Water column stratification, phytoplankton diversity and consequences for resource use and productivity

NTNU Sletvik Fieldstation
NTNU Sletvik Field Station Phytoplankton Diversity and Water Column Stratification

EC contract no. 261520

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<tr>
<td>Lead Author</td>
<td>Maren Striebel</td>
</tr>
<tr>
<td>Email</td>
<td><a href="mailto:striebel@limnology.eu">striebel@limnology.eu</a></td>
</tr>
<tr>
<td>Contributors</td>
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1. Scientific aim and background:

Stratification and diversity

The seasonal stratification of water columns determines the general availability of the resources light and nutrients for phytoplankton growth (Diehl 2002; Diehl et al. 2002). Experiments manipulating the depth of mixing layers and/or the mixing intensity showed that these physical parameters strongly affect phytoplankton primary production by influencing phytoplankton light exposure and affecting phytoplankton mortality by sedimentation (Diehl 2007; Jäger et al. 2008). However, seasonal stratification can be affected and disturbed by aseasonal effects such as strong rain and wind events. Hence, disturbances of water column stratification imply disturbances for phytoplankton dynamics since it causes alterations in the relative supply of light and nutrients (Flöder & Sommer 1999). According to ecological theory, the frequency of disturbance strongly affects the diversity of biological communities (Huston 1994). Whether disturbances increase or decrease the diversity of a community also depends on the productivity and the resource supply rate (Huston 1994). In environments with low nutrient supply, the same disturbance may have opposing effects on phytoplankton communities as compared to environments with high nutrient supply. This important interaction between disturbances and nutrient supply rate is, however, seldom considered in investigations of disturbance effects on plankton communities.

Diversity and resource use efficiency

Environmental effects on phytoplankton diversity will have extensive consequences extending beyond changes in species composition. A recent metaanalysis including about 3000 freshwater and brackish phytoplankton samples shows that diversity is the best predictor for the resource use efficiency (and thereby carbon production) and the stability of the resource use efficiency in phytoplankton communities (Ptacnik et al. 2008). Consequences of these findings are that in less diverse communities resources may be more easily monopolized by bloom forming species and that
phytoplankton – zooplankton interactions are less stable, possibly hampering trophic transfer (Ptacnik et al. 2008). Based on data from experiments with natural algal communities from 46 lakes and 30 laboratory cultures we demonstrated experimentally that the efficiency of using the resource light, the carbon production and the biomass composition (carbon to nutrient ratio) of freshwater phytoplankton communities is indeed related to diversity (Striebel et al. 2009a, 2009b). The carbon to nutrient ratio of phytoplankton in turn is an important parameter determining nutrient recycling, transfer efficiency between phytoplankton and zooplankton, stability of phytoplankton - zooplankton interactions and diversity of zooplankton communities (Urabe & Sterner 1996; Sterner et al. 1997; Urabe et al. 2002). Therefore, disturbance mediated effects of diversity on resource use and biomass stoichiometry of phytoplankton communities can have major impacts on the functioning of the entire pelagic ecosystem.

We proposed to analyze the above described un-investigated links between disturbances of water column stratification and diversity and its consequences for marine plankton dynamics in a gradient of disturbances at different nutrient supply rates in a large scale mesocosm experiment. We hypothesized that experimental disturbances of water column stratification will have consequences for phytoplankton diversity and thereby affect the resource use efficiency and carbon production of phytoplankton and phytoplankton – zooplankton interactions.

The objectives of our study were as follows:

1) To analyze the relationship between disturbance of water column stratification and phytoplankton diversity

The relationship between water column stratification, rate of disturbance and phytoplankton diversity has been studied to some detail in freshwater environments. However, there is a considerable lack of evidence for marine environments. Closing this gap of knowledge will allow generalizing possible relationships between stratification disturbances and phytoplankton diversity in pelagic environments.

2) To analyze the relationship between phytoplankton diversity and diversity dependent resource use efficiency, the stability of resource use efficiency and carbon production

Data from meta-analyses and experiments clearly demonstrate that species diversity is one of the best predictors of the resource use efficiency and the carbon dynamics of phytoplankton communities in freshwater and brackish environments. It is surprising that, despite the global importance of marine phytoplankton (responsible for about 50% of global carbon production), the relationship between phytoplankton diversity and carbon dynamics has not been investigated in marine environments. Our experiments will result in a first data set showing how species diversity, resource use efficiency and carbon production are linked within a marine phytoplankton community.

3) To analyze the relationship between diversity dependent carbon dynamics of phytoplankton and zooplankton growth

The carbon content and the carbon to nutrient ratio of phytoplankton biomass are most important for zooplankton growth. In freshwater experiments it has been shown that phytoplankton diversity influences carbon assimilation and nutrient uptake unequally. This results in phytoplankton diversity dependent shifts in the carbon to nutrient ratio within phytoplankton biomass, influencing phytoplankton food quality for zooplankton. We investigate the link between disturbances of the water column, phytoplankton diversity and its consequences for zooplankton growth in a marine pelagic community.

4) To analyze the relationship between disturbance and the growth and diversity of ciliates
Ciliates have population growth rates equaling or exceeding those of phytoplankton. As a result, the response to disturbance of phytoplankton in ciliate-edible size classes may be masked by changes in abundance and diversity of their ciliate grazers. Our experiments show how rapid changes in the grazer community can influence the impact of disturbance on primary producers.

5) To analyze the relationship between disturbance of water column stratification and the abundance and diversity of mixotrophic protists

The exact mechanisms controlling the abundance and diversity of mixotrophic protists and their contribution as producers and consumers to the carbon flow are still poorly understood. Changes in water column stratification and the resulting (hypothesized) abiotic and biotic changes are likely to also affect the mixotrophs in the mesocosms. These direct and indirect effects were investigated in our experiments.

References:


We studied the responses of a natural coastal phytoplankton community to manipulations of the stratified water column. We installed 24 enclosures (10m depth) and disturbed the stratification of the water column by artificially mixing the water column with a Secci-plate with different time intervals (1-16 days). Undisturbed mesocosms (mixed every 32 days) acted as the least disturbed
mesocosms in the gradient. We performed the experiments at two nutrient levels, a unfertilized and a moderate supply level (0.5 µg P l⁻¹ d⁻¹; Si:N:P 16:16:1) compared to the natural loading of the system (Vadstein et al. 2004). We followed the response of phytoplankton, protist (ciliate and flagellate) and zooplankton communities to stratification disturbances for about 4 weeks. We were especially interested in the consequences of stratification disturbances for phytoplankton diversity and thereby phytoplankton resource use efficiency and carbon dynamics.

3. Highlights important research results:

At the moment we are still analyzing samples, phytoplankton, ciliates, and zooplankton samples that are very time-consuming. Thus, we hope that we will finish these analyses until the beginning of 2010. Then we will be able to investigate the relationship between disturbance of water column stratification and phytoplankton and ciliate diversity. Additionally, we just finished the nutrient analysis (C, N, P) and after gaining the phytoplankton and ciliate data we will be able to analyse the relationship between marine phytoplankton diversity and diversity dependent resource use efficiency, the stability of resource use efficiency and carbon production. Moreover, we will analyse the relationship between diversity dependent carbon dynamics of phytoplankton and zooplankton growth and analyse the relationship between disturbance and the growth and diversity of ciliates.

4. Publications, reports from the project:

Counting of plankton samples and final analyses of the results will need until beginning of 2010. A first paper will be submitted at the end of 2009 year for the proceedings of the HYDRALAB II Joint user meeting. Additionally, we plan to publish a first paper originating from the experiment within one year after its completion (2010) in an international peer reviewed journal such as Limnology and Oceanography or Marine Ecology Progress Series


5. Description:

5.1. Description:

Mesocosm experiments with natural algal communities

We studied the responses of a natural coastal phytoplankton community to manipulations of the stratified water column. We installed 24 enclosures (10m depth) and disturbed the stratification of the water column by artificially mixing the water column (with a Secci-plate) using different time intervals (1-16 days). Undisturbed mesocosms (mixing after 32 days) acted as the least disturbed mesocosms in the gradient. We performed the experiment at two nutrient levels,
unfertilized treatments and treatments with a moderate supply level (0.5 μg P l⁻¹ d⁻¹; Si:N:P 16:16:1) compared to the natural loading of the system (Vadstein et al. 2004). We followed the response of phytoplankton, protist (ciliate and flagellate) and zooplankton communities to stratification disturbances for about 4 weeks. We were especially interested in the consequences of stratification disturbances for phytoplankton diversity and thereby phytoplankton resource use efficiency and carbon dynamics. Measurements included phytoplankton, ciliate, and zooplankton biomass, composition, and dynamics, nutrient dynamics, phytoplankton stoichiometry and resource use efficiency.

Figur 5.1.1 Scheme of the experimental setup. Arrangement of mesocosms observed from the raft. Red numbers display fertilized treatments.

Table 5.1.1 Summary of the treatments: mixing frequency, unfertilized, and fertilized treatments.

<table>
<thead>
<tr>
<th>Mixing frequency (days)</th>
<th>Enclosure numbers, unfertilized</th>
<th>Enclosure numbers, fertilized</th>
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<tr>
<td>1</td>
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<td>13, 14</td>
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<td>21, 22</td>
</tr>
<tr>
<td>32</td>
<td>11, 12</td>
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Analyses
Phytoplankton species composition, phytoplankton stoichiometry (particulate organic carbon (POC) particulate organic nitrogen (PN) and particulate organic phosphorus (PP); filtration with GF-F filters) and nutrients were analyzed at the start of the experiment and afterwards every third day. Phytoplankton will be enumerated from samples fixed with Lugol’s iodine with an inverted microscope using Utermöhl chambers until beginning of 2010. Phytoplankton biovolume was determined during the experiment using a cell counter (Casy® Counter). Primary productivity of the different phytoplankton communities was determined with the dialysis method (see below). Detailed pigment analyses will be performed with HPLC (November-
December 2009) to see whether taxonomic diversity is coupled with pigment diversity (functional diversity). Zooplankton and ciliate abundance and species composition and zooplankton biomass composition (POC, PP, PN) was analysed every third day and samples will be counted until January 2010.

Figure 5.1.3 Experimental setup: (1) daily mixing, (2) experimental setup, (3) daily mixing and fertilization, and (4) raft with enclosures.

Figure 5.1.4 Enclosure with bottle for ciliate growth experiments (5) and setup for dialysis experiments to determine phytoplankton primary production and loss rates (6).

Phytoplankton growth and loss rates
To estimate phytoplankton growth and loss rates (mainly grazing by micro- and mesozooplankton), in situ, different techniques were developed within the last centuries. Disadvantages of these techniques were their often complicated enforcement and the neccessarity to use potential harmful substances such as radioactive tracers. Due to safety regulations, it is not always possible to use such methods in the field. Additionally, radioactive tracer methods do not allow quantifying grazing rates on individual phytoplankton groups or species.

Thus, we used a modification of the dilution method and used dialysis bags to estimate growth and loss rates of phytoplankton instead of non permeable glass bottles (Stibor et al. 2006b). Dialysis membranes possess the advantage to be permeable for nutrients and thereby allow an in situ estimation of phytoplankton gross growth rates. Dialysis bags also allow simultaneously the estimation of microzooplankton grazing by dilution of plankton communities.

Bags with a volume of 250 ml were constructed using dialysis membrane tubes with a molecular weight cut-off of 6000. This allowed diffusion of molecules smaller than proteins which equilibrilate rapidly with ambient water. Dialysis tubes were hydrated by soaking them in deionised water for 12 h prior to use. Dialysis cultures consisted of depth integrated samples from fertilized enclosures. Samples were taken with a tube sampler and filtered through a 200 μm mesh to exclude macrozooplankton.

The original sample was diluted with GF/F filtered water from the same water body in 5 steps. The share unfiltered water was 12.5 %, 25 %, 50 %, 75% and 87.5 %.

Figure 5.1.5 Scheme of the dilution steps for the experimental setup of dialysis experiments.

Samples were incubated for 48 hours and this incubation period resulted in a clear and measurable growth response of phytoplankton in all experiments. After incubation, dialysis tubes were opened and from sub samples chlorophyll-a concentration (using a fluorometer), cell numbers, and total cell volume (using a Casy® Counter) were determined. Additionally 100 ml sub-samples were fixed with Lugol’s iodine. These samples will be counted until beginning of
2010 according to Utermöhl’s inverted microscope technique (Utermöhl 1958). Net growth rates, grazing rates by microzooplankton, and grazing rates by mesozooplankton will be calculated.

Figure 5.1.6 First results from biovolume data from the dialysis experiment (measured with the Casy® Counter). A: Relationship between mixing intensity and phytoplankton growth in the different treatments. B: Relationship between mixing intensity and micrograzing calculated after Stibor et al. (2006).

Ciliate growth experiments
In order to measure ciliate growth rates, water samples were taken from every mesocosm at the surface (about 30 cm depth). Samples were filtered through 100 μm mesh to exclude zooplankton. A starting sample (95 mL sample + 5 mL Bouins) was taken from every mesocosm. The rest of the water was filled in a polycarbonate bottle (Nalgene, transparent with a volume of 640 mL) and incubated in the mesocosm at a depth of 30 cm. After 24 hours the bottles were taken out of the mesocosms and end samples were taken (95 mL sample + 5 mL Bouins). The samples will be counted and determined under microscope and growth rates will be calculated under the assumption of exponential growth.

The experiments will provide estimates of ciliate growth in the absence of predation. When compared with ciliate growth rates in the mesocosms themselves, the effects of the experimental manipulations on gross and net growth rates can be compared.

Lipid analysis
The FlowCAM® (Fluid Imaging, Portland) is a continuous imaging flow cytometer being used for monitoring of microorganisms and particles in water. It combines microscopy, flow cytometry, imaging and fluorescence technologies. A laser interacts with a high resolution digital camera to capture images and data of passing cells or particles. It offers cell counts, size data, pattern recognition, organism classification and image management. Hence, there are two measurement modes that can be used with the FlowCAM®: auto-trigger mode and fluorescence mode.

To estimate the cell specific lipid content of marine phytoplankton we use the fluorescence mode. For staining the algal cells we use the fluorescent lipophilic dye Nile Red with a shift of emission from red to yellow. After staining 5ml algal sample with 20μl Nile Red solution followed an incubation of 30min in the dark.
Fluorometric analysis ensued immediately with the FlowCAM® with an excitation wavelength of 532nm and an emission wavelength of 645±20nm (green laser). In terms of the imaging technology it is feasible getting images and the information of fluorescence of each detected cell. Thus, it is possible to estimate the lipid content of each algal cell even in diverse communities.

5.2. Definition of the coordinate systems used:

5.3. Instruments used:

5.4. Definition of time origin and instrument synchronisation:

6. Definition and notation of the experimental parameters:

6.1. Fixed parameters:

For definition of parameters see part 5.1

6.2. Variable independent parameters:

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Table 6.2.1

6.3. Derived parameters and relevant non-dimensional numbers:

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Table 6.3.1

7. Description of the experimental campaign, list of experiments:

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<thead>
<tr>
<th>Experiment Name</th>
<th>Experiment Date</th>
<th>Remarks</th>
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Table 7.1

8. Data processing:

All analysis will be done until the beginning of 2010 and all data will be collected by the group leader.

We will obtain data from nutrient analysis, HPLC, lipid measurements, phytoplankton composition and biomass, ciliate composition and biomass, zooplankton composition and biomass and data
gained from the dialysis experiment with phytoplankton and growth data from the experiments with ciliates.

9. Organisation of data files:

Data will be stored as:

Exel files: nutrient data, HPLC data, lipid (FlowCAM®) measurements, and for Casy® Counter measurements.

Exel files: Phytoplankton, ciliate, and zooplankton counting’s.

Photos of the experimental setup.

Exel files for additional experiments (3 dialysis experiments and cilate growth experiments).

Word files: documentation and reports.

10. Remarks about the experimental campaign, problems and things to improve:

Everything was very good and there are no remarks concerning the experimental facility and the assistance was perfect. The experimental setup was proven and everything necessary was on site or organized quickly.

The disadvantage of such a large-scale experiment is that a lot of samples have to be analyzed after the experiment and that these analyses are very time-consuming. That’s the reason why at the present moment we are not able to present clear result and we just can show preliminary results.