POSEIDON Berichte

Biofilm-like habitat at the sea-surface: A mesocosm study

Cruise No. POS537

14.09.2019 – 04.10.2019,
Malaga (Spain) – Cartagena (Spain)
BIOFILM

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1 Cruise Summary

1.1 Summary in English

Biofilm-like properties can form on sea surfaces, but an understanding of the underlying processes leading to the development of these biofilms is not available. We used approaches to study the development of biofilm-like properties at the sea surface, i.e. the number, abundance and diversity of bacterial communities and phytoplankton, the accumulation of gel-like particles and dissolved tracers. During the expedition POS537 we used newly developed and free drifting mesocosms and performed incubation experiments. With these approaches we aim to investigate the role of light and UV radiation as well as the microbes themselves, which lead to the formation of biofilms. With unique microbial interactions and photochemical reactions, sea surface biofilms could be biochemical reactors with significant implications for ocean and climate research, e.g. with respect to the marine carbon cycle, diversity of organisms and ocean-atmosphere interactions.

1.2 Zusammenfassung

Biofilme-ähnliche Eigenschaften können sich auf Meeresoberflächen ausbilden, aber ein Verständnis der zugrundeliegenden Prozesse, welche zur Entwicklung dieser Biofilme führen, ist jedoch nicht vorhanden. Wir nutzten Ansätze um die Entwicklung von biofilm-ähnlichen Eigenschaften an der Meeresoberfläche zu verfolgen, d.h. die Anzahl sowie Abundanz und Diversität von bakteriellen Gemeischaften und Phytoplankton, die Anreicherung von gel-artigen Partikeln sowie gelösten Tracersubstanzen. Während der Expedition POS537 haben wir neuentwickelte Treibmesokosmen eingesetzt sowie Inkukationsexperimente durchgeführt. Mit diesen Ansätzen haben wir das Ziel, die Bedeutung von Licht und UV-Strahlung sowie die der Mikroben selbst zu untersuchen, welche zur Bildung der Biofilme führen. Mit einzigartigen mikrobiellen Wechselwirkungen und photochemischen Reaktionen, könnten Biofilme an der Meeresoberfläche biochemische Reaktoren sein, welche bedeutende Auswirkungen auf die Ozean- und Klimaforschung haben, z.B. in Bezug auf den marinen Kohlenstoffkreislauf, auf die Diversität der Organismen und auf Wechselwirkungen zwischen dem Ozean und der Atmosphäre.

2 Participants

2.1 Principal Investigators

Name	Institution
Wurl, Oliver, Prof.	University Oldenburg
Striebel, Maren, Dr.	University Oldenburg
Ferrera, Isabel, Dr.	Spanish Institute of Oceanography
Montserrat Sala, Maria, Dr.	Institut de Ciències del Mar
Simo, Rafael, Dr.	Institut de Ciències del Mar

2.2 Scientific Party

Name	Discipline	Institution
Wurl, Oliver, Prof.	Marine Biogeochemist/ chief Scientist	Uni OL
Mustaffa, Nur Ili Hamizah, Dr.	Marine Chemist, scientist	Uni OL
Tiera-Brandy, Robinson, Dr.	Marine Biogeochemist, scientist	Uni OL

Ferrera, Isabel, Dr.	Marine Microbiologist, senior scientist	IEO
Ruiz Gazulla, Carlota	Marine Microbiologist, graduate student	ICM
Hoppe, Jennifer	Marine environment, student	Uni OL
Jaeger, Leonie	Marine environment, student	Uni OL
Heinrichs, Anna-Lena	Marine environment, student	Uni OL
Hennings, Laura Margarethe	Marine environment, student	Uni OL
Rodrigo, Gonçalves	Planktologist, senior scientist	CESIMAR

2.3 Participating Institutions

Uni OL University Oldenburg, Germany
IEO Instituto Español de Oceanografía, Spain
ICM Institut de Ciències del Mar, Spain
CESIMAR Centro Para el Estudio de Sistemas Marinos, Argentina

3 Research Program

3.1 Description of the Work Area

It has been essential to conduct the proposed work in an oceanic regime with the calmest sea state to be expected and preferably at oligotrophic conditions to have low background signals from biological activity. The Balearic Sea typically fullfills these conditions for the late summer (August to September), indicated by the analysis of wind data (Zecchetto and Biasio, 2007; Wurl et al., 2016) and personal experience by colleagues from ICM. Despite these careful pre-assessment, higher sea states and strong winds during the expedition POS537 limited the time for the deployment of the mesocosm and the formation of biofilm-like sea surface. Mesocosms were deployed according to the local current and weather regime as outlined in section 3.3.

3.2 Aims of the Cruise

The sea-surface microlayer (SML) is the boundary interface between the atmosphere and ocean, covering about 70% of the Earth's surface. With a typical thickness of 60-100 μm, the SML has physicochemical and biological properties that are measurably distinct from underlying waters. Because of its unique position at the air-ocean interface, the SML is central to a range of global biogeochemical and climate-related processes. More recent studies indicate that the SML is an aggregate-enriched biofilm environment with distinct microbial communities (Stolle et al., 2010, Wurl et al., 2016). The redeveloped SML paradigm pushes the SML into a new and wider context that is relevant to many ocean and climate sciences, including marine carbon cycle (Reinthaler et al., 2010), air-sea gas exchange (Mustaffa et al., 2020) and aerosol production (Wilson et al., 2015).

The overall objective of this expedition has been to gain a mechanistic understanding of the formation of a biofilm-like habitat at the sea surface. In a time series (several days), we have investigated the dynamics of biofilm formation and light conditions as primary force (i.e. natural dark/light cycle and controlled dark conditions). We have modified old lift rafts as floating mesocosms and covered them with UV transmitting and UV blocking foil. Another set of mesoscosm served as dark control. In addition, we have conducted deck incubations to gain additional information on the influence by UV radiation and light on chemical and biological

composition of natural SML and near-surface water. Specifically, we will address the following objectives:

Objective A: We aim to elucidate that visible light, UV radiation, and microbial communities are essential for the accumulation of gel-like particles in the SML, i.e. formation of biofilm-like habitats at the sea surface**.** We have monitored the accumulation of gel-like particles in the SML and changes in chemical composition within the mesocosms.

Objective B: We have investigated if the bacterial communities in the biofilm-like SML are unique in terms of abundance, diversity and physiology (i.e. enzyme activities, carbon acquisition). We have compared free-living and particle-associated communities between biofilm-like SMLs (within mesocosms), natural SML (outside mesocosms), and underlying bulkwater. We have monitored the development and changes of the community in time, also in context of light and UV radiation, as well accumulation of gel-like particles.

Objective C: We have addressed the abundance and biodiversity of phytoplankton communities residing in the biofilm-like habitat of the SML and underlying bulkwater**.** We will show how the harsher environment (intense UV radiation, greater temperature and salinity fluctuations) controls their abundance and diversity in the SML and near-surface layer. We will provide detailed characterization of taxonomic and trait (pigments, nutrient stoichiometry) diversity under different light conditions.

In addition, we have also performed a comparison between the mesocosms and natural slicks, the latter likely occurring frequently in the proposed study area. It will support the relevance of made observations to natural conditions, at least to the time point at which natural slicks typically persist. Beyond this point, it is of our particular interest to explore if through continuing UV radiation to the SML environment, the diversity of microbes further decreases and to identify rare or unknown species typically low in abundance for detection, i.e. part of hypotheses B and C. Overall, with this exploratory study, we aim to fill the lack on relevant data for the near surface ocean and SML, and its importance as gateway for air-sea interactions. Showing its relevance, we hope to encourage colleagues to consider the very top of the ocean as an important compartment of the ocean in future studies.

3.3 Agenda of the Cruise

The aim of this cruise is to investigate the development of biofilm-like properties at the sea-surface, it was essential to conduct the proposed work in an oceanic regime with the calmest sea state to be expected and preferably at oligotrophic conditions to have low background signals from biological activity. The Balearic Sea fullfills these conditions for the late summer (August to September), indicated by the analysis of wind data (Zecchetto and Biasio, 2007; Wurl et al., 2016) and personal experience by colleagues from ICM.

We started the cruise in Malaga, Spain, and streamed northward to identify an area with low ship traffic and good weather forecasts for the upcoming days to deploy the mesocosms for at least 3 days (Figure 3.1).

Figure 3.1: General track chart for R/V Poseidon 537. Dashed box indicates area of deployments of free floating mesocosms.

General timing:
• 14th of September: Arrival of participants in Malaga
• 15th of September: setup of scientific equipment in the labs and the mesocosm
• 16th of September: Leaving Malaga port heading north to our study area.
• 29th of September: Disembarkation of scientist in Barcelona
• 4th of October: arrival in Cartagena and end of POS537

Due to technical issues, the DShip system was not recording for POS537, and only limited data are available for meteorological and underway system. The limited data set included data part of the mails sent every 10 minutes with navigation and some weather data (position, speed of vessel, heading absolute wind direction, absolute wind speed, air pressure, air temperature, humidity, water temperature, water salinity, water cChlorophyll). However, these "mailed" data are only available for the duration from 26.9.2019 bis 3.10.2019. That is unfortunate, as some meteorological data, especially wind speed, are critical in the evaluation of our field data. We installed a hyperspectral radiance and irradiance sensors for the UV/VIS range on the deck above the bridge to obtain information on the atmospheric UV and light conditions.

Incubation experiments
We collected water samples from the upper surface layer with the CTD to conduct incubation experiments (Figure 3.2). Seawater samples from 10 different depths were collected and filled 200 cell cultures bottles with and were treated with three light treatments, i.e., blue, red and green and a control treatment (grey) for > 2 weeks. This experiment aims to understand how phytoplankton community at different depths respond to different light penetration in the ocean.

An additional incubation experiments were set up at a later point of the cruise, to investigate the effect of grazing by zooplankton on transparent exopolymer particles; the latter an essential component of biofilms observed on the sea surface.

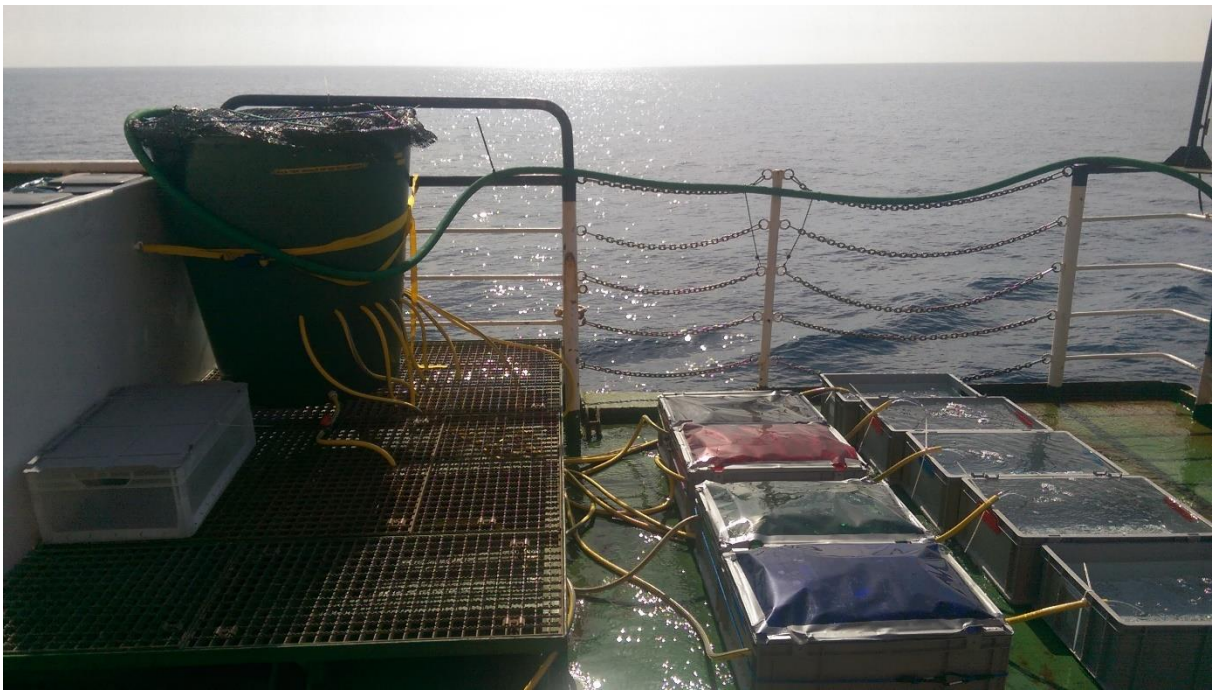


Figure 3.2: Incubation experiments set up to the begin of the cruise.

Mesocosm setup

Six mesocoms, modified from decommissioned lift rafts were deployed. The bottom of the lift rafts was cut out to have a floating oval shape with a site of approximately 2.8 meter x 1.5 meter. The roof structure, also inflatable, were used to attach UV transmitting and UV blocking foil (each two mesocosm), two mesocosms remain their original cover as dark control. In total we deployed six mesocosms (Figure 3.3). The mesocosms were attached to each other, randomly, in a chain. The free floating mesocosm were sampled when the weather permitted, but typically in the morning and the afternoon, from the small boat. The SML were collected using the glass plate technique (Figure 3.4), which was proven to be challenging from the small boat, especially when the sea states were unfavourably higher than hoped for. At the time, bulk water samples were collected from 1 meter depth as reference.

Figure 3.3: Deployment of six free floating mesocosms during POS 537.

Figure 3.4: Sampling mesocosms from the small boat for the SML and underlying bulk water.

Tows of Manta net

Tows with the manta net were conducted to collect zooplankton from the sea surface, i.e. the upper few centimeters of the water column. The collected zooplankton was sorted, identified and used for incubations experiments.

4 Narrative of the Cruise

The narrative is based on the three weekly reports of the expedition POS537.

14th + 15th September 2019, Saturday
All scientists arrived in Malaga. We received a warm welcome from the Captain and the crew onboard. The science team unpacked their boxes and setup the labs. Filtration system and all instruments were setup in the laboratories. Everyone is happy with the space provided. It was discovered that no onboard Milli Q system was available and thus approximately 20L of Milli Q was brought from Malaga institute for Oceanography.

16th September, Monday
Leaving Malaga port at 10:00 am local time and heading north to our study area. Everyone is excited for the science to begin. All six mesocoms were inflated on the deck. At 15:20, everyone gathered on the main deck for safety training by the second officer. After dinner one of the scientist, Rodrigo Goncalves from Argentina gave a short presentation about his project.
17th September, Tuesday
Second day at sea. Planktology team prepared their bottles and covered their boxes with different colored foils for the on-board incubation experiment. The boxes were left overnight on the deck and the water temperature was checked every 20 minutes. They found that water temperature in the incubation boxes increased by 4°C during mid-day and decided to increase the water inflow. Together with the Planktology team, Rodrigo prepared his boxes for zooplankton incubation experiment.

Meanwhile the mesocosm team discovered a leak in one of the mesocosms and it was patched in time for it's planned deployment the following day.

18th September 2019, Wednesday
Third day at sea. After breakfast, first CTD cast was deployed. Seawater samples from 10 different depths were collected and filled 200 cell cultures bottles with and were treated with three light treatments, i.e., blue, red and green and a control treatment (grey). This experiment aims to understand how phytoplankton community at different depths respond to different light penetration in the ocean. As a group who study the upper sea surface layer, deployment of CTD is not within our normal routine. We thank the crew for helping us with the deployment.
The mesocosms were cleaned and UV foils were prepared to be attached on top.

19th September 2019, Thursday
Phytoplankton nets were deployed between 40 and 50 m depth followed by manta net to collect plankton at the surface. Meanwhile, planktology team measured the optical density of each incubated bottles in the chemical lab. First deployment of all six mesocosms in the water for initial testing (Figure 4.1), due to incoming bad weather the mesocoms had to be deployed ahead of schedule and whether they can and will be recovered in the morning is unclear and depends on weather conditions.

Figure 4.1: Deployment of mesocosms: (photo: Tiera-Brandy Robinson)

20th September 2019, Friday
06:00 am, second CTD cast was deployed. Dr Isabell Ferrera collected water at two depths (100 meter and surface) for micro plastic analysis. One day mesocosm experiment started. Two scientists went on a small boat and collected the sea surface microlayer (SML) and one-meter bulk water at five different time points. Scientists from Marine Interface group (ICBM University Oldenburg) collected water for TEP analysis and optical density measurement. Meanwhile Dr Ferrera and her team collected water for DNA analysis, flow cytometry and enzyme activity. Dr Mustaffa from ICBM University Oldenburg filtered the collected water for extracellular carbonic anhydrase (eCA) analysis. Despite the limitation of sampling volume using dipping glass plate technique, we manage to provide everyone with sufficient volume they needed. Everyone is happy and enjoy every meal onboard.

21st September 2019, Saturday
After a successful one day mesocosm on Friday, we had to abort our first 3-day mesocosm experiment due to incoming bad weather. With the help from the crew, all mesocosm were successfully recovered onboard, but the mesocosms had minor damage.

22nd September 2019, Sunday
Weather condition did not permit to deploy any mesocosm or small boat. Science team used time to repair the damaged mesocosm. All scientist worked together as a team to repair the damage, by making new eye lids and learned new knots to secure the UV foils (Figure 4.2).
As a backup plan for a bad weather, we also did an alternative incubation experiment onboard to look at photolysis of TEPs. 20 Liter of bulk water was collected from the ship using bucket, pre-filtered through 200 µm and 50 µm filters to remove any predators and phytoplankton, and filled into 1 Liter cubicle bottles. The bottles were incubated with dark, UV transmitting and UV radiation treatments. Samples for TEP, optical density and flow cytometry were measured from each bottles every day after sunset.

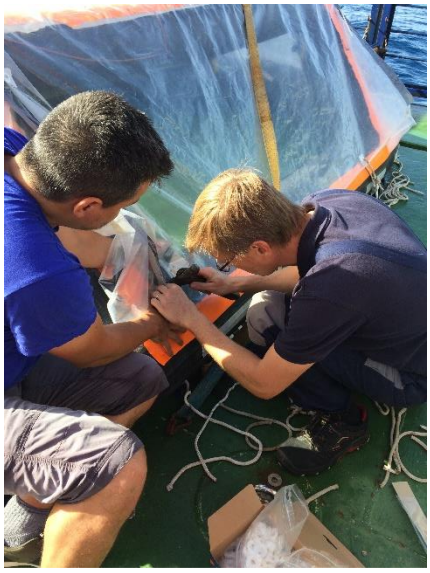


Figure 4.2: Preparing new eye lids for UV foils (photo: Mustaffa NIH)

23rd September 2019, Monday
Planktology team measure daily the optical density from their phytoplankton incubation experiment. The weather was still bad postponing mesocosm deployments or sampling from a small boat. Meanwhile other scientists involved in the TEP incubation experiment sampled and filtered the water for their measurements.

24th September 2019, Tuesday
We went out on a small boat to sample high volume natural sea surface (SML) for RNA and DNA analyses (Figure 4.3). Together with the SML, seawater from 20 cm and 1 meter depths were sampled as reference. These samples were collected and analysed for comparison to those from our mesocosms.

Figure 4.3: SML sampling from a small boat. (Photo: L. Jeager)

25th September 2019, Wednesday
Bad weather day. Scientists took their time to rest, recovered from sea sickness or got prepared for upcoming mesocosm experiment. Meanwhile after sunset, scientists who were involved in TEPs incubation experiment sampled and filtered the seawater for their measurements.

26th September 2019, Thursday
The weather is on our side today. All six mesocosm were successfully deployed (Figure 4.4). The sea surface water and bulk water at 1-meter depths were collected at three time points daily.

Scientists take turns to go on the small boat and experience surface water sampling inside the mesocosm.

Figure 4.4: Calm sea and all mesocosm in the water for 2 days' experiment (photo: Mustaffa NIH)

After lunch, manta net was deployed to collect zooplankton at the surface water. Approximately 100 of blue copepods were collected and sorted accordingly in the dry lab. We thank the bridge for their flexibility to deploy manta net allowing for effective use of the time of calm seas.

27th September 2019, Friday
Second day of mesocosm experiment. The sampling activity went smoothly as planned. Mesocosm were left overnight and final sampling will be done on the next day.
After lunch, the CTD was deployed to collect water from 5-meter depth for 24 hours' zooplankton incubation experiment. The zooplankton collected from previous day were incubated in the surface and subsurface water (5 meter) and left under UV transmitting and UV radiation treatments for 24 hours.

28th September 2019, Saturday
Morning sampling was cancelled due to higher wind speeds. After lunch, we finally went out for a final sampling followed by mesocosm recovery. Meanwhile in the wet lab, scientists were all busy filtering samples from mesocosm and 24 hours' zooplankton incubation experiment. We are now steaming towards Barcelona to drop off the chief scientists (Oliver Wurl) for an impoirtant meeting in Bruessel. Dr. Mustaffa took the role as chief scientist for the last week. Despite having a bad weather week, we had a good trip so far.

29th September 2019, Sunday
Final week at sea. During 8 hours steaming towards the study area, we used the time to repair minor damage and inflated the mesocosm for the next deployment. After arrival, all mesocosm were deployed in the water and we went out on a small boat to sample high volume natural sea surface (SML) for RNA and DNA analyses. Together with the SML, bulk water from 1 meter depths were sampled as reference. Samples were filtered in the wet labs for DNA, RNA, TEPs and optical density analyses.

30th September 2019, Monday
The high wind speed did not permit mesocosm sampling or small boat operation in the morning. Therefore, the first sampling was postponed until after lunch. Scientists take turns to go on a small boat for surface water sampling. Mesocosms were left overnight and will be sampled tomorrow.

1st October 2019, Tuesday
Final sampling for mesocosm experiment was done in the morning and afternoon. All mesocosm were recovered onboard, rinsed with freshwater, deflated, and ready to be packed. Meanwhile in the labs, scientists were all busy filtering the finalsamples for their measurements

2nd – 4th October 2019
We were informed by the Captain for incoming bad weather on Wednesday. Therefore,we decided to leave the study area and ended our science activities. We use the time to clean the labs and packed the lab wares/instruments into boxes. Meanwhile, planktology team started filtering the samples from their incubation experiment. We are now heading towards Cartagena and are expected to arrive on Thursday, 3rd October in the evening.

5 Preliminary Results

5.1 Mesocosms I (carbon)

(T.B. Brandy)

5.1.1 Methodologies

Sampling method for discrete samples: Samples were collected from the Sea Surface Microlayer (SML) via the glass plate technique (Harvey and Burzell 1972), from six mesocosms, bulk samples were collected using a syringe tube that was weighted and placed at a depth of 1 meter. A total of three mesocosm experiments were run (BFL1, BFL4, BFL6) with additional *in situ* samples take to contextualise the mesocosm experiments (BFL2, BFL3, BFL5).

Transparent exopolymer particles (TEP): TEP samples were filtered via low vacuum (<200mm Hg) filtration onto polycarbonate filters with 25mm diameter and 0.2 µm pore size (Nuclepore, Whatman), which had been soaked in 1mol HCl solution for 24 hours for cleaning and reducing background staining of the filter. After filtration, TEP were stained with 1 mL of Alcian blue 8GX (Sigma-Aldrich) solution at pH 2.5 (0.02% in 0.06% acetic acid) (Passow and Alldredge, 1994). The dye was left on the filter for 6 seconds in order to stain the TEP that was retained, then it was drawn through the filter at low vacuum. Milli Q water was used to rinse the filter and remove excess dye. Working solutions of Alcian Blue were prepared fresh each day by making a 1/50 dilution from the stock solution. The working solution was pre-filtered to counter spontaneous aggregation of the stain. All filters were prepared in triplicate and stored in Eppendorf tubes at -20°C until analysis. TEP will be analyzed using the spectrophotometric method (Passow and Alldredge, 1995). Stained TEP filters will be placed in 25mL glass vials and 5ml of extraction solution (80%H_2SO_4) added. Vials will be incubated for 2 hours with gentle shaking to reduce bubble formation Concentrations of TEP are then shown in relation to a xanthan gum standard and reported as micrograms of xanthan gum equivalents per liter.

Biolog-Ecoplates: *Carbon utilization assay:* The utilization of different carbon sources by microbial communities was investigated with Biolog ®Ecoplates in samples from the BFL4 experiment. The plates contain 31 carbon sources in triplicates together with tetrazolium violet to indicate substrate oxidation, and we inoculated each well with 150 lL unfiltered seawater from the SML and underlaying water (ULW). All plates were incubated in a temperature-controlled box, in the dark, for seven days. A microplate reader (ELX800 BIOTEK Instruments, Inc.,Winooski, VT, United States) was used to monitor colour development in each well every 24hrs at a wavelength of 590 nm. Guild classes were created by assigning the substrates into

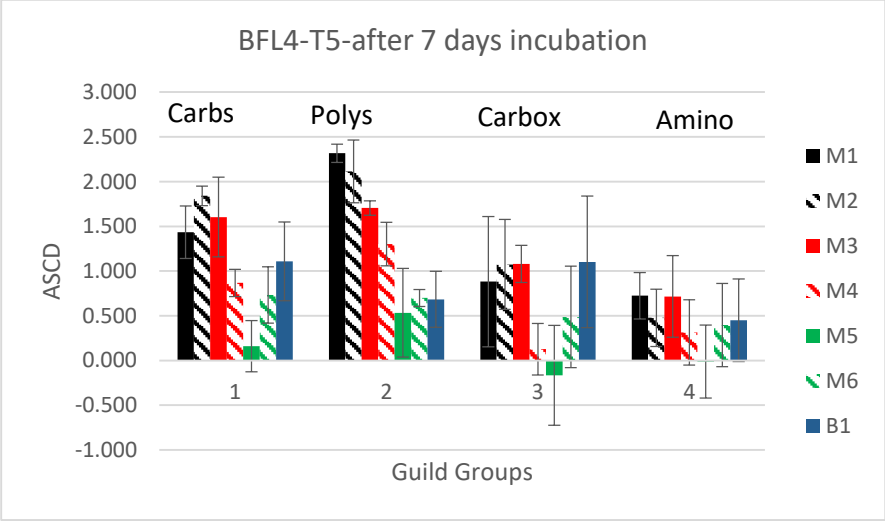
five different biochemical classes and their guild colour development (AGCD) was calculated (Leflaive et al., 2008). While such an approach has limitations in terms of identification, they have been found to be useful for comparing communities (Preston-Mafham et al. 2002). We followed this recommendation and used Biolog ®Ecoplates to compare SML and ULW communities before, during and after experiment BFL4 and between the different mesocosm light conditions.

Optical density: The optical density of samples was taken at each sampling time point, for all experiments, and were analysed at 190-400nm wavelength on a spectrophotometer. The wavelength of the peak absorbance (208nm) was used to calculate Optical Density (OD), a range of 190-400nm was chosen instead of 600nm since natural seawater was being analysed and is clearer than yellow culture media that is often analysed. Optical density is calculated by the following equation: OD=-log(I/Io) where I is the intensity of light that passes through the sample and Io is the initial light intensity.

5.1.2 Preliminary results

Transparent exopolymer particles (TEP): No preliminary results exits due to a broken spectrophotometer required for analysis, and restricted usage of laboratories during Covid19 pandemic. Samples will be run and analysed in the next few months when our spectrophotometer is repaired and returned.

Biolog assay: Utilization of carbon sources were only conducted for experiment BFL4 but showed an increase over incubation time for all guild groups (Figure 5.1). Initial analysis of AGCD shows similar increases for all sampling time points, suggesting that carbon utilisation was relatively constant within each mesocosm during the experiment. AGCD was in general highest in samples from the dark mesocosms (M1, M2), then mesocosms allowing all light, UV+ (M3, M4) and then the mesocosms which blocked UV light, UV- (M5, M6).



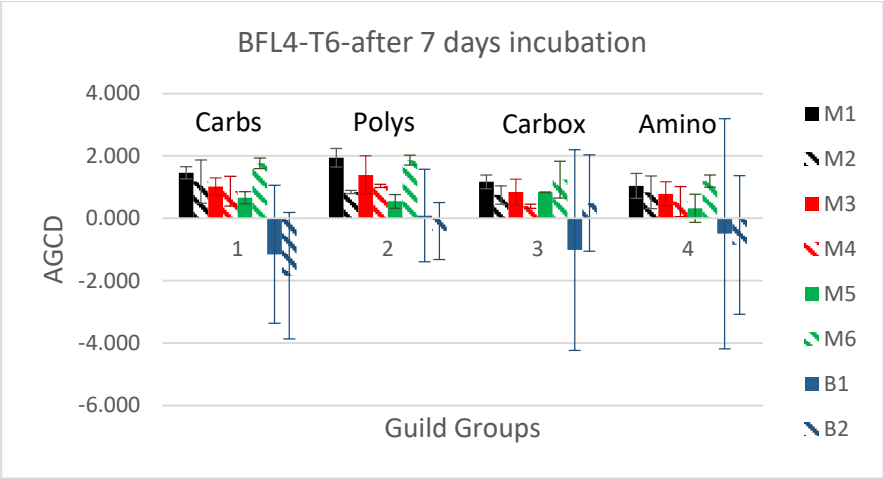


Figure 5.1: Average guild colour development (AGCD) for sampling time point 5 (upper panel) and 6 (lower panel) from experiment BFL4 after 7 days incubation. Samples are from six mesocosms with light blocking (M1, M2) UV transmitting (M3, M4) and UV blocking (M5, M6) material on top, as well as two ULW samples (B1, B2). Guild groups were split into; carbohydrates (carbs), Polymers (polys), carboxylic and acetic acids (carbox), and amino acids (amino).

Optical density: The absorbance at 208 nm (optical density) were highest in the incubated SML (M1 to M6), i.e. the SML within the mesocosms, compared to the bulk water samples (B1 and B2) (Figure 5.2). Highest absorbance was measured in the dark treatment (M1 and M2) and in one UV treatment (M3), but the duplicated UV treatment was as lower and at a level of the samples not radiated with UV light.

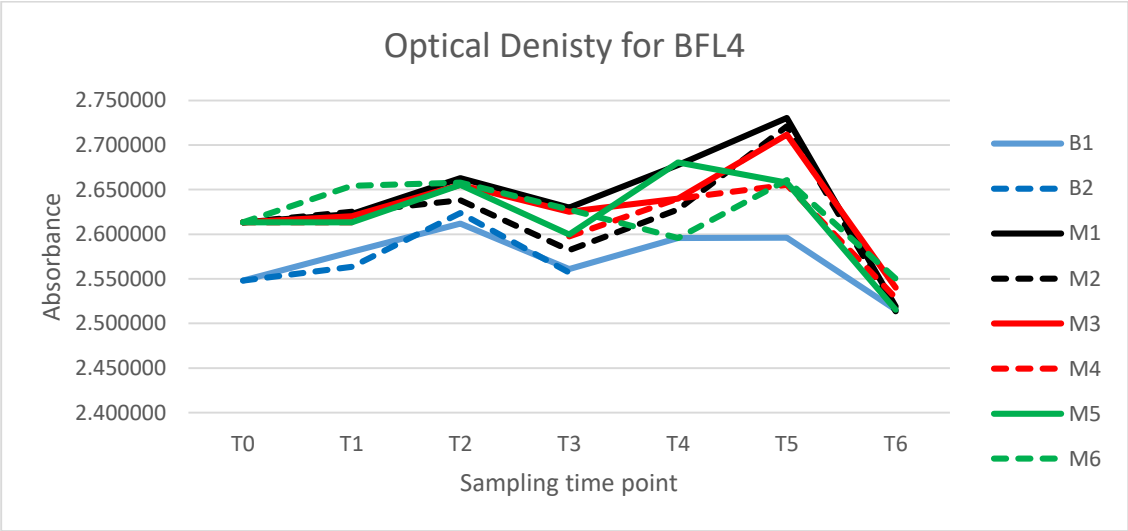


Figure 5.2: Optical density development over time during experiment BFL4 for each mesocosm. Samples are from six mesocosms with light blocking (M1, M2) UV transmitting (M3, M4) and UV blocking (M5, M6) material on top, as well as two ULW samples (B1, B2). (M1-M6) and ULW water samples (B1, B2).

5.2 Mesocosm 2 (microbiology)

(I. Ferrera)

5.2.1 Methodologies

Samples for microbial abundance, activity and diversity were taken in three mesocosms experiments (BFL1, BFL4, BFL6). Additionally, *in situ* sampling of the surface microlayer and bulk water were collected three times (BFL2, BFL3, BFL5) to contextualize the mesocosm experiments. To estimate the abundance of photosynthetic picoplankton and heterotrophic bacteria (i.e., Bacteria and Archaea or prokaryotes), flow cytometry samples were taken over time during the incubations. Extracellular enzyme activities were measured on board in the same mesocosms. To estimate single-cell activity, we used the technique Bioorthogonal non-canonical amino acid tagging (BONCAT). Samples for genomic analyses (DNA and RNA) were collected by filtering samples onto 0.2 µm polycarbonate filters using a peristaltic pump and immediately preserved in liquid nitrogen and transferred to a -80ºC freezer once in the lab. Additionally, samples for inorganic nutrient analyses were also collected and frozen immediately. Further details on sample collection and processing are indicated below.

Flow cytometry counts of picoplankton. Samples (1.2 ml) were immediately fixed with 1% paraformaldehyde plus 0.05% glutaraldehyde (final concentrations), incubated for 10 min at room temperature, flash frozen in liquid nitrogen and kept at -80°C until analysis in the laboratory. These samples allow the estimation of photosynthetic picoplankton and heterotrophic bacteria. So far, only heterotrophic bacteria have been counted. For that purpose, prior to analysis, heterotrophic bacteria were stained with SYBER green (SIGMA-ALDRICH) at 10x final concentration and counted in a FACSCalibur flow cytometer (Becton Dickinson) based on their relative green fluorescence (FL1, 511 nm) and SSC (side scatter) signals. Absolute counts are calculated by estimating the flow rates using TruCount™ bead suspensions prepared by adding deionised water to TruCount™ tubes (Becton Dickinson).

Extracellular enzyme activities. Leu-aminopeptidase, chitobiase, β-glucosidase, and alkaline phosphatase activities, were estimated using the following fluorogenic substrates: L-leucine-7-amino-4-methylcoumarin, 4-methylumbelliferyl α -D-glucoside, 4-methylumbelliferyl β-D-glucoside, and 4-methylumbelliferyl phosphate, respectively (all purchased at Sigma-Aldrich) following the method developed by Hoppe (1983). Assays were performed as described in Sala et al., (2016). Briefly, each sample (350 µl) was pipetted in quadriplicate into 96 black well plates, and substrates were added to obtain a final concentration of 125 µM. This concentration has been found to be saturating in previous experiments and was thus chosen to estimate potential activities, to facilitate comparison among treatments. The fluorescence of the 96 well plates was measured with a Modulus microplate reader at 365 nm excitation and 450 nm emission wavelengths, at the beginning and after 24 hours of incubation close to *in situ* temperature conditions. Activity was derived from the increase in fluorescence in each well over time, using a standard curve prepared with the fluorophores 4-methylumbelliferone (MUF) or 4-methylcoumarinyl-7-amide 4 (Sigma-Aldrich).

Single cell activity. Single-cell activity was estimated using the technique Bioorthogonal non-canonical amino acid tagging (BONCAT), following the protocol detailed in Leizeaga et al. (2017). This is a promising technique for detecting and quantifying translationally active bacteria in the environment. Two 10 mL replicates were incubated with L-homopropargylglycine (HPG) (100 nM final concentration). After the incubation, samples were fixed with 0.2 µm-filtered paraformaldehyde [final concentration 1% (v/v)]. Killed controls (samples fixed before the HPG addition) were included with all sets of samples to correct for

background fluorescence from naturally occurring azides. The samples were then gently filtered through a 0.2 µm pore size polycarbonate filter, washed with sterile milliQ water, and kept frozen at −80◦C until further processing. Once in the lab, dfter thawing, attachment of the cells to the filters, permeabilization and and click chemistry was performed as detailed in Leizeaga et al. (2017).

5.2.2 Preliminary results

Counts of heterotrophic bacteria. Cell abundance was measured *in situ* at the surface microlayer (SML), 20 cm and 1m below at three different samplings (BFL2, BFL3, BFL5). Overall, the three depths sampled showed no remarkable differences in the abundance of heterotrophic bacteria Figure 5.3a. This observation was maintained in the onset (time 0) of the three mesocosm experiments (Figure 5.3b-c). While in BFL1, an increase in cell abundance was observed overtime, the opposite trend occurred in BFL4 and BFL6 incubations. In any case, no clear differences in cell abundance were observed among the different light treatments (Dark, UV+ and UV-).

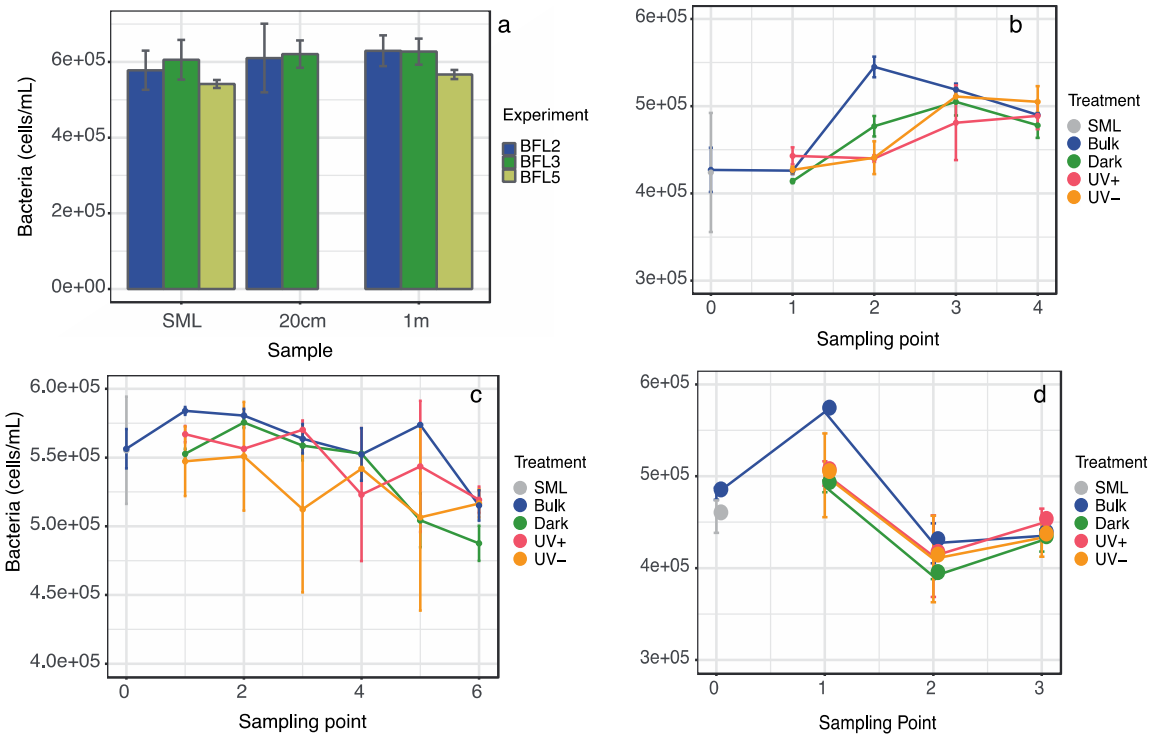


Figure 5.3: Heterotrophic bacterial counts in the three *in situ* samplings conducted (a) and in the three mesocosm experiments (b: BFL1, c: BFL4, d: BFL6). Bars represent the standard deviation. Panel a shows data from the SML as well as 20 cm and 1m below the SML. SML: Surface microlayer; Bulk: 1m below SML; Dark: mesocosms under dark; UV+: mesocosms under light with UV radiation; UV-: mesocosms under light without UV radiation

Extracellular enzyme activities. We focused on the activities of ß-glucosidase (GLU), which is involved in the utilization of dimers of cellulose, chitobiase (CHI) for the degradation of chitin, leucyl aminopeptidase (AMA), which degrades the proteinaceous components of DOM, and alkaline phosphatase (APA), involved in the hydrolysis of organic phosphoesters. All 4 activities were measured both in the *in situ* sampling and the three mesocosms incubations.

Figure 5.4 shows the results of the 4 enzymes in the *in situ* samplings. Results show clear differences in activity between the SML and the underwater samplings for BFL5, while the differences were less remarkable for BFL3, while the activities seemed comparable in BFL2.

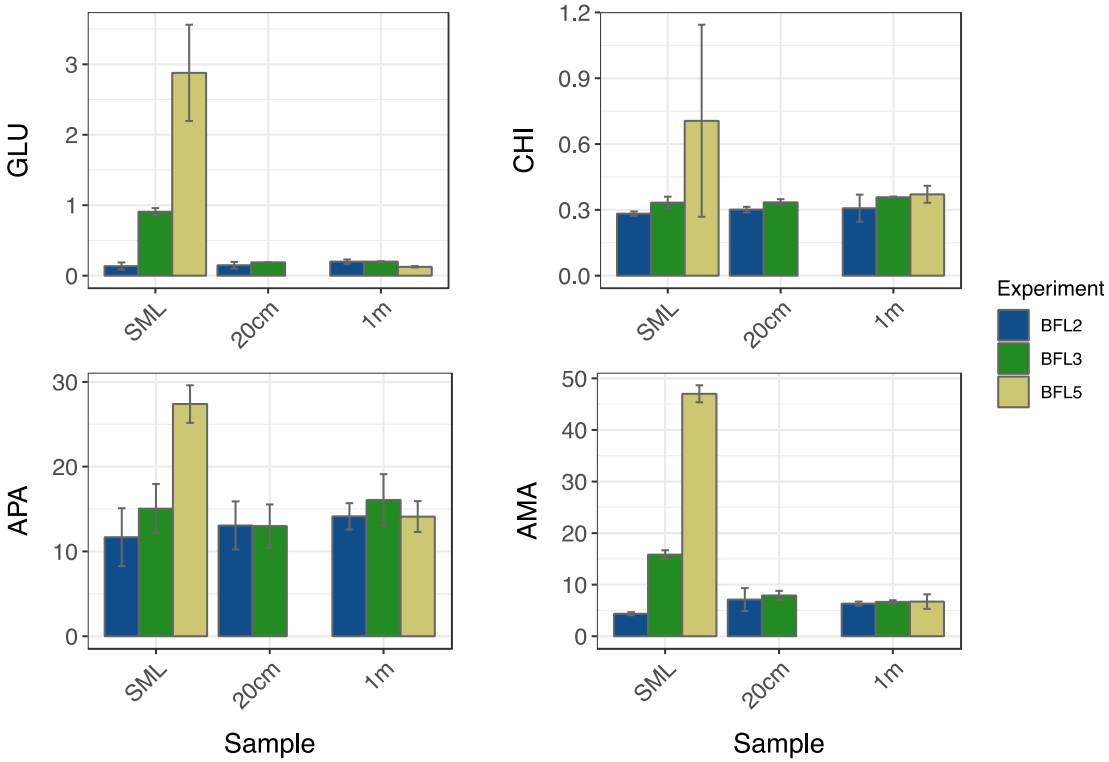


Figure 5.4: Extracellular enzymatic activities in the three *in situ* samplings. Activity units are shown in nmol · L^{-1} h^{-1}.

Regarding the incubation experiments, in which various time points were measured, differences between SML and Bulk samples as well as among light treatments were varying depending on the mesocosm experiment and the enzyme measured, as shown in Figures 5.5, 5.6 and 5.7.

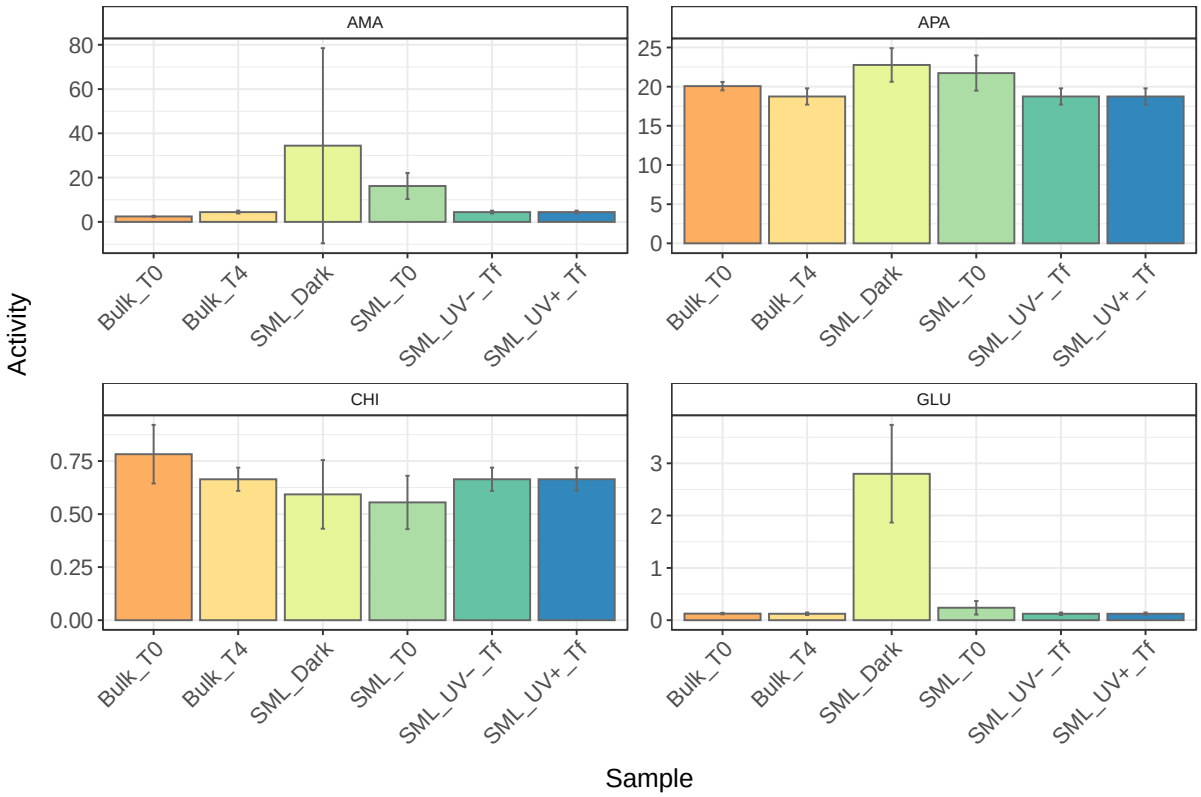


Figure 5.5: Extracellular enzymatic activities in the BFL1 mesocosm study. Activity units are shown in nmol · L^{-1} h^{-1}.

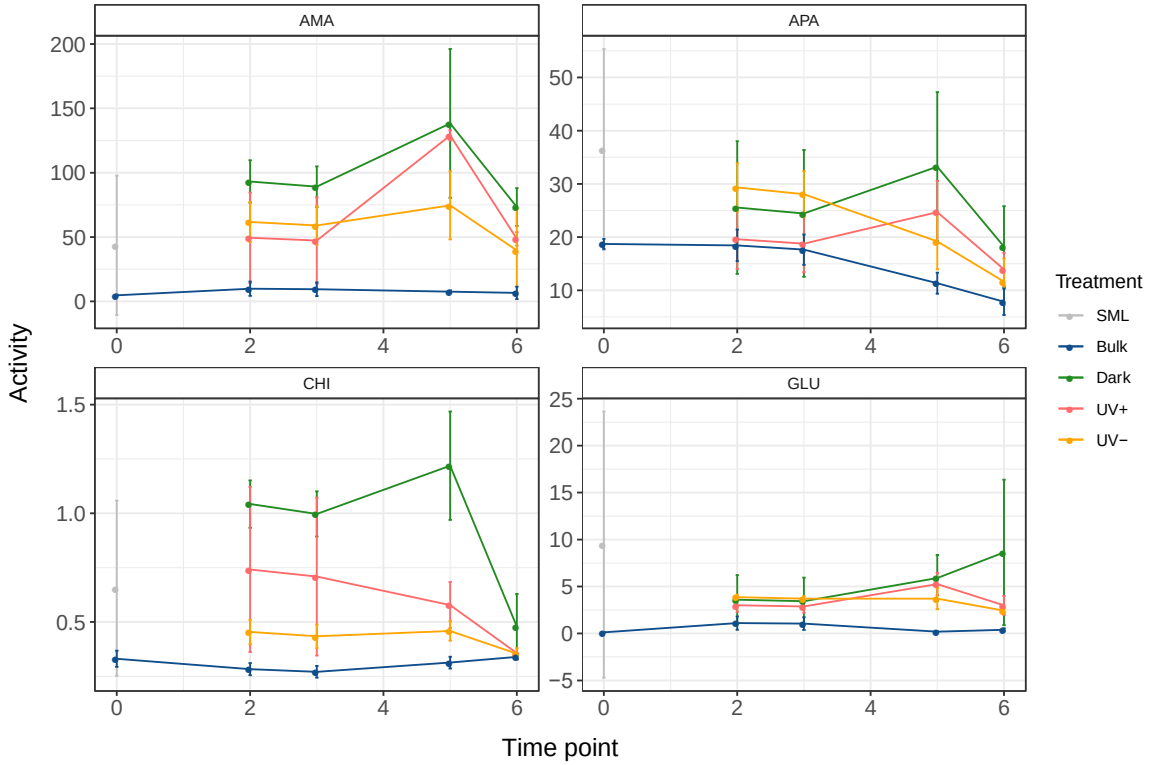


Figure 5.6: Extracellular enzymatic activities in the BFL4 mesocosm study. Activity units are shown in nmol · L^{-1} h^{-1}.

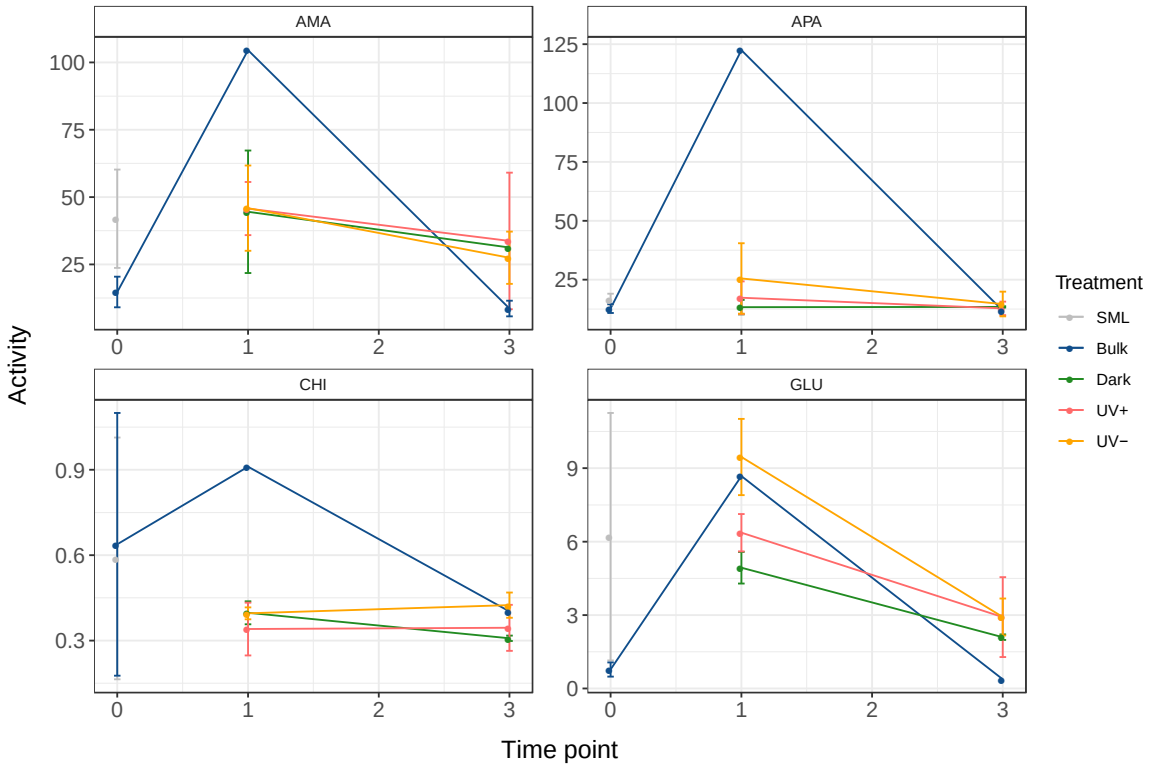


Figure 5.7: Extracellular enzymatic activities in the BFL6 mesocosm study. Activity units are shown in nmol · L^{-1} h^{-1}.

5.3 Incubation experiment I (Phytoplankton)

(Nur Ili Hamizah Mustaffa†, Anna Lena Heinrichs†, Laura Hennigs and Maren Striebel)
†both authors contributed equally to this work

5.3.1 Methodologies

We conducted on-board an incubation experiment from September 16 to October 4, 2019. The seawater samples from 10 different depths (i.e., 5 m, 8 m, 12 m, 15 m, 25 m, 35 m, 45 m, 60 m, 70 m, and 80 m) were collected on September 18, 2019 (39°14’ 54’N, 001° 19’ 71’ E) using rinsed 10 L Niskin bottles mounted on a rosette. The collected samples were prefiltered through a 200 µm mesh nylon screen to remove zooplankton. Nitrogen and phosphorus (concentrations based on f/2 growth medium, Guillard and Lorenzen 1972) were added before transferred into 200 ml cell cultures flask (T-75 CytoOne®) to avoid nutrient limitation. The samples were incubated under four different light spectra foils (LEE color filtres), i.e., control (425-770nm), blue (430-530nm), red (600-750nm) and green (425-600nm). Light intensity was reduced to a mean of 43.7% of ambient light using the foils but was assured to be the same for all treatments (sd=0.45% intensity). Every treatment was replicated five times resulting 200 bottles in total. During the experiment, the cell flasks were placed in boxes covered with the respective light foil and filled with pumped through seawater to maintain the temperature. Temperature and light were monitored continuously using data loggers (HOBO Pendant ®, Onset, Bourne, MA, USA). The average temperature in the boxes conducted 23.6°C (sd=0.95°C). The bottles were gently shaken daily. The optical density (OD) of each bottle were measured every second day of the experiment using a custom-made device as a proxy for biomass (Frank et al. 2020; Mustaffa et al. 2020). All treatments were sampled after day 13. Samples for pigment analyses were obtained by filtering 150mL samples onto acid-washed and pre-combusted glass

microfiber filter (Whatman GF/C, UK) and determined using the spectral deconvolution method as described in Thrane et al. (2015).

Statistical analyses
All statistical analyses and graphs were done using R studio 3.6.2 (R Core Team 2019). The growth rate (μ) was calculated with the following equation:

$$\mu = \frac{\ln(\mathrm{N\,t1}) - \ln(\mathrm{Nt0})}{\Delta t}$$

where Nt_0 is the optical density at day 7, Nt_1 is the optical density of cells at the end of the experiment (day 13) and Δt is the difference in days. The Shannon Index (H') was calculated using package '*vegan*' using the following equation:

$$H' = -\sum_{i} \mathrm{pi} * \ln \mathrm{pi}$$

where p_i is the proportion of individuals belonging to the *i*th species ($\mathrm{pi} = \frac{\mathrm{ni}}{\mathrm{N}}$) and N the sum of species. The effects of light treatment and water depth on growth, pigment concentration and Shannon diversity index were analyzed using a linear mixed model and a simple linear model followed by ANOVA. The growth rate was log-transformed for the analysis (Table 5.1).

Table *5.1*: ANOVA of light spectra and depths effect on the linear growth rate (R), total pigment concentration, Shannon Index of pigment diversity (H').

		Log R		*Total Pigment*		*H' Pigment*	
Effect	Df	*F*	*P*	*F*	*P*	*F*	*P*
Light Treatment	3	44.2	<.0001*	148.7	<.0001*	133.5	<.0001*
Depth	9	1.6	0.129	12.5	<.0001*	4.91	<.0001*
Depth x Light	27	1.7	0.033*	2.7	<.0001*	7.8	<.0001*

Note*: R was log-transformed for the analyses*

5.3.2 Preliminary Results

Different light treatments affect significantly the growth rate of phytoplankton (F=28.11, p<0.001). Phytoplankton incubated under blue light treatment showed the highest growth rate, meanwhile phytoplankton grew under green light had the lowest growth rates (Fig. 5.8A). However, growth rate was not significantly different between control and red light treatments (p>0.5). Although the effect of light treatment was the same at most water depth, we found significant interaction between water depth and light treatment (F=1.67, p=0.03).

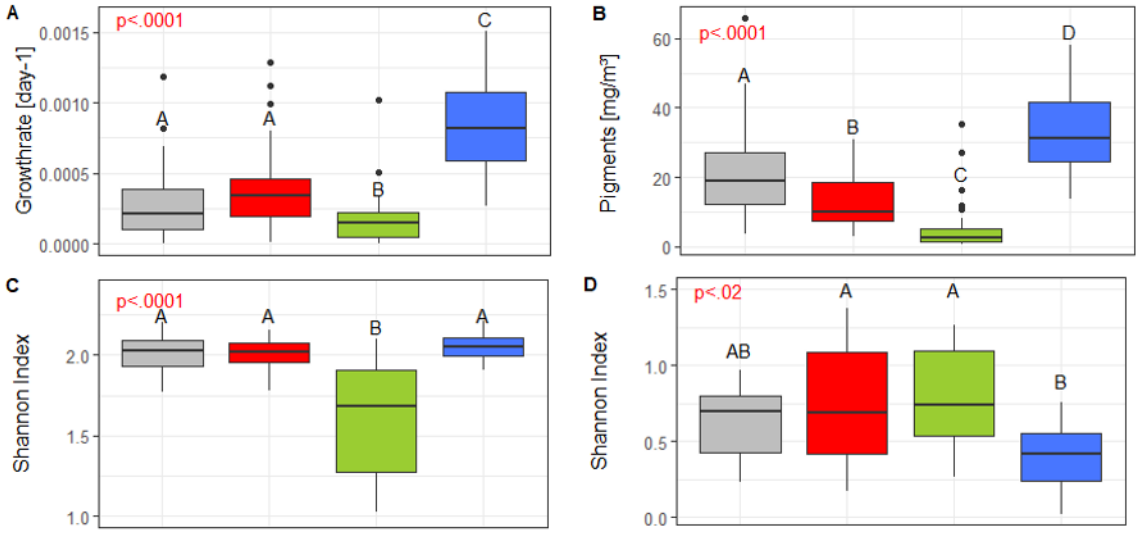


Figure 5.8: Effect of different light treatment considering from all water depths together on (A) growth rate, (B) total pigment concentration, (C) Shannon Index of phytoplankton pigments, (D) Shannon Index of phytoplankton community composition. Colors represents light treatments, grey indicates control.

Considering samples from all depths, total pigment concentration was significantly affected by the light treatments ($F_{1334.6}$=148.71, p<0.0001). Phytoplankton under blue light treatment showed the highest total pigment concentration, followed by phytoplankton which grew under control light (Figure 5.8B). By contrast, the lowest total pigment content was observed in the green light treatment (Fig. 5.8B). Furthermore, we found a significant interaction between water depth and light treatments (Table 5.1). Total pigment concentration of the communities grown under blue light tend to increase with increasing water depth and showed the highest total pigment concentration in 80 m and the lowest in 5 m. Meanwhile, phytoplankton which grew under red and green light showed a unimodal trend with increasing depth (Fig. 5.9).

Pigment diversity and composition

The Shannon index of the pigment diversity was significantly affected by the light treatments ($F_{43,361}$=133.48, p<0.0001), water depth ($F_{43,361}$=4.91, p<.0001) and their interaction ($F_{43,361}$= 2.7, p<.0001) (Figure 5.8C, 17C)). In general, diversity was significantly lower in samples grown under green light treatment (p<0.0001) (Fig. 5.8C). Samples under green light treatment showed the most differences between the depths ($F_{2592.8}$=8.9, p<0.0001), while the pigment diversity under other lighting conditions showed little or no significant differences between the depths ($p_{blue+red}$>0.05, $p_{control}$=0.001) (Fig. 5.8C). The highest pigment diversity and total pigment concentrations were observed in the samples collected from 25 m under green light treatment (Fig. 5.9B, C).

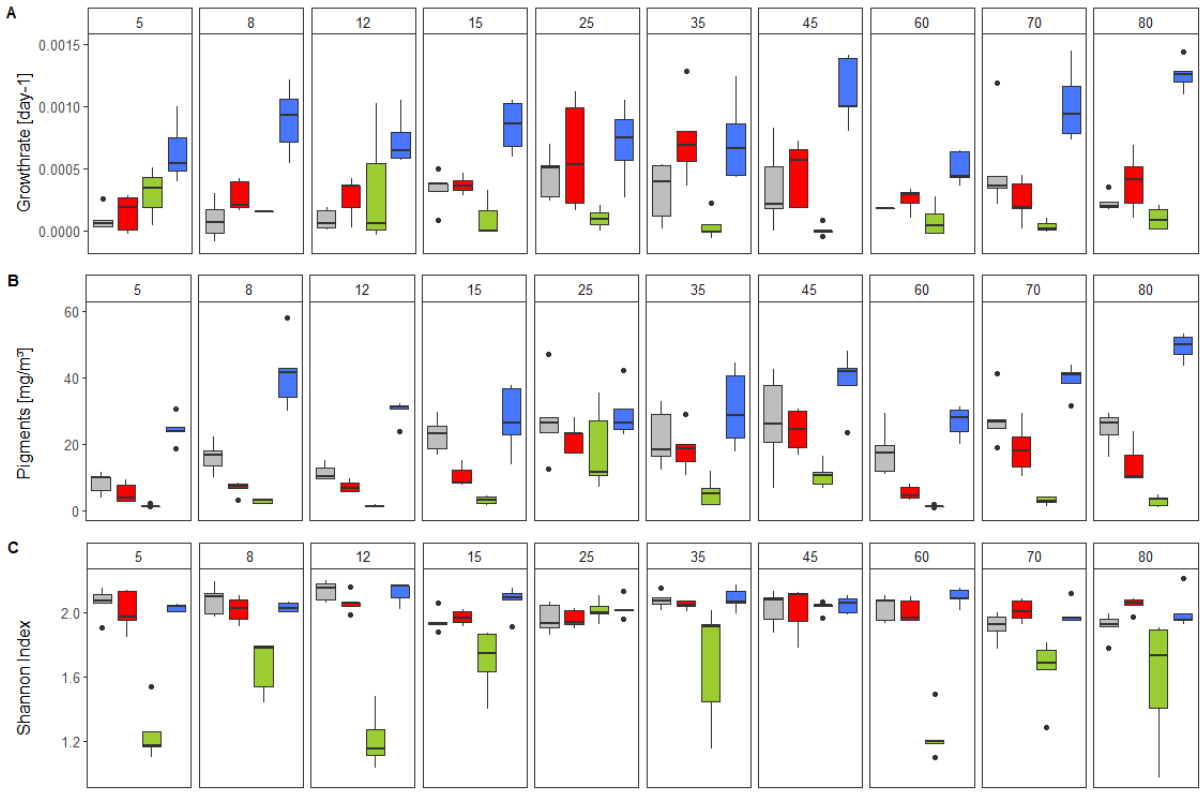


Figure 5.9: Effect of different light treatments and water depth. (A) Linear growth rate (µ), (B) Total pigment concentration, (C) Shannon Index of pigment diversity. Grids represent the water depth [m] and colours represent light treatments, grey indicates control.

In conclusion, our experiment demonstrate that different light sources lead to changes in community growth rate, pigment composition and content. Although our results showed an interaction of light and water depth, our expectation, that communities from different water depths show different adaptions to the light treatments in terms of their growth, pigment composition and content could not be confirmed. In all water depths, the blue light benefits the growth and pigment content most and green light less. Investigations on the effect of light sources on the community abundances and composition are still in progress.

5.4 Incubation experiment II (Zooplankton)

(R. Gonçalves)

5.4.1 Methodologies

The overal methodology consisted in an onboard incubation of zooplankton (~24 h) in surface (SW) and bulk (BW) seawater, under two light treatments: with (UV+) and without (UV-) solar ultraviolet radiation. Surface seawater was collected in plastic containers from the deck. This included water from the first 1-10 cm of the surface layer, which is the usual range of the studied copepods. Bulk water was collected from 1-2 m depth sampling using Niskin bottles. The surface and bulk water are referred herein to as SW and BW, respectively. Zooplankton was collected (date) from surface waters using a manta net (300 µm pore size). Samples were taken to analyse zooplankton community composition and abundance. The target group was pontellid copepods which live associated to the surface layer. This were removed from the sample and sorted out in the laboratory. Healthy, moving individuals were gently picked one by one using wide-mouth plastic pipetes and put into incubation bottles. Samples of SW and BW were used for measurements of initial conditions. The copepods were put in groups of 5 individuals into each of 24 units of 1-L plastic (HDPE) bottles (Cubitainers, ThermoFisher). The bottles were

placed on deck inside a running-water bath to keep a stable temperature. Temperature inside the water bath was monitored with HOBO sensors. Half of the bottles were covered with plastic film, which blocks solar ultraviolet radiation (UVR, wavelenghts between 280 and 400 nm).

Using the above setup, 2 factors (water origin and light conditions) were configured, each one with 2 levels (SW vs BW, UV+ vs UV-, respectively). Three replicates were used for all combinations, and initial measurements were done before starting the incubation. Thus, the overall treatments layout was a 2 x 2 factorial design, as shown in the following figure 5.10. Initial and end times are denoted by To and Tf. The labels such as ZS1, B1, etc. are to reference individual replicates. The bottles under the UV- treatment had a plastic film covering the incubation container, so these replicates did not receive UVR (but did receive visible light). Bottles under UV+ received the full spectrum of solar radiation at all times. Bottles were gently moved periodically to avoid settlement of particles. After the incubation period, copepods were gently removed (ie individually picked using a plastic pipette) from each bottle, checked for mortality and frozen for further analysis. The water from the corresponding bottle was then used for the rest of the analysis, as detailed below.

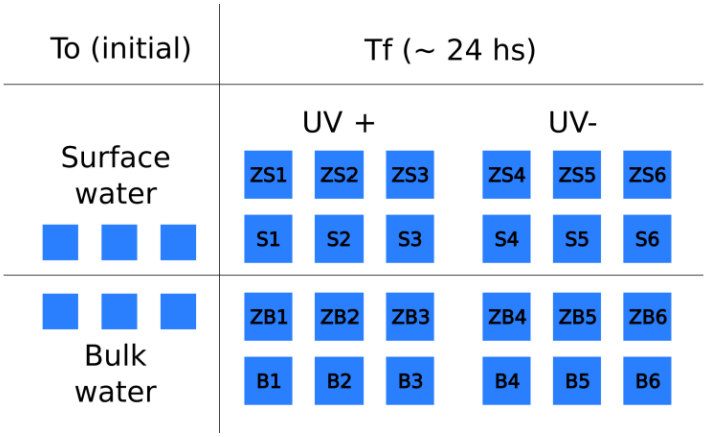


Figure 5.10: Treatments as a 2x2 factorial design with To and Tf as initial and end times. S indicates surface samples, B bulk water samples, and Z treatments with zooplankton.

A water (both BW and SW) sample from the inital conditions was fixed with Lugol‘s solution to analyse phytoplankton community abundance and composition. After incubation, survival of copepods was calculated by counting dead animals in each bottle. Zooplankton samples were taken for pigment analysis. Between 2 and 20 individuals (depending on the species size) were put into paper filters and kept at -20. In the lab, the frozen filter containing the individuals was put into plastic tubes with pure methanol and sonicated to disrupt tissues and cells. Then each tube was vortexed and kept overnight in the fridge. The content of the tubes was then pured into quartz cubettes and an absorbance spectra (wavelenghts between 300 and 700 nm) was obtained. This was done for zooplankton before and after incubations. A sample from each incubation bottle was taken for the following measurements: a) flow cytomery: 1-2 mL were preserved into Eppendorf tubes using glutharaldehide and run in a flow cytometer to estimate bacterial and picoeukariote abundances. b) optical density: (as a proxy for chl-a abundance): 10 mL were put into vials and *in vivo* optical density (as absorbance) was measured onboard with a scaning spectrophotometer (wavelengths betwen 190 and 750 nm). In addition, sub samples for pigments (for Chl-a and phaeopigments), dissolved organic carbon (DOC), total dissolved

nitrogen (TDN) and transparent exopolimeric partcles (TEP) were taken. The ambient solar radiation was monitored using a radiometer (TriOS irradiance sensor) installed on the top deck.

5.4.2 Preliminary results

Zooplankton survival: Less than 5% mortality in all replicates occurred. Analysis of zooplankton pigments and taxonomy (counts and identification) are still in progress.

Phytoplankton composition: Samples have been analysed and its taxonomic composition is listed in the following table 5.2:

Table 5.2. Phytoplankton composition in incubated sea water samples.

Scientific name	Mean (cells/L)	Median	Std. dev.	Min	Max
Bacteriastrum	1125	1125	375	750	1500
Ceratium furca	40	40	0	40	40
Ceratium fusus	20	20	0	20	20
Ceratium lineatum	20	20	0	20	20
Chaetoceros	1437.5	1500	872.765002735558	250	2500
Ciliophora	50	50	30	20	80
Cylindrotheca closterium	1875	1875	375	1500	2250
Dinophyceae	6204.33333333333	2000	8664.48732214177	250	24860
Dinophysis	625	625	375	250	1000
Guinardia striata	500	500	250	250	750
Gyrodinium	250	250	0	250	250
Hemiaulus	500	500	0	500	500
Leptocylindrus danicus	1125	1125	125	1000	1250
Lohmanniella oviformis	250	250	0	250	250
Pennales	416.666666666666	250	235.702260395515	250	750
Prorocentrum	250	250	0	250	250
Protoperidinium	20	20	0	20	20
Pseudo-nitzschia	1250	1250	0	1250	1250
Rhizosolenia	250	250	0	250	250
Strombidiidae	50	50	10	40	60
Thalassionema	20	20	0	20	20
Flagellates species incertae sedis	47692	52480	21077.7934328999	11050	71810

Flow cytometry: Bacterial densities are plotted in Figure 5.11. Pico eukaryotes are still being analysed.

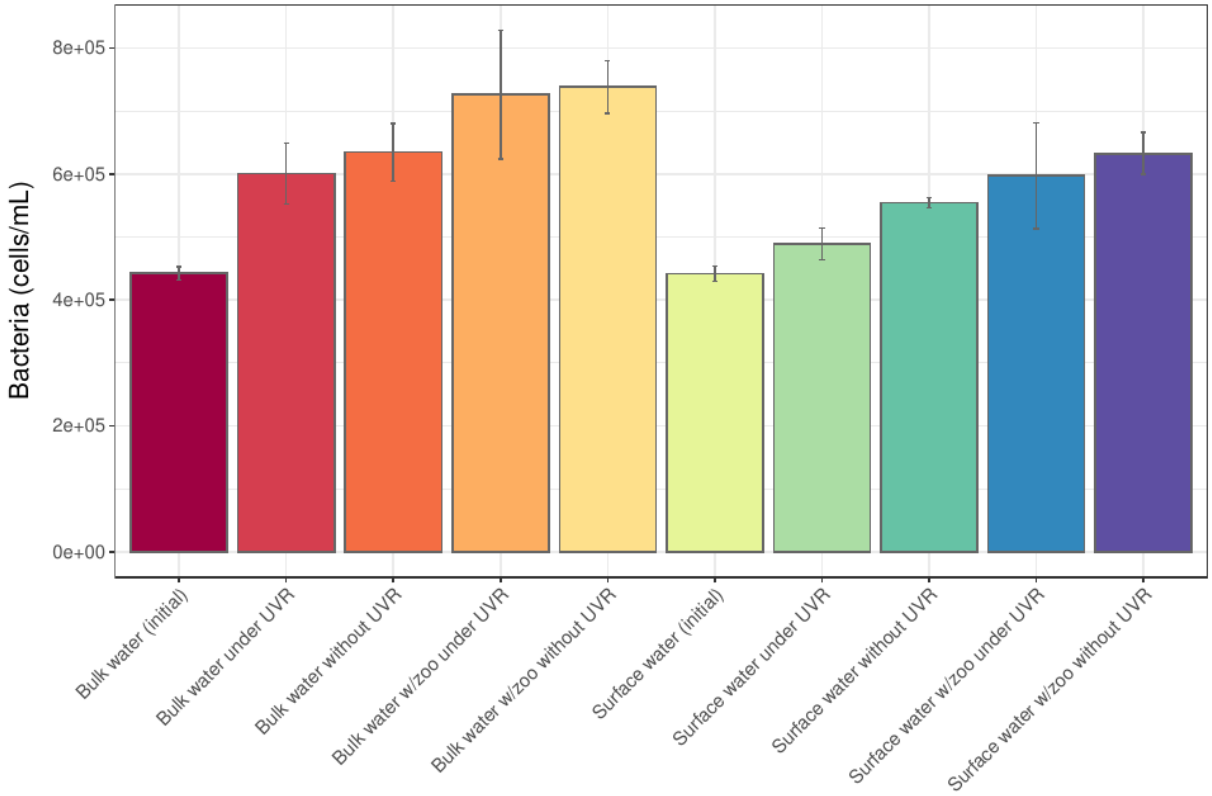


Figure 5.11: Bacterial densities in incubated bulk and surface water samples with (w/zoo) and without zooplankton, and under UV and without UV radiation.

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) samples have been analysed and are shown in Figure 5.12.

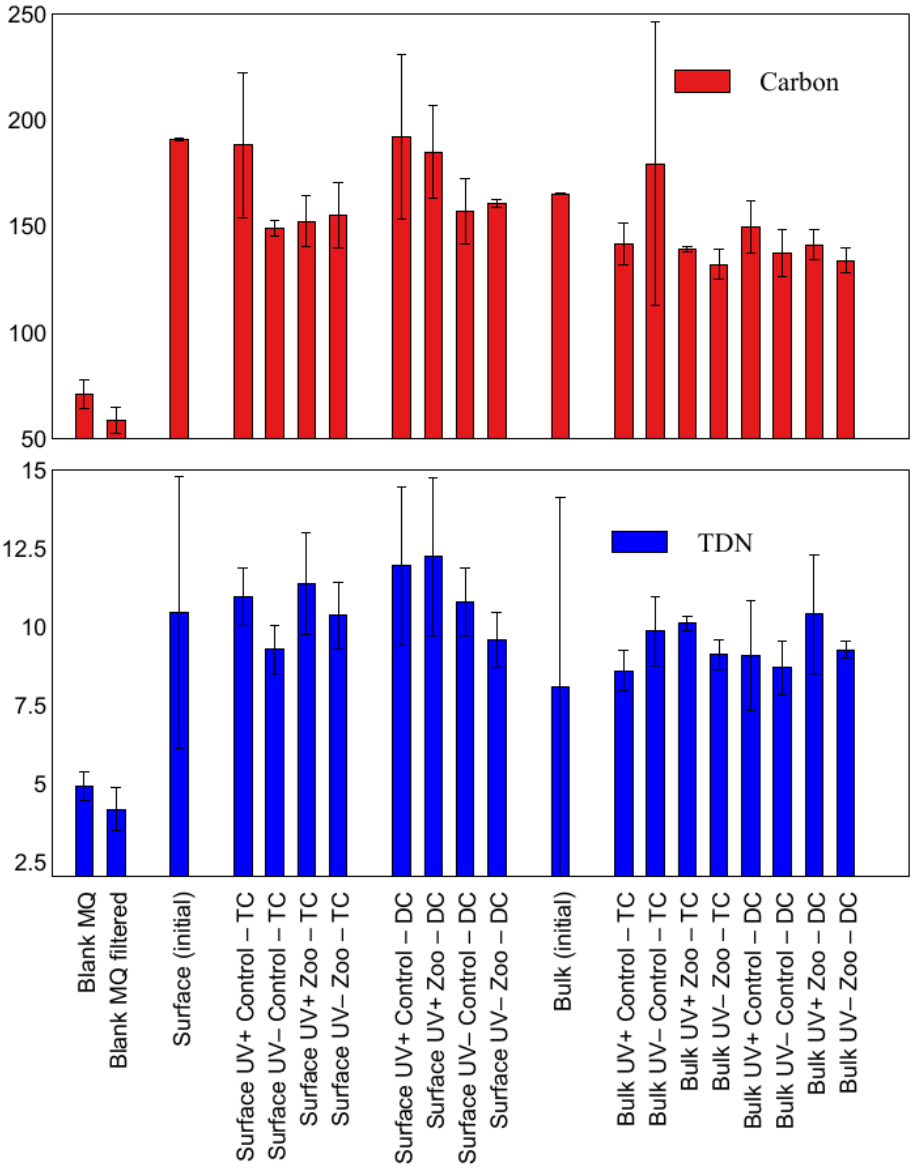


Figure 5.12: Total dissolved carbon and nitrogen for the zooplankton incubation experiment.

5.5 Expected Results

(Shipboard Scientific Party)

TEP samples will be extracted and analyzed via spectrophotometric method in the next few months. Current data on all parameters will be further analyzed for all experiments in addition to BFL4 which is shown here. Comparison to other parameters will also be conducted once all samples have been run. Data from the TrioS Radiometer will be evaluated for the interpretation of the preliminary data. Photosynthetic picoplankton will be enumerated by flow cytometry in the next weeks. Inorganic nutrients will be also processed soon. Likewise, BONCAT data from BFL4 will be available before the end of the year. All data will be further analysed and compared with other variables measured. Moreover, a selection of samples will be processed for genomic analyses in order to determine the diversity of microbial communities in the *in situ* samplings and mesocosms experiments. For the zooplankton incubation experiment, optical

density, transparent exopolimeric particles (TEP) and pigments of post-incubation samples have been collected, but currently subjected for analysis.

With the outstanding completion of analysis, mainly delayed through restricted work in laboratories due to Covid19 pandemic, we expect a comprehensive biogeochemical data set. Analysis of radiance data will be completed in the next few months and being linked the biogeochemical observations. However, weather conditions did not allow for the planned mesocosms studies over a time period of 3 days, and we may have a more limited time frame. In addition, missing meteorological data (DShip system was not running throughout POS537) limits the evaluation of the data in respect to atmospheric condition and sea states.

6 Ship's Meteorological Station

Not applicable

7 Station List POS537

7.1 Overall Station List

Station No.		Date	Gear	Time	Latitude	Longitude	Water Depth	Remarks/Recovery
Poseidon		2020		[UTC]			[m]	
POS537_1-1		18.9.	ROS/CTD	06:28	39.24283	1.33062	596	Collected seawater samples for incubation experiment
POS537_2-1		19.9	Plankton net	06:13	41.30184	3.64083	2360	Collection of zooplankton
POS537_2-2		19.9	Plankton net	06:22	41.30189	3.64174	2360	Collection of zooplankton
POS537_2-3		19.9	Plankton net	06:31	41.30170	3.64286	2360	Collection of zooplankton
POS537_2-4		19.9	Plankton net	06:37	41.30180	3.64397	2366	Collection of zooplankton
POS537_2-5		19.9	Manta trawl	06:52	41.30265	3.64785	2365	Collection of surface-dwelling zooplankton
POS537_2-6	BFL1	19.9	Mesocosm	07:52	41.30214	3.63991	2360	First mesocosm experiment. Recovered 21.09
POS537_3-1		19.9	Manta trawl	13:24	41.28443	3.64298	2321	Collection of surface-dwelling zooplankton
POS537_4-1		20.9	ROS/CTD	06:00	41.31446	3.66630	2378	Collection of two samples at 100 m for micro plastic analysis (I. Ferrera)
POS537_5-1	BFL2	24.9	Small boat	06:16	41.30232	3.64046	2358	Collection of natural SML and bulk water samples
POS537_5-2		24.9	Manta trawl	07:49	41.30950	3.651321	2372	Collection of surface-dwelling zooplankton
POS537_5-3	BFL3	24.9	Small boat	12:09	41.29604	3.65579		Collection of natural SML and bulk water samples
POS537_6-1	BFL4	26.9	Mesocosm	06:23	41.30248	3.63559	2355	Second mesocosm experiment. Recovered 28.09
POS537_7-1		26.9	Manta trawl	10:46	41.41648	3.52261	2095	Collection of surface-dwelling zooplankton for incubation experiment
POS537_8-1		26.9	Manta trawl	12:36	41.34613	3.52261	-1975	Collection of surface-dwelling zooplankton for incubation experiment

POS537_9-1		27.9	ROS/CTD	10:46	41.41648	3.78230	2303	Collection of seawater samples for zooplankton incubation experiment
POS537_10-1	BFL 5 /BFL6	29.9	Small boat (BFL5) , and mesocosm (BFL6 on following day)	12:32	41.30382	3.64112	2360	Collection of natural SML and bulk water samples (BFL5) and third mesocosm experiment (BFL6, 30.09 to 01.10.2019)

7.2 Sample Station List for Mesocosms

Station No.	Sample Station No.	Date	Time UTC	Latitude	Longitude	Water Depth (0 = SML)	Parameter	Sampling remarks
Poseidon		**2019**	**h**	**Represents ship's position at give date and time**		**[m]**		
POS537_2-6	BFL1-T0_0	20.9.	07:00	41.305373	3.67555	0	DNA, flow cytometry, nutrients, enzyme activity, TEP, chlorophyll-a	From small boat outside mesocosms
POS537_2-6	BFL1-T0_1	20.9.	07:00	41.305373	3.67555	1	DNA, flow cytometry, nutrients, enzyme activity, TEP, chlorophyll-a	From small boat outside mesocosms
POS537_2-6	BFL1-T1_0	20.9.	08:30	41.309258	3.672893	0	Flow cytometry, TEP	all six mesocosms
POS537_2-6	BFL1-T1_1	20.9.	08:30	41.309258	3.672893	1	Flow cytometry, TEP	Duplicated bulk water
POS537_2-6	BFL1-T2_0	20.9.	11:30	41.326857	3.666612	0	Flow cytometry, TEP, chlorophyll-a	all six mesocosms
POS537_2-6	BFL1-T2_1	20.9.	11:30	41.326857	3.666612	1	Flow cytometry, TEP, chlorophyll-a	Duplicated bulk water
POS537_2-6	BFL1-T3_0	20.9.	14:30	41.345553	3.679307	0	Flow cytometry, TEP, chlorophyll-a	all six mesocosms
POS537_2-6	BFL1-T3_1	20.9.	14:30	41.345553	3.679307	1	Flow cytometry, TEP, chlorophyll-a	Duplicated bulk water
POS537_2-6	BFL1-T4_0	20.9.	17:00	41.36636	3.687527	0	DNA, flow cytometry, nutrients, enzyme activity, TEP, chlorophyll-a	all six mesocosms
POS537_2-6	BFL1-T4_1	20.9.	17:00	41.36636	3.687527	1	DNA, flow cytometry, nutrients, enzyme activity, TEP, chlorophyll-a	Duplicated bulk water
POS537_5-1	BFL2_0	24.9	08:00	41.311778	3.638405	0	RNA, flow cytometry, nutrients, enzymes, TEP, optical density	From small boat; natural condition, i.e., no mesocosms
POS537_5-1	BFL2_0.2	24.9	08:00	41.311778	3.638405	0.2	RNA, flow cytometry, nutrients,	From small boat; natural condition,

							enzymes, TEP, optical density	i.e., no mesocosms
POS537_5-1	BFL2_1	24.9	08:00	41.311778	3.638405	1	RNA, flow cytometry, nutrients, enzymes, TEP, optical density	From small boat; natural condition, i.e., no mesocosms
POS537_5-3	BFL3_0	24.9	12:15	41.296593	3.647783	0	Boncat-FISH-AAPs, flow cytometry, nutrients, enzymes, TEP, optical density	From small boat; natural condition, i.e., no mesocosms
POS537_5-3	BFL3_0.2	24.9	12:15	41.296593	3.647783	0.2	Boncat-FISH-AAPs, flow cytometry, nutrients, enzymes, TEP, optical density	From small boat; natural condition, i.e., no mesocosms
POS537_5-3	BFL3_1	24.9	12:15	41.296593	3.647783	1	Boncat-FISH-AAPs, flow cytometry, nutrients, enzymes, TEP, optical density	From small boat; natural condition, i.e., no mesocosms
POS537_6-1	BFL4_T0_0	26.9	07:00	41.301235	3.625682	0	DNA, flow cytometry, nutrients, enzymes, Boncat-FISH-AAPs, cultures, TEP optical density, Biolog assay, chlorophyll-a	From small boat outside mesocosms
POS537_6-1	BFL4_T0_1	26.9	07:00	41.301235	3.625682	1	DNA, flow cytometry, nutrients, enzymes, Boncat-FISH-AAPs, cultures, , TEP optical density, Biolog assay, chlorophyll-a	From small boat outside mesocosms
POS537_6-1	BFL4_T1_0	26.9	10:00	41.31418	3.587018	0	Flow cytometry, TEP optical density, Biolog assay, chlorophyll-a	all six mesocosms
POS537_6-1	BFL4_T1_1	26.9	10:00	41.31418	3.587018	1	Flow cytometry, TEP optical density, Biolog assay, chlorophyll-a	, TEP optical density, Biolog assay, chlorophyll-a Duplicated bulk water
POS537_6-1	BFL4_T2_0	26.9	14:00	41.380733	3.612713	0	Flow cytometry, enzymes, Boncat-FISH-AAPs, , TEP optical density, Biolog assay, chlorophyll-a	all six mesocosms
POS537_6-1	BFL4_T2_1	26.9	14:00	41.380733	3.612713	1	Flow cytometry, enzymes, Boncat-FISH-AAPs, , TEP optical density,	Duplicated bulk water

							Biolog assay, chlorophyll-a	
POS537_6-1	BFL4_T3_0	27.9	06:30	41.39097	3.713615	0	DNA, flow cytometry, enzymes, , TEP optical density, Biolog assay, chlorophyll-a	all six mesocosms
POS537_6-1	BFL4_T3_1	27.9	06:30	41.39097	3.713615	1	DNA, flow cytometry, enzymes, , TEP optical density, Biolog assay, chlorophyll-a	Duplicated bulk water
POS537_6-1	BFL4_T4_0	27.9	10:00	41.41475	3.77045	0	Flow cytometry, , TEP optical density, Biolog assay, chlorophyll-a	all six mesocosms
POS537_6-1	BFL4_T4_1	27.9	10:00	41.41475	3.77045	1	Flow cytometry, , TEP optical density, Biolog assay, chlorophyll-a	Duplicated bulk water
POS537_6-1	BFL4_T5_0	27.9	17:00	41.401505	3.84913	0	DNA, flow cytometry, enzymes, TEP, optical density	all six mesocosms
POS537_6-1	BFL4_T5_1	27.9	17:00	41.401505	3.84913	1	DNA, flow cytometry, enzymes, TEP, optical density	Duplicated bulk water
POS537_6-1	BFL4_T6_0	28.9	17:00	41.399177	3.963255	0	DNA, flow cytometry, nutrients, enzymes, Boncat-FISH-AAPs, cultures, TEP, optical density	all six mesocosms
POS537_6-1	BFL4_T6_1	28.9	17:00	41.399177	3.963255	1	DNA, flow cytometry, nutrients, enzymes, Boncat-FISH-AAPs, cultures, , TEP, optical density	Duplicated bulk water
POS537_10-1	BFL5_0	29.09	13:00	41.308738	3.651882	0	RNA, flow cytometry, enzymes, , TEP, optical density	From small boat; natural condition, i.e., no mesocosms
POS537_10-1	BFL5_0	29.09	13:00	41.308738	3.651882	1	RNA, flow cytometry, enzymes, , TEP, optical density	From small boat; natural condition, i.e., no mesocosms
POS537_10-1	BFL6_t0_0	30.09	11:00	41.264558	3.635798	0	DNA, flow cytometry, enzymes, Boncat-FISH-AAPs, , TEP, optical density	From small boat outside mesocosms
POS537_10-1	BFL6_t0_0.2	30.09	11:00	41.264558	3.635798	0.2	DNA, flow cytometry, enzymes, Bocat-FISH-AAPs, ,	From small boat outside mesocosms

							TEP, optical density	
POS537_10-1	BFL6_t0_1	30.09	11:00	41.264558	3.635798	1	DNA, flow cytometry, enzymes, Boncat-FISH-AAPs, , TEP, optical density	From small boat outside mesocosms
POS537_10-1	BFL6_t1_0	30.09	15:00	41.25704	3.587518	0	Flow cytometry, nutrients, , TEP, optical density	all six mesocosms
POS537_10-1	BFL6_t1_1	30.09	15:00	41.25704	3.587518	1	Flow cytometry, nutrients, , TEP, optical density	Duplicated bulk water
POS537_10-1	BFL6_t2_0	01.10	06:00	41.282297	3.560958	0	Flow cytometry, , TEP, optical density	all six mesocosms
POS537_10-1	BFL6_t2_1	01.10	06:00	41.282297	3.560958	1	Flow cytometry, , TEP, optical density	Duplicated bulk water
POS537_10-1	BFL6_t3_0	01.10	10:30	41.303327	3.546718	0	DNA, flow cytometry, enzymes, Boncat-FISH-AAPs, , TEP, optical density	all six mesocosms
POS537_10-1	BFL6_t3_1	01.10	10:30	41.303327	3.546718	1	DNA, flow cytometry, enzymes, Boncat-FISH-AAPs, , TEP, optical density	Duplicated bulk water

8 Data and Sample Storage and Availability

Datasets will be transferred into the PANGAEA data center (Table 8.1) and released for every user by the time of publication in a journal. All data from subsequent data analyses in the laboratories will be stored in PANGAEA within 24 months' time. Public access will be activated by the time of publication.

Table 8.1 Overview of data availability

Type	Database	Available	Free Access	Contact
		Date	Date	E-Mail
Inorganic nutrients	PANGAEA	Dec 2021	Upon availability	Isabel.ferrera@ieo.es
Bacterial abundance	PANGAEA	Already	Upon publication	Isabel.ferrera@ieo.es
Zooplankton incubations	PANGAEA	Dec 2022	Upon publication	patagoniaplankton@gmail.com
Phytoplankton abundance and optical densities	PANGAEA	Dec 2021	Upon publication	nur.ili.hamizah.mustaffa@uni-oldenburg.de
TEP, Optical Density, Biolog Plate	PANGAEA	Dec 2021	Upon publication	Tiera-brandy.robinson@uol.de
Radiometer, Light and Temperature sensor	PANGAEA	Dec 2021	Upon publication	Tiera-brandy.robinson@uol.de

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