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Colourful coexistence : a new solution to the plankton paradox

Stomp, M.

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Appendix C

Chemostat conditions

We used a nutrient-rich mineral medium to avoid nutrient limitation (Stomp *et al.* 2004). NaCl was added to obtain a salinity of ~ 12 g/L, resembling the brackish waters of the Baltic Sea. The chemostat vessels were bubbled with compressed air enriched with CO₂ to avoid carbon limitation. The CO₂ inflow was controlled by mass flow controllers (BROOKS Smart Mass Flow) maintaining the pH between 8 and 8.5. The chemostat vessels were maintained at a constant temperature of 21 °C by water jackets placed between the light sources and the chemostat vessel, and connected to a Coloura thermocryostat.

Background turbidity

The absorption spectrum of the background turbidity, $K_{bg}(\lambda)$, was calculated from the spectrum of the incident light intensity, $I_{in}(\lambda)$, and the spectrum of light penetrating through the chemostat vessels, $I_{out}(\lambda)$, when filled with mineral medium only:

$$K_{bg}(\lambda) = \frac{\ln(I_{in}(\lambda)) - \ln(I_{out}(\lambda))}{z_M} \quad (C1)$$

The background absorption in our shallow chemostat vessels was largely independent of wavelength, and thus could be treated as a wavelength-independent parameter K_{bg} .

Population densities

Samples were taken from the chemostat experiments daily, and were fixed with a solution of 10% glutaraldehyde and 1% formaldehyde (Tsuji & Yanagita 1981). The population densities of the species were counted in duplicate with a MoFlow flowcytometer (Dako Cytomation, Fort Collins, CO, USA), equipped with a blue laser (488 nm) and a red laser (633 nm). The flow cytometer could distinguish between red and green cyanobacteria based upon their different fluorescence signals (Jonker *et al.* 1995; Stomp *et al.* 2004). Cells rich in phycoerythrin emitted yellow-orange light (550–620 nm) when excited by the blue laser, whereas cells rich in phycocyanin emitted far red light (> 670 nm) when excited by the red laser. The flow cytometer distinguished between the single-celled picocyanobacteria and the much larger filaments of *Pseudanabaena* by their size (using side scattering). Since the picocyanobacteria were much smaller than the *Pseudanabaena* filaments, the counts obtained by flow cytometry were not a good indicator of the biomasses of the three species. For comparison, therefore,

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the population densities of the species were converted to the total light absorption (per cm) by each species, A_i , which can be expressed as:

$$A_i = \frac{1}{z_m} \ln \left(\frac{\int_{400}^{700} I_{in}(\lambda) d\lambda}{\int_{400}^{700} I_{in}(\lambda) e^{-k_i(\lambda) N_i z_m} d\lambda} \right) \quad (C2)$$

The fluorescence signals of the *Pseudanabaena* filaments obtained from the blue and red laser provided information on the relative content of phycoerythrin and phycocyanin in *Pseudanabaena*. Using monoculture experiments of *Pseudanabaena* grown under different red-light and green-light conditions, we calibrated these fluorescence signals with absorption spectra measured with the AMINCO DW-2000 double-beam spectrophotometer. This enabled estimation of the fraction phycoerythrin (i.e., the parameter x_i in Equation 6.4 of the main text), and hence the absorption spectrum of *Pseudanabaena* during competition could be reconstructed from the flow-cytometer measurements.