Colourful coexistence : a new solution to the plankton paradox

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Chapter 2

Adaptive divergence in pigment composition promotes phytoplankton biodiversity

Abstract

The dazzling diversity of the phytoplankton has puzzled biologists for decades (Hutchinson 1961; Tilman 1982; Sommer 1985; Huisman & Weissing 1999; Irigoien *et al.* 2004). The puzzle has been enlarged rather than solved by the progressive discovery of new phototrophic microorganisms in the oceans, including picocyanobacteria (Waterbury *et al.* 1979; Chisholm *et al.* 1988), pico-eukaryotes (Moon-Van der Staay *et al.* 2001), bacteriochlorophyll-based (Kolber *et al.* 2000; Kolber *et al.* 2001; Béjà *et al.* 2002) and rhodopsin-based phototrophic bacteria (Béjà *et al.* 2000; Venter *et al.* 2004). Physiological and genomic studies suggest that natural selection promotes niche differentiation among these phototrophic microorganisms, particularly with respect to their photosynthetic characteristics (Moore *et al.* 1998; Béjà *et al.* 2001; Rocap *et al.* 2003). Here, we analyze competition for light between two closely related picocyanobacteria of the *Synechococcus* group that we isolated from the Baltic Sea (Ernst *et al.* 2003). One of these two has a red colour because it contains the pigment phycoerythrin, whereas the other is blue-green because it contains high contents of the pigment phycocyanin. Theory and competition experiments reveal stable coexistence of the two picocyanobacteria, owing to partitioning of the light spectrum. Further competition experiments with a third marine cyanobacterium, capable of adapting its pigment composition, show that the latter species persists by investing in the pigment that absorbs the colour not utilised by its competitors. These results demonstrate the adaptive significance of divergence in pigment composition of phototrop[1]hic microorganisms, which allows an efficient utilisation of light energy and favours species coexistence.

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Introduction

Phytoplankton harvest light with photosynthetic pigments (Kirk 1994; Falkowski & Raven 1997). These pigments absorb photons in specific regions of the light spectrum, while reflecting or scattering photons in other regions of the spectrum. The latter determines the colour of the pigment. The combination of pigments in a species determines which part of the light spectrum the species can utilise for photosynthesis. Figure 2.1a and 1c show chemostat cultures of two *Synechococcus*-type picocyanobacteria, called BS4 and BS5 that we isolated from 10 m depth during a cruise on the Baltic Sea in August 1996. The two picocyanobacteria are genetically very similar, with less than 1% sequence divergence in their ITS-1 sequences (Ernst *et al.* 2003), yet differ remarkably in colour. The blue-green colour of BS4 is a result of the pigment phycocyanin, which absorbs photons in the orange-red part of the spectrum (620-630 nm; Figure 2.1b). The red colour of BS5 is due to the pigment phycoerythrin, which absorbs photons in the green-yellow part of the spectrum (560-570 nm; Figure 2.1d). The absorption spectra of BS4 and BS5 both show additional peaks in the blue and the red part of the spectrum (at ~430 and ~680 nm, respectively), caused by the pigment chlorophyll *a*, shared by all oxygenic phototrophic organisms.

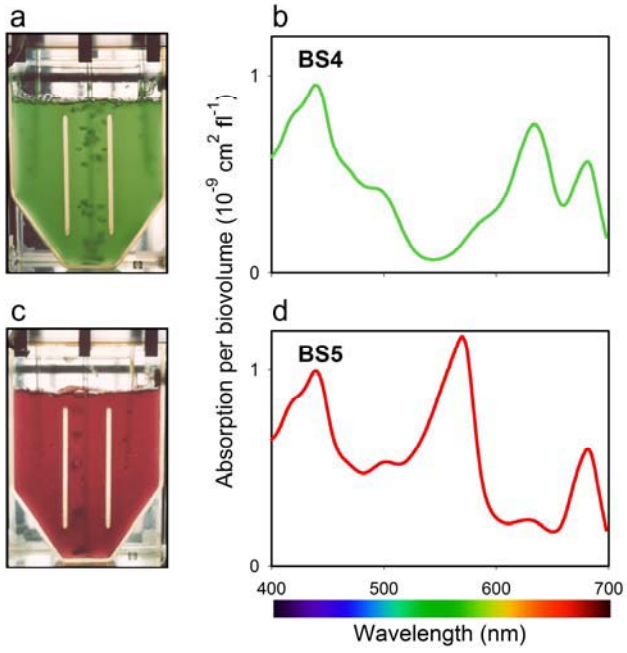

Figure 2.1 Optical characteristics of the picocyanobacteria BS4 and BS5. Monocultures of BS4 (**a**) and BS5 (**c**) grown in chemostats, and the light absorption spectra of BS4 (**b**) and BS5 (**d**).

Would the differences in pigment composition allow coexistence of the two picocyanobacteria, through a subtle form of niche differentiation? Existing theory and experiments on phytoplankton competition for light predict competitive exclusion (Huisman & Weissing 1994; Huisman *et al.* 1999*a*; Huisman *et al.* 2004). That is, photons absorbed by one species are not available for photosynthesis by other species. As a result, species interact by shading each other, and only the strongest competitor for light survives. However, this previous work neglected the spectral aspects of light. Here, we develop a competition model that does include the light spectrum. Consider a mixture of n phytoplankton species. Let N_i denote the population density (in biovolume/mL) of phytoplankton species i, and let $I(\lambda,z)$ denote the light intensity (in μmol photons m^{-2} s^{-1}) of wavelength λ at depth z. According to Lambert-Beer's law, the underwater light spectrum can be described as:

$$I(\lambda, z) = I_{in}(\lambda) \exp\left(- \sum_{i=1}^{n} k_i(\lambda) N_i z - K_{bg}(\lambda) z \right) \tag{2.1}$$

where $I_{in}(\lambda)$ is the spectrum of the incident light intensity, $k_i(\lambda)$ is the specific light absorption spectrum of phytoplankton species i, and $K_{bg}(\lambda)$ is the background light absorption spectrum.

The number of photons absorbed over the photosynthetically active range of 400-700 nm by a single phytoplankter of species i at depth z, which will be denoted as $\gamma_i(z)$, can be quantified as (Sathyendranath & Platt 1989):

$$\gamma_i(z) = \int_{400}^{700} I(\lambda, z) k_i(\lambda) d\lambda \tag{2.2}$$

In photosynthesis, one absorbed photon can excite at most one electron. Hence, the number of photons absorbed by species i is the relevant quantity for photosynthesis, and thus for population growth of species i. Accordingly, if all populations are uniformly distributed over the surface mixed layer, the population dynamics of n competing species in the surface mixed layer can be described as:

$$\frac{dN_i}{dt} = \frac{\phi_i}{z_m} \int_0^{z_m} \gamma_i(z) N_i dz \; - \; L_i N_i \qquad i=1,...,n \tag{2.3}$$

where z_m is the mixing depth, ϕ_i is the photosynthetic efficiency of species i (i.e., the efficiency by which absorbed photons are utilised for population growth), and L_i is the specific loss rate of species i due to factors like grazing, viruses, or other forms of cell death. We note that these differential equations are coupled via Equation 2.1. Thus, the species interact by shading each other in specific regions of the underwater light spectrum.

To test the competition model defined by Equations 2.1-2.3, we ran various experiments with BS4 and BS5 in light-limited chemostats. First, we used monoculture experiments under white light to estimate the model parameters of the two species (see Methods section). The model predictions fitted very well to the monoculture data (Figure 2.2a and b). Parameter values thus obtained are given in Table 2.1. In a next step, we ran competition experiments with BS4 and BS5 under red light, green light, and white light (full spectrum). The population dynamics in these competition experiments are compared against model simulations of competition using the parameter values estimated from the monocultures. Under red light, only BS4 (the green species) was able to survive (Figure 2.2c). Under green light, BS5 (the red species) was the only survivor (Figure 2.2d). In both cases, the experimentally observed population densities remained somewhat below the predicted population densities, presumably because photosynthesis is generally less efficient when all available light is concentrated on a single pigment (Kirk 1994). Under white light, competition yielded stable coexistence of BS4 and BS5 for at least 60 days, in excellent agreement with the model predictions (Figure 2.2e). Simulation of the model over a longer timespan predicted that BS4 and BS5 would maintain almost equal abundances on the long run. These findings demonstrate that partitioning of the light spectrum favours species coexistence.

Some species are able to adapt their pigment composition to the prevailing light spectrum. *Tolypothrix tenuis*, for example, is a marine filamentous cyanobacterium that is able to adjust the ratio of its phycocyanin (PC) to phycoerythrin (PE) content while keeping the total amount of these two pigments constant. This phenomenon is known as complementary chromatic adaptation (Tandeau de Marsac 1977; Ohki *et al.* 1985). We incorporated this ability of chromatic adaptation in the model by the assumption that the ratio between phycoerythrin and phycocyanin changes in a direction that results in an increased specific growth rate under the prevailing light conditions (i.e., an increased fitness) (Metz *et al.* 1992; Abrams 1999). That is, if x_T denotes the fraction PC/(PC+PE) in *Tolypothrix*, then the adaptive dynamics of the pigment composition in *Tolypothrix* can be described as

$$\frac{dx_T}{dt} = \alpha_T \frac{\phi_T}{z_m} \int_0^{z_m} \frac{\partial \gamma_T(x_T, z)}{\partial x_T} dz \qquad (2.4)$$

where the constant of proportionality α_T is a measure of the rate of chromatic adaptation of *Tolypothrix*.

To test these adaptive dynamics, we ran competition experiments with *Tolypothrix* against BS4 and BS5 under white light. *Tolypothrix* and BS4 were able to coexist (Figure 2.3a), because *Tolypothrix* produced the complementary pigment phycoerythrin (Figure 2.3b). That is, *Tolypothrix* turned red in the presence of the green cyanobacterium BS4. In competition with BS5, *Tolypothrix* was driven to low abundance but BS5 was not able to exclude *Tolypothrix* (Figure 2.3c). Hence, *Tolypothrix* and BS5 coexisted as well, because in this case *Tolypothrix* produced the complementary pigment phycocyanin (Figure 2.3d). That is, *Tolypothrix* turned green in the presence of the red cyanobacterium BS5. The model predictions agreed well with the experimental results (Figure 2.3a and c).

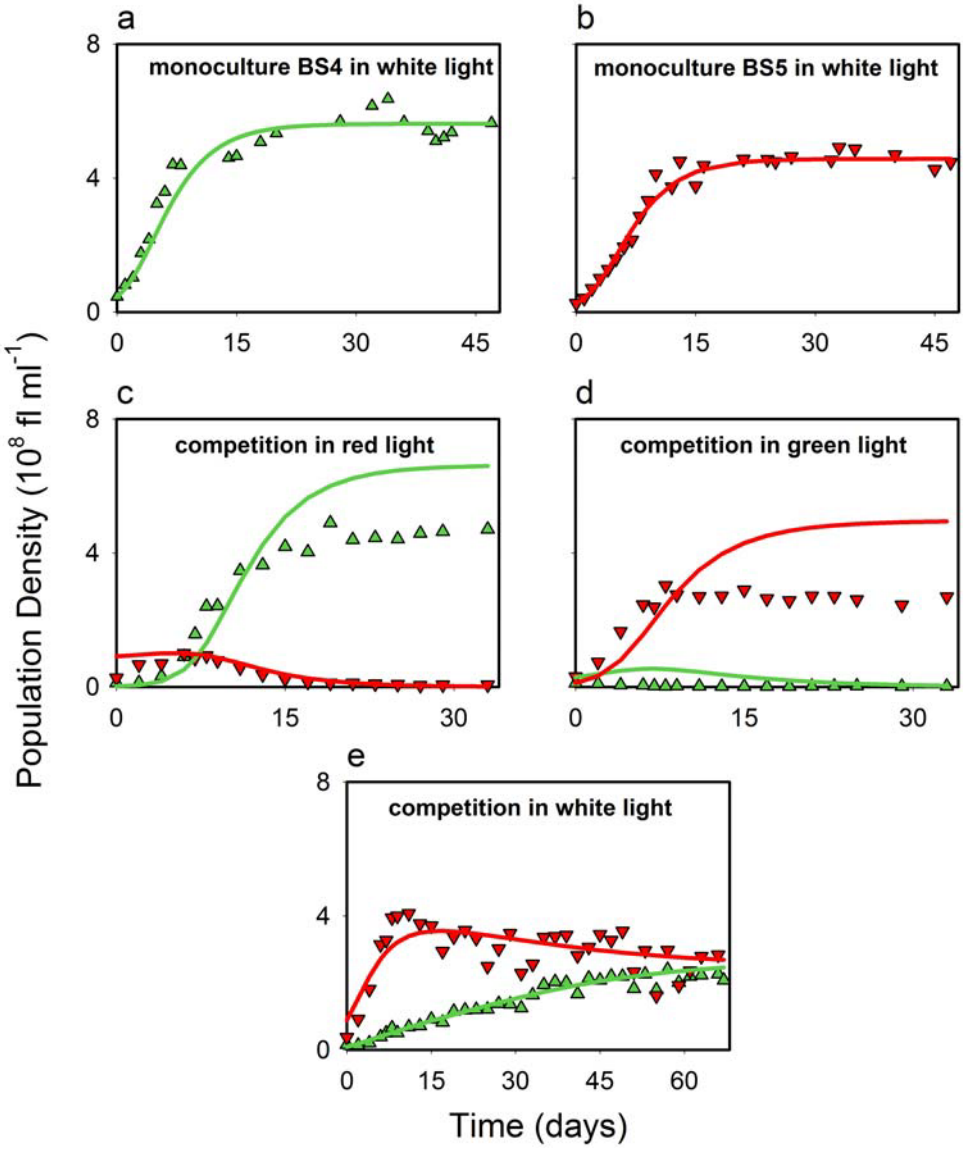

Figure 2.2 Monoculture experiments and competition experiments with the picocyanobacteria BS4 and BS5. Time course of monocultures of BS4 (**a**) and BS5 (**b**) in white light. Competition between BS4 and BS5 in red light (**c**), green light (**d**), and white light (**e**). Symbols represent the observed population densities of BS4 (▲) and BS5 (▼). Lines represent the population densities predicted by the model: green solid line for BS4, red dashed line for BS5. Population densities are expressed in biovolumes (in femtolitres) per millilitre of water.

Furthermore, simulation of the model over a longer timespan predicted that stable coexistence would be maintained on the long run. These findings show that complementary chromatic adaptation favours divergence in pigment composition of competing species. The diversity of the phytoplankton is commonly attributed to a variety of factors, including differential nutrient utilisation, predation resistance, spatial and temporal heterogeneity, and complex dynamics (Hutchinson 1961; Tilman 1982; Sommer 1985; Huisman & Weissing 1999; Irigoien *et al.* 2004). Recent discoveries of various new groups of phototrophic microorganisms in the oceans (Waterbury *et al.* 1979; Chisholm *et al.* 1988, Moon-Van der Staay *et al.* 2001, Kolber *et al.* 2000; Kolber *et al.* 2001; Béjà *et al.* 2002, Béjà *et al.* 2000; Venter *et al.* 2004) have spurred the novel hypothesis that differences in photosynthetic characteristics may offer subtle opportunities for niche differentiation of the plankton as well (Moore *et al.* 1998; Béjà *et al.* 2001; Rocap *et al.* 2003; Ernst *et al.* 2003).

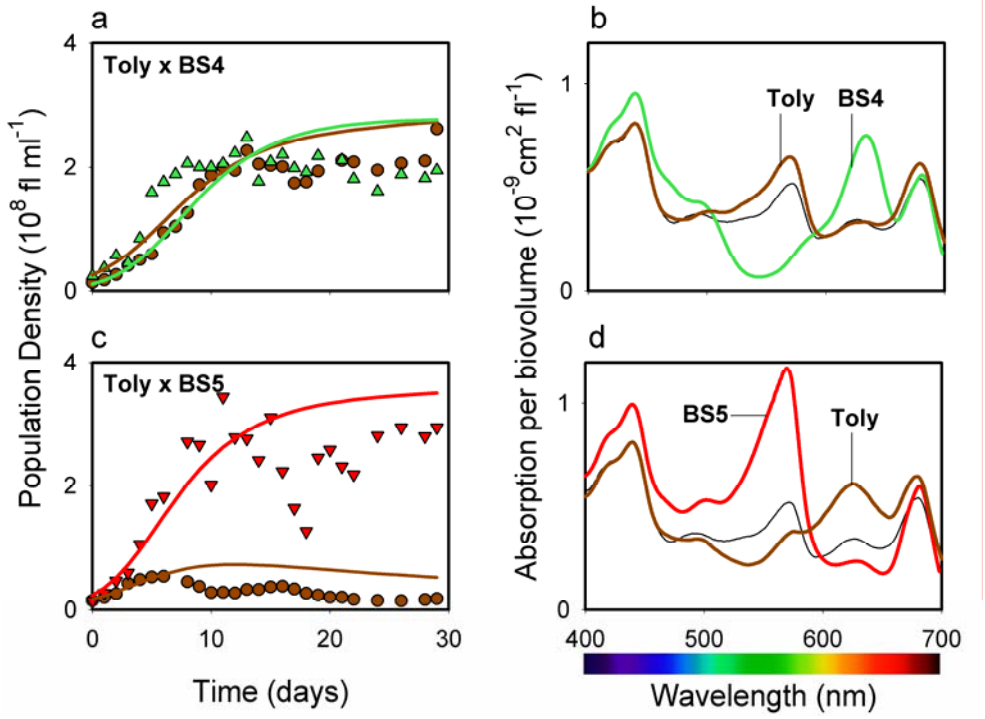

Figure 2.3 Competition between the filamentous cyanobacterium *Tolypothrix* and the picocyanobacteria BS4 and BS5. (**a**) Competition between *Tolypothrix* and BS4. (**b**) Absorption spectra of BS4 (green solid line), and of *Tolypothrix* at day 0 (thin black line) and at day 30 (brown dash-dotted line) of the competition experiment. (**c**) Competition between *Tolypothrix* and BS5. (**d**) Absorption spectra of BS5 (red dashed line), and of *Tolypothrix* at day 0 (thin black line) and at day 30 (brown dash-dotted line) of the competition experiment. In (**a**) and (**c**), symbols represent the observed population densities of BS4 (▲), BS5 (▼), and *Tolypothrix* (●); lines represent the population densities predicted by the model: green solid line for BS4, red dashed line for BS5, and brown dash-dotted line for *Tolypothrix*. Population densities are expressed in biovolumes (in femtolitres) per millilitre of water.

Our findings provide firm evidence for this hypothesis. The theory and experiments demonstrate that selective forces promote partitioning of the light spectrum, which favours divergence in pigment composition. This divergence allows a more efficient utilisation of the available light energy, and contributes to the unexpected biodiversity of phototrophic microorganisms in aquatic ecosystems.

Methods

Experiments

Experiments were performed in chemostats specifically designed to study light-limited growth (Huisman *et al.* 1999*a*). Fluorescent tubes with a white spectrum (Philips TLD 18W/965) were used as light source in all experiments. Green and red light was obtained by placing coloured filters between the light source and the chemostat vessels (Lee filters, Andover, England, #124 dark green filter for green conditions and Lee #26 red filter for red conditions). In all experiments, the light intensity incident upon the chemostat vessels was set at 40 μmol photons m^{-2} s^{-1} (integrated over PAR range), as measured with a Licor LI-190SA quantum sensor. The spectrum of the incident light, $I_{in}(\lambda)$, was measured by a Licor LI-1800 spectroradiometer. To resemble the salinity of the Baltic Sea, we used the following brackish mineral medium: NaCl (8.25 g l^{-1}), $MgCl_2.6H_20$ (0.66 g l^{-1}), KCl (0.17 g l^{-1}), $MgSO_4.7H_2O$ (1.16 g l^{-1}), $CaCl_2.2H_2O$ (0.17 g l^{-1}), Na_3-citrate (4.98 mg l^{-1}), Na_2-EDTA (0.83 mg l^{-1}), $NaNO_3$ (1.25 g l^{-1}), trace metal mix (1.0 mg l^{-1}), Na_2CO_3 (46.0 mg l^{-1}), $K_2HPO_4.3H_2O$ (33.2 mg l^{-1}), Fe-NH_4-citrate (4.8 mg l^{-1}). Given the maximal specific growth rates of BS4, BS5 and *Tolypothrix* in the range 0.025 – 0.030 h^{-1}, the chemostats were run at a dilution rate of 0.014 h^{-1}. Samples were taken daily and population densities were determined. The spherical cells of BS4 and BS5 were counted with a Coulter Elite flow cytometer (Coulter, Florida, USA), which discriminated between the two species based on their pigment fluorescence (Jonker *et al.* 1995). The filamentous cyanobacterium *Tolypothrix* was counted by microscope using image analysis software (Leica Qwin standard, Version 2.5). The absorption spectra of the species, $k_i(\lambda)$, were measured by an Aminco DW-2000 double-beam spectrophotometer. To monitor the changes in the absorption spectra of *Tolypothrix* during the competition experiments with BS4 and BS5, *Tolypothrix* was isolated from the species mixture by percoll-gradient centrifugation (Amersham Biosciences, England). The absorption spectrum of the background, $K_{bg}(\lambda)$, is calculated based upon the spectrum of the incident light and the spectrum of light that leaves the chemostat vessels when filled with mineral medium only.

Simulations

Numerical simulations are based on a 4th order Runge-Kutta procedure for time integration, and Simpson's rule for depth integration. Parameter values are given in Table 2.1. The spectra of incident light and background absorption, the absorption spectra of the species, and the PAR-integrated incident light intensity were measured as described above. The depth of the

water column equalled the depth of the chemostat vessel. The specific loss rate of the species was set equal to the dilution rate of the chemostat. The photosynthetic efficiencies of the species were estimated by fitting the model to monoculture experiments. The chromatic adaptation parameter of *Tolypothrix* was estimated by fitting the model to experiments with *Tolypothrix* under a changing light spectrum. These model fits were obtained by minimisation of the residual sum of squares by means of the Gauss-Marquardt-Levenberg algorithm. This was performed by a software package called PEST (Watermark Numerical Computing, Brisbane, Australia). The parameters thus estimated were used to predict the population dynamics in the competition experiments.

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Table 2.1 Parameter values and their interpretation

Symbol	Interpretation	Units	Value
Independent variables			
t	time	hr	-
z	depth	cm	-
λ	wavelength	nm	-
Dependent variables			
N_i	Population density of species *i*	fl cm^{-3}	-
$\gamma_i(z)$	Absorbed photons by species *i*	µmol photons s^{-1} fl^{-1}	-
$I(\lambda,z)$	Underwater light spectrum	µmol photons $m^{-2}s^{-1}nm^{-1}$	-
x_T	Fraction of phycocyanin of *Tolypothrix*	dimensionless	-
Parameters			
$I_{in}(\lambda)$	Spectrum of incident light	µmol photons $m^{-2}s^{-1}nm^{-1}$	m
$\int_{400}^{700} I_{in}(\lambda)d\lambda$	PAR-integrated incident light intensity	µmol photons m^{-2} s^{-1}	40 m
$K_{bg}(\lambda)$	Spectrum background absorption	cm^{-1}	m
$k_i(\lambda)$	Absorption spectrum of species *i*	cm^2 fl^{-1}	m
z_m	Depth of the water column	cm	7.7 m
L_i	Specific loss rate of species *i*	h^{-1}	0.014 m
ϕ_i	Photosynthetic efficiency, of	fl (µmol photons)$^{-1}$	
	- BS4		2.2×10^6 e
	- BS5		1.6×10^6 e
	- Tolypothrix		1.7×10^6 e
α_T	Chromatic adaptation parameter of *Tolypothrix*	dimensionless	0.12 e

fl = femtolitre, denoting the biovolume of the species.
m = measured parameter, e = estimated parameter (see Methods).