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# Studying plankton community dynamics – an optimized mesocosm design

## Abstract

An optimized mesocosm setup was designed for experimental investigation of the internal mechanisms (trophic relations and interactions between species and trophic levels) that drive plankton dynamics under the constant external conditions. This setup was used in two consecutive one year-long mesocosm experiments, each carried out in four replicates. In both experiments, all replicates had similar initial conditions; moreover, the abiotic parameters (temperature, irradiance and water movement) were kept stable throughout the experiment and were similar in all replicates. The experimental setup, including techniques for water mixing and control of temperature and light conditions, sampling procedures and methods of data analyses are described in detail. Some results for the starting period of the first experiment are shown (e.g., dynamics of total nutrients and seston properties). In one replicate, deviation of the measured parameters from those in the other three replicates was revealed which was not attributed to the external drivers. The overall results of the experiments demonstrated high degree of long-term stability of external drivers for plankton dynamics (light, temperature and salinity) as well as acceptable similarity of these parameters in the mesocosm replicates. The applicability of this approach and experimental design for unraveling the internal mechanisms of plankton dynamics is discussed.

**Keywords:** Experimental setup, mesocosm, plankton dynamics, salinity, irradiance, temperature, variability

## 1 Introduction

Plankton consists mainly of short-living, small organisms that demonstrate large ecological, physiological and taxonomic diversity. Consequently, plankton communities exhibit high variability in time and space, which is difficult to register and sample with adequate frequency and spatial resolution in nature, where abiotic triggers such as irradiance, water movement and temperature fluctuate at different timescales ranging between milliseconds (irradiance) and hours (temperature). Being a part of the overall matter cycle, plankton organisms interact using intra-trophic

mechanisms (i.e. relations within one trophic level), e.g., competition for nutrients, as well as experience trophic interactions (grazing or predation), the latter processes back coupling to nutrient availability. As a result, plankton dynamics are hard to describe empirically due to sampling restrictions. Therefore, causal analysis of plankton dynamics, trophic and other interactions like competition, symbiosis etc. is only possible on a very rough scale for natural communities, explaining at the very best just the cumulative plankton reactions to master factors when they are dominating for longer periods of time.

Meanwhile, laboratory investigations of plankton dynamics at the species and community levels can provide an insight into a fascinating world of those interactions in plankton, including the complex triggering, controlling and feedback mechanisms that drive plankton dynamics. Moreover, results of experiments with plankton communities can even raise a question whether or not chaotic behavior is a part of plankton dynamics, since BENINCÁ et al. (2008) put forward strong arguments in favor of chaotic behavior of the species-reduced plankton communities in a long-term mesocosm experiment.

The inspiring results published by BENINCÁ et al. (2008), however, are vulnerable in two aspects. First, the analysis was based on a single experiment and, therefore, no replicates were available. The second problem was the lack of long-term environmental stability in the mesocosm during the experimental phase. In the experiment by BENINCÁ et al. (2008), which lasted during ca. 10 years, temperature was kept constant only in a very rough manner, by using aquarium heaters for a long period of time; additionally, the effect of sampling, which might have shifted the nutrient regime over time, was not considered.

Therefore, one of the targets of the approach presented in this study was to develop an experimental setup allowing for establishing the replicate plankton communities that can be investigated under the identical conditions with respect to initial environment as well as the abiotic conditions throughout the experimental phase. The second target of this study was to develop a mesocosm setup allowing for maximum long-term stability of the main abiotic triggers of plankton dynamics such as temperature, irradiance and water movement.

This paper describes in detail the optimized mesocosm experimental design aimed at solving both problems mentioned above. The obtained results are discussed in terms of long-term stability of the experimental conditions and comparability of abiotic parameters in different replicate mesocosms.

# 2 Material and methods

## 2.1 Description of the mesocosm setup

A set of four identical plastic barrels with a volume of 120 L each and a water level of 72 cm formed the mesocosm compartments for plankton incubation. Each mesocosm was surrounded by a water jacket for temperature control. The water jacket consisted of a 300 L vessel for each mesocosm; the individual water jackets were interconnected by tubes and formed a closed thermostabilizing system (Figure 1), driven by an immersion pump (AquaMedic, OR3500) and controlled by a cryostat

(AquaMedic, SK2). The aim was to keep water temperature at about 20°C throughout the entire 1 year-long experimental phase inside all mesocosms.

**Fig. 1:** Mesocosms setup with 4 replicates, each mesocosm (blue barrel) is surrounded by a water jacket (green barrel), which are interconnected by tubes and a pump.

To prevent formation of abiotic gradients and biotic stratification, each mesocosm was equipped with a stirrer (MFA/Como Drills; 919D Series connected to a 9.5 cm-plastic-propeller), placed at a water depth of 37 cm (Figure 2). A slow rotating speed of 72 rounds/min and a stirring regime of 5 min on and 5 min off resulted in gentle circulation of water in the mesocosms without resuspension of the sediment layer.

The mesocosms were covered with transparent acryl plates in order to diminish evaporation and heat losses. Illumination under a 12 h/12 h dark/light cycle was provided by halogen energy-efficient lamps (OSRAM DuluxStar Mini Twist 23W/825), allowing for a maximum of about 135-140 µmol photons $m^{-2}$ $s^{-1}$ incident irradiance at the uncovered water surface.

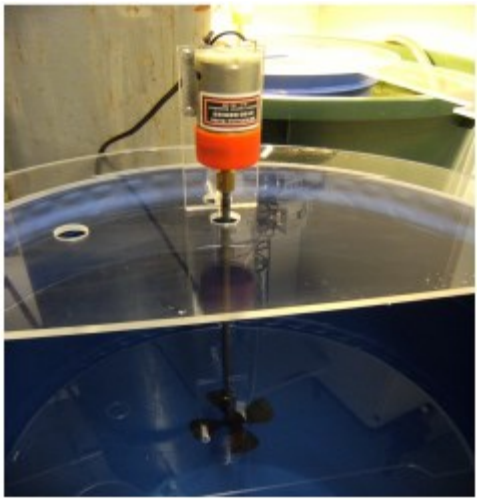

**Fig. 2:** Stirring system. Above the transparent cover plate, an electronically controlled motor drives a 4-paddle propeller at 37 cm water depth.

## 2.2 Inoculation procedure

The four mesocosms were used for each of the two experiments, the first starting in July 2010 and lasting until August 2011, and the second – in December 2012 through December 2013.

The first experiment (2010-2011) was started with a 1:4 mix of water obtained from a previously constructed species-poor mesocosm setup (started in 2008 with water initially collected in a coastal lagoon of the Baltic Sea, the Darß-Zingst Bodden, site Zingster Strom) mixed with natural water taken in July 2010 from the same site. These 3 parts of “new” brackish water contained the natural plankton community. Additionally, 4 L of sediment were collected at Fuhlendorf (9 km distance from the site Zingster Strom) for adding to the barrels. The sediment was first homogenized gently, then divided into equal portions and added into the replicate mesocosms. The resulting thickness of the bottom sediment layer in each barrel was 2.5 cm. From the sediment, resting eggs of zooplankton species and phytoplankton cysts could develop, thus adding more species to the initial plankton complex.

The second experiment (2012-2013) was initiated by splitting the content of one of the previously used mesocosms (vessel C in the first experiment) and dividing the water equally to fill in the four new mesocosms. The inside walls of all vessels were carefully cleaned with brushes prior to filling them in. Each mesocosm was supplied with 2.2 L of sediment after retrieving and carefully homogenizing the sediment from the previously used mesocosms. The remaining water from three other mesocosms left from the first experiment was filtrated through gauze (mesh size 20 µm), well mixed and redistributed to four new mesocosms. This procedure should have equalized plankton composition and biomass as well as nutrient content in the four new replicate mesocosms. Water losses were filled up with filtrated (20 µm) biotope water from the same site as for the first experiment. A reserve of filtrated biotope water was kept in the laboratory in darkness to compensate water lost by sampling during the experiment. We assumed that the 20 µm filtrates should have contained most of the phytoplankton. Zooplankton from the initial vessel C was redistributed equally between 4 new replicate mesocosms and thus its biomass was reduced to ¼ of the initial zooplankton biomass.

## 2.3 Sampling and measuring regime

Sampling in both experiments was carried out twice a week (every Monday and Thursday); separate sampling equipment was used for each mesocosm to prevent an interchange of organisms. Brushing the barrel walls for removing microbial layers was performed weekly. Replacement of water losses due to sampling was done monthly using filtered (GF92, Whatman) inoculation water from a reservoir which was kept in darkness since the installation of the respective experiment. Since evaporation from the barrels was efficiently reduced by the covering plates, the water volume replaced monthly (mean of 1.3 L $month^{-1}$; PAUL 2011) matched well the amount of water taken from the vessels by sampling (1.2 -1.4 L $month^{-1}$).

During the experiments, irradiance was measured at a defined place in the middle of the transparent cover plate by means of a LiCor250 PAR-meter. Additionally, the sensor was placed first to the center and then directly at the wall of the mesocosm, to estimate shadowing effects of the barrel walls.

Salinity and water temperature were measured by means of a conductivity meter (LF320, WTW); pH was measured by a pH-meter (pH90, WTW). These parameters were evaluated in the samples taken for nutrients and plankton analysis.

For nutrient analysis, 50 mL of water from each mesocosm were filtered (GF/F, Whatman), from which 17.5 mL were used immediately for ammonia determination and 20 mL were frozen at -20°C until determination of o-phosphate, nitrate and nitrite. The remaining amount of sampled water was used for bacteria (including picocyanobacteria) counting after fixation by glutardialdehyde (25% solution) added to reach a final concentration of 1%. Bacterial counting was performed within 2 months; the samples were stored in darkness in a refrigerator until analysis.

In addition to the measurements that were carried out twice a week, samples for determination of total nitrogen and total phosphorus, chlorophyll content, phytoplankton biomass and species composition, phytoplankton growth experiments, protist abundance and biomass were taken monthly. For chlorophyll *a* (Chl *a*) determination, 50 mL of sample water were filtered (GF/F, Whatman), the filters were frozen at -20°C until analysis. Total nitrogen (TN) and total phosphorus (TP) were determined from the unfiltered samples (ca. 50 mL). Phytoplankton samples (20 mL) were fixed with Lugol's solution and stored refrigerated in darkness. Phytoplankton growth experiments required additional 20 mL of the unfiltered sample, and samples for protists determination – 15 mL. However, all those samples were taken sporadically but not regularly throughout the entire experiment. TP and TN were quantified from November 2010 to February 2011, Chl *a* – from September 2010 to March 2011, phytoplankton growth – from November 2010 to March 2011, phytoplankton composition – from July 2010 to March 2011, and protists – from July 2010 to June 2011.

## 2.4 Nutrient concentrations

All nutrients, elements, and pigments (as well as plankton organisms) were estimated by procedures, which are described in the HELCOM Manual "COMBINE" (HELCOM COMBINE 2015) and established by the respective analytical norms developed by the DIN or ISO institutions as well as the quality measures proposed by the BMLP (German Federal and State Monitoring Programme). Here, we only cite

the COMBINE manual and the most often used analytical textbook (GRASSHOFF et al. 1999), since all documents mentioned above are in good agreement. In addition, we acknowledge some of the earliest method descriptions (see below). All nutrients were estimated without replicates; therefore, the combined standard deviations were calculated using the results of the other samples from the respective nearest data set, which usually contained 3-4 replicates.

Ammonium concentration was determined as an indophenol-blue dye (LUBOCHINSKY & ZALTA 1954 cited in GRASSHOFF et al. 1999). Pre-heated glass tubes (4 h at 180°C) were used. Blank values were determined in de-ionized ultrapure water (conductivity <0.055 µS $cm^{-1}$). Absorbance was measured at 630 nm in a UV-mini 1240 (Shimadzu) spectrophotometer. Reagents were replaced every 4 weeks. Usually, a 5 cm cuvette was used; 1 cm cuvettes were chosen at higher concentrations ensuring that a maximum absorbance value of 0.6 never got exceeded. Limit of quantification (LOQ) was 0.7 µmol $L^{-1}$. The combined standard deviation of samples and standards amounted to 8.0 % (in 2011).

Phosphate was determined as a molybdenum blue complex (MURPHY & RILEY 1962) always in a 5 cm cuvette in a Hach photometer (DR 3900). LOQ was 0.15 µmol $L^{-1}$. LOQ was much better as this procedure was re-transferred to a segmented analyzer (MALCOLM-LAWES & WONG 1990). The combined standard deviation of samples and standards amounted, nevertheless, to 10.6% in 2013, which is attributed to the dominance of very low phosphate concentrations near the LOQ in lagoon waters.

Nitrate was reduced over cadmium particles to nitrite, which resulted in a value of $NO_x$ (GRASSHOFF 1970 and many other methods cited in GRASSHOFF et al. 1999). Nitrite was determined as an azo dye (BENDSCHNEIDER & ROBINSON 1985). Nitrite was subtracted from $NO_x$; the result was nitrate. Both procedures took place in a (segmented) continuous flow analyzer (Alpkem PFA300). LOQ was 0.3 µmol $L^{-1}$. Samples standard deviation was 9.3% for concentrations <10 µmol $L^{-1}$, which reflects the majority of field samples and samples from the mesocosms presented here.

## 2.5 Total phosphorus and nitrogen in seston

Total phosphorus and nitrogen in seston was digested by oxidation with peroxodisulphate (HANSEN & KOROLEFF 1999). A spoon full of a commercial powder mixture (Oxisolv® Merck) was added to 15 mL seston samples (TP) or 1:4 diluted samples for TN in Teflon tubes. Samples were digested at high temperature and pressure in a microwave (Miele M688) at 450 W for 2 times. Samples were cooled down to <50°C and transferred into glass tubes.

A set of samples (for TP) was neutralized with nitrophenol as an indicator, which turns yellow after adding some droplets of ammonia solution. Samples were titrated back to normal (colorless) by 1 N HCl. Samples were stocked up to 20 mL and stored frozen until phosphate quantification.

The other digested samples (TN) were neutralized after acidification with 1 mL 0.9 N $H_2SO_4$ with phenolphthalein as an indicator upon titration with 0.12 n NaOH. Then, 1 mL of 5 N $NH_4Cl$ was added; samples were restocked to 20 mL and stored frozen for nitrate determination.

We do not provide LOQs and standard deviations for these methods here since they were not used anymore. Nevertheless, we chose the best estimates to describe seston properties here roughly. The main problem with single vessel digestion in a normal microwave was poor control by blanks or standards, since energy input strongly depends on the vessel position in the oven. Later, we applied a so called sub-boiling digestion (normal oven at 90°C) in Teflon vessels (HUANG & ZANG 2009) that do not need to be pressure-stable. All quality parameters for this TP determination are published in BERTHOLD et al. (2015) and are likewise satisfactory for TN (not shown).

## 2.6 Chlorophyll *a*, phytoplankton and bacteria

Chlorophyll *a* was extracted only from one subsample during each sampling procedure, in order to save plankton biomass in the mesocosms. Frozen samples were extracted in 3 mL 96% ethanol for 12-14 h at 5°C (HELCOM COMBINE 2015). Ethanolic extraction was proposed especially for cyanobacteria and green algae (e.g. SARTORY & GROBBELAAR 1984). Filters and cell debris were removed by centrifugation. The supernatant was measured in a Shimadzu UV 2401 PC in halfmicrocuvettes. Chl *a* concentration was calculated from absorbance at 665 and 750 nm (PARSONS 1966). Sample reproducibility was 3.4% in samples from the Zingster Strom in 2011 (own data).

Phytoplankton composition was determined in Lugol-fixed samples (LUND et al. 1958, UTERMÖHL et al. 1958) under an inverted microscope (Carl Zeiss Telaval) at magnifications of 200 x or 400x. Phase contrast was used always. At least 100 individuals (cells, filaments or colonies) were counted for 3-5 dominating species at more than 20 separate areas of a chamber. Alternatively, 500 individuals were counted in total. Determination of cyanobacteria followed the taxonomic concepts of KOMÁREK & ANAGNOSTIDIS (1999 and 2005), and PANKOW (1990) was used for identification of green algae and diatoms. If the genera names are mentioned, the respective latest synonyms are used according to algaebase (http://www.algaebase.org), which is used by monitoring agencies. In special cases, these names are cross-referenced by the literature given in the HELCOM COMBINE (2015) manual as well as the recent determination key for Nostocales (KOMÁREK 2013).

There is one major difficulty is calculation of cell numbers and biomass of cyanobacterial colonies which is crucial to mention here, because such cyanobacteria dominated the experimental phytoplankton community, at least initially. Three major colony groups were defined: rod shaped cells (*Aphanothece, Cyanonephron, Cyanodictyon*), spherical cells (*Microcystis*), and larger spherical and ovoid forms on gallert stalks (*Gomphosphaeria, Snowella, Woronichinia*). Cells per colony of the "*Aphanothece*" type were enumerated in 10 colonies per sample at high magnification using their autofluorescence (see below). The other colony types were only represented by few individuals in the samples.

Solitary cyanobacterial cells and heterotrophic bacteria were counted under an epifluorescence microscope (Olympus BX51 objective UPlan FL 100 NA 1.3 Oil). Usually, more than 100 cells were counted per sample at green excitation (filter cube U-MWG2). Samples with very low abundances were screened for about 30 cells.

Bacteria were stained with DAPI (PORTER & FEIG 1980, 91 µmol $L^{-1}$, 5 min), filtered onto black stained isopore track edged membranes (polycarbonate, 0.2 µm pore size), and mounted between slide and cover slip by immersion oil. In most samples, more than 400 cells were counted per slide under UV excitation (filter cube U-MWU2). Samples with very low bacterial abundances were screened for at least 150 cells per filter. In both cases, ocular grids were used to adjust counting area to cell number.

## 2.7 Protists (ciliates and some flagellates)

Ciliates and larger flagellates (mostly dinoflagellates) were counted in rafter chambers at a magnification of 32x (Euromex lab microscope). There were as many replicates of 0.5 – 1 mL counted as needed to examine at least 30 individuals in total. Cells were measured for their main axes (length and width) with an increment of 5 µm. Genera or higher taxonomic groups of ciliates were determined according to TELESH et al. (2009).

Flagellates <15 µm were counted in Bürker chambers (volume 0.9 µL) at a magnification of 100x. Six to 8 replicates were investigated so that total cell numbers added up to at least 30 cells. Taxonomic work was not possible for these living samples except for identification of the group Crytophyceae. All cells were measured with an increment of 2 µm.

Biovolume was calculated from length and width using sphere's or spheroid formulas. Fresh mass was calculated applying a cell density of 1.04 g $cm^{-3}$ (HEERKLOSS & VIETINGHOFF 1981).

## 2.8 Metazooplankton

Metazooplankton samples were taken twice a week by filtering 10 L of water through gauze with mesh width 56 µm. The filtrate was returned into the respective mesocosm. This procedure intended to mimic grazing by organisms from the higher trophic levels, which were not included in the experimental plankton communities in the mesocosms. On a volume basis, 17% of zooplankton larger than 56 µm was removed per week.

The extracted zooplankton was transferred into 18 mL of tap water, fixed with 2 ml formaldehyde (37%, buffered with borax) and stored in 20 mL tubes. For counting, the samples were concentrated using gauze (56 µm mesh width) into a volume of 2 mL and transferred into two 1 mL cylindrical counting chambers. Both chambers (= one sample) per date were analyzed; zooplankton was counted under a light microscope (Olympus BH2) at 100x magnification.

Two most common copepod crustacean species in the mesocosms were *Eurytemora affinis* and *Acartia tonsa*, both – typical zooplankters in the brackish inner coastal waters. Cyclopoid copepods were not determined to the genus level. All copepods were counted in the following units: nauplii I-III, nauplii IV-VI, copepodits I-III, copepodits IV-VI, adult males and females. Rotifers were identified to the lowest possible taxonomic level and counted; specimens with eggs were counted separately. Genus and species determination of rotifers and copepods was based on TELESH & HEERKLOSS (2002, 2004). Biomass values per animal and stage were adopted from FEIKE & HEERKLOSS (2009).

# 3 Results

## 3.1 Irradiance

In the case of using a single bulb as a light source, horizontal gradients in surface irradiance are unavoidable. In order to compare these gradients between the mesocosms, measurements were done in the center as well as close to the walls of the barrels and percent of reduction of irradiance was calculated (Figure 3).

For all mesocosms, irradiance close to the barrel walls was reduced by about 80% if related to the irradiance at the center of the cover plate. Whereas irradiance reduction in barrels A and B was comparable (79.5% ± 1.7 and 78.1% ± 1.6, respectively), barrel C showed a less pronounced reduction (75.2% ± 0.96) and barrel D – an enhanced reduction of irradiance (80.5% ± 0.57).

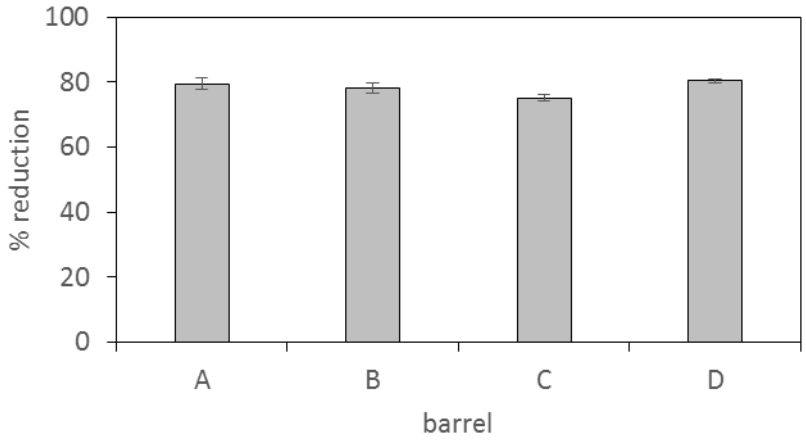


**Fig. 3:** Irradiance homogeneity. Shown is reduction of irradiance, expressed as percent of irradiance measured close to the barrel walls *versus* the maximum irradiance value in the center of the cover plate (mean values with standard deviation, *n*=3); A, B, C and D – four mesocosm barrels.

With respect to long-term stability, ageing of light sources leads to changes in spectral composition as well as brightness. Figure 4 shows the mean irradiance and its standard deviation, calculated from the measurements on top of the 4 mesocosms. Irradiance was reduced intentionally by changing the position of lamps after the first two months of the experiment. The reduction was done because one of the barrels (D, see Figure 5) received less light (for the unknown reason) after the first adjustment. Within a week, incident irradiance decreased by about 20% and then became stabilized. This effect could only be adjusted by reducing irradiance for all mesocosms to the level of barrel D. After this adjustment, the position of lamps was kept constant.

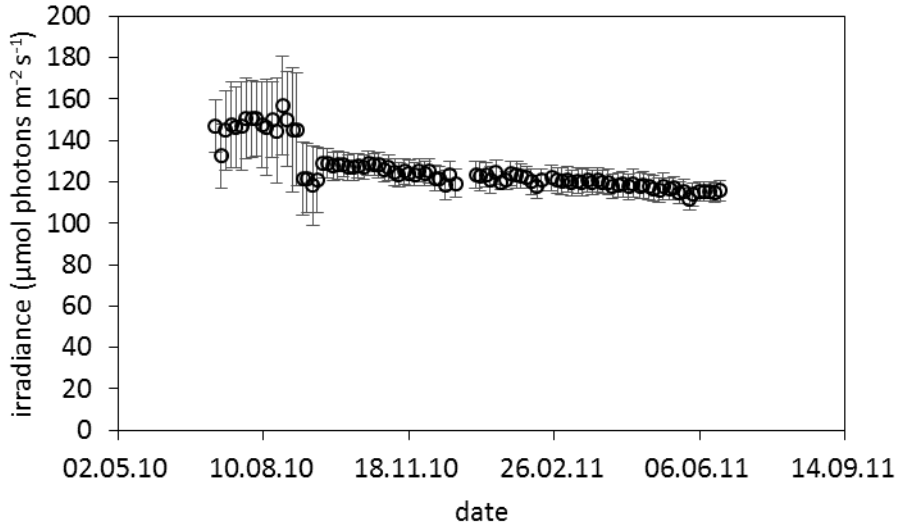


**Fig. 4:** Incident irradiance. Shown are mean irradiance values measured in the center of the cover plates of the 4 mesocosms, and standard deviations (of the measurements of the 4 mesocosms) for the first experiment.

As can be seen from the standard deviation values, differences between the mesocosms decreased after reducing incident irradiance on September 2, 2010 (Figure 4). The adjustment was done by lifting the lamps individually until comparable irradiance was measured in all mesocosms. Figure 5 shows the deviation from mean irradiance for the individual barrels at the respective sampling date.

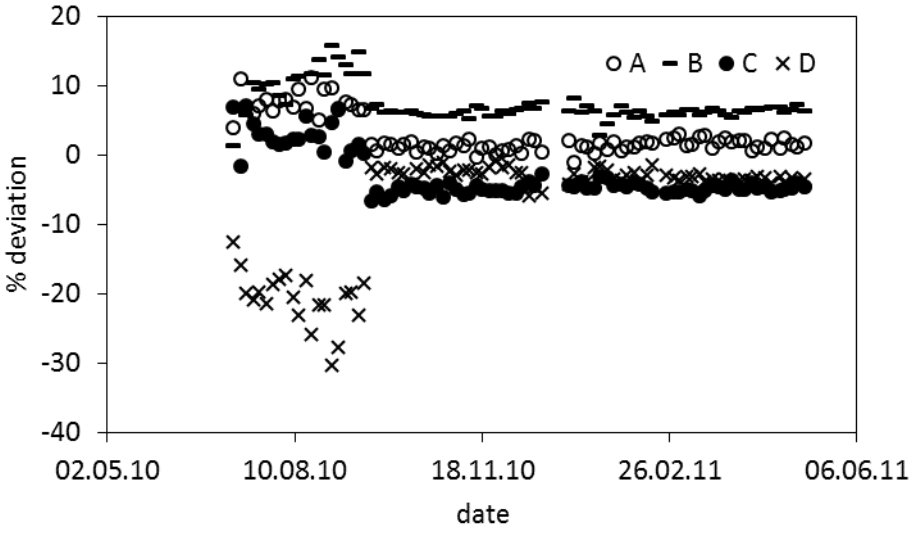


**Fig. 5:** Deviation (%) of incident irradiance measured on top of the individual barrels from mean value measured at the respective sampling date of the first experiment; A, B, C and D – mesocosms.

It can be seen in Figure 5, that the large standard deviations before reduction of irradiance were mainly caused by barrel D (crosses) receiving less light than the others. After the adjustment, consistently small differences in the incident irradiance were measured throughout the first experimental phase.

During the second experiment, irradiance was adjusted again after a first initial period, allowing the lamps to adjust themselves. For this, after the first month irradiance was reduced to a level comparable to the first experiment for all 4 mesocosms on 10.01.2013.

Figure 6 shows the mean irradiance measured on top of the 4 mesocosms together with the standard deviation. After the initial shift, mean irradiance still fluctuated, whereby the fluctuations were not uniform for all compartments.

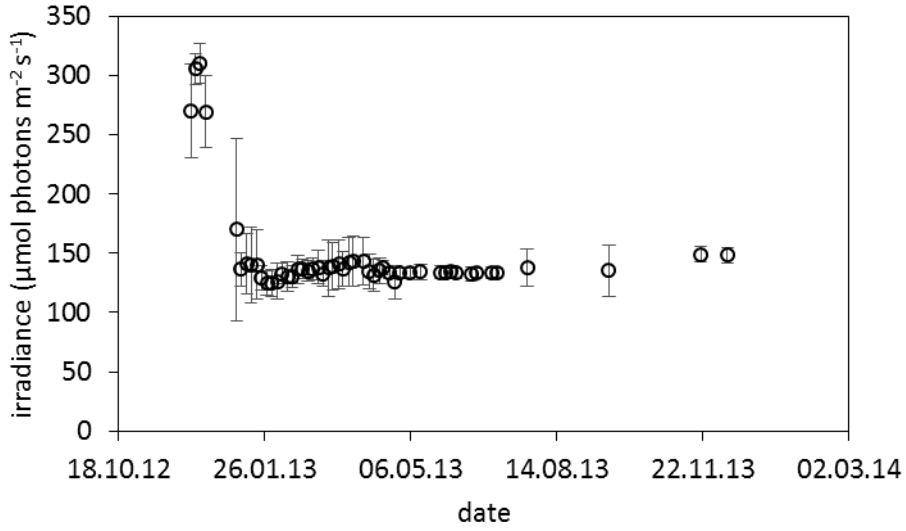


**Fig. 6:** Incident irradiance. Shown are mean irradiance values measured in the center of the cover plates of the 4 mesocosms and standard deviations (of the measurements of the 4 mesocosms) for the second experiment.

As can be seen in Figure 7, showing the percent of deviation of the irradiance measured for the individual barrels from the mean value for the respective sampling, barrel A had a continuous tendency for higher values; barrel C showed lowest values throughout almost the entire experimental phase.

Figure 8 summarizes the differences between incident irradiance measured for all mesocosms. It becomes obvious, that mean irradiance differed between the experiments as well as between mesocosms, which has to be taken into account when discussing differences between the replicates.

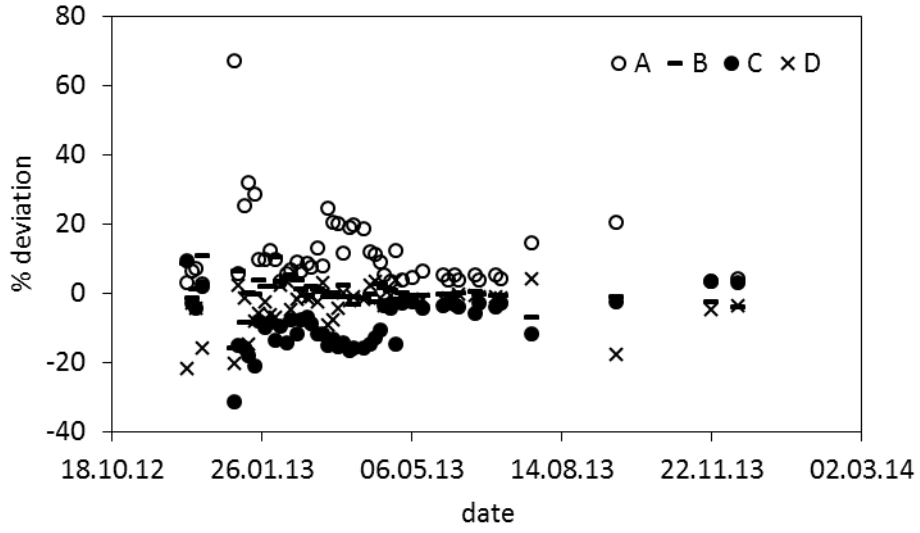


**Fig. 7:** Deviation (%) of incident irradiance measured on top of the individual barrels from mean values measured at the respective sampling dates of the second experiment; symbols – see legend to Figure 5.

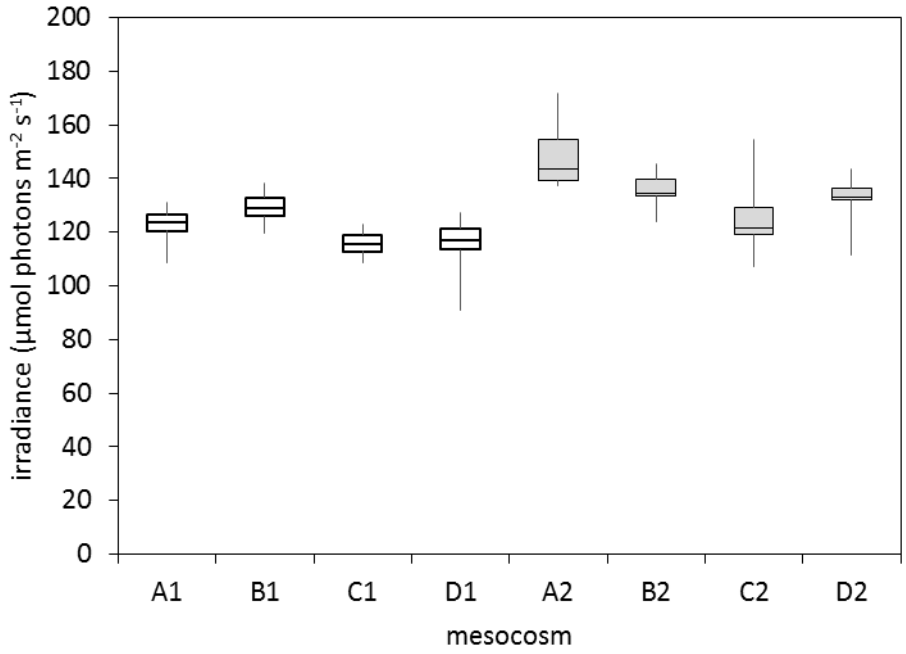


**Fig. 8:** Box-Whisker plot of irradiance values of the individual mesocosms in both experiments. Shown are minimum, median and maximum values as well as the 25% and 75% quartiles of the values measured after the initial phase of two (first experiment) or one (second experiment) month of enhanced illumination. Box-Whiskers were plotted by Excel and the Quartile.Inkl function. A1, B1, C1 and D1 – mesocosms of the first experiment; A2, B2, C2 and D2 – mesocosms of the second experiment.

## 3.2 Temperature

Figure 9 summarizes the temperature readings for the first experiment. Mean temperature was kept constant successfully, with a slight increase towards the end of the experiment. There were slight differences between the replicates; only barrel A showed a specific trend to slightly enhanced temperatures. This barrel was also responsible for the tendency of the increasing mean temperatures for the whole experiment towards the end of the experiment. However, overall difference between the barrels was pretty low, reaching 3.8% (i.e. less than 0.5°C) at the outermost.

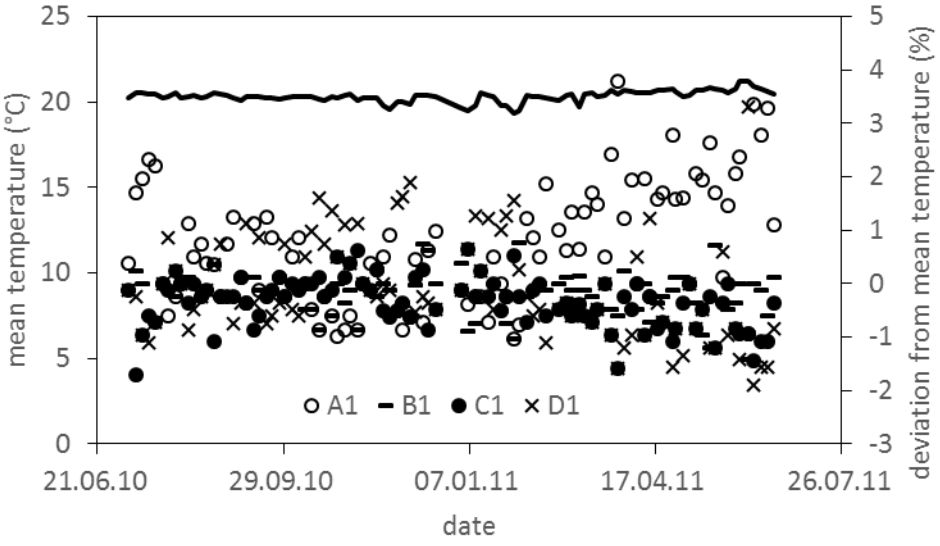


**Fig. 9:** Temperature data for the first experiment. Shown are mean temperature values (black line, left Y-axis) for all 4 mesocosms as well as deviation (%) from the mean temperature (symbols, right Y-axis, see legend to Figure 5) for the individual mesocosms at the respective sampling dates. A1, B1, C1 and D1 – see legend to Figure 8.

In the middle of the second experiment, a sudden shift in temperature occurred for about a week due to overheating of the cryostat (Figure 10). Temperature was peaking at 25.3°C instead of the mean 20.6°C. Except for this period of time, mean temperature was kept rather stable, but by far not that exact as in the first experiment. However, differences between the mesocosms were smaller compared to the first experiment irrespective of the fact that barrel A still had a tendency to the enhanced values.

Figure 11 summarizes the temperature readings for both experiments. Except for the peak during the second experiment, temperature fluctuations were kept well below 3°C for all mesocosms for the entire experimental phase; differences between the mesocosms were below 0.5°C.

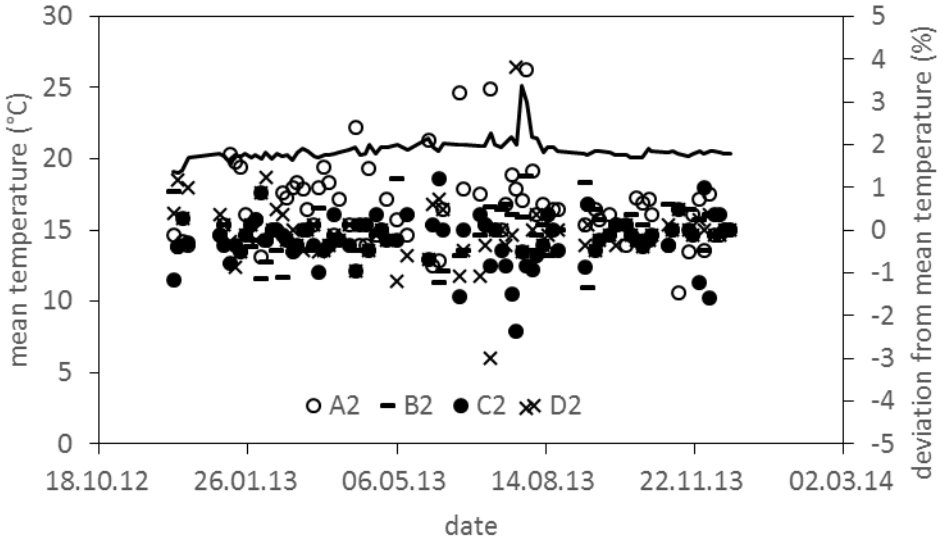


**Fig. 10:** Temperature data for the second experiment. Shown are mean temperature values (black line) for all 4 mesocosms as well as deviation (%) from the mean temperature for the individual mesocosms at the respective sampling dates. A2, B2, C2 and D2 – see legend to Figure 8; symbols – see legend to Figure 5.

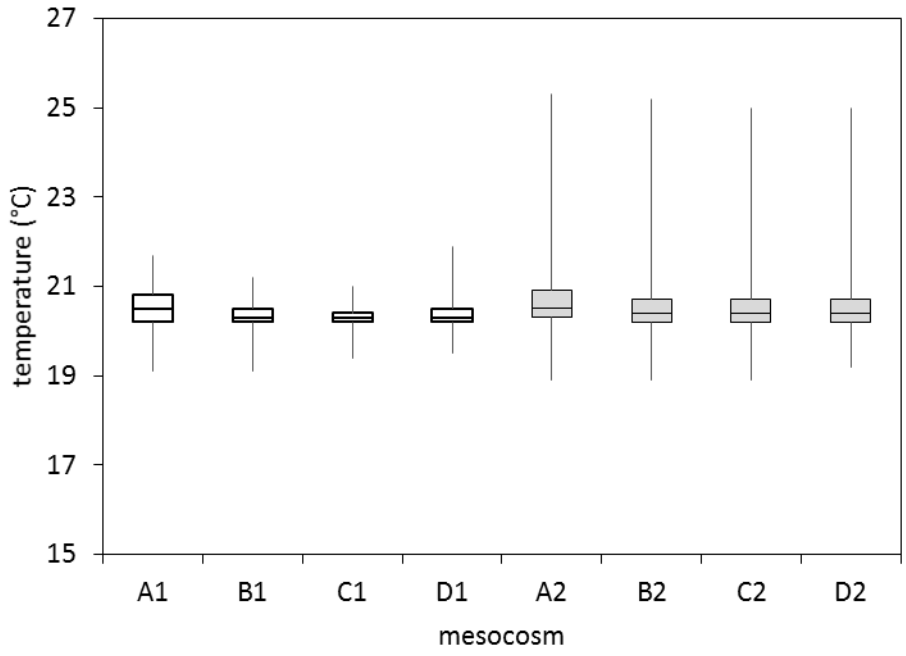


**Fig. 11:** Box-Whisker plot of temperature values of the individual mesocosms in both experiments. Shown are minimum, mean and maximum values as well as the 25% and 75% quartiles. Y-Axis stretched.

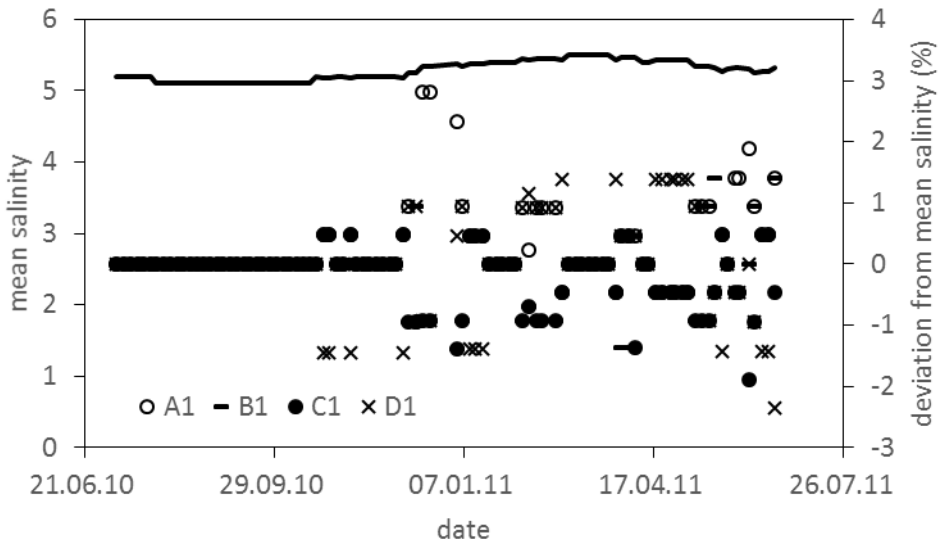


**Fig. 12:** Salinity data for the first experiment. Shown are mean salinity values (black line, left Y-axis) for all 4 mesocosms as well as deviation (%) from the mean temperature (symbols, right Y-axis, see legend to Figure 5) for the individual mesocosms at the respective sampling dates.

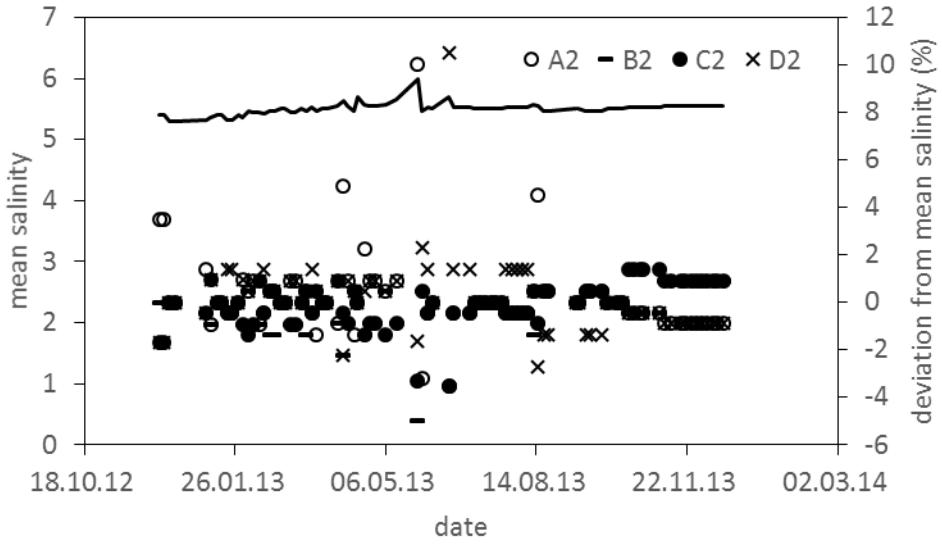


**Fig. 13:** Salinity data for the second experiment. Shown are mean salinity values (black line) for all 4 mesocosms as well as deviation (%) from the mean salinity (symbols, right Y-axis, see legend to Figure 5) for the individual mesocosms at the respective sampling dates.

### 3.3 Salinity

Since replacement of sample volume was unavoidable, salinity, which is also a potential master factor, had to be measured. Figure 12 gives an overview about salinity measurements for the first experiment, and Figure 13 – for the second one.

In both experiments, salinity showed only small changes in the course of time. In the first experiment, the slight increase in salinity with time might indicate evaporation, which has led to an increase of the mean salinity via refilling of the barrels with brackish water. However, all mesocosms stood completely uniform during the entire first experiment; differences between them were still minor until the end. Two peaks in the middle of the second experiment were caused by outlier values of barrels A2 and D2, which were, most probably, erroneous readings, because the following values were back to the mean again.

Figure 14 gives an overview about salinity for both experiments. Except for the outliers A2 and D2, mentioned above, salinity was kept stable within a range of 0.5, without consistent differences between the mesocosms of each experiment.

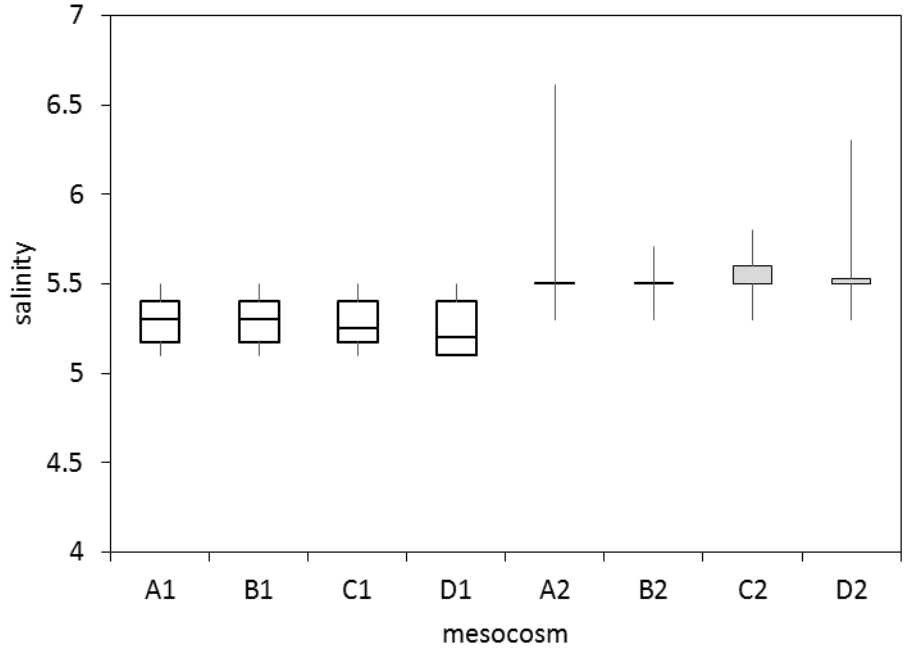


Fig. 14: Box-Whisker plot of salinity values of the individual mesocosms in both experiments. Shown are minimum, mean and maximum values as well as the 25% and 75% quartiles. Y-Axis stretched.

### 3.4 Example of peculiar results on seston and phytoplankton

Here we describe a specific situation which refers to the period of time 2-3 months after the initial splitting of sediments and seston into the 4 mesocosms as well as the adjustment of light conditions during the first experiment. A peculiarity of that time span consisted of the fact that phytoplankton declined in all 4 mesocosms during just that period, in spite of the considerable amount of nutrients (see the percentage of readily available plant nutrients in Table 1). The data for barrel D was

particularly surprising since concentration of nutrients there was even higher than in the other 3 barrels.

The TN:TP ratios (total concentrations) were rather high compared to the demand of algae (Redfield ratio 16:1) and ranged between 42:1 and 100:1 (Table 1). Those high ratios were caused by comparably low TP values in barrels A-C compared to field samples. Phytoplankton communities in all 4 mesocosms were declining fast. The differences between barrels on the first date observed (23rd of September) were attributed only to different speed of the phytoplankton breakdown. Thus, in barrel D the TP values being almost 3 times higher than elsewhere, caused a very low biomass to TP ratio, which was 5 to 11 times lower than in barrels A-C (Table 1).

**Tab. 1:** Average (median) concentrations of total nutrients (µmol $L^{-1}$), portion of free dissolved nutrients (%, DIN, dissolved inorganic nitrogen), phytoplankton biomass as chlorophyll *a* (µg $L^{-1}$) and ratios of TN:TP (on a mol basis) and Chlorophyll *a*:TP (g:mol) in 4 mesocosms (A-D) during the first experiment; n – number of observations over the winter period 2010/2011.

| | A | B | C | D | n |
|---|---|---|---|---|---|
| TP | 1.1 | 1.2 | 1.3 | 3.0 | 4 |
| % phosphate | 33 | 20 | 26 | 45 | 4 |
| TN | 105 | 109 | 106 | 136 | 5 |
| % DIN | 5.4 | 6.8 | 4.9 | 23.6 | 5 |
| TN:TP | 100 | 88 | 82 | 42 | 4 |
| Chl *a* on 23. Sep | 55 | 17 | 16 | 103 | 1 |
| Chl *a* | 12 | 17 | 19 | 12 | 7 |
| Chl *a*:TP | 48 | 54 | 100 | 9 | 4 |

# 4 Summary

Unraveling the role of internal mechanism contributing to plankton dynamics observed under field conditions requires stability of potential external triggers, especially irradiance, temperature and salinity, which have been shown to be master factors for plankton communities by several authors (e.g. SAGERT et al. 2008 and references therein).

For this reason, an experimental mesocosm setup targeted on achieving maximum long-term stability of the above-mentioned factors has been designed and tested. Irrespectively of the considerable effort spent for maintaining stability of these parameters at a constant level, certain fluctuations still occurred. Irradiance, as almost the sole energetic base for the whole food web in this kind of long-term experiments, shifted due to ageing of the bulbs in the first experiment by almost 10%. This fact must be taken into account when discussing the results with respect to phytoplankton species composition as well as food web dynamics. Moreover, in both experiments differences between the mesocosms were observed. So, mesocosm D1 received almost 13% less energy compared to B1. In the second experiment, differences between the mesocosms were even larger: C2 and A2 differed by 17% of the received energy, as calculated for the whole experimental period.

Variability of temperature was successfully maintained stable in a range of less than 3K for total variation, except for a short period during the second experiment. Consequently, the results of the second experiment must be interpreted with caution, because in that case, temperature was elevated accidentally by 5K for about a week and this event, most probably, would have influenced the biotic processes. So, this specific period of time must be regarded as an external disturbance, after which biotic interactions had to re-adapt. With respect to comparability between the replicates, the mesocosm A1 differed consistently from B1-D1 by slightly higher temperatures.

Salinity was not expected to change much in a closed system. Replacing the water volume (lost due to sampling) with the original habitat water should have prevented gradual changes, which otherwise might have driven some species to the edge of their physiological limits (TELESH et al. 2011). However, even if the noise caused by technical limitations is ignored, a slight increase in salinity was observed in both experiments. This increase can only be explained by evaporation still going on irrespective of presence of the cover plates. The resulting shift in salinity by about 0.3 (first experiment) or 0.2 might be minor and it is less likely that this might have caused major biological effects. Moreover, comparability between the replicates with respect to salinity was achieved in full during both experiments.

Nevertheless, there was still one replicate (barrel D in experiment 1), which deviated in its element concentrations from all other replicates, and this deviation could not be explained by abiotic drivers that differ so little elsewhere.

## 5 Conclusions

The optimized mesocosm setup, designed for experimental investigation of the internal mechanisms that drive plankton dynamics under the constant external conditions, was successfully used in two consecutive mesocosm experiments, each one year long, that were carried out in four replicates. However, irrespective of all efforts, only partial long-term stability of the external factors was achieved in this study. This result gives a warning for interpretation of the long-term experiments, especially when the experimental parameters are sampled with low frequency, or when the conclusions rely only on start-end measurements of the abiotic characteristics. Moreover, our results highlight the importance of repeated measurements of the experimental parameters in all replicate mesocosms, irrespective of similarity (or even identity) of light source and automated temperature control. The residual differences in abiotic conditions between the replicates as well as through time must be tested for their impact on the biotic interactions in the long-term experiments, as it will be done in a forthcoming paper dealing with the biotic components of the experiments described here. We can also draw the conclusion about the necessity of setting up more mesocosm replicates than the minimum needed number; in such a case, the outliers can be easily identified and excluded from the analysis in order to enhance the accuracy of experimental results.

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