



Water sampling, preservation and processing protocol



Prepare CTD

1. Prepare CTD and Niskin bottles, and take a profile, after 3 minutes of acclimatizing in the seawater, and register this action in MIDAS

2. Once the CTD carrousel is on deck, water from the Niskin Bottles will be used to sample for water descriptors: five types of samples originate from these bottles.

- A) Pigments: Important: we will filter as much seawater as possible in order to measure precise pigment concentrations!!
 - 1) Connect vacuum pump to filter unit, place GF/F Whatmann filter and cup.
 - 2) Fill cup with known amount of collected sample water (eg 500 mL)
 - 3) Note total volume of filtered water in excel report
 - 4) Once the filter runs dry, flush sides of cup clean with distilled water and remove cup
 - 5) Remove filter, fold and dry filter on paper tissue (filter has to be very dry!)
 - 6) Store the filter in the specifically designed storage unit and label with cryopen: 'VLIZ', 'Date', 'Station', 'Chla', 'Volume filtered' (e.g. VLIZ 20141218_130_500ml_ChIA)
 - 7) Wrap tape around the label to ensure it stays attached.
 - 8) Store in -24°C and clean all used equipment properly by rinsing 3 times with Milli-Q water.
 - Nutrients: Filter about 200ml seawater with 0.2 µm cellulose-acetate filter for residual water for nutrient analysis
 - 1) Connect vacuum pump to Erlenmeyer and place cellulose acetate filter and cup on filter unit
 - 2) When the filter runs dry, pour the filtered water into a nutrient-recipient, label with 'VLIZ', 'station', 'date' (e.g. VLIZ 20141218_130).
 - 3) Store at -24° C and clean the Erlenmeyer and all other equipment properly: rinse **3 times** with Milli-Q water.

C) Suspended matter:

- a. Fill a 1-liter container for SPM determination by VMM (prelabeled), store at 4° C
- b. In the papers supplied with the VMM box, fill in station (should be done already), time and secchi depth.

D) Metagenomics

B)

- 1. Isolate 10-30L of seawater using a Niskin bottle from the surface (0-2m depth) of the water column.
- 2. Collect microbial community by passing as much of the sampled seawater through a 0.22µm filter, using Sterivex cartridges. No prefiltration step needed. Please try to filter as much as possible until the filter clogs.
- 3. Filtration for Sterivex should be done using either a peristaltic pump or a hand pump (e.g. 50mL sterile syringe). In either case you will need a leur lok adapter enabling an attachment to the Sterivex.
- 4. The Sterivex should be pumped free of standing water following filtration but does not need to be dry.
- 5. Seal the Sterivex filter using a sticky tac, e.g. Blu Tac or similar.
- 6. Label your filters as follows: 'VLIZ', 'Date', 'Station', 'dna, 'Volume filtered' (e.g. VLIZ 20141218_130_670ml_dna) and store the entire filter in a plastic 200ml recipient. Make sure you label both, the filter and the plastic recipient!!
- 7. To protect the label from running please seal the label on the filter with transparent adhesive tape (also known as Scotch tape, Sellotape or Tesafilm).
- 8. For short-term storage a -20°C freezer can be used. For transport from sea to the land for a period of time shorter than one hour samples can be stored in sealed bag buried in ice. In the MSO metagenomic samples will be stored at -86 °C

E) eDNA

- 1. Collect two Niskin bottles at the bottom, and two Niskin bottles at 3m depth (= surface).
- 2. Once Niskin bottles are on deck, fill two 1-liter bottles from bottom water, and fill two 1-liter bottles from surface water.
- 3. Label with 'bottom/surface', eDNA, date, station. Store these 4 bottles at -24° C

	On board	On land	Partner laboratory
Pigments	-24°	-86 ° in MSO	UGent PAE (Be)
Nutrients	-24°	-24 ° in MSO	NIOZ (NL)
Suspended matter	in dark, room temperature	4 ° in MSO	VMM (Be)
eDNA	-24°	-24 ° in MSO	Bioarchive VLIZ
Prokaryotic DNA	-24°	-86 ° in MSO	Bioarchive VLIZ