

MarineBasisDisko

The Disko Marine Monitoring program



Sampling manual for the field program

MarineBasisDisko

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Introduction

A long-term monitoring program that holds data on water temperature and salinity dates back to 1924 and data on nitrate and phosphate goes back to 1973 (Hansen et al., 2012). Measurements of water column conductivity, salinity and temperature (CTD) by means of an automatic CTD-profiler (Seabird SBE 19 Plus) have been performed on a regular basis since fall 2001 (Hansen et al. 2012).



Figure 1. The location of monitoring station “Fast station” (●) in Disko Bay, Greenland.

The Monitoring program became part of MarineBasis (as MarineBasisDisko) in 2018. The seasonal program then involves a single (Fast station) for intensive plankton samplings (Figure 1). This includes samples taken for molecular analysis. Apart from the seasonal study, the program includes two, four-day cruises in Disko Bay to describe the variability in the hydrography in the area. The MarineBasisDisko also includes macroalgal part, which study depth distribution of macroalgae in both tidal and subtidal stations as well growth estimates.

1. Sampling program

1.1. Marine plankton monitoring programme

1.1.1. Station location and parameters sampled for the seasonality

The monitoring station “Fast station” in Disko Bay (Figure 1)

- Position: 69° 11.1' N, 53° 31.1' W
- Depth: 300 m

Parameters measured with CTD (obtained with AML-6 CTD; a Seabird SBE19plus CTD functions as backup)

- Temperature, salinity, density, fluorescence, light and depth

Water sample measurements:

Chemical measurements:

- pH, DIC, NO₃, NO₂, NH₄, PO₄ and SiO₄

Biological parameters:

- Total Chl_a and fractionated Chl_a (20, 200 µm)
- Bacteria
- Nanoplankton
- Phytoplankton
- Microzooplankton
- Zooplankton (100 m to top; 45 µm mesh size net)
- Fish larvae

1.1.2. Sampling frequency

CTD cast and collection of water samples are carried out from the permanent station monthly from Porsild except for the periods where Porsild cannot operate due to ice. When the sea ice is safe, sampling will be carried out from the ice.

1.1.3. Water sampling

Water samples are taken at depths of 1, 10, 20, 50, 100, 200 and 300 m with a 10 or 20 L Niskin-sampler. In most cases, the water samples can be taken directly from the Niskin on the boat and gently transferred with a tube to the sample bottles (for salt, nutrients, pH, DIC/alkalinity). From depths: 1, 10, 20 and 50 m water is transferred into two 10 L canisters (filled to capacity). From these canisters samples for chl_a, fractionated chl_a and barcoding are taken as fast as possible for chemical and biological analysis.

For barcoding: Mix 5 L from each of the 20 L samples from 1, 10, 20 and 50 m in a 25 L canister. Keep dark and at water temperature until lab (can be done on the boat or later in the lab).

7 depths (1, 10, 20, 50, 100, 200 and 300 m): **Salt, nutrients (N, P and Si)**

5 depths (1, 10, 20, 50 and 100 m): **pH, DIC/Alkalinity, total chl a**

4 depths (1, 10, 20, and 50m): **Fractionated Chl a (20 and 200 μ m)**

2 depths (1, 20 m): **Bacteria/picoplankton, phyto/microzooplankton**

1.1.4. *Phytoplankton net tows*

One net tow from ~50 m to surface until a dense concentration is achieved. Fix in Lugols (final conc. 2%). Two times 250 ml are fixed with Lugol's on the boat; both marked "Phytoplankton net sample", and one of them specifically marked "Barcoding". The live net sample should be kept alive (cold and dark) until lab and use for barcoding.

1.1.5. *Zooplankton net tows*

Three vertical net tows (WP2 net, mesh size 45 μ m) are taken from 100 m to surface. Mount a weight in the bottom of the net to keep the vertical position. Samples are fixed in borax buffered formalin (final conc. 4 %) in 1 L plastic beakers with a lid. Borax buffered formalin is made by adding 30 g sodium tetraborate to 1L Formalin (40%) to make Formalin pH-neutral.

1.1.6. *Fish larvae net tows*

Samples are taken with a Bongo twin net 300 and 500 μ m mesh. Perform a V haul to 100 ms, wire out with 25 m per min (0.42 m/s), take it in with 12 m per min (0.2 m/s) when sailing 2 knots per hour. The 500 μ m sample fixed in 3% buffered formalin (final conc), while the 300 μ m sample should be dried for water and fixed in 96% ethanol.

1.1.7. *Procedures on ship*

pH: pH is measured as soon as possible after collection on ship.

Inorganic nutrients: Well mixed waters samples (60 ml) are taken and passed through a 0.22 μ m filter using a syringe directly into plastic centrifugation tubes for later measurements of inorganic nutrients (NO_3 , NO_2 , NH_4 , PO_4 , SiO_3). Samples are thereafter frozen at -20°C and later analyzed at the Section for Freshwater Biology, University of Copenhagen. Contact person: Per Juel Hansen (pjhansen@bio.ku.dk).

DIC/Alkalinity: Six 40 ml Exetainers are filled up to capacity with the water sample from each depth and the lips are fastened tightly. 600 μ l saturated HgCl_2 have been added to each container the day before. Store the Exetainers on ice on the ship in the dark. Make sure that the solution is well mixed. Store the Exetainers in the dark at the lab.

Salt: Add a water sample of to brown bottles (250 ml) without headspace from 1, 10, 20, 50, 100, 200 and 300 m. Store without freezing. Will be analyzed in Denmark. Contact person: Torkel Gissel Nielsen (tgin@aqua.dtu.dk)

Bacteria/nanoplankton. Add sterile filter borax buffered Glutaraldehyde (4 ml 37%) to each 50 ml falcon tube and top up with the water sample from the selected depths. If this is not possible due to rough sea, then add the formalin upon arrival to the lab. Keep samples on ice or in the refrigerator for 3-4 hours before the samples are stored at -80°C upon arrival at the laboratory.

Phyto/microzooplankton. Add 6 ml Lugol's to the 250 ml brown glass bottles prior to the addition of the water sample.

Zooplankton: Samples are stored cold (but NOT frozen) before shipment to Denmark. Contact person: Torkel Gissel Nielsen (tg@aquadtlu.dk)

Fish Larvae. Samples are stored cold (but NOT frozen) before shipment to Denmark. Contact person: Torkel Gissel Nielsen (tg@aquadtlu.dk)

1.1.8. Procedures in the laboratory

1.1.8.1. Total Chla and fractionated Chla

Total Chla (5 depths: 1, 10, 20, 50 and 100 m): Well mixed samples (200-1000 ml, depending on season) from depths 1, 10, 20 50 and 100 m, are filtered separately onto GFF/F glass fiber filters, transferred to 15 ml falcon tubes and extracted in 10 ml 96% ethanol. The tubes are kept at 4 °C for 24 h and measured in the Turner Trilogy flourometer at AS. Save the remaining extracts in the freezer as backup. If measurements must be postponed the samples are frozen at -20°C for later measurements. Filters must show some green color.

Save some of the filtered water for rinsing purposes under Barcoding.

Contact person: Per Juel Hansen (pjhansen@bio.ku.dk).

Fractionated Chla (4 depths 1, 10, 20 and 50 m)

2 L of the sampled water from 1, 10, 20 and 50 m are passed through either 1) a 200 µm or 2) a 20 µm net gauze. The water from each fraction (< 200 µm and <20 µm) is then filtered onto GFF/F glass fiber filters, transferred to 15 ml falcon tubes and extracted in 10 ml 96% ethanol, and kept at 4 °C for 24 h and measured in the flourometer at AS. Save the remaining extracts in the freezer as backup. If measurements are postponed the samples are frozen at -20°C for later measurements. Filters must show some green color. Contact person: Per Juel Hansen (pjhansen@bio.ku.dk).

1.1.8.2. Metabarcoding

Plankton net sample in laboratory (keep cold): - approx. 1000ml

- *For toxins and backup:* Filter two times 150 ml of plankton-net sample on 3µm Polycarbonate filters, fold filters, wrap in Alu foil or put in cryovials. Freeze immediately at -80 °C in a plastic bag. #Label: toxins+current date + net sample

Prefilter the net sample (200 µm)

- *For DNA:* Filter one times 150 ml of plankton-net sample on 3µm Polycarbonate filters, fold filters, wrap in alufoil or put in cryovials. Freeze immediately at -80 °C in a plastic bag.
#Label: DNA+current date + net sample + < 200 µm

Niskin-samples in laboratory (keep cold): (estimated time 1-1.5 h)

Add 5L of the collected water from the 4 depths (1, 10, 20 and 50 m) to a 25-Liter bottle. Mix it well.

- 1) Run the mixed water sample over the filter tower into the next 25 L bottle (first 200µm (one dot) then 20 µm (three dots).
- Rinse the 20 µm size fraction into a 50 ml Falcon tube with filtered seawater (leftovers from the total chl a samples). Take this sample, split it into two and filter these over two 3 µm polycarbonate filters and wrap these in alu foil or add them to cryovials. Freeze immediately at -80 °C in a plastic bag. #Label: current date + niskin + > 20 µm
- 2) Take three times 5 L out of the 25 L bottle (20 µm prefiltered = from [1]) and filter (as much as possible, depending of the biomass 1-3 L) over 3 µm polycarbonate filter. Wrap the filter in alu foil or add them to cryovials). Freeze immediately at -80 °C in a plastic bag. #Label: current date + niskin + < 20 µm
- 3) Take the filtrate (3 µm prefiltered from [2]) and add to the three beakers (approx. 1.5L each). Filter three times 0.5L (or less depending on season) through a 0.2 µm polycarbonate filter, and wrap this in alu foil or add to cryovials. Freeze immediately at -80 °C in a plastic bag. #Label: current date + niskin + < 3 µm + > 0.2 µm

Contact person: Nina Lundholm (nlundholm@snm.ku.dk)

1.2.1. Station locations and parameters sampled

Parameters measured with CTD:

- ### Water sample measurements:

- pH, NO₃, NO₂, NH₄, PO₄ and SiO₄

1.3. Macroalgae

1.3.1 Parameters and rationale

Macroalgae are sensitive indicators of climate change and have many important ecosystem functions including habitat provision, climate change mitigation and adaptation. Macroalgae are expected to grow faster and expand their distribution and associated ecosystem functions in a warmer future with longer open water periods, but increased turbidity due to glacier melt may also affect the light climate of subtidal macroalgae (Lebrun et al. 2023). The macroalgal monitoring targets both the subtidal and the intertidal vegetation where a set of key parameters are measured (Table 1, see also GEM annual research card 2018). The specific rationale for the monitored parameters is summarized below.

The macroalgal monitoring program shares links to MarinBasis Zackenberg that also monitors growth of kelp, and to MarinBasis Nuuk that also monitors tip growth of *Ascophyllum*.

Subtidal vegetation (kelp)

Cover and depth distribution: Kelps such as *Saccharina latissima*, sugar tangle, are expected to expand their distribution and grow deeper in response to longer open water periods unless turbidity reduces light availability at the seafloor (Krause-Jensen et al. 2012, Lebrun et al. 2023). Kelp forests grow particularly deep at offshore sites in the Disko Bay where depth limits deeper than 61 m have occasionally been observed (Krause-Jensen et al. 2019).

Growth of kelp (size of blade): Kelps such as *Saccharina latissima*, sugar tangle, are expected to grow faster in response to longer open water periods and higher temperatures in the Arctic. The size of the annual blade is a proxy for the annual growth (Krause-Jensen et al. 2012).

Intertidal vegetation

Cover and depth distribution. Intertidal Arctic vegetation is expected to act as sentinels of climate change by e.g. increasing cover and extending higher in the tidal zone in a warmer setting; species composition/dominance may also change. As an additional component (not part of standard monitoring), the area distribution of the intertidal vegetation at the study sites in Fortuna Bay and Kronprinsens Island has been recorded by drone.

Tip growth of *Ascophyllum* (Knotted wrack): Knotted wrack is, together with *Fucus* spp., a key species of the intertidal community in protected settings and responds positively to increases in Arctic temperature by increasing growth. Knotted wrack has its northernmost documented distribution limit at Qeqertarsuaq and Kronprinsens Ejland, and these sites also hold the longest known record of tip growth rate of this species. The growth rate of this tidal vegetation increases with warming and the distribution is also expected to expand with warming (Marbà et al. 2017).

Supporting parameters: Temperature is being logged in the intertidal zone.

Table 1. Overview of sampling sites and parameters for the DiskoBasisDisko monitoring program on macroalgae

Station	Subtidal vegetation (kelp) (S)		Intertidal vegetation (I)		
	Cover and depth distribution	Growth of kelp (size of blade)	Cover and depth distribution	Tip growth of Ascophyllum	Temperature
<i>Kronprinsens Ejland</i>					
St. Sub-Kr1 (Engelskmandens Havn) 69° 01.74' N, 53° 19.72' W	X	X			
St. Int-Kr1 69° 00.512' N, 53° 19.464' W			X		
St. Int-Kr2 69° 00.617' N, 53° 19.441' W			X		
St. Int-Kr3 69° 00.620' N, 53° 19.434' W			X		
St. Int-Kr4 69° 00.689' N, 53° 19.573' W				X	X
<i>Qeqertarsuaq</i>					
St. Sub-Q1 (Køddøen) 69° 14.272' N, 53° 34.751' W	X	X			
St. Int-Q1 (Dump) 69° 14.24' N, 53° 32.32' W				X	
<i>Fortuna Bay</i>					
St. Int-F1 69° 15.377' N, 53° 45.262' W			X		X
St. Int-F2 69° 15.380' N, 53° 45.274' W			X		
St. Int-F3 69° 15.349' N, 53° 45.270' W			X		

1.3.2 Frequency and stations

All macroalgal parameters are measured once per year in late August-early September.

Temperature loggers (Hobo) mounted in the mid-intertidal zone are read/replaced annually during the vegetation survey. Loggers are set to log every 30 min.

1.3.3 Procedure

General equipment: Safety suits, gloves, bookings, workplan/guidelines/station list, tidal table (and app: Tidevand), permission to sample algae, GPS (or smartphone), camera, taxonomy literature on algae, pencils, markers, tweezers, notebook, zip lock bags – small/medium/large

Subtidal vegetation - Cover and depth distribution

Sampling equipment/personnel: A dinghy with boat driver and two additional persons to conduct the surveys. Underwater video system with a built-in depth sensor connected by a 50 m (or longer) cable to a screen on the boat allowing online view of the video signal. The video system must also have built-in GPS and time logger. Charged batteries and file storage space must be ensured; box to transport/cover the equipment; tub for placing the cord; information on site location and GPS to locate it if needed.

Measurements:

Three replicate video transect lines are produced around the study site. Time and start position of each transect line is noted. The camera system, connected to a 50 m (or longer) cable, is carefully lowered (ensuring distance from the engine) from the dinghy to about one meter above the sea floor, above the kelp canopy, and towed/directed from the shore to the deepest occurrence of erect macroalgae, which can be followed on the screen. The speed of the video survey must allow proper identification of macroalgal depth distribution. Recordings are logged as e.g. '.MP4' files.

The depth at the time of sampling should be corrected for tides and expressed relative to the average annual tidal level (MWL) using tidal information from the nearest harbour (we found that the app "Tidevand" is more reliable than the tidal information in DMI's tidal tables, - and also provides information on tide level (relative to MWL or chart zero) for any given time. .

Subtidal vegetation - Size of blade as proxy for annual growth of *Saccharina latissima*

Sampling equipment: KC plant rake, Rope for rake + bucket for rope + 2 extra buckets (for seawater), plastic bags/containers for collecting kelp, scissors/knife. This sampling is typically done in connection with the video surveys, but if not, the following supplementary items should be brought: information on site location and GPS to locate it if needed; notebook; pencil.

Lab equipment: Tape measure, folding ruler, white sorting trays tissue paper, scissors, scale, oven (60 °C) (or freezer -18 for storage to subsequent analysis), trays for oven drying of samples; zip lock bags/paper bags for storage of dried samples.

Measurements:

(1) 10-20 individuals of *Saccharina latissima* are collected with e.g. a KC plant rake at 5-10 m depth at each station. (NB. In the MarinBasisZackenbergl program, sampling targets *Saccharina latissima* individuals >1.5 m in length, which are easily sampled as they are attached to small stones on a soft seafloor. Ideally, MarinBasisDisko should apply similar requirements, but it has proved difficult to obtain such long individuals by rake at the current Disko study sites, where slender kelp individuals are firmly attached to the rocky seafloor, and where frequent ice scour probably keeps the population relatively young and shorter-leaved Hence shorter individuals are included in the sampling).

(2) In the field, the individuals are rolled up from the tip towards the stipe and placed carefully in plastic bags/containers to protect the blade from breaking. They are kept moist, cold and protected from direct sunlight until measurements in the lab.

(3) The length of the individuals (cf. Figure X1) is measured by a folding ruler/measuring tape to the nearest 5 mm. The central measure is the length of the annual blade, which extends from the blade base to the constriction, if any ("Length 1"). If there is no constriction, which is the typical case at the Disko stations, the entire blade represents the annual growth. In case of a constriction,

the extremity represents the old blade (Length 2-Length 1), which is then also measured. “Length 3” is the length of the stipe and the haptera, which should be measured separately. It should be indicated whether the plant parts (blade, stipe, haptera) are entire or broken; the latter often being the case for haptera and stipe, and in some cases for the blade. In addition, the width of the blade is measured across the center of the blade.

(4) The wet weight is measured for the blade section corresponding to “Length 1”, the blade section corresponding to the old blade “Length 2 minus Length 1” and each of the stipe and haptera is measured.

(5) For ww-dw conversion and subsequent analysis of CN content a cross-section including both the central flat part and the peripheral wavy part is cut at the center of the new blade (length 1). The cross-section should be ca 2-3 cm broad for the flat part, widening at the wavy periphery. The samples are weighed and then dried to constant weight at 60 °C (to avoid the samples from sticking to the base, they are airdried before placing in the oven). The dried material is weighed and stored. The mean ww:dw relation for all blades is used for estimating blade . If there is no oven available, the algal tissue can be frozen at -18 °C and stored for later analysis.

(6) The remaining part of the algal tissue is discarded.

(7) A minimum of 5 subsamples of the dried algal tissue (the cross sections) is analyzed for carbon and nitrogen content using a CN analyzer to obtain a measure of variability.

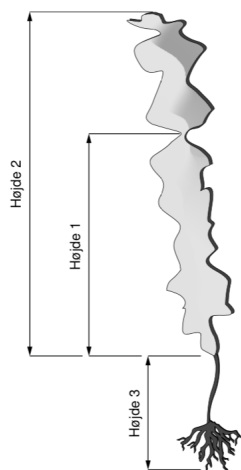


Figure X1: *Saccharina latissima* – measures to be taken: The central measure is the “Length of new blade” (“Højde 1/Length 1”). In addition, we measure “Total blade length” (“Højde 2/Length 2”). Moreover, we measure “Højde 3/Length 3”, that includes the length of the stipe and of the haptera (quantified separately), with indication of whether they were entire or not.

Intertidal vegetation - Cover and depth distribution

Sampling equipment: Paint to mark the rock to locate sampling site, handheld niveller to identify tidal heights, tidal table, 3 x marked rope (ca 5 m long, marked every 25 cm) for location of intervals along the tidal gradient, field scheme, 2 x folding rulers, 3 x white sorting trays, camera, zip lock bags.

Measurements: To be conducted at low tide! The vegetation is measured along 3 replicate transect lines extending seaward on the rocky shore. The upper starting point is marked by paint on the rock. One end of the marked rope is placed here, and the rope is extended to reach the lower part of the intertidal zone. Once the ropes are placed, each transect line is photographed both in its full extension and for each 25 cm interval. Subsequently for every 25 cm along the rope the identity and cover of macroalgal and macroinvertebrates is recorded the following way: a folded folding ruler (20 cm long, ca 1 cm broad) is placed transversally on the rope at each mark and the identity and cover of macroalgal and macroinvertebrates below the ruler is recorded.

Intertidal vegetation - Tip growth of *Ascophyllum* (Knotted wrack)

Sampling equipment: Scissors for cutting tips, Zip lock bags, marker, ruler, plastic cups (20) for drying of *Ascophyllum* tips.

Measurements:

Sampling is done in the mid intertidal zone. 20 tips (actively growing, light brown) are sampled randomly in the population to measure annual tip growth. The tips are cut below the 3rd bladder (see Figure X2). All samples should represent parts that continue as frond at least down to the 4th bladder.

In the lab, each of the youngest 3 segments (year 0 incl. first bladder, year 1 incl. second bladder, year 2 incl. third bladder) and each of the associated bladders are measured to nearest mm, and the number of branches are indicated (0: no branch/division, just an undivided segment; 1: one branch/division, i.e. a double segment; 2: two branches/divisions, i.e. a triple segment) for each of the segments. (As each of the 20 tips contain several young segments, the number of observations for assessing the latest years growth will be >20). See Table X2.

Subsequently all the measured segments of a given age class are pooled in a small bag, their total length quantified and the sample dried to constant weight at 60 °C and weighed to establish a relationship between thallus length and biomass for each age class. This results in three bags: one for year 0 segments incl. first bladder, one for year 1 segments incl. second bladder, and one for year 2 segments incl. third bladder.

The dried tips (year 1 segments, 3 subsamples) are subsequently analysed for carbon and nitrogen content using a CN analyzer.

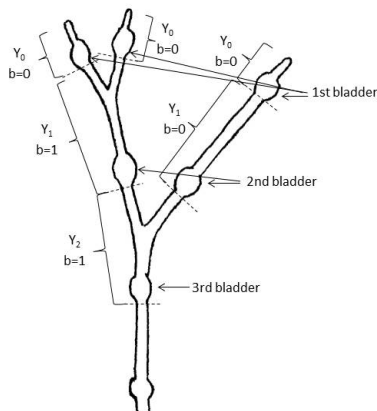


Figure X2. Sketch of *Ascophyllum* tip-section showing the three youngest segments: year 0 (y_0), year 1 (y_1) and year 2 (y_2) and the associated bladders. Branching is indicated as $b=0$: no branch/division, just an undivided segment; $b=1$: one branch/division, i.e. a double segment.

Table X2. Example of data entry for measurements of tip growth of *Ascophyllum*

Site: Kobbe Fjord - monitoring site

All samples are from parts that continue as frond at least down to the 4th bladder

Data for gDW/cm conversion

All measured segments including length of segment has a branch, the branch is included in the measurement (i.e. is measured).

EXAMPLE

Tidal zone	Date	prim shoot no.	no. of branches year 0	Length year 0 incl. 1st bladder (cm)	no. of branches year 1	Length year 1 incl 2nd bladder (cm)	no. of branches year 2	Length year 2 incl 3rd bladder (cm)	total cm year 0 dried for gDW/cm conversion	total cm year 1 dried for gDW/cm conversion	total cm year 2 dried for gDW/cm conversion
Upper	23-08-2012	1	1	2,9	1	4,2	1	4,3	63	68,4	51,4
Upper	23-08-2012	1	1	2,7							
Upper	23-08-2012	1	1	3,2	0	4,3					
Upper	23-08-2012	2	1	3,3	1	5,8	1	7,7			
Upper	23-08-2012	2	0	2,9							
Upper	23-08-2012	2	0	3,5	0						
...									
...	...	20									
Sum upper				63		68,4		51,4			
Mid											
...	...										
...	...										
Sum mid											
Lower											
...	...										
...	...										
Sum lower											

Supporting parameters

Temperature logging:

Equipment: Hobo loggers - 4 pieces (2 to be placed in the mid intertidal of Fortuna Bay and Kronprinsens Ejland, respectively); plastic strips (bright color for easier detection) to tie loggers to *Ascophyllum*/*Fucus* (line and small anchors + floats for alternative attachment of logger; loggers must be pre-programmed to log temperature every 30 min; Hobo control unit, camera.

Measurement: A pair of loggers, preprogrammed to log every 30 min, is placed in a protected setting, attached to the base of *Fucus*/*Ascophyllum*, near the rock. The bright color of the cable tie facilitates detection the following year.

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1.4. *Suspended material and dating*

1.4.1. *Sampling for Suspended Particulate Matter, Loss On Ignition (LOI) and grain size*

Location: “Fast station”

- Position: 69° 11.1' N, 53° 31.1' W
Depth: 300 m

Frequency:

In conjunction with the general MarinBasisDisko sampling (approximately monthly) from May to September.

Sampling at the station:

CTD-profile:

- CTD-profile to the bottom. Preferably profile right from the surface, not from 1 m depth which is often considered to be surface.

Water sampling:

- 4 L at 1m, 10m and 300 m depths. Each sample is transferred to two 5 L bottles after stirring.
- 4 L from 300m below surface. This sample is transferred to one 5 L bottle.

Filtration in the laboratory:

The samples (approx. 4 l) is filtered onto GFF glass-fibre filters (mesh size 0.7 µm).

The water samples should be well-mixed during pouring and the volume filtered on each filter is noted on the annotation sheet. Volume can be determined by weighing of the bottles with and without water (accuracy 1 g).

Each filter is flushed for salt by filtering approximately 150 ml of fresh water onto the filter after filtration of the seawater. The exact volume of this flushing water is not important.

Filtration of blank filters:

After filtration of a batch of water samples, approximately 100ml fresh water is filtered through three control filters.

Drying and storing

Filters are preferably dried for two hours at 60 degrees after filtration and then weighed (accuracy: g with four decimals)

Alternatively, the filters are stored cold and in darkness.