NPI Cruise Kongsfjorden July 12-17, 2018

Katrine Husum and cruise scientists

Norwegian Polar Institute

Contents

Introduction

Norwegian Polar Institute has since the mid-1990s carried out annual monitoring and research cruises to Kongsfjorden, Svalbard. This cruise has evolved including more parameters as technology and knowledge improved, and more stations from the shelf and eastern Fram Stratit have been added to the original stations inside Kongsfjorden (Figure 1). A new parameter was added to the 2018 cruise as sediment samples were collected to determine whether they contained any microplastic. The cruise was carried out with M/V "Lance" rented from Northshore A/S with a new crew.

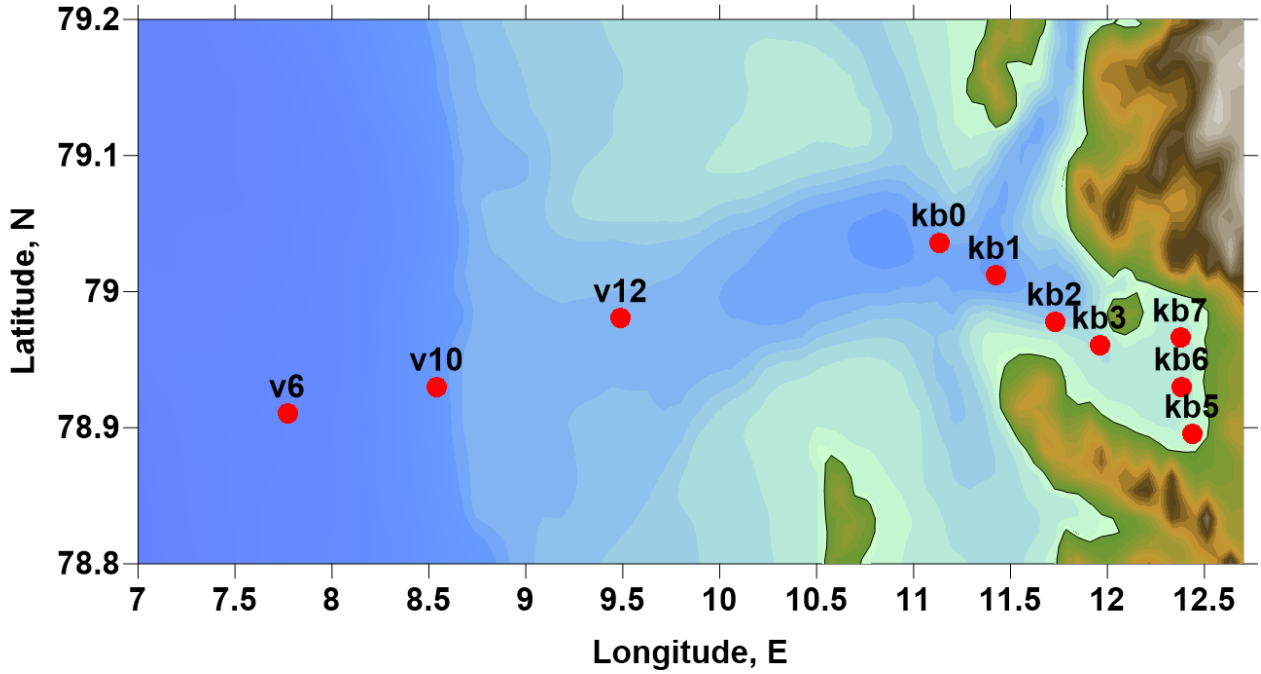


Figure 1. Transects locations and station positions (red filled circles).

Programmes and projects

MOSJ pelagic survey - Kongsfjorden

Responsible: Haakon Hop (NPI), Lasse Mork Olsen (NPI) & Anette Wold (NPI)

MOSJ (Environmental monitoring – Svalbard and Jan Mayen, www.mosj.npolar.no/) is a program to monitor changes in the Arctic Ecosystem at Svalbard and Jan Mayen. As a part of MOSJ, the survey in Kongsfjorden - Fram Strait (KongHau) is used by NPI to monitor long-term changes in phytoplankton and zooplankton as well as trophic structure of this ecosystem. The survey in Kongsfjorden and Fram Strait which includes CTD, nutrients, phytoplankton, zooplankton and benthos is maintained through joint efforts by Akvaplan-niva (Apn), the University of Tromsø (UiT), the University Centre in Svalbard (UNIS), the Norwegian Polar Institute (NPI), the Polish Institute of Oceanology (IOPAS) and the Alfred Wegener Institute of Polar and Marine Science (AWI).

- The transect consists of seven stations inside the fjord (Kb7, Kb6, Kb5, Kb3, Kb2, Kb2, Kb1, Kb0), three stations on the shelf (V12, V10, V6) and three stations off the shelf (KH, HG-I, HG-IV).
- The transect is sampled every year in July, additional seasonal studies was done in 2002, 2006 and 2007.
- CTD and zooplankton sampled since 1996.
- Chlorophyll, nutrient, phytoplankton, sampled since 2009
- Fatty acid and stable isotope of phytoplankton, zooplankton, fish, benthos and seabirds are also analysed but not on a regular basis.
- All phytoplankton and zooplankton data are analyzed to species level in cooperation with the Institute of Oceanology in Sopot, Poland.
- All data is stored in the Marine database at NPI.

Ocean Acidification (OA)

Responsible: *Agneta Fransson (NPI) & Melissa Chierichi (IMR)*

Investigate the natural calcium carbonate saturation state (Ω) in the area around Svalbard.
Influence of water mass composition (Atlantic, polar, fjord water) and freshwater (glacier, sea-ice and river) on Ω. Distribution of *Calanus*, *Limacina helicina* will be related to Ω state.
Pterodpods should be collected from Multinet and MIK when present and stored in 95% alcohol.

Planktonic microbial communities (PMC)

Responsible: *Pedro Duarte (NPI), Lasse Mork Olsen (NPI) & Catarina Magalhães (CIIMAR, Portugal)*

Genomics

We aim to proceed with a comprehensive study on the diversity and functional aspects of planktonic microbial communities at the genomic and biogeochemical levels. The data generated will complement the sampling effort being performed by the NPI team on board. To accomplish our goals water samples that will be collected during **KR Cruise 2017** will be processed on board by concentrating it through filtration and preserved on board for later DNA extraction, and genomic and metagenomic analysis. After successful filtration of 3-5L, sterivex filter units with concentrated microbial planktonic communities will be stored at -80°C, for latter sequence analysis at CIIMAR

Biochemistry: Nitrogen

We are also planning to perform some incubations to do Nitrogen activity-based measurements using isotope-paring techniques (IPT). Water samples will be amended with ^{15}N-^{14}N isotopic mixtures proceeded with time series incubations. After this procedure on board samples will be preserved (at minus 80 °C) and the different N_2 produced ($^{28}N_2$, $^{29}N_2$ and $^{30}N_2$) will be quantified by isotope ratio mass spectrometry (IRMS) at CIIMAR.

ARISE: Isotopes (carbon and nitrogen) and HBIs

Responsible: *Haakon Hop (NPI) & Raja Ganeshram (University of Edinburgh, UK)*

ARISE

ARISE – Seeks to identify pan-Arctic variability in the baseline of stable isotopes of carbon and nitrogen. Water samples will be collected, fractionated and analysed as part of a larger initiative that will simultaneously collect samples from the eastern Barents Sea and Canadian Arctic to quantify Arctic-wide baseline variability in stable isotopes. This data will be complemented by time series analyses of harp and ringed seals as well as satellite telemetry tracking of seals. Combined, it will explore how baseline variability might be propagated to Arctic marine mammals, affecting interpretations and comparisons of pan-Arctic trophic structure.

ARISE will be collecting water samples for 15N nitrate and 15N DON and a smaller subset samples for 30Si. They will also be be filtering for particles for 13C, 15N 30Si (small subset). Very similar to what was done in 2017.

Marine geology and paleoceanography (MGP)

Responsible: *Katrine Husum (NPI) & Arto Miettinen (NPI)*

Benthic foraminiferal monitoring in Kongsfjorden and Rijpfjorden

This project aims to monitor and quantify the response of benthic foraminifera to different physical environmental forcing in the Arctic, e.g. with regard to variations of the influence of Atlantic Water in Kongsfjorden. Benthic foraminifera are good monitors of environmental changes because they are very abundant in the surface sediments and very diverse even in Arctic coastal and fjord settings. Furthermore, they have a short life cycle enabling to

respond quickly to changes. After death, the shells of the benthic foraminifera are preserved in the sediment enabling baseline studies of pre-impacted conditions back in time. A CTD cast/data will also be necessary on all these stations in order compare the modern foraminiferal data with the modern hydrographic data. The transect consists of five stations inside the fjord (Kb3, Kb2, Kb1, Kb0, FM).

Paleo-records – reconstructions of sea ice and water mass variability

In order to obtain data on temperature, salinity etc. in the water masses from the past when it was not possible to get instrumental measurements of these factors; we study fossil micro-fauna (foraminifera) and micro-flora (diatoms) in the sediments in addition to their chemical composition (biomarkers). Their composition and abundance depend on many environmental factors like temperature and salinity of the sea water, thus showing how the marine environment and water masses were back in time (paleo-oceanographic proxy data).The second aim of the project is to improve the proxies of ocean temperature and sea-ice and apply them to down core sediment samples from the multicores (fossil data). Hence, the monitoring data will also serve as modern analogue data for interpretation of fossil data. The modern analogue data will also be utilized when developing statistical models making quantitative reconstructions (transfer functions based on benthic foraminifera and diatoms). The paleo-records will be established at selected stations in Kongsfjorden.

Field Sampling and Laboratory Methods for the Analysis of Microplastics in Sediments from Kongsfjorden, Svalbard (MiP)

Responsible: Ingeborg Hallanger (NPI), Katrine Husum (NPI) & Geir Wing Gabrielsen (NPI)

This project will collect sediments from Kongsfjorden and Rijpfjorden, Svalbard in order to investigate the levels of microplastics and toxicants in samples. Furthermore, we want to build upon and validate new methods of sampling and analyzing sediments for microplastics and toxicants. Recently the presence and potential hazardous effects of plastic materials were identified in the Arctic and deep-sea sediments demonstrating the ubiquitous distribution of this newly identified anthropogenic pollution on a global scale. There is a severe lack of methods for identification, characterization and quantification of micro- and nanoplastics in the environment. Owing to the small size and relatively large surface area of the microplastics, waterborne organic pollutants can adsorb onto the particles. In addition, the leaching of toxic plasticisers, such as phthalates, in addition to various persistent organic pollutants (POPs) and heavy metals are of concern.

Diving: collecting bivalves

Responsible: Haakon Hop (NPI)

This project aims to investigate and establish bivalves as records of seawater conditions around Svalbard. Two dives were carried out during the cruise in order to collect bivalve samples from Kongsfjorden (Gluudneset and). These sites have been sampled before and are known to host bivalves. Additional bivalves were also collected in order to identify if they contained any microplastic.

NCAOR ocean mooring rig

Responsible: *Arild Sundfjord (NPI), Divya David (NCAOR), G. Raguraman (NIOT) & A. Thirunavukkarasu (NIOT).*
Since 2014 NCAOR has had a mooring rig in Kongsfjorden measuring various oceanographic parameters troughout the year. The rig contains CTD sensors, nitrate sensors, ADCP (Acoustic Doppler Current Profiler) etc at different

depths. The last day of the cruise was dedicated for retrieving and deploying the mooring rig. Two participants came onboard from Ny-Ålesund and left again before the vessel left for Longyearbyen.

Work on board and results

From the start it was clear that the outermost station HG-IV would not be sampled during this cruise as one day (July 16) had been dedicated to the Indian mooring in Kongsfjorden. However, departure from Longyearbyen was delayed as M/V "Lance" arrived later than planned in Longyearbyen. Additionally, overall operations during the cruise took much longer than usual due to a new crew onboard. It was not possible to carry out all planned sampling; not all stations were visited, and only a reduced sampling scheme was obtained at the two last station V10 and V6 (Figure 1).

Area	Station	Latitude	Longitude	Depth(m)	Sampling
Kongsfjorden	Kb7	78° 57.98'	12° 22.60'	64	Pelagic
	Kb6	78° 55.81'	12° 23.11'	83	Pelagic
	Kb5	78° 53.79'	12° 26.45'	96	Pelagic; Marine geology
	Kb3	78° 57.24'	11° 57.38'	329	Pelagic; Marine geology
	Mooring				NCAOR Mooring
	Kb2	78° 58.68'	11° 43.91'	330	Pelagic; Marine geology
	Kb1	79° 00.67'	11° 25.66'	352	Pelagic; Marine geology
	Kb0	79° 02.78'	11° 08.36'	315	Pelagic; Marine geology
	FM	79° 03.05'	11° 05.17'	328	Marine geology
	V12	78° 58.79'	09° 29.77'	224	Pelagic
	V10	78° 55.96'	08° 32.82'	291	Pelagic
	V6	78° 54.39'	07° 46.24'	1125	Pelagic

Table 1. List of stations visited during the cruise.

Hydrographic survey

Responsible: Olga Pavlova (NPI) & Gary Griffith (NPI)

One of the aims of the cruise was do targeted oceanographic measurements covering the following key topics: distribution hydrography and other physical parameters of water mass in Kongsfjorden, Svalbard.

Data were collected using a range of instruments. CTD are used for collection of vertical profiles of conductivity (salinity), temperature, and associated parameters like chlorophyll, radiance etc. The CTD rosette is also equipped with water sampling bottles. PAR sensor was not available in the cruise (not installed). The CTD used an SBE911+ unit. Niskin bottles were closed using the bottle fire command within the Sea-Bird acquisition software so that a .bl file was created for each deployment when bottles were fired. No direct NMEA feed (depth, latitude and longitude) in all stations. Headers (.hdr and .hex files) were filled. Cast starting times were automatically added to the header of

all data files. A paper log sheet was completed at each CTD station. Log sheets list the depths at which bottles were fired and the samples taken from each bottle. Times and positions manually recorded on log sheets are indented as a backup in the case of a problem with the data acquisition, not a replacement for logged time and position data.

CTD data acquisition

In order to obtain 'round the clock' CTD data the scientific personnel was divided into two groups. Team 1 (Tomas Torsvik and Olga Pavlova with the two persons from the crew) ran the 00 – 06 shift whilst Team 2 (Gary Griffith and Marius Bratrein with the two persons from the crew) performed the 06 to 12 shift.

Once in the water the logging was initiated using SeaSave the Seabird data acquisition software. The salinity and temperature values were monitored until they were stable and then the CTD was lowered at about 1m/s. Water samples were 'fired' on the upcast.

At the end of a station the CTD was taken back on deck, wheeled into the shelter where it could be fastened to the ship before 'Lance' headed to the next station. After which the data was downloaded, changed to ASCII using Data Conversion (DatConv) on the SBE data processing software. The resulting CNV file was used for plotting of TS vertical profiles and transects. At this stage no other processing was performed to the data.

Hydrographic structure

Vertical transects

Positions of the CTD stations and transects for Kongsfjorden (KF) are presented in Figure 1 and Table 1.

Figures 2 and 3 show temperature and salinity distribution at the KF transect. The major physical features found in the section include the area of high water temperature and low salinity (especially in the eastern part of KF) in the surface layer. The high water temperature in the surface layer (T>6.4^{o}C) was formed due to spreading of the Atlantic waters (AW) from west and summer warming (Fig. 2). The salinities in the western part of the transect have typical values for AW in the surface layer S>35 psu (Fig. 3). Thickness of AW at the stations V10 and V6 is about 170m.

Low salinities in the eastern part of the fjord are result of glacier melting. Well pronounced salinity frontal zone is presented near the station KB0. This front separates low salinity melting waters and waters of the ocean origin with relatively high salinities. Figure 4 shows fluorescence distribution at the KF transect. Largest values of the fluorescence here are in the western part of the KF transect (v12, v10, v6), in the layer from 10 to 40 m, with the maximum (up to 14 mg/m^3) near the station v10. Deeper 50-80 m fluorescence practically is equal zero.

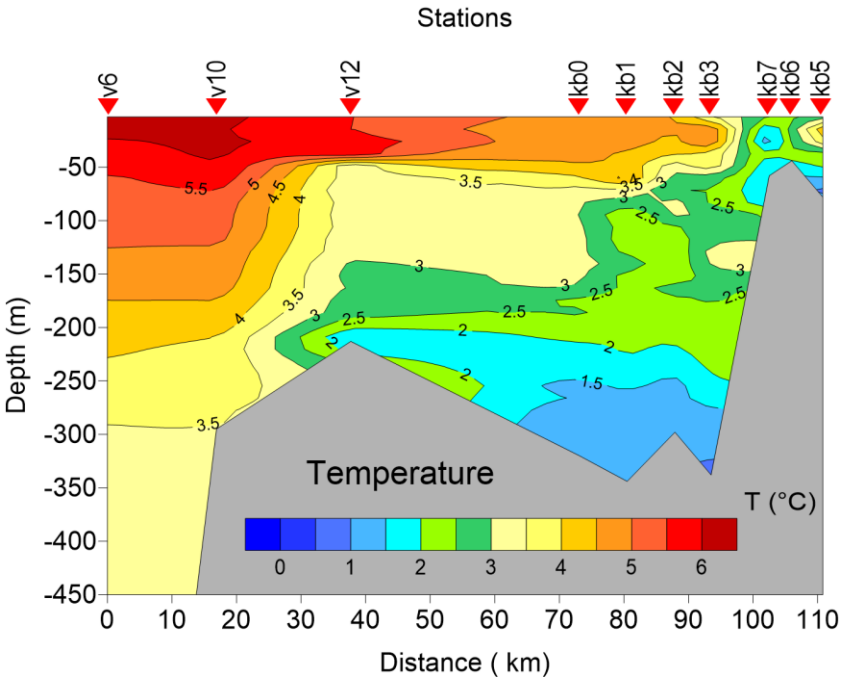


Figure 2. Temperature distribution at the transect in Kongsfjorden (profiles V6, V10, V12, KB0, KB1, KB2, KB3, KB7, KB6 and KB5). Station positions are shown in Figure 1 and Table 1.

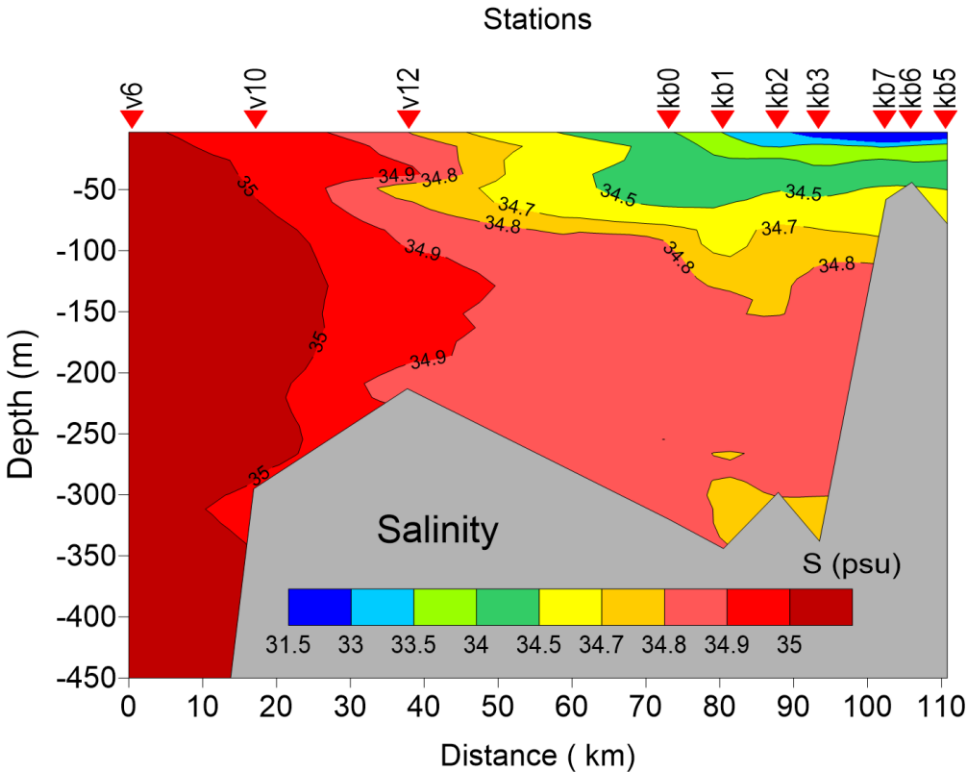


Figure 3. Salinity distribution at the transect in Kongsfjorden (profiles V6, V10, V12, KB0, KB1, KB2, KB3, KB7, KB6 and KB5). Station positions are shown in Figure 1 and Table 1.

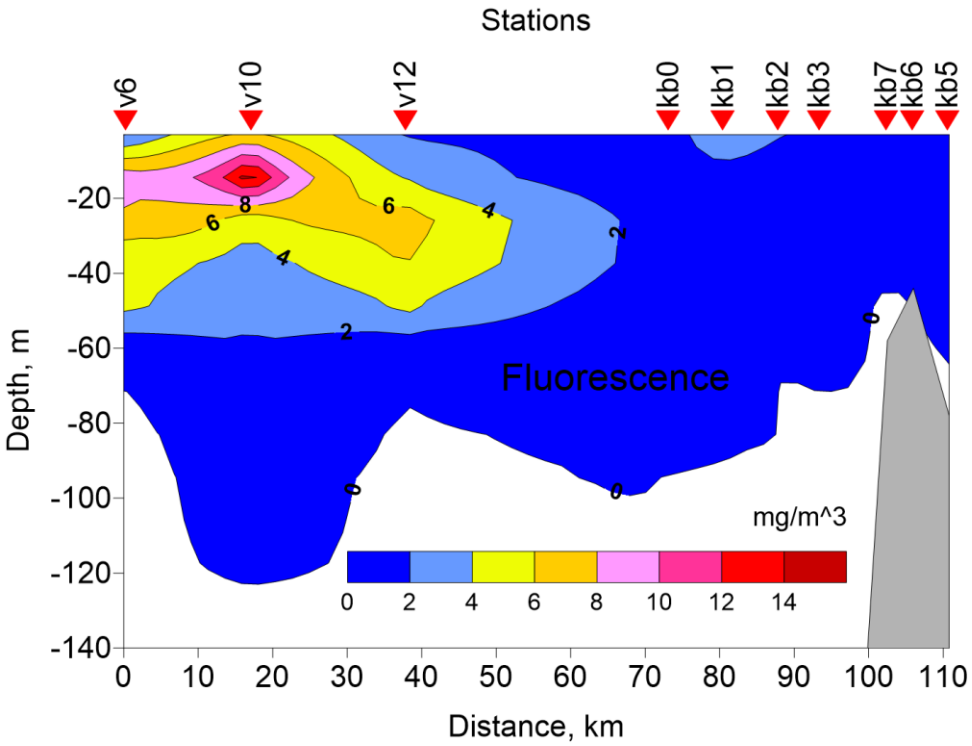


Figure 4. Fluorescence distribution at the transect in Kongsfjorden (profiles V6, V10, V12, KB0, KB1, KB2, KB3, KB7, KB6 and KB5). Station positions are shown in Figure 1 and Table 1.

Biogeocehmical variables and phytoplankton

Responsible: *Jozeph Wictor (IOPAS), Agnieska Tatarek (IOPAS), Svein Kristiansen (UiT) & Anette Wold (NPI)*

Water samples were collected from Niskin bottles from the CTD rosette. Chlorophyll and ammonium samples were analyzed directly on board. Samples for phyto- and microplankton taxonomy will be sent with RV *Oceania* and will be analyzed at IOPAS in Poland. Particulate organic carbon and nitrogen (POC/PTDN) will be analysed at SYKE in Helsinki by Hermanni Kartokallio. Nutrient samples will be analyzed at IMR by Kjell Gundersen.

Zooplankton

Responsible: *Anette Wold (NPI)*

Mesozooplankton was sampled with multiple plankton sampler (MPS, Hydro-Bios Kiel), consisting of five closing nets with 0.25 m^2 opening and 200 µm mesh size. Macrozooplankton was sampled with a Midwater Isaak Kit Trawls (MIK) with 3.14 m^2 opening and 1500 µm mesh from the total water column. The standard depths for MPS samples was as follows:

Bottom depth <600m; bottom-200m, 200-100m, 100-50m, 50-20m, 20-0m

Bottom depth >600m; bottom-600m, 600-200m, 200-50m, 50-20m, 20-0m

All MPS samples were preserved immediately after sampling and stored on 4% formaldehyde solution buffered with hexamintetrahydrat. The MIK samples were splitted in two, half of the samples were frozen and half were stored on 4% formaldehyde solution. The frozen samples will be sent to Padmini Dalpadado, IMR for examination of gonad status of krill while the taxonomical sampled will be analysed either at NP or at IMR.

The zooplankton community in the inner part of Kongsfjorden was dominated by krill while the *Calanus* was dominating from Kb3 and further out.

Long-term environmental monitoring using benthic foraminifera in Kongsfjorden

Responsible: *Katrine Husum (NPI) & Pedro Duarte (NPI)*

Surface sediment samples were collected at 5 stations in Kongsfjorden (Kb3, Kb2, Kb1, Kb0, FM) as part of the annual monitoring of Kongsfjorden using benthic foraminifera. The samples were preserved in ethanol with Rosa Bengal stain and will be analyzed at NPI.

Reconstructions of natural sea-ice and water mass variability

Responsible: *Katrine Husum (NPI), Arto Miettinen (NPI)*

xx stations (Kbxx) were sampled for both surface sediments and down core sediment samples. Both surface and paleo-samples will be investigated for fossil micro-fauna (foraminifera) and micro-flora (diatoms).

Ocean Acidification

Responsible: *Agneta Fransson (NPI) & Melissa Chierichi (IMR)*

Investigate the natural calcium carbonate saturation state (Ω) in the area around Svalbard. Influence of water mass composition (Atlantic, polar, fjordwater) and freshwater (glacier, sea-ice and river) on Ω. Distribution of *Calanus*, *Limacina helicina* will be related to Ω state. Pterodpods should be collected from Multinet and MIK when present and stored in 95% alcohol

Appendix 1

Participants

Function	Name	Affiliation	Email	#
Cruise leader/chief scientist	Katrine Husum	NPI	Katrine.husum@npolar.no	1
Engineer	Marius Bratrein	NPI	Marius.Bratrein@npolar.no	2
Marine geology	Arto Miettinen	NPI	Arto.Miettinen@npolar.no	3
Marine geology	Lisa Orme	NPI	Lisa.Orme@npolar.no	4
Microplastic	Ingeborg Hallanger	NPI	Ingeborg.Hallanger@npolar.no	5
Microplastic	France Collard	NPI	France.Collard@npolar.no	6
Microplastic	Zhibo Lu	Uni Tongji, China	zhibolu@126.com	7
Microplastic	Chen Liulin	PRIC, China	chenliulin@pric.org.cn	8
15N Nitrate and DON (ARISE)	Raja Ganeshram	Uni Edinburgh, UK	R.Ganeshram@ed.ac.uk	9
15N Nitrate and DON (ARISE)	Adam Francis	Uni Edinburgh, UK	s1436927@sms.ed.ac.uk	10
Nitrogen cycling & genomics	Catarina Magalhães	CIIMAR, Portugal	cmagalhaes@ciimar.up.pt	11
Nitrogen cycling & genomics	Antonio Sousa	CIIMAR, Portugal	antonio.sousa@ciimar.up.pt	12
Oceanography, CTD	Olga Pavlova	NPI	olga.pavlova@npolar.no	13
Oceanography, CTD	Gary Griffith	NPI	Gary.Griffith@npolar.no	14
Oceanography	Tomas Torsvik	NPI	Tomas.Torsvik@npolar.no	15
Phytoplankton	Lasse Mork	NPI	lasse.mork.olsen@npolar.no	16
Phytoplankton & diving	Pedro Duarte	NPI	Pedro.Duarte@npolar.no	17
Phytoplankton	Josef Wiktor	IOPAS, Poland	wiktor@iopan.gda.pl	18
Phytoplankton	Agnieszka Tatarek	IOPAS, Poland	derianna@iopan.gda.pl	19

Water chemistry (ocean acidification)	Claire Mourgues	NPI	claire.mourgues@hotmail.fr	20
Zooplankton	Anette Wold	NPI	anette.wold@npolar.no	21
Zooplankton & diving	Haakon Hop	NPI	haakon.hop@npolar.no	22
Zooplankton & diving	Mikko Vihtakari	NPI	Mikko.Vihtakari@npolar.no	23
Zooplankton & diving	Peter Leopold	NPI	peter.leopold@uit.no	24
Hydrophone deployment	G.Raguraman,	NIOT, India	ragoo@niot.res.in	Mon July 16
Hydrophone deployment	A.Thirunavukkarasu,	NIOT, India	vathiru@niot.res.in	Mon July 16

NPI: Norwegian Polar Institute. PRIC: Polar Research Institute of China. IOPAS: Institute of Oceanology of the Polish Academy of Sciences, Poland. CIIMAR: xxxx. NIOT: National Institute of Ocean Technology.

Appendix 2

Sampling protocols

A sample-log on an excel sheet will be used during cruise to register each sample
A unique sample ID will be provided to label each sample
UTC time and date will be used in the sample log
Excel sheet will be uploaded to database after cruise

CTD

- 11 x 8L Niskin bottles (bottle #1 is from the deepest depth)
- Sensors: Chl, O_2, transmissometer 660 nm, PAR, sPAR
- One cast whole water column for biology & chemistry
- One extra cast for microplankton >20 µm
- One hand-net (20 µm) 25-0m

Order of sampling from ship CTD

1. **Methane** 160 ml serum bottles (OA team)
2. **DIC/AT** 250 ml glass bottle (OA team)
3. **$\delta^{18}O$** 15 ml DDPE vials (OA team/ Phytoplankton team)
4. **Nutrients** 20 ml acid washed vials (Phytoplankton team)
5. **Chlorophyll and phaeopigments** 50 -1000 ml al. covered bottles (Phytoplankton team)
6. **POC/PON** 200 -1000 ml plastic bottles (Phytoplankton team)
7. **Stable isotopes of carbon and nitrogen Isotopes** xxx ml bottles (ARISE team)
8. **Phytoplankton taxonomy** 250 ml brown glass bottles (Phytoplankton team)
9. **Microplankton (separate CTD cast)** 100 ml bottles (Phytoplankton team)

SAMPLING DEPTHS BIOLOGY (Chl, Part. absorption, POC/PON, BSi, Phytoplankton)
Upper 100m: 100, 50, 25, 10, 5m + Chl max

SAMPLING DEPTHS CHEMISTRY (Methane, DIC/AT, $\delta^{18}O$, Nutrients, Ammonium)
Entire water column: Bottom, intermediate layers (e.g. 200, 500, 1000m), 100, 50, 25, 10, 5m

SAMPLING DEPTHS STABLE ISOTOPES ARISE (carbon, nitrogen)
Upper 100m: 100, 50, 25, 10, 5, 0m

SAMPLING DEPTHS MICROPLANKTON (filter through) 20 µm
Below Chl max, Chl max, above Chl max

Methane

Responsible: Agneta Fransson (NPI) & Melissa Chierichi (IMR)

Methane is a volatile and relatively insoluble trace gas and so it is concentration in the seawater sample will be affected by prolonged contact with a headspace of air. It should therefore be sampled after SF6/CFCs and before dissolved oxygen and DIC/Alkalinity, or first if no SF6/CFC samples are drawn.

Sampling equipment
Silicone sampling tube (about 30 cm long)
160 mL serum bottles
Butyl rubber septa and metal crimp seals (best to assemble before sampling)
Crimping pliers
Eppendorf dispensing pipette (set to dispense 50 µL) and spare tips,
Gloves
☠ Saturated solution of mercuric chloride ☠

Sampling procedure

- Attach the flexible tubing to the Niskin bottle spigot and flush the tube with seawater to expel all air bubbles.
- Rinse a 160 mL serum bottle with the sample water then place the end of the tube in the bottom of the bottle and allow it to fill and then overflow by at least 2 volumes (as for dissolved oxygen samples) Slowly withdraw the tubing from the bottle, pinching it to reduce flow as the end reaches the neck of the bottle. The aim is to have a completely full bottle free of bubbles with a slight convex meniscus over the opening.
- Immediately add 50 µL (2 drops) of saturated mercuric chloride solution then seal the bottle with a metal seal and butyl rubber septum using the crimping tool.
- Store samples in a refrigerator at 4 ºC. Do not allow the samples to warm up to room temperature before analysis.
- Please rinse crimping pliers in fresh water after sampling.

Caution
Saturated mercuric chloride solution is highly toxic. Wear gloves during the sampling/poisoning procedure and wash hands afterwards

Dissolved Inorganic Carbon (DIC) and total alkalinity (AT)

Responsible: Agneta Fransson (NPI) & Melissa Chierichi (IMR)

- Samples for DIC and total alkalinity should be sampled right after the dissolved oxygen samples to avoid contamination from air.
- Fill the 250 ml Duran glass bottle from the bottom to the top and squeeze the tubing as to enable a **bubble free and controlled filling of sample**.
- Overfill the bottle with at least twice the bottle volume (keep the tubing to the bottom of the bottle). Fill up the bottle, and towards the end of the filling, slowly move the tubing out of the bottle. Close the cap.
- After all bottles have been filled, add 60 µL saturated mercuric chloride ($HgCl_2$) to each sample by submerging the pipette tip into the sample. Close the bottle with the blue cap. Do not shake or mix. Some headspace in the bottle (a few mL) is OK.
- Store the samples well marked, in cold and dark place. However, make sure they do not freeze. Best place is a cooling room at 4-6°C.

Oxygen Isotope Ratio ($\delta^{18}O$)

Responsible: Mats Granskog, Agneta Fransson (NPI) & Melissa Chierichi (IMR)

Samples of $\delta^{18}O$ are collected to determine the fractions of river water and sea-ice meltwater in the ocean. Concurrent samples for salinity, oxygen isotope ratio and dissolved nutrients must be collected from the same

sample volume. Ratios of ^{16}O to ^{18}O in the H_2O molecule are measured to a very high accuracy. This sample has nothing to do with dissolved oxygen.

Collecting the sample:

Sample evaporation and/or moisture condensation in the sample bottle are the principal enemies to these samples!

1) Rinse the vial and cap with sample ***three*** (3) times. This removes any water than may have condensed inside the bottle/cap.

2) Fill the vial and cap with seawater from the Niskin bottle.

3) Apply the cap to the vial without touching the inside.

4) Turn the bottle upside down and check for a small air bubble. It should look like the picture below. If the bubble is too small loosen the cap and tighten it again.

5) When all the $\delta^{18}O$ samples have been collected from one CTD, dry the vials, tighten the caps and seal with Parafilm **following the instructions below / on the next page**.

6) Store the $\delta^{18}O$ samples at room temperature or in a fridge.

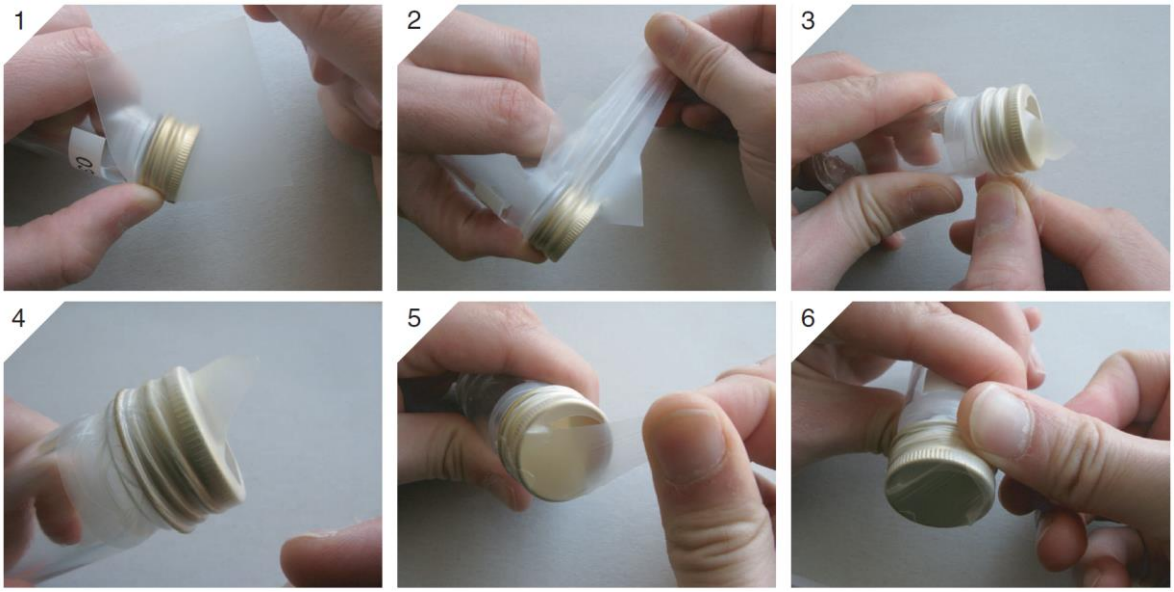


Figure 5: Applying a 5 cm ×5 cm square of Parafilm in a warm dry place. If the Parafilm is cold or wet, it will not stretch and will not stick to the bottle. It is better to wait a day than to apply Parafilm in the cold. Practise on an empty vial until you can do it neatly and securely.

Nutrients

Responsible: Lasse Mork Olsen (NPI)

- Samples should be filled right from the Niskin-bottle into acid-washed 20 ml scintillation vials. Rinse the vial with water from Niskin.
- Allow some head space for the addition of chloroform.
- Add 0.2 ml (200 µl) of chloroform with the dispenser
- Close the cap tight (chloroform is very volatile) and do not shake or turn the vial.
- Put the sample in the fridge inside the tray they were delivered. Keep the trays because they are used to ship the samples to IMR in Bergen.

Figure 6: Acid washed 20 ml plastic scintillation vials used for nutrient samples.

Chlorophyll & Phaeopigments

Responsible: Lasse Mork Olsen (NPI), Joseph Wiktor (IOPAS) & Agnieszka Tatarek (IOPAS)

Filtration

- Filter ca. 50-2000 ml (depending on biomass – a light colour on the filter is enough) from each depth through 25 mm GF/F filters.
- Use plastic sampling bottles wrapped in aluminium foil because Chl *a* is sensitive to light.
- **If no time for extraction**: Filters are placed in extraction tubes (10 ml PP-tubes) and frozen as cold as possible (liquid nitrogen, dry shipper or -80°C) immediately after filtering (if immediate analysis on board is impossible). Wrap samples in aluminium foil.

Extraction

- Work as dark as possible.
- Fold the filter once and place it in Chl *an* extraction vial.
- Add 5 ml methanol to the vial using a dispenser, put a lid on the vial and cover with aluminium foil.
- Extract it "over night" (12 hours) in a refrigerator. NB: Note the start and end time of extraction.
- Turn on Turner Design fluorometer at least 10 min before taking the first measurement.
- Vortex the tube for a few sec and let the sample adjust to room temperature before fluorometer reading.
- Transfer the sample to a clean borosilicate cuvette, and dry the cuvette on the outside.
- Place the cuvette in the cuvette holder of the fluorometer and wait until readings have stabilized. Press ***** button on fluorometer (see picture below), it will first show **Delay**, then **Average** and finally **Done** on the fluorometer display. Read the value on the fluorometer. This is the Rb value (Reading before acid addition) to get the total chlorophyll.
- Take the cuvette out of the cuvette holder and add 2 drops of 5% HCl, cover the cuvette with parafilm and mix it gently 3 times. Read the value on the fluorometer. This is the Ra value (Reading after acid addition) to get the phaeopigment concentration.
- Wash cuvette with clean methanol between every sample and let it dry.
- Before start, and in between, use a methanol blank to check that the cuvette is clean and that the fluorometer is zero for methanol.

Calculations

- Concentrations are calculated on the basis of calibrating data (see xls-file Chlorophyll_N-ICE_2015)
- In the same xls-file under the measurement spread sheet, type in the methanol and filtered volumes and the Rb and Ra values. This will give you the chlorophyll and phaeopigment concentrations.

Particulate organic carbon and nitrogen (POC/PON)

Responsible: Lasse Mork Olsen (NPI)

- Filter 200 - 2000 ml, depending on particle concentration, on pre-combusted 25 mm GF/F filters (the filters were combusted at 450°C for 12 hours and are stored in aluminium foil).
- After filtration, each GF/F filter should be directly placed into Pall filter slides and dried at 60°C in a drying oven and thereafter stored at room temperature. Wrap filter slides from one station in aluminium foil and keep them in a labelled Ziploc bag.
- For each sampling day or event, prepare a reference filter by filtering MilliQ water through a filter (similar volume than sea water for the samples) and treating it the same way than the samples. The reference filters get normal sample running number (but make an additional note "MilliQ" on the sample label/analysis slide), and are noted in the filtering protocol and log sheet. Make also a note on CTD log sheet to avoid confusion with numbering on following casts.

Stable isotopes of carbon and nitrogen Isotopes

Responsible: ARISE team, Haakon Hop

Water (0, 5, 10, 25, 50 and 100m water depth from all stations);

- 250 ml of filtered seawater for stable isotopes of nitrate, oxygen and DON.
- 3 to 5L filtered through a glass fibre filter for d15N-PON and d13C-POC analysis.

Phytoplankton

Phytoplankton taxonomy - CTD

Responsible: Lasse Mork Olsen (NPI), Joseph Wiktor (IOPAS) & Agnieszka Tatarek (IOPAS)

- 190 ml of seawater from each depth are filled into 200 ml brown glass bottles. Fill 200 ml measuring cylinder up to 190 ml mark directly from Niskin bottle and decant into brown glass bottle.
- Under the fume hood, phytoplankton samples are fixed with an aldehyde mixture: First add 0.8 ml of 25% glutaraldehyde and fix for approx. 5 min. Thereafter add 10 ml of 20% hexamine-buffered formaldehyde (to achieve a final concentration of 0.1% and 1%, respectively).
- Store the samples dark and cold, **do not freeze**!

Microplankton (>10µm) CTD extra cast

Responsible: Lasse Mork Olsen, Joseph Wiktor, Agnieszka Tatarek

- One extra CTD cast is sampled for microplankton >10 µm at three depths in the upper 50 m (5, 25 and 50 m).
- Three Niskin bottles (24 L) are closed per depth and the entire contents from each depth drained into 25 L carboys. Note the volume in the carboy.
- The contents of the 25 L carboys are then filtered over 10 µm mesh via a filtration tower (see picture below).
- Samples are concentrated to 90 ml (measure with 100 ml cylinder) and filled into 100 ml brown glass bottles.
- Under the fume hood, samples are first fixed with 3 ml strontiumchloride stock solution and then 10ml of 20% hexamine-buffered formaldehyde (final concentration of 2%).
- Store the samples dark and cold, **do not freeze**!

- 

Figure 8: Filtration tower to concentrate microplankton samples

Rare taxa 20 µm hand-net

Responsible: Lasse Mork Olsen (NPI), Joseph Wiktor (IOPAS) & Agnieszka Tatarek (IOPAS)

Vertical hauls (upper 20 m) with a 20 µm hand-net for taxonomy of rare taxa, biomass measurements, SEM/TEM material and HBIs' material. **Never** tow hand-net with the winch!

Figure 9: Phytoplankton hand net 20 µm

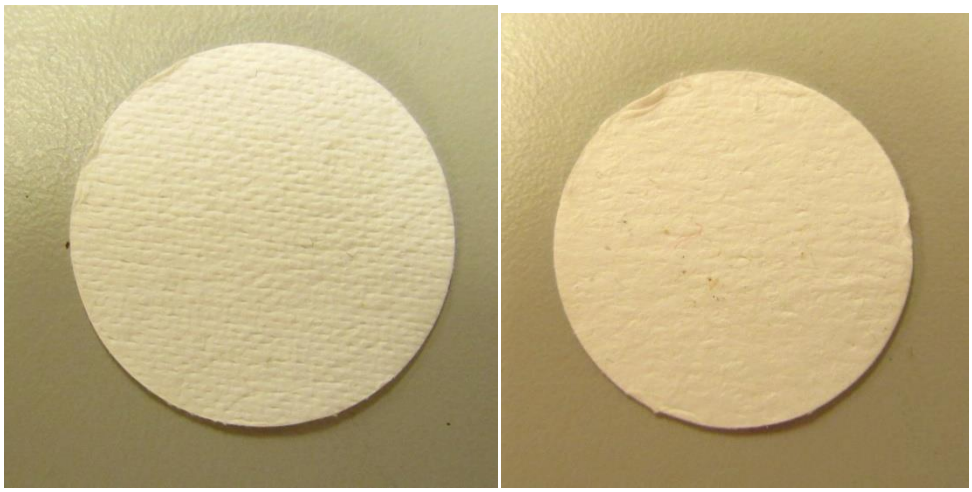
- Connect the hand-net to the provided blue rope and attach a 1-2 kg shackle (ask crew) below the cod end.
- Make sure that the valve of the cod end is closed when lowering the hand-net.
- Wait until <90 ml are left in the cod end (less than half of the cod end volume), open valve and drain hand-net sample into 100 ml measuring cylinder. Fill up measuring cylinder to 90 ml by flushing the mesh of the cod end with squeeze bottle. Fill content of the measuring cylinder into 100 ml brown glass bottle.

- Under the fume hood, fix hand-net samples first with 3 ml strontiumchloride stock solution and then 10 ml of 20% hexamine-buffered formaldehyde (final concentration of 2%).
- Store samples dark and cold, **do not freeze**!
- Collect 50-100 ml phytoplankton material from R1-R8 for HBIs, use sampling tubes from Plymouth University (Lukas Smik). Freeze them at -20 C.

Figure 10: Brown glass bottles 200 ml (phytoplankton taxonomy) and 100 ml (microplankton >20µm & hand-net taxonomy).

General filtration guideline for particulates

- Collect seawater from the Niskin bottles from standard depths down to 100 m. Rinse the plastic bottle with sample water (~100 ml) before collecting the sample.
- Remember to **gently** mix the bottle (turn upside down for a couple of times) before filtering to ensure that no particles settle down (results in uneven concentration if subsampling the sampling bottle).
- Be sure to have placed the filter in the middle of the filter holder and that the funnel is thoroughly placed on top of it (if something is leaking the exact volume filtered or the filtration area on the filter is not known – these are needed for the calculations later).
- The two sides of the GF/F filter are not identical – for particulate absorption it is important to place it the right way (see photos below) – to make it simple use the same orientation for all parameters.
- Use low vacuum pressure (about -30 kPa). Always have the valve of the filtering funnel closed before turning the pump on or off.
- Cover the funnels with aluminium foil when filtering. If you expect to filter 1 L or 2 L, you can place the respective plastic bottle into the funnel and let it run. Please also note that the 0.5 and 1 L mark are indicated on the plastic sampling bottles.
- Rinse the funnel with filtered seawater (collect filtered sea water from previous filtrations) once the sample has been filtered. Do not let the filters dry out, close the valve.
- Use forceps to lift filters onto the sample containers (analysis slides (POC/N), petri dishes (BSi), cryovials (HPLC), white dishes/petri slides (particle absorption), plastic tubes (Chl)). Note that some filters are folded before placing them into the containers, whereas others remain flat.
- Rinse the filtration equipment with MilliQ before the next sample. Cover filtration funnel with aluminium foil when not used.



Mesh side – PARTICLES ON THIS SIDE. Soft “wave” side – back side.

Figure 11: Showing the different sides of the filters.

Zooplankton

Mesozooplankton- Multinet

Responsible: Anette Wold (NPI) & Haakon Hop (NPI)

Zooplankton is sampled with the Multinet from 5 standard depths:
Bottom depth <600m: bottom-200-100-50-20-0 m
Bottom depth >600m: bottom-600-200-50-20-0 m

Prepare Multinet:

- Check that the small pin on the rotating cylinder is in the right position (if not see instructions below).

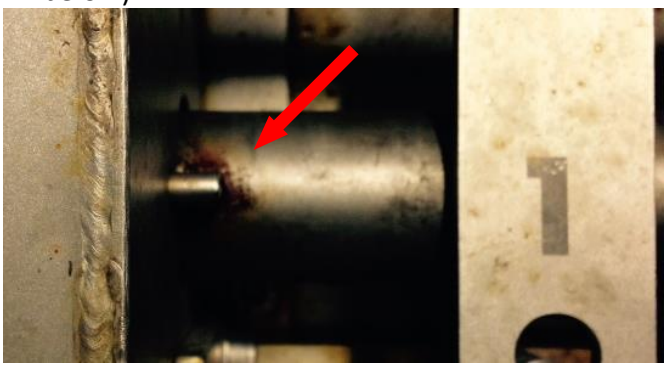


Figure 12: There is a small pin on the rotating cylinder which should point straight at the pin seen here.

- Turn on the Multinet
- Connect Multinet to the Toughbook, open program "OceanLab3" from desktop.
- Check the battery status (should be >7)
  - Connect to Multinet using *connect symbol* (1st from left in menu bar)

Figure 13: Menu bar of the Ocean Lab software

  - Open the *control mode* (6th from left in menu bar)
  - Choose *Pressure programming* to program the releasing depth. Remember that the Unlock depth must be minimum 1 m below the depth of the 1st net. The nets do not open if the Multinet has not been set below the unlock depth. Always go at least 10 m below to make sure it opens (if not the cast needs to be repeated).
  - Send to Multinet.

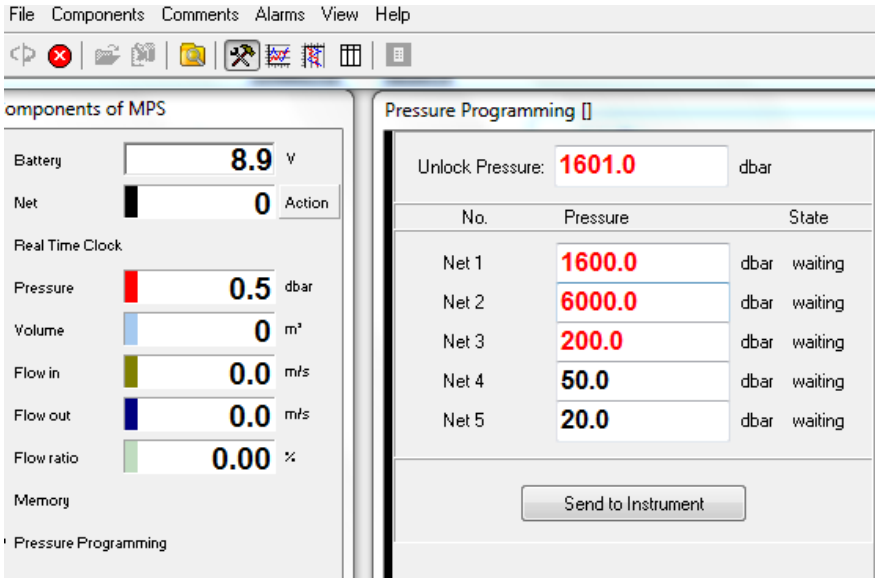


Figure 14: Pressure programing. Unlock pressure needs to be deeper than opening depth of Net 1 (depth of Net 2 is wrong in this picture, should be 600m).

- Disconnect Multinet. **Remember to put dummy plug back on.**

If not all the net opened during the previous deployment, check that the small pin of the rotating cylinder that controls the opening of the nets, is in the right position, pointing straight up towards the pin at the net (see picture above). If not mark *Net* and reset the rotating cylinder either by using *reset counter* or *half step*.

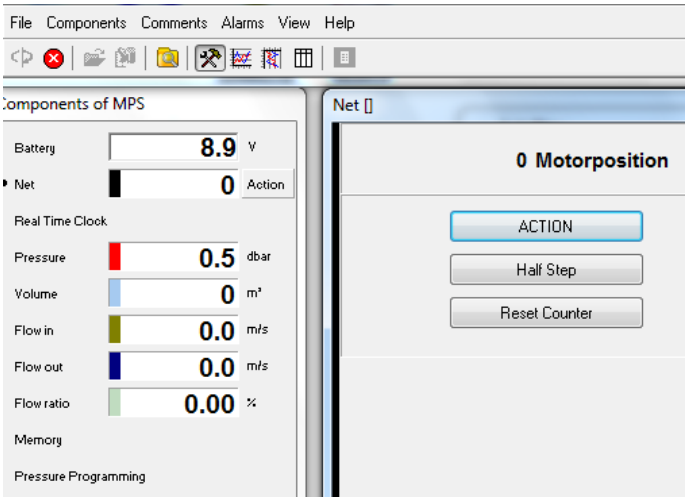


Figure 15: Mark *Net* in the right menu and use *Half step* to reset rotating cylinder

- Prepare the nets (strain the spring by using the bar to lift them into position).

- Net #1 samples the deepest layer, Net# 5 the surface layer.
- Make sure that cod ends are placed at the right net (check numbers)!
- Make sure that the nets are not twisted before the Multinet is lowered into the water.
- The weight of the lower part of the Multinet should be entirely supported by the ropes and not by the nets! (The nets have to hang loose, the ropes should be straight).

Depth sensor /Scanmar

The Scanmar has to be mounted to the frame of the net and the receiver has to hang overboard. There are two Scanmars on board and they are located in the back of the steer house (loading station). They are attached to the nets using solid rubber cords.
The Scanmar works down to approx. 1500 m. When deeper use the Ek 60 to check the depth of the Multinet.

Receiving net:

- Flush net with water hose when it comes on deck.
- Before removing the cod end, make sure that water level is low enough (can been seen through the mesh of the cod end), otherwise part of the sample will spill over when cod end is removed! (This may be a problem when nets are clogged by high abundance of phytoplankton in the water).

Sample treatment Abundance samples:

- Filter contents of cod end through a sieve (mesh size 200 µm) placed over a white plastic tray (in case something gets spilled). Flush cod end with wash bottles filled with sea water.
- Larger jellies (ctenophores and cnidarians) should be removed prior to preservation (as they disintegrate in formalin, which makes enumeration of other zooplankton species, especially small

ones, much more difficult). The removed species names and abundances should be recorded in the sample-log.
- The sample is then transferred from the sieve into 125 ml bottles (or larger if samples are dense).
- Fill bottles to the neck with sea water (red arrow)
- Add 10 ml formalin and a bit of hexamine
- Label bottles from the outside and place label

Macrozooplankton - MIK net

Responsible: Anette Wold (NPI) & Haakon Hop (NPI)

Samples are taken from bottom to surface

Equipment & fixatives:
- 250 or 500 ml bottles
- Zip bags
- 37% formaldehyde solution buffered with hexamintetrahydrat

Sampling:
- Flush the net with the hose before removing the cod end.
- Transfer sample into large bucket.
- Split sample in two using a plankton splitter.
- 1 part is fixed in formalin (same as for Multinet).
- 1 part is frozen at -80°C.
- Label bottles from the outside and place label inside.

Sediment sampling

Surface sediment sampling (marine geology) – box corer

Responsible: Katrine Husum (NPI) & Arto Miettinen (NPI)

The upper 0-1 and 1-2 cm will be sampled immediately (100 ml of sediments). The foraminiferal samples will be preserved with ethanol and Rosa Bengal stain and kept in small plastic sediment containers/bottles. All samples should be stored cold (maximum 5-10° C). Further processing and analysis will be carried out onshore at NPI.

Surface sediment sampling (microplastic MiP project) – box corer

Responsible: Ingeborg Halanger (NPI), France Collard (NPI), Katrine Husum (NPI) & MiP project members

Three casts of the box core will be carried out in order to obtain enough samples. Avoid using furry clothing during sampling and handling of samples! Cover open sample surfaces with clean aluminum foil as quickly as possible.
Equipment
- Box corer (50*50*50 cm)
- Large plastic bucket to place sediment core in
- Large metal spoon
- Filtered SW or distilled water in squirt bottles for cleaning spoon between samples
- 1 l glass jars for storage

Surface sediment sampling (microplastic MiP-China project) – box corer

Responsible: MiP-China project members, Katrine Husum (NPI) & Ingeborg Halanger (NPI)

Two casts of the box core will be carried out. One box core will be sampled using 1 short pvc tube which have to be stored at -20 C in addition to sampling of the upper 5 cm of the box core (kept in aluminum foil + plastic bag and stored at 5C). The upper 5 cm of the other box corer (30 x 30 cm) will also be sampled (kept in aluminum foil + plastic bag and stored at 5C). These samples will be analyzed with regard to microplastic content in the sediments using project partners' lab facilities in China.

Diving

Collection of bivalves and sediments

Responsible: Haakon Hop (NPI)