Dynamic species interactions in phototrophic biofilms

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Chapter 3

Temperature and light dependent performance of the cyanobacterium *Leptolyngbya foveolarum* and the diatom *Nitzschia perminuta* in mixed biofilms
Chapter 3

Abstract

This paper investigates the role of species interactions as a mechanism determining the changing seasonal abundance of microphytobenthic species. Different kinds of interactions can occur between benthic algal species, e.g. interference competition (a species directly hindering the growth of another) or resource competition. If such interactions are strong, the capacity of species to exploit parts of the seasonal spectrum of temperature and light conditions could be greatly affected.

A model system of two freshwater benthic algae, the cyanobacterium *Leptolyngbya foveolarum* and the diatom *Nitzschia perminuta*, was used to study the capacity of each species to grow in ranges of temperature (7, 15 and 25 °C) and light (5, 40 and 200 μmol m⁻² s⁻¹) conditions in single-species and two-species cultures.

Growth was followed for 14 - 17 days by measuring chlorophyll a and maximum photosynthetic capacity using spectrophotometry and PAM fluorimetry. A PHYTO-PAM fluorimeter facilitated simultaneous observations in mixed cultures on the two species.

In mixed cultures, the diatom appeared to be a “cool season species” (low temperature and low light intensity) and the cyanobacterium a “summer or autumn species” (higher temperature and light intensities). This is different than predicted by monoculture experiments, where a wide range of optimal growth conditions was found.

Two-species biofilm tests indicated inhibitory effects of the cyanobacterium on the diatom species, especially under conditions favorable to the cyanobacterium. High or low light intensities, increase of local pH caused by depletion of inorganic carbon, and limitation of other inorganic nutrients (resource competition) were examined as factors contributing to diatom inhibition, but none provided an acceptable explanation for observed growth patterns. Our results pointed towards interference competition.
**Introduction**

The seasonal succession of micro-algal communities in the temperate zone shows distinct patterns of species abundance and of taxonomic groups, such as diatoms, green algae and cyanobacteria for both plankton (e.g. Pick and Agbeti 1991) and benthos (e.g. McCormick and Stevenson 1991). Experimental studies of this phenomenon have often been focused on species-specific differences in the capacities of microalgae to exploit a certain range of conditions defined by e.g. nutrient concentration or light regime (Reynolds 1997). However, if interspecific interactions between species are strong, the physiological capacities of the species to exploit parts of the seasonal spectrum of conditions could be greatly affected.

It is well established that the exploitation of nutrients, the availability of which varies strongly through the seasons, differs among species and thus might generate succession (Tilman 1982). However, competition for resources may be modified by direct biotic interactions leading to ‘interference competition’ (sensu McCormick 1996): competition in which one species directly kills, overgrows, or inhibits another species. Interference competition between microalgal species is likely to be more important in benthic communities where cells are packed together tightly on the surface of the substrate, than in planktonic communities. Strong interactions between biofilm forming heterotrophic bacteria (Wimpenny et al. 2000) and between bacteria and algae (Murray et al. 1986) have been proven, however for benthic microalgae such knowledge is still lacking.

In this study, we investigated if interference competition could be one of the mechanisms determining changing seasonal abundance of algal species in a biofilm. To test this hypothesis, we chose as a model system two freshwater benthic phototrophic species, the cyanobacterium *Leptolyngbya foveolarum* and the diatom *Nitzschia perminuta*. We studied their coexistence under a range of light and temperature conditions, knowing that microalgal species exhibit a large flexibility to these environmental factors (Eppley 1972, Reynolds 1997).

The model algae were observed in single species and two-species cultures to establish their capacity to persist in ranges of light or temperature, reflecting the range of seasonal changes. A novel application of a method for recording the abundance and photosynthetic efficiency of the two species in mixed culture was applied.
Methods

Algal cultures

Two benthic freshwater microalgae were used for the experiments: the motile pennate diatom *Nitzschia perminuta* (Grunow) M. Peragallo and the filamentous cyanobacterium *Leptolyngbya foveolarum* (Rabenhorst *ex* Gomont) Anagnostidis *et* Komárek. These algae were collected from coarse sandy sediments at two floodplain lakes of the river Rhine at a water depth between 10 and 50 cm. They were isolated as single cells and filaments under a dissecting microscope.

Non-axenic unicellular stock cultures were kept in 100 ml Erlenmeyer flasks on 75 ml nutrient-rich WC medium (Guillard and Lorenzen 1972) modified as follows: the concentration of 1.0 mg l⁻¹ H₃BO₃ was lowered to 0.006 mg l⁻¹, and molybdenum was added as sodium salt (same molybdenum concentration as in original WC medium). HEPES (2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid) buffer was used to stabilize pH at 7.0. The flasks were closed with cellulose plugs to allow for CO₂ exchange with the air.

The cultures were illuminated from above with fluorescent cool-white tubes (TL Philips 65W/33 or Massive 36W/33) following a light:dark regime of 16:8 hours at 35 ± 5 μmol m⁻² s⁻¹ (measured with a LICOR LI-185B) in a temperature controlled chamber at 20 °C. An inoculum of the stock culture was transferred bi-monthly to new medium to ensure continuous growth.

To obtain enough biomass for the experiments, inocula from the stock were transferred to 1000 ml Erlenmeyer flasks with 300 - 400 ml of medium, using glass beads as substrate for biofilm development. From preliminary experiments, it was concluded that a layer of ca. 3 mm of glass beads (Ø 490 - 700 μm) was a more suitable artificial substrate for benthic algae for this kind of growth experiments compared to other sizes of beads, glass discs or analytically clean sand. Both algae grew well on this substrate, which provided enough space for the algae to colonize (generating a two-fold enlargement of the growth surface). Algae were easily removed from the substrate for sampling by shaking the flask for 20 min (350 r.p.m.) homogenizing the culture.

Measurements on cultures

In pilot experiments comprising wide ranges of temperature and light, the growth of the two test algae in monocultures was recorded with three techniques: cell number and cell volume (Coulter Counter), pigment determinations (spectrophotometry) and PAM fluorimetry (PHYTO-PAM).
Cell number and cell volume were measured as follows: after homogenizing the culture, triplicate subsamples were stabilized with 30 µl Lugol's iodine to avoid sticking of the benthic algae to the vials. Subsequently, these subsamples were diluted with a saline solution and cell densities (ml⁻¹) and total cell volume (µm³ ml⁻¹) were determined using the Coulter Multisizer II (70 µm aperture). Total cell volume was used to ensure uniform inoculation at the start of the experiments.

Pigments were measured as follows: chlorophyll a and phaeopigments were extracted overnight with 90% acetone from filtered (GF/C, 1.2 µm) subsamples of the homogenized cultures and measured with a Shimadzu UV-1601 spectrophotometer. Phaeophytin corrected concentrations were calculated according to Lorenzen (1967) and were expressed as µg I⁻¹ chlorophyll a.

PAM fluorimetry was used as a measure of chlorophyll a concentration and maximum photosynthetic capacity. In vivo chlorophyll fluorescence was determined using a PHYTO-PAM (pulse amplitude modulated fluorimeter) equipped with a special fiberoptics-Emitter-Detector-Unit PHYTO-EDF and PhytoWin software v1.03b (Walz, Effeltrich, Germany). The special EDF-unit features a 50 mm long, 5 mm Ø quartz rod, which allowed measurement on surfaces (Schreiber et al. 2002) as well as in homogenized cultures.

The fluorescence parameters used in this study were: $F_0$, the minimal fluorescence signal of dark-adapted cells and $F_m$, the maximal fluorescence signal after a saturating light pulse in dark-adapted cells. These two parameters allow the calculation of the maximum quantum yield of photosystem II ($\Phi_0$), which is a measure of the maximum photochemical efficiency of PSII, using the equation $\Phi_0 = (F_m-F_0)/F_m$. The fluorescence nomenclature is in accordance with Van Kooten and Snel (1990). $\Phi_0$ can be used as an indicator for the general level of fitness of the photosynthetic organisms under non-steady state conditions (Kolber and Falkowski 1993, Schreiber et al. 1995, Parkhill et al. 2001, Lippemeier et al. 1999).

The PHYTO-PAM fluorimeter can distinguish between differently pigmented algal groups (such as diatoms, cyanobacteria and green algae) by applying 4 different excitation wavelengths (650, 590, 525 and 470 nm). This allows a separate measurement of the fluorescence signal of each algal group in a mixture. Fluorescence signals from the four wavelengths were deconvoluted to the two algal groups, using reference spectra from the two test species. The reference spectra were recorded in high-density suspensions, this allowed differentiation of the fluorescence signal in the mixtures.

Because the $F_0$ values from the PHYTO-PAM are relative units, a calibration using actual chlorophyll a concentrations was performed for each species, using $F_0$ and spectrophotometrically derived chlorophyll a values from monocultures. With similar deconvoluted fluorescence, chlorophyll a values for *L. foveolarum* were 1.5 times the chlorophyll a value of *N. perminuta* for our PHYTO-PAM.
Table 3.1
Survey of experimental conditions for the temperature experiments and the light experiments (PAR: Photosynthetically Active Radiation during the light period of 16h per day). Initial cell densities in each Erlenmeyer flask are given as biovolumes and added up for two species in mixed cultures.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Culture</th>
<th>Species</th>
<th>T (°C)</th>
<th>PAR (µmol m⁻² s⁻¹)</th>
<th>Initial cell density (µm³ ml⁻¹)</th>
<th>Sampling days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature Mono</td>
<td>N. perminuta</td>
<td>7, 15, 25</td>
<td>35 ± 5</td>
<td>2.70*10⁵</td>
<td>4, 7, 10, 12, 14</td>
<td></td>
</tr>
<tr>
<td>Mono</td>
<td>L. foveolarum</td>
<td>7, 15, 25</td>
<td>35 ± 5</td>
<td>2.70*10⁵</td>
<td>4, 7, 10, 12, 14</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>Both</td>
<td>7, 15, 25</td>
<td>35 ± 5</td>
<td>5.40*10⁵</td>
<td>4, 7, 10, 12, 14</td>
<td></td>
</tr>
<tr>
<td>Light Mono</td>
<td>N. perminuta</td>
<td>20</td>
<td>5, 40, 200</td>
<td>6.90*10⁵</td>
<td>3, 8, 10, 14, 17</td>
<td></td>
</tr>
<tr>
<td>Mono</td>
<td>L. foveolarum</td>
<td>20</td>
<td>5, 40, 200</td>
<td>6.90*10⁵</td>
<td>3, 8, 10, 14, 17</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>Both</td>
<td>20</td>
<td>5, 40, 200</td>
<td>1.38*10⁶</td>
<td>3, 8, 10, 14, 17</td>
<td></td>
</tr>
</tbody>
</table>

The detection limit of the PHYTO-PAM was 13.5 and 15 µg chlorophyll a l⁻¹ for L. foveolarum and N. perminuta respectively. Maximum photosynthetic capacity, (Φ₀), of all N. perminuta cultures was significantly higher than Φ₀ of the L. foveolarum cultures, but these values were not corrected for species-specific differences, as Φ₀ values are independent of biomass.

Experimental setup

The algae were precultured at all different temperature and light conditions, during 9 and 11 days (in the temperature and light experiments respectively) prior to the start of experiments. Part of the medium was replaced in these precultures every 3 - 4 days to avoid nutrient limitation.

The initial algal density in the monocultures was measured as total cell volume, as described above. The mixed cultures contained the same inoculum of each of the two algae, so the initial algal density on the basis of biovolume was twice as high as biovolume in the monocultures (Table 3.1). The inoculum size was such that biofilms were kept thin during the time span of the experiments to minimize formation of extreme gradients within the biofilm. The start inocula from the mono- and mixed cultures were taken on the same day from the same preculture, but at different times for the temperature and the light experiment.

Adapted cells of each species were transferred to sterile 300 ml Erlenmeyer flasks with cellulose plugs containing 100 ml fresh and sterile modified WC medium and 20 g glass beads. Both species were grown simultaneously as monocultures and
mixed cultures of the two species for a period of 14 to 17 days. The experiment started with 15 replicate flasks per treatment and at each sampling date three flasks per treatment were removed to sample for chlorophyll a and maximum photosynthetic capacity measurements (Table 3.1). The position of replicate flasks was randomly changed every other day to eliminate any location effect due to minor changes in external conditions.

As the total pool of nutrients in the medium was sufficient to reach very high cell densities, the medium was not replaced during the experiments, allowing the growth of an undisturbed biofilm. As a consequence of high algal densities, pH (measured with a glass Sentix 41 pH electrode on the colonized bead surface) rose towards the end of the experiments (first observed in the temperature experiment). Therefore, the Erlenmeyer flasks were swirled gently to neutralize the CO₂ gradient, to slightly lower the pH without breaking up the biofilm. This was only done when pH exceeded 8.5 in the light experiment.

On each sampling date, maximum photochemical efficiency of photosystem II (Φ₀) of the algae was measured in triplicate in the Erlenmeyer flasks 1 mm above the glass bead surface, after dark adaptation for 30 min at 20 °C, using the PHYTO-PAM signal, calibrated and deconvoluted as described above. Because it was difficult to position the PAM measuring fiber exactly 1 mm from the glass bead surface, these measurements were used only for the biomass independent Φ₀ calculations. For more accurate biomass estimation, the Φ₀ data from homogenized samples were used, although Φ₀ data from both methods compared reasonably well.

To dislodge the biofilm from the substrate and homogenize the culture as a suspension, cultures were shaken for 20 min on a rotating shaking device (350 r.p.m.), efficiently removing both algal species from the glass beads. Algal density was determined by fluorescence measurements with the calibrated PHYTO-PAM. Minimum fluorescence (Φ₀) of a dark-adapted 3 ml subsample of the homogenized culture was measured in triplicate in an acrylic cuvette, at a distance of 10 mm from the bottom of the cuvette to obtain a reliable chlorophyll a estimation of the whole culture. Subsamples of homogenized cultures were analyzed for chlorophyll a with the spectrophotometer as described above.

Data analyses

Maximum specific growth rates (μ_max) were calculated as:

\[ \mu_{\text{max}} = \frac{\ln (B_t / B_0)}{\Delta t} \]  

(3.1)

where \( B_0 \) is the density of chlorophyll a at the start of the exponential phase, \( B_t \) is the density at time \( t \) in the exponential phase and \( \Delta t \) is the time interval. Values of \( \mu_{\text{max}} \) are given as mean specific rate of increase of chlorophyll a \( (d^{-1}) \pm \) standard error.
Data points belonging to the exponential growth phase were selected from the log-linear regression plots of the whole growth curve for each treatment individually.

Differences between treatments (species, temperature, light, mono- or mixed cultures) were tested for significance \( p > 0.05 \) with two-factor analyses of variance (ANOVA), with biomass concentration as dependent factor and treatment and time as fixed factors (GLM univariate procedure). Decrease of maximum photosynthetic capacity was tested using linear regression and analysis of variance. Statistical analyses were done using SPSS v. 11 software.

**Results**

Figure 3.1 shows growth curves of *Leptolyngbya foveolarum* and *Nitzschia perminuta* in monocultures, measured as chlorophyll fluorescence (PHYTO-PAM) and extracted chlorophyll \( a \).

![Figure 3.1](image-url)

Figure 3.1

Growth curves of *Leptolyngbya foveolarum* (panels a, c) and *Nitzschia perminuta* (panels b, d) in monoculture at the three light intensities (5, 40 and 200 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), measured as chlorophyll fluorescence (PHYTO-PAM, panels a, b) and extracted chlorophyll \( a \) (Spectrophotometrically measured, panels c, d). Averages \( \pm \text{SD} \) (\( n = 3 \)).
Growth was measured under widely different light intensities that led to different pigment concentrations per cell. *N. perminuta* contained 0.025, 0.013 and 0.005 pg chl a µm⁻³ at respectively 5, 40 and 200 µmol m⁻² s⁻¹. For *L. foveolarum*, chlorophyll content averaged to 0.010 ± 0.004 pg chl a µm⁻³ for the three light intensities, with large margins of uncertainty due to difficulties in counting filaments with the Coulter Counter.

Despite variable chlorophyll a concentration per volume, PHYTO-PAM-derived and spectrophotometer-derived chlorophyll a showed almost identical growth curves in monocultures for both species (Figure 3.1), which is in accordance with previously found positive correlations between fluorescence and biomass in field studies (Serodio *et al.* 1997, Honeywill *et al.* 2002). Despite variable chlorophyll a contents of adapted algae, similar growth curves can effectively be established with both methods. Species-specific growth in mixtures can only be measured with PHYTO-PAM, therefore only the observations made with PHYTO-PAM were presented.

**Effect of temperature**

The growth of the cyanobacterium *L. foveolarum* and the diatom *N. perminuta* in monoculture differed between species grown at different temperatures (Figure 3.2). The cyanobacterium showed its highest maximum growth rate (0.40 ± 0.01 d⁻¹) in monoculture at 25 °C, decreasing to 0.19 ± 0.03 (d⁻¹) at 15 °C and marginal growth at 7 °C. Similarly, increase of algal biomass was highest at 25 °C, lower at 15 °C and minimal at 7 °C (Figure 3.2a, b, c).

The diatom showed its highest maximum growth rate in monoculture at 25 °C (0.65 ± 0.14 d⁻¹). Although this rate was lower at 15 °C (0.30 ± 0.10 d⁻¹), two-way ANOVA showed no significant differences between these two treatments (Figure 3.2e, f). In contrast to the cyanobacterium, the diatom grew well at 7 °C (µmax 0.26 ± 0.03 d⁻¹). However, the lag phase was extended and as a result, the incubation period of 14 days was too short to obtain a full growth curve (Figure 3.2d).

The overall differences in growth between the species were larger when they coexisted in a mixture (Figure 3.2), than when grown in monoculture. On the one hand, the density of *L. foveolarum* was unaffected by coexistence at 15 and 25 °C but decreased earlier at 7 °C compared to its monocultures (Figure 3.2b, c, a). On the other hand, the diatom response was significantly different in the mixture than in monoculture: at 25 °C the growth of *N. perminuta* was terminated after 7 days in the mixture, while at 15 °C it still showed continuous growth but at lower densities than in monoculture (Figure 3.2f, e). At 7 °C, this species reached significantly higher densities than in monoculture (Figure 3.2d).
Temperature experiment: changes of biomass in time recorded as fluorimetrically measured chlorophyll a increase. Chlorophyll a values in the mixtures derived simultaneously for N. perminuta and L. foveolarum from PAM fluorimetry (see Methods). Panel a, b and c represent data from L. foveolarum, panel d, e and f represent data from N. perminuta. Closed symbols represent monoculture data; open symbols represent data from mixed cultures. Averages ± SD (n = 3).
Effect of light

Observations from pilot experiments with monocultures showed similar growth of both species at 20 °C. Therefore this temperature was selected for experiments on light.

In monoculture, the cyanobacterium densities were not significantly different at the three light intensities (Figure 3.3a, b, c) however, the calculated maximum growth rates at 5 and 200 μmol m² s⁻¹ (0.22 ± 0.03 and 0.21 ± 0.01 d⁻¹ respectively) were lower than at 40 μmol m² s⁻¹ (0.30 ± 0.02 d⁻¹). The diatom showed a significantly lower increase in density at the highest light intensity (200 μmol m² s⁻¹), compared to the two lower light intensities (Figure 3.3d, e, f). Nevertheless, maximum growth rates at 5 and 200 μmol m² s⁻¹ (0.28 ± 0.03 and 0.29 ± 0.02 d⁻¹ respectively) were lower than at 40 μmol m² s⁻¹ (0.39 ± 0.04 d⁻¹).

In mixture, the cyanobacterium was unaffected (no significant differences) by the coexistence at the two higher light intensities (Figure 3.3b, c), however, at 5 μmol m² s⁻¹ the growth was slightly but significantly lower than in monoculture (Figure 3.3a). The growth of the diatom was significantly affected at all light intensities in the mixture, compared to the monoculture. At the highest light intensity, the growth of the diatom stopped in the mixture after some initial growth. A decrease in chlorophyll a concentration was detected during the remaining nine days of the experiment.

The time course of suppression of N. perminuta by L. foveolarum under high light (Figure 3.3f) resembled the suppression that occurred at high temperature (Figure 3.2f). At very low light intensities the growth of both species was significantly lower in mixed cultures than in monocultures (Figure 3.3a, d). Specific growth rates at these low light intensities were also lower compared to the monocultures (0.22 ± 0.03 to 0.17 ± 0.01 d⁻¹ for L. foveolarum and 0.28 ± 0.03 to 0.21 ± 0.02 d⁻¹ for N. perminuta).
Chapter 3

$L. foveolarum$

$N. perminuta$

**Figure 3.3**

Light experiment: changes of biomass in time recorded as fluorimetrically measured chlorophyll a increase. Chlorophyll a values in the mixtures derived simultaneously for $N. perminuta$ and $L. foveolarum$ from PAM fluorimetry (see Methods). Panel a, b and c represent data from $L. foveolarum$, panel d, e and f represent data from $N. perminuta$. Closed symbols represent monoculture data; open symbols represent data from mixed cultures. Averages ± SD (n = 3).
**pH**

In monocultures of *N. perminuta*, pH never exceeded 8.4 in the temperature experiment and 7.8 in the light experiment (results not shown). In contrast, in monocultures of *L. foveolarum* pH rose up to 9.7 at 25 °C and to pH 8.5 at 200 μmol m⁻² s⁻¹ (results not shown). Figure 3.4 shows the development of pH in the mixed cultures.

The pH was measured with a macro-electrode and thus might have underestimated the extreme pH values developing within the biofilm. Yet, it is clear that extreme pH values (over 9) concurred with cyanobacterial dominance in the temperature experiment (Figure 3.4a), while values between 7.9 and 8.9 accompanied such events in the light experiment (Figure 3.4b).

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**Figure 3.4**

pH values measured in the culture medium on the colonized bead surface during the experiments in the mixed cultures. Panel a: temperature experiment, panel b: light experiment with light intensities of 5, 40 and 200 μmol m⁻² s⁻¹.
Maximum photochemical efficiency ($\Phi_0$)

Under optimal temperature conditions (25 °C, Figure 3.2c) the average values of $\Phi_0$ in the cyanobacterial monoculture showed no significant change in time (ranging from 0.17 ± 0.08 on day 4 to 0.24 ± 0.03 on day 14), while wider variations were observed at low densities in monoculture (e.g. at 7 °C from 0.25 ± 0.15 at day 4 to 0.34 ± 0.01 at day 14, not significantly different). In accordance with published observations (e.g. Campbell et al. 1998) the diatom showed higher $\Phi_0$ values (between 0.57 and 0.72 for all treatments), with only minor differences between the temperature and light conditions tested and hardly changing in time.

The $\Phi_0$ values of diatoms and cyanobacteria in mixed cultures were measured simultaneously and were compared to their respective monocultures. $N. perminuta$, in coexistence with $L. foveolarum$ at 25 °C, showed average $\Phi_0$ values of 0.60, with only 0.05 unit change in time. Thus, the consistent inhibition of growth observed for the diatoms (Figure 3.2d, e, f) did not result in a decrease of the maximum photochemical efficiency. Similarly, inhibition of diatom growth at 200 μmol m$^{-2}$ s$^{-1}$ (Figure 3.3f) was not matched with any systematic change in $\Phi_0$. For $L. foveolarum$ such changes were not evident either.

Discussion

The present study, together with Watermann et al. (1999), shows that cyanobacterial dominance at high temperatures (25 °C) can be mimicked in synthetic multi-species biofilms. Diatoms have shown large increases in density, both at high temperature in monocultures in laboratory experiments (this study, Admiraal 1977) and in the field in a population composed only of diatoms (Blanchard et al. 1997).

Nevertheless, our observations in mixtures of a diatom and a cyanobacterium fully agree with previous studies showing diatoms as “cool season species” and cyanobacteria as “summer or autumn species” in natural mixed communities (e.g. Davison 1991, Snoeijis 1990, Coles and Jones 2000). We also showed that even though our diatom has the capacity to grow at a wide range of light intensities, high light intensities promote cyanobacterial dominance in mixture. Therefore it can be concluded that diatoms are potentially able to grow well at high temperatures and light intensities, but cannot compete well with cyanobacteria.

The mechanisms responsible for seasonal distribution of diatoms and cyanobacteria are still subject of discussion (Shapiro, 1990). Most of the arguments for the succeeding consortia of diatoms and cyanobacteria stem from observations of phytoplankton: resource competition (e.g. for light and nutrients, Reynolds 1997, Tilman 1982) is assumed to be a dominant mechanism, but interference competition is probably also strong, especially in benthic algae consortia. Experimental proof of
Temperature and light interactions caused by interference competition in benthic algal assemblages is still lacking (McCormick 1996).

Resource competition could be one explanation of the interactions found in this study. Resource competition for temperature is not possible, but competition for light could have promoted cyanobacterial dominance at high light intensities in this study. Although it is known that several planktonic cyanobacteria prefer lower light intensities (Scheffer et al. 1997) and photoinhibit sooner than diatoms (Coles and Jones 2000, Litchman 2000), benthic algae generally have higher photoacclimation parameters ($I_0$) than planktonic algae (Barranguet et al. 1998). This allows them to grow at much higher light intensities, as was shown for *L. foveolarum* in this study.

If light were the key factor in these interactions, it should be limiting for one of the two species. Yet, the excess dose of light in our study had larger consequences than a limiting dose: low light permitted *N. perminuta* to reach optimum growth and to coexist with *L. foveolarum*, while excess light enabled *L. foveolarum* to dominate. Because algal density at low light intensity was lower for both species in mixture compared to in monoculture, self-shading could have occurred, favoring the success of the dominant species in mixed culture when algal density increased. However, at very low algal density in the initial exponential growth phase, where self-shading was improbable, specific growth rates were also lower compared to growth rates in the monocultures. Therefore, light as a limiting resource could not explain the dominance of the cyanobacterium found at high light intensities.

Competition for nutrients could be another explanation for cyanobacterial dominance. At high cell densities (at high temperature and high light intensities) the inorganic carbon resources were depleted as indicated by the rise of pH. The capacity of cyanobacteria in general (and also the present *L. foveolarum*) to exhaust DIC could have deprived coexisting diatoms from essential substrate. The prominent role of CO$_2$ and pH for cyanobacteria in general is advocated by Shapiro (1990), particularly in the case of plankton. Earlier observations of coexisting diatom species (De Jong and Admiraal 1984) showed the relevance of CO$_2$ to diatom films, while a biofilm model predicted the key role for diffusion of carbon substrates and their different exploitation by cyanobacteria and diatoms (Ludden et al. 1985). The present observations indicate that pH values over 9 concur with the “loosing” of the diatom (in the temperature experiment) but inhibition of the diatom could already be seen on day 7, where pH was not that high and hence not under conditions of carbon limitation. Moreover, in the better-mixed light experiment pH values of 7.9 - 8.9 provoked a similar inhibition.

Although biofilms were kept thin during the experiments to diminish the chance of strong gradients within the biofilm, environmental conditions within the biofilm were not measured; this would have required micro-electrodes. Barranguet et
al. (1997) found spatial replacement of diatoms by cyanobacteria at high temperatures in tidal sediments without extreme increase of pH.

Moreover, maximum photochemical efficiency did not decrease, indicating no proof of limitation for other inorganic nutrients like nitrogen, silicate or phosphorus (Parkhill et al. 2001, Lippemeier et al. 1999). Thus, it cannot be concluded that competition for a depleted resource is the key factor for the exclusion of the tested diatom species.

Benthic algal species occupy space that cannot be used for settlement of co-existing algae. Tujii (2000) demonstrated that successional stages of biofilms harbor morphologically different forms (enabling vertical structures in the biofilm) differing in their exploitation of limiting light. The vigorous growth of a filamentous alga, such as *L. foveolarum*, may create overstories expanding the overall space of the biofilm (Steinman et al. 1992). At the same time, the gelatinous structures, produced by dominant phototrophic organisms may limit the options for settlement of co-existing species (Chapter 6).

The present experiments provide proof of inhibitory effects of a cyanobacterium on a diatom species, especially under conditions that favor the cyanobacterium. An explanation of these effects could not be found in the concept of exploitative competition. It is possible that in our case interference competition played an important role. It is tempting to suggest that allelopathic interaction is involved, in accordance with many reports of anti-microbial compounds released by cyanobacteria (Von Elert and Juttner 1997, Smith and Doan 1999, Ray and Bagchi 2001); the impaired cell multiplication, which was shown in our experiments already from day 7 on, possibly points towards a chemical warfare between two phototrophs.

Under natural conditions, earlier observations (De Jong and Admiraal, 1984) of the effects of salinity on estuarine benthic diatoms in monoculture and mixed culture indicated that the physiological range at which maximum growth rates occurred in mixed cultures was greatly restricted compared to monocultures. In the present study, the coexistence of two phototrophic species led to a similarly restricted range of conditions permitting dominance: i.e. low light, low temperature for the diatom and high light, high temperature for the cyanobacterium.

From our results it appeared also that ranking species according to their physiological range for temperature and light does not allow an accurate prediction of their seasonal occurrence in mixed biofilms. Other mechanisms, like interference competition, may seriously impair a more detailed reconstruction of the seasonal succession in the microphytobenthos.
In summary, while it is quite possible that interference competition rules the interaction of the cyanobacterium *L. foveolarum* and the diatom *N. perminuta* in a model system, with possible consequences for their seasonal distribution, caution is still needed in concluding on a single key factor ruling microalgal coexistence in biofilms: chemical warfare, competition for the resources light and DIC or space.

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