fully adapted to green light conditions. The horizontal dashed lines in (c) indicate the predicted rates of competitive exclusion in case the flexible phenotype would have been unable of chromatic adaptation (assuming a fixed phycoerythrin fraction of $x=0.5$).

Discussion

Time scale of phenotypic plasticity

In this study, we have shown that the time scale of phenotypic plasticity affects competition in fluctuating environments. Theory often assumes a cost to phenotypic plasticity. Therefore, ideally one would like to perform experiments in which the fixed phenotype is a better competitor under constant conditions, while the flexible phenotype is a better competitor in a variable environment. In practice, however, one has to work with the species one can find. Our experiments were based on cyanobacteria isolated from the same environment. In the Baltic Sea, we found a colourful mixture of red and green picocyanobacteria with a constant pigment composition (fixed phenotypes) and filamentous cyanobacteria that can tune their pigments to the prevailing light colour (flexible phenotypes). In our experiments, it turned out that the flexible phenotype was the strongest competitor under all experimental conditions. Yet, the experiments clearly revealed that the rate of competitive exclusion depended on the time scale of phenotypic plasticity in relation to the frequency of environmental fluctuations (Figure 6.7). When environmental fluctuations were fast compared to the rate of chromatic adaptation, the flexible phenotype displayed an intermediate pigment composition and, hence, could not benefit from its plasticity. In contrast, when environmental fluctuations were slow, the flexible phenotype had sufficient time to fully adjust its pigmentation to the prevailing light colour. In this case, the flexible phenotype benefited from its phenotypic plasticity, as it performed better than a fixed phenotype of intermediate pigment composition (compare solid lines and dashed lines in Figure 6.7c).

Model studies of Padilla & Adolph (1996) and Gabriel (2005, 2006) compared the fitness of a phenotypically fixed individual to that of a flexible phenotype switching from one phenotypic state to the other state after a certain time lag. They showed that the advantage of phenotypic plasticity decreased with increasing environmental variability, and also decreased with an increasing time lag in its phenotypic adjustment. In highly variable environments, plasticity could even be disadvantageous due to a permanent mismatch between the flexible but time-lagged phenotype and its environment.

Our results confirm these findings. Although the flexible phenotype was the strongest competitor, it benefited from its phenotypic plasticity only if environmental fluctuations were sufficiently slow compared to the rate of phenotypic adjustment. However, in contrast to the model studies of Padilla & Adolph (1996) and Gabriel (2005, 2006), rapid fluctuations in environmental conditions did not have a negative impact on the fitness of the flexible phenotype. In their model studies, the flexible phenotype switched abruptly from one phenotypic state to the other state. In our system, the flexible phenotype gradually adjusted its pigment composition to the prevailing light conditions. As a result, the flexible phenotype displayed an intermediate pigment composition when fluctuations were too fast for full adjustment. This intermediate pigment composition prevented a complete mismatch between

the flexible phenotype and its variable environment, and thereby explains why rapid fluctuations never had a negative fitness impact in our study system.

Summarizing, when is reversible phenotypic plasticity advantageous during species interactions in fluctuating environments? Let us define the time scale of phenotypic adjustment as the time required to change from one phenotype to the other. According to our results, phenotypic plasticity is advantageous only if the period of the environmental fluctuations (T_f) exceeds twice the time scale of phenotypic adjustment (T_a):

$$\frac{T_f}{2T_a} \gg 1 \quad (6.7)$$

In this case, there is sufficient time for the flexible phenotype to fully adjust its phenotype to both the ups and downs in the prevailing environmental conditions.

The ecological significance of chromatic adaptation

Complementary chromatic adaptation (CCA) of cyanobacteria is extensively studied at the molecular and physiological level (Tandeau de Marsac 1977; Kehoe & Gutu 2006). Yet, surprisingly little is known about the ecology and biogeographical distribution of species capable of CCA. Can we predict, based on the findings of this study, in which environments CCA will be advantageous?

In natural waters, phytoplankton species are exposed to fluctuating light conditions when cells change their vertical position in the water column. In the Baltic Sea, from which our *Pseudanabaena* strain and picocyanobacteria originated, the underwater light spectrum changes from white light at the surface to green light at greater depths (Stomp *et al.* 2007a; Haverkamp *et al.* 2008). At the water surface, in white light, we would expect that the flexible phenotype will adjust its pigment composition to complement the pigments of competing phytoplankton species, consistent with earlier competition experiments (Stomp *et al.* 2004). Accordingly, its pigment composition at the surface may depend on the pigment composition and population dynamics of its competitors. Deeper down in the water column, the prevailing green light conditions in the Baltic Sea perfectly match the absorption peak of the phycoerythrin pigment (Stomp *et al.* 2007a, 2007b). Hence, we expect that phenotypically flexible species like our *Pseudanabaena* strain will turn red (i.e., will produce the red pigment phycoerythrin) when mixed to greater depth in the Baltic Sea. This may also explain why *Pseudanabaena* was biased towards red pigmentation in our experiments (Figure 6.6, Figure 6.7), because it can encounter green light but not purely red light in its natural habitat.

Although green light is the predominant underwater light colour in coastal seas like the Baltic Sea, other light colours may prevail in other aquatic ecosystems. For instance, blue light is the predominant colour in the open ocean, whereas red light penetrates the deepest in peat lakes (Stomp *et al.* 2007a, 2007b). These different underwater light environments are likely to select for chromatic adaptation by specific sets of pigments. For example, in clear ocean

waters, it would be advantageous for cyanobacteria to tune pigments active in the blue part of their light absorption spectrum. Indeed, some marine *Synechococcus* strains from oceanic environments are flexible in their absorption of blue versus green light. They use two alternative chromophores in the phycoerythrin pigments (Palenik 2001). The chromophore phycoerythrobilin (PEB) has its absorption peak in green light (550 nm), while the chromophore phycourobilin (PUB) has its absorption peak shifted towards blue light (495 nm). These flexible phenotypes have a low PUB/PEB ratio when exposed to white light at the water surface, but a high PUB/PEB ratio when exposed to blue light at greater depths (Palenik 2001; Everroad *et al.* 2006).

What is the ecological significance of the time scale of chromatic adaptation? Some cyanobacteria are neutrally buoyant. They are passively dispersed throughout the euphotic zone by turbulent mixing. The time scale of turbulent mixing may vary from several hours to several days (Denman & Gargett 1983; Huisman *et al.* 1999b), depending on the intensity of turbulent mixing and the depth of the euphotic zone. When mixing through the euphotic zone occurs on a time scale of hours, cells will experience fluctuations in the underwater light colour that will be too fast for CCA. In contrast, when mixing through the euphotic zone occurs on a time scale of several days, cells will experience slower fluctuations in the underwater light colour that can be tracked by CCA. The latter condition may apply to stratified open ocean waters, where turbulent mixing can be relatively weak and the euphotic zone may extend over more than 100 m depth. Here, CCA by marine *Synechococcus* species with flexible PUB/PEB ratios could be advantageous compared to *Synechococcus* species with a fixed pigment composition (Everroad *et al.* 2006).

CCA may be particularly advantageous for positively buoyant cyanobacteria that can escape from turbulent mixing. For instance, cyanobacteria with gas vesicles may float upwards, forming blooms near the water surface as long as the weather conditions remain stable (Huisman *et al.* 2004; Jöhnk *et al.* 2008). When these surface blooms are disrupted by storm events, their light environment may change completely as they are entrained by intense mixing to the deeper waters below. Storm events occur irregularly, at intervals of, say, several weeks or months. Our results indicate that, at these time scales, CCA would provide a strong selective advantage. We therefore hypothesize that this scenario of intermittent mixing, which seems to apply to the Baltic Sea (Walsby *et al.* 1997), will favor chromatic adaptation in buoyant cyanobacteria such as our *Pseudanabaena* strain.

Coexistence versus competitive exclusion

Temporal variability can promote species coexistence, depending on the frequency and amplitude of the environmental fluctuations (Hutchinson 1961; Connell 1978; Armstrong & McGehee 1980). This is confirmed by many experimental studies, which have shown that a fluctuating resource supply yields a higher species diversity in phytoplankton communities than a constant resource supply (Sommer 1984; Gaedeke & Sommer 1986; Grover 1991; Litchman 1998; Flöder *et al.* 2002, Litchman 2003). Likewise, theoretical studies predict

competition for two fluctuating resources may allow coexistence of a generalist and two specialist species (Wilson & Yoshimura 1994; Egas *et al.* 2004; Abrams 2006*a*, 2006*b*). These theoretical studies may resemble our study, where the flexible phenotype can be interpreted as a generalist species capable of growing on both resources, while the red pico and green pico can be considered specialists growing mainly on green light and red light, respectively.

However, in our competition experiments and parameterized model simulations we did not find coexistence under fluctuating conditions. An explanation for the lack of coexistence could be the absence of suitable trade-offs. Model studies have shown that coexistence in fluctuating environments strongly depends on the parameter combinations of competing species (Grover 1990; Litchman & Klausmeier 2001). In particular, a strong trade-off is required such that each species has an advantage during at least part of the competitive process. In our experiments, analogous to the R^* concept of competition for nutrients (Tilman 1982), the species with the lowest critical light intensity for a particular colour of light will be the superior competitor for that colour of light (Huisman & Weissing 1994). In view of the critical light intensities Table 6.1), the flexible phenotype and the green pico were equally strong competitors for red light, as confirmed by their neutral coexistence in continuous red light (Figure 6.5a). In addition, the flexible phenotype was a slightly stronger competitor for green light than the red pico (Figure 6.5b). This makes the flexible phenotype a superior competitor under fluctuating light conditions. As a result, in our experiments, fluctuating light conditions did not promote coexistence; the flexible phenotype always won.

Hypothetically, we may expect many situations in which there will be trade-offs in the competitive ability for different colours of light. In particular, it seems plausible that flexible phenotypes will often be weaker competitors for a single colour of light than species specialized on that light colour. We simulated such a situation, in which we used our estimated model parameters, but modified the photosynthetic efficiencies of the species to mimic this trade-off. With this new parameter setting, the flexible phenotype was a weaker competitor for red light than the green pico and a weaker competitor for green light than the red pico. In this case, with fast fluctuations of red and green light, the model predicts that the flexible phenotype will not be able to catch up with the changing light conditions, and is excluded by the joint forces of the coexisting red and green picos (Figure 6.8a). However, at slower fluctuations of red and green light, the model predicted coexistence of all three species (Figure 6.8b). Additional model simulations (not shown) revealed that the transition from competitive exclusion of the flexible phenotype to coexistence of all three species occurred at a periodicity of ~ 14 days; the same periodicity at which the flexible phenotype starts to benefit from chromatic adaptation in our experiments (Figure 6.7). These model results support the earlier model prediction that two specialists and one generalist can coexist on two fluctuating resources (Wilson & Yoshimura 1994; Egas *et al.* 2004; Abrams 2006*a*, 2006*b*). Moreover, coexistence of all three species requires that the flexible phenotype has sufficient time to fully adapt to the prevailing light colours.

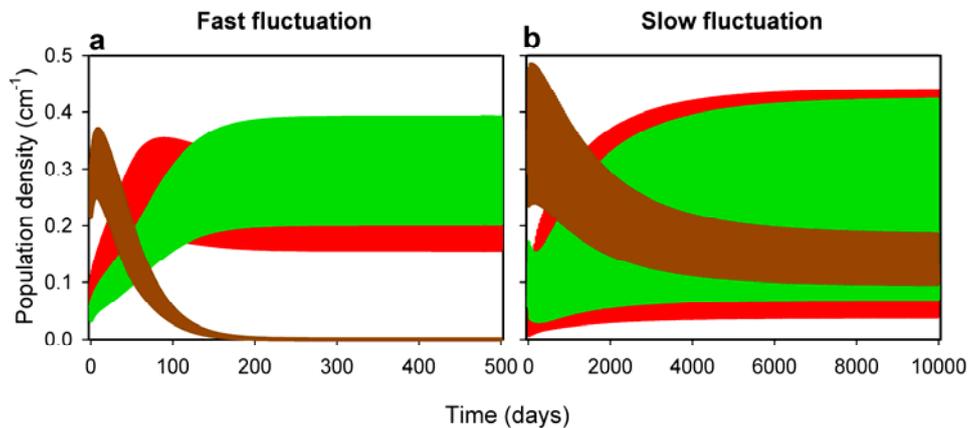


Figure 6.8 Model simulations exploring the possibility of coexistence in a fluctuating light environment. (a) When fluctuations are fast ($T=0.5$ days), the model predicts coexistence of the red pico (red line) and green pico (green line), which jointly exclude the flexible phenotype (brown line). (b) When fluctuations are slow ($T=40$ days), the model predicts coexistence of all three species. In both panels, the species fluctuations are fast compared to the time scale of the x-axis, so that the species abundances are merged into bands. Population abundances are expressed in terms of the light absorption by each species. The model parameters in these simulations differed from the species parameters estimated in the monoculture experiments. In particular, these simulations assumed that the flexible phenotype was a weaker competitor for green light than the red pico, and a weaker competitor for red light than the green pico. Model parameters are the same as in Table 6.2, except for the photosynthetic efficiency of the red pico ($\varphi = 1.3 \times 10^6 \text{ cells } (\mu\text{mol photons}^{-1})$) and the flexible phenotype ($\varphi = 3.7 \times 10^5 \text{ cells } (\mu\text{mol photons}^{-1})$).

Conclusions

In conclusion, our results confirm that the underwater light colour is an important selective factor in phytoplankton competition. Phytoplankton species which have tuned their photosynthetic pigments to the prevailing light colour have a clear competitive advantage. More generally, our results show that the time scale of phenotypic plasticity can be decisive for species interactions in fluctuating environments. In particular, the interplay between the time scale of phenotypic plasticity and the time scale of environmental fluctuations influences the rate of competitive exclusion, may reverse competitive hierarchies, and can affect the species composition of ecological communities.

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Table 6.2 Parameter values and their interpretation

Symbol	Interpretation	Units	Value
Independent variables			
t	time	h	-
z	depth	cm	-
λ	wavelength	nm	-
Dependent variables			
N_i	Population density of species i	cells cm^{-3}	-
A_i	Light absorption by species i	cm^{-1}	-
$\gamma(z)$	Absorbed photons by species i	$\mu\text{mol photons s}^{-1} \text{ cell}^{-1}$	-
$I(\lambda, z)$	Underwater light spectrum	$\mu\text{mol photons m}^{-2} \text{ s}^{-1} \text{ nm}^{-1}$	-
x_i	Fraction phycoerythrin of species i	dimensionless	-
Parameters			
$I_m(\lambda)$	Spectrum of incident light	$\mu\text{mol photons m}^{-2} \text{ s}^{-1} \text{ nm}^{-1}$	m
I_{inPAR}	PAR-integrated incident light intensity	$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$	40 ^m
I_{outPAR}	PAR-integrated penetrating light intensity	$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$	m
$K_{bg}(\lambda)$	Absorption spectrum of background turbidity	cm^{-1}	m
$k_i(\lambda)$	Absorption spectrum of species i	$\text{cm}^2 \text{ cell}^{-1}$	Figure 6.1 ^m
$k_{PE}(\lambda)$	Absorption spectrum of phycoerythrin	$\text{cm}^2 \text{ cell}^{-1}$	e
$k_{PC}(\lambda)$	Absorption spectrum of phycocyanin	$\text{cm}^2 \text{ cell}^{-1}$	e
$K_{other}(\lambda)$	Absorption spectrum of chlorophyll and carotenoid pigments	$\text{cm}^2 \text{ cell}^{-1}$	e
z_m	Depth of the water column	cm	5.0 ^m
L_i	Specific loss rate of species i	h^{-1}	0.014 ^m
$p_{max,i}$	Maximum specific growth rate of species i	h^{-1}	0.080 [*]
ϕ_i	Photosynthetic efficiency	cells $(\mu\text{mol photons})^{-1}$	
	- Green pico		2.4×10^6 ^e
	- Red pico		1.0×10^6 ^e
	- Flexible phenotype		4.2×10^5 ^e
α_G	Chromatic adaptation parameter from red to green pigmentation	dimensionless	0.57 ^e
α_R	Chromatic adaptation parameter from green to red pigmentation	dimensionless	0.30 ^e

m = measured parameter, e = estimated parameter (see Methods). * = The iterative fitting procedure led to an estimate of p_{max} that diverged to infinity. This indicated that the light intensities encountered were still in the linear part of the $p(I)$ -curve, such that p_{max} was not reached. Hence, p_{max} of the species was arbitrarily set to a high (but not unrealistic) value of 0.080 h^{-1} to guarantee that the modeled $p(I)$ values remained in the linear part.