Colourful coexistence: a new solution to the plankton paradox

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Publication date
2008

Citation for published version (APA):

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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:

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

(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% gluteraldehyde 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,
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:

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

(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.