

R/V Heincke (HE492) Report

**POLFJORD: Contrasting plankton diversity and interactions with organic matter flux in Arctic fjord systems subject differential climatic forcing mechanisms along the coast of Svalbard**

Cruise No. HE492

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## 1 Summary

The POLFJORD cruise (Heincke HE492) focused on the comparison of plankton diversity and species interactions among Arctic fjord systems subject differential climatic forcing mechanisms along the coast of Svalbard. The primary transects originated from the Norwegian coast, at Trondheim, to the Svalbard archipelago, where more comprehensive studies were conducted within five major fjord systems along the western and northern coasts of the main island of Spitsbergen: Wijdefjord, Van Mijenfjord, Woodfjord, Kongsfjord, and Isfjord. A detailed investigation of temporal and spatial relationships among members of plankton communities within and outside polar fjords was coordinated and compared with prevailing oceanographic regimes defined by temperature and salinity gradients, and the profile of dissolved organic carbon (DOC), solid-phase extracted organic matter (SPE-DOM), dissolved macronutrients and chlorophyll from various depths. The CTD measurements of temperature and salinity profiles essentially matched what would be expected of fjord circulation at polar latitudes during the summer, i.e. high stratification with low surface salinity within the fjords and a sharp pycnocline. Plankton samples were collected by net-tows (30 m vertically integrated), and in Niskin bottles or via high volume pumping from fixed depths, followed by size-fractionation. Live samples containing micrograzers were retained for grazing experiments on board within a temperature- controlled growth chamber. Archived plankton samples are now being subjected to meta-genomic and meta-transcriptomic analysis to compare biodiversity patterns within and among these fjords and adjacent waters. Extensive DNA bar-coding and single-cell PCR was applied to cells isolated directly from the field plankton at various stations. In general, microscopic analysis of the micro- and nano-plankton revealed a post-bloom community, with extremely low chlorophyll concentrations, low plankton biomass and a relatively high abundance of copepods and micrograzers (heterotrophic dinoflagellates, tintinnids and other ciliates, in particular). Expected genera of centric diatoms were present, e.g., *Thalassiosira*, *Chaetoceros*, *Skeletonema*, but never at bloom concentrations; pennate diatoms were typically less common, but at a few stations they contributed substantially to the composition (e.g., *Pseudonitzschia* spp.). The haptophyte *Phaeocystis*, a nuisance HAB genus, was occasionally quite abundant, particularly in the outer stations. Large thecate phototrophic dinoflagellates, comprising the typical toxic HAB targets (e.g., *Alexandrium*, *Protoceratium* spp.), were never dominant nor abundant in the water column, and except for *Dinophysis* spp., were seldom found. Attempts were made to isolate and successfully bring into culture many of these taxa, particularly diatoms and haptophytes for further morphological and molecular diversity studies. The POLFJORD cruise yielded an abundance of new knowledge on the association of plankton biodiversity in Svalbard coastal and fjord systems, but the most important

information awaits further samples and data analysis. These efforts are directed towards providing the linkage of biodiversity patterns to environmental variables and potential driving forces resulting from climate-driven regime shifts in the Arctic and sub-Arctic.

## 2 Zusammenfassung

Die POLFJORD-Forschungsausfahrt (Heincke HE492) konzentrierte sich auf den Vergleich von Planktondiversität und Spezies-Interaktionen zwischen arktischen Fjord-Systemen, die an der Küste von Spitzbergen zum Teil unterschiedlichen klimaverändernden Mechanismen ausgesetzt sind. Die primären Transekte reichten von der norwegischen Küste, von Trondheim, bis nach Spitzbergen, wo umfangreichere Studien innerhalb von fünf großen Fjord-Systemen entlang der West- und Nordküsten der Hauptinsel von Spitzbergen durchgeführt wurden: Wijdefjord, Van Mijenfjord, Woodfjord, Kongsfjord, und Isfjord. Es wurde eine detaillierte Untersuchung der zeitlichen und räumlichen Beziehungen zwischen Vertretern von Planktongemeinschaften innerhalb und außerhalb der polaren Fjorde koordiniert und mit den vorherrschenden ozeanographischen Regimen verglichen, die durch Temperatur- und Salinitätsgradienten definiert wurden sowie dem Profil von gelöstem organischen Kohlenstoff (DOC), Festphasen-extrahierter organischer Substanz (SPE-DOM), gelösten Makronährstoffen und Chlorophyll aus unterschiedlichen Tiefen. Die CTD-Messungen von Temperatur- und Salinitätsprofilen stimmten im Wesentlichen überein, was von der Fjord-Zirkulation in polaren Breiten während des Sommers erwartet werden würde, d.h. eine hohe Schichtung mit einem niedrigen Oberflächen-Salzgehalt innerhalb der Fjorde und einer scharfen Pyknokline. Planktonproben wurden durch Netzzüge (30 m vertikal integriert) und in Niskin-Flaschen oder über Hochvolumenpumpen (Membranpumpen) aus festgelegten Tiefen gesammelt, gefolgt von einer Größenfraktionierung. Lebendproben, die Mikrograzer enthielten, wurden für grazing Experimente an Bord mit einer temperaturgesteuerten Wachstumskammer zurückbehalten. Archivierte Planktonproben unterliegen nun einer meta-genomischen und meta-transkriptomischen Analyse, um die Biodiversitätsmuster innerhalb und zwischen diesen Fjorden und angrenzenden Gewässern zu vergleichen. Eine umfangreiche DNA-Barcodierung und single cell PCR wurde an Zellen durchgeführt, die direkt aus dem Feldplankton an verschiedenen Stationen isoliert wurden. Im Allgemeinen zeigte die mikroskopische Analyse des Mikro- und Nano-Planktons eine „post-bloom“ Gemeinschaft mit extrem niedrigen Chlorophyll-Konzentrationen, einer niedrigen Plankton-Biomasse und einer relativ hohen Anzahl von Copepoden und Mikrograzern (insbesondere heterotrophische Dinoflagellaten, Tintinniden und anderen Ciliaten). Die erwarteten Gattungen zentrischer Diatomeen waren vorhanden, wie z. B. *Thalassiosira*, *Chaetoceros*, *Skeletonema*, aber nicht in Konzentrationen einer Blüte; Pennate Diatomeen waren typischerweise weniger häufig vorhanden, aber an einigen Stationen trugen sie wesentlich zur Arten-Zusammensetzung bei (z. B. *Pseudo-nitzschia* spp.). Die Haptophyte *Phaeocystis*, eine HAB-Gattung, war gelegentlich sehr häufig vertreten, vor allem an den äußeren Stationen. Große thecate phototrophe Dinoflagellaten, die typischen

toxischen HAB-Targets (z. B. *Alexandrium*, *Protoceratium* spp.), waren in der Wassersäule nicht dominant, und mit Ausnahme von *Dinophysis* spp. wurden sie selten nachgewiesen. Es wurden Versuche unternommen, viele dieser Taxa, insbesondere Diatomeen und Haptophyten, für weitere morphologische und molekulare Diversitätsstudien zu isolieren und erfolgreich in Kultur zu bringen. Die POLFJORD-Ausfahrt lieferte eine hohe Anzahl neuer Erkenntnisse über die Assoziation der Plankton-Biodiversität an Spitzbergens-Küsten- und Fjord-Systemen. Die wichtigsten Informationen werden nach weiteren Proben- und Datenanalysen erwartet. Diese Bestrebungen richten sich darauf, die Verknüpfung von Biodiversitätsmustern mit Umgebungsvariablen und potenziellen Triebkräften aus klimabedingten Veränderungen (regime-shifts) in der Arktis und Subarktis zu verbinden.

### 3 Participants

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## 4 Research Program

The ecosystem component of the HE492 cruise **POLFjord** addressed interactions and feedback among hydrographic regimes, biogeochemical and bio-optical signatures and phytoplankton species diversity and molecular activities. The effects of global climate change are particularly pronounced in the Arctic and yield fundamental environmental changes as a consequence. The rising temperature causes higher water temperatures in summer and especially for melting of Arctic glaciers, the freshwater input inevitably leads to changes in Arctic fjord systems. Therefore, the proposed study had two main objectives to address: (1) change in the biological diversity of the plankton communities (zoo-, phyto- and bacterioplankton) along salinity and temperature gradients in Arctic fjords, and (2) the potential occurrence of toxic phytoplankton species in the Svalbard fjords due to the changing climatic conditions. Accordingly, 5 different fjord systems in the north and west of Svalbard were studied (van Mijenfjorden, Wijdefjorden, Woodfjorden, Kongsfjorden, Isfjorden); among these fjords, only Kongsfjorden and Issfjorden are relatively well investigated, but they are differentially strongly influenced by Arctic and Atlantic water and differ further in their size, the influx of glacial freshwater and the annual ice-cover compared to the other three systems. Detecting and analyzing temporal and spatial phytoplankton communities and their blooms with respect to the corresponding influx of organic matter is extremely important to understand pelagic ecology within such fjord systems and predict the effects of climate change.

These objectives were addressed by the following specific elements: 1) establishing an inventory of the phytoplankton diversity in various size-fractions; 2) determining metabolic activities of the planktonic communities in different size-fractions and sampling areas; and 3) determining the water chemistry, including the composition of DOM in the water column. By combining data from the oceanographic, biogeochemical and bio-optical components with conceptual modeling, we contributed to major advances in understanding coastal and fjord ecosystems with regard to the distribution and dynamics of biodiversity under the influence of climate change stressors.

## 5 Narrative of the Cruise

The research cruise HE492 started on 29.07.2017 at 8:00h from Trondheim, Norway. We conducted a test station (St 01) at the Lofoten to perform a full sampling protocol. When we continued towards Spitzbergen we experienced harsh weather conditions and had to proceed at reduced speed (3-4 kn) for the first few days while heading northeast towards

Spitzbergen. The cruise duration was 21 days over a total distance of 1728 sm. The cruise terminated when we arrived Longyearbyen on 18.08.2017 at 9:00h.

The overarching aim of this cruise was to study plankton functional biodiversity in the context of comparative fjord ecosystems in a climate change scenario. This mission aspect was only partially successful due to the late cruise timing and therefore many expected target phytoplankton species were not abundant in the water column anymore, or had even completely disappeared perhaps due to strong copepod grazing dominance in the western fjords. We sampled for phytoplankton with a 20 µm mesh plankton net and a pump system, and for nutrients, dissolved organic matter (DOM) and phycotoxins with a rosette sampler equipped with Niskin bottles. In addition to the water column, we also investigated the sediment for potential resting-cyst stages of the key dinoflagellates and diatoms. These samples are currently under taxonomic examination.

**Van Mijenfjord:** On 03.08.2017 we reached the second sample station (Table 1). We had five stations to sample and moved at the fourth station to the next transect. The salinity values indicated Atlantic water which showed at the tip (close to the glacier) a clearly defined low salinity water layer <30. The surface water temperature of the five stations was between 6 and 8°C, and slightly higher at the entrance of the fjord (St 02). The microplankton was dominated by heterotrophs and mixotrophs, such as tintinnid ciliates (e.g., *Parafavella denticulata* and *Ptychocylis obtusa*) and heterotrophic dinoflagellates (*Protoberidinium pallidum*, *P. depressum*, *Phalacroma rotundatum*). Among the dinoflagellates, the phototrophic *Tripos* spp. and the toxic *Alexandrium ostenfeldii*, in addition to some diatoms (*Chaetoceros*, *Thalassiosira*, *Rhizosolenia* spp.), were mainly present in the outer fjord. The eugleophyte *Eutrepetiella* sp. was also prominent in this fjord.

**Wijdefjord:** The Wijdefjord (06.08-07.08.2017) was up to 400 m deep and exhibited an Atlantic water layer on top of polar deep water. At the entrance of the fjord the surface layer was not as well defined. A salinity of 31.8 (surface) to 34.8 (deep) was measured. At the fjord entrance, the temperature was 2-4 °C at the surface. Within the fjord, the surface was 6°C, decreasing at the glacier front to <2°C and in the deep layer below 0°C. Five stations were sampled along a transect from the outer to inner fjord (St 09-14). At the outer station (St 09) the microplankton showed high diversity and was dominated by phototrophs, particularly by large diatoms, such as members of the genera *Chaetoceros* (e.g., *C. borealis*, *C. concavicornis*, *C. convolutes*, *C. decipiens*) and *Thalassiosira*, forming long chains, and *Proboscia alata*, *Rhizosolenia hebetata*, as well as a high diversity of heterotrophic and phototrophic dinoflagellates (e.g., *Protoberidinium* spp, *Tripos* sp.) and diverse ochrophytes

(e.g. *Dinobryon baltica*, *Dictyocha speculum*). This community suggests bloom conditions with good nutrient availability, possibly from the melting ice edge. At the innermost station (St 13) the microplankton community was dominated by heterotrophs, such as tintinnid ciliates (*Parafavella*, *Ptychocystis*, *Acanthostomella* and *Tintinnopsis* spp.) and heterotrophic dinoflagellates (*Protoberidinium* spp., *Phalacroma rotundatum*, *Gymnodinium* spp.). Some diatoms were also present (*Rhizosolenia*, *Proboscia*, *Thalassiosira* spp.). High abundance of copepods and fecal pellets suggests strong grazing pressure.

**Woodfjord:** The fjord showed similar water layering as in Wijdefjord, with higher salinity and lower temperature at the outermost station. Five stations were sampled (St 16-21). The microscopically identified plankton community composition at the inner stations was similar to that of the inner Wijdefjord, and was dominated by heterotrophic tintinnid ciliates (see above). Here the heterotrophic dinoflagellates *Prorocentrum pelagicum*, *Cochlodinium pulchellum*, *Protoberidinium* spp, and mixotrophic *Dinophysis ruudi* and *Gymnodinium* sp. were recorded.

**Kongsfjorden:** On 11-12.08.2017 sampling of the water column within the Kongsfjord indicated layer formation in the upper 10 m with salinity around or slightly below 32. The surface temperature was between 3 - 6 °C. As at the other stations, the water masses close to the glaciers were colder, with less chlorophyll and much silt in the water column, thereby coloring the water red. At the three stations sampled (St 23-26) the microplankton diversity in the inner part again was poor and dominated by heterotrophs, such as ciliates and some heterotrophic dinoflagellates (e.g., *Phalacroma rotundatum*). High abundance of copepods and fecal pellets also suggests a strong grazing pressure.

On 12-13.08.2017 we entered the Ny-Alesund harbor and took cooled samples over from the station to bring them to Bremerhaven.

**Hausgarten system:** The Hausgarten transect was sampled on 14.08.17. The winch was not functioning towards the end of the sampling and therefore only limited CTD and water rosette sampling could be conducted. The water temperature was between 6 - 8°C within the first 50 m and then dropped to 2 - 4°C. The chlorophyll max layer was around 20m and at salinity 35. The microplankton showed high diversity and consisted of both phototrophic phytoplankton dominated by various diatoms (particularly *Chaetoceros*, Thalassiosiraceae, *Leptocylindrus*, *Fragilariopsis*, *Pseudo-nitzschia*, *Skeletonema*) and some dinoflagellates (*Tripos arctica*), and with notable presence of heterotrophic dinoflagellates (*Protoberidinium* and *Phalacroma* spp.) and ciliates. The microplankton composition suggested strong Atlantic

influence and good availability of inorganic nutrients for phytoplankton growth.

**Isfjord:** The Isfjord was sampled on 15 – 16.08.17 at three stations (St 30-32). On August 15 we met the *RV Polarstern* to transfer samples to transport to AWI, Bremerhaven. The temperatures at the surface were around 18°C and only declined below 10°C at St 31. The surface salinity of 20 to 22 increased at greater depths, where the highest value (32.9) was measured at 30.7 m at St 31. The phytoplankton diversity was low and the plankton biomass dominated by zooplankton (copepods) and ciliates. High abundance of copepods and fecal pellets again suggested a strong grazing pressure.

In summary, although we lost some work time after the bad weather conditions during the first few days on the way from Trondheim to Spitzbergen, we were able to compensate and adjust the sampling regime and station plan such that most scientific objectives were accomplished. We gained important comparative insights into plankton dynamics and diversity in coastal/fjord systems of Spitzbergen.

## **6 Methodology and Instrumentation**

### **6.1 Physical oceanography and bio-optics**

(Voß, Henkel)

#### **6.1.1 Oceanographic parameters from the ship CTD**

The CTD (Figure 1) casts were performed with a Seabird 'sbe911+' CTD probe with sampling rosette (onboard device) at each station, as an initial activity at the station to determine further key discrete sampling depths, e.g., to locate chlorophyll maxima. Live data acquisition was carried out via CTD-client onboard and data post-processing with Seasoft V2. Salinity and depth were calculated from pressure values (UNESCO, 1983), and temperature was corrected to ITS-90 (Preston-Thomas, 1990). The CTD was equipped with additional sensors for turbidity, fluorescence and oxygen. All CTD data will be published via Pangaea@ ([www.pangaea.de](http://www.pangaea.de)). CTD data are under processing and will be published as soon as possible as also linked to already published track.



Figure 1 Onboard device Seabird 'sbe911+' CTD probe with sampling rosette for station work on R/V Heincke.

### 6.1.2 Chlorophyll, SPM and CDOM from water samples

Water samples were collected at each station from defined depths to measure colored dissolved organic matter (CDOM), suspended particulate matter (SPM) and chlorophyll *a* (Chl *a*). Immediately after sampling, CDOM and FDOM samples were filtered under low vacuum through 0.2  $\mu\text{m}$  membrane filters (Sartorius, Germany). The filtration unit had been pre-rinsed with Milli-Q dionized water (Millipore, USA) to avoid contamination, followed by sample water ( $\sim 100$  mL). Samples were directly analyzed onboard (Figure 2).

In a 0.01 m quartz cuvette, pre-rinsed twice with filtered seawater, fluorescence excitation-emission matrices (EEM) for FDOM were measured with a spectrofluorometer (LS55®, Perkin Elmer, Germany). Measurements were performed using Milli-Q water as reference. The scan ranged from 240 to 750 nm with an excitation increment of 5 nm. The EEM data were scanned for peaks according to Coble (2007).

For SPM determination, water samples (up to 8 L volume) were filtered through pre-combusted and pre-weighed Whatman GF/F filters, pre-washed and rinsed with Milli-Q water. Filters were frozen immediately at  $-25$  °C and reweighed in the laboratory after the cruise. The SPM concentrations were normalized to 1 L.

Chl *a* was determined (up to 6 L volume) after sample filtration through Whatman GF/F filters, pre-washed and rinsed with 0.2  $\mu\text{m}$  filtered seawater. Filters were stored onboard at  $-80$ °C and analyzed later in the laboratory after pigment extraction with acetone followed by fluorometric measurements and calculation of chlorophyll *a* concentrations according to EPA method 445 (Arar & Collins, 1997).



Figure 2 Large-scale DOM-SPM tank, filter and solid phase extraction for 600 L, 20 L and 0.5 L DOM concentration

### 6.1.3 Bio-optical parameters from the profiling system

A HyperPro II profiling system (Satlantic, Halifax, Canada) was used to acquire bio-optical data for different parameters (Figure 3). The profiler consists of one hyperspectral irradiance and one hyperspectral radiance sensor, as well as fluorescence and backscatter sensors and an integrated CTD. A second hyperspectral irradiance sensor was mounted on the research vessel for reference measurements. On the profiler, the irradiance sensor measured downwelling and the radiance sensor upwelling light.

Profiler measurements were conducted at selected stations depending on sea and weather conditions. At these stations, three casts were typically performed. At each cast, the profiler was lowered until the downwelling light values were of the same order of magnitude as the background noise level of the sensor.



Figure 3 Hyperspectral profiling system Satlantic Inc. (Canada) to acquire bio-optical data during free fall mode from *R/V Heincke*.

#### 6.1.4 Underway data from the Ferry-Box and ship systems

The FerryBox is a flow-through system deployed as an underway device for ship expeditions and for attendant measurements during stationary operations. The system provides basic data at high spatial and temporal resolution for various parameters, e.g., salinity, temperature (at the intake and inside the system), chlorophyll-fluorescence, CDOM-fluorescence, turbidity, dissolved oxygen. For multi-parameter sensing, water was pumped from the moonpool of the ship. Measurements were performed at a sampling interval of 1 min.

#### 6.1.5 Ocean color sensing

##### *Secchi depth & Forel-Ule observations*

Water transparency measurements were performed with a 30 cm Secchi disc at each daytime station to determine the penetration of light. The Forel-Ule (FU) color comparator scale is a device that is composed of 21 colors, from ‘indigo blue’ to ‘cola brown’, and represents the range of colors that can be found in the open sea, coastal, and continental waters. Based upon a historical background, this provides an estimation of the present water constituents influencing the water color. The color of the water was determined over a Secchi disc at half the disc’s depth (where the disc disappears from sight) at each day station (Figure 4).



Figure 4 Secchi disc above and in water (left, middle) and Forel-Ule (FU) color comparator scale to determine the water color (right).

##### *Hyperspectral radiometric observation*

Above-water hyperspectral radiometric observations were conducted during the whole cruise (Figure 5). A radiometer setup with a RAMSES-ACC hyperspectral cosine irradiance meter to measure  $ES(\lambda)$  (downwelling solar irradiance), and two RAMSES-ARC hyperspectral radiance meters (one set-up at starboard, one at the port side of the ship) to measure  $L_{sfc}(\theta_{sfc}, \Phi, \lambda)$  (upwelling water-leaving radiance) and  $L_{sky}(\theta_{sky}, \Phi, \lambda)$  (sky-leaving radiance) were installed on the ships’ foremast (TriOS GmbH, Germany). Hyperspectral

measurements were collected at 5 min intervals over a spectral range  $\lambda = 320 - 950$  nm. Data processing was done according to Garaba & Zielinski (2013).

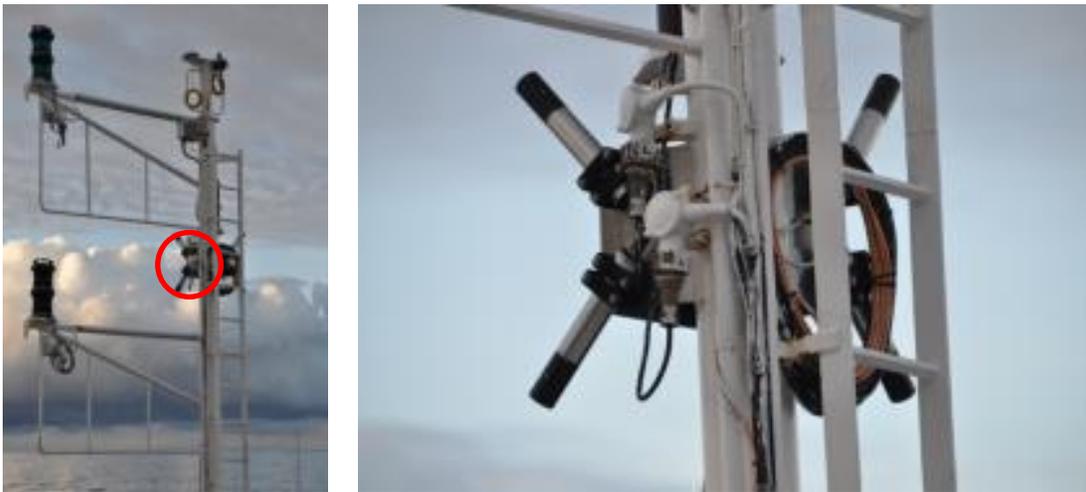


Figure 5 Radiometric set-up on the foremast of *R/V Heincke*. One RAMSES-ACC hyperspectral cosine irradiance meter was installed at the top of the mast to measure total downwelling solar irradiance (red circle).

## 6.2 Nutrients and DOM

(Kemper, Tebben)

All stations was sampled (except for sediment only St 10-12) for dissolved organic carbon (DOC), solid-phase extracted organic matter (SPE-DOM), particulate organic carbon (POC), and nutrients from various depths.

### *Nutrients*

Subsamples of seawater were directly collected from the Niskin bottles of the CTD-rosette-system and stored at  $-20^{\circ}\text{C}$  until further analysis in the laboratory. Nitrate, nitrite, ammonium, phosphate and silicate were measured with an autoanalyzer system (Evolution III, Alliance instruments) by standard seawater methods (Kattner and Becker 1991). All samples were analyzed in duplicate; the accuracy was set by running three standards at the beginning and two standards after each 8 samples. The analytical precision of replicates was approximately  $0.05 \mu\text{M}$  for nitrate, silicate and ammonium and  $0.01 \mu\text{M}$  for phosphate and nitrite for the range of concentrations in this study.

### *DOM extraction and molecular characterization*

The DOC concentration of the original glass fiber-filtered samples (Whatman GF/F, pre-combusted at  $450^{\circ}\text{C}$  for 5 h) was determined by high temperature catalytic oxidation

(TOC-V<sub>CPN</sub> analyzer, Shimadzu). For SPE-DOM measurements, subsamples of the filtrates were acidified to pH 2 with HCl (32%, p.a.) and passed through SPE cartridges (PPL, Varian), rinsed with acidified ultrapure water. The 0.5 L SPE-DOM was eluted with methanol according to Dittmar et al. (2008). The SPE-DOM fraction was roughly characterized according to its polarity distribution in a water/methanol gradient by reverse-phase liquid chromatography (Koch et al., 2008). In addition, samples will be analyzed with an FT-ICR mass spectrometer (Apex Qe, Bruker Daltonics, Billerica, MA), equipped with a 12 T refrigerated actively shielded superconducting magnet (Bruker Biospin, Wissembourg, France). An Apollo II dual electrospray source (ESI, Bruker) will be used in negative ion mode (for details see Lechtenfeld et al., 2013).

For the mid-scale SPE-DOM samples (20 L) the filtrate of the RNA particle collection (5.4.1.1) were used, adjusted to pH 2 with hydrochloric acid (32 %) and concentrated by solid phase exchange columns (PPL, 5 g, Varian) by gravity flow. Large scale (600 L) DOM samples were pumped from approximately Chl *a* maximum depth (2 - 40 m) with a Teflon membrane pump and pre-filtered over 5 and 1 µm filter socks into 600 L acid washed LDPE water tanks . Peristaltic pumps were used to filter this water through 0.2 µm cartridges (AcroPak 1000 Capsules, Pall Corporation, NY) and solid phase exchange columns (14 g, Bond Elut ENV, Agilent, Santa Clara, CA). These samples will be measured with a HR-LCMS Orbitrap mass spectrometer (QExactive-Plus, Thermo-Fisher, Bremen, Germany) and NMR (600 MHz, Bruker Biospin, Billerica, MA) and analyzed for low concentration bio-active molecules (e.g. trace-metal ligands).

### ***POC sampling and extraction***

For the measurement of particulate organic carbon (POC) two litres of sampled seawater were filtered (Whatman GF/F, pre-combusted at 450°C for 5 h). The filters were kept in Petri-dishes and stored in -20°C until further extraction in the lab.

## **6.3 Microplankton species diversity**

(Edwardsen, Kalita, John)

At every station, except the last one because of very strong winds, plankton was sampled by vertical net tows through the upper water column (usually 30m but a few times to 40m) by a 20 µm-mesh size phytoplankton net (Hydrobios). Plankton communities were characterized by onboard microscopic examinations of live or freshly preserved subsamples of these net tows. At each station 90 mL of a diluted net tow subsample was transferred into

a 200 mL brown medicine flask and with added 5 mL formalin (1% final conc.) and 0.5 mL glutaraldehyde (0,1% final conc.) for light microscopy (to IOPAS, PL).

Microscopic observations of live material were conducted with an inverted microscope (Leitz Aristoplan), and a standard orientation optical microscope (Zeiss AxioStar), both with an attached camera for documentation. The main aims were to establish species identity and identify dominant taxa, and documentation by photomicrography. Furthermore, interesting samples for cell isolation of target taxa for single-cell genetic analyses and for culturing from the microplankton community were identified.

When species of interest were detected in the net tow, single cells were isolated by microcapillary pipette thereby establishing monoalgal cultures. The cells were placed into single wells of 96-well plates. Plates were incubated on-board at 5°C and at 50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  photon flux density. The taxonomic groups of major focus were dinoflagellates and diatoms, with additions of some silicoflagellates and haptophytes. After the cruise, plates were brought back to the laboratory and processed for clonal culture establishment at UiO, as part of the RCN project TaxMArc.



Figure 6 Harvesting of surface plankton by vertical net tow (20  $\mu\text{m}$ -mesh) and subsequent analysis of net tow concentrate by stereo-dissecting and phase interference (Nomarski) microscopy.

## 6.4 Molecular biodiversity

(Wohlrab, Edvardsen, Kalita, Kühne, John)

Assessing the eukaryotic and prokaryotic biodiversity and link their composition and activity to the chemical and physical environment was one of the prime targets of the research cruise (Figure 7).



Figure 7 Seawater sampling devices used for size fractionation during the cruise.

### 6.4.1 Onboard sampling

Sampling for molecular biodiversity (Metabarcoding), metatranscriptomics and single-cell gene-sequencing and transcriptomics was achieved by three approaches (Figure 8): 1) CTD/water-rosette; 2) plankton net (20 $\mu$ m); 3) membrane pumping at discrete depth from around the Chl *a* maximum layer. Collected water samples were size-fractionated into >200 $\mu$ m, 200-100 $\mu$ m, 100-20  $\mu$ m with gravity-filtration over filter towers and 20-3  $\mu$ m and 3-0.2  $\mu$ m size-fractions in a series of tripod filtration units by peristaltic pumping to collect the plankton on polycarbonate filters.

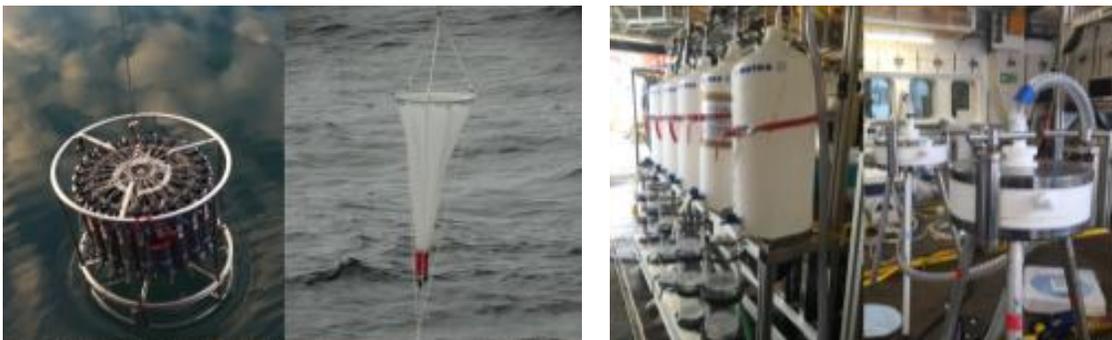


Figure 8 Sampling and filter devices for molecular diversity and metatranscriptomics.

In brief, plankton samples were divided for DNA and RNA extraction, as well for phycotoxin and metabolomic analysis. Polycarbonate filters were cut into four equal pieces, which were dedicated for DNA, RNA, metabolomics and one backup sample. Filters for metabolomics were immediately extracted with methanol and stored at  $-20^{\circ}\text{C}$  for analysis in the home laboratory. 40 stations were sampled, and 79 RNA samples will be extracted and analyzed for quantity, purity and integrity. DNA extractions and analyses were done onboard. Additionally, we isolated 104 single cells for the gene amplification of 18S and 28S rRNA regions and 42 single cells for a novel transcriptomic approach.

#### **6.4.1.1 Metabarcoding**

PCR gene amplification of the V4 regions of the 18S rRNA, and for the 3-0.2  $\mu\text{m}$  size fraction also of 16S rRNA, will be performed in the laboratory. Generated libraries will be sequenced by Illumina MiSeq and the obtained data analyzed with the established bioinformatics pipelines at the AWI and MPI. Short sequences (reads) combined with metadata will be immediately published in public databases such as NCBI. Data will be analyzed with multifactorial statistics to elucidate the linkage of biodiversity and environmental parameters.

#### **6.4.1.2 Metatranscriptomics**

Three net tows (20  $\mu\text{m}$  mesh size, 50 cm diameter) were carried out from 30 m depth, with the net raised to the surface at  $0.5\text{ m s}^{-1}$ . Cells larger than 20  $\mu\text{m}$  were collected from the cod-end, rinsed with 0.2  $\mu\text{m}$ -filtered seawater, adjusted to 1 L with sterile-filtered seawater and fractionated through a sequential filtration-tower (200  $\mu\text{m}$ , 50  $\mu\text{m}$  and 20  $\mu\text{m}$ ). Additional water was collected from 3, 15 and 30 m depth in Niskin bottles with the rosette sampler then collected into 25 L tanks and filtered through the filter tower (200  $\mu\text{m}$ , 50  $\mu\text{m}$  and 20  $\mu\text{m}$ ). The water was collected and further filtered through 3  $\mu\text{m}$  (for nanoplankton) and 0.2  $\mu\text{m}$  (for picoplankton and bacteria) filters mounted on tripods.

For the net tow and the Niskin bottle samples, the size-fraction retained on 20  $\mu\text{m}$  Nitex mesh (for microplankton) was rinsed off with sterile-filtered seawater and transferred into centrifugation tubes. The centrifugation tubes were topped off to a total volume of 45 mL and split into four 15 mL centrifugation tubes for further analyses (RNA and DNA). The tubes for DNA and RNA-extraction were centrifuged and the supernatant was discarded. The pellet was resuspended in 400  $\mu\text{L}$   $60^{\circ}\text{C}$  warm AP1 lyse-buffer (DNA) or 400  $\mu\text{L}$   $60^{\circ}\text{C}$  warm Tris-Reagent (RNA) and transferred into a 2 mL cryovial with three small spatula-tip aliquots of acid-washed glass beads and stored at  $-80^{\circ}\text{C}$  until further analysis. Filters from the tripods were cut into four pieces (2 for DNA; 2 for RNA), transferred to a centrifugation tube and

rinsed with 1 mL AP1 lysis-buffer (DNA) or 400 µL Tris-Reagent (RNA) and transferred into a 2 mL vial with three small spatula-tip loads of acid washed glass beads and stored at 80°C until further analysis.

For the micro- and nano-plankton fractions, as well as for the picoplankton, DNA was extracted as Genomic DNA, using the soil-kit to obtain DNA from eukaryotes and bacteria (Macherey-Nagel, 2011).

#### **6.4.1.3 Single-cell-PCR (18S and 28S rDNA)**

Here we isolated 104 cells of “phytoplankton” or heterotrophic protists, with focus on heterotrophic dinoflagellates and ciliates, from net and CTD rosette samples by capillary pipetting under the light microscope. The morphology of each cell was documented by micrography. The cells were washed in sterile seawater and transferred to a tube with PCR solutions and frozen. Further, PCR gene amplification for 18S and 28S rRNA regions was performed and the results checked via gel electrophoresis on board.

#### **6.4.1.4 Single-cell transcriptomics**

To be able to link a DNA sequence of a marker gene to a morpho-species, we need reference sequences. These sequences are, however, missing for a large number of protists, such as of heterotrophic organisms which are difficult to grow (depending on the right prey), or from species from remote non-sampled locations. Similar to the single-cell-PCR, we isolated 42 cells of unknown, abundant, and/or heterotrophic protists from net and CTD samples by capillary pipetting under the light microscope. The morphology of each cell was documented by micrography. Again, the cells were washed and transferred to a single tube, but then directly processed by an adapted SMARTSeqV4 (Takara Clontech) protocol. The enzyme of the SMARTSeqV4 kit distinguishes mRNA from other expressed RNAs and uses the mRNA specific poly(A) tail as a primer site for reverse transcription that subsequently allows a direct cDNA synthesis followed by an amplification. We can thereby recover a great number of true mRNA transcripts for the creation of marker genes as mentioned above.

### **6.4.2 Onboard Data/First results**

#### *Single-cell PCR (18S and 28S)*

Gene amplification by PCR was performed with two primer sets for two molecular markers (18S and 28S rRNA genes) for each cell. Amplicons were analysed with gel electrophoresis and gels inspected and documented under UV light onboard.

Nine PCR reactions and corresponding gels were performed onboard. Of 104 analysed cells nearly 50% yielded one or two bands on the gel, indicating successful amplification of one or both gene markers (Figure 9).

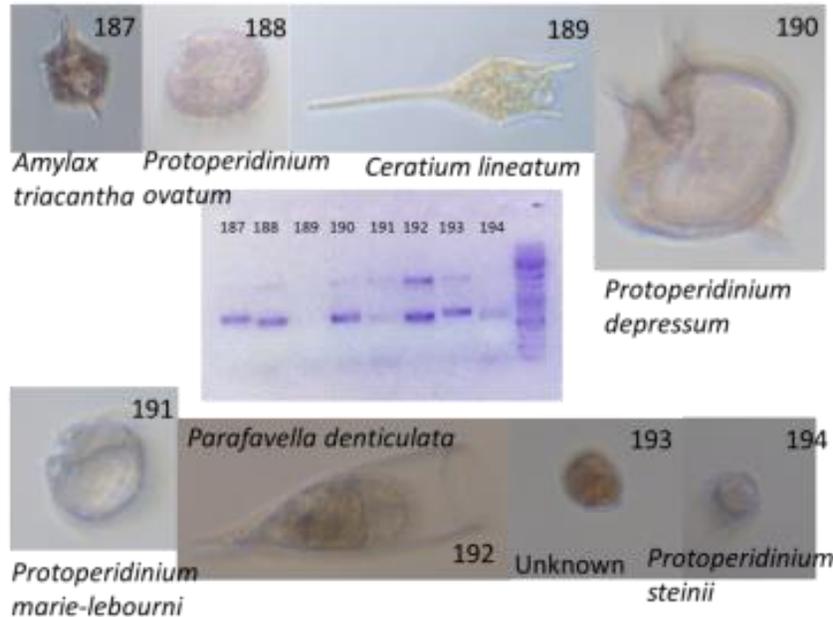


Figure 9 Light microscope images of selected plankton cells which have been single cell selected and used in the PCR. Numbering indicated the number of picked and photographed and PCR attributed organism, if feasible species names are provided. Agarose image show the amplified SSU (upper) and LSU (lower) amplicon of the selected single cells generated by PCR on-board.

### 6.4.3 Expected results

#### ***Metabarcoding***

Illumina MiSeq sequences of the libraries will be analyzed bioinformatically and then published in public databases such as NCBI. Multifactorial statistics will elucidate the linkage of biodiversity and environmental parameters.

#### ***Metatranscriptomics***

RNA samples of the five fjord systems and the Hausgarten will be used for cDNA library generation, which will be paired end sequenced at an in house (AWI) Illumina NextSeq platform. Reads from each cDNA-Library (representing one station and size fraction) will be mapped against a reference database assembled from public available transcriptome sequences and in house curated databases (Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) at iMicrobe:

<https://www.imicrobe.us/#/projects/104>; NCBI transcriptome data from various organisms (<https://www.ncbi.nlm.nih.gov/>). Each sample will therefore contain information about reads per contig, with the respective contigs bearing information about the taxonomic identity and function of the gene. Results will be used for a multivariate analysis to identify traits expressed by different species in different environment.

### ***Single-cell-PCR (18S and 28S)***

The positive amplicons will be reamplified by PCR in the laboratory and prepared for Sanger sequencing. The sequences will then be edited, aligned and phylogenetic analyses performed to infer their relationships and taxonomic placements.

### ***Single cell transcriptomics***

SMARTSeqV4 (Takara Clontech) single-cell transcriptomic samples will be quality checked via the Agilent 2100 Bioanalyzer and ligated with individual Illumina adapter sequences as stated in the Nextera XT protocol (Illumina). The cDNA-Library will then be paired end sequenced at an in house (AWI) Illumina NextSeq platform. The obtained data will then be imported into the in house bioinformatic pipeline to identify new marker genes for taxon-omics approaches. With the possibility of single-cell sequencing we will improve the sequence database for Arctic microplankton and be able to get further information about the real biodiversity, particular for heterotrophic protists.

## **6.5 Abundance and distribution of phytoplankton functional groups**

(K. Brandenburg)

Samples for phytoplankton functional type counts were taken at all stations and depths, and analysed by means of flow cytometry. A 20 mL sample was tapped from the CTD bottles and directly processed by an Accuri C6 flow cytometer (BD Biosciences). Subsamples were also fixed as back-up for phytoplankton, as well as for later analysis of bacteria counts, and stored at -80°C.

Phytoplankton functional groups were differentiated based on their fluorescence and scatter signal (LARSEN et al., 2001; Figure 10). Abundances of the respective groups were determined, and using the FL3-A (red fluorescence) signal from the Accuri C6 measurements, the importance of the phytoplankton groups relative to each other was estimated using Equation 1 where X stands for one of the phytoplankton groups (Figure 10, top middle subplot, e.g. picoeukaryotes), “con” refers to contribution of this particular group to the total FL3-A signal, abundance is the number of cells within gate X, FL3-A is the average red fluorescence of gate

X, and “All” refers to a gate which includes all phytoplankton groups (Figure 10, top right subplot).

Equation 1 Fluorescence conversion for the determination of phytoplankton abundances during PolFjord cruise.

$$X_{con} = \frac{X_{abundance} \times X_{FL3-A}}{All_{abundance} \times All_{FL3-A}}$$

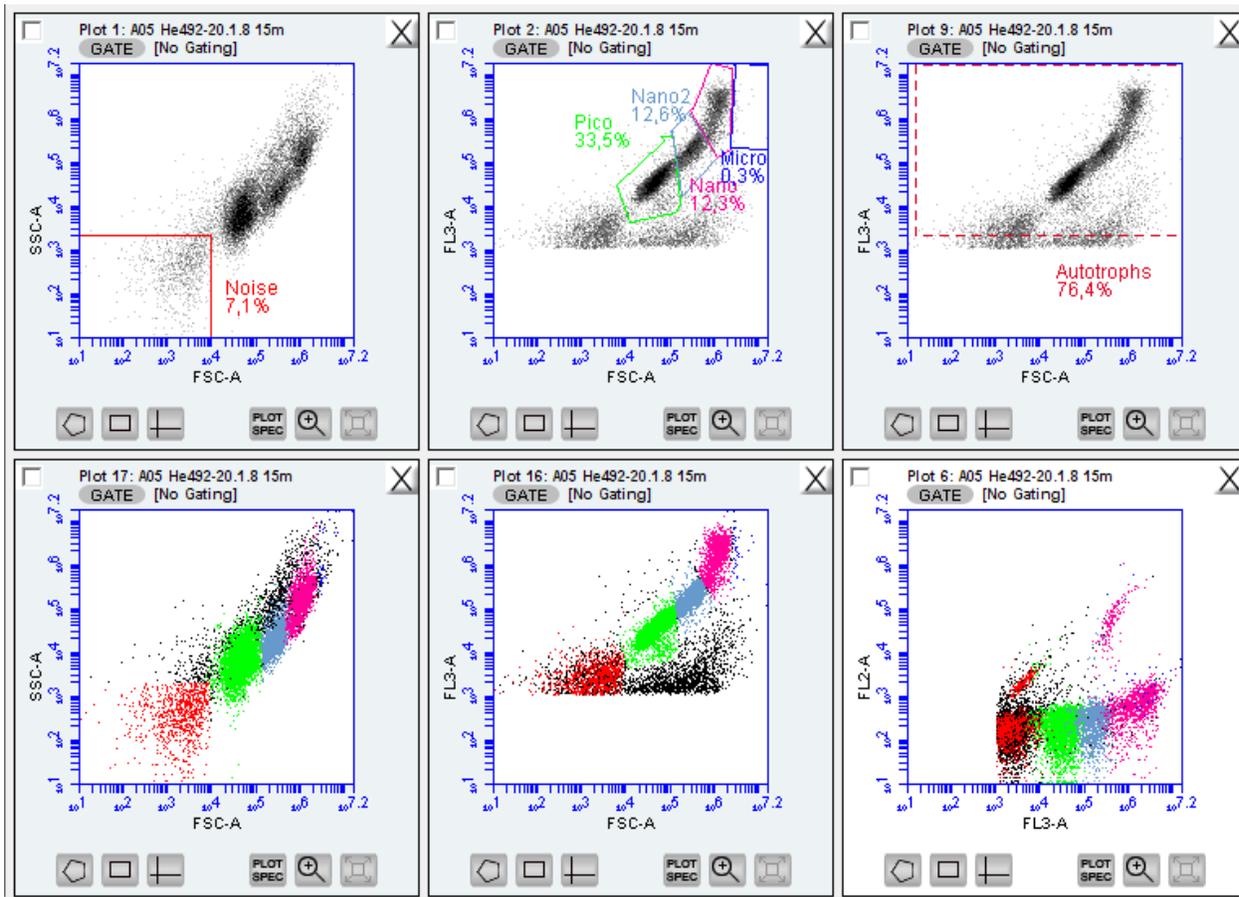


Figure 10 Cytograms from the Accuri C6 flow cytometer counts. The gates in the top right and left subplot were used to determine the phytoplankton fraction, and the gates in the top middle subplot were most important ones for differentiating the functional groups.

## 6.6 Micrograzing

(Philipp Wenta, Uwe John)

Microzooplankton plays a major role in most marine habitats. As grazers of phytoplankton and food for larger zooplankton it acts as an important trophic link channeling primary

production up the food web. However, there have been few studies evaluating the role of microzooplankton grazing in Arctic food webs.

The dilution technique is the most common method to simultaneously estimate phytoplankton growth and microzooplankton grazing rates in marine waters. The basic version of this experimental design (Landry & Hassett, 1982) involves serial dilutions of whole water samples to alter the encounter rates between predator and prey and therefore the predation rate of the predators and the net growth rate of the prey. The slope of a regression of proportion whole water against net growth rate of the prey (measured as change in abundance from beginning to end of a 3 days experiment) gives the predation rate, and the intercept of the regression line (at a theoretical 0% whole water) gives the prey growth rate in the absence of predation. To conduct as many experiments as possible, we minimized the workload and amount of water needed by applying a simplified version of the dilution technique termed the “two-point” dilution technique. This method has recently been approved to deliver reliable rate-estimates (Chen, 2015; Morison & Menden-Deuer, 2017).

Water for the experiments was sampled with the CTD/ Rosette water sampler at the chlorophyll maximum in four fjords – Wijdefjorden, Woodfjorden, Kongsfjorden and Van Mijenfjorden. The respective stations are listed in Table 1

Table 1 Sampling coordinates for the grazing experiment.

<b>Station</b>	<b>Fjord</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Date</b>
HE492_7_7	Van Mijenfjord	77° 36.868' N	013° 39.208' E	04.08.2017
HE492_10-8	Wijdefjord	79° 44.067' N	015° 21.697' E	06.08.2017
HE492_20_10	Woodfjord	79° 46.350' N	014° 08.076' E	10.08.2017
HE492_24_6	Kongsfjord	79° 00.730' N	011° 25.962' E	11.08.2017

The sample water was gently collected from the Niskin bottles and transferred into polycarbonate carboys using funnels and silicone tubing as shown in Figure 11, which minimizes handling derived loss of abundances and diversity. Bigger zooplankton was excluded by placing a 150 µm mesh filter hut inside the funnel. Particle free seawater acquired from the same station was used for the required dilutions of sample water.

The experiments were run in 2.3L acid rinsed Duran glass bottles, incubated at near in situ conditions in a temperature controlled thermo-lab. Light sources inside the lab were turned off and UV lamps with a 20:4 light:dark cycle were installed above the plankton wheels (Figure 11). The 4 experiments all had 3 replicates of undiluted sample water and 3 replicates of diluted (10%) sample water. Because the method requires nutrients not to be limiting

(more dilute samples have more nutrients per phytoplankter, which under limiting conditions would result in higher net growth independent of grazing pressure), nutrients in the form of f2-algal growth medium ( $3 \mu\text{mol NO}_3 \text{ L}^{-1}$  final concentration) were added to all but 3 additional bottles with 100% whole water. Comparing algal growth rates in bottles with and without nutrients allows the degree of nutrient limitation to be ascertained.

The following samples were taken at the beginning and end of each experiment: 3 x 2.3L of sample water filtered onto GF/F filters and frozen for later extraction and chlorophyll *a* quantification, 3 x 1L filtered onto polycarbonate filters and frozen for later DNA extraction, 3 x 1L fixed in 5% Bouin's fluid for later enumeration and morphological identification of microplankton, 3x <50ml for quantification of pico- and nanoplankton using flow cytometry. Directly after incubation further samples were taken from each bottle: Around 0.5-1L for chlorophyll *a* quantification, 500-750ml for DNA extraction, 475ml for fixation in Bouin's fluid and <50ml for flow cytometry. Considering microzooplankton and phytoplankton as a uniform groups in terms of their grazing impact or vulnerability to predation is commonly done in food web studies, but can result in a very misleading picture. Therefore the samples will be analyzed using a combination of flow cytometry, DNA-based molecular techniques and quantitative protargol staining (QPS) to investigate species compositions in all size classes. This will also enable us to compare results obtained by morphological and molecular methods. Additionally, a non-invasive monitoring of photosynthetic activity and respiration was implemented during the experimental incubations by using  $\text{O}_2$ -sensitive planar optodes which were attached to the inside walls of the incubation bottles.

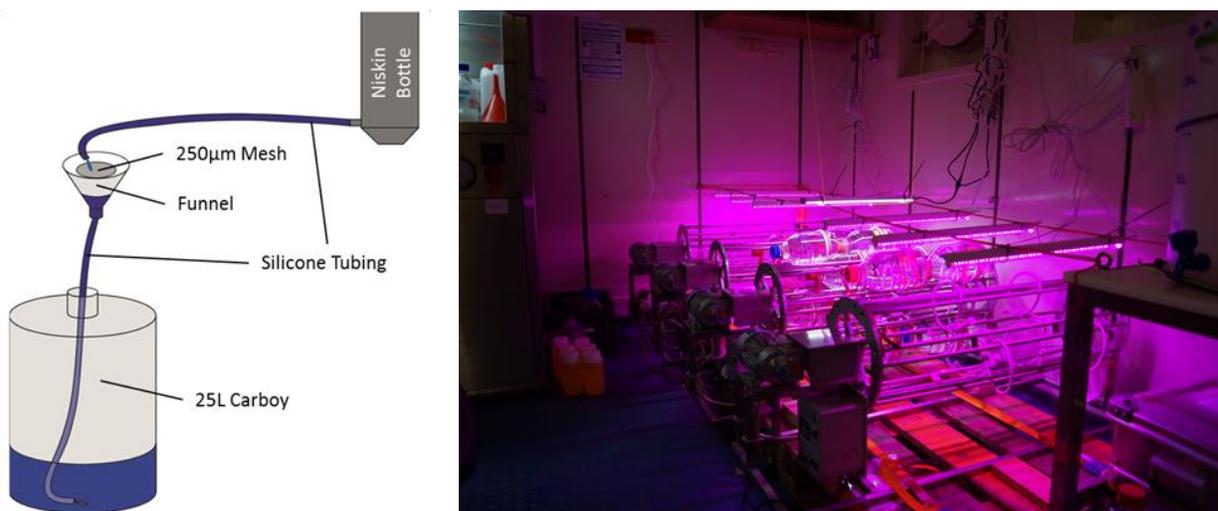


Figure 11 The left image: Sample water collection from the Niskin bottles. Right image: Incubation of grazing experiments in the thermo-lab.

A major goal of these experiments was to measure microzooplankton grazing and algal growth rates in different fjords and relate these rates to species compositions of the respective plankton communities as well as physical-optical and biogeochemical key variables.

## 6.7 Cyst distribution

(Cembella)

Benthic sediment samples were collected (when possible) with a Van Veen-type sediment grab sampler (Figure 12), to examine the abundance and distribution of sexual cysts of marine dinoflagellates along the cruise track. These cysts represent the dormant resting stage of the life history of many planktonic species, in particular of certain large thecate dinoflagellates associated with HABs. Stations were selected based upon inferences of expected cyst abundances from historical knowledge of the location of blooms of cyst-forming HAB species, expectations of the nature of the sedimentary regime (e.g., sandy, gravelly, or silty) and water depth.

Casts were sometimes repeated in cases where the initial cast was only partially successful in retrieving sediments. Mini-cores of the sediment substrate were collected in 50 ml Falcon tubes, and examined microscopically within several hours of collection. Preliminary processing involved dilution of the sediment suspension with a 10:1 V:V ratio of seawater to sediment. The slurry was vortex mixed for several seconds and then shaken vigorously to disaggregate the cysts from sediment particles, then allowed to sediment until the supernatant was clear. The surface layer was aspirated by Pasteur pipette and transferred dropwise to a glass depression slide for microscopic analysis by contrast interference (Normarski) optics (Lietz Diaplan).

For selected stations with ideal sediment consistency (silty, clay of fine sand) where cyst retention was assumed to be optimal, samples were probe-sonicated for 1 min for disaggregation from sediment particles. Subsequently the cyst-containing slurry was processed by resuspension with Nalco silica gel and centrifuged at 5,000 x g for 10 min. The cyst enriched layer was harvested from the round bottom centrifuge tubes and examined in a Sedgewick-Rafter counting chamber by contrast interference microscopy and documented by photomicrography.

In general, the sediment samples were poor in dinoflagellate cysts, but occasionally cysts of thecate dinoflagellates (*Operculodinium*, *Spiniferites*, *Scrippsiella*, *Protoperidium*, *Protoceratium reticulatum*) were present in low numbers. More numerous were usually

resting stages of centric diatoms (auxospores) at most stations, but the distributional pattern was not consistent. Samples exhibiting high numbers of cysts of target species, e.g., *Alexandrium* spp., were noted for future isolation of cysts for germination in culture. Whole sediment samples were stored in the dark at 4°C for further processing. Detailed analyses of promising samples is still pending.



Figure 12 Benthic sediment samples collected with a Van Veen-type sediment grab sampler; left side grabber hived on board and right side detailed sampling of the sediment.

## 7 Preliminary Results

### 7.1 Physical oceanography and bio-optics

#### 7.1.1 Oceanographic parameters from the ship CTD

In this chapter the CTD profiles for the different fjord areas are shown (Figure 13). For all sections, temperature, salinity, fluorescence, transmission and oxygen profiles are plotted over the Latitude or the distance of each station [km] (Figure 14 - Figure 18).

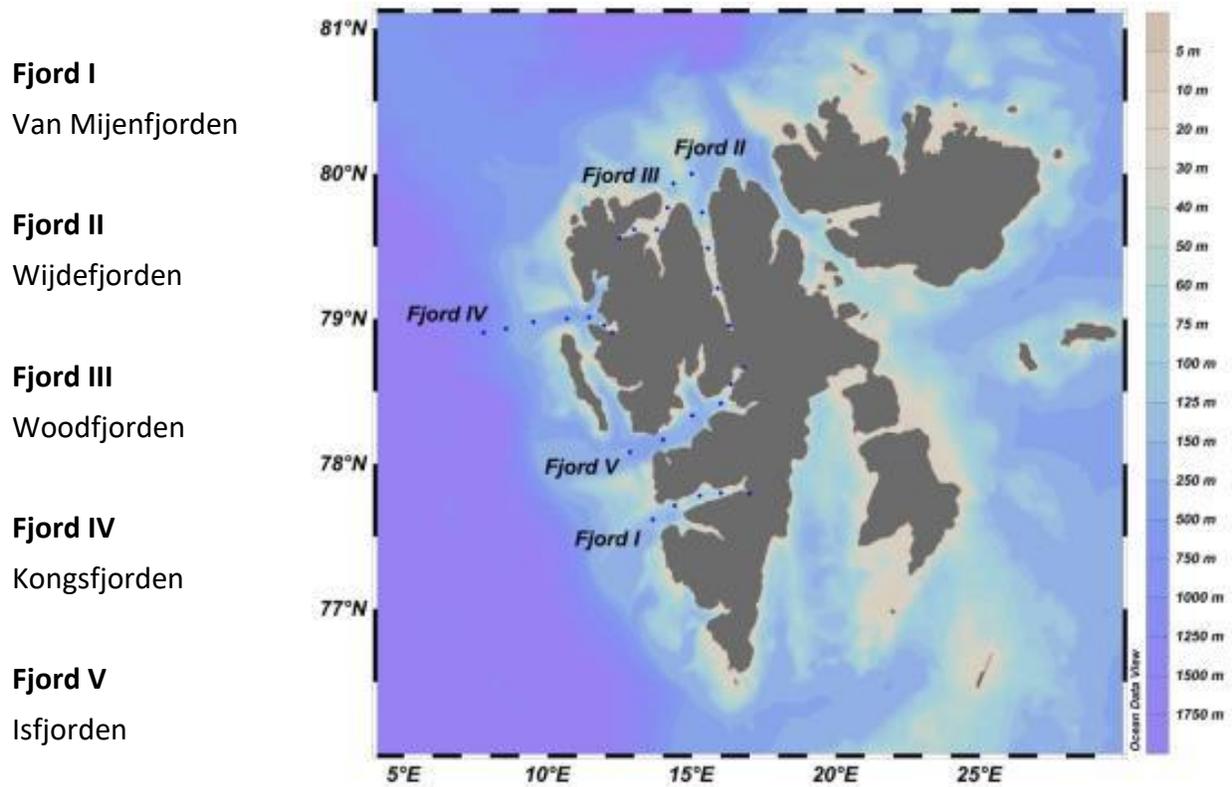


Figure 13 Map of the investigated areas during the cruise HE492 aboard *R/V Heincke*.

### Fjord I Van Mijenfjorden

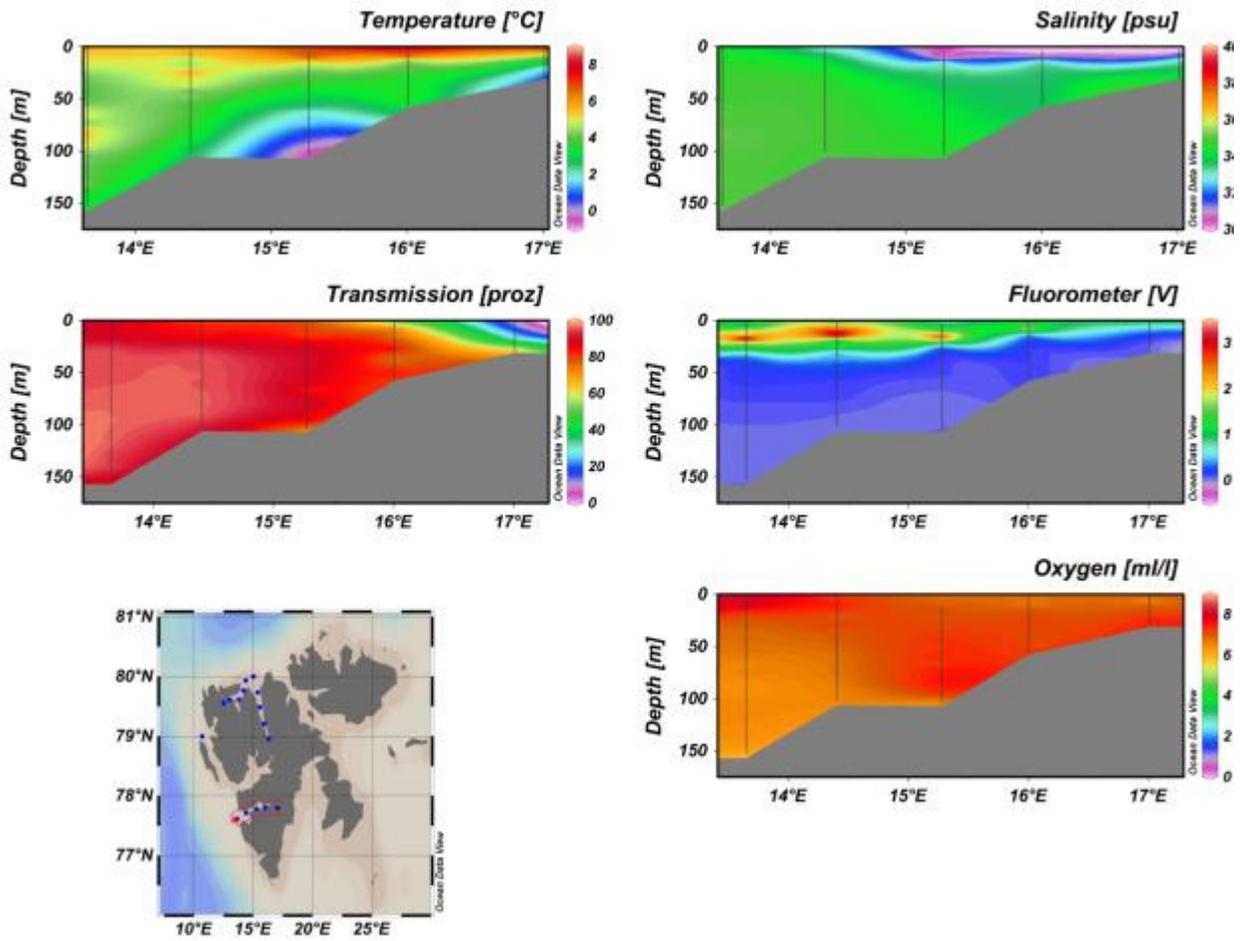


Figure 14 CTD profiles for temperature [°C], salinity [psu], transmission [%], fluorescence [V], and oxygen [ml/L] for the Van Mijenfjorden from outside to inside stations.

## Fjord II Wijdefjorden

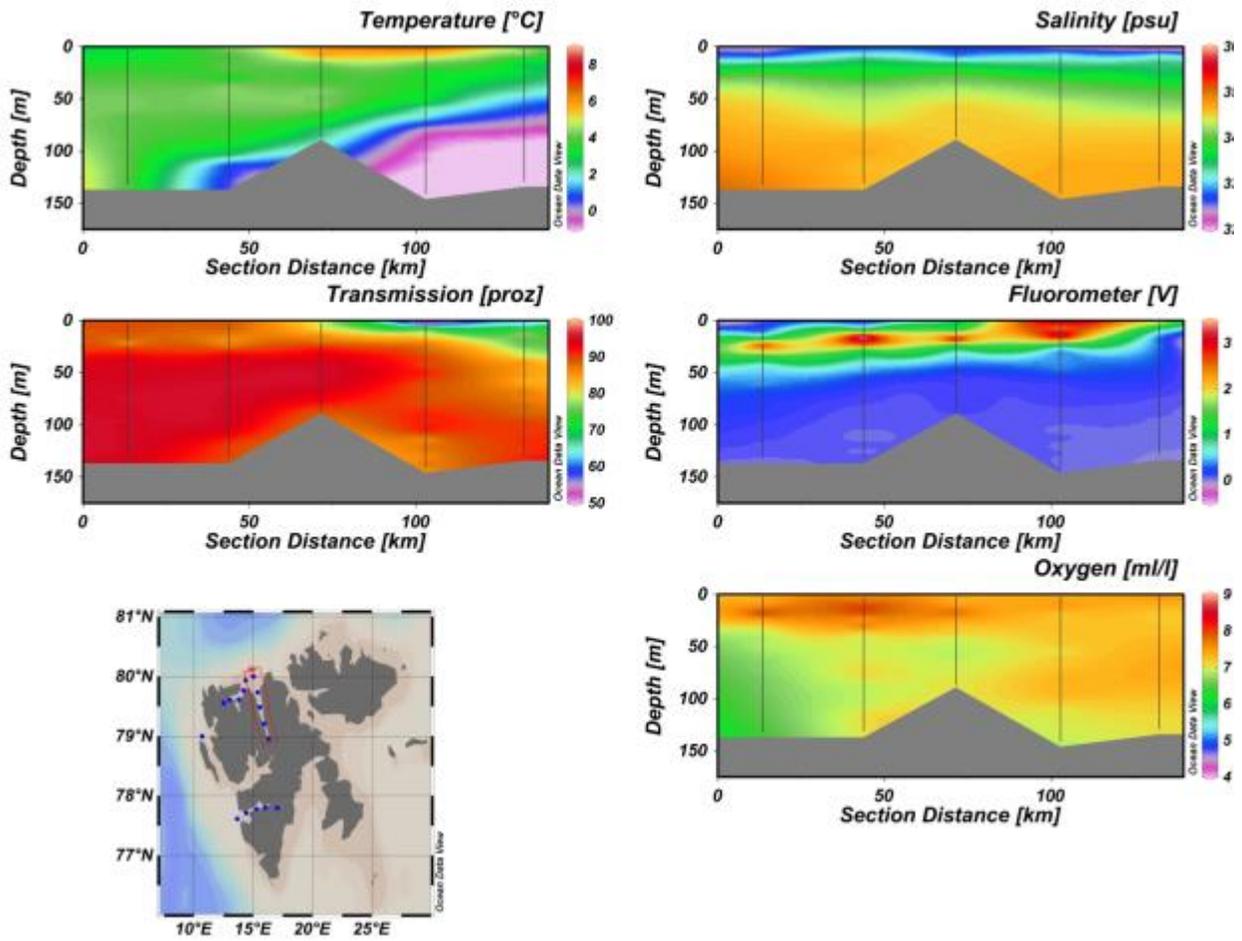


Figure 15 CTD profiles for temperature [°C], salinity [psu], transmission [%], fluorescence [V], and oxygen [ml/L] for the Wijdefjorden from outside to inside stations.

### Fjord III Woodfjorden

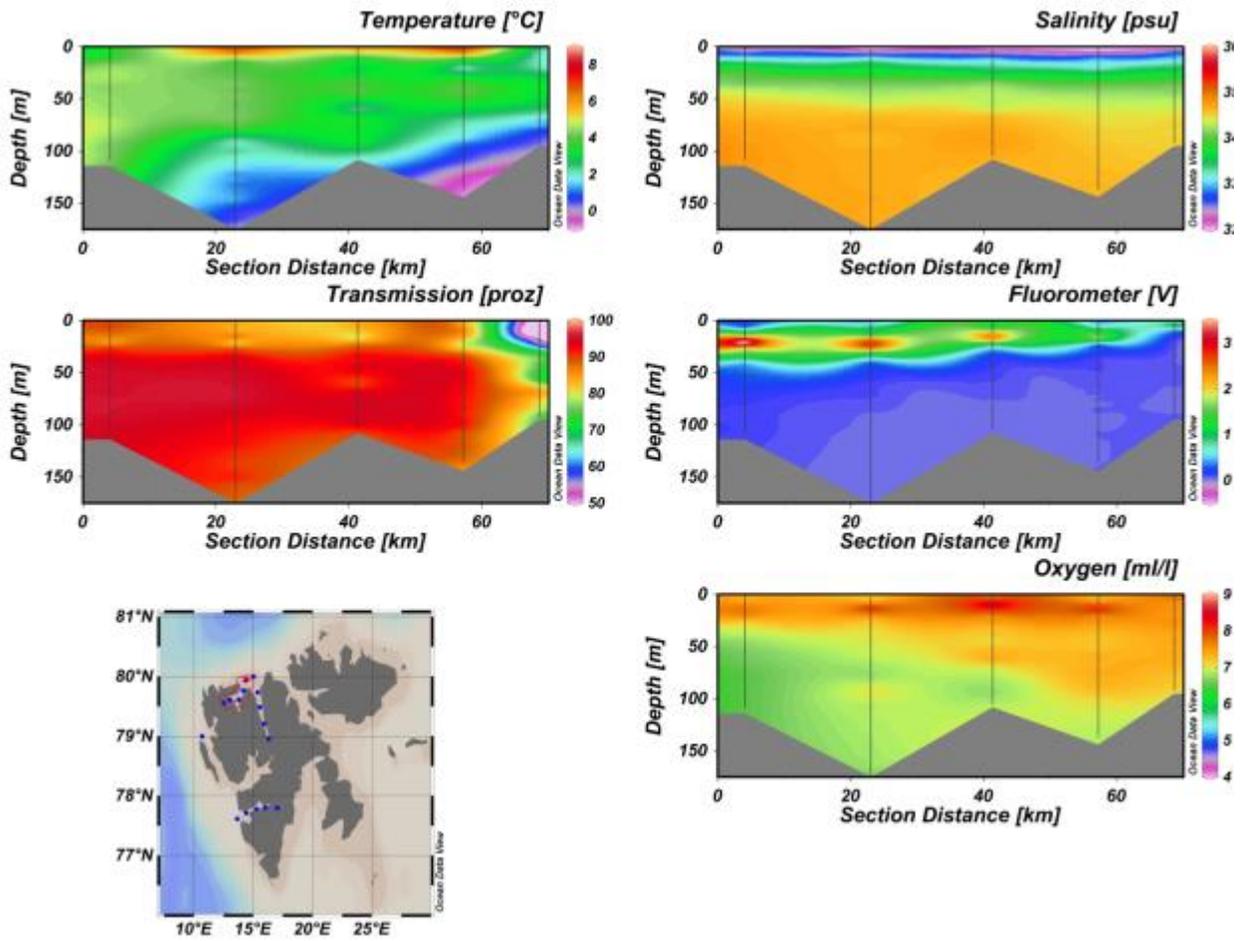


Figure 16 CTD profiles for temperature [°C], salinity [psu], transmission [%], fluorescence [V], and oxygen [ml/L] for the Woodfjorden from outside to inside stations.

### Fjord IV Kongsfjorden

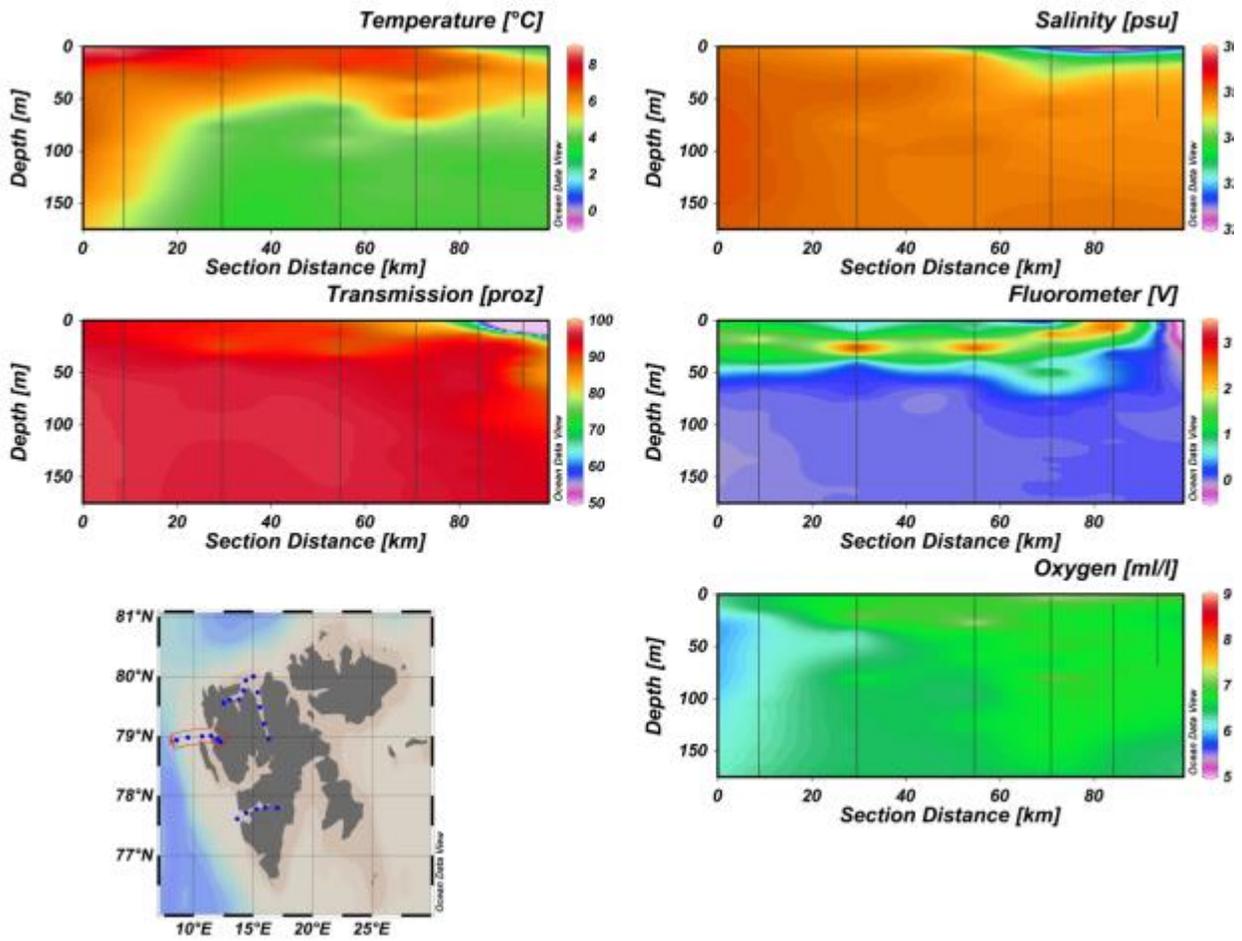


Figure 17 CTD profiles for temperature [°C], salinity [psu], transmission [%], fluorescence [V], and oxygen [ml/L] for the Kongsfjorden from outside to inside stations.

## Fjord V Isfjorden

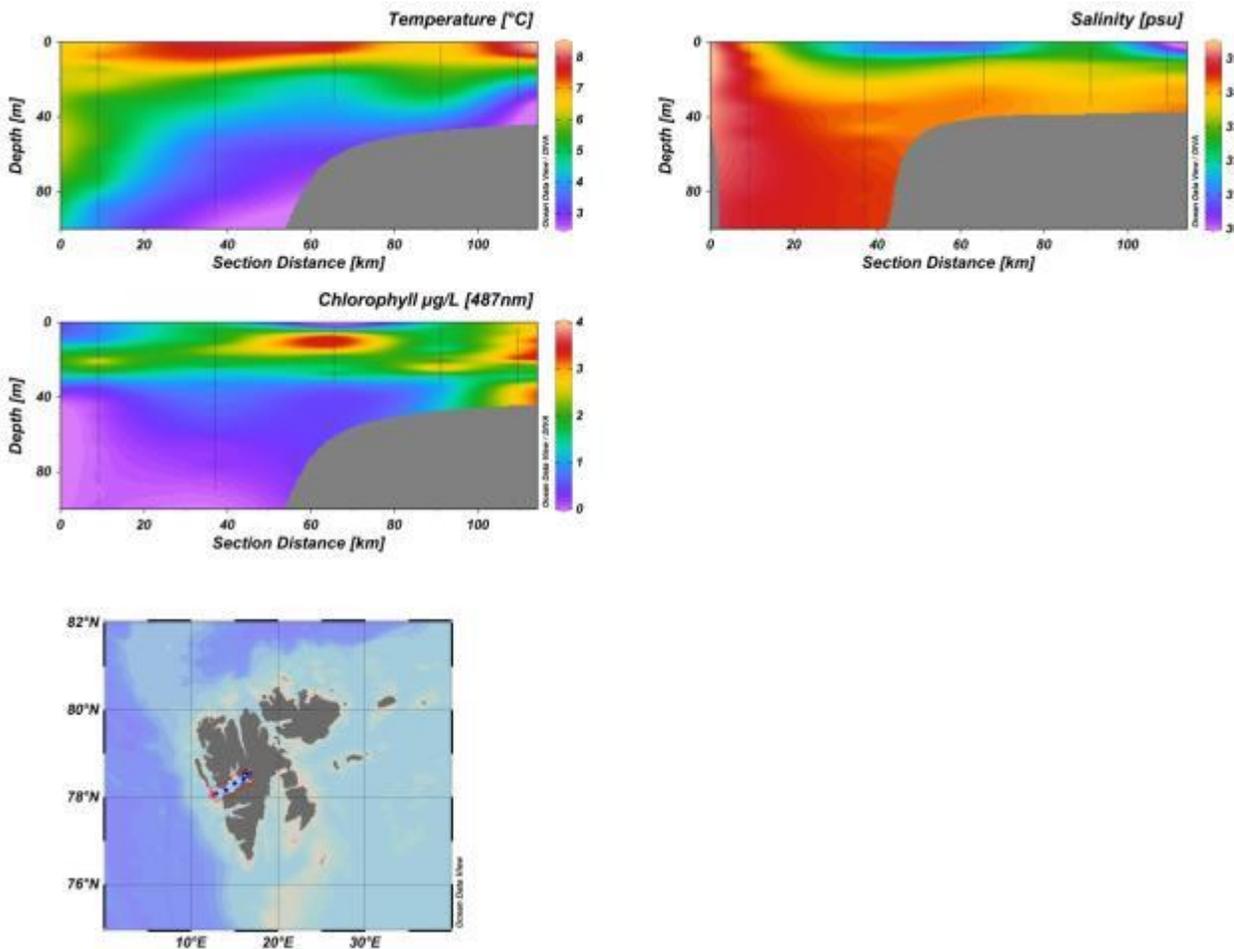


Figure 18 CTD profiles for temperature [°C], salinity [psu], transmission [%], fluorescence [V], and oxygen [ml/L] for the Isfjorden from outside to inside stations.

Due to technical problems with the winch of the ship, parameters for the Isfjorden were determined with the bio-optical profiling system which is equipped with an additional CTD. Therefore, only temperature [°C], salinity [psu] and Chl at 487 nm [µg/L] are shown for this fjord system.

For all other stations, the comparison of both probes of the CTD for temperature and salinity showed good agreement over the whole cruise.

### 7.1.2 Bio-optical parameters from the profiling system

At all daytime stations free-falling profiler measurements were conducted to determine light penetration in the water column. As a preliminary result for each fjord system the photosynthetically active rate [PAR, log] is shown from outside to inside stations (Figure 19 - Figure 23).

#### Fjord I Van Mijenfjorden

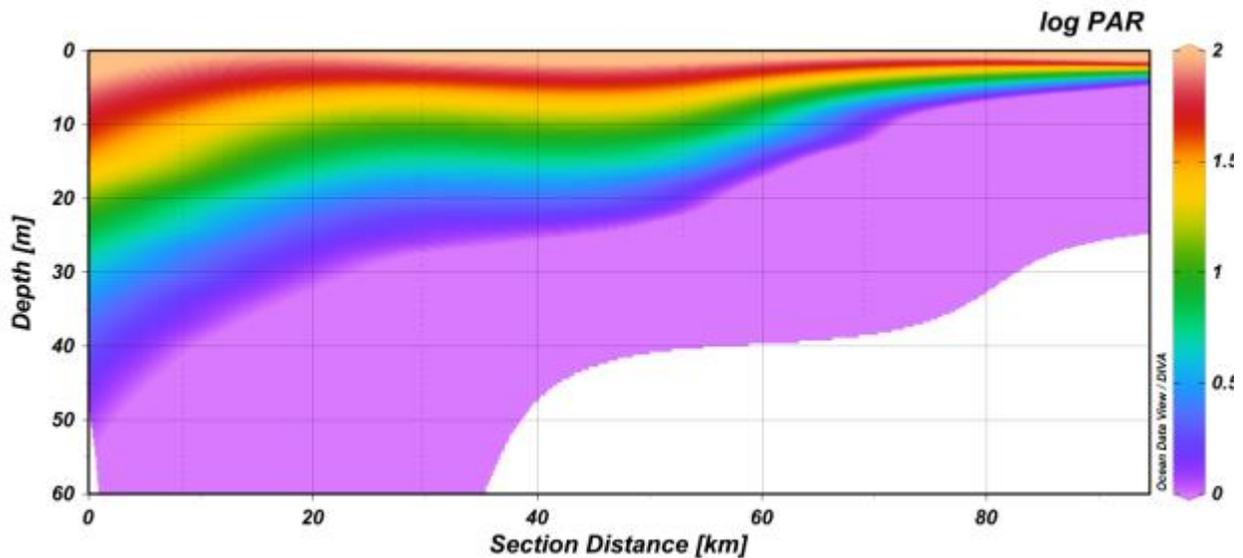


Figure 19 Photosynthetically active rate (PAR) profiles for the Van Mijenfjorden.

#### Fjord II Wijdefjorden

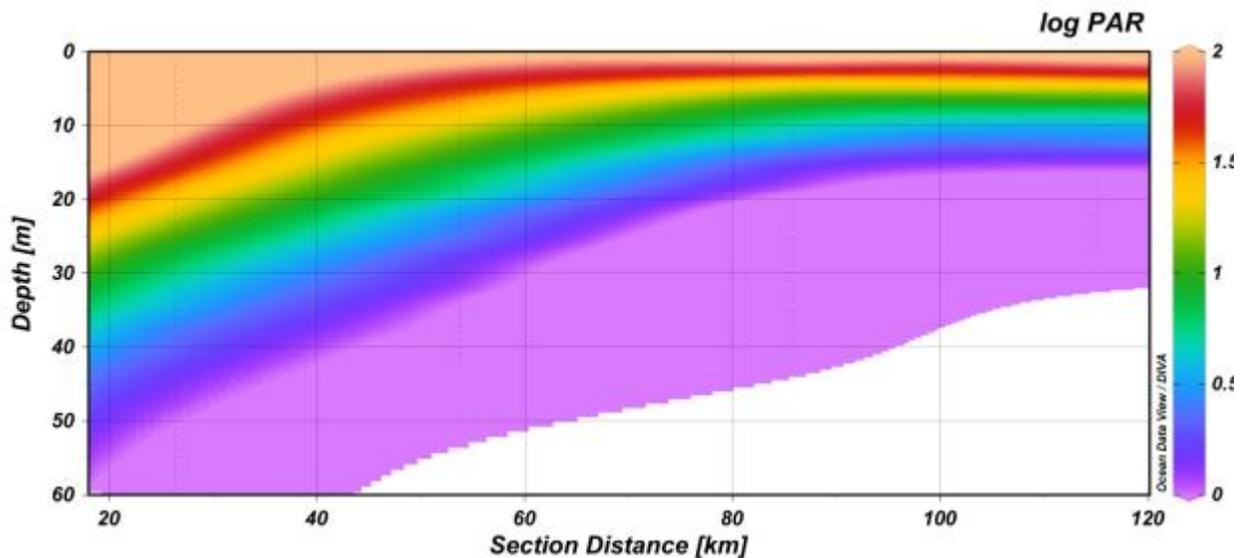


Figure 20 Photosynthetically active rate (PAR) profiles for the Wijdefjorden.

### Fjord III Woodfjorden

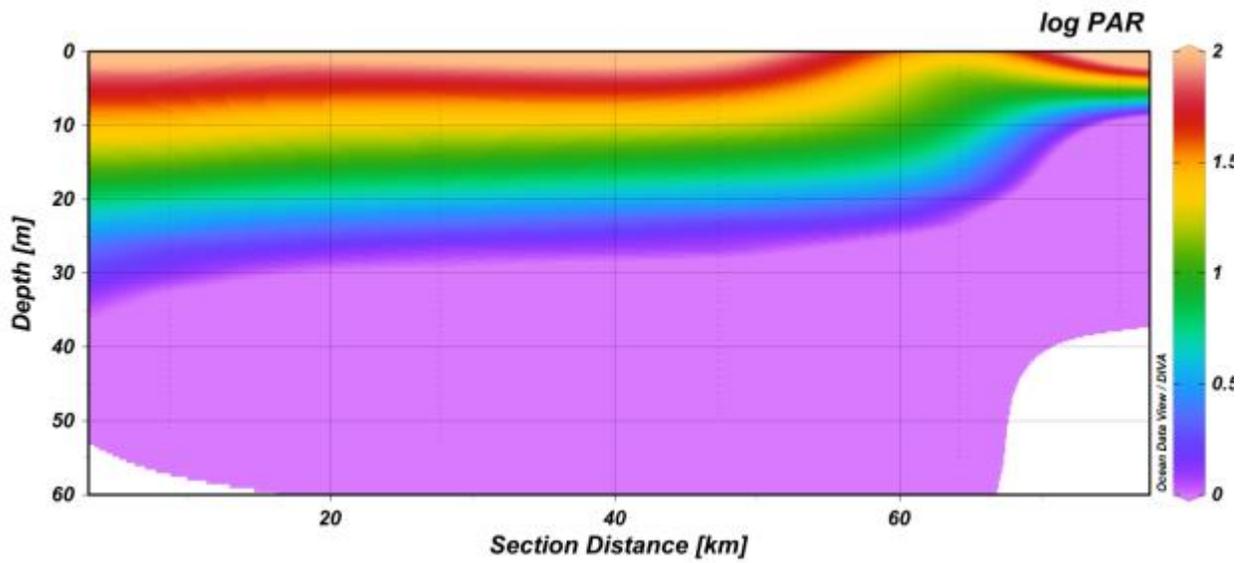


Figure 21 Photosynthetically active rate (PAR) profiles for the Woodfjorden.

### Fjord IV Kongsfjorden

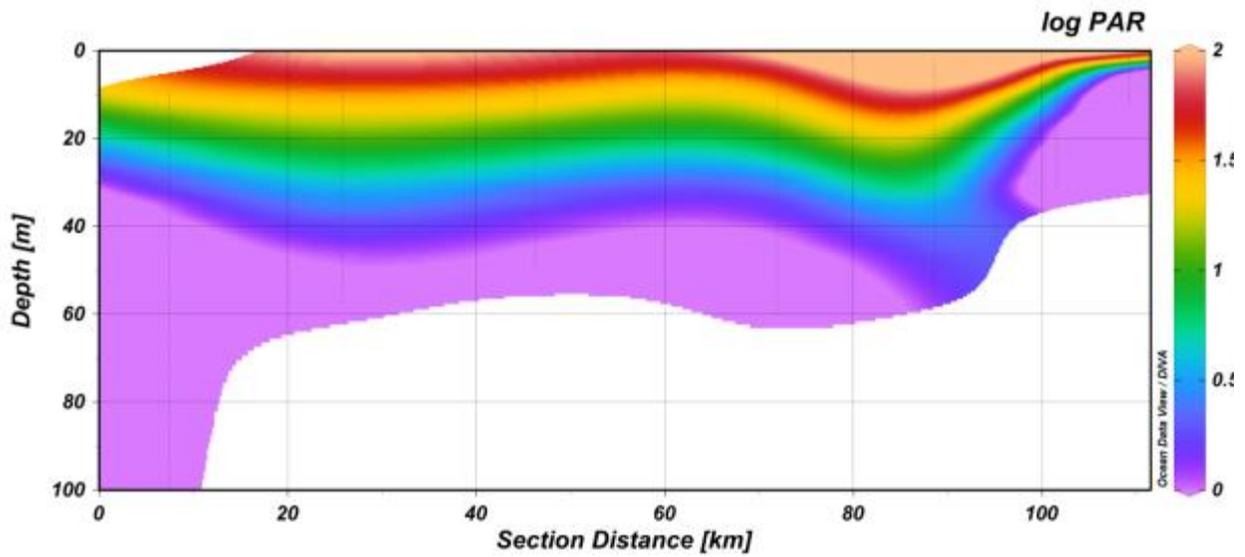


Figure 22 Photosynthetically active rate (PAR) profiles for the Kongsfjorden.

### Fjord V Isfjorden

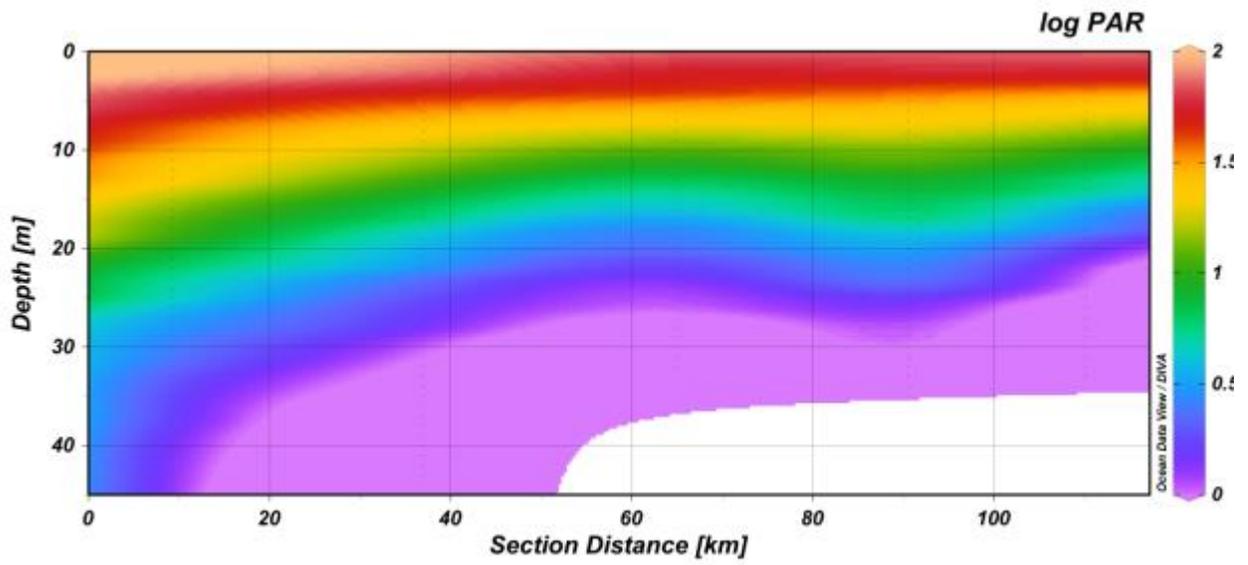


Figure 23 Photosynthetically active rate (PAR) profiles for the Isfjorden.

### 7.1.3 Ocean color sensing

Results from the Secchi disc measurements can be seen in Figure 24. The results of Forel-Ule indices (FUI) are shown in Figure 25, representing open sea and coastal regions. High penetration depths correspond well with lower FUI values. With further processing, more conclusions about existing components in the water (e.g., as an index of phytoplankton biomass in clearest waters) can also be drawn. Both data sets are needed as a reference for hyperspectral radiometric observations, as one goal is the determination of ocean color by radiometric measurements (compare Garaba et al, 2014). Analysis is still in progress.

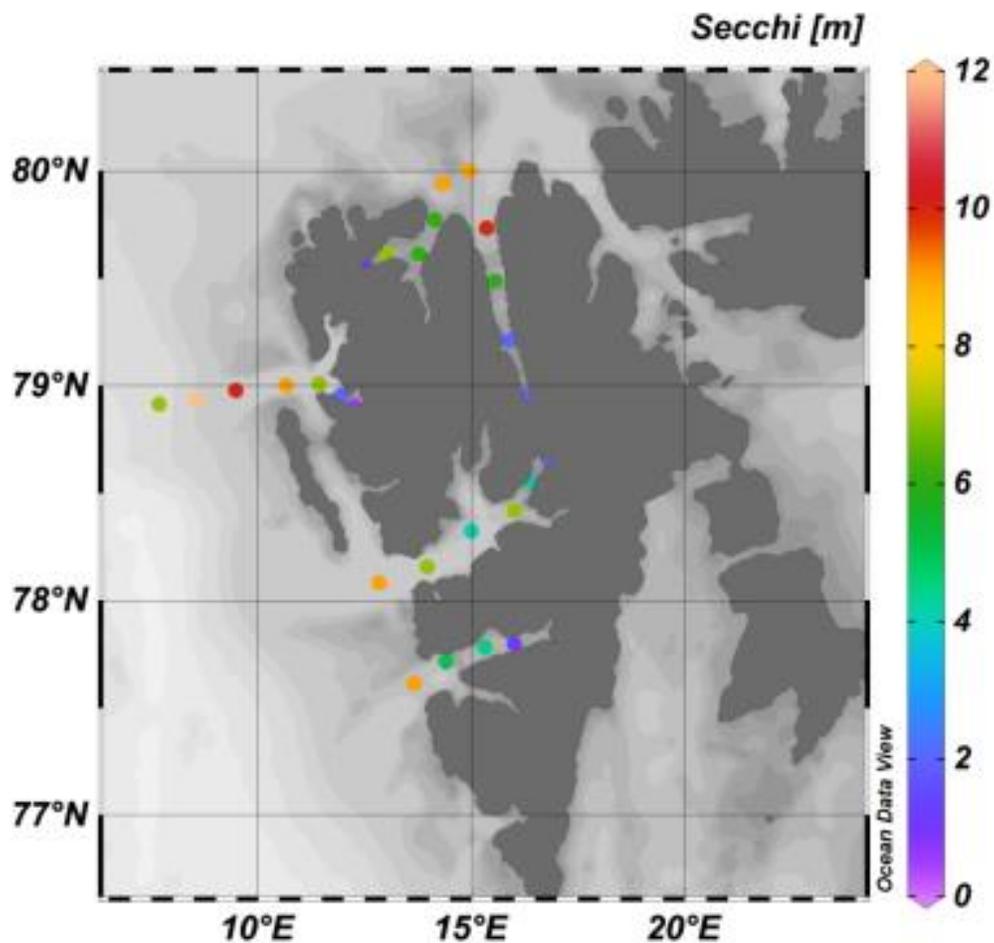


Figure 24 Measured Secchi disc depth [m] at daytime determined for investigated fjord areas around Spitzbergen.

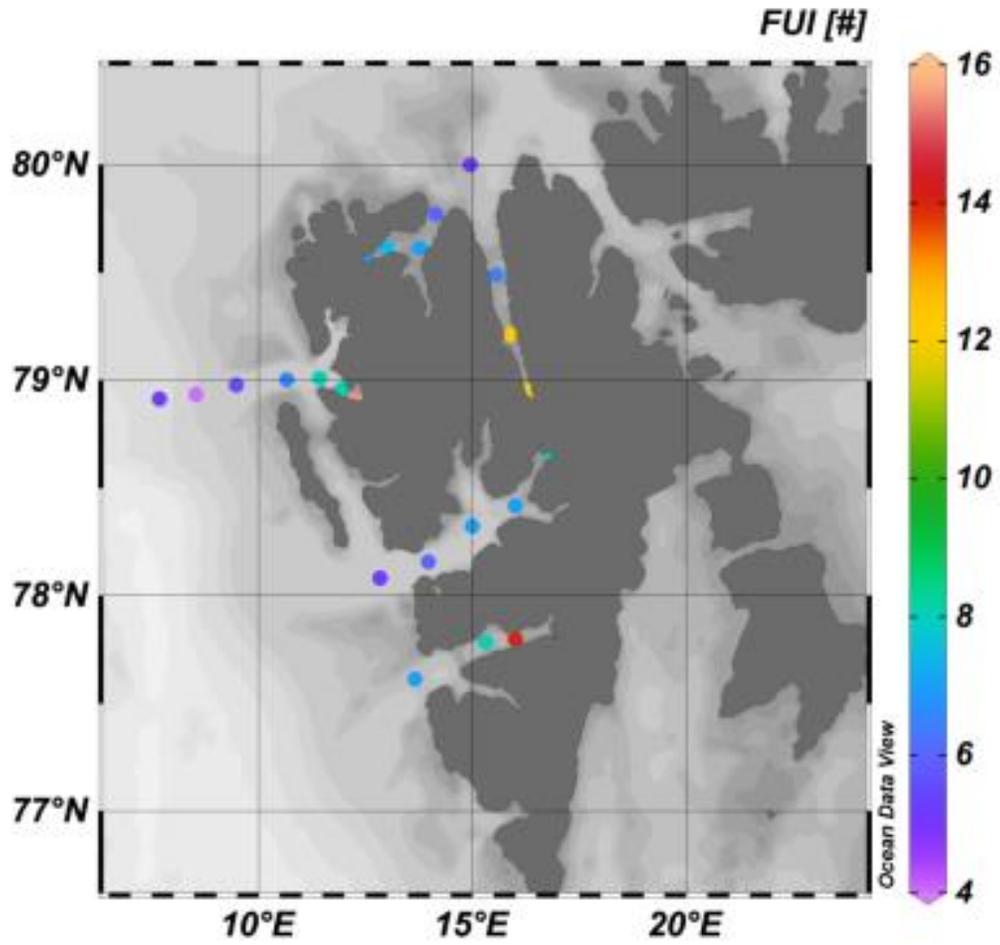


Figure 25 Measured Forel-Ule indices [#] determined for investigated fjord areas around Spitzbergen.

## 7.2 Microplankton species diversity

(Edvardsen)

See the text earlier which present the results.

Identification of the >20 µm size-fraction plankton composition from vertical phytoplankton net tows showed dominance of diatoms and dinoflagellates. Among the diatoms, the dominant genera were *Chaetoceros*, Thalassiosiraceae, *Pseudo-nitzschia*, *Rhizosolenia*, *Pseudo-nitzschia*, *Fragilariopsis*.

### **Cultures**

At University of Oslo, more than 50 pure strains of diatoms have been isolated and identified in light and electron microscopy, mainly from Widjefjorden and the Hausgarten stations, where the highest phytoplankton diversity. The strains are presently characterised genetically in the 18S and 28 rDNA gene. The culture work is performed as part of an assisiated RCN-funded project TaxMarc.

## 7.3 Molecular biodiversity

(Wohlrab, Kühne, John)

### **Metabarcoding**

Samples are extracted libraries are under production.

### **Metatranscriptomics**

Samples are extracted libraries are under production.

### **Single- cell-PCR (18S and 28S)**

The positive amplicons are in the process of being reamplified by PCR in the laboratory and prepared for sequencing via Sanger.

### **Single cell transcriptomics**

SMARTSeqV4 (Takara Clontech) single-cell transcriptomic samples have been quality checked via the Agilent 2100 Bioanalyzer and ligated with individual Illumina adapter sequences as stated in the Nextera XT protocol (Illumina). The cDNA-Library was recently paired end sequenced at an in house (AWI) Illumina NextSeq platform. Obtained reads have been trimmed and assembled (CLC Genomics Workbench) and are currently being annotated via Trinotate, to identify new marker genes for taxon-omics approaches.

### 7.4 Micrograzing

All samples from the grazing experiments are currently under analysis. Preliminary results from the O<sub>2</sub>-monitoring for the first experiment (Van Mijenfjord) are exemplarily shown in Figure 26.

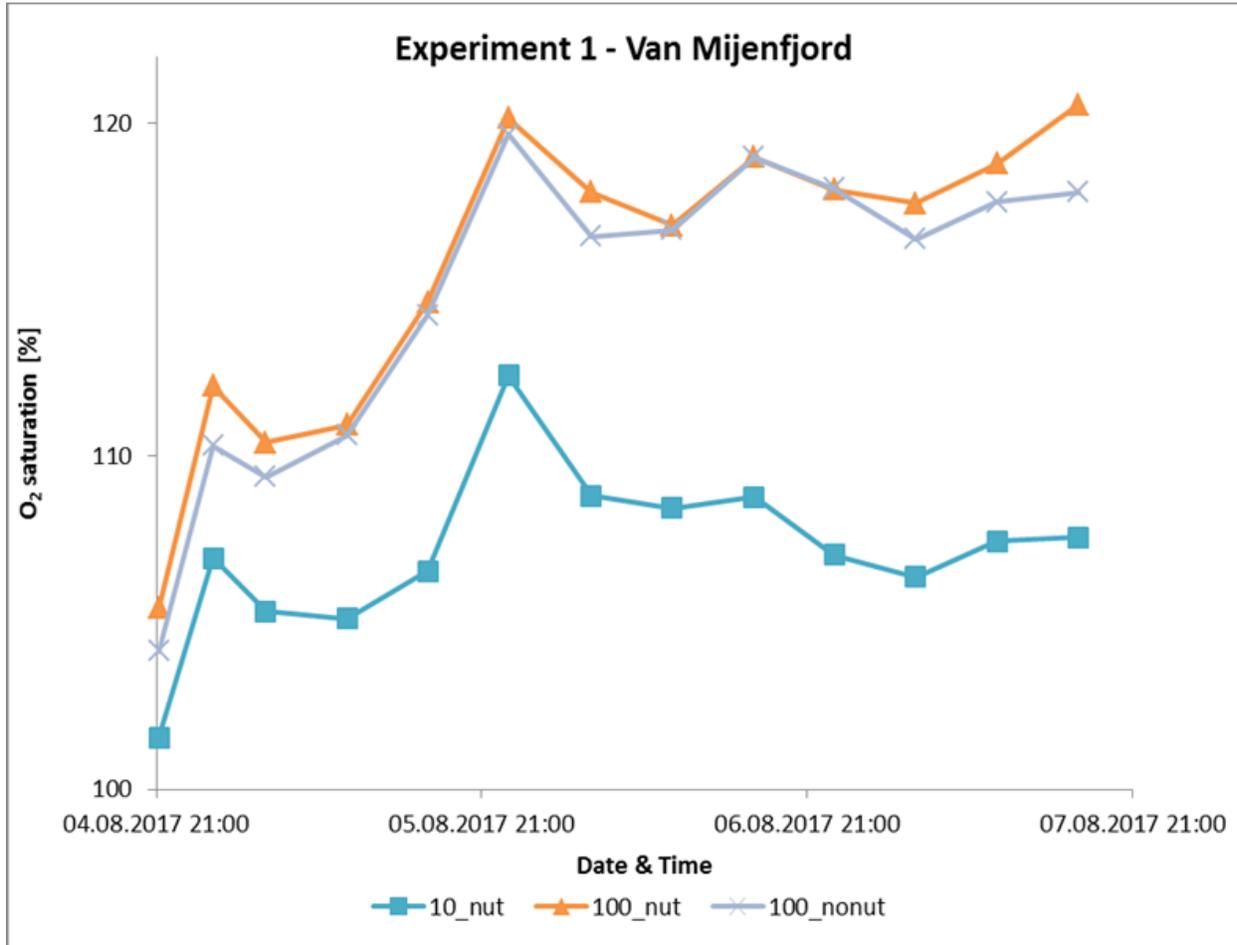


Figure 26 Diagram of oxygen saturation development over time of incubation in the first grazing experiment (Van Mijenfjord). 10\_nut: 10% sample water with nutrient addition; 100\_nut: 100% sample water with nutrient addition; 100\_nonut: 100% sample water without nutrient addition.

## 8 Station List

Stations 4, 8, 12, 15, 18, 22, 33 were sediment samples close to the next station, the short distance to the original sample station was conducted to avoid fate of sediments in the water column, the position where (st4) lat 77.800.020, long16.001.069; (st8) lat 80.010.865, lon15.039.333; (St12 lat79.220.357, lon 15.887.168; (St15) lat 79.607.366, lon 12.746.763; (St18) lat 79.619.088, lon 13.814.134; (St22) lat 78.998.269, lon 10.610.385; (St33) lat 78.419.865, lon 15.954.642, respectively (Table 2).

Table 2 Stations list of the PolFjord cruise (HE492).

Station	Date		Latitude	Longitude
1	30.07.2017	Lofoten	67,072454	13,013178
2	03.08.2017	Van Mijenfjord	77,7945	17,0115333
3	03.08.2017		77,7997667	16,0005333
5	04.08.2017		77,7826333	15,32055
6	04.08.2017		77,7154	14,4055
7	04.08.2017		77,6118167	13,6465167
9	06.08.2017	Wijdefjord	80,0006667	14,9009
10	06.08.2017		79,7334167	15,3606167
11	06.08.2017		79,4880833	15,55615
13	07.08.2017		79,20885	15,9080667
14	07.08.2017		78,9549167	16,3147
16	09.08.2017	Woodfjord	79,5622333	12,4622167
17	09.08.2017		79,6139333	12,97975
19	10.08.2017		79,61475	13,7603833
20	10.08.2017		79,7731	14,13285
21	10.08.2017		79,9409833	14,3343833
23	11.08.2017	Kongsfjord	79,0052667	10,6523167
24	11.08.2017		79,0122667	11,43505
25	11.08.2017		78,9599167	11,9663333
26	12.08.2017		78,9118833	12,2373
27	14.08.2017	Hausgarten	78,9791	9,4613
28	14.08.2017		78,9349333	8,52681667
29	14.08.2017		78,91545	7,66811667
30	15.08.2017	Isfjord	78,0827167	12,8502667
31	15.08.2017		78,1664833	14,0014333
32	15.08.2017		78,3184	15,0022333
34	16.08.2017		78,4180167	16,0042167
36	16.08.2017		78,5489	16,3852833
37	16.08.2017		78,661658	16,80762

## **9 Data and Sample Storage and Availability**

All data will be transferred to the PANGAEA database as soon as they are available and quality checked. Depending on data type and progress of sample analysis, this will be done within 2-3 years. Already several datasets were submitted to PANGAEA, allocated by the cruise identifier HE492. The following compilation names the scientists who are responsible for access to the different data and sample sets.

CTD and bio-optics data are held at the ICBM (Oldenburg) and were analyzed by the group of Prof. Dr. O. Zielinski. CTD data and accompanying information were already submitted to PANGAEA.

Nutrient and DOM data are archived at AWI (Bremerhaven) (Prof. Dr. B. Koch, U. John).

Most algal strains isolated during this cruise are maintained at U Oslo (Prof. Dr. B. Edvardsen) and are available on request.

Genomic analysis was performed by AWI (Dr. S. Wohlrab, Dr. U. John). Information will be stored in the AWI repository and (where applicable) transferred to PANGAEA.

Cyst isolation and distribution data were analyzed by AWI (Prof. Dr. A. Cembella) and will be uploaded to PANGAEA. No cultured isolates were successfully obtained from cysts due to the low abundance and sub-optimal maturation stage of dinoflagellate cysts, but there may be single-cell PCR confirmation of identification in a few cases.

## **10 Acknowledgements**

The scientific team is very grateful to Captain Hays Diecks and crew of the *RV Heincke* for cooperative and efficient assistance with operation and deployment of scientific equipment, and for the friendly and positive working and social atmosphere on board. The HAB research was conducted in the framework of the IOC/SCOR program GLOBALHAB, as successor to the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) Core Research Project on HABs in Fjords and Coastal Embayments. The AWI contribution was provided by the HGF Program Earth and Environment under PACES Theme 2 (Coast) Workpackage 3.

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