BIOcean5D

MARINE BIODIVERSITY ASSESSMENT AND PREDICTION ACROSS SPATIAL, TEMPORAL AND HUMAN SCALES

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List of Acronyms

acronym	meaning
BORAX	Tetraborate de Potassium
CDOM / FDOM	Colored Dissolved Organic Matters / fluorescence of dissolved organic materials.
CHL or Chl or Chla	Chlorophyll-a
CTD	Conductivity Temperature Density probes
DIC	dissolved inorganic carbon
DNA	deoxyribonucleic acid
DOC	Dissolved organic carbon
DOC	dissolved organic carbon
eDNA	environmental DNA
eDNA	Environmental deoxyribonucleic acid
EEMs	excitation-emission Matrixes
EMBL	European centres in molecular/cell biology
EMBRC	European marine biology/ecology
EMO BON	European Marine Omics Biodiversity Observation Network
EtOH	Ethanol
F-Store	front storage area
FC	flow-cytometry
FNSW	filter-sterilized natural seawater
FOI	Fraction organic/inorganic
FRG	Fridge
FRW	rinse with fresh water
FRZ	freezer

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FSW	Filtered Sea Water
Gluta	Glutaraldehyde
GPS	Global Positioning System
HCL	Hydrochloric Acid
HDPE	High-density polyethylene
HG	Mercure
HgCl2	Mercuric Chloride
HLB	hydrophilic and lipophilic bounding for use as a sorbent material in solid phase extractions (SPE)
HTSRB	Hyperspectral Tethered Spectral Radiometer Buoy
LDPE	Low-density polyethylene
LN	Liquid nitrogen
LSI	Land Sea Interface
MQW or MQ	MilliQ water
MSDS	material safety data sheet
MTE	Marine Trace elements
NOT	do Not dO it at any Time
NRT	near real-time
PA (or ap)	Particulate absorption
PAR	Photosynthesis Active Radiation
PAR	photosynthetically active radiation
PBS	Phosphate-Buffered Saline
PC	Polycarbonate
PCR	polymerase chain reaction
PES	Poly Ethylène Sulfate
PETG	Polyethylene Tetra phthalate
PFA	Perfluoroalkoxy

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PFA	Para Formaldehyde
POC-PON	Particulate organic matter (in Carbon or Nitrogen units)
PPL	Priority PolLutant
PSU	Practical salinity units
PVDF	Poly(vinylidene fluoride)
RNA	Ribonucleic acid
RT	room temperature
RT	Room Temperature
SAL	Salinity
SPE	solid phase extractions
SW	shallow waters
TEM/SEM	Transmission / surface electron microscopy
TOC	Total Organic carbon
TREC	TRaversing European Coastlines
TSG	Termo salinograph

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Executive summary

The deliverable D1.1 provides the BIOcean5D Handbook of Protocols describing the suite of technologies and protocols used for a holistic assessment of marine biodiversity and contextual data across pelagic and benthic organisms and ecosystems. It contains all standardised methodologies used across the project to generate consistent and quantitative datasets from existing and novel marine samples covering the taxonomic, spatial, and time scales explored in BIOceant5D.

This Handbook involves BIOcean5D standards and best practices related to:

- Sampling strategy
- Sampling protocols
- Environmental and metadata collection
- Samples processing and Biobanking
- Flow charts

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Introduction

Marine biodiversity sustains ecosystem services for planetary and human health. Recent surveys of marine ecosystems have unveiled our ignorance of the richness and functioning of marine life, which is changing at a faster rate than terrestrial life in the Anthropocene. BIOcean5D unites major European centres in molecular/cell biology (EMBL), marine biology/ecology (EMBRC), and sequencing (Genoscope), together with 26 partners from 11 countries, to build a unique suite of technologies and protocols allowing holistic re-exploration of marine biodiversity, from viruses to mammals, from genomes to holobionts, across exceptional spatial and temporal scales from pre-industrial to modern times. A focus is given on understanding pan-European biodiversity *land-to-sea gradients* and ecosystem services, including marine exposomes, with notably the TREC expedition that will deploy in 2023/24 mobile labs, research vessels including the schooner *Tara*, and innovative citizen science tools, through 21 coastal countries and 35 marine labs from the Mediterranean to Arctic seas.

The BIOcean5D Handbook of Protocols assembles all protocols used in the project to sample and measure *in situ* the biological, (bio)chemical, and physical parameters allowing holistic assessment of marine ecosystems (waters, sediments, atmosphere) and habitat-keystone holobionts (e.g. kelp forests, seagrasses, invertebrates, microalgae, sponges, *etc*). It is arguably the most complete guide to date to sample marine life across taxonomy and levels of biological organisation, with state-of-the-art protocols for metagenomics, metabarcoding, metatranscriptomics, metaproteomics, metabolomics, chemical profiling, environmental sensing, or eDNA and bioacoustics to detect larger animals.

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1. Marine waters and sediments sampling and processing

1.1. Marine water column

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1.1.2. Introduction

The sampling of the water column is conducted on board the schooner *Tara* along her ***Tara* EUROPA** expedition (April 2023 – July 2024; https://fondationtaraocean.org/en/expedition/tara-europa/). *Tara* EUROPA is the ocean part of a larger program, **TREC** – TRaversing European Coastlines. In this framework, a team of 6 international researchers and engineers on board *Tara* realise the sampling of European coastal waters and aerosols in synchronisation with the land team who samples shallow waters, superficial sediments, soils, aerosols and selected species. *Tara* EUROPA is therefore integrated into the systematic and standardised cross-sectional sampling of European coastlines.

1.1.3. Sampling Strategy

162 sampling stations at 120 sites (Land-Sea Transects) across 20 European countries have been pre-planned for the *Tara* EUROPA sampling, covering the water column part of the TREC expedition during 2023 and 2024. The *Tara* stations are located along the TREC land-sea transects, in continuity to the terrestrial sampling. The 120 sites represent pristine and a variety of human impacted sites (such as agricultural sites, city, ports and touristic places), as well as river outputs. There are three types of *Tara* EUROPA 'sites' : (i) the '*coastline sites'* with a single ocean station to characterise local Land-Sea transects; (ii) the '*estuary sites'* with 2 or 3 stations to assess ecosystems in and out river plumes; (iii) the '*super sites'* with 3 to 4 stations to characterise highly coastal to oceanic biogeochemical areas.

At each "coastline site" and "estuary site" *Tara* Europa station, the team onboard realise a suite of 54 protocols, and collect c.a. 100 samples stored in appropriate conditions for future analyses in laboratories. At 'Super sites', additional protocols are carried out for a total of around 190 samples. These protocols allow in depth characterization of the biological content and diversity present in the water (from viruses to animals, from genomes, expressed genes, metabolites, proteins, to cellular and organismal features), together with contextual physical, biophysical, chemical and biochemical properties. At pre-selected harbours, three subsites were sampled for port and aliens surveys.

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Optimising *Tara* EUROPA sampling station locations using Satellite data and tide information

The positions of the coastal stations are pre-adjusted based on historical remote-sensing (ocean colour) and oceanographic (bathymetry, currentology) data, and can be slightly modified depending on real-time remote sensing data and weather/navigation conditions. Overall, the *Tara* Europa stations represent a patchwork of coastal water masses with different degrees of impact from the land ecosystems.

Default station locations are pre-planned based on monthly climatological means of CHL concentration. Such a climatological analysis highlights spatial biogeochemical patterns on the statistical ground and allows safe recognition of target sampling area. Sampling stations are then re-adjusted based on high-resolution (300 m), site-specific satellite information, to ensure that water sampling occurs within well defined and strategic biogeochemical coastal patterns. When available (i.e., in case of cloud-free conditions) location of sampling stations in each site are proposed based on near real-time (NRT) satellite images of Chlorophyll-a (CHL) and total suspended matter concentration as a proxy of phytoplankton biomass and resuspended sediments, collected on the sampling day and during the previous three to five days preceding the sampling day, continuously. These satellite maps capture the main biogeochemical spatial patterns across the target site and the fine-scale variability associated with the coastal environment.

Finally, information on tidal currents is provided to set water sampling activities (i.e., CTD rosette cast, water pumping, net deployments, processing of collected water, etc.) during the slack water period of low tide, when there is little or no horizontal motion of the water due to tidal currents and influence of the land.

Following the observed gradient of CHL concentration, mainly from the inner coast towards the open sea, the locations of the sampling stations will be arranged as follow:

a. In the case of one single station at "**coastal sites**" (no time to explore biogeochemical gradient along a cross-shore transect), this will be located within the inner coastal/shallow biogeochemical pattern that marks a well-defined coastal ecosystem. Such a sampling station will be at least around 1 km from the coastline and be at a bathymetric depth of at least 10 metres.
b. In the case of two to four stations along cross-shore transects in "**estuary sites**" and "**super sites**", they will be relocated/refined within diverse biogeochemical patterns (i.e., from the coastal/shallow biogeochemical areas to most offshore waters).

Organisation and distribution of protocols on-board

Fifty-four protocols are realised in one Tara Europa regular station (see the step-by-step protocols below). in order to collect approximately 100 samples stored in appropriate conditions for future analyses in laboratories. The 54 protocols are divided into 6 pre-determined series of protocols.

The science team on board is composed of 6 individuals who are each responsible for one series of protocols. The work is therefore divided into six "profiles":

1. Oceanography engineer
2. Biology engineer – Aerosol, Biogeochemistry, Trace metals and Flowcam
3. Omics protocols

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4. Carbon cycle and chemical profiling protocols
5. Decknet-5 µm, flow cytometry, genetics, single cell genomics and eDNA protocols
6. Biogeochemistry, pigments, Decknet-20 µm & Plankton nets processing

Operator A coordinates the deployment of all sampling gear and instruments. During stations, he/she works closely with everyone on deck and helps in particular the other operators with pumping water out of the Niskin bottles and rinsing the nets.

Operator B, **C** and **D** work closely together on deck and in the different labs to coordinate the various filtrations and the sampling of the rosette for biogeochemical protocols.

Operator B is in charge of the Flowcam protocol and also coordinates the aerosol surveys and the MTE sampling. He/she helps **operator F** in the deployment of the different nets. He/she is also responsible for all the science activities, materials and science supplies.

Operators D works on the various filtrations for chemical profiling and biogeochemistry protocols.

Operator E and **F** work closely together to coordinate the deployment and processing of the various nets, as well as processing seawater from the rosette for biology.

Sampling plan for one station

1. Cleaning process depending of the protocols and preparation of filters/capsules/various sampling set-up
2. Deployment of the Rosette in surface (0-5m)
3. Starting of filtrations for OMICS protocols and eDNA
4. Boarding of the Rosette
5. Filtration of water from the Rosette (Biogeochemistry, biology and chemicals profiling)
6. Deployments of the 200-µM and 680-µM nets
7. Treatment and sorting of the nets
8. Filtration of water with 5-µM decknet and fixation for various protocols
9. Filtration of water with 20-µM decknet
10. Processing of the 20-µM sample (filtration on different filters, fixation with different preservatives and Flowcam)
11. BowPole for MTE protocol
12. Mercury protocols using a niskin bottle in the S-Lab only in estuaries
13. Alien protocol only on pre-selected harbours

1.1.4. Sampling tools and equipment

- **Pump A20:** tubing system in sub-surface waters, connected to a peristaltic pump installed in the wet laboratory on *Tara*'s deck. Water is then filtered through large membranes to concentrate plankton biomass for genetic analyses.
- **Net:** various types of plankton nets with specific mesh-sizes (5 µm, 20 µm, 200 µm or 680 µm), either on *Tara*'s deck (Decknets, 5 µm, 20 µm) or overboard (200 µm or 680 µm).

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- **Cast:** Rosette sampler (holding 5x12L and 8x8L XL Niskin bottles and sensors) to collect a suite of biophysical data and water samples along the water column.
- **Bow pole:** long stick for clean, contamination-free collection of small volumes of surface water stored for laboratory analyses of trace elements (Manual handled).
- **HTSRB (Hyperspectral Tethered Spectral Radiometer Buoy):** floating gear with sensors to measure optical properties (hyper-spectral radiometry) of surface seawater.
- **Aliens in port:** *in-situ* pumping system (*Watera* capsules) to concentrate biomass from 30L of subsurface water for eDNA analyses.

1.1.5. Metadata collection

Metadata collected by site includes:

- GPS coordinates
- Date
- Station number
- Bathymetry
- Salinity
- Seawater temperature
- Turbidity
- Fluorescence
- PAR
- Tides information
- Sea state
- Wind speed
- Wind direction

Logsheets 'Event' for each deployments included all the metadata of the station site (GPS coordinates, station number, bathymetry, salinity, seawater temperature, turbidity, fluorescence, and other). A second type of logsheets "Samples" include all the barcode is also used for the labelling of the samples and recording any other information about the sampling and sampling processing.

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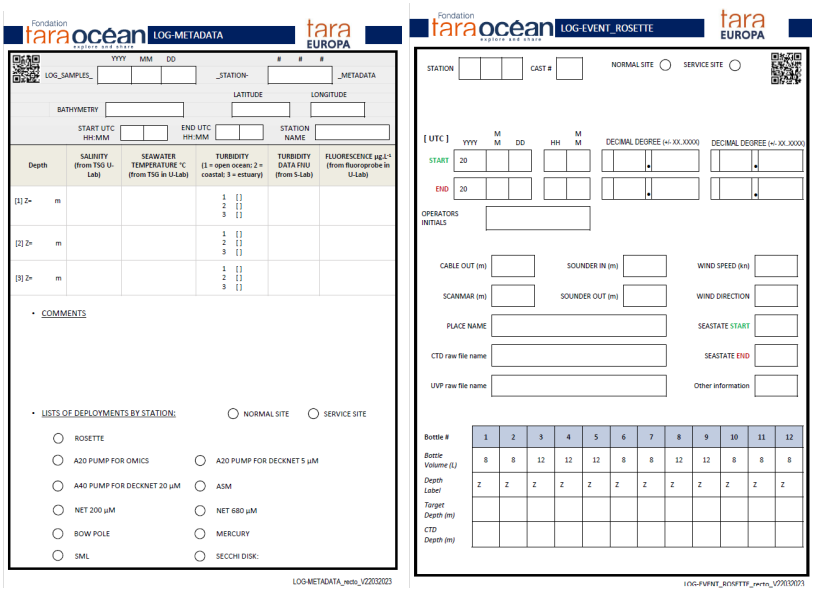
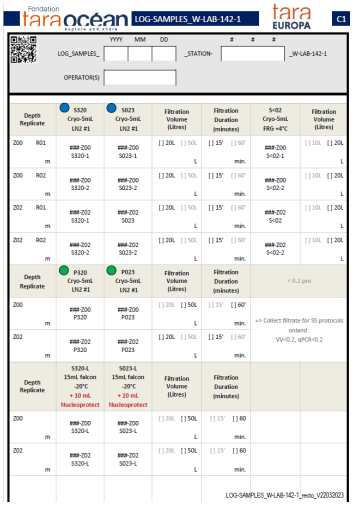


Examples of Event Logsheets



Example of Samples Logsheet

1.1.6. Environmental parameters measurement

On Tara, an underway laboratory with an in-line system is dedicated to the measurements of sea parameters. All the instruments and their associated parameters are listed as following:

- TSG: Measures temperature and conductivity
- ACS: Measures absorption and attenuation of visible light by particles (400-750 nm). Provide information on quantity and types of particles in the water. It can be used to measure chlorophyll-a concentration, phytoplankton biomass, average of particles and phytoplankton sizes, and estimate the phytoplankton “species” that dominate the community.

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- Hyper-BB: Measures backscattering of visible light by particles (430-750 nm). Provide information on quantity and types of particles in the water.
- SUVF (CDOM): Colored Dissolved Organic Matters measures fluorescence of organic materials.
- IFCB: Automated microscopy of phytoplankton from 7-140 micrometres in size. Collect thousand images every 25 min from a 5ml volume.
- CTD on the Rosette: Measures the temperature, conductivity (salinity), dissolved oxygen, CDOM, fluorescence, turbidity, PAR, and transmittance of the sea.
- Cytosense: automated measurements of phytoplankton cell size and fluorescence from less than 1 to 600 micrometres in size. Microscopy images are also acquired to facilitate cell identification.
- Fluoroprobe: Measures the quantity and concentration of chlorophyll-a present in phytoplankton and enables identification of the main planktonic communities present in the water.
- PAR: Photosynthesis Active Radiation provides information on photon fluxes received from the sun.
- Radiometers (HTSRB/HyperPro and So-Rad): measures the intensity and colour of light coming into the water from the sun and light reflected by the sea water. Provides a link between the colour/intensity of the water as seen from satellite and the composition and concentration of materials in the water
- HSP above-water hyperspectral reflectance: Records the colour of the sea to compare with satellites data. Particularly near the coast, where the colour of the sea can change with tides, river outflow, wind mixing and algal bloom.

1.1.7. Sampling protocol

The different sampling protocols are organised in four categories (a. to d.): Oceanography/biogeochemistry, chemical profiling, nucleic acids/sequencing and imaging.

a. Preparation of samples for Oceanography-biogeochemistry analysis

DICTA – DIC & Total Alkalinity

Contact: Frederic Gazeau (frederic.gazeau@imev-mer.fr)

CAUTION – This protocol is subject to contamination and uses toxic substances

- Smoking is not allowed on deck during sampling
- Mercuric Chloride ($HgCl_2$) is toxic; Handle with gloves (see MSDS sheet)

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- $HgCl_2$ will contaminate Mercury samples; DICTA samples and HgCl2 must be stowed away before the HG samples are collected

Step-by-Step

1. Install a clean silicone tube on the Niskin spigot, and open the spigot
2. Rinse 3x times the 500-mL sample bottles with ca. 50 mL of sample
3. Let the clean silicone tube reach the bottom of the bottle and fill it slowly, avoiding bubbles
4. Count in your head the time required to fill the bottle and let the sample water overflow by at least one full volume, counting the filling time once the bottle starts to overflow
5. Close the Niskin spigot, pinch the silicone tube and slowly remove it from the sample bottle, thus leaving an air space at the top (0.5 cm below the base of the neck); if this does not work, give the bottle a quick "flick" or use a pipette to get the desired result
6. Bring the bottle into the "poison box" on the deck table and add 300 µL of HgCl2, using the dedicated pipette
7. Put three diagonal thin strips of carbon-free Apiezon grease extending 2/3 of the way from the top towards the bottom of the ground portion of the stopper; and twist it in the neck of the bottle to squeeze the air out of the grease and make a good seal.
8. Secure the stopper with a rubber band and invert the bottle gently to disperse the mercuric chloride solution thoroughly.
9. Store in the dark (sample box) at room temperature (RT).

SAL – Salinity

Contact: Gilles Reverdin (reve@locean-ipsl.upmc.fr), LOCEAN, Paris, France

CAUTION – Glass bottles can break

- Separate bottles in the storage box to avoid choc during sailing and transport

Step-by-Step

1. Fill the glass bottle, keeping 1 cm of air at the top to allow for water expansion
2. Close the glass bottle with the plastic cap insert and the cap

<!> If cap inserts are not available, secure the outside of the cap with parafilm

3. Store in the sample box at room temperature (RT) in the front storage area (F-Store)

NB: *This protocol is done every three days and only outside estuaries*.

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MTE-T – Marine Trace Elements – Total & T-HG – Total Mercury

Contact: MTE, Seth John (sethjohn@usc.edu); T-HG, Lars-Eric Heimbürger-Boavida (lars-eric.heimburger@mio.osupytheas.fr); University of Southern California and Mediterranean Institute of Oceanography (MIO), Marseille

This protocol is under the responsibility of **Operator B** (**blue**) and the metadata is recorded on one (**blue**) logsheet.

Bow-pole sampling procedure

Unfiltered seawater is collected using a custom-made hand-held bow-pole at each sampling station to determine total dissolvable iron (Fe), zinc (Zn), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), and lead (Pb). Samples are collected from the bow of the boat while oriented towards the wind to minimise contamination.

Low-density polyethylene (LDPE) bottles (125 mL) were cleaned on land by soaking overnight in 1% Citranox detergent, rinsed thoroughly, then soaked for at least 1 week in 10% HCl, followed by at least 8 rinses in ultrapure water, and individually enclosed in plastic bags to reduce contamination during transport and storage.

CAUTION - This protocol is extremely sensitive to contamination:

- Use polyethylene gloves
- If the wind is coming from backward, ask the sea man if it's possible to luff during this sampling (turn the boat to go towards the wind)
- Do not sample if the sea is too rough. Wear your life jacket and security ropes

Step-by-Step

This protocol requires 2 people, i.e. the ***Clean*** scientists who touches ONLY the sampling bottle; and the ***Dirty*** scientist who uses the bow-pole

Open the grey box where IRON bottles are stored on the deck

1. ***Dirty & Clean***: Put on your gloves without touching the outside of the gloves
2. ***Dirty***: Open the MTE-S box; take one zip-lock; and open the zip-lock
3. ***Clean***: Grab the sample bottle in the opened zip-lock and do not touch anything else
4. ***Dirty***: Close the MTE-S box; and grab the carbon stick in the grey box
5. ***Dirty & Clean***: Go to the bow without touching anything except the bottle and the bow-pole, respectively; and Take position toward wind direction
6. ***Dirty***: Attach both security ropes to the boat
7. ***Clean***: Place the closed bottle in the receptacle at the end of the bow-pole; Open the bottle and hold the cap without touching the inside
8. ***Dirty***: Unfold the bow-pole up-wind at the maximum length; Fill the bottle with water that has not been in contact with the boat; Bring back the full bottle
9. ***Dirty***: fill the 40-mL vials for T-HG protocol
10. ***Dirty:*** Refill the MTE bottle as indicated line 8
11. ***Clean***: Carefully close the bottle with its cap; and take the bottle off the bow-pole

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12. ***Dirty***: Detach both security ropes
13. ***Dirty & Clean***: Go back to the A-deck without touching anything

Clean: Place the bottle into the zip-lock bag; close the zip-lock; label the outside of the zip-lock bag (not the bottle and not inside the bag); and store the zip-lock the MTE-S box where you took it from.

p.MeHg, f.MeHg, p.THg, f.THg, uf.THg – Mercury

Contact: Mark C Marvin-DiPasquale (mmarvin@usgs.gov); Jacob A Fleck (jafleck@usgs.gov) and Lars-Eric Heimbürger-Boavida (lars-eric.heimburger@mio.osupytheas.fr); USGS Water Resources Mission Area, California and Mediterranean Institute of Oceanography (MIO), Marseille.

Step-by-step:

p.MeHg and f.MeHg protocols

1. With the water flow OFF, screw on one preloaded filter holder to the end of the PFA line.
2. Slowly open the water control valve and begin collecting the filter-passing water into the collapsible cubitainer.
3. Note: it is important to capture all of the water passing through the filter so that the volume passing through the filter can be measured at the end of each filter collection
4. After some water has been filtered (e.g. 15-20 ml), switch from collecting water into the container (or into the graduated cylinder) and begin collecting water into the 125 ml PETG plastic bottle. Rinse the bottle 3 times with 20ml for each rinse (cap and shake) and dump the rinse water into the cubitainer. Then fill the PETG container to the 100 ml mark. After collecting this f.MeHg sample, continue collecting any additional filtered water in the cubitainer until the filter begins showing signs of clogging.
5. After filling the 125ml PETG bottle, add 1ml of 6N HCL. Mix gently, place the bottle back into the original ziplock and store in the dark (refrigerated).
6. Once the filter is beginning to clog, turn the water pressure off, upstream of the filter holder and allow a few moments for the back-pressure to naturally drop.
7. Very gently loosen the PFA nut at the top of the filter holder and remove the filter holder from the PFA line keeping the filter in an upright position
8. Take the 60ml syringe and pull back the plunger, filling the barrel with air, before attaching the syringe onto the upstream end of the filter-holder. Gently push the remaining water through the filter.
9. Gently unscrew the two valves of the filter holder. Using the plastic forceps, remove the filter and place it (dirty side up) into the small plastic petri dish. Retape the petri dish, return the petri dish to the zip-lock and freeze immediately.
10. After the first filter is safely stored, use the graduated cylinders to measure the quantity of water filtered and write it on the logsheet. Do not add into the recorded value the 100ml volume collected into the sample bottle.

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p.THG and f.THg protocols

This process is almost exactly the same as the one described above for the p.MeHg and f.MeHg sample collection, with the following exceptions

1. Re-used the PFA nut to secure the THg filter holder
2. Sample collected into 40ml glass vial (instead of 125 ml PETG bottle). Ensure bar code with 'f.THg' analysis type is used
3. Do not preserve the f.THg sample with acid !
4. All other steps are the same as for the p.MeHg pair , including: the collection and volume measurement of filter-passing water, the collection of the filter and preservation of the filter, and the need to push through the water sitting on top of the filter prior to opening the filter holder, …

Unfiltered THg (uf.THg) sample

1. At the end of the processing
2. With NO FILTER-HOLDER attached to the PFA line, simply turn back on the flow from the continuous main unfiltered water source- and rinse (3x) the 40ml glass vial.
3. Do not add acid. Store in a cool dark place
4. There is no need to measure of record water volumes used in the collection of this unfiltered sample

TOC – Concentrations of total organic carbon

Contact: Chiara Santinelli, CNR-IBF (chiara.santinelli@ibf.cnr.it)

CAUTION – this protocol is extremely sensitive to contamination

- Do not smoke on deck during sampling
- Wear polyethylene gloves or nytril gloves without powder
- Wash the gloves with MQW

Step-by-Step

1. Collect the sample directly from the Niskin bottle.
2. Rinse 3 times the 40 mL glass vial with the sample: fill the 40mL glass bottle about a third full (or better half full); close the bottle, shake to rinse and discard the content (REPEAT this step three times).
3. Fill 3 x 40 mL vials, until 1 cm below the base of the neck.

<!> Be careful not to touch the inside of the cap or the bottle.

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4. Dispense 150 µL of 2M High purity HCl in the vials, close the cap and mix. There is a small box dedicated with the HCl already diluted and the pipette with tips for this operation.

<!> Do not use the HCl from other bottles, do not use other pipette or tips to spike the HCL. The HCl and the pipette is dedicated ONLY to DOC and TOC acidification.

5. Store the bottle at 4°C in the front storage (F-Store).

NB: Not necessary to spike the vials one by one. They can be spiked all together once filled or together with those for DOC.

Filtration with portable peristaltic pump – DOC/CDOM/FDOM/NUT-1 protocols

Contact: Chiara Santinelli, CNR-IBF (chiara.santinelli@ibf.cnr.it) and Giancarlo Bachi, CNR-IBF (giancarlo.bachi@ibf.cnr.it)

Setup of the portable peristaltic pump

Filtration for DOC and CDOM/FDOM will be carried out by using a homemade portable peristaltic pump (Figure 1) with silicon platinised tubes and Sterivex filters. One end of the tube will be attached directly to the Niskin bottle, the other end will be attached to the Sterivex filter (Figure 1).

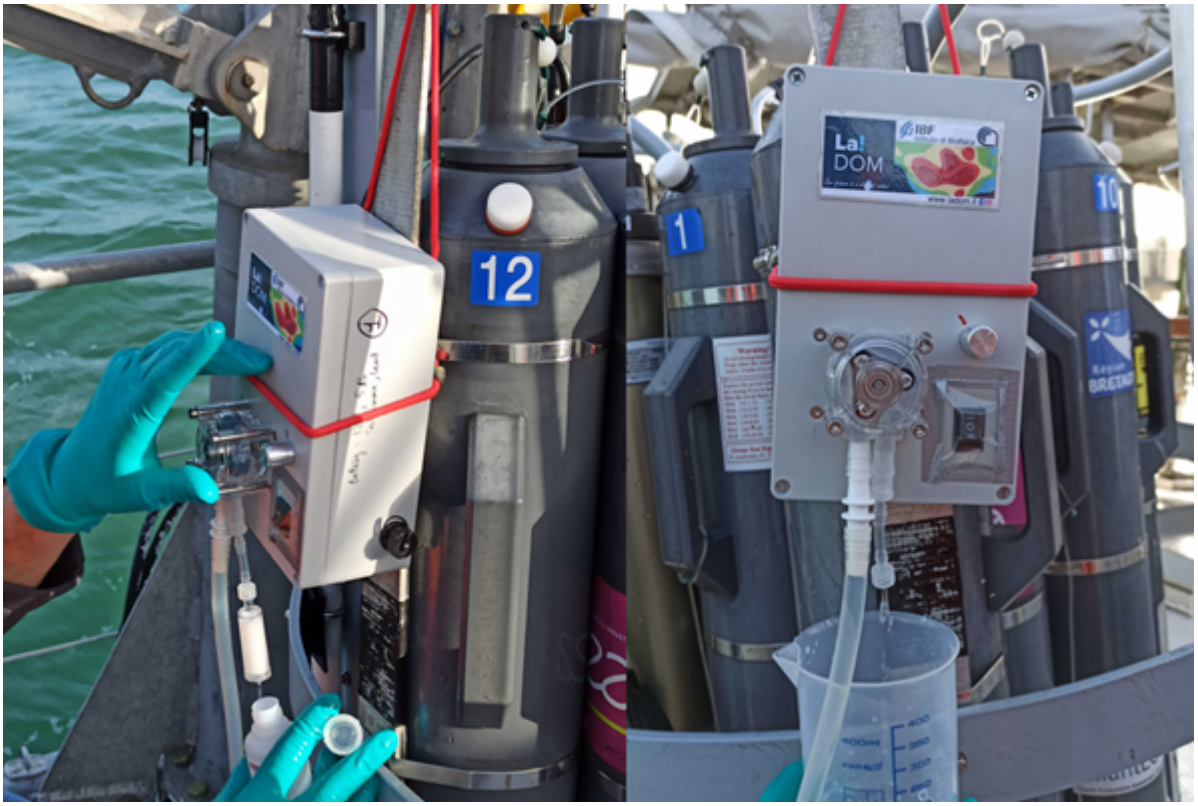

Figure 1. *Homemade portable peristaltic pump connected with the Niskin bottle and Sterivex filter.*

CAUTION

<!>Charge the battery of the pump before each station.

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<!>Always wear gloves (polyethylene or nitrile, without powder!) while handling the tubes, the bottles and the filter.

<!>Do not smoke when the system is filtering and near the rosette.

<!>Before preparing the setup and anytime you change your gloves, wash your gloves with MQW.

BEFORE FILTRATION

1. Turn on the pump.
2. Select the right pump rotational direction using the black button.
3. Put the long end of the tube in the 2 L polycarbonate Nalgene bottle (you will find a dedicated bottle in the protocol box) with MQW.
4. Start the pump and fill the tube with MQW.
5. Open a Sterivex filter and keep the bag.

<!> be careful not to touch the filter edges.

6. Attach and screw the Sterivex filter to the adapter.

<!> Be careful not to touch the edge of the adapter.

7. Start the pump flow and wash the sterivex with 500 mL of MQW.
8. Put the Sterivex filter back in its bag.

<!> You can clean the filter a few hours before to use it, no need to clean it immediately before the filtration.

9. Start the pump and empty the tube from the MQW water.

FILTRATION

10. Attach the long end of the tube to the Niskin bottle.
11. Start the pump and wash the tube with at least 300 mL of sample.
12. Stop the pump.
13. Attach and screw the MQW cleaned Sterivex filter.

<!> Be careful not to touch the edge of the adapter

14. Start the pump flow.

<!> The speed should not be changed, probably the flow of filtered water will decrease during filtration, change the filter if filtration speed decreases too much, but do not change the speed of the pump.

15. Wash the Sterivex with the sample (few mL are enough to remove the MQW from the filter).
16. Collect samples in this order: nutrients, DOC and CDOM/FDOM.

<!> If the filter is clogged, change it and wash it with 500 ml of MQW and a few mL of sample before using it.

AFTER FILTRATION

After filtration, wash the tube with 2 L of (you will find a dedicated bottle in the protocol box) to clean the system. To do this, wash the long end of the tube with and insert it inside the 2 L bottle.

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<Important!> If the filtration system is not used for more than two days, wash the tube with ~200 mL of HCL at 1%. For the dilution dispense 10 mL of HCl 25% in 1 L of MQW (you will find a cylinder and a 1L polycarbonate Nalgene dedicated bottle in the protocol box).

<!> Do not clean the system with ethanol or other chemicals.

<!> Do not use Bleach to clean the system

NUT - Nutrients

Contact: Hugo Berthelot (hugo.berthelot@ifremer.fr)

CAUTION – This protocol is subject to contamination

- DO NOT smoke on deck during sampling

Step-by-Step

1. FILTRATION by portable peristaltic pump, follow the protocol for filtration with portable peristaltic pump (1-14).
2. Rinse the 60-ml bottles; Close the vial and shake to rinse it and discard the content (REPEAT this step a second and a third time)

Collect the sample:

1. Dispense the sample into the bottles (leave ~1 cm air)
2. Repeat the previous step until all bottles and vial are filled

<!> DO NOT touch or wet the neck of the vial or the thread of the cap; Salt crystals could otherwise form and breach the seal, letting air in, which can contaminate the sample

<!> DO NOT fill to the brim to allow sample water to expand in the freezer, without breaching the seal and leaking brine

3. Store upright in the sample box at -20°C in the freezer (FRZ)

DOC – Concentrations of dissolved organic carbon

Contact: Chiara Santinelli, CNR-IBF (chiara.santinelli@ibf.cnr.it)

CAUTION – this protocol is extremely sensitive to contamination

- Do not smoke on deck during sampling

Co funded by the European Union (GA# 101059915). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

- Wear polyethylene gloves or nytril gloves without powder
- Wash the gloves with MQW

Step-by-Step

1. FILTRATION by portable peristaltic pump, follow the protocol for filtration with portable peristaltic pump (1-16).
2. Rinse 3 times the 40 mL glass vial with filtered sample: fill the 40 mL glass bottle about a quarter full, close the bottle, shake to rinse and discard the content (REPEAT this step 3 times).
3. Fill 3 x 40 mL vial, leaving 1 cm of head space.

<!> Be careful not to touch the inside of the cap or the bottle.

4. Dispense 150 µL of 2 M High purity HCl in the vial, close the cap and mix. There is a small box dedicated with the HCl already diluted and the pipette with tips for this operation.

<!> Do not use HCl from other bottles, do not use other pipette or tips to spike the HCL. The HCl and the pipette is dedicated ONLY to DOC and TOC acidification.

5. Store in the sample box at 4°C and in the dark in the front storage area (F-Store).

NB: Not necessary to spike the vials one by one. They can all be filtered and spike together at the end of the filtration.

CDOM/FDOM – Chromophoric/Fluorescent Dissolved Organic Matter

Contact: Chiara Santinelli, CNR-IBF (chiara.santinelli@ibf.cnr.it)

CAUTION – this protocol is extremely sensitive to contamination

- Do not smoke on deck during sampling
- Wear polyethylene gloves or nytril gloves without powder
- Wash the gloves with MQW

Step-by-Step

1. FILTRATION by portable peristaltic pump, follow the protocol for filtration with portable peristaltic pump (1-16).
2. Rinse 3 times the 60 mL polycarbonate bottle with filtered sample: fill the 60 mL Nalgene bottle with ~20mL of filtered sample; close the bottle, shake to rinse and discard the content (REPEAT this step three times).
3. Fill 3 x 60 mL bottles until the base of the neck of the bottle.

<!> Be careful not to touch the inside of the cap or the bottle.

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4. Store the bottle in the dark and at 4°C in the front storage (F-Store).

Filtration for HP, PM, FOI & PA protocols

Water turbidimeter step-by-step protocol:

The turbidity (T, in Formazin Nephelometric Unit (FNU)) of the water filtered for PM is measured on the bench using portable HACH 2100P and 2100QIS turbidimeter.

1. Power on the HACH turbidity sensor
2. Fill one 10-ml vial with freshwater and rinse it three times.
3. Make sure the water sample is homogeneous, fill the 10-ml vial with the water sample and rise twice.
4. Fill the 10-ml vial with the water sample, dry the vial with optical cleaning tissue and ensure it is clean
5. Place the filled vial into the turbidity sensor, close and press 'measurement', read and note on the logsheet the measured turbidity (in FNU)
6. Repeat steps 3 to 5 3 times to measure the water turbidity in triplicates.
7. Rinse the vial with freshwater then empty it.
8. Switch off the HACH turbidimeter.

Taxonomy – HP – pigments (HPLC)

Contact: Céline Dimier (SAPIGH; celine.dimier@imev-mer.fr)

TRIPLICATE ONCE A MONTH

CAUTION - Cells & pigments may be modified, degraded or lost by:

- ***Time spent in the 2L-bottle*** - Filter as soon as water is drawn from the rosette. If some bottles have to wait, keep them cool and in the dark space.
- ***Light*** - Either close the lights in the S-Lab or cover the bottles with black plastic cover.
- ***Time spent on a dry filter*** – Immediately package & store filters once the filtration is finished.
- ***Too much suction*** – The vacuum should not exceed 0.04 cm Hg (0.0005 bar) (0.008 PSI).

<!> Regularly homogenise the sample of water during the filtration

Step-by-Step

1. ***Clean the filtering device***: Use the rinse-bottle with MQW to clean the inside of the filter holders and funnels while the suction is on. Cover each funnel with aluminium foil.

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2. ***Position the filters***: Use clean tweezers (tips standing in EtOH and rinsed with MQW before use) to handle the filters. The GF/F filters must be well centred to avoid leaks. Position them on the filtration head, always with the unsquared side up. Switch on the pump and open the valves; the suction will "stick" the filters in the right position; install the funnels on top; switch off the pump.
3. ***Fill the 2L-bottles up to the top*** and close them with the caps: The 2L-bottles are pre-labelled with a sampling depth label (Z0-Z10). From cast to cast, the Niskin Bottles (numbered 1 to 12) collect water from different depths. You must refer to the sampling plan (see Oceano engineer) to determine which Niskin bottle corresponds to which depth label.
4. ***Start filtrations***: Put on the caps with the siphon system; Position and secure the 2L-Bottles on the filtration system; Switch on the pump; Start the chronometer or note the start-time on the logsheet.
5. ***End filtrations***: Close a valve as soon as its filter dries out; Stop the chronometer and note the duration on the logsheet or note the end-time on the logsheet; Remove the 2L-Bottle and the funnel; Make sure to have in hand the 2mL-cryotube that has the corresponding label; Fold the filter in four, always using clean tweezers, and place it in the cryotube.
6. ***<!> Do not rinse the filtration cups*** before removing the filters
7. ***<!> Do not rinse the filter with*** MQW
8. ***Store the filters***: temporarily in the -20° freezer in the S-Lab and later transfer them in the liquid nitrogen (Dewar #2) labelled “HPLC” in the W-Lab. Do not leave the filtrations unattended to do this. Do not leave tubes with filters on the filtration bench.
9. ***Time limit***: The standard protocol is to filter the entire 2L-Bottle (2.27 L), but this volume can be reduced if the water is highly charged with particles. As a rule, end the filtration after maximum 45 minutes. If so, close the syphon system with its clamp and proceed to end the filtration and store the filter; Measure the volume remaining in the 2L-Bottle with a graduated cylinder; note the volume filtered on the logsheet; Discard the remaining volume.

Rinse the filtering device: Use the rinse-bottle with MQW to clean the inside of the filter holders and funnels while the suction is on. Cover each funnel with aluminium foil.

PM – Particulate Matter (PN/POC/d13C/d15N) & particulate light absorption (ap)

Contact: David Doxoran (david.doxaran@imev-mer.fr)

CAUTION – This protocol is sensitive to carbon contamination

This protocol uses pre-combusted and pre-weighed GF/F filters

<!> Regularly homogenise the sample of water during the filtration

Step-by-Step

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1. The same filters are used to measure PM, PN, POC and isotopes, in triplicates; pre-combusted and pre-weighed GF/F filters are provided in their petri slide with a name and a weight written on it
2. Open the three filter holders and rinse the inside generously with MilliQ water
3. Using two clean tweezers, place a Dacron pre-filter and three pre-combusted 25-mm GF/F filter (one per filter holder), and close the filter holder
4. Filter 0.5 to 1 L of sample material (depending on water turbidity)
5. Stop the pump and note the filtered volume on the logsheet
6. Rinse the filter with MilliQ water (1/3 of the sample volume)
7. Using one clean tweezer, place each of the three filters in inside its petri slide, face up
8. Place the petri slides in the cold and in the -20°C freezer
9. Repeat steps 2 to 8 with 2 other pre-combusted and pre-weighed filters in order to measure the PM organic/inorganic fractions
10. Rinse the filter holders with freshwater

<!> Note on the logsheet the volume seawater filtered

FOI – Fraction organic/inorganic

Contact: david.doxaran@imev-mer.fr

CAUTION – This protocol is sensitive to carbon contamination

This protocol uses pre-combusted and pre-weighed GF/F filters

<!> Regularly homogenise the sample of water during the filtration

Step-by-Step

1. Use 3 pre-combusted and pre-weighed 25mm GF/F filters provided in their petri slide with a name and a weight written on it.
2. Open the three filter holders and rinse the inside generously with MilliQ water
3. Using two clean tweezers, place the 3 (three) pre-combusted 25-mm GF/F filter (one per filter holder), and close the filter holder
4. Filter the exact same volumes of water samples as for particulate matter (PM)
5. Stop the pump and note the filtered volume on the logsheet
6. Rinse the filter with MilliQ water (1/3 of the sample volume)
7. Using one clean tweezer, place each of the three filters in inside its petri slide, face up
8. Place the petri slides in the cold and in the freezer (-20°C)
9. Repeat with 2 other pre-combusted and pre-weighed filters in order to measure the PM organic/inorganic fractions

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10. Rinse the filter holders with freshwater

<!> Note on the logsheet the volume seawater filtered

PA – Particulate absorption (ap)

Contact: david.doxaran@imev-mer.fr

CAUTION – This protocol is sensitive to carbon contamination

<!> Regularly homogenise the sample of water during the filtration

1. Rinse with MilliQ water the central filter holder and funnel and use new GF/F 25 mm filter directly from their box, i.e., DO NOT use pre-combusted and pre-weighed filters
2. Using a clean tweezer, place a filter with its regular gridded face up
3. Filter 250 mL of water sample, and repeat adding 250 mL of water sample until the filter is coloured
4. Rinse the filter with MilliQ water (1/3 of the sample volume)
5. Using a clean tweezer, carefully remove the filter from the filter holder, put it on a new petri slide, face up, and write the station name and date on both sides using a cryopen
6. Rinse the filter holders with freshwater

No replicate for this protocol

b. Preparation of samples for chemical profiling analyses

MB320/MB033/HLB/DOM-PPL– targeting unicellular eukaryotes, prokaryotes, exometabolites and geoexometabolome

Contact: Georg Pohnert (georg.pohnert@uni-jena.de) University of Jena, Germany and Jessika Füssel (jessika.fuessel@uol.de) University of Oldenburg, Germany

Filtration system setup and sampling:

<!> Filtrations for these protocols are done using peristaltic pumps and 142 mm filter holders. The piece of tube that goes in the pump head MUST be Tygon tubing (light beige colour, opaque). No Silicon tubing can be used anywhere in the setup. The inlet & outlet tubes must be securely fixed. It may be necessary to put adjustable metal collars.

- Always wear gloves when handling the tubing, filters, cartridges etc.

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- Clean tubing and adapters with Ethanol every 3 stations when sampling is finished, use MilliQ water and MQW pH 2 (MQW pH2) after cleaning with ethanol
- Before preparing the setup and anytime you change your gloves, wash you gloves with MQW or seawater directly from the rosette if MQW is limited
- Avoid applying any beauty products (especially sprays), such as sunscreen or deodorant while sampling or sample processing
- No smoking in the vicinity of the CTD rosette while sampling

Before each set of filtrations:

1. Place a 2.8-µm Cytiva Whatman™ Binder-Free Glass Microfiber Pre-fiters, Grade GF/D on the first tripod of the line
2. Place a 0.3-µm Advantec GF75142MM Grade GF75 Glass Fiber filter filter on the second tripod of the line and close all tripods tightly (Wetlab)
3. Rinse the outlet tubing with MQW, wipe with Kimwipe and insert into the first 10L clean carboys
4. Place the outlet lines of the extraction setup (Tardis) into the funnel at the back of the tardis so the flowthrough can be collected in the large 20 L carboy underneath
5. Clean surfaces, tweezers, intake (sample side) and outflow (filtrate side) tubes, and everything you use with MQW before starting filtration

During filtrations

<!> Always wear gloves and wash your gloves vigorously with MQW or SW from the Rosette before starting, handle filters carefully; Pick them by the edge and do not touch the part of the filter that will be in contact with the sample; Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface

Step-by-Step:

<u>Sample collection and Filtration</u>

1. Rinse the first 20L carboys with ~ 0.5 L Seawater from the Niskin bottle 3 times
2. Fill the carboy with ~ 20 L of water from the Niskins using a clean piece of tygon tubing
3. Insert the funnel with the 20 µm nylon mesh into the mouth of the second carboy
4. rinse the second carboy three times with pre-filtered SW
5. Pour the entire content through the 20 µm mesh into the second carboy carefully
6. Remove the aluminium cover from the intake tubing of the filtration setup and insert into the 20 L carboy, make sure the tubing reaches the bottom of the carboy
7. Fix the tubing with pre-combusted aluminium foil
8. Open all bleeding valves before starting the pumps
9. Start the first pump (flow= 450 mL/min) at a speed of 10, filtration will take ~ 30 min
10. Make sure the outlet tubing is safely inserted into the two 10L receiving carboys
11. Close the bleeding valves once sample water comes out without air

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12. Filter 8 L litres into each 10L carboy
13. Exchange the carboys and filter 8L into the second carboy
14. Lift the tubing from the inflow carboy so it does not reach the water anymore and pump air through the system until no more water flows out
15. Open the bleeding valves of each tripod until no water flows out
16. Stop the pump
17. Open the bleeding valves on top of the two filter holders to release the pressure and open the first tripod and hang the 'lid' onto the provided hook
18. With clean tweezers, carefully fold the first filter into a small triangle and place it in the pre-labelled 50 ml Falcon tube
19. Repeat with the second tripod and filter
20. Immediately freeze the Falcon tubes at -20
21. Add 11.48 ml of 25% HCl to the PPL carboy and shake well
22. Prepare one 2L PC bottle with 2L MQW and one 2L PC bottle with 2L MQW pH2

Extraction

1. Transfer 2 L acidified sample into 3 x 2L polycarbonate (PC) bottles and 2 L neutral samples into 3x 2L PC bottles
2. Remove the aluminium cover from the inlet tube of pump
3. Pull the first three lines of tubing through the hole in the lid of the PC bottles that contain the neutral sample
4. rinse each tube with MQW from a squeeze bottle and make sure the tube reaches the bottom of the bottle
5. Do the same with the fourth line and insert it into the MQW blank
6. Remove the aluminium foil from the next 3 lines of inlet tubing and insert each line as above into the 3 2L PC bottles that contain the acidified sample
7. Insert the last line to a 2L PC bottle containing 2 L MQW pH 2
8. Make sure the outlet tubes are safely inserted into the receiving funnel for the waste carboy
9. Start the pump (flow= 16 mL/min)
10. make sure all lines are filled with sample and let a small amount run through
11. Stop the pump
12. Have pre-cleaned Luer adapters ready for HLB and PPL cartridges (different sizes). They can be stored in a pre-combusted beaker covered with pre-combusted aluminium foil)
13. One by one, remove the caps of the HBL and PPL cartridges and replace them with the clean Luer adapters that you take out with clean tweezers
14. Save the caps from the PPL cartridges in a ziplock bag for cleaning
15. Attach 4 HBL cartridges (directly from the package) to the first 4 lines of the peristaltic pump

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16. Attach 4 pre-conditioned PPL cartridges to the remaining 4 lines of the peristaltic pump
17. Start the pump (flow= 16 mL/min)
18. Run for ca. 2-3 h until all water has run through the cartridges
19. When all water has run through, and the cartridges are dry, stop the pump
20. Fill one 0.250 L HDPE bottle with 100 mL MQW pH2 from the "MQW pH2 for cartridge rinsing" bottle (250 ml PC bottles(in "Jessika's box in the forepeak) and one 0.25 L HDPE bottle with 100 mL MQW
21. Remove the first 3 lines of inflow tubing from the carboy and insert it into the HDPE bottle with MQW (non-acidified) (blank does not need to be rinsed)
22. Start the pump (flow= 15 mL/min)
23. Rinse the cartridges
24. When the bottle is empty, remove the tubing an insert it into a clean 2 L PC bottle
25. Let the pump run until the cartridges are dry (for about 2 min)
26. Repeat the same rinsing and drying steps for the 3 PPL cartridges using the HDPE bottle containing MQW pH2
27. Remove all cartridges one by one from the tubing and transfer them to their corresponding Falcon tube
28. Immediately freeze the samples at-20°C

After each station:

1. Empty the collection carboys and rinse with fresh water (FRW)
2. Rinse the stainless steel funnel and the 20 µm mesh with FRW and then with some MWQ and let it dry
3. Rinse the inside of all carboys with FRW
4. Rinse the filtration system wit 8-10 L of FRW
5. After each site (~ every 3 samples) rinse the filtration and extraction system as well as all bottles and adapters with 100% Ethanol
    - Fill 500 ml of ethanol into a PC bottle and pump it through the filtration system while collecting the flow through in another PC bottle
    - Take the ethanol to subsequently rinse the 2 L PC bottles
    - Fill the ethanol into one PC bottle and pump through the extraction system
    - Collect all adapters in a precombusted beaker and cover with ethanol, soak for ~ 1 h
    - Discard the ethanol
    - Rinse the adapters thoroughly with MQW
    - Soak the adapters in MQW pH2 for at least 4 h (or longer)
    - Transfer the adapters to MQW and soak for at least 4 h (or longer)
    - Transfer the adapters to a clean pre-combusted beaker and cover with aluminium foil until used again
6. Use the pre-prepared MQW pH2 in the 10 L carboy and run 2 L through the filtration system

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7. collect the MQW pH2 in a carboy cleaned with FRW and use it to clean all carboys vigorously by successively pouring it from one to the next
8. rinse 1 10L carboy with some MWQ and fill it with 4-5 L of MQW from the onboard system
9. Rinse the filtration system with 2 L of MQW
10. Collect the MQW and use it to clean all carboys vigorously by pouring from one to the other successively
11. Rinse all 2 L PC bottles with MQW pH2 2 x and MQW 2x by pouring it from one bottle to the next successively
12. Fill 2 L of MQW pH 2 into a clean 2 L PC bottle
13. Rinse the extraction system with 2 L of MQW pH2
14. Fill another clean 2 L PC bottle with 2 L of MQW and rinse the system
15. Rinse the outside of the tubing that will be inserted into the sample with some MQW from a squeeze bottle, wipe with Kimwipe and wrap the ending in pre-combusted aluminium foil
16. Leave the tripods open to dry for a few hours
17. Collect the Luer adapters for a few stations in a plastic bag, rinse them with MQW and bath them in MQW pH2 over night and then in MQW for at least 4 h using a pre-combusted beaker
18. Rinse the tweezers with MQW, and store them in the 50 ml Falcon for the next station
19. Store everything in its place in the tardis, fixed securely with the rubber bands attached to the right side ready for the next station

c. Preparation of samples for nucleic acids/sequencing analyses

MB20 – targeting unicellular eukaryotes

Contact: maia.henry@uni-jena.de

CAUTION – This protocol is very sensitive to contamination from the skin

- *Wear Nitrile gloves at all time*
- *Always clean tweezers with EtOH and then MilliQ water before use*

Step-by-Step

1. Thoroughly rinse one 47-mm filter holder and cup with MQW
2. Place a 2.7-µm GFD filter on the filter holder and place the cup on top
3. Gently but thoroughly mix the 250-mL glass bottles and pour ca. 100 mL in the filtration cup
4. Switch on the suction; Gradually add more volume, always mixing the glass bottle before pouring; Not too much at a time in case the filter clogs

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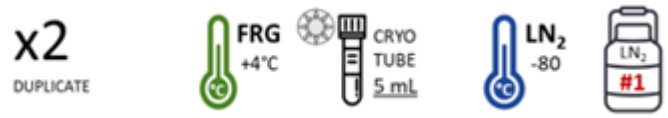
5. ***<!> Do not exceed 15 minutes*** for the filtrations; keep the remaining volume in the glass bottles to measure the unfiltered volume in a graduated cylinder
6. Switch off the suction as soon as the filter gets dry
7. Thoroughly rinse the cup with filtered sea water, using a little suction to drain; Repeat as needed and remove the cup
8. Using two dissecting tweezers, fold the filter in half without touching the sample with the tweezers and without "crushing" the filter too much.
9. Insert the filter into a 4-ml glass vial and store the vial at -20 °C
10. ***<!> write on the logsheet the total volume filtered***

Thoroughly rinse the 47mm filter holder, cup and cylinders with FRW; re-assemble the filter holders and cups; rinse with EtOH

Genetics – SG – single-organism-genomics pico/nano

Contact: Flora Vincent (flora.vincent@embl.de)



CAUTION – Glycine Betaine is not a toxic substance

- ***Wear gloves, handle with care, and read the safety sheet (MSDS)***
- ***The pre-aliquoted cryotubes are kept at +4°C in the fridge (FRG) in the front storage area (F-Store)***

Step-by-Step

1. Dispense 4 mL of whole seawater into a 5-mL cryotube (star-shaped cap) pre-aliquoted with 600 µL of 48% Glycine Betaine.
2. Close the cryotube and mix gently by inversion
3. Temporarily place in the thermos container for max 15 minutes
4. Store in Liquid Nitrogen Dewar #1 in the W-Lab

Genetics – HC – virus Hi-C sequencing

Contact: Matthew Sullivan, Ohio State University, USA (mbsulli@gmail.com)

Step-by-Step

1. Dispense 3 mL of whole seawater into a 5 mL cryotube (x8 replicates).
2. Close the 8 cryotubes
3. Temporarily place in the thermos container for max 15 minutes

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4. Store in Liquid Nitrogen Dewar #1 in the W-Lab.

Genetics - HC-G - virus HI-C sequencing

Contact: Matthew Sullivan Ohio (USA, mbsulli@gmail.com)

Step-by-Step

1. Dispense 3 mL of whole seawater into a 5 mL cryotube containing pre-aliquoted 0.75mL glycerol (x8 replicates).
2. Close the 8 cryotubes and mix by inversion.
3. Temporarily place in the thermos container for max 15 minutes.
4. Store in Liquid Nitrogen Dewar #1 in the W-Lab.

Genetics - CP-G - Cryopreservation

Contact: Shinichi Sunagawa (ETHZ, ssunagawa@ethz.ch)

Step-by-Step

1. Dispense 3 mL of whole seawater into a 5 mL cryotube containing 0.75 mL pre-aliquoted glycerol (x3 replicates).
2. Close the 3 cryotubes and mix several times by inversion.
3. Temporarily place in the thermos container for max 15 minutes.
4. Store in Liquid Nitrogen.

MetaBGTomics – eDNA – Environmental DNA

Contact: Sophie Arnaud (sophie.arnaud-haond@umontpellier.fr), MARBEC, Ifremer, Montpellier, France and Frédérique Viard (frederique.viard@umontpellier.fr), ISEM, CNRS, Montpellier, France)

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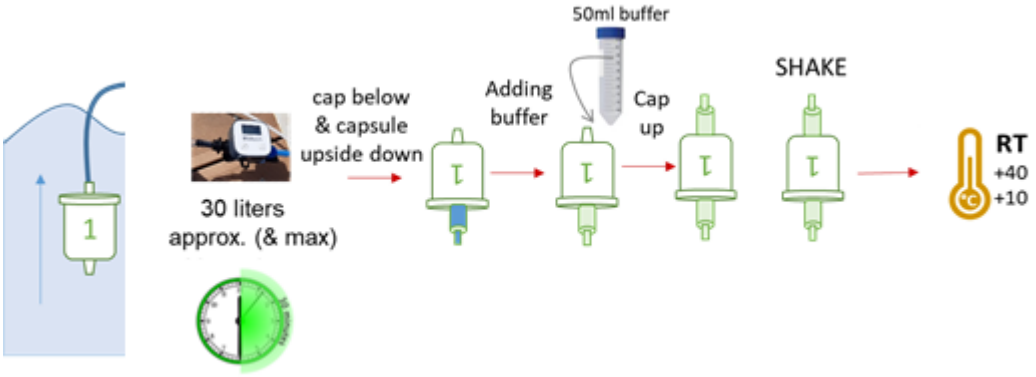

CAUTION – This protocol is sensitive to DNA contamination

Step-by-Step

1. Put on gloves (preferably two pairs to ease the change of gloves if needed).
2. Before opening the bag, put the blue plug on the curved end of the water capsules (without pushing it in as they are difficult to remove once fully pushed). Open the bag with a scissor. Take the filter capsule out of its plastic bag (KEEP THE BAG AND BLUE PLUGS FOR LATER).
3. Place a Masterflex tubing at the outlet of the capsule (on the flat side: SIDE MATTERS) and attach it with Serflex.
4. Fix the pole/rod with the hose on the edge of the boat and connect the hose to the Masterflex tubing linked to the capsule. Secure it with Serflex.
5. Place the capsule below the surface in the water (immersed by ca. 50 cm) as far as possible from the hull using the pole/rub.
6. Turn on the pumps and filter 30 litres
7. After 30 litres are pumped, put the capsules upside down, without stopping the pump, in order to suck air to empty the water remaining in the capsule. Make sure you have emptied the capsule.
8. Place the capsule in the plastic packaging and remove the Masterflex. Avoid touching the capsules with your hands, even with gloves.
9. Place a blue plug at the capsule no.1 outlet (flat side)
10. Place a small 10 cm-long silicone tube on a syringe to retrieve the buffer from a Falcone tube, then remove the small silicone tube. Screw the white adapter onto the syringe and insert it in the capsule to fill in the capsule with the buffer.
11. Place the second cap (blue plug) and shake vigorously for 30 seconds to 1 minute the capsule with the shaker or by hand.
12. Put the capsule in its bag and in a Ziploc. Put 1 label on the capsule + 1 label on the Ziploc
13. Store the capsule preferably in the fridge (although room temperature might be fine for 1-2 weeks)

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MetaBGTomics – S320-S and S023-S– targeting unicellular eukaryotes and prokaryotes

Contact: Julie Poulain (poulain@genoscope.cns.fr), Genoscope

CAUTION – This protocol is sensitive to RNA degradation - 15 min max

Caution before filtration:

· Proceed with the "quick cleaning" before the first filtration at the beginning of each station

· Clean surfaces, tweezers, intake (sample side) and outflow (filtrate side) tubes, and everything you use with bleach 10% using a spray and then rinse with filtered sea water using a spray.

· Always wear gloves and change them regularly as needed

· Handle filters carefully; Pick them by the edge and do not touch the part of the filter that will be in contact with the sample.

· Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface**.**

Step-by-Step:

1. Humidify all clean filter holders generously with filtered sea water
2. Place Dacron pre-filters on all the tripods, then a 3-µm filter on the first tripods of each line, and a 0.2-µm filter on the second ones, and close all tripods tightly with the key
3. Wipe the intake (sample side) and outflow (filtrate side) Masterfelx tubing with bleach 10% using a spray and then rinse with filtered sea water using a spray, and place the tubes in their respective 10-L, 20-L or 50-L carboys
4. Ensure that flowmeters are in place at the outlet of the second tripods of each line. Make sure the flowmeters countdowns are initialised at 20 L. Start the countdown.
5. Open all bleeding valves before starting the pumps
6. Start the pumps (<15 psi) and start the timer for each filtration line.
7. Close the bleeding valves once sample water comes out without air.

When all water went through the filters OR <!> no longer than 15 minutes

8. ***Pull the intake Masterfelx tubing off the water (or above the water level if there is still water remaining in the carboy)***.
9. Keep the pump running until no more remaining water in all the lines and stop the timer (Do not reset the timer. Filtration duration will be needed at the end.)
10. ***<!> Proceed as quickly as possible to collect filters in order to avoid cell damage and DNA & RNA degradation*** by following the 4 steps below:
    - Make sure there is no more water remaining in the tripods. It is important to allow the filters to dry. To do that: Keep the pump on to resume pumping on the line until air goes through the two filters and no more water comes out of the second filter holder

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<!> If there is still water in the second filter holder you can open the bleeding valves on top of the two filter holders to release the pressure; unplug the top tube connectors from the two filter holders; connect the tube coming from the pump directly on the second filter holder and continue pushing air through it.

- Open the tripod filter holders. Avoid touching the inside of the filter holder area.
- Use the forceps to handle the filters. Soak forceps in the bleach 10% tube and then in the MilliQ water before every use
- Place the filters in their respective 5-mL cryotube

11. Store immediately in Liquid nitrogen (Dewar #1)

When all filtrations are ended AND <!> all samples are stored in liquid nitrogen

12. Read the filtered volume on each flowmeter (see flowmeter memo).
13. ***<!> Note on the logsheet*** the filtration time and volume of filtrate obtained from all filtrations

Caution after the last filtration of each station:

- Proceed to the “full cleaning” at the end of each station

MetaBGTomics – S<02 – targeting viruses

Contact: Matthew Sullivan, Ohio State University, USA (mbsulli@gmail.com)

Caution before filtration:

- Proceed of the "quick cleaning" before the first filtration at the beginning of each station
- Clean surfaces, tweezers, intake (sample side) and outflow (filtrate side) tubes, and everything you use with bleach 10% using a spray and then rinse with filtered sea water using a spray.
- Always wear gloves and change them regularly as needed
- Handle filters carefully; Pick them by the edge and do not touch the part of the filter that will be in contact with the sample.
- Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface.

Step-by-Step:

1. Add 1 mL of the stock solution of Iron Chloride (2.82 g $FeCL_3$ in 50 mL ultrapure water) to each filtrate, shake to mix, and add an additional 1 mL of Iron Chloride stock solution.
2. Shake vigorously the 20-L carboy and leave it to incubate 1 hour in the W-Lab

<After 1 hour>

3. Humidify all clean filter holders generously with filtered sea water

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4. Place Dacron pre-filters on all the tripods, then a 0.8-µm filters on the tripods, and close all tripods tightly with the key
5. Wipe the intake (sample side) and outflow (filtrate side) Masterfelx tubing with bleach 10% using a spray and then rinse with filtered sea water using a spray, and place the tubes in their respective 20-L filtrate carboys
6. Ensure that flowmeters are in place at the outlet of the second tripods of each line. Make sure the flowmeters countdown are initialised at 20 L. Start the countdown
7. Open all bleeding valves before starting the pumps
8. Start the pumps and start the timer for each filtration line
9. Close the bleeding valves once sample water comes out without air

When all water went through the filters OR <!> no longer than 60 minutes

10. ***Pull the intake Masterfelx tubing off the water (or above the water level if there is still water remaining in the carboy)***.
11. Keep the pump running until no more remaining water in all the lines and stop the timer (Do not reset the timer. Filtration duration will be needed at the end.)
12. ***<!> Proceed as quickly as possible to collect filters*** by following the 4 steps below:
    - Make sure there is no more water remaining in the tripods. It is important to allow the filters to dry. To do that: Keep the pump on to resume pumping on the line until air goes through the two filters and no more water comes out of the second filter holder

      <!> If there is still water in the second filter holder you can open the bleeding valves on top of the two filter holders to release the pressure; unplug the top tube connectors from the two filter holders; connect the tube coming from the pump directly on the second filter holder and continue pushing air through it.
    - Open the tripod filter holders. Avoid touching the inside of the filter holder area.
    - Use the forceps to handle the filter. Soak forceps in the bleach 10% tube and then in the MilliQ water before every use
    - Place the filters in their respective 5-mL cryotube
13. Store at +4°C in the Fridge (FRG) in the front storage area (F-Store)

CAUTION after the last filtration of each station:

- Proceed to the “full cleaning” at the end of each station

MetaBGTomics – S320-L and S023-L – targeting unicellular eukaryotes and prokaryotes

Contact: Julie Poulain (poulain@genoscope.cns.fr), Génoscope

CAUTION before filtration:

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- Proceed with the "quick cleaning" before the first filtration at the beginning of each station
- Clean surfaces, tweezers, intake (sample side) and outflow (filtrate side) tubes, and everything you use with bleach 10% using a spray and then rinse with filtered sea water using a spray.
- Always wear gloves and change them regularly as needed
- Handle filters carefully; Pick them by the edge and do not touch the part of the
- filter that will be in contact with the sample.
- Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface.

Step-by-Step:

1. Humidify all clean filter holders generously with filtered sea water
2. Place Dacron pre-filters on all the tripods, then a 3-µm filter on the first tripods of each line, and a 0.2-µm filter on the second ones, and close all tripods tightly with the key
3. Wipe the intake (sample side) and outflow (filtrate side) Masterfelx tubing with bleach 10% using a spray and then rinse with filtered sea water using a spray, and place the tubes in their respective 10-L, 20-L or 50-L carboys
4. Ensure that flowmeters are in place at the outlet of the second tripods of each line. Make sure the flowmeters countdowns are initialised at 20 L. Start the countdown.
5. Open all bleeding valves before starting the pumps
6. Start the pumps (<15 psi) and start the timer for each filtration line.
7. Close the bleeding valves once sample water comes out without air.

***When all water went through the filters** OR <!> no longer than 15 minutes*

8. ***Pull the intake Masterflex tubing off the water (or above the water level if there is still water remaining in the carboy)***.
9. Keep the pump running until no more remaining water in all the lines and stop the timer (Do not reset the timer. Filtration duration will be needed at the end.)
10. ***<!> Proceed as quickly as possible to collect filters in order to avoid cell damage and DNA & RNA degradation*** by following the 4 steps below:
    - Make sure there is no more water remaining in the tripod. It is important to allow the filters to dry. To do that: Keep the pump on to resume pumping on the line until air goes through the two filters and no more water comes out of the second filter holder

    <!> If you feel that there is still water in the second filter holder you can open the bleeding valves on top of the two filter holders to release the pressure; unplug the top tube connectors from the two filter holders; connect the tube coming from the pump directly on the second filter holder and continue pushing air through it.

    - Open the tripod filter holders. Avoid touching the inside of the filter holder area.

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- Use the forceps to handle the filter. Soak forceps in the bleach 10% tube and then in the MilliQ water before every use.

Fold the filter in half with two dissecting tweezers and then fold it in half again lengthwise without touching the sample with the tweezers and without "crushing" the filter too much.

- Place the filters in their respective 15-mL tube. Dispense 10 ml of Nucleoprotect using a propipette and 10 ml pipet. Make sure the filter is well submerged by the Nucleoprotect) and mix by inversion 3 times.

11. Store in the freezer -20°C in the front storage area

When all filtrations are ended AND <!> all samples are stored in freezer

12. Read the filtered volume on each flowmeter (see flowmeter memo).
13. ***<!> Note on the logsheet*** the filtration duration and volume of filtrate obtained for all filtrations

Caution after the last filtration of each station:

- Proceed to the “full cleaning” at the end of each station

Metaproteomics – PT320 and PT023– targeting unicellular eukaryotes and prokaryotes

Contact: Mak Saito, Woods Hole Oceanography Institute, USA (msaito@whoi.edu)

Step-by-Step:

1. Humidify all filter holders generously with filtered sea water
2. Place Dacron pre-filters on all the tripods, then a 3-µm filters on the first tripods of each line, and a 0.2-µm filter on the second ones, and close all tripods tightly with the key
3. Wipe the intake (sample side) and outflow (filtrate side) tubes with EtOH in your glove and then rinse with filtered sea water, and place the tubes in their respective 10-L, 20-L or 50-L carboys
4. Open all bleeding valves before starting the pumps
5. Start the pumps and start the timer for each filtration line
6. Close the bleeding valves once sample water comes out without air
7. ***<!> if 10-L carboys are used to collect the filtrate***, either let filtrate overflow once full, or close the carboy with its cap and collect the filtered sea water filtrate in a dedicated carboy used for rinsing

When all water went through the filters OR <!> no longer than 60 minutes

8. Open the pump head to pause pumping on the line, and keep the pump running
9. Leave the intake (sample side) tube in its carboy, ***OR <!> lift it above the water level*** if some sample remains in the carboy
10. ***<!> Cell damage and degradation happens when the material dries on the filter***; Do not proceed to end a filtration unless you are ready to collect the filter; better wait that all filtrations are finished before proceeding to end a filtration

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11. Proceed to end the filtration (see above)
12. Place the filters in their respective 5-mL cryotube
13. Store immediately in Liquid nitrogen (Dewar #1)
14. If applicable, measure the volume of sample left in any of the carboys

<!> Note on the logsheet the filtration time and volume of filtrate obtained

MetaBGTomics – S20-L, S-200-L and S-680-L – targeting eukaryotes

Contact: Julie Poulain (poulain@genoscope.cns.fr)

CAUTION – This protocol is sensitive to RNA degradation - 15 min max

Caution before filtration:

- Clean surfaces, tweezers, and everything you use with bleach 10% using a spray and then rinse with filtered sea water using a spray.
- Always wear gloves and change them regularly as needed
- Handle filters carefully; Pick them by the edge and do not touch the part of the filter that will be in contact with the sample.
- Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface.

Step-by-Step

1. Thoroughly rinse one 47-mm filter holder and cup with filtered sea water
2. Place a 10-µm PC filter on the filter holder and place the cup on top
3. Gently but thoroughly mix the 250-mL glass bottles and pour ca. 100 mL in the filtration cup
4. Switch on the suction; Gradually add more volume, always mixing the glass bottle before pouring; Not too much at a time in case the filter clogs
5. If filter clogs; keep the remaining volume in the glass bottles to measure the unfiltered volume in a graduated cylinder
6. Switch off the suction as soon as the filter gets dry
7. Thoroughly rinse the cup with filtered sea water, using a little suction to drain; Repeat as needed and remove the cup
8. Using two dissecting tweezers, fold the filter in half without touching the sample with the tweezers and without "crushing" the filter too much.
9. Place the filters in their respective 5-mL tube. Dispense 5 ml of Nucleoprotect using a propipette and 10 ml pipet and mix by inversion 3 times.
10. Check that the entire filter is fully submerged in the buffer and store the tube at -20 °C in the front storage area
11. ***<!> write on the logsheet the total volume filtered***
12. Thoroughly rinse the 47mm filter holder, cup and cylinders with filtered sea water; re-assemble the filter holders and cups

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Caution after the last filtration of each station:

- Proceed to the “full cleaning” protocol of 47 mm filter holder, cup and cylinders at the end of each station

MetaBGTomics- S20-S and S-200-S – targeting eukaryotes

Contact: Julie Poulain, Genoscope (poulain@genoscope.cns.fr)

CAUTION – This protocol is sensitive to RNA degradation - 15 min max

Caution before filtration:

- Clean surfaces, tweezers, and everything you use with bleach 10% using a spray and then rinse with filtered sea water using a spray.
- Always wear gloves and change them regularly as needed
- Handle filters carefully; Pick them by the edge and do not touch the part of the filter that will be in contact with the sample.
- Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface

Step-by-Step

1. Thoroughly rinse one 47-mm filter holder and cup with filtered sea water
2. Place a 10-µm PC filter on the filter holder and place the cup on top
3. Gently but thoroughly mix the 250-mL glass bottles and pour ca. 50-100 mL in the filtration cup. Start the timer.
4. Switch on the suction; Gradually add more volume, always mixing the glass bottle before pouring; Not too much at a time in case the filter clogs
5. ***<!> Do not exceed 15 minutes*** for the filtrations; keep the remaining volume in the glass bottles to measure the unfiltered volume in a graduated cylinder
6. Switch off the suction as soon as the filter gets dry. Stop the timer. (Do not reset the timer. Filtration duration will be needed at the end.)
7. Thoroughly rinse the cup with filtered sea water, using a little suction to drain; Repeat as needed and remove the cup.
8. Using two dissecting tweezers, fold the filter in half without touching the sample with the tweezers and without "crushing" the filter too much.
9. Package it in a 5-mL cryotube and immediately store the cryotube in liquid nitrogen Dewar #1
10. ***<!> write on the logsheet the total volume filtered and filtration duration.***
11. Thoroughly rinse the 47mm filter holder, cup and cylinders with filtered sea water; re-assemble the filter holders and cups.

Caution after the last filtration of each station:

- Proceed to the “full cleaning” protocol of 47 mm filter holder, cup and cylinders at the end of each station

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d. Preparation of samples for imaging analyses

Taxonomy – FC – flow-cytometry pico/nano

Contact: Josep "Pep" Gasol, CSIC, Barcelona (pepgasol@icm.csic.es)

CAUTION – Glutaraldehyde and paraformaldehyde are toxic substances

- Wear gloves, handle with care, and read the safety sheet (MSDS)
- The pre-aliquoted cryotubes are kept at -20°C in the freezer (FRZ) in the front storage area (F-Store).

Step-by-Step

1. FC-P: Dispense 1.5 mL of whole seawater into a 2-mL cryotube (red cap-insert) pre-aliquoted with 50 µL of a solution of paraformaldehyde 32%
2. FC-G: Dispense 1.5 mL of whole seawater into a 2-mL cryotube (blue cap-insert) pre-aliquoted with 30 µL of a solution of Glutaraldehyde 25%
3. Close the cryotube, and mix gently by inversion
4. Incubate for **15 min in the dark at +4°C** in the thermos container
5. Store in Liquid Nitrogen Dewar #3 in the W-Lab

Morphology – FM5 – e-HCFM(/SEM/exM nano)

Contact: Rainer Pepperkok, EMBL (pepperko@embl.de) and Hugo Berthelot, Ifremer (hugo.berthelot@ifremer.fr)

CAUTION – This protocol uses toxic substances

Read PFA & Gluta MSDS

Wear gloves and work in the fume hood

Pre-aliquoted 50-mL Falcon tubes are stored at -20°C in the freezer (FRZ)

Step-by-Step

1. Thoroughly rinse a 50-mL graduated cylinder with MQW
2. Measure 45 mL of the gently mixed sample material from the 250-mL bottle into the cylinder
3. Pour the 45-mL into a 50-mL Falcon tube pre-aliquoted with 1.4 mL of 32% PFA + 225 µl of 50% glutaraldehyde
4. Gently invert the tube a few times and wash the outside of the falcon with fresh water and dry

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5. Thoroughly rinse the graduated cylinder with FRW and then with EtOH
6. Store the tubes horizontal at +4°C in the Fridge

Morphology/Genetics - E20 - single-organisms-genomics micro

Contact: Flora Vincent (flora.vincent@embl.de)

CAUTION:

EtOH may rub-off the information written by hand on the label.

The maximum volume of EtOH in a sample is limited to 15 mL for international shipping

Step-by-Step

1. Pour the content of the 200-mL glass jar onto a sieve of 20µm
2. Gently transfer the bio-material from the sieve to a 15-mL Falcon tube using EtOH
3. Bring the volume of EtOH to 15mL and close the cap tightly
4. Store the 15-mL Falcon tube(s) at -20°C in the Freezer

<!> If the ETOH turns green after 24h, change it using a small 20µm sieve to keep all of the organisms

Morphology – FCAM20 – FlowCam 4x

Contact: Fabien Lombard, LOV (fabien.lombard@imev-mer.fr)

Step by step

1. Load the context file : « setup », « context », on « Load a Context File... » Choose
2. “autoimagemode_current_context_tara_europa” then click on “Load From Database”.
3. *To change for every leg change to define a new raw folder* : In VisualSpreadsheet, click on “Preferences” à “Advanced Settings” à “Settings…” ; “Choose…” and define the new leg pathway.
4. Deactivate Clean and Rinse : « setup », « fluidics », "Uncheck « clean » and « rinse' ' . Close the window.
5. Fill the pipette with MilliQ water and remove the air bubble using the tip.
6. Run « autoimage mode no save » to check the flowcell and let some water run.
7. Fill in some samples and check the quality of the image. Click on « recalibrate » if necessary.
8. Once ready, start « Autoimage mode » : *Stop with 7000 particles count and time after 45 minutes.* Name : **flowcam_taraeuropa_stationnumber_sampleid**

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-do not hesitate to use tool/recalibrate if objects get attached to the glass (or get unstuck)

-do not hesitate to pinch the tube between the flow cell and the pump to unstuck objects

(ideally should be done every 2-3 minutes to be sure not plankton clogs are created)

-Homogenise the sample regularly in the funnel using the pipetman.

-Check that there are no clogs or bubbles that would block the passage of organisms. If

it is blocked, squeeze the tube between your fingers to change the pressure and release the clog.

-Monitor the quality of the images: if you observe objects repeated x times or images containing no objects, click on "Tools" > "Recalibrate".

9. When finished, export the context file : “File” à “Context…” à check “autoimagemode_current_context_projectname” à choose
10. the export directory by clicking on “…” and going into the « raw » folder of the run.
11. Recover all the samples from the pipette tip using the pipetman.
12. Remove the funnel and clean it under the sink with freshwater, and then with MilliQ water.
13. Reconnect it and fill it with some MilliQ water, then run « autoimage mode no save » to let the MilliQ water run in the system, to be flushed. Once you don’t see cells passing anymore, you can go to « setup », « fluidics », « flush », 2 cycles.
14. Cleaning: check the user manual if bottles of flowcam are emptied. « setup », « fluidics », « clean/rinse ». After the cleaning, empty the flowcell by pumping
15. A little water (using camera view). Remove the funnel and put the red cover back on the flowcam.
16. Enter the Metadata in the excel sheet on the computer, and on zooprocess. Process the pid-vignettes overnight. Make the back-up.

Morphology – FM20 – e-HCFM(/SEM/exM nano)

Contact: Rainer Pepperkok, EMBL (pepperko@embl.de) and Hugo Berthelot, Ifremer (hugo.berthelot@ifremer.fr)

CAUTION – This protocol uses toxic substances

- Read PFA & Gluta MSDS
- Wear gloves and work in the fume hood
- Pre-aliquoted 50-mL Falcon tubes are stored at -20°C in the freezer (FRZ)

Step-by-Step

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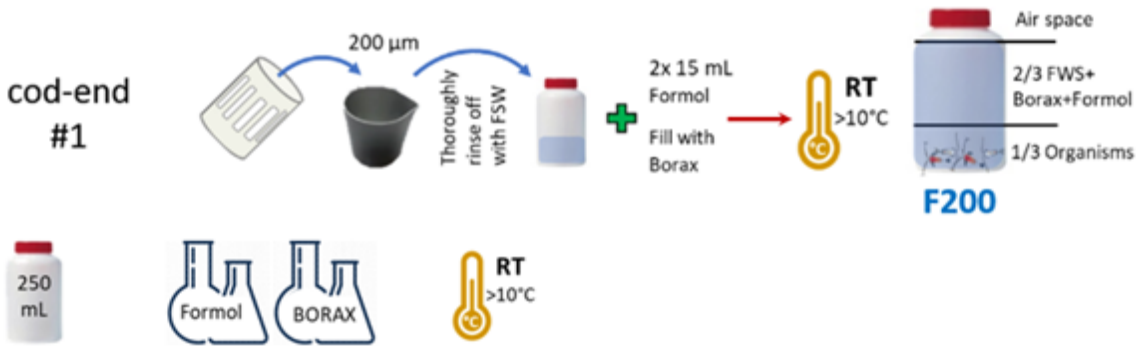
1. Thoroughly rinse a 50-mL graduated cylinder with MQW
2. Measure 45 mL of the gently mixed sample material from the 250-mL bottle into the cylinder
3. Pour the 45-mL into a 50-mL Falcon tube pre-aliquoted with 1.45 mL of 32% PFA + 225 µl of 50% glutaraldehyde
4. Gently invert the tube a few times and wash the outside of the falcon with fresh water and dry
5. Thoroughly rinse the graduated cylinder with FRW and then with EtOH
6. Store the tubes horizontal at +4°C in the Fridge

Morphology – F200 – ZooScan

Contact: Fabien Lombard, LOV (fabien.lombard@imev-mer.fr)



CAUTION: Toxic substance

Step-by-Step

1. Thoroughly rinse the 200-µm sieve with fresh water
2. Progressively pour the content of the cod-end into the 200-µm sieve
3. Rinse the cod-end with FSW until no more plankton is visible in the cod-end
4. Concentrate sample on one side of the sieve using the FSW squeeze bottle
5. Pour the sample in a 250-mL red-cap bottle, and rinse the sieve upside down directly into the bottle using BORAX
6. ***<The quantity of material should never exceed 1/3 of the bottle capacity>*** If it is the case, divide the sample in several 250-mL bottles and make a clear note on the Logsheet.
7. Add 30mL of non-buffered formol using the dispenser in the Fume Hood in the W-Lab
8. Complete with BORAX to a final volume of 250 mL, leaving an air space below the neck of the bottle (see flowchart)
9. Close the bottle with its inside cap liner and the red cap, and rinse it with fresh water
10. Store the sample at room temperature (RT) in the front storage area (F-Store)

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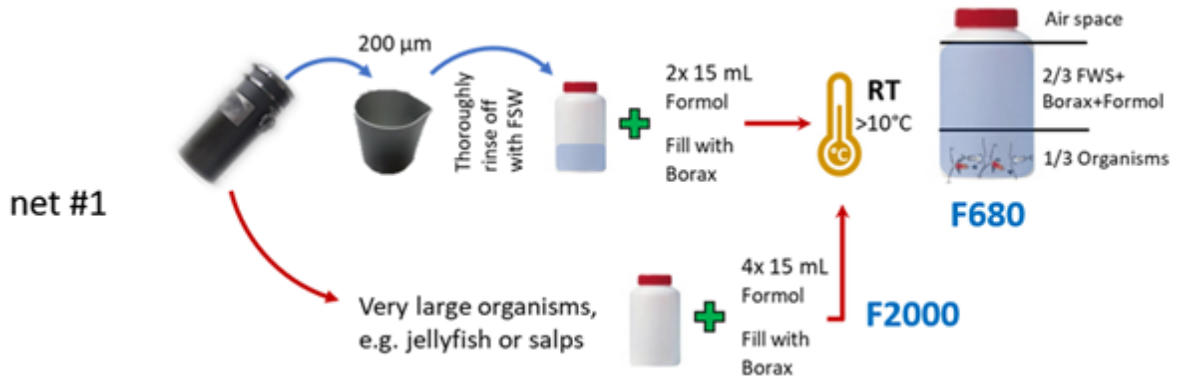
Morphology – F680 & F2000 – ZooScan

Contact: Fabien Lombard, LOV (fabien.lombard@imev-mer.fr)



CAUTION: Toxic substance

Step-by-Step F680

1. Thoroughly rinse the 200-µm sieve and a small funnel with MQW
2. Pour the content of the cod-end into the 200-µm sieve
3. Rinse the cod-end with FSW until no more plankton is visible in the cod-end
4. Concentrate sample on one side of the sieve using the FSW squeeze bottle
5. Pour the sample in a 250-mL red-cap bottle, and rinse the sieve upside down directly into the funnel using the BORAX squeeze bottle
6. ***<The quantity of material should never exceed 1/4 of the bottle capacity>*** If it is the case, divide the sample in several 250-mL bottles and make a clear note on the Logsheet.
7. Rinse the 200-µm sieve and funnel with Borax until no plankton sticks on the side
8. Add 30mL of non-buffered formol using the dispenser in the Fume Hood in the W-Lab
9. Complete with Borax to a final volume of 250 mL, leaving an air space below the neck of the bottle; Close the bottle and rinse it with fresh water
10. Label the bottle with station #, depth, and size fraction, e.g. “MM-001”, “0-150m” and “>200”
11. Store the sample at room temperature in the F-Store

Aliens – at selected sites (ports)

Contact: Frédérique Viard (frederique.viard@umontpellier.fr) ISEM (Institut des Sciences de l’Evolution de Montpellier), CNRS; Montpellier, France; Sophie Arnaud (sophie.arnaud-haond@umontpellier.fr) MARBEC (Marine Biodiversity, Exploitation and Conservation), Univ. Montpellier, Ifremer, IRD, CNR; Sète, France;

CAUTION – This protocol is sensitive to DNA contamination

Step-by-Step

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For each station, three pumps located in a waterproof carrying case are operated simultaneously (less than a minute apart). They are piled- up and secured with a rope to the boat.

1- Switch on each pump before piling them.
2- Install the battery in the flow metre (for measuring the volume of water that has been filtered)
3- Set the flowmeter to 30L. Before mooring the three cases, connect the battery of the pumps and close each case.
4- Place the flowmeter at the pump outlet.
5- Place the end of the silicone tubing (one metre) after the flow metre.
6- Repeat steps 2 to 4 for pump 2 and pump 3.

7- Put on gloves (preferably two pairs to ease the change of gloves if needed).
8- **Before opening the bag**, put the blue plug on the curved end of the capsules (without pushing it in as they are difficult to remove once fully pushed). Open the bag with a scissor. Take the filter capsule out of its plastic bag (KEEP THE BAG AND BLUE PLUGS FOR LATER).
9- Put the three capsules in the basket – clip them and secure them with a colson or rubber band.
10- Take one rigid tube, cut the end of the tube with parafilm and place it along a pole and attach it with nylon collars (cable tie), leaving about 50 cm of it at the end of the pole/rod and attach the other end to the pump inlet.
11- Insert the rigid pipe located at the end of the pole/rod at the outlet of the capsule (**on the side which is flat**: **SIDE MATTERS**), for each of the three rigid tubes (3 capsules = total). (KEEP THE BLUE PLUG FOR LATER). Repeat with 2 other rigid tubes.
12- Place the pole/rod so that the filter/basket is below the surface in the water (immersed by ca.50cm). The pole is used to maintain together the three rigid tubes (attached to the three capsules put together within a basket), and a rope is attached between the boat and the basket containing the three capsules and lested with laids (1 to 3 kilos) to secure the capsules. The capsules should be placed on the bow or the side of the boat, as far as possible from the hull, to avoid contamination from the boat. Note: This implies a static sampling , for i) the pole is not robust enough to cope with water motion when perpendicular to the boat traject and ii) if mowing forward even at 2 to 3 and speed, capsules may end up drifting on the water surface preferably from the bow of the boat to avoid contamination.
13- Plug each of the 3 rigid tubes to one of the 3 pumps.

Summarised flowchart (3 samples per station; 3 stations per sites)

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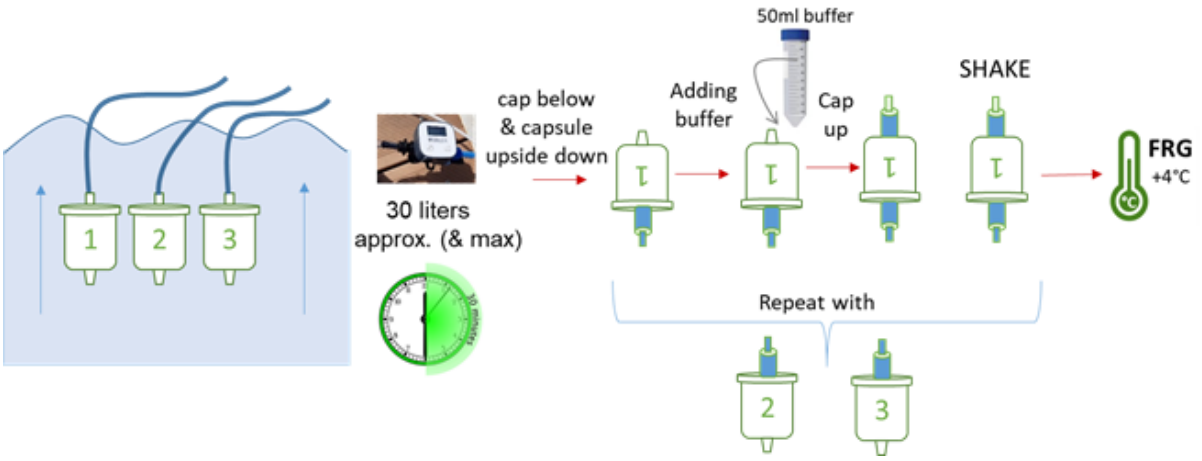

14- Turn on the pumps with the switch on the outside and filter 30 litres. While the water is passing through the filter, the flowmeter indicates decreased volume. When the flowmeter indicates zero, you retrieve the pole and thus the capsules out of the water. **Caution:** the pump will not stop when the flow metre indicates 0; **you have to remove the capsules from the water when the volume on the flowmeter is 0**.

Note: Allow about 20-25 minutes to filter 30 L if the water is not too heavy with organic matter. Stop: it is possible to stop the pump if it takes too long. In that case, note the volume which has been filtered.

15. Put the capsules upside down**, without stopping the pump**, in order to suck air to empty the water remaining in the capsule. Make sure you have emptied the capsule.

16. Place the capsules in their plastic packaging and remove the rigid tube. Avoid touching the capsules with your hands, even with gloves.

17. Place a blue plug at the capsule no.1 outlet (flat side)

18. Place a small 10 cm-long silicone tube on a syringe to retrieve the buffer from a Falcone tube, then remove the small silicone tube. Screw the white adapter onto the syringe and insert it in the capsule to fill in the capsule with the buffer.

Note: in case adaptor and syringe are lacking, for step 18: place a blue plug at the capsule no.1 outlet (flat side), then place a small silicone tube (5 cm) at the capsule inlet and a small serflex within the small silicone tube, then gently pour the buffer along the serflex into the capsule through the silicone tube. This step has to be made very slowly.

Change the silicone tube, adapter and syringe between each site of one port. Keep the silicone tube and the syringe adaptor for recycling. Throw the syringe.

19. Place the second cap (blue plug) and shake vigorously for 30 seconds to 1 minute the capsule with the shaker or by hand.

20- Put the capsule in its bag and in a Ziploc. Put 1 label on the capsule + 1 label on the Ziploc.

21. Repeat steps 17 to 20 for the two other capsules.

22. Store the capsule preferably in the fridge (at 4°C) (although room temperature might be fine for 1-2 weeks)

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23. **If a new sampling is made in a new station of the same site**, leave the rigid tube on the pole for the following stations sampling but protect the tip with a glove or parafilm to avoid contamination. In between stations, although the rigid tube is not to be changed, it is cut by a few cm (ab. 3 cm) to avoid contamination (**before putting it in a glove**). In addition, when arriving at the next sampling point, pump water directly into the tube (without capsule) for ca. 30 seconds, in order to 'clean' it before inserting a new capsule. Set the flowmeter at 30L and repeat the protocol from step 10 (if the rigid tubes are still attached in the pole).
21. **After the last station**, remove the battery from the flowmeter of each pump, remove all rigid tubes. The silicone tube is kept for other sites. Charge the battery by using the battery charging system provided. All the equipment used (e.g. scissors) needs to be disinfected with bleach as frequently as necessary and rinsed with milliQ water.

    Rinse the pumps with bleach (10%) and milliQ water.

Blank (field control):

Every three sites, make an additional sampling (one capsule only) to make a field control: after completing the last sampling (should be in the port) repeat the exact same protocol but by filtering ultrapure water with a single pump and a single capsule (No triplicates needed). For this purpose, use the rigid tube that has been used for the sampling of the day, cut a few cm of the rigid tube then filter pure water (18.2 megohm) without a capsule for ca. 30 seconds, then place a capsule and filter 2 litres of pure water. The capsule is then retrieved from the water, the pump still on to discard all the water inside, and then the pump is shut down and the capsule removed and filled with the buffer (as for step 18).

Sampling tools and equipment:

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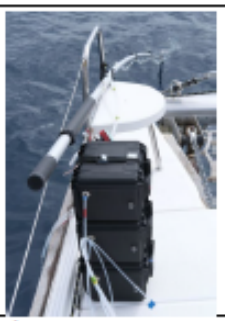
Waterproof carrying case with pump, battery and flowmeter (©Argaly)		N=3
Pole and cable ties ca. 9) (to attach tubes/hose)		N=1
Basket for capsules with leads (1-2g) attached at the bottom and one rope (©Argaly)	No image	N=1
Capsule's shaker (©Argaly)		N=1
1 m silicon hose/tube attached to the pump outlet	See image above (line 1)	N=3
4m rigid hose attached to the pump inlet (and capsules)	See image above (line 1 and 2)	N=3
50 ml tube filled with Longmire buffer		N=9
Capsule ©Waterra		N=9
Adaptor (for filling capsules; from Argaly)		N=9
Syringes (60ml, luer)		N=9
5 cm silicone tube (to retrieve Longmire with the syrynge)		N=9
Pairs of gloves		N=12
Permanent marker - Labels		N=1
20 cm Parafilm		N=9
Cissor		N=1

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1.1.8. Samples processing transient storage, shipping conditions and Biobanking

Protocol code	Protocol name	Sample processing		Transient storage and T° at boat	Responsibles (PI) and biobanking
		sample	Chemical		
DICTA	total alkalinity	500 mL glass bottle with mercuric chloride	HeCl2	RT	Fred Gazeau LOV
SAL	salinity	125 mL glass bottle		RT	Giles Reverdin, LOCEAN
NUT	dissolved nutrients (organic and inorganic)	60 mL HDPE bottle pre washed HCl	na	-20°C	Hugo Berthelot (DYNECO, IFREMER)
MTE	Marine trace Elements - Bow Pole (Co, mn, pb, cu, Ni, Cd, Zn, Fe)	125 mL bottle pre washed HCl		RT	Seth John, USC (california)
FC-P	Flow cytometry - Paraformaldehyde	2 mL cryotube	PFA	LN2	Josep Gasol CSIC Barcelona
FC-G	Flow cytometry - Glutaraldehyde - virus	2 mL cryotube	Glutaraldehyde	LN2	Josep Gasol CSIC Barcelona
HP	HPLC - High performance liquid chromatography	2 mL cryotube		LN2	David Doxaran LOV Villefranche
HC	virus Hi-C	5 mL cryotube		LN2	Matthew Sullivan Ohio USA
HC-G	virus Hi-C glycerol	5 mL cryotube pre aliquoted	glycerol	LN2	Matthew Sullivan Ohio USA
CP-G	cryopreservation in glycerol, same as HC-G	5 mL cryotube pre aliquoted	glycerol	LN2	Shinichi Sunagawa, ETH Zurich
SG	single cell genomics	5 mL cryotube pre aliquoted	Glycine Bétaïne	LN2	Flora Vincent, EMBL, Germany
ASM	aerosol sampling mast	slide	No	-20°C	Michel Flores, Weizman

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					institute, Shinichi Sunagawa ETHZ
CDOM FDOM	Colored/Fluorescent organic matter	60 mL bottle pre washed with HCl		4°C	Chiara Santinelli, CNR-IBF, Italy
DOC	Dissolved organic carbon	40 mL vial pre washed and combusted	HCl (High purity)	4°C	Chiara Santinelli, CNR-IBF, Italy
TOC	Total organic carbon	40 mL vial pre washed and combusted	HCl (High purity)	4°C	Chiara Santinelli, CNR-IBF, Italy
PM	PM/POC/PON Particulate matter/ Particulate organic carbon (and Nitrogen)	filter pre combusted and pre weighted		-20°C	David Doxaran LOV Villefranche/mer
FOI	Fraction organic/inorganic (same proto as PM, optionnal)	filter pre combusted and pre weighted		-20°C	David Doxaran LOV Villefranche/mer
PA	Particulate Absorption	filter pre combusted and pre weighted		-20°C	David Doxaran LOV Villefranche/mer
S<0.2 (Virus)	sequencing virus	5 mL cryotube	FeCl3	4°C	Matthew Sullivan Ohio USA
S023-S	sequencing short read prokayotes	5 mL cryotube		LN2	Patrick Wincker, Julie Poulain CEA/Genoscope
S320-S	sequencing short read eukaryotes	5 mL cryotube		LN2	Patrick Wincker, Julie Poulain CEA/Genoscope
eDNA	sequencing short read eDNA	watera capsule		RT	Sophie Arnaud, IFREMER
ALIEN	Sequencing short read; eDNA	Watera capsule		+4°C	Frederique Viard, CNRS

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S023-L (long read)	sequencing long read	15 mL falcon tube	nucleoprotect	-20°C	Patrick Wincker, Julie Poulain CEA/Genoscope
S320-L (long read)	sequencing long read	15 mL falcon tube	nucleoprotect	-20°C	Patrick Wincker, Julie Poulain CEA/Genoscope
S20-S	sequencing short read	5 mL cryotube		LN2	Patrick Wincker, Julie Poulain CEA/Genoscope
S20-L	sequencing long read	5 mL falcon tube	nucleoprotect	-20°C	Patrick Wincker, Julie Poulain CEA/Genoscope
S200-S	sequencing short read	5 mL cryotube		LN2	Patrick Wincker, Julie Poulain CEA/Genoscope
S200-L	sequencing long read	5 mL falcon tube	nucleoprotect	-20°C	Patrick Wincker, Julie Poulain CEA/Genoscope
S680-L	sequencing long read	5 mL falcon tube	nucleoprotect	-20°C	Patrick Wincker, Julie Poulain CEA/Genoscope
P023	proteomics	5 mL cryotube		LN2	Mak Saito, WHOI USA
P320	proteomics	5 mL cryotube		LN2	Mak Saito, WHOI USA
SG5	single cell genomics	5 mL cryotube pre aliquoted	Glycine Bétaïne	LN2	Flora Vincent, EMBL, Germany
E20	single cell genomics	15 mL falcon tube	ethanol	-20°C	Flora Vincent, EMBL,

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					Germany
SG20	single cell genomics	5 mL cryotube pre aliquoted	Glycine Bétaïne	LN2	Flora Vincent, EMBL, Germany
FM5	fluorescent microscopy / eHCFM	50 mL falcon tube pre aliquoted onboard	Glutaraldehyde, Paraformaldehyde	4°C	Rainer Pepperkok
FM20	fluorescent microscopy / eHCFM	50 mL falcon tube pre aliquoted onboard	Glutaraldehyde, Paraformaldehyde	4°C	Rainer Pepperkok
LV 20	Flowcam / imagery	images		data	Fabien Lombard
F200	formol 200	250 mL bottle with formol	Formaldehyde + Borax	RT	Fabien Lombard LOV, France
F680	formol 680	250 mL bottle with formol	Formaldehyde + Borax	RT	Fabien Lombard LOV, France
MB320	Metabolomics	50mL falcon with GFF filter	no	-20°C	Michael Zimmermann
MB033	Metabolomics	50mL falcon with GFF filter	no	-20°C	Michael Zimmermann
PPL	chemical profiling / DOM	cartridge SPE	HCl to acidify sample before extraction, and MilliQ pH2 after extraction for desalinisation of cartridge	-20°C	Michael Zimmermann
HLB	chemical profiling / Metabolomics	cartridge SPE	no	-20°C	Michael Zimmermann
W<0.3	chemical profiling / DOM, back up for all	glass vial or falcon		-20°C	Michael Zimmermann
WHCl<0.3	chemical profiling / DOM back up for all	glass vial or falcon		-20°C	Michael Zimmermann
MB20	metabolomics	4 mL vial with filter		-20°C	Georg Pohnert, Lena

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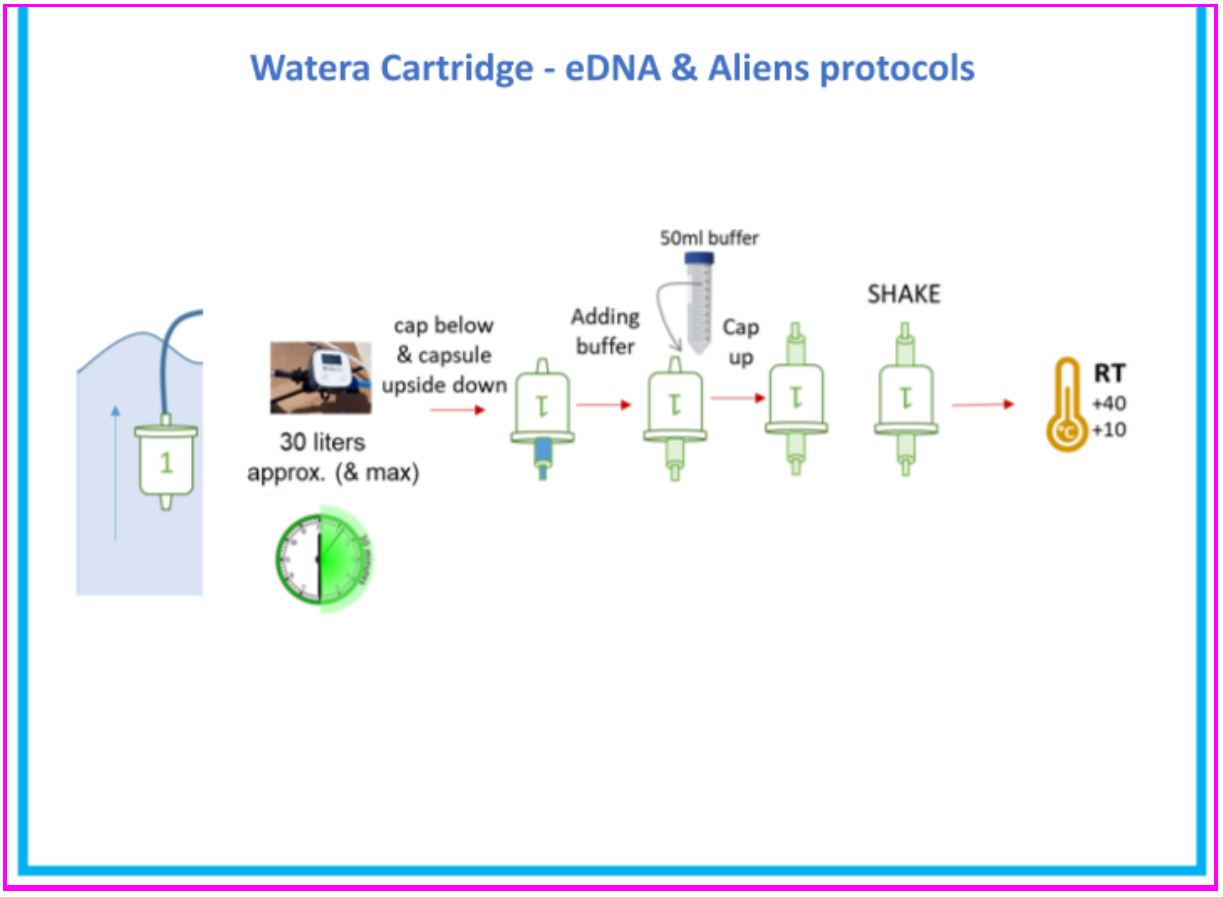
					Germany
pHG-T	Mercury				Lars Eric Heimburger
fHG-T	Mercury				Lars Eric Heimburger
pHG-M	Mercury				Lars Eric Heimburger
fHG-M	Mercury				Lars Eric Heimburger
HG-T	Mercury				Lars Eric Heimburger

1.1.9. Flow charts



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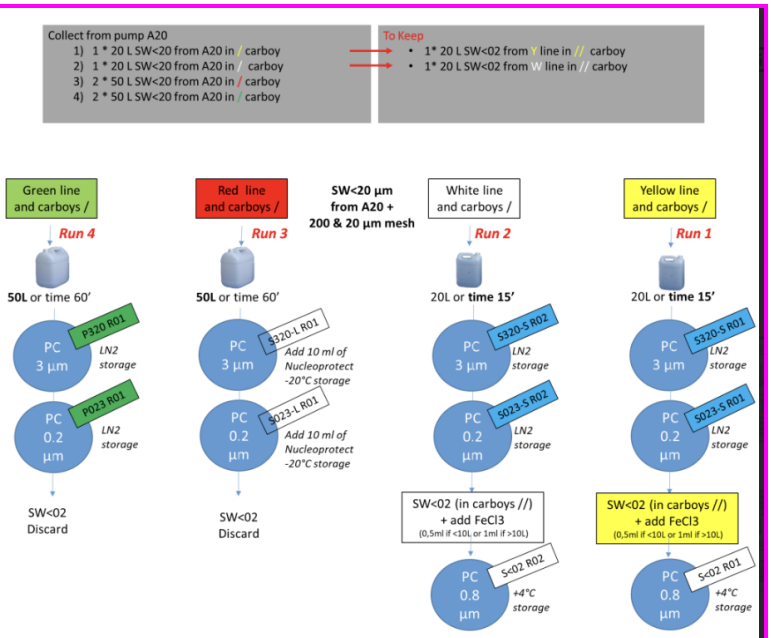
OMICS Flow Chart – operator C – Normal Site
S023-S/S320-S/S<02/S023-L/S320-L/P023/P320

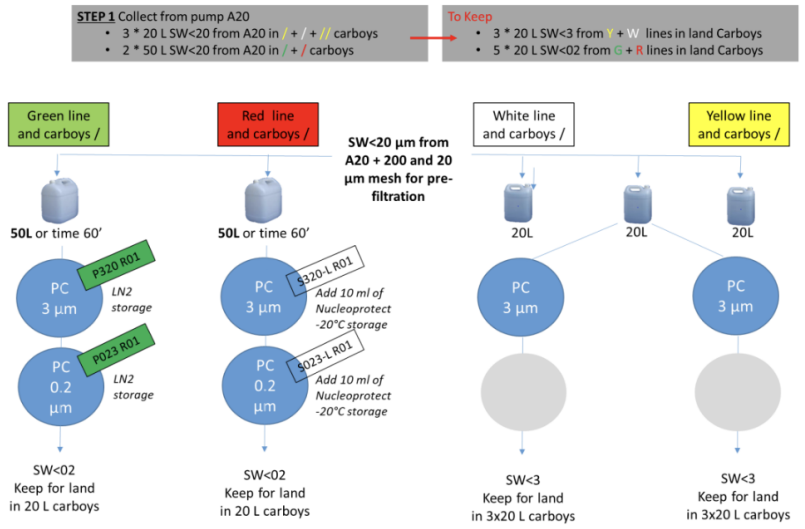
- Consumables
  - Cryotube 5 ml x8
  - Falcon 15 ml x2
  - Nucleoprotect 10 ml x2 (in the chemical hood)
  - Dacron filter x 8
  - Membrane 0.2 µm x 4
  - Membrane 3 µm x 4
  - Membrane 0,8 µm x2
  - FeCl3 0,5ml or 1ml depending of the volume of SW<02 x2 (solution ready to use in wetlab-stock in forepeak)
- Materials
  - Carboys / + / + / + / + // + //
  - Funnel + mesh 20 µm
  - Net 200 µm
  - Javel 10%
  - Spray javel 10%, MQW, FSW, EthOH 70%
  - Tweezers x2
- Instruments used
  - Pump A20
  - FSW
  - Fresh Water
  - Peristaltic pump and tripodes
  - Switch on the circuit-breaker on electric board



OMICS Flow Chart – operator C-Service Site
S023-L/S320-L/P023/P320

STEP 1

- Consumables
  - Cryotube 5 ml x10
  - Falcon 15 ml x2
  - Nucleoprotect 10 ml x2 (in the chemical hood)
  - Dacron filter x 8
  - Membrane 0.2 µm x 6
  - Membrane 3 µm x 6
  - Membrane 0,8 µm x2
  - FeCl3 0,5 to 1 ml x2 (solution ready to use in wetlab-stock in forepeak)
- Materials
  - Carboys / + / + / + / + // + //
  - Carboys for land SW<3 + SW<02
  - Funnel + mesh 20 µm
  - Sieve 2000 µm
  - Net 200 µm
  - Javel 10%
  - Spray javel 10%, MQW, FSW, EthOH 70%
  - Tweezers x2
- Instruments used
  - Pump A20
  - FSW
  - Fresh Water
  - Peristaltic pump and tripodes
  - Switch on the circuit-breaker on electric board



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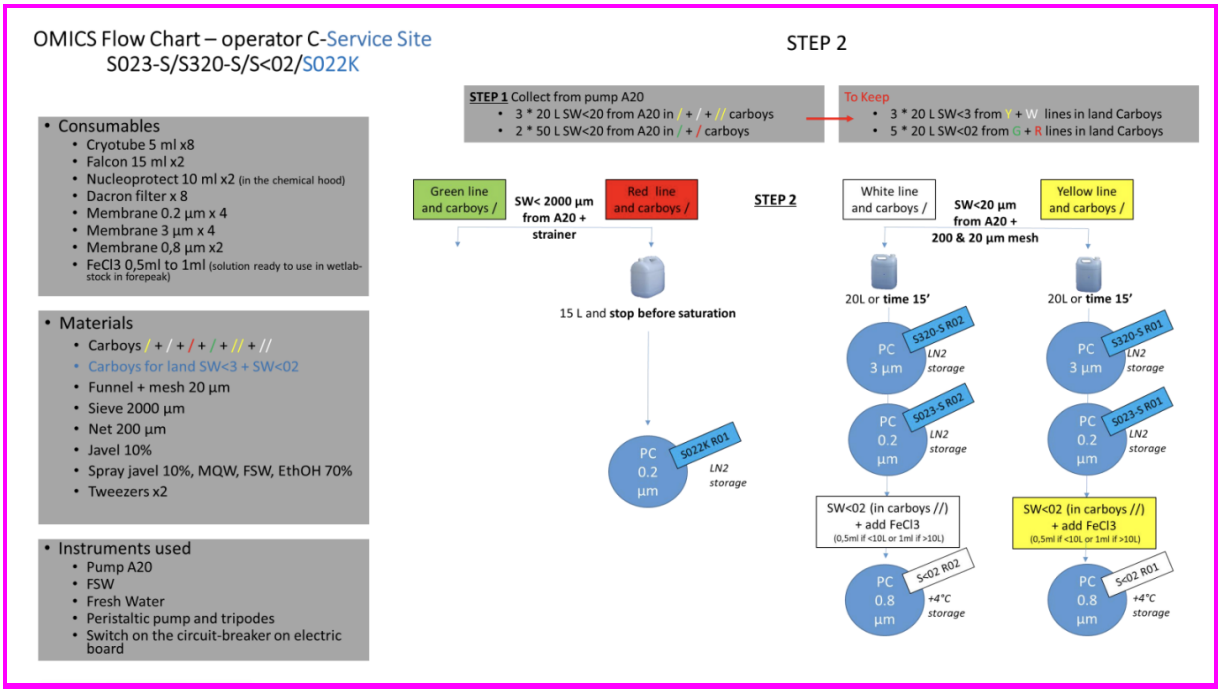

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NET WPII-20

FCAM20  1/8 200mL → Process immediately on the FlowCam

S20 x2  1/8 200mL →  PC 10 µm 47 mm →  →  5-ml cryotube →  LN_2 -80 #1 Store immediately

S20-L x1  1/8 200mL →  PC 10 µm 47 mm →  Falcon 5-ml + 5 mL Nucleo-protect Let soak for 15 – 30 min → FRZ -20°C

If possible, filter more than 200 mL using the « extra for sequencing » bottle.

MB20 x1  1/8 200mL →  GF/D 47 mm →  GLASS VIAL 4 mL →  FRZ -20°C

E20 x1  → Sieve 20 µm  →  Falcon 15-mL + 15 mL EtOH Molecular grade ethanol →  FRZ -20°C After 24h replace EtOH if coloured

FM20 x2  45mL →  Pre-aliquoted PFA + GLUTA +  →   FRG +4°C →  FRG +4°C

** Golden rules **

- Filtrate one filter at a time if it is too complex to manage many filtrations at the same time..
- Never let the filter dry, unless at the end before storing it.
- S20 must go direclty in the LN2 after collection to be able to study RNA => RNA expression is time sensitive.
- S20-L can wait to let nucleoprotect react with DNA.

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NET WPII-200

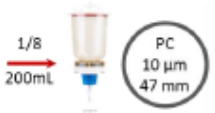
S200 x2



Store Immediately !!

If possible, filter more than 200 mL

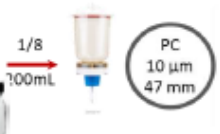
S200-L x1

If possible, filter more than 200 mL.

NET REGENT-680

S680-L x1

If possible, filter more than 200 mL.

** Golden rules **

- Filtrate one filter at a time if it is too complex to manage many filtrations at the same time..
- Never let the filter dry, unless at the end before storing it.
- S20 must go direclty in the LN2 after collection to be able to study RNA => RNA expression is time sensitive. DO NO WAIT !
- S20-L can wait to let nucleoprotect react with DNA.

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25 mm Filtration Units

USE THE TURBIDIMETER TO ESTIMATE THE VOLUME TO FILTER

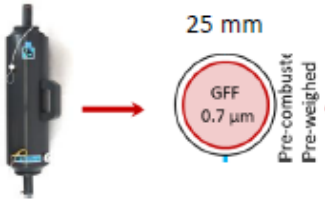
GFF filters : gridded face up !

HP

IN DARK

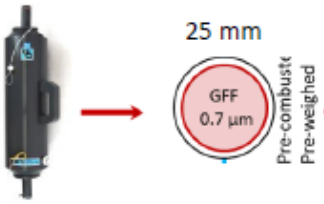
Triplicate once a month

PM x3



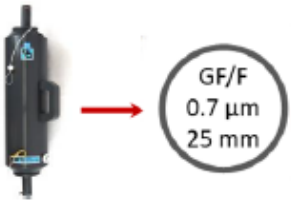
Rinse the filter with MilliQ (1/3 of the volume sampled)

FOI x3



Rinse the filter with MilliQ (1/3 of the volume sampled)

PA x1



Rinse the filter with MilliQ (1/3 of the volume sampled)

=> /!\ Please store the petri upright in the -20°C boxes / don't let the filter stand on the biomass side /!

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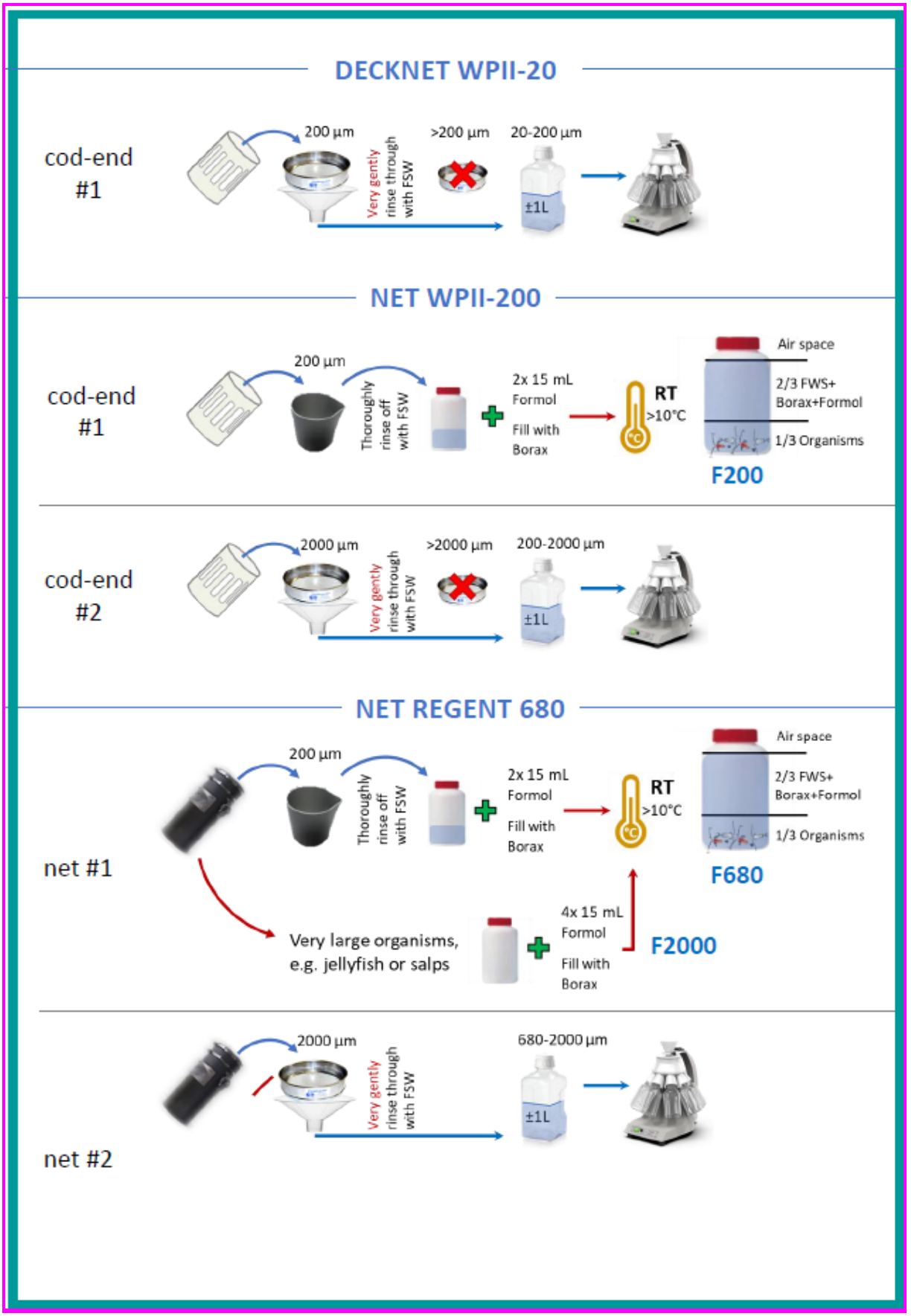

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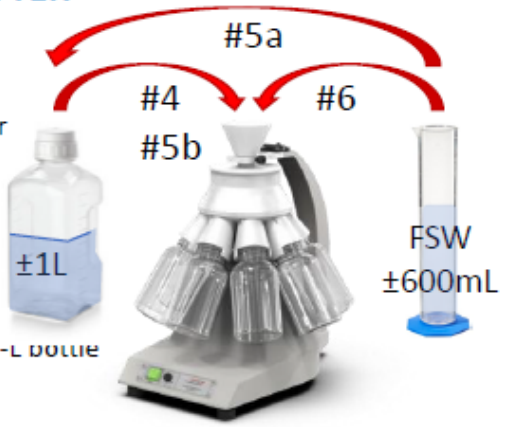
SAMPLE SPLITTER



Preparing the sample splitter

1. Remove the funnel and the lid from the splitter
2. Put back the funnel in the OFF-center configuration (used to split liquids)

Splitting the sample material in 8 aliquots

1. Measure the volume of sample material in the 2-L bottle
   - It should be around 1000 mL
2. Pour Filtered Sea Water (FSW) in a graduated cylinder
   - The total volume (sample + FSW) must be 1600 mL
   - Measure the required amount of FSW; It should be around 600 mL
   - This way each aliquot will be 200 mL
3. Switch the sample splitter ON and wait for it to reach a regular speed
4. Thoroughly, but gently mix the sample and pour it in the sample splitter
   - In 2-3 shots, re-mix each time and pour with a regular flow
5. Rinse the 2-L bottle with half the FSW (keep the other half for the next step)
   a) Pour half the FSW in the 2-L bottle and thoroughly rinse the inside
   b) Pour it in the sample splitter
6. Pour the remaining FSW directly in the sample splitter to rinse the splitting funnel
7. Switch the sample splitter OFF
8. Refer to the labels on the 8 bottles to process the aliquots

When all the aliquots are processed : clean the bottles and the splitter !

1. Wash with freshwater the sample bottles. Remove the splitter and wash it in the sink with freshwater. Put the bottles back in place on the sample splitter (align the arrows and turn clockwise)
2. Switch the sample splitter ON and wait for it to reach a regular speed
3. Pour 2L of freshwater with bleach in the sample splitter, let soak for 15 min.
4. Empty the bottles and splitter and wash again with 2L of milliQ water.
5. Empty the bottles and splitter and put aluminium foil on the top of the splitter.

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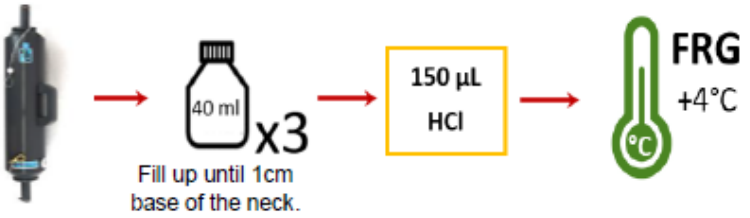
BioGeoChemistry

/!\ Be careful : protocols highly sensitive to carbon contamination /!
Wash your gloves with milliQ water before starting.
All samples to be rinsed 3 times with sample water (1/3 full).

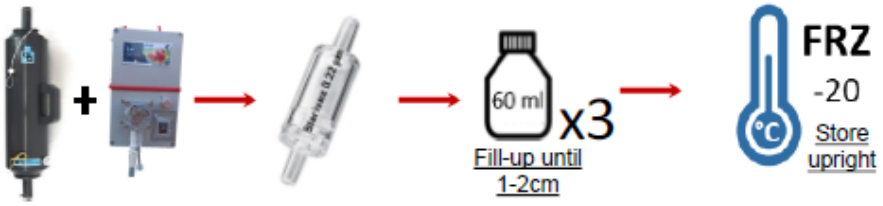
1) TOC x3
Directly from Niskin bottle



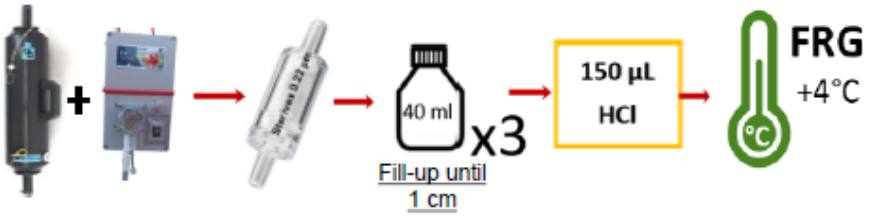
Portable pump protocols

⇒ The sterivex must be rinsed with 500 mL of MilliQ before using it.
⇒ Pump 300 mL of sample run in the line to rinse it, and then attach the sterivex to the line.
⇒ Pump 20 - 30 mL of seawater runs through the sterivex to rinse the MilliQ out.

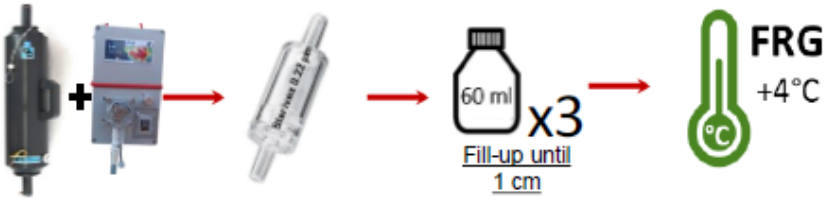
2) NUT x3



3) DOC x3



4) CDOM/FDOM x3



After use of the portable pump : wash the line with 2L MilliQ water.
If not used for more than 24h : wash it with 200 mL of MilliQ-HCl-1% and then with 2L of MilliQ.

NO ETHANOL / NO BLEACH / NO OTHER CHEMICAL

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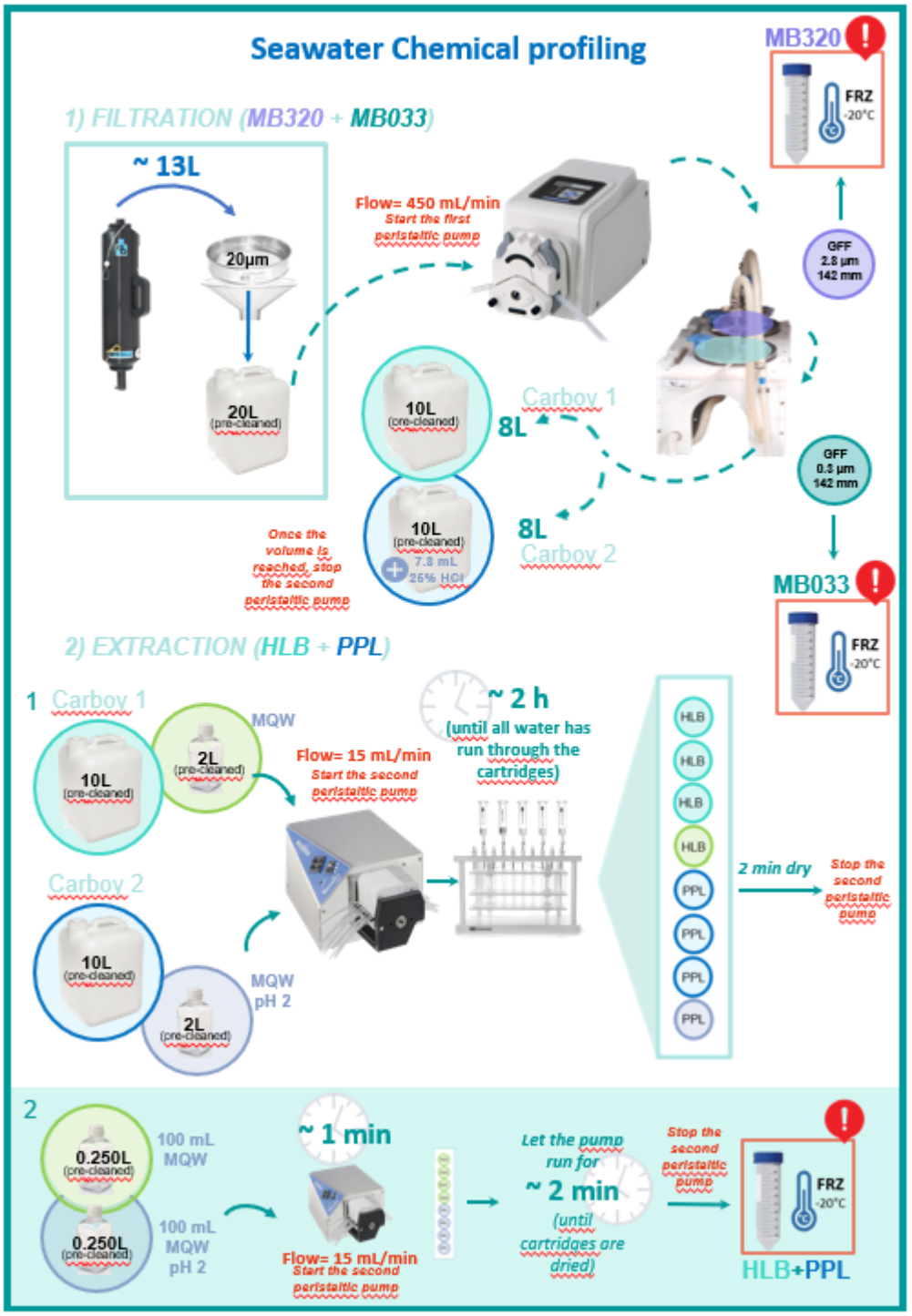

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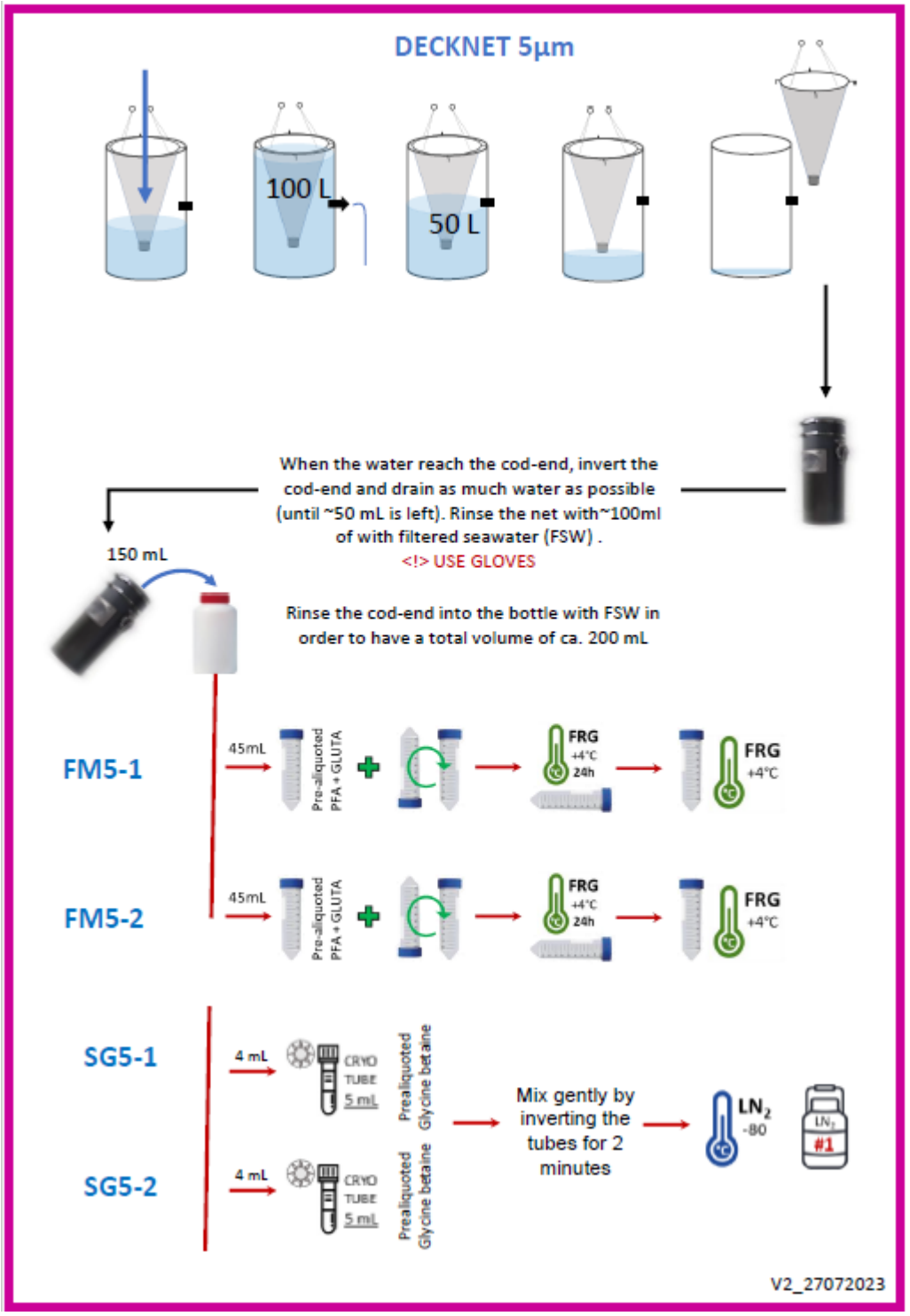

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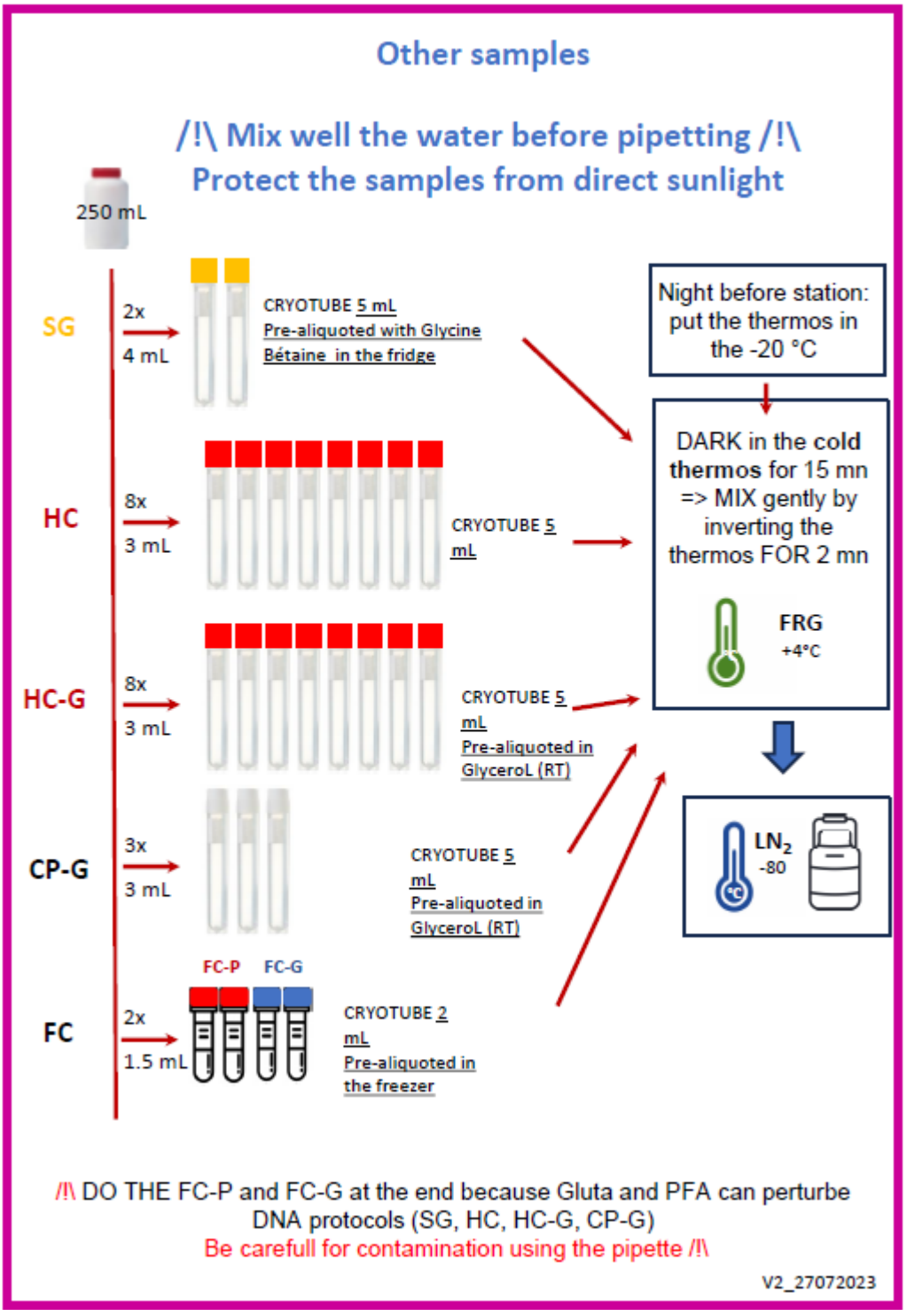

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1.2. Water to unveil (sub)mesoscale processes shaping coastal marine biodiversity along land-sea gradients

1.2.1. Authors and affiliations

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1.2.2. Brief introduction to the protocols and analysis that will be carried out with the collected samples.

A seasonal sampling strategy is applied over 2 years (April 2023-January 2024) along land-to-sea transects in two contrasting systems, the Roscoff coastal zone (Fig. 1) and the Gulf of Naples to disentangle the role of interacting local chemical and biological processes in shaping marine biodiversity. Sampling is conducted along each transect to measure environmental parameters, ecosystem processes and characterise (macro)benthic and pelagic communities (see Macrobenthic & pelagic community analyses across the target LSGs in the section 2. Selected species and holobionts sampling and processing).

1.2.3. Sampling Strategy

In Roscoff (**Figure 1a**), sampling is performed at 6 stations at ebb tide and at high tidal coefficients (> 75) that allow water and sediments collection in the Penzé river. Due to time and tidal constraints and on board space limitations, sediment and surface water samplings along the Penzé river (stations Penzé 1, 2 and 3) and in the Bay of Morlaix (stations Figuier, Pierre Noire, and Basse Plate) are performed on different days (but within a week).

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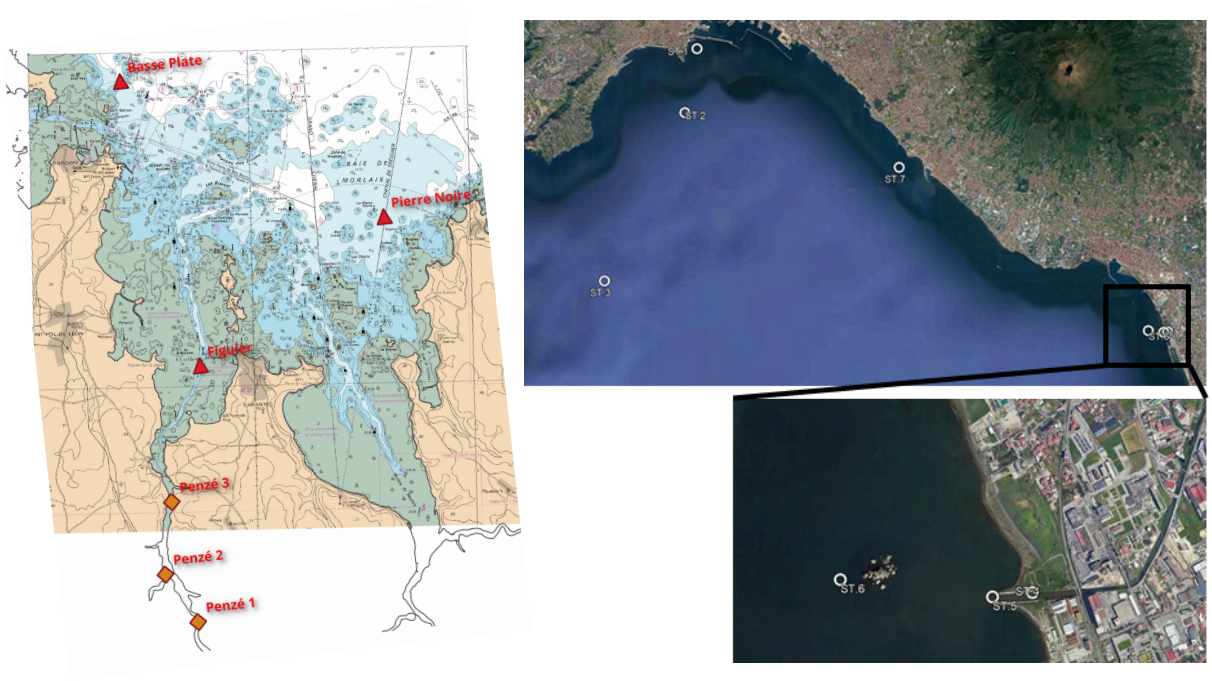

In Napoli, (**Figure 1b**) 7 stations are sampled along 2 land-to-sea transects.The first transect (MC) surveys the effects of urban runoff from the city and harbour, and includes the long term monitoring site Marechiara, where extensive previous knowledge is present. The transect includes 3 stations (S1 to S3). The first point is just outside the harbour, the second is Marechiara, the third explores potential effects of the coast at the start of the continental shelf dropoff (Canyon Ammontatura). The second transect (Sarno) surveys the effects of river runoff from the Sarno river. It includes 4 stations (S4 to S7), catching the freshwater input (Salinity 0), the plume, the water masses interface (NEREA monitoring site, previous knowledge present), and the offshore. Samples consist of water (2 depths, surface and bottom), sediments, and net tows.

1.2.4. Sampling tools and equipment

Chlorophyll a

- Nisikin bottles
- Silicon tube: 0.5cm in diameter and 50 cm in length
- 12mL borosilicate Exetainers
- 7M ZnCl2 solution
- Micropipette (p200)
- Pipette tips

DGAS

- Nisikin bottles
- Silicon tube: 0.5cm in diameter and 50 cm in length
- 12mL borosilicate Exetainers

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- 7M ZnCl2 solution
- Micropipette (p200)
- Pipette tips
- Gloves

Dissolved methane in marine and estuarine waters

- NISKIN Bottles (water sample collection)
- 20 ml, crimp-sealed headspace glass vials
- 20mm aluminium caps + septa
- Crimping tool
- Silicone tubing for the NISKIN bottles
- Saturated solution of Hg(Cl)2
- 20 µL syringe and needle

$\partial^{13}C\text{-}CH_4$ and $\partial^{13}C$-DIC in seawater and estuarine waters

- NISKIN Bottles (water sample collection)
- 12 ml Exetainer® vials (1 for d13C-CH4, 1 for d13C-DIC)
- Silicone tubing for the NISKIN bottles
- Saturated solution of Hg(Cl)2
- 20 µL syringe and needle

Simultaneous measurement of C and N2 fixation rates in planktonic communities

- Niskin bottles
- 25L tank
- 12 x 2L Nalgene bottles
- Incubator with adjustable temperature and light
- 15N2 tracing solution (50 mL 15N2 gas in 2L filtered sea water)
- 13C-DIC tracing solution (1,2 g NaH13CO3 in 50 mL MilliQ water)
- Filtration unit 4 holders (25mm ∅ filter holders) + pump
- Measuring cylinders (1000 and 2000 mL)
- Pipettes and pipettes tips (p1000 and p200)
- 36 Pre-combusted GF/F filters 25mm ∅ (4.5 hours at 450°C) – for biomass samples
- 36 GF/F filters (25mm ∅) – for chlorophyll samples
- 6 x 6 wells tissue culture plates
- 96 wells tissue culture plates
- 2 mL cryotubes
- 60 x 12mL borosilicate Exetainers – for gas samples
- 12 X 24mL borosilicate vials - for DIC samples
- Zinc chloride 7M solution

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- HgCl2 saturated solution
- Tin foil and black plastic bags (for dark incubations)
- 10x 20 mL syringes with plastic tubes
- Small forceps for GF/F filter handling
- 200um mesh
- Desiccator
- Fume hood
- Oven
- Tin caps (12 x 6 mm)

Dissolved nutrients

- Nisikin bottles
- Silicon tube: 0.5cm in diameter and 50 cm in length
- 20mL HDPE vials
- 0.2 µm PES syringe filters
- Gloves

Dissolved Organic Carbon and Chromophoric/Fluorescent Dissolved Organic Matter

- Nisikin bottles
- Homemade portable peristaltic pump
- Sterivex filters
- 2 L polycarbonate Nalgene bottles
- MQW water
- 40 mL glass bottles
- 2 M High purity HCl
- 60 mL Nalgene bottles
- Gloves

Flow cytometry for pico and nanoplankton diversity and abundance

- Automatic pipettes (P1000 or similar)
- Tips
- Cryovials with 100 ml of fixative
- Falcon tubes (15 ml) wrapped with aluminium foil
- Racks
- Gloves
- Niskin bottles
- Liquid Nitrogen

Natural stable isotope analyses for trophic web reconstructions

- MQ water

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- Sieve 20 µm
- Sieve 200 µm
- HDPE containers (vol 500ml)
- 10L tank (acid-cleaned)
- Pre-combusted Whatman GF/F filters (25 mm diameter)
- Filtration system
- Tweezers for filters
- Spatula
- Falcon tubes (15 ml)
- 6-well culture plate

Meta-omics analyses on 3 size-fractions (0.22-3; 3-20; 20-200 µm)

Before starting, ensure all materials equipment and tools are available by checking the following table:

- Niskin bottles
- Stainless steel filtration apparatuses (tripods), 142 mm
- Polycarbonate hydrophilic membranes, 3 µm pore size, 142 mm in diameter
- Polycarbonate hydrophilic membranes, 0.2 µm pore size, 142 mm in diameter
- Peristaltic pump and pump heads
- Appropriate silicone tubes
- 20 µm mesh size sieve
- 200 µm mesh size sieve
- Sample containers of appropriate volume (10 L graduated tank with tap as in-tank and 10 L graduated tank as out-tank)
- Funnel
- Forceps
- Beakers
- Gloves
- Table paper
- 5 ml cryotubes
- Permanent marker
- Distilled water
- Ethanol
- Dewar for liquid N2
- Access to -80°C

Particulate Organic Matter (POC-PON)

- Nisikin bottles
- Large carboys
- Filtration ramp 25 mm

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- Precombusted GF/F filters (0.7 µm) 25 mm
- 6-wells plates

Pigments

- Nisikin bottles
- Large carboys
- Filtration ramp 47 mm
- GF/F filters (0.7 µm) 47 mm
- 2ml cryotubes
- Dewar with Liquid Nitrogen

1.2.5. Metadata collection

Metadata collected by site includes:

- GPS coordinates
- Date
- Station number
- Bathymetry
- Salinity
- Seawater temperature
- Tides information
- Weather conditions (raining conditions for dissolved methane and $\partial^{13}C\text{-}CH_4$ *and* $\partial^{13}C\text{-}DIC$)
- Sample ID
- Replicates when needed

1.2.6. Environmental parameters measurement

- CTD measures the temperature, conductivity (salinity), in vivo fluorescence, O2, turbidity, and PAR.

1.2.7. Sampling protocol (step by step, detailed and simple)

Chlorophyll a

Contacts: Marta Furia (marta.furia@szn.it), Francesca Margiotta (francesca.margiotta@szn.it), SZN

The collected samples will be used to analyse chlorophyll a concentrations. Collect samples directly from the Niskin bottles and filter them on board or as soon as possible in a laboratory.

Step by step

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1. Collect water samples from the Niskin bottles into a dark tank to prevent light exposure by using a silicon tube; rinse and discard a small volume of samples (3 times) Fill the tank with not less than 1 litre of the sample.
2. Filter the water by using a filtration system equipped with Whatman GF/F filter (25mm) and connected to a vacuum pump, obtaining one replicate for each sample.
3. PUT the filter inside a 1.2 mL cryotube, labelled with sampling cruise, station, date and depth.
4. Immediately store the cryotubes into a cryogenic storage filled with liquid nitrogen (e.g. standard cryogenic dewar) until analysis.

Dissolved gases in seawater (DGAS)

Contacts: Maira Maselli (maira.maselli@szn.it), Francesca Margiotta (francesca.margiotta@szn.it), Ulisse Cardini (ulisse.cardini@szn.it), SZN

Water samples are collected to determine the concentration of gases that are affected by biological and physical processes in the water column. Dissolved O2:Ar ratios are indicative of net community production (the difference of photosynthesis and respiration), because O2 and Ar share similar solubility properties, but only O2 is biologically influenced. For similar reasons, the N2:Ar ratios can be used to estimate net N2 production as contributed primarily by benthic and water column denitrification. Additionally, CO2, Methane and dimethyl sulfide (DMS) are measured, which are climate-relevant gases.

N2, O2, Ar, Methane, CO2 and DMS are measured on a High-precision Membrane Inlet Mass Spectrometer (MIMS, Bay Instrument) at the Zoological Station Anton Dohrn. The instrument pumps water at < 1 mL min-1 through a semipermeable microbore silicone tubing positioned inside an inlet vacuum line of a quadrupole mass spectrometer.

Samples are collected along land-to-sea transects on 6 sampling stations which have an incremental distance from the coast. The sampling is repeated seasonally (with a 4 months' frequency) for two years. Surface water is collected from the research vessel by Niskin bottles. Triplicate samples are collected at each sampling station. In case of water column stratification, water will also be collected at one selected depth below the thermocline.

CAUTION: this protocol uses toxic substances and is subject to contamination and degassing.

- Smoking is not allowed during sampling,
- Zinc chloride (ZnCl2) is toxic, use gloves (see MSDS)

Step by step

1. Collect the CTD profile of the sampling station
2. Collect water with a Niskin bottle
3. Attach the silicon tube to the release valve of the Niskin bottle while it is still closed
4. Place the other end of the silicon tube at the bottom of a 12 mL Exetainer and open the valve slowly to avoid the formation of air bubbles

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5. Fill the Exetainer and allow the water to overflow by at least half of its volume then start to slowly extract the tube while the water is still flowing.
6. Ensure the Exetainer is full and contains no air bubbles. A water meniscus should be visible on the top of it.
7. Close the Exetainer and turn it upside down to verify the absence of air bubbles in the water sample.
8. Repeat point 4 to 7 to get triplicate samples
9. After all samples have been collected, fix them by adding 100 uL of ZnCl2
10. Fixed samples should be kept in the dark at 4°C until the analysis.

Dissolved methane in marine and estuarine waters

Contacto: Cédric Boulart, CNRS UMR 7144, cedric.boulart@cnrs.fr

This protocol describes the sampling procedure to determine the concentrations of dissolved CH4 in natural waters, including marine and estuarine waters.

For estuarine waters:

sampling along a salinity gradient, at least 3 stations at S=10, S=20, S=30, below surface (1m) and above the bottom (ideally 1m above).

For marine waters:

sampling below surface (1m) and above the bottom (1-2m above). It is recommended to start the sampling at the beginning of ebb tide to limit the influence of offshore water.

CAUTION: this protocol uses toxic substances and is subject to contamination and degassing.

- Smoking is not allowed during sampling,
- Mercuric chloride (HgCl2) is toxic, use gloves (see MSDS)

Step by step

1. CTD Vertical profile
2. NISKIN sampling
3. Install a silicone tube on the NISKIN spigot
4. Rinse 3 times without air bubbles the headspace vials
5. Let the silicone tube reach the bottom of the vials and fill it slowly without air bubbles
6. Let the sample water overflow for a few seconds
7. Slowly remove the silicone tube from the vials and close the NISKIN spigot. Leave a meniscus.
8. Place a septum and an aluminium on the vial and crimp the vial with the crimping tool
9. With the small syringe and the needle, inject 20µl of saturated HgCl2 solution through the septum (use gloves)

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10. For estuarine waters, take note of the salinity and temperature directly in the NISKIN bottle.
11. Dissolved CH_4 samples (i.e. 20ml headspace glass vials) are stored at 4°C until analysis if properly poisoned. If not poisoned, samples should be analysed within a few hours.

$\partial^{13}C$-CH_4 and $\partial^{13}C$-DIC in seawater and estuarine waters

Contact: Cédric Boulart, CNRS UMR 7144, cedric.boulart@cnrs.fr

This protocol describes the sampling procedure to determine the carbon isotopic composition of Dissolved Inorganic Carbon and methane.

For estuarine waters:

sampling along a salinity gradient, at least 3 stations at S=10, S=20, S=30, below surface (1m) and above the bottom (ideally 1m above).

For marine waters:

sampling below surface (1m) and above the bottom (1-2m above). It is recommended to start the sampling at the beginning of ebb tide to limit the influence of offshore water.

CAUTION: this protocol uses toxic substances and is subject to contamination and degassing.

- Smoking is not allowed during sampling,
- Mercuric chloride (HgCl2) is toxic, use gloves (see MSDS)

Step-by-step

1. CTD Vertical profile
2. NISKIN sampling
3. Install a silicone tube on the NISKIN spigot
4. Rinse 3 times without air bubbles the Exetainer® vials
5. Let the silicone tube reach the bottom of the vials and fill it slowly without air bubbles
6. Let the sample water overflow for a few seconds
7. Slowly remove the silicone tube from the Exetainer® and close the NISKIN spigot. Leave a meniscus.
8. Place the cap on the Exetainer® and tighten
9. With the small syringe and the needle, inject 20µl of saturated HgCl2 solution through the septum (use gloves)
10. For estuarine waters, take note of the salinity and temperature directly in the NISKIN bottle.
11. Samples are stored at 4°C until analysis if properly poisoned. If not poisoned, samples should be analysed within a few hours.

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Simultaneous measurement of C and N2 fixation rates in planktonic communities

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Incubation experiments are conducted to determine carbon and nitrogen fixation rates of natural planktonic communities. Water samples collected at desired locations are incubated in the lab under controlled light and temperature conditions and with the addition of stable isotopes of inorganic carbon (13C) and nitrogen gas (15N). These isotopes are used as tracers: C and N fixation rate is calculated based on their abundance in the plankton biomass at the end of the incubation. The incubation is performed in 12 replicates: 4 of them follow a complete daily cycle of 24 hours in which light and dark periods alternate. Shorter incubations of 8 hours are conducted simultaneously in the light (4 replicates) and in the dark (4 replicates). The longer incubation (24h) informs about the net daily carbon fixation (photosynthesis – respiration), while the shorter incubations in the light inform about the hourly carbon fixation rate, after correction for the passive incorporation measured in the dark incubations. Other than biomass samples for isotopes analysis, dissolved inorganic carbon (DIC) is measured to calculate carbon fixation rate. Chlorophyll a is measured to normalise rates on chlorophyll units. Gas samples are measured to monitor the abundance of N2 and O2 along the incubation.

Surface water samples are collected along land-to-sea transects on 3 sampling stations which have an incremental distance from the coast. The sampling is repeated seasonally (with a 4 months' frequency) for two years.

CAUTION: this protocol uses toxic substances and is subject to contamination and degassing.

- Smoking is not allowed during sampling,
- Zinc chloride (ZnCl2) is toxic, use gloves (see MSDS)

Step by step

1. Collect the CTD profile of the sampling station
2. Collect 25L of surface water at each site and transport to the lab
3. Pre-filter with 200um mesh to remove large meso-zooplankton
4. Divide in 12 incubation bottles (4 light+4 dark+4 for 24 hours)
5. Add 1,7 mL 13C-DIC tracing solution in each bottle
6. Add 200mL 15N2 tracing solution in each bottle
7. Take T0 samples for dissolved gases (one sample/each incubation chamber)
8. Fix with 100uL of ZnCl2.
9. Refill the bottles with no bubbles no headspace
10. Cover 4 bottles with thin foil and black plastic bags (for dark incubation) and start the incubation by placing all bottles in the incubation chamber

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11. Divide the remaining pre-filtered water in 4 replicates.
12. Take samples for DIC: 24mL in borosilicate vials. Fix with 10 uL of HgCl,
13. Take samples for chlorophyll: 200-500mL. Filter on a GF/F filter. Fold the filter twice and store in a 2mL cryotube.
14. Collect biomass samples on filters to measure isotopes abundance: on a pre-combusted GF/F filter. Filter as much water as possible. Store filters in a 6 well tissue culture plate.
15. At the end of the light period, stop the incubation of 4 light and 4 dark incubated bottles
16. From each bottle, take samples for gases (point 7-8) and biomass (point 14)
17. At the end of the 24 hours do the same on the remaining 4 bottles
18. Gas samples: store and ship in the dark at 4°C.
19. DIC samples: store and ship in the dark at 4°C.
20. Chlorophyll samples: freeze and store in liquid nitrogen. Ship in dry ice.
21. Biomass samples: Store at -20°C and process as follow before shipping:
    - Decarbonate the filters by placing them in a desiccator together with a small beaker containing approximately 20 mL of 37% fuming HCl;
    - Leave for 4 hours;
    - Take the filters out from the desiccator and place them in a fume hood for 4 hours to allow the acid vapours to evaporate;
    - Dry in the oven overnight at 50°C;
    - Encapsulate in tin caps (12 x 6 mm) and place the capsules in a 96 well plate, keeping track, for each sample, of the corresponding position in the plate.

Dissolved inorganic nutrients

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The collected samples will be used to analyse dissolved inorganic nutrient concentrations.

Collect samples directly from the Niskin bottles and filter them on board.

CAUTION:

- RISK OF CONTAMINATION
- Beware of boarding fumes
- Do not smoke during sampling
- Use only swinnex filter holders and tweezers washed with HCl 10% and rinsed with MQ

Step by step

1. Rinse the tweezers in MQ, place the filter (cellulose acetate filter; 0.47 µm pore; 47 mm diam) into the swinnex;

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2. Rinse the filtration system (syringe, swinnex and filter) 3 times with MQ;
3. Connect the syringe to the Niskin bottle and collect 60 ml of water;
4. Connect the swinnex to the syringe, filter the water and discharge it;
5. Connect the syringe to the Niskin bottle and collect 60 ml of water;
6. Connect the swinnex to the syringe, RINSE 20ml HDPE vials with a small volume of the filtered sample; close the vial, shake it and discard the water (3 times)
7. FILL the vials, making sure to leave enough space to contain the increased volume of water after freezing (fill up to ~ ¾ of the bottle), collect 2 replicates for each sample.
8. Immediately store upright the samples into the freezer on board
9. Transport samples from the ship to the fixed storage by using a cooler box with ice packs inside
10. Store the samples at -20°C until the analysis

Dissolved Organic Carbon (DOC) and Chromophoric/Fluorescent Dissolved Organic Matter (CDOM/FDOM)

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With this protocol we measure the concentration of Dissolved Organic Carbon (DOC) in the water column as well as the optical properties of dissolved organic matter: absorption (CDOM) and fluorescence (FDOM). DOC is the largest and most complex reservoir of reactive carbon on Earth, playing a central role in the carbon cycle. DOC is produced at all the levels of the food web and its oxidation by the microbial communities is responsible for oxygen consumption. DOC concentration is measured by a Shimadzu TOC analyzer (TOC-Vcsn) by high-temperature catalytic oxidation. CDOM and FDOM are indicators of water quality and have important implications for aquatic ecosystems. CDOM and FDOM determine the underwater light availability in the open ocean and coastal waters and they can have a contrasting effect, they can protect organisms by ultraviolet radiation in the upper layer, but they can also reduce the visible light, limiting photosynthesis. CDOM is optically measurable and therefore an excellent candidate for quantification by remote sensing techniques. Understanding the spatial and temporal variability of CDOM and FDOM is important to the study of water quality, global carbon budgets, and climate change. CDOM is measured through absorption spectra by using a SHimadzu UV-visible spectrophotometer while FDOM is measured through Fluorescence excitation-emission Matrixes (EEMs) obtained using an Aqualog spectrofluorometer (Horiba).

Step by step

Setup of the portable peristaltic pump

Filtration for DOC (and CDOM/FDOM) will be carried out by using a homemade portable peristaltic pump (Figure 1) with silicon platinised tubes and Sterivex filters. One end of the tube will be attached directly to the Niskin bottle, the other end will be attached to the Sterivex filter (Figure 1).

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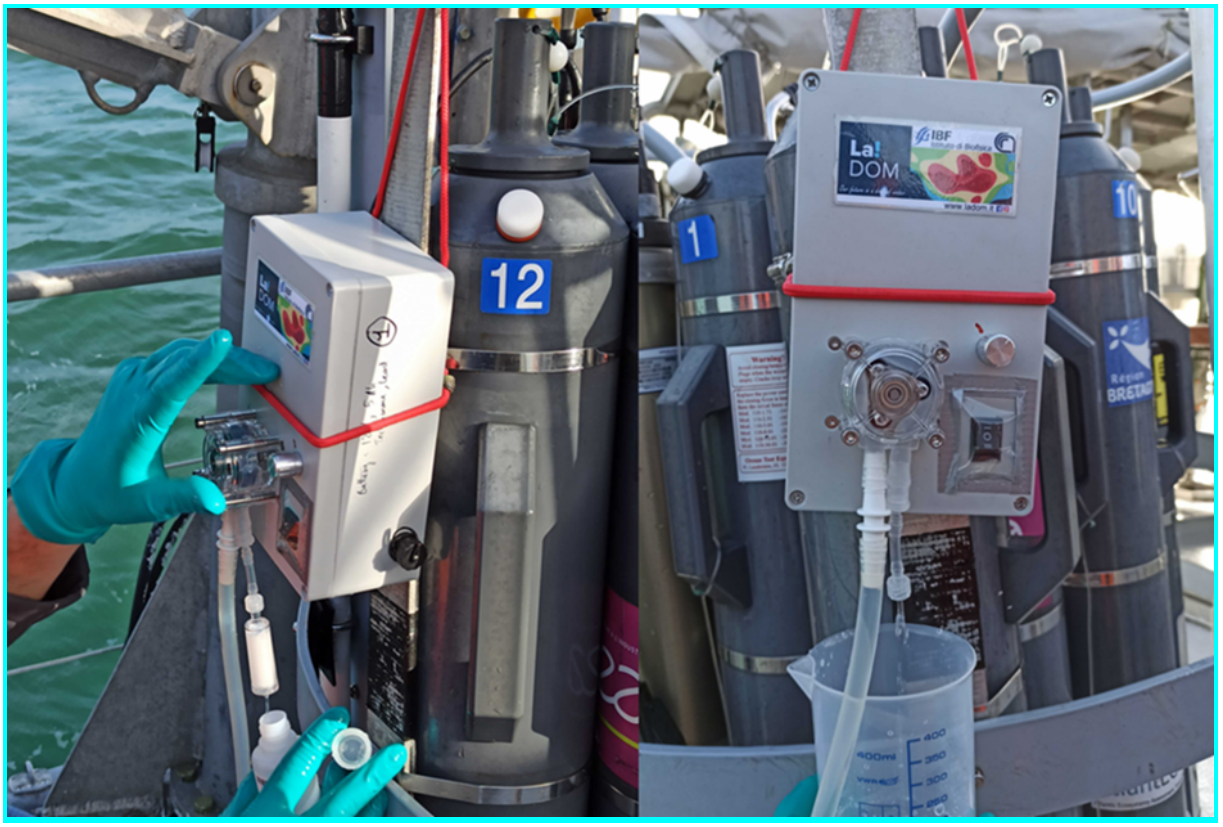

Figure 1. Homemade portable peristaltic pump connected with the Niskin bottle and Sterivex filter.

<!>Charge the battery of the pump before each station.

<!>Always wear gloves (polyethylene or nitrile, without powder!) while handling the tubes, the bottles and the filter.

<!>Do not smoke when the system is filtering and near the rosette.

<!>Before preparing the setup and anytime you change your gloves, wash your gloves with MQW.

Before filtration

1. Turn on the pump.
2. Select the right pump rotational direction using the black button.
3. Put the long end of the tube in the 2 L polycarbonate Nalgene bottle (you will find a dedicated bottle in the protocol box) with MQW.
4. Start the pump and fill the tube with MQW.
5. Open a Sterivex filter and keep the bag.

   <!> be careful not to touch the filter edges.
6. Attach and screw the Sterivex filter to the adapter.

   <!> Be careful not to touch the edge of the adapter.
7. Start the pump flow and wash the sterivex with 500 mL of MQW.
8. Put the Sterivex filter back in its bag.

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<!> You can clean the filter a few hours before to use it, no need to clean it immediately before the filtration.

9. Start the pump and empty the tube from the MQW water.

Filtration

1. Attach the long end of the tube to the Niskin bottle.
2. Start the pump and wash the tube with at least 300 mL of sample.
3. top the pump.
4. Attach and screw the MQW cleaned Sterivex filter.

   <!> Be careful not to touch the edge of the adapter
5. Start the pump flow.

   <!> The speed should not be changed, probably the flow of filtered water will decrease during filtration, change the filter if filtration speed decreases too much, but do not change the speed of the pump.
6. Wash the Sterivex with the sample (few mL are enough to remove the MQW from the filter).
7. Collect samples in this order: nutrients, DOC and CDOM/FDOM.

   <!> If the filter is clogged, change it and wash it with 500 ml of MQW and a few mL of sample before using it.

DOC filtration

Filtration by portable peristaltic pump, follow the protocol for filtration with portable peristaltic pump (1-16).

1. Rinse 3 times the 40 mL glass vial with filtered sample: fill the 40 mL glass bottle about a quarter full, close the bottle, shake to rinse and discard the content (REPEAT this step 3 times).
2. Fill 3 x 40 mL vial, leaving 1 cm of head space.
3. Dispense 150 µL of 2 M High purity HCl in the vial, close the cap and mix. There is a small box dedicated with the HCl already diluted and the pipette with tips for this operation.

   <!> Do not use HCl from other bottles, do not use other pipette or tips to spike the HCL. The HCl and the pipette is dedicated ONLY to DOC and TOC acidification.
4. Store in the sample box at 4°C and in the dark in the front storage area (F-Store).
5. NB: Not necessary to spike the vials one by one. They can all be filtered and spike together at the end of the filtration.

CDOM/FDOM filtration

1. FILTRATION by portable peristaltic pump, follow the protocol for filtration with portable peristaltic pump (1-16).
2. Rinse 3 times the 60 mL polycarbonate bottle with filtered sample: fill the 60 mL Nalgene bottle with ~20mL of filtered sample; close the bottle, shake to rinse and discard the content (REPEAT this step three times).

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3. Fill 3 x 60 mL bottles until the base of the neck of the bottle.
   <!> Be careful not to touch the inside of the cup or the bottle.
4. Store the bottle in the dark and at 4°C in the front storage (F-Store).
5. After filtration, wash the tube with 2 L of MQW (you will find a dedicated bottle in the protocol box) to clean the system. To do this, wash the long end of the tube with MQW and insert it inside the 2 L bottle.
   <Important!> If the filtration system is not used for more than two days, wash the tube with ~200 mL of HCL at 1%. For the dilution dispense 10 mL of HCl 25% in 1 L of MQW (you will find a cylinder and a 1L polycarbonate Nalgene dedicated bottle in the protocol box).
   <!> Do not clean the system with ethanol or other chemicals.
   <!> Do not use Bleach to clean the system

Flow cytometry for pico and nanoplankton diversity and abundance

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The protocol is aimed at preserving picoplankton (autotrophs and heterotrophs) and their optical properties (scatter and fluorescence) so as to be amenable to following flow cytometry analyses. Final data are cell concentrations (cell ml-1) and optical parameters expressed as units relative to internal standards (beads)

Seawater samples are collected by Niskin bottles at different depths along the water column. Full sets of physical, chemical and biological data are collected at the same time.

Collect water samples directly from the Niskin bottles and FILTER them on board or as soon as possible in a laboratory.

CAUTION

- Glutaraldehyde and paraformaldehyde are toxic substances
- Wear gloves, handle with care, and read the safety sheet (MSDS)
- The pre-aliquoted cryotubes are kept at -4°C

Step by Step

1. Dispense 1 mL of whole seawater into two 2-mL cryotube pre-aliquoted with 100 µL of a solution of paraformaldehyde 1% mixed with glutaraldehyde 0.05%
2. Close the cryotube, and mix gently by inversion
3. Incubate for 15 min in the dark at +4°C
4. Deep in Liquid Nitrogen dewar
5. Store at -80°C once in the lab
6. Once thawed, one replicate sample is run unstained for the analysis of pico and nano phytoplankton with the addition of 5 l of 1 m Polysciences polystyrene beads as

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internal standard. A BD FACSVerse flow cytometer is used, with standard laser and filter settings.

7. The second replicate sample is thawed, added with 1 ml of SYBRGreen I for 15 min in the dark and run with the same instrument for the analysis of heterotrophic bacteria.
8. Cell concentrations are calculated directly by the instrument (absolute count), but in order to make sure that the calculations are correct, one tube of TruCount beads (BD BioSciences) is run before every batch of samples. Populations are discriminated according to their scatter vs fluorescence properties and analysed using FCSexpress software.

Natural stable isotope analyses for trophic web reconstructions

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The protocol is aimed at collecting different size fractions of plankton from which to acquire natural stable isotope compositional data, useful for trophic web reconstructions.

Water samples are collected from the Niskin bottles and from nets. Samples should be processed on board or as soon as possible in a laboratory in order to acquire data of δ13C and δ15N on three different size classes (< 20 µm; 20-200 µm and >200 µm).

CAUTION:

RISK OF CONTAMINATION;

Beware of boarding fumes

Step by step

Sampling protocols for the three size fractions are described as follow:

<20 µm size fraction

1. Water samples are collected from the Niskin bottles into acid-clean tank (~ 6-10 litres) using a silicon tube
2. Rinse and discard a small volume of samples (3 times)

20-200µm size fraction

1. Perform a horizontal net tow (20 µm mesh size)
2. Collect the sample in an acid-cleaned HDPE container
3. Keep on ice or at +4°C until processing

>200µm size fraction

1. Perform a vertical (horizontal if the site is too shallow) net tow (200 µm mesh size)
2. Collect the sample in an acid-cleaned HDPE container

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3. Keep on ice or at +4°C until processing

- **Always wear gloves and rinse spatulas, tweezers, sieves and every tool with MQ Water before each use.**

Samples processing for the three size fractions are described as follow**:**

<20 µm size fraction

1. Pre-filter the sample using a 20 µm nylon mesh;
2. Split the water into 3 sub-samples (same volume);
3. Filter the 3 sub-samples on pre-combusted Whatman GF-F filter (25mm) using a filtration system (3 replicates);
4. Put the filter into a 6-well culture plate;
5. Add a blank filter (only pre-combusted filter) for each plate;
6. Immediately store the samples into the freezer on board;
7. Transport samples from the ship to the fixed storage using a cooler box with ice packs inside;
8. Store the samples at -20°C until the analysis.

20-200µm size fraction (Phytoplankton and microzooplankton)

1. Pre-filter sample through 200 µm nylon mesh;
2. Concentrate the sample onto a 20 µm sieve;
3. Transfer the concentrated sample into a Falcon tube using the spatula;
4. Immediately STORE the samples into the freezer on board;
5. Transport samples from the ship to the fixed storage using a cooler box with ice packs inside;
6. STORE the samples at -20°C until the analysis

>200µm size fraction (Mesozooplankton)

1.Filter the sample on a 200 µm sieve;

2.Collect the concentrated sample and transfer into a Falcon tube using the spatula;

3.Immediately STORE the samples into the freezer on board

4.Transport samples from the ship to the fixed storage using a cooler box with ice packs inside

5.STORE the samples at -20°C until the analysis

Meta-omics analyses on 3 size-fractions (0.22-3; 3-20; 20-200 µm; > 200 µm)

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Co funded by the European Union (GA# 101059915). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

The protocol allows marine prokaryotes and eukaryotes in 3 size classes (0.2-3, 3-20 and 20-200 m) in order to subsequently extract and sequence their DNA and RNA. Plankton are separated according to their size so that the genetic material of the larger but less abundant organisms is not overshadowed by that of the smaller and more abundant organisms. Subsequent sequencing is conducted using amplicon sequencing as well whole genome and transcriptome sequencing, so as to obtain a complete and detailed analysis of total diversity (amplicon sequencing) and of genetic potential (metagenomics) and potential metabolic activities (metatranscriptomics).

Seawater is collected by Niskin bottles at different stations and at different depths to gain a better understanding of biodiversity and function of planktonic communities in the Gulf of Naples. The whole seawater is sequentially filtered through 200 and 20 m nylon sieves and the resulting fraction is then filtered sequentially through 3 and 0.22 mm polycarbonate filters. The resulting size fractions are therefore 0.2-3; 3-20; 20-200 m, (and > 200 µm at Roscoff) taken in duplicate after filtration, the filters are placed in 5 ml cryovials, appropriately labelled, and flash frozen in liquid nitrogen and then stored at -80°C until further analysis.

Filtration of seawater must be performed immediately after sampling or as soon as it reaches the laboratory, especially when RNA is to be sampled. For RNA sampling, the filtration step is limited to 15 min).

The volume of seawater required to ensure adequate biomass collection in all size fractions may vary by location. For the Gulf of Naples which includes coastal as well as offshore oligotrophic stations, 5 L are filtered for each replicate. It is crucial that the same volume of seawater is used for every sampling, in order to achieve consistency and comparability between samples. For Roscoff which includes river, estuarine, coastal and offshore stations, the filtration volumes vary by location and by season (variable weather conditions).

Step by step

1. Collect seawater from the selected depth using Niskin bottles previously thoroughly rinsed with tap water and then with seawater while on use;
2. Rinse the funnel, and the sample in-tank (previously clean with 10% HCl) three times with seawater from the sampling site;
3. Make sure that the tap of the sample container (carboy, later named in-tank) is closed and turned up, before collecting the water;
4. Place the 200 µm sieve on top of a 20 µm sieve inside a funnel and place it on the sample container mouth.
5. A total of 10 L seawater are collected from the Niskin bottle after sieving through 200 µm and 20 µm mesh size nylon sieves, sequentially;
6. After sieving 10 L seawater, place the 20 µm sieve in a beaker with some filtered seawater to keep it hydrated;
7. Following filtration should be initiated as soon as possible after sampling. Sampled seawater may be transported to the laboratory if filtration in the field is not possible. Keep each of the sample containers in a dark and cool place until filtration.

Preparation and cleaning

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1. Always wear gloves and change them regularly;
2. Filtration apparatuses and tubes are cleaned at the end of each sampling event, allowed to dry and stored properly;
3. After each filtration sequence, the system is cleaned by passing purified water (Milli-Q) through;
4. Never touch the filter (not even with gloves on);
5. Avoid touching the inside of the filtration apparatus;
6. Clean sampling equipment using tap water and purified water (Milli-Q);
7. Rinse the 20 and 200 µm mesh and funnels with tap water and purified water (Milli-Q);
8. Clean the sample containers (tanks) by incubating overnight with 10% HCl. The following day, wash twice using distilled water and twice using purified water (Milli-Q).

Filtration and storage

Size fractions 0.2-3 and 3-20 micron

The filtration apparatus consists of: the container with the sampled seawater, called in-tank, a peristaltic pump that passes the seawater through a 142 mm filtration apparatus equipped with a 3 µm PC filter and then through a 142 mm filtration apparatus equipped with a 0.2 µm PC filter. The seawater is transported through special silicone tubes that connect the seawater container to the peristaltic pump and filtration apparatuses. Filters are Millipore Isopore TSTP 14250 (3 µm) and Millipore Isopore GTTP14250 (0.22 µm).

Step by step

1. Open the tripod filter holders. Avoid touching the inside of the filter holders;
2. Humidify filter holder area of filtration tripods using distilled water;
3. Place a 3 µm pore size PC filter membrane (142 mm diameter) on the first tripod to collect 3-20 µm organisms and a 0.2 µm pore size PC filter membrane (142 mm diameter) on the second tripod using forceps to collect 0.2- 3 µm organisms. Each PC filter is separated by 2 blue inter-filters in the filter boxes which need to be removed. Handle the filter with the forceps not by hand.
4. Close the tripods, tighten the hand knobs and connect the inlet and outlet silicone tubes;
5. Connect the inlet tube of the first filtration tripod (3 µm filter membrane) to the peristaltic pump and to the in-tank tap;
6. Connect the outlet tube of the first filtration tripod (3 µm filter membrane) to the inlet of the second filtration tripod (0.2 µm filter membrane);
7. Connect the outlet of the second filtration tripod (0.2 µm filter membrane) to a tube and place the tube in the out-tank;
8. Turn on the peristaltic pump, checking the pumping speed and flow direction. The flow must be smooth and delicate;
9. 5 L of prefiltered seawater will be sequentially filtered through two filter membranes, first through the 3 µm and subsequently through the 0.2 µm pore size membrane;

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10. Always initiate the filtration with the filter holder vents open. Wait until air bubbles are expulsed and close the vents;
11. Check the tubing system and the filtration tripod connections regularly. If the pressure is too high, decrease the flow rate to avoid damaging the cells;
12. Check for the presence of bubbles and use the filter holder vents to expel them;
13. Make sure that the filter membranes always remain moist during filtration;
14. After filtration, allow the filters to dry, then keep the pump on and allow the system to pump air;
15. Gently shake each tripod to make sure there is no water trapped in the filter holder and open both vents while turning off the peristaltic pump;
16. Open the tripod filter holders. Avoid touching the inside of the filter holder area;
17. Use the forceps to handle the filter;
18. Fold the filter and insert it into 5 ml cryotubes (previously marked with a permanent marker);
19. Transfer tubes into liquid N2 for flash freezing and subsequently store at -80°C;
20. Close the tripod and clean the filtration system with purified water (Milli-Q). Place new filters in the filtration tripods and repeat the filtration steps for the remaining 5 L (second replicate);
21. Each filtration produces one replicate per fraction; therefore, after 2 filtrations, 2 replicated samples per fraction will be produced.

Size fraction 20-200 µm

Step by step

1. Once terminated the procedure described above:
2. Always wear gloves;
3. Clean the filtration system and tripods with purified water (Milli-Q);
4. Use only the 3 µm tripod;
5. Place a new 3 µm filter membrane in the filtration tripod using clean forceps;
6. Take the beaker with the 20 µm sieve from the initial collection;
7. Rinse the 20 µm nylon sieve using the filtered water from the out-tank;
8. Pour the remaining filtered water from the out-tank into the in-tank, together with the water used to rinse the nylon sieve;
9. Close the tripod, tighten the hand knobs and connect the inlet and outlet silicone tubes;
10. Connect the inlet tube of the tripod to the peristaltic pump and to the in-tank tap;
11. Connect the outlet tube of the tripod to a silicone tube and place the tube in the out-tank;
12. Turn on the peristaltic pump, checking the pumping speed and flow direction;
13. Always initiate the filtration with the filter holder vents open. Wait until air bubbles are expulsed and close the vents;

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14. Filter 5 L, then close the tap of the in-tank and let all the remaining water to be filtered through;
15. Check for the presence of bubbles and use the filter holder vent to expel them;
16. Make sure that the filter membrane always remains moist during filtration;
17. After filtration, allow the filters to dry, then keep the pump on and allow the system to pump air;
18. Gently shake the tripod to make sure there is no water trapped in the filter holder and open the filter holder vent while turning off the peristaltic pump;
19. Open the tripod filter holder. Avoid touching the inside of the filter holder area;
20. Use the forceps to handle the filter;
21. Fold the filter and insert it into 5 ml cryotubes (previously marked with a permanent marker);
22. Transfer tubes into liquid N2 for flash freezing and subsequently store at -80°C;
23. Repeat for the remaining 5L (second replicate).

Particulate organic matter (POC-PON)

Contacts: Marta Furia (marta.furia@szn.it), Francesca Margiotta (francesca.margiotta@szn.it), SZN

The collected samples will be used to analyse particulate organic matter elemental composition (POC-PON). Collect water samples directly from the Niskin bottles and filter them on board or as soon as possible in a laboratory.

CAUTION

- RISK OF CONTAMINATION
- Beware of boarding fumes
- Do not smoke during sampling
- Use only swinnex filter holders and tweezers washed with HCl 10% and rinsed with MQ

Step by step

1. Wear protective gloves to avoid contamination
2. Collect water samples by using a silicone tube from the appropriate Niskin bottles into acid-clean plastic tanks avoiding contamination; rinse and discard a small volume of samples to assure the elimination of previous residual inside the tank (3 times);
3. Fill the tank with 2-4 litres (the filtration volume depends on station, depth and season);
4. Filter the samples by using a filtration system equipped with pre-combusted Whatman GF-F filter (25mm), respecting the following steps:
5. Precombusted Glass Fiber Filters (Whatman GF/F, 25 mm in diameter) are dipped in HCl 10% and placed in the inline custom-built filtration system;

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6. Filters are rinsed with MilliQ Water (MQ);
7. The sample is homogenized by hand-mixing
8. A variable volume (0.5-4 L) is filtered
9. Filters are then washed with 20 mL of DDW, and immediately stored in a polycarbonate Petri dish appropriately marked at –20 °C until analysis.
10. A blank filter (with no sample filtered through) is placed in a Petri dish at the same time, so as to estimate the filter contribution.
11. 2.Immediately store the samples into the freezer on board
12. 3.Transport samples from the ship to the fixed storage by using a cooler box with ice packs inside
13. 4.STORE the samples at -20°C until the analysis

Pigment analysis

Contacts: Marta Furia (marta.furia@szn.it), Francesca Margiotta (francesca.margiotta@szn.it), SZN

The collected samples will be used to analyse pigments in seawater. Collect water samples directly from the Niskin bottles and filter them on board or as soon as possible in a laboratory.

CAUTION <!> Risk of cell and pigment degradation!

Step by step

1. Water samples are collected from the Niskin into dark high-density polyethylene bottles by using a silicon tube; rinse and discard a small volume of water (3 times);
2. Fill the tank with not less of 2-4 litres of the sample; the filtration volume depends on station, depth and season);
3. A variable volume (0.5 to 4 l) is filtered under low vacuum in dim light onto Glass Fiber Filters (Whatman GF/F, 47 mm in diameter), obtaining ONE replicate for each sample.
4. Filters are then folded using clean tweezers and placed in 2 mL cryotubes, appropriately labelled with sampling cruise, station, date and depth.
5. Immediately STORE the cryotubes into a cryogenic storage filled with liquid nitrogen (e.g. standard cryogenic dewar) until analysis.

1.2.8. Samples processing, transient storage, shipping conditions and Biobanking

Protocol code	Protocol name	Sample processing		Transient storage and T° at boat	Responsibles (PI) and biobanking
		sample	Chemical		
CHLa	Chlorophyll a	1.2 mL cryotube		-80°C	Francesca Margiotta

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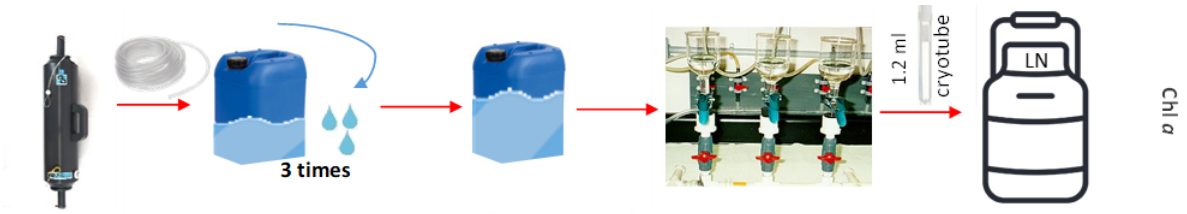
					(SZN)
DGAS	Dissolved gases	12 mL exetainer	ZnCl2	+4°C	Ulisse Cardini (SZN)
DCH4	Dissolved methane	20 mL glass vial	HgCl2	+4°C	Cédric Boulart (CNRS)
∂13C-CH4-DIC	∂13C-CH4 and ∂13C-DIC	12 mL exetainer	HgCl2	+4°C	Cédric Boulart (CNRS)
C&N2FIX	C and N2 fixation rates	GFF filters		-20°C	Ulisse Cardini (SZN)
DIN	Dissolved inorganic nutrients	25 mL HPDE		-20°C	Francesca Margiotta (SZN)
DOC	Dissolved Organic Carbon	40 mL glass vial	HCl	+4°C	Chiara Santinelli (CNR)
CDOM/FDOM	Chromophoric/Fluorescent Dissolved Organic Matter	60 mL Nalgene		+4°C	Chiara Santinelli (CNR)
FLOWC	Flow cytometry for pico and nanoplankton	2-mL cryotube	paraformaldehyde; glutaraldehyde	-80°C	Raffaella Casotti (SZN)
SIA	Natural stable isotope analyses	GFF filters Falcon tubes		-20°C	Francesca Margiotta (SZN)
METAOM	Meta-omics analyses on 3 size-fractions	polycarbonate membranes		-80°C	Raffaella Casotti (SZN)
POM	Particulate organic matter	GFF filters		-20°C	Francesca Margiotta (SZN)
PIGM	Pigment analysis	GFF filters		-80°C	Francesca Margiotta (SZN)

1.2.9. Flow charts

Chlorophyll a



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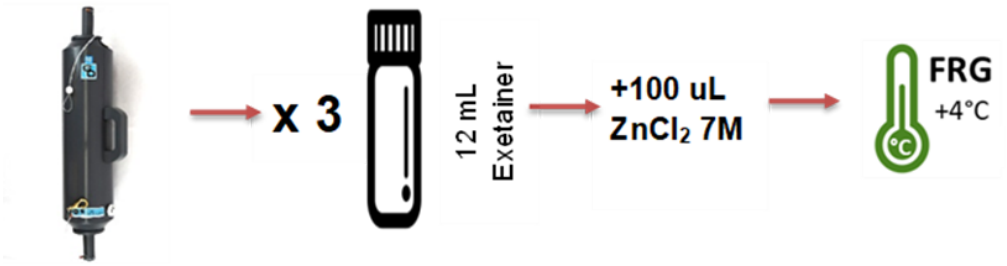
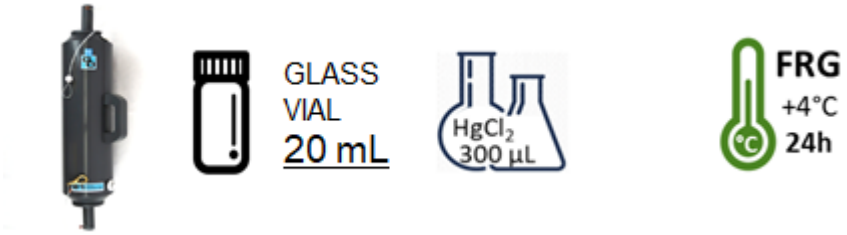
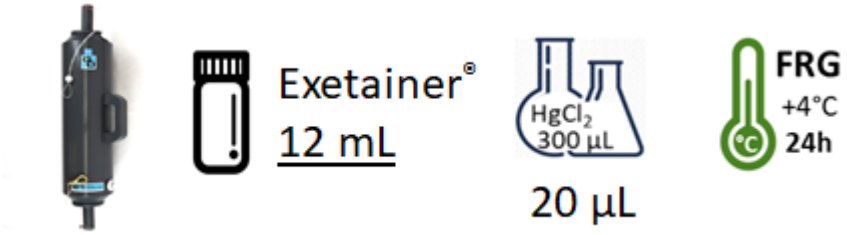
Dissolved gases in seawater (DGAS)



Dissolved methane in marine and estuarine waters



∂^{13}C-CH_4 and ∂^{13}C-DIC in seawater and estuarine waters



Simultaneous measurement of C and N2 fixation rates in planktonic communities

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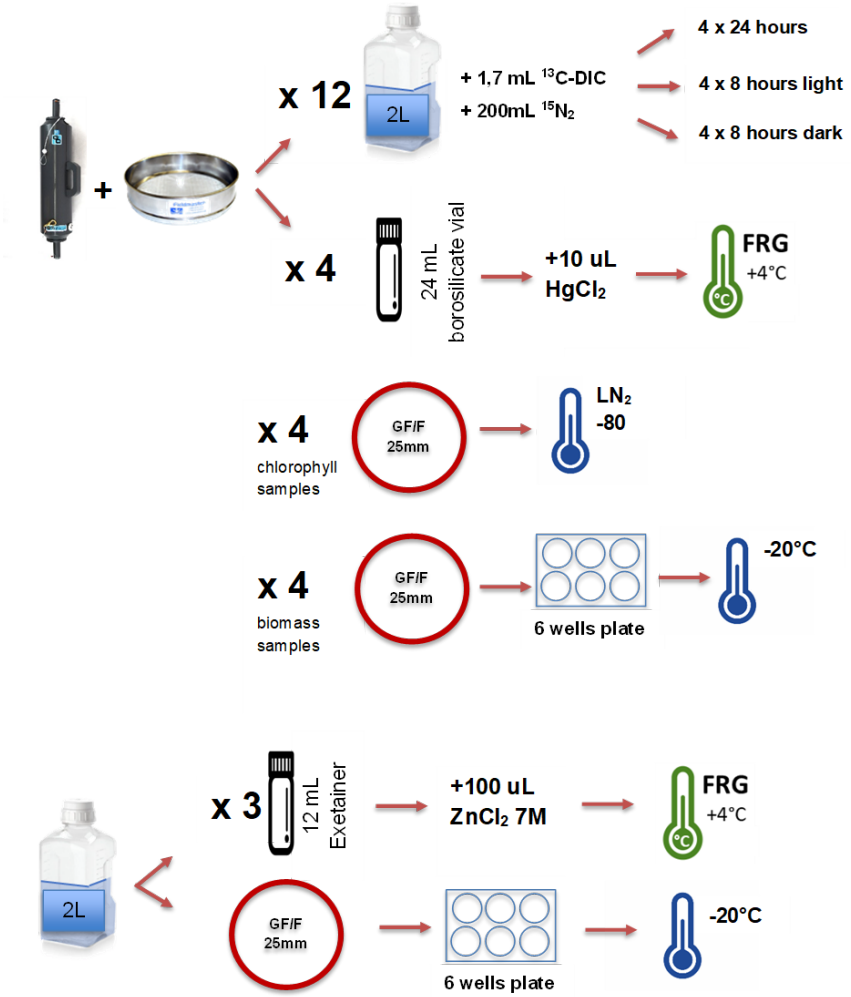

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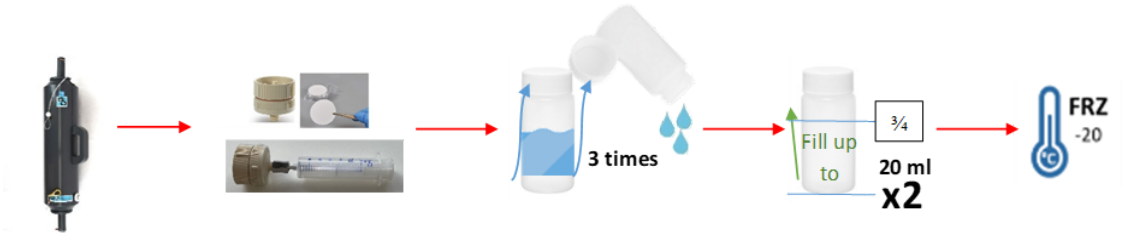
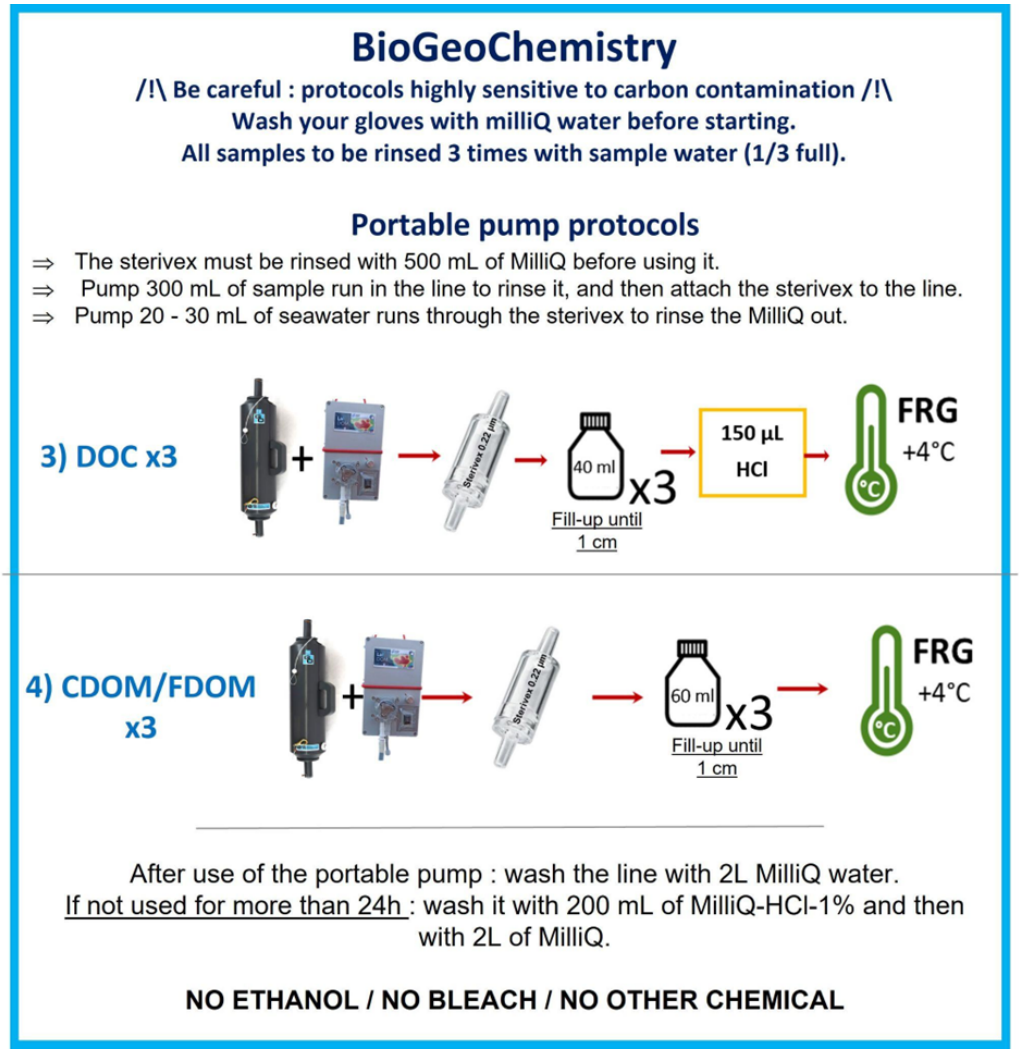
Dissolved inorganic nutrients



Dissolved Organic Carbon (DOC) and Chromophoric/Fluorescent Dissolved Organic Matter (CDOM/FDOM)



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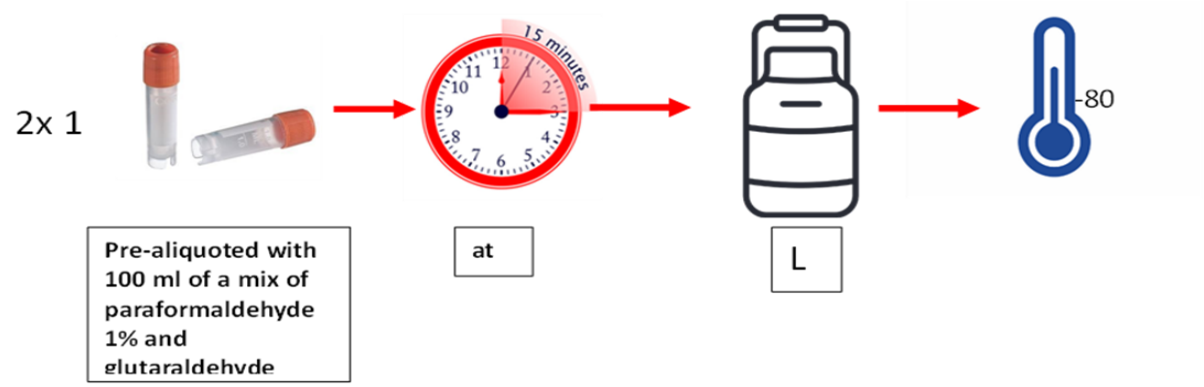
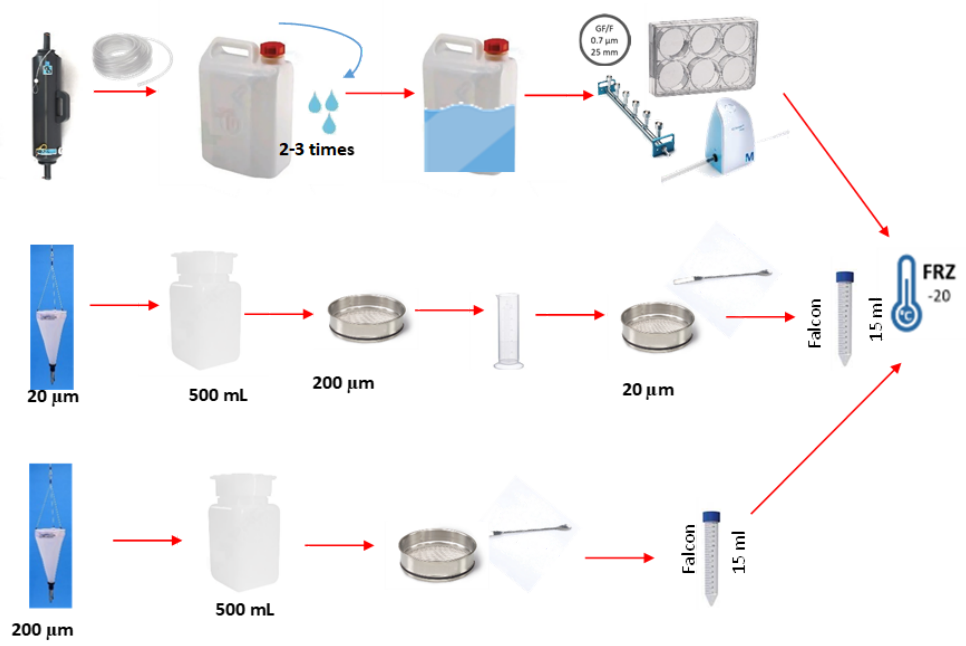
Flow cytometry for pico and nanoplankton diversity and abundance



Natural stable isotope analyses for trophic web reconstructions



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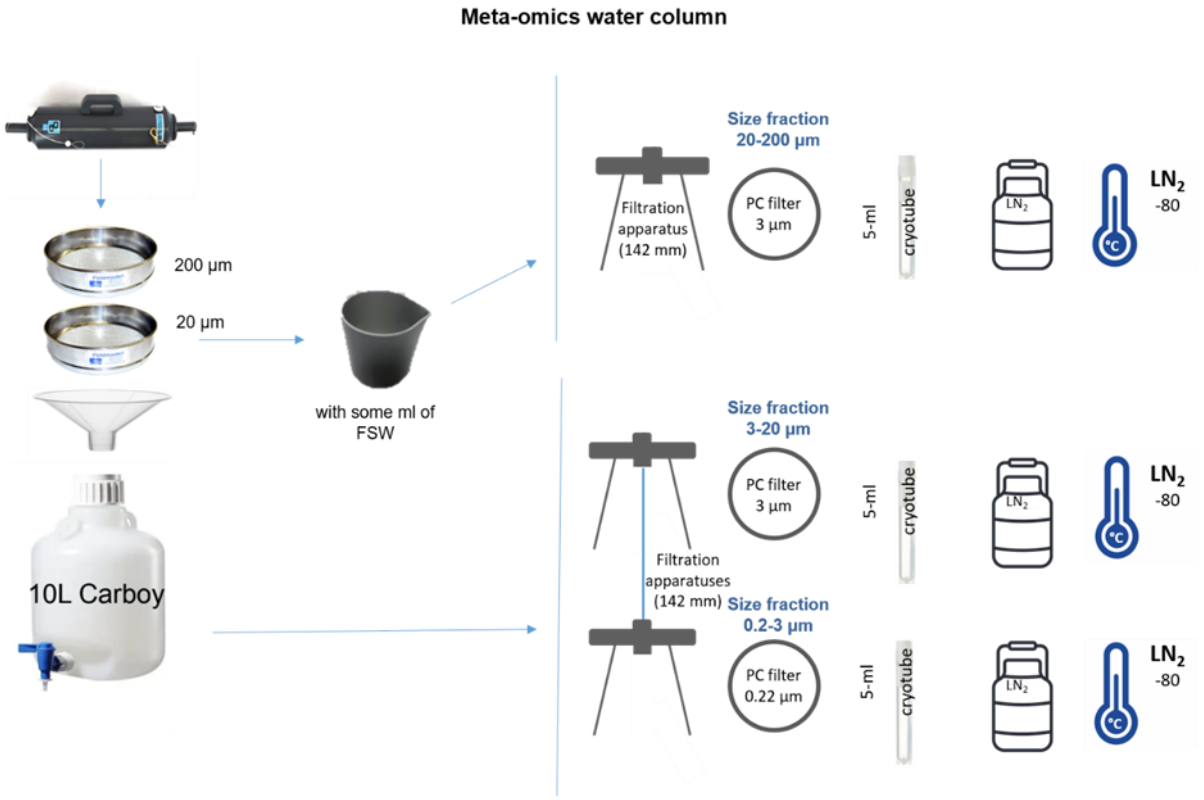
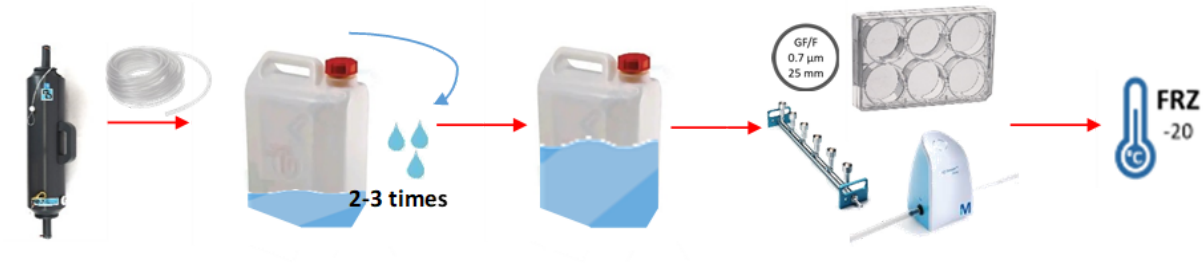
Meta-omics analyses on 3 size-fractions (0.22-3; 3-20; 20-200 µm)



Particulate Organic Matter (POC-PON)



Pigments

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1.2. 1.3. Shallow waters

1.3.1. Authors, affiliations and contact

Flora Vincent (vicent@embl.de) EMBL, Hugo Berthelot (hugo.berthelot@ifremer.fr) IFREMER

1.3.2. Introduction

Within the land-sea continuum, shallow waters (SW) are the interface between sediment, coastal waters and soil. The encounter of these contrasted biomes make SW a place for complex and intense biogeochemical transformations. Yet, this complexity makes SW difficult to interpret and is greatly under-sampled. The strength of TREC is the transbiome strategy where both soil, sediment, aerosols and coastal waters are systematically sampled. This is a unique opportunity to sample SW in a contextualised sampling strategy to better understand the fluxes and the nature of biological and chemical compounds exchanged and transformed within the land-sea continuum.

1.3.3. Sampling strategy

Shallow waters are collected at 120 sampling sites along the TREC land-sea transects, parallel to the terrestrial and water column sampling. The sites represent pristine sites and a variety of human impacted sites (such as agricultural sites, city, ports and touristic places) and river outputs. Water samples are taken by walking into the waters until a depth of 30-100cm and collecting 20-50 litres that are pre-filtered and size fractionated on site. When possible, samples are processed in the same standardised way as on Tara to insure intercompatibility.

1.3.4. Sampling tools and equipment

Sampling equipment

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Niskin bottle	
RBR CTD probe (with its buoy and mooring)	
2 x 20L carboys	
8 x 2L Nalgene	
200mL bottles	

200µm and 20µm sieves	
Funnel	
Gloves	
2 * Washing bottle	
Portable filtration unit+bucket	

Consumables per site

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Parameter	Type	Number /Volume	Preconditioned	
NUT/CDOM/DOC	Sterivex	1	No	
NUT	Nalgene 60ml	3	Yes	
CDOM	60ml PC	3	No	
DOC	Glass bottle 40ml	3	Yes *(Baked 450°C)*	
HP	25mm GF/F filter	1	No	

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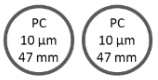
HP	2ml cryotube	1	No	
PM	25mm GF/F filter	3	Yes *(Baked 450°C)*	
PM	Alu Foil	3	No	
PM	Small Whirl Bag	1	No	
FC-P	2ml cryotube	1	No	
FC-G	2ml cryotube	1	No	
SG	5 ml cryotube	3	Yes *(Glycine Betaine)*	
CP-G	47mm 0.2µm PC filter	1	No	
CP-G	5ml cryotube	3	Yes (*glycerol*)	
S20	47mm 10µm PC filter	2	No	
S20	5ml cryotube	2	No	
S320-S	Dacron filter	2	No	

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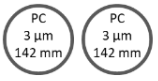
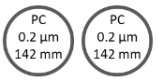
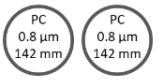
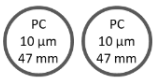
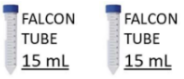
S320-S	142mm 3.0µm PC filter	2	No	
S320-S	5ml cryotube	2	No	
S023-S	Dacron filter	2	No	
S023-S	142mm 0.2µm PC filter	2	No	
S023-S	5ml cryotube	2	No	
S<02	Dacron filter	2	No	
S<02	142mm 0.8µm PC filter	2	No	
S<02	FeCl3 stock solution	1 mL	No	
S<02	5ml cryotube	2	No	
FM20	50mL conical tube	2	Yes *(Glu+PFA mix)*	
FM5	50mL conical tube	2	Yes *(Glu+PFA mix)*	
E20	Custom made 5µm filter	2	No	
E20	15mL conical tube	2	No	

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SG5	5mL cryotube	3	Yes *(Glycine Betaine)*	
MB20	47mm GF/D 2.7um	1	No	
MB20	5ml cryotube	1	No	
MB320	142mm GF/D filter	1	Yes *(baked 450°C)*	
MB320	50mL conical tube	2	No	
MB033	142mm GF75 filter	1	Yes *(baked 450°C)*	
MB033	50mL conical tube	2	No	
MB-HLB	50mL conical tube	4	No	
MB-HLB	50mL conical tube	4	No	
MB-HLB	SPE-HLB cartridge	4	No	
MB-PPL	SPE-PPL cartridge	4	Yes (methanol)	
MTE	Nalgene 60mL	1		

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eDNA	eDNA sampling kit	1	Yes	

List of chemicals

Washing	MilliQ grade water	20L	NA	
LN2	Liquid N2 (in a dry shipper)		NA	
Fixation	Ethanol	0.5L	96%	
Washing	Bleach 10 %			
Acidification	HCL 25%	300ml	200ml HCL 37% plus 100ml MQ	
Acidification	HCl 1%		80ml HCL 25% to 2L MQ	
Washing	pH2	2L	Adjust MQ to pH 2 using pH paper Use HCl 25% to acidify (1.3ml per L or 1,5 to 2L)	

Equipment	**Chemicals**
• Niskin bottle • RBR CTD probe (with its buoy and mooring) • 2 x 20L carboys • 8 x 2L Nalgene • 200mL bottles • 200µm and 20µm sieves • Funnel • 2 * Washing bottle	• 20 L MilliQ grade Water • Liquid Nitrogen and dry shipper • 0,5 L 70% Ethanol • Bleach 10% • 300 mL HCl 25% • HCl 1% • 2 L pH2

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• Portable filtration unit+bucket	• Iron Chloride (2.82 g $FeCIL_3$ in 50 mL ultrapure water)

1.3.5. Metadata collection

Site log-sheet :

- Site ID
- Date
- operator
- Check CTD deployment
- Starting time CTD deployment
- CTD retrieve Check
- End time
- Starting collection water
- End time collection water
- Starting time processing water
- End time processing water

Niskin bottle log sheet :

- Number of sterivex
- Time Particulate matter
- Volume Particulate matter
- Edna volume

Carboy 1 log sheet :

- Time MB320/023
- Volume MB320/023
- Time MB20
- Volume MB20
- Cartridge PPL extraction Time

Carboy 2 log-sheet :

- Time S320/023
- Volume S320/023
- Time S20
- Volume S20
- Time S<20

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- Volume S<20

1.3.6. Environmental parameters measurement

At the same time as the sampling team is collecting water, a CTD is deployed just a few metres from the water collection point. This CTD allow us to measure various environmental parameters:

- Temperature,
- Salinity/conductivity,
- Depth,
- CDOM/FDOM,
- Dissolved O2 concentration,
- Fluo (Chla),
- Backscatter (turbidity)

1.3.7. Sampling protocols

Water collection

CTD Acquisition

1. Assemble the CTD
2. **CAUTION**: make sure the floats are secured to the CTD
3. Moor the CTD in close vicinity to the sampling point using an anchor. If the anchor can't be deployed (e.g. rocky bottom, high current) the CTD is held by an operator on a line.
4. Turn on the CTD by turning clockwise the red cap, vibrations signal the CTD is running
5. Retrieve CTD after shallow water sampling
6.

In ideal conditions, the CTD acquisition should span over the whole water sampling. In degraded conditions (CTD not moored) the minimum acquisition time is 10 minutes.

<!> If there is a water current, place the CTD upstream of the sampling point to avoid contaminating the CTD acquisition with resuspended sediment that might be caused by the sampling process.

Make filtered sea water on site

Before proper sampling, make filtered sea water using the portable filtration device (Katadyn Hiker Pro).

1. Plunge the inlet in seawater and connect the outlet to 5L filtered sea water carboy
2. Rinse carboy 3x with filtered water
3. Fill 5L filtered sea water carboy
4. Fill the two squirt bottles

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<!> Pay attention not to contaminate filtered sea water with drops coming from your hands and/or filtration device

Sample using the 5L Niskin bottle

<!> Avoid contamination with artificially resuspended sediments by staying downstream of the Niskin. Minimise stepping in the sampling area

<i> If the conditions are not met for Niskin sampling (e.g. water too shallow, outgoing tide….) use a 10L bucket (degraded mode)

1. Place the Niskin bottle with both sides open at sub-surface (~20-30 cm deep).
2. Let some water flow through the Niskin bottle to rinse it (especially for the first water collection).
3. Pour ~1L of the Niskin content to each of the carboys to rinse them three times. Use the series of 200µm and 20µm sieves and the funnels to keep only the <20µm size fraction. To speed up the process use the bottom Niskin lid instead of the Niskin spigot. Open the lid gently to control the flow.

<!> Pay attention to not let water overflow the sieve.

4. Close the carboys lids, shake and empty them. Repeat this rinsing operation a second time.
5. After rinsings, clean the sieve to remove all particulate matter by dipping them in the water and quickly washing them with filtered sea water.
6. Fill all the carboys to 20 L by four Niskin samplings and transfer the water to the carboys through the 200µm and the 20µm sieves.
7. Recover the 20-200µm size fraction after each two Niskin bottles (each 10L). Concentrate the particles collected on the 20 µm sieve on one side using the freshly on-site collected filtered sea water (in the washing bottle). Pour the particles to the dedicated 20-200µm 200 mL bottles. Complete the bottle to 200 mL using the filtered sea water.

<!>The last filled carboy is the one dedicated to MetaBGT.

8. Fill two Niskin bottles to be brought back to the filtering van
9. Recover the CTD probe and stop the data acquisition.
10. Bring the 2 x 20 L carboys, the 4 x 200 mL 20-200 µm bottles and the 2 Niskins bottles to the filtering van for processing.

1.3.8. Samples processing, transient storage, shipping conditions and Biobanking

The samples are processed on site in a mobile laboratory set up in the TREC sampling van.

a. Preparation of samples for Metabarcoding, Metagenomics, Metatranscriptomics analysis

During filtrations:

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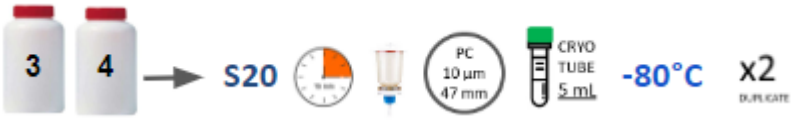
- Always wear gloves and change them as often as needed
- Handle filters carefully; Pick them by the edge and do not touch the part of the filter that will be in contact with the sample.
- Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface.

Protocol S20 - MetaBGTomics targeting eukaryotes

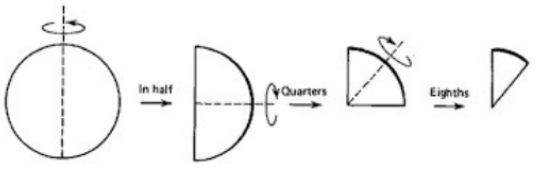
Contact: Julie Poulain, Genoscope (poulain@genoscope.cns.fr)



<!> CAUTION – This protocol is sensitive to RNA degradation - 15 min max

Step-by-step

1. Thoroughly rinse two 47-mm filter holder and cup with MilliQ water
2. Place a 10-µm PC filter on the filter holder and place the cup on top
3. Gently but thoroughly mix the 200mL red cap bottles and pour 50mL in the filtration cup. Start the timer.
4. Switch on the vacuum. Gradually add more volume, always mix the bottle before pouring. Do not pour too much at a time in case the filter clogs
5. <!> Do not exceed 15 minutes for the filtrations. Keep the remaining volume in the red cap bottles to measure the unfiltered volume in a graduated cylinder.
6. Switch off the vacuum as soon as the filter gets dry. Stop the timer. Do not reset the timer. Filtration duration will be needed at the end.
7. Thoroughly rinse the cup with filtered sea water, using a low vacuum to drain; Repeat as much as needed and remove the cup.
8. Fold the filter in half using tweezers. Don't touch the sample with the tweezers and don't crush the filter too much.



9. Place the filter in a 5-mL cryotube and immediately store it in liquid nitrogen.
10. <!> write on the logsheet the total volume filtered and filtration duration.
11. Thoroughly rinse the 47mm filter holder, cup and cylinders with MillQ water and reassemble the filter holders and cups.

Protocol S320-S - MetaBGTomics targeting unicellular eukaryotes

Contact: Julie Poulain, Genoscope (poulain@genoscope.cns.fr)

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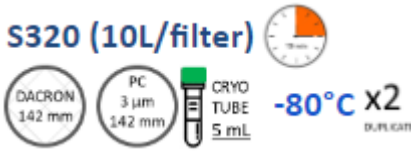

<!> CAUTION – This protocol is sensitive to RNA degradation - 15 min max

<!> Caution before filtration: Clean surfaces, tweezers, intake (sample side) and outflow (filtrate side) tubes, and everything you use with bleach 10% using a spray and then rinse with MilliQ water using a spray.

Step-by-step

1. Humidify all clean filter holders generously with Milli Q water
2. Place Dacron pre-filters on all the tripods, a 3 µm filter on the first tripod, and close tripods tightlt with the key
3. Wipe the intake (sample side) and outflow (filtrate side) Masterflex tubing with bleach 10% using a spray and then rinse with MilliQ water using a spray. Attach a 10 mL pipette (remove filter first) and place the tubes in their respective 20 L carboys. Break off tip as well as filter end to increase the inlet opening. Use the virus canisters to collect the filtrate, it will be used for the next step (protocol S<02 - viruses).
4. Open all bleeding valves before starting the pumps
5. Start the pumps (<17 psi) and start the timer for each filtration line.
6. Close the bleeding valves once sample water comes out without air. Make sure tubing is connected to the bleeding valves in the collection cups to prevent flooding the bench. When all water went through the filters OR <!> no longer than 15 minutes:
7. **Pull the intake Masterflex tubing off the water** (or above the water level if there is still water remaining in the carboy).
8. Keep the pump running until no more remaining water in all the lines and stop the timer (Do not reset the timer. Filtration duration will be needed at the end.)
9. <!> Proceed as quickly as possible to collect filters in order to avoid cell damage and DNA & RNA degradation by following the 4 steps below :

a. Make sure there is no more water remaining in the tripods. It is important to allow the filters to dry. To do that:

Keep the pump on to resume pumping on the line until air goes through the two filters and no more water comes out of the second filter holder

<!> If you feel that there is still water in the second filter holder you can open the bleeding valves on top of the two filter holders to release the pressure. Unplug the top tube connectors from the two filter holders; connect the tube coming from the pump directly on the second filter holder and continue pushing air through it.

b. Open the tripod filter holders. Avoid touching the inside of the filter holder area.

c. Use the forceps to handle the filters. Soak forceps in the bleach 10% tube and then in the MilliQ water before every use

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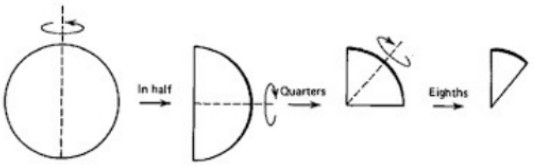
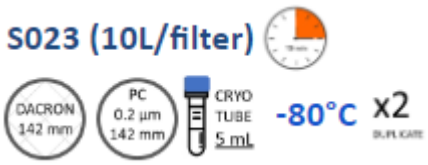


d. Place the filters in their respective 5-mL cryotube

10. Store immediately in Liquid nitrogen.
11. When all filtrations are ended AND <!> all samples are stored in liquid nitrogen
12. <!> Note on the logsheet the filtration time and volume of filtrate obtained from all filtrations.
13. Keep the filtrate for the next step (protocol S<02 - viruses)

Protocol S023-S - MetaBGTomics targeting prokaryotes

Contact: Julie Poulain, Genoscope (poulain@genoscope.cns.fr)



<!> CAUTION – This protocol is sensitive to RNA degradation - 15 min max

<!> Caution before filtration: Clean surfaces, tweezers, intake (sample side) and outflow (filtrate side) tubes, and everything you use with bleach 10% using a spray and then rinse with MilliQ water using a spray.

Step-by-step

14. Humidify all clean filter holders generously with Milli Q water
15. Place a 0.2-µm filter on the second ones, and close all tripods tightly with the key
16. Wipe the intake (sample side) and outflow (filtrate side) Masterflex tubing with bleach 10% using a spray and then rinse with MilliQ water using a spray. Attach a 10 mL pipette (remove filter first) and place the tubes in their respective 20 L carboys. Break off tip as well as filter end to increase the inlet opening. Use the virus canisters to collect the filtrate, it will be used for the next step (protocol S<02 - viruses).
17. Open all bleeding valves before starting the pumps
18. Start the pumps (<17 psi) and start the timer for each filtration line.
19. Close the bleeding valves once sample water comes out without air. Make sure tubing is connected to the bleeding valves in the collection cups to prevent flooding the bench. When all water went through the filters OR <!> no longer than 15 minutes:
20. **Pull the intake Masterflex tubing off the water** (or above the water level if there is still water remaining in the carboy).

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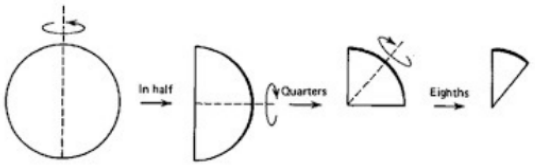
21. Keep the pump running until no more remaining water in all the lines and stop the timer (Do not reset the timer. Filtration duration will be needed at the end.)
22. <!> Proceed as quickly as possible to collect filters in order to avoid cell damage and DNA & RNA degradation by following the 4 steps below :

a. Make sure there is no more water remaining in the tripods. It is important to allow the filters to dry. To do that:

Keep the pump on to resume pumping on the line until air goes through the two filters and no more water comes out of the second filter holder

<!> If you feel that there is still water in the second filter holder you can open the bleeding valves on top of the two filter holders to release the pressure. Unplug the top tube connectors from the two filter holders; connect the tube coming from the pump directly on the second filter holder and continue pushing air through it.

b. Open the tripod filter holders. Avoid touching the inside of the filter holder area.
c. Use the forceps to handle the filters. Soak forceps in the bleach 10% tube and then in the MilliQ water before every use



d. Place the filters in their respective 5-mL cryotube
23. Store immediately in Liquid nitrogen.
24. When all filtrations are ended AND <!> all samples are stored in liquid nitrogen
25. <!> Note on the logsheet the filtration time and volume of filtrate obtained from all filtrations.
26. Keep the filtrate for the next step (protocol S<02 - viruses)

Protocol S<02 - MetaBGTomics targeting viruses

Contact: Matthew Sullivan, Ohio State University, USA (mbsulli@gmail.com)

Step-by-Step:

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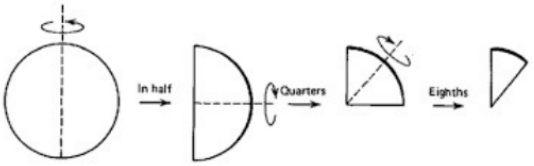
1. Add 1 mL of the stock solution of Iron Chloride (2.82 g $FeCl_3$ in 50 mL ultrapure water) to each filtrate from the previous filtration (previous protocols S320 and S023), shake vigorously and let it incubate at least 1 hour (up to 24h) in the dark
2. ***<After 1 hour> (1-24h)***
3. Humidify all clean filter holders generously with Filtered Sea Water
4. Place Dacron pre-filters and a 0.8-µm PC filter on the first tripod of the MetaBGT line. Close all tripods tightly with the key.
5. Wipe the intake (sample side) and outflow (filtrate side) Masterflex tubing with bleach 10% using a spray and then rinse with MilliQ water using a spray. Attach a 10 mL pipette (remove filter first) and place the tubes in their respective 10 L carboys.
6. Open all bleeding valves before starting the pumps
7. Start the pumps and start the timer for each filtration line
8. Close the bleeding valves once sample water comes out without air

When all water went through the filters OR <!> no longer than 60 minutes

9. ***Pull the intake Masterflex tubing off the water (or above the water level if there is still water remaining in the carboy).***
10. Keep the pump running until no more remaining water in all the lines and stop the timer. Do not reset the timer. Filtration duration will be needed at the end.
11. ***<!> Proceed as quickly as possible to collect filters*** by following the 4 steps below:
    a. Make sure there is no more water remaining in the tripod. It is important to allow the filters to dry. To do that:

       Keep the pump on to resume pumping on the line until air goes through the two filters and no more water comes out of the second filter holder

       <!> If you feel that there is still water in the second filter holder you can open the bleeding valves on top of the two filter holders to release the pressure; unplug the top tube connectors from the two filter holders; connect the tube coming from the pump directly on the second filter holder and continue pushing air through it.
    b. Open the tripod filter holders. Avoid touching the inside of the filter holder area.
    c. Use the forceps to handle the filter. Soak forceps in the bleach 10% tube and then in the MilliQ water before every use



    d. Place the filters in their respective 5-mL cryotube
12. Store at +4°C in the Fridge (FRG).

b. Preparation of samples for Metabolomics analyses

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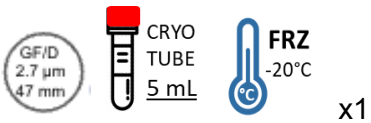
<!> Filtrations for these protocols are done using peristaltic pumps and 142 mm filter holders. The piece of tube that goes in the pump head MUST be Tygon tubing (light beige colour, opaque). No Silicon tubing can be used anywhere in the setup. The inlet & outlet tubes must be securely fixed. It may be necessary to put adjustable metal collars.

During filtrations

- Always wear gloves when handling the tubing, filters, cartridges etc.
- Always wear gloves and wash your gloves vigorously with MilliQ water before starting, handle filters carefully; Pick them by the edge and do not touch the part of the filter that will be in contact with the sample; Make sure that the filters are positioned in the centre of the filter holders and cover the entire filtration surface
- Avoid applying any beauty products (especially sprays), such as sunscreen or deodorant while sampling or sample processing
- No smoking in the vicinity while sampling and during sample processing
- Clean surfaces, tweezers, intake (sample side) and outflow (filtrate side) tubes, and everything you use with MillQ water before starting filtration
- Use PINK tweezers only for the metabolomics protocol!

Protocol MB20 - Metabolomics targeting unicellular eukaryotes

Contact: Georg Pohnert (georg.pohnert@uni-jena.de) University of Jena, Germany



x1

Step-by-step

1. Thoroughly rinse one 47-mm filter holder and cup with MillQ water.
2. Place two 2.7 µm GF/D filters on the filter holders and place the cups on top.
3. Gently but thoroughly mix the 200mL red cap bottles and pour 50mL in the filtration cups. Start the timer.
4. Switch on the suction. Gradually add more volume, always mix the bottle before pouring. Do not pour too much at a time in case the filter clogs
5. ***<!> Do not exceed 15 minutes*** for the filtrations. Keep the remaining volume in the glass bottles to measure the unfiltered volume in a graduated cylinder.
6. Switch off the suction as soon as the filter gets dry. Stop the timer. (Do not reset the timer. Filtration duration will be needed at the end.)
7. Thoroughly rinse the cup with MilliQ water, using a little suction to drain; Repeat as needed and remove the cup.
8. Cut the filter in half for the two MB20 replicates. Fold each half carefully as shown below and place in a 5 mL cryotube.

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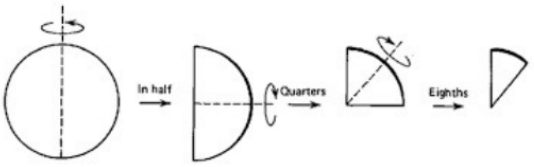

9. Store the cryotubes at -20°C..

10. <!> write on the logsheet the total volume filtered and filtration duration.

Protocol MB320 - Metabolomics targeting unicellular eukaryotes

Contact: Georg Pohnert (georg.pohnert@uni-jena.de) University of Jena, Germany

x2 (½ filter)

Step-by-step

1. Place a 2.8-µm Cytiva Whatman™ Binder-Free Glass Microfiber Pre-filters, Grade GF/D on the first tripod of the line. Keep the foil in which the filters are wrapped for wrapping the Particulate Matter sample filters later (see protocol PM).
2. Rinse the outlet tubing with MilliQ water, wipe with Kimwipe and insert into the first 5 L sample carboy
3. Remove the aluminium cover from the intake tubing of the filtration setup, attach a 10 mL pipette (remove filter first) and place the tubes in their respective 20 L carboys. Make sure the tubing reaches the bottom of the carboy
4. Fix the tubing with pre-combusted aluminium foil
5. Open all bleeding valves before starting the pumps
6. Start the pump (flow= 450 mL/min) at a speed of 17 <!> Filtration will take ~ 25-30 min.
7. Make sure the outlet tubing is safely inserted into the 10 L receiving carboys
8. Close the bleeding valves once sample water comes out without air
9. Filter 5 L into the first 5 L carboy
10. Exchange the sample carboys and filter 5 L into the second carboy.
11. <!> Lift the tubing from the inflow carboy so it does not reach the water anymore and pump air through the system until no more water flows out
12. Open the bleeding valves of each tripod until no water flows out
13. Stop the pump. Record the time of filtration and add it to the logsheet.
14. Open the bleeding valves on top of the two filter holders to release the pressure and open the first tripod and hang the 'lid' onto the provided hook
15. Cut the filters in half for the two replicates of previous protocols MB320 and MB023.
16. With clean tweezers, carefully fold and cut in two the filter halves and place them in their respective 50 mL falcon tubes.
17. Repeat with the second tripod and filter.

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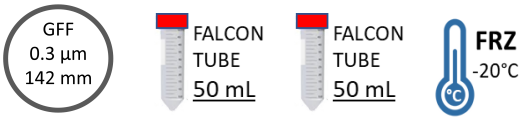
18. Immediately freeze the Falcon tubes at -20°C

Protocol MB033 - Metabolomics targeting prokaryotes

Contact: Georg Pohnert (georg.pohnert@uni-jena.de) University of Jena, Germany



x2 (½ filter)

Step-by-step

1. Place a 2.8-µm Cytiva Whatman™ Binder-Free Glass Microfiber Pre-filters, Grade GF/D on the first tripod of the line. Keep the foil in which the filters are wrapped for wrapping the Particulate Matter sample filters later (see protocol PM).
2. Place a 0.3-µm Advantec GF75142MM Grade GF75 Glass Fiber filter filter on the second tripod of the line and close all tripods tightly. Keep the foil in which the filters are wrapped for wrapping the Particulate Matter sample filters later (see protocol PM).
3. Rinse the outlet tubing with MilliQ water, wipe with Kimwipe and insert into the first 5 L sample carboy
4. Remove the aluminium cover from the intake tubing of the filtration setup, attach a 10 mL pipette (remove filter first) and place the tubes in their respective 20 L carboys. Make sure the tubing reaches the bottom of the carboy
5. Fix the tubing with pre-combusted aluminium foil
6. Open all bleeding valves before starting the pumps
7. Start the pump (flow= 450 mL/min) at a speed of 17 <!> Filtration will take ~ 25-30 min.
8. Make sure the outlet tubing is safely inserted into the 10 L receiving carboys
9. Close the bleeding valves once sample water comes out without air
10. Filter 5 L into the first 5 L carboy
11. Exchange the sample carboys and filter 5 L into the second carboy.
12. <!> Lift the tubing from the inflow carboy so it does not reach the water anymore and pump air through the system until no more water flows out
13. Open the bleeding valves of each tripod until no water flows out
14. Stop the pump. Record the time of filtration and add it to the logsheet.
15. Open the bleeding valves on top of the two filter holders to release the pressure and open the first tripod and hang the 'lid' onto the provided hook
16. Cut the filters in half for the two replicates of previous protocols MB320 and MB023.
17. With clean tweezers, carefully fold and cut in two the filter halves and place them in their respective 50 mL falcon tubes.
18. Immediately freeze the Falcon tubes at -20°C

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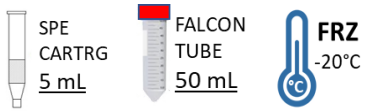
19. Add 1.3 mL 25% HCl per litre to the PPL carboy (red) (6.5 mL to 5 L sample) and shake well.

Protocol MB-HLB - Metabolomics targeting exometabolites

Contact: Georg Pohnert (georg.pohnert@uni-jena.de) University of Jena, Germany



Step-by-step

1. Prepare one 2L PC bottle with 1.6 L MilliQ water (blue, blank)
2. Transfer 3 x 1.6 L neutral samples (blue) into 3x 2L PC bottles.
3. Remove the aluminium cover from the inlet tube of pump
4. Rinse each tube with MillQ water from a squeeze bottle. Add the sample inlet tubings into the respective sample bottles and the blank insert tubings into the respective blank bottles (MilliQ water and MilliQ water pH 2). Make sure that the tubes for acidified samples/blanks are in the acidified bottles! Make sure the tube reaches the bottom of the bottle.
   (Tubes 1-3: neutral sample. Tube 4: MilliQ water-blank. Tube 5-7: acidified sample. Tube 8: MillQ water pH 2 blank).
5. You can fix each tube and close the bottles with pre-combusted aluminium foil to avoid contamination and secure the tubing.
6. Make sure the outlet tubes are safely inserted into the receiving funnel for the waste carboy.
7. Start the pump (flow= 16 mL/min or 45 rpm for the actual pump in the van).
8. Make sure all lines are filled with samples and let a small amount run through.
9. Stop the pump.
10. Have pre-cleaned Luer adapters ready for HLB and PPL cartridges (different sizes). They can be stored in a pre-combusted beaker covered with pre-combusted aluminium foil). Large adapters are for HLB and smaller ones for PPL.
11. One by one, remove the caps of the HBL and PPL cartridges and replace them with the clean Luer adapters that you take out with clean tweezers.
12. For the PL cartridges, please collect the lids and plugs in a separate bag.
13. Attach 4 HBL cartridges (directly from the package) to the first 4 lines of the peristaltic pump.
14. Attach 4 pre-conditioned PPL cartridges to the remaining 4 lines of the peristaltic pump. In case you run out of PPL cartridges you can avoid the blank.
15. Start the pump (flow= 45 mL/min). You can adjust the flow for each tube individually by increasing the pressure on each tube by clicking the tube holders up. You should do this slowly and only for tubes that are not dripping yet. Make sure that the pump is always running smoothly. If you increase the pressure on all tubes too much, the motor might not be able to run properly.

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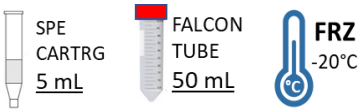
16. Run for ca. 2 h or until all water has run through the cartridges.
17. Stop the pump when all water has run through and the cartridges are dry.
18. Use the 8 rinsing bottles (4x MilliQ water pH2 (red)and 4x MilliQ water (blue). Preparation is described in final cleaning and preparations for the next site).
19. Remove the first four inflow tubings from the sample/blank bottles and add them to the rinsing bottles with MilliQ water . Remove the first four inflow tubings from the sample/blank bottles and add them to the rinsing bottles with MillQ water pH2.
20. Start the pump (flow= 45 mL/min).
21. Rinse the cartridges.

Steps 18-21 can be done later in case of running late.

22. When the bottle is empty, remove the tubing and insert it into a clean 2 L PC bottle.
23. Let the pump run until the cartridges are dry (for about 2 min).
24. Remove all cartridges one by one from the tubing and transfer them to their corresponding Falcon tube. Collect the adapters in a ziploc bag (or similar).
25. Place tubes with cartridges in the cooler while in the field. Immediately freeze the samples at-20°C when back at the Sampling Processing Lab.

Protocol PPL - Metabolomics

Contact: Jessika Füssel (jessika.fuessel@uol.de) University of Oldenburg, Germany



Step-by-step

26. Prepare one 2L PC bottle with 1.6 L MilliQ water pH2 (red, blank).
27. Transfer 3x 1.6 L acidified (red) samples into 3 x 2L polycarbonate (PC) bottles.
28. Remove the aluminium cover from the inlet tube of pump
29. Rinse each tube with MillQ water from a squeeze bottle. Add the sample inlet tubings into the respective sample bottles and the blank insert tubings into the respective blank bottles (MilliQ water and MilliQ water pH 2). Make sure that the tubes for acidified samples/blanks are in the acidified bottles! Make sure the tube reaches the bottom of the bottle.
    (Tubes 1-3: neutral sample. Tube 4: MilliQ water-blank. Tube 5-7: acidified sample. Tube 8: MillQ water pH 2 blank).
30. You can fix each tube and close the bottles with pre-combusted aluminium foil to avoid contamination and secure the tubing.
31. Make sure the outlet tubes are safely inserted into the receiving funnel for the waste carboy.
32. Start the pump (flow= 16 mL/min or 45 rpm for the actual pump in the van).

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33. Make sure all lines are filled with samples and let a small amount run through.
34. Stop the pump.
35. Have pre-cleaned Luer adapters ready for HLB and PPL cartridges (different sizes). They can be stored in a pre-combusted beaker covered with pre-combusted aluminium foil). Large adapters are for HLB and smaller ones for PPL.
36. One by one, remove the caps of the HBL and PPL cartridges and replace them with the clean Luer adapters that you take out with clean tweezers.
37. For the PL cartridges, please collect the lids and plugs in a separate bag.
38. Attach 4 HBL cartridges (directly from the package) to the first 4 lines of the peristaltic pump.
39. Attach 4 pre-conditioned PPL cartridges to the remaining 4 lines of the peristaltic pump. In case you run out of PPL cartridges you can avoid the blank.
40. Start the pump (flow= 45 mL/min). You can adjust the flow for each tube individually by increasing the pressure on each tube by clicking the tube holders up. You should do this slowly and only for tubes that are not dripping yet. Make sure that the pump is always running smoothly. If you increase the pressure on all tubes too much, the motor might not be able to run properly.
41. Run for ca. 2 h or until all water has run through the cartridges.
42. Stop the pump when all water has run through and the cartridges are dry.
43. Use the 8 rinsing bottles (4x MilliQ water pH2 (red)and 4x MilliQ water (blue). Preparation is described in final cleaning and preparations for the next site).
44. Remove the first four inflow tubings from the sample/blank bottles and add them to the rinsing bottles with MilliQ water . Remove the first four inflow tubings from the sample/blank bottles and add them to the rinsing bottles with MillQ water pH2.
45. Start the pump (flow= 45 mL/min).
46. Rinse the cartridges.

Steps 18-21 can be done later in case of running late.

47. When the bottle is empty, remove the tubing and insert it into a clean 2 L PC bottle.
48. Let the pump run until the cartridges are dry (for about 2 min).
49. Remove all cartridges one by one from the tubing and transfer them to their corresponding Falcon tube. Collect the adapters in a ziploc bag (or similar).
50. Place tubes with cartridges in the cooler while in the field. Immediately freeze the samples at-20°C when back at the Sampling Processing Lab.

c. Preparation of samples for BioGeoChemistry analyses

Protocol MTE - BioGeoChemistry – Marine Trace Elements total

Contact: Seth John (sethjohn@usc.edu)

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CAUTION - This protocol is extremely sensitive to contamination: proceed ideally before CTD deployment

- This protocol requires a polyethylene glove, IRON bottles in 2 plastic bags which are in a grey box named MTE protocol.
- This protocol requires 2 people, i.e. the clean scientist who touches ONLY the sampling bottle; and the dirty scientist who uses the MTE-pole

Step-by-Step:

1. Open the grey box where IRON bottles are stored with MTE label
2. Dirty & Clean: Put on your gloves without touching the outside of the gloves
3. Dirty: Open the MTE-S box; take one zip-lock bag ; and open the zip-lock bag
4. Clean: Grab the sample bottle in the opened zip-lock bag and do not touch anything else
5. Dirty: Close the MTE-S box; and grab the MTE-pole
6. Dirty & Clean: walk into the water to the SW collection area
7. Clean: Place the closed bottle in the receptacle at the end of the bow-pole; Open the bottle and hold the cap without touching the inside
8. Dirty: Fill the bottle with water in the SW, Bring back the full bottle
9. Clean: Carefully close the bottle with its cap; and take the bottle off the MTE-pole
10. Clean: Place the bottle into the zip-lock bag; close it; label the outside (not the bottle and not inside the bag); and store it in the MTE-S box where you took it from.

Protocol for filtration with portable peristaltic pump from Niskin bottle

Contact: Chiara Santinelli, CNR-IBF (chiara.santinelli@ibf.cnr.it),Giancarlo Bachi, CNR-IBF (giancarlo.bachi@ibf.cnr.it)

During filtrations

- Make sure that the battery of the pump is charged. Check this before the day of sampling!
- Always wear gloves (polyethylene or nitrile, without powder!) while handling the tubes, the bottles and the filter.
- Please use the dedicated polycarbonate bottles!!
- **Do NOT clean the system with ethanol or other chemicals.**
- **Be careful to never touch the edges of the Sterivex filter.**

Filtration for nutrients (NUT), dissolved organic carbon (DOC) and coloured dissolved organic matter/fluorescent organic matter (CDOM and FDOM)is carried out by a custom made portable peristaltic pump (Figure 1) with silicon platinized and Teflon tubes and Sterivex filter. One end of the tube is connected to a rigid Teflon tube and inserted in the polycarbonate bottle containing the sample, the other end is attached to the Sterivex filter (Figure 1).

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Figure 1: Filtration system with the homemade portable peristaltic pump and Sterivex filter (left panel). Detail of the Teflon tube inside a river sample. (right panel) Credit: Chiara Santinelli

Step-by-step

1. Rinse the 2 L polycarbonate Nalgene bottle 3x with the sample.
2. Fill the 2 L polycarbonate Nalgene bottle with the sample. <!> Be careful not to touch the inside of the cup nor the bottle.
3. Insert the Teflon tube in the polycarbonate bottle containing the sample. <!> Be careful to completely empty the tube before inserting it into the sample bottle.
4. Fill the tube with the sample. <!> Be careful, no air bubbles should be inside the tube.
5. Attach and screw the Sterivex filter washed with 200ml MilliQ water.
6. Let 20 drops of sample run through the filter.
7. Start filtration
8. Proceed to NUT, DOC and CDOM/FDOM protocols
9. To save time, do the cleaning of the pump directly after

<!> The speed should not be increased too much, probably the flow of filtered water will decrease during filtration. Change the filter if the filtration speed decreases too much or stops. In case you change the filter, you need to wash it with at least 200 mL of MillQ water (see points 5-8).

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Protocol NUT - Nutrients

Contact: Hugo Berthelot (hugo.berthelot@ifremer.fr)

CAUTION – This protocol is sensitive to contamination

- DO NOT smoke during sampling

Step-by-step

1. Rinse the 60-mL bottles with the filtered sample (filled to a third); Close the vial, shake and discard the content (REPEAT this step a second and a third time).
2. Dispense the sample into the bottles (leave a head space of ~1 cm below the neck).
3. Repeat the previous step until all bottles and vials are filled.
4. Store upright in the sample box at -20°C in the dark.

<!> DO NOT touch or wet the neck of the vial or the thread of the cap; Salt crystals could otherwise form and breach the seal, letting air in, which can contaminate the sample.

<!> DO NOT fill to the brim to allow sample water to expand in the freezer, without breaching the seal and leaking brine.

Protocol DOC - Concentrations of dissolved organic carbon

Contact: Chiara Santinelli, CNR-IBF (chiara.santinelli@ibf.cnr.it)

CAUTION – this protocol is extremely sensitive to contamination

- <i> Do not smoke during sampling
- <i> Wear polyethylene gloves or nitrile gloves without powder
- <i>Wash the gloves with MilliQ Water

Step-by-step

1. Rinse the 40 mL glass vial three times with a filtered sample: fill the 40 mL glass bottle about a third full (or better half full), close the bottle, shake to rinse and discard the content (REPEAT this step 3 times). <!> Be careful not to touch the inside of the cup nor the bottle.
2. Fill 3 x 40 mL vials, leaving 1 cm of head space. <!> Be careful not to touch the inside of the cup nor the bottle.

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3. Dispense 150 µL of 2M high purity HCl in the glass vials, close the cap and mix. <!> Please use a dedicated pipette to avoid any contamination. This can be done at the end of all the operations.
4. Store in the sample box at 4°C and in the dark

Protocol CDOM/FDOM – Chromophoric/Fluorescent Dissolved Organic Matter

Contact: Chiara Santinelli, CNR-IBF (chiara.santinelli@ibf.cnr.it)

CAUTION – this protocol is extremely sensitive to contamination

- <i> Do not smoke during sampling
- <i> Wear polyethylene gloves or nitrile gloves without powder
- <i>Wash the gloves with MilliQ water

Step-by-step

1. Rinse the 60 mL polycarbonate Nalgene bottles three times with the sample: fill the 60 mL polycarbonate Nalgene bottles about a third full (or better half full), close the bottle, shake to rinse and discard the content (REPEAT this step 3 times). <!> Be careful not to touch the inside of the cup nor the bottle.
2. Fill 3 x 60 mL bottles until the base of the neck of the bottle. <!> Be careful not to touch the inside of the cup nor the bottle.
3. Store all the samples at 4°C and in the dark.

Protocol PM - Particulate Matter (POC/PON/d13C/d15N)

Contact: david.doxaran@imev-mer.fr

CAUTION – This protocol is sensitive to carbon contamination

This protocol uses pre-combusted GF/F filters

Step-by-step

1. The same filters are used to measure PON, POC and isotopes in triplicates ; pre-combusted and GF/F filters are packed in aluminium foil capsules.
2. Rinse the three filter holders generously with filtered sea water

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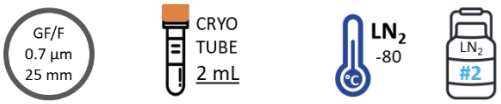
3. Using tweezers cleaned with MilliQ water, place three pre-combusted 25-mm GF/F filters on the filter holder.
4. Start the pump and start the timer for the filtration.
5. Filter 0.5 L(?) of sample material (depending on water turbidity) for 30 min maximum until the filter is clogged and water does not flow through.
6. Stop the pump and the timer; note the time and filtered volume on the logsheet
7. Rinse with 25 mL of MilliQ water
8. Using two clean tweezers, fold each of the three filters in half and put them inside a combusted aluminium foil capsule. You can use the aluminium foil in which the GF/F filters from the metabolomics protocol were stored if applicable

9. Put each foil wrapped filter into a small whirlpack which is labelled with a barcode and the site ID, the sample type and replicate.
10. Store the samples at -20°C

<!> Note on the logsheet the filtration time and volume seawater filtered - 30 minutes maximum.

Protocol HP - Taxonomy pigments (HPLC)

Contact: Joséphine Ras, Céline Dimier (SAPIGH; celine.dimier@imev-mer.fr)



CAUTION - Cells & pigments may be modified, degraded or lost by:

- Filter as soon as water is drawn from the Niskin bottle
- Time spent on a dry filter – Immediately package & store filters once the filtration is finished.
- Too much suction – The vacuum should not exceed 100 mm Hg (0.13 bar).

Step-by-step

1. Clean the filtering device: Use the rinse-bottle with MilliQ water to clean the inside of the filter holder and tulip while the suction is on. Cover the tulip with aluminium foil.
2. Position the filters: Use clean tweezers (rinsed with MilliQ water before use) to handle the filters.

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3. From the Niskin bottle fill the 500mL black bottle up to 500mL and close with the cap.
4. Start filtrations: pour water into the filtering tulips and switch on the vacuum pump. Start the timer and note the time on the logsheet.
5. End filtrations: Close a valve as soon as its filter dries out. Record the time of filtration on the logsheet. Fold the filter in four, always using clean tweezers, and place it in the cryotube.
6. <!> Do not rinse the filtration cups before removing the filters
7. Store the filters: Store the filter in liquid nitrogen. Do not leave tubes with filters on the filtration bench.
8. Time limit: The standard protocol is to filter the entire 500mL-Bottle, but this volume can be reduced if the water is highly charged with particles. As a rule, end the filtration after a maximum of 45 minutes. If so, close the syphon system with its clamp and proceed to end the filtration and store the filter; Measure the volume remaining in the bottle with a graduated cylinder; note the volume filtered on the logsheet; Discard the remaining volume.
9. Rinse the filtering device: Use the rinse-bottle with MilliQ water to clean the inside of the filter holders and tulip while the suction is on. Cover each tulip with aluminium foil.

Protocol FC - Taxonomy flow-cytometry pico/nano

Contact: Josep “Pep” Gasol, CSIC, Barcelona (pepgasol@icm.csic.es)

CAUTION – Glutaraldehyde and paraformaldehyde are toxic substances

- Wear gloves, handle with care, and read the safety sheet (MSDS)
- The pre-aliquoted cryotubes are kept at -20°C in the freezer (FRZ)

Step-by-step

1. FC-Paraformaldehyde: Dispense 1.5 mL of whole seawater into a 2-mL cryotube (red cap-insert) pre-aliquoted with 50 µL of a solution of paraformaldehyde 32%.
2. FC-Glutaraldehyde: Dispense 1.5 mL of whole seawater into a 2-mL cryotube (blue cap-insert) pre-aliquoted with 30 µL of a solution of Glutaraldehyde 25%.
3. Close the cryotube, and mix gently by inversion.
4. Incubate for 15 min in the dark at +4°C.
5. Store in Liquid Nitrogen dewar.

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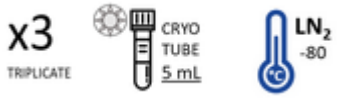
Protocol SG - Genetics – single-organism-genomics pico/nano

Contact: Flora Vincent (flora.vincent@embl.de)



CAUTION – Glycine Betaine is not a toxic substance

- Wear gloves, handle with care, and read the safety sheet (MSDS)
- The pre-aliquoted cryotubes are kept at +4°C in the fridge (FRG) in the front storage area (F-Store)

Step-by-step

1. Dispense 4 mL of the sample into each 5-mL cryotube pre-aliquoted with 600 µL of 48% Glycine Betaine.
2. Close the cryotubes and mix gently by inversion
3. Temporarily place at +4°C for 10-15 minutes
4. Store in Liquid Nitrogen dewar

Protocol CP-G - Genetics - Glycerol stocks

Contact: Michael Zimmerman (michael.zimmerman@embl.de)

Step-by-Step

1. From the Niskin bottle, pre-filter 200 mL of seawater on a 20 µm mesh (same used for the thereafter protocol E20) directly into the tulip
2. Concentrate on 0.2µm 47mm vacuum manifold and keep the filter
3. Insert filter in a 15mL Falcon tube and resuspend by shaking in 10 mL filtered sea water.
4. Discard the filter
5. Aliquot in triplicate different replicates with 2.250 mL of the resuspended bacteria and 0.750mL of 100% glycerol (final concentration 25%) into three labelled 5mL cryotubes
6. Quickly close tubes and mix by inverting
7. Transfer and store in liquid nitrogen

d. Preparation of samples for Single cell ecology analyses

Protocol FM20 - Morphology – e-HCFM(/SEM/exM nano)

Contact: Rainer Pepperkok, EMBL (pepperko@embl.de) and Hugo Berthelot, Ifremer (hugo.berthelot@ifremer.fr)

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CAUTION – This protocol uses toxic substances

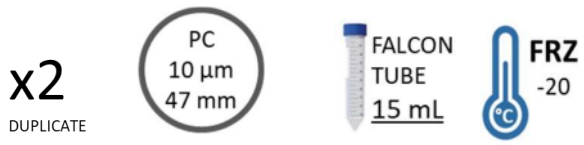
- Read PFA & Gluta MSDS.
- Wear gloves and work in the fume hood.
- Pre-aliquoted 50-mL Falcon tubes are stored at -20°C in the freezer (FRZ).

Step-by-step

1. Fill a 50-mL Falcon tube pre-aliquoted with 1.45 mL of 32% PFA + 225 µl of 50% glutaraldehyde up to 50 mL with gently mixed sample material from the 250-mL bottle (20-200µm).
2. Gently invert the tube a few times and wash the outside of the falcon with fresh water and dry.
3. Repeat step 2 one more time.
4. Store the tubes horizontal at +4°C in the Fridge.

Protocol E20 - single cell genomics

Contact: Flora Vincent, EMBL (flora.vincent@embl.de)



CAUTION:

- EtOH may rub-off the information written by hand on the label.
- The maximum volume of EtOH in a sample is limited to 15 mL for international shipping.

Step-by-step

1. Pour 2 x half of the content of the 200-mL bottle (20-200µm fraction, leftover from previous protocol FM20) onto a 20-µm-pore-size membrane. > Black filter cup in the plastic drawer at the very left of the lab bench.
2. Wash the material off the sides of the filtration cup with 100% molecular grade EtOH and rinse it into the 15 mL falcon tube.
3. Bring the volume of 100% molecular grade EtOH to 10 mL and close the cap tightly
4. Store the 10 mL Falcon tube(s) at -20°C in the Freezer (the sample is temporarily stored in the cooler before getting in the actual -20°C storage)

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Protocol - Cell concentration of the 5-20 µm size fraction

Contact: Hugo Berthelot, Ifremer (hugo.berthelot@ifremer.fr)

Step-by-step

1. Back to the filtering van, hang the 5µm net.
2. Slowly transfer the content of the carboy 4 and filter by gravity through the 5µm net. Wash the walls of the net using water freshly collected on site (filtered sea water).
3. Carefully "massage" the net to keep the outside of the net wet and therefore increasing the filtering surface.
4. The concentrate (200mL) is transferred to a 200 mL red cap bottle (x50 concentration factor) and manually homogenised.
5. Proceed to aliquoting from net for protocols FM5, SG5 thereafter

<!> Cells are fast sinking. Homogenise the bottles before each sub sampling.

Protocol SG5 - Single Cell Genomics

Contact: Flora Vincent (flora.vincent@embl.de)

CAUTION – Glycine Betaine is not a toxic substance

- Wear gloves, handle with care, and read the safety sheet (MSDS)
- The pre-aliquoted cryotubes are kept at +4°C in the fridge (FRG) in SPL

Step-by-step

1. Dispense 3x 4 mL of gently mixed 5-20µm bottle into three 5-mL cryotubes (star-shaped cap) pre-aliquoted with 600 µL of 48% Glycine Betaine.
2. Close the cryotubes and mix gently by inversion.
3. Temporarily place the cryotubes at 4 °C/cooler for max 15 minutes.
4. Store triplicates in Liquid Nitrogen.

Protocol FM5 - Morphology e-HCFM(/SEM/exM nano)

Contact: Rainer Pepperkok, EMBL (pepperko@embl.de) and Hugo Berthelot, Ifremer (hugo.berthelot@ifremer.fr)

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CAUTION – This protocol uses toxic substances

- Read PFA & Gluta MSDS.
- Wear gloves and work in the fume hood.
- Pre-aliquoted 50-mL Falcon tubes are stored at -20°C in the freezer (FRZ).

Step-by-step

1. Fill a 50-mL Falcon tube pre-aliquoted with 1.45 mL of 32% PFA + 225 µl of 50% glutaraldehyde up to 50 mL with gently mixed sample material from the 200-mL bottle (5-20µm fraction).
2. Gently invert the tube a few times and wash the outside of the falcon with fresh water and dry.
3. Repeat step 2 one more time
4. Store the tubes horizontal at +4°C in the Fridge

Shallow waters eDNA analyses

Contact: Prof. Dr. Kristy Deiner (ETH Zurich) (kristy.deiner@usys.ethz.ch)

Step-by-step

1. Put on nitrile gloves to avoid contaminating the water sample with human DNA.
2. If sampling water directly from the surface using the syringe is not possible, then sample the water by filling a decontaminated bottle (1 L). Avoid contact with the water (e.g., standing, falling in, etc...) to limit contamination of the sample.
3. If water is collected, then transport water samples to a suitable site for filtration, pour water into a sample kit plastic bag if filtering from the bottle does not work well (too narrow of an opening).
4. Fill 50-60 mL syringe with sample water and attach to the female luer end of the filter
5. Push sample water through the filter.
6. Repeat steps 4 and 5 until the entire sample is filtered or if it becomes too hard to push the water through, record volume filtered on the sample logsheet.
7. IMPORTANT: Once all the water is filtered. Remove 50-60 mL syringe from filter, pull the plunger on the syringe back, re-attach syringe to filter housing and push air through the

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filter until no water comes through. This ensures that excess water is removed to allow proper preservation.

8. Remove the 60 mL syringe from the filter housing and set aside.
9. Remove the red cap from the 5 mL syringe and attach the 3-5 mL syringe with lysis buffer to the filter housing on the female luer lock side. Hold the filter housing with the female luer lock side down (i.e., the side that attaches to the syringe).
10. Hold the filter up and push the lysis buffer from the prefilled 3-5mL syringe into the filter housing until 0.5 mL is inside or until bubbles start to come out the top from the other end of the filter housing. Cap the male side of the filter housing. Push the remaining preservation buffer into the housing. Turn the housing and filter right side up
11. Remove the 5 mL syringe and cap the female end using the other cap and ensure a secure fit for both.
12. Dry the outside of the filter housing. Label the capped filter housing with the site name/replication number and date with the permanent pen.
13. Place the capped filter housing inside the dry plastic bag from the sample kit (label the outside of the bag and mail this back to the final recipient of the sample. The sample in the filter is room temperature stable for 6 months, but does not store in direct sunlight or above 45°C.
14. Record all information on the provided sample logsheet.

Cleaning procedures

Quick cleaning after sampling

- Rinse filter used for producing filtered sea water and the sieves with FRW before it dries/as soon as possible.
- Rinse tripod and manyfold filtration system with FRW.

Full cleaning of the NUT/DOC/DOM pump

Do this right after sampling to save time.

- Insert the Teflon tube into the polycarbonate bottle containing 1% HCl and wash the tube with 500 mL 1%HCl using the custom made pump.
- Insert the Teflon tube into the polycarbonate (2L) bottle containing MilliQ water and wash the tube with at least 2 L of MilliQ water to clean the system. Please, use dedicated polycarbonate bottles. <!> Do not clean the system with ethanol or other chemicals.
- Rinse 3 times the 2 L polycarbonate sample Nalgene bottle with MilliQ water, fill with 1% HCl and leave with HCl 1% for at least 1 night. Empty the HCl and rinse 3 times with MilliQ water before.

Full cleaning of MetaBGT tripode filtration system

- Fill the filtration system with all intake tubing in a single 10-L carboy filled with 5 L of diluted (10%) bleach (labelled filtrate “BLEACH”), stop the pump and let the bleach act for at least 10 minutes. Restart the pump and collect the filtrate in one 10-L carboy

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- Rinse the filtration system with all intake tubing in a single 5-L carboy filled with 5L of MilliQ water
- Dry the filtration system with all intake tubing by pumping air for 3-5 min
- Cover the tubing with aluminium foil and close tripods once all is dry

Cleaning of the carboys:

- Distribute the BLEACH filtrate from the peristaltic pump in the carboys and close all carboys with their cap.
- Shake all carboys vigorously and let the bleach act for at least 10 min
- Rinse vigorously the inside of all 20-L carboys with 5L of MilliQ water, successively pouring from one carboy to the next, and repeat a second time with another 5L of MilliQ water.
- Make sure that no residual water remains in the carboys

Full cleaning of the manyfold

- Fill the 47mm filter holder, cup and cylinders with BLEACH 10%. Let the bleach act for at least 10 minutes. Switch on the suction. (Only brown tulips)
- Fill the 47mm filter holder, cup and cylinders with MilliQ water and switch on the suction.
- Once dry, re-assemble the filter holders and cups.
- Clean the blue tulips with MilliQ water.

1.3.8. Samples processing, transient storage, shipping conditions and Biobanking

Protocol name	Field Storage	Shipping storage	Long-term storage	Analysis institut/ Recipient's premises	Biobanking at EMBL SHUB
MTE	RT	RT	RT		RT
CDOM	4°C	4°C	4°C	**IBF Pisa**	4°C
DOC	4°C	4°C	4°C	**IBF Pisa**	4°C
S<0.2 (Virus)	4°C	4°C	4°C	**Ohio State University**	4°C
FM5	4°C	4°C	4°C	**Ifremer Brest**	4°C
FM20	4°C	4°C	4°C	**Ifremer Brest**	4°C
eDNA	4°C	4°C	4°C	**ETH Zurich**	4°C
E20	- 20°C	- 20°C	- 20°C	**EMBL HD**	- 20°C
PM	- 20°C	- 20°C	- 80°C	**Ifremer Brest**	- 80°C
MB320	- 20°C	- 20°C	- 80°C	**Jena University**	- 80°C
MB033	- 20°C	- 20°C	- 80°C	**Jena University**	- 80°C
PPL	- 20°C	- 20°C	- 80°C	**University of Oldenburg**	- 80°C

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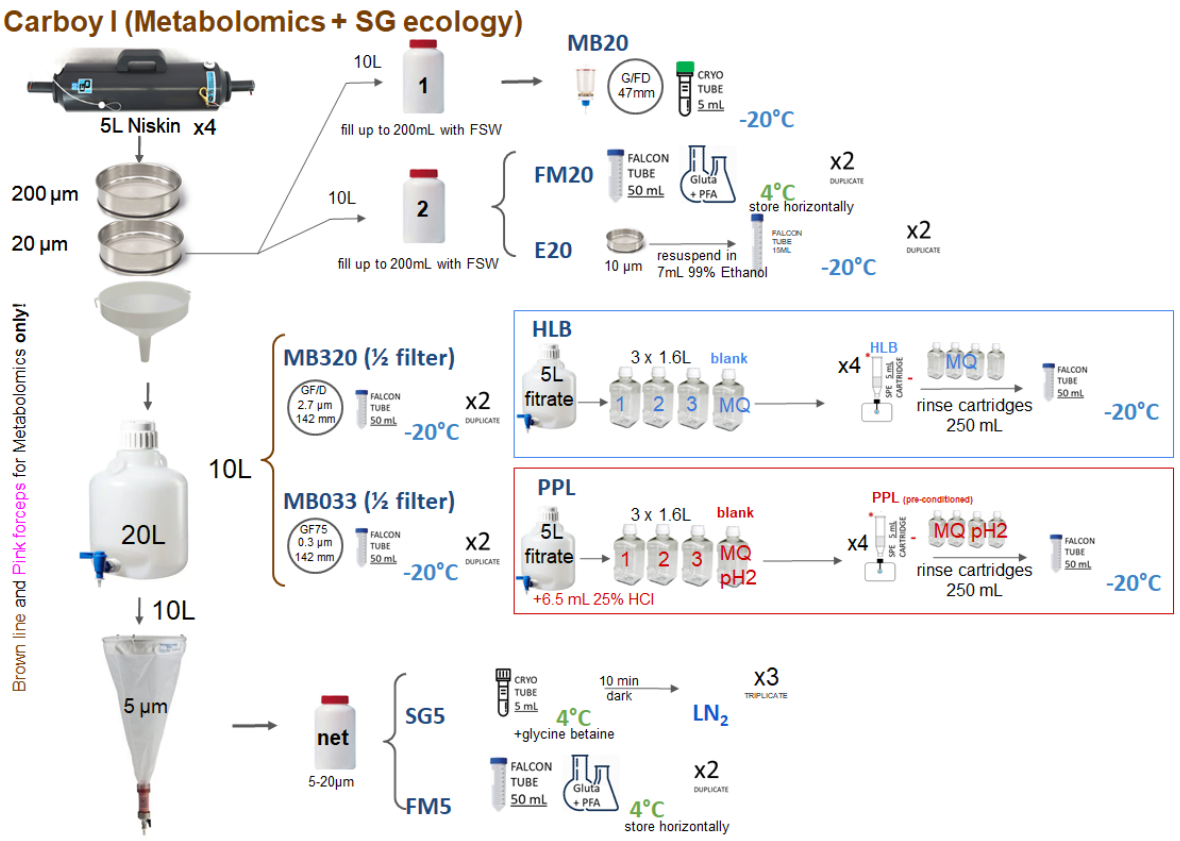
HLB	- 20°C	- 20°C	- 80°C	**Jena University**	- 80°C
MB20	- 20°C	- 20°C	- 80°C	**Jena University**	- 80°C
NUT	- 20°C	- 20°C	- 20°C	**Ifremer Brest**	- 20°C
FC-P	Dryshipper	Dryshipper	- 80°C	**CSIC Barcelona**	- 80°C
FC-G	Dryshipper	Dryshipper	- 80°C	**CSIC Barcelona**	- 80°C
HPLC	Dryshipper	Dryshipper	- 80°C	**IMEV**	- 80°C
CP-G	Dryshipper	Dryshipper	- 80°C	**EMBL HD**	- 80°C
SG	Dryshipper	Dryshipper	- 80°C	**EMBL HD**	- 80°C
S023-S	Dryshipper	Dryshipper	- 80°C	**EMBL HD**	- 80°C
S320-S	Dryshipper	Dryshipper	- 80°C	**EMBL HD**	- 80°C
S20-S	Dryshipper	Dryshipper	- 80°C	**EMBL HD**	- 80°C
SG5	Dryshipper	Dryshipper	- 80°C	**EMBL HD**	- 80°C

1.3.9. Flow charts



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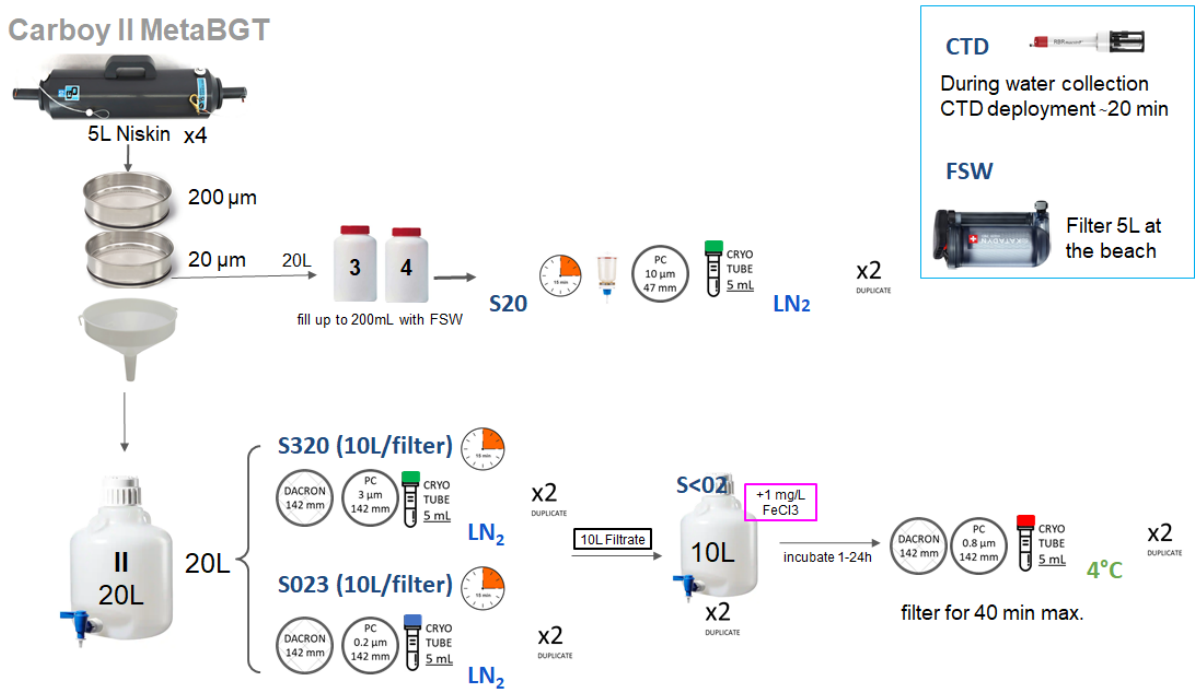
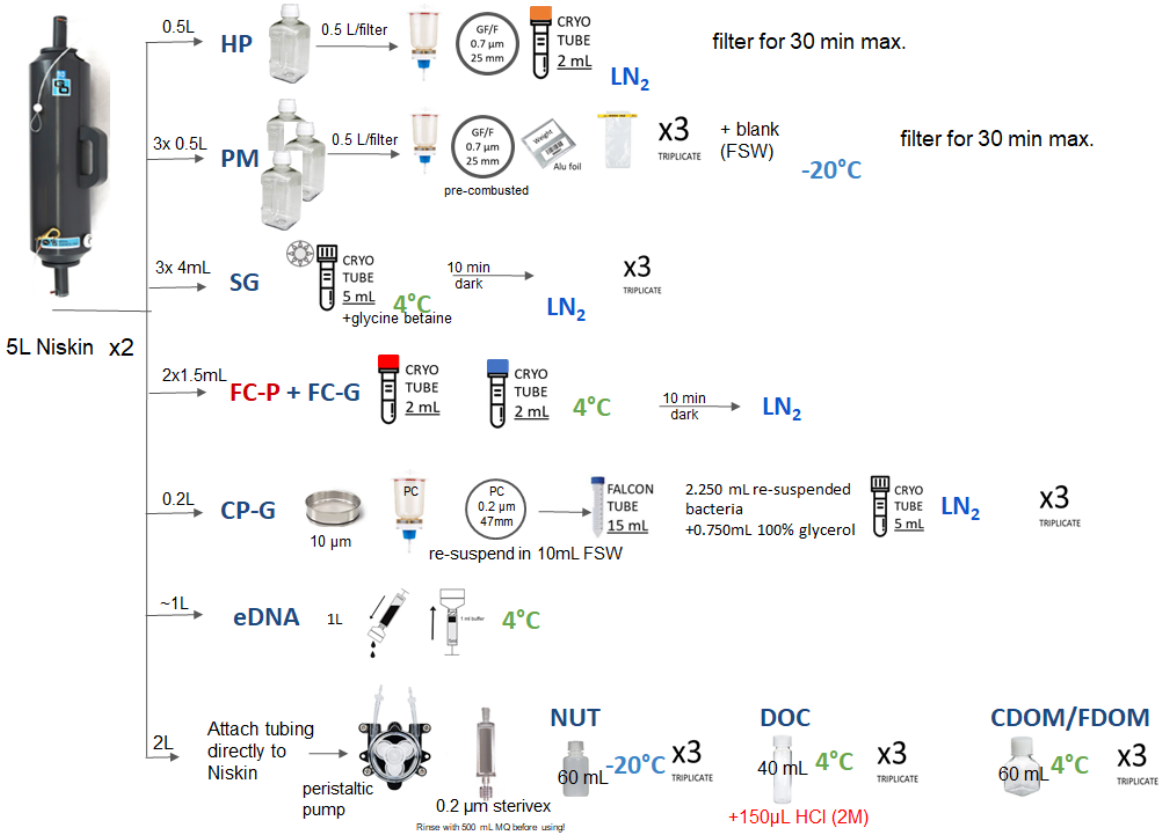


Time sensitive protocol! Prioritise it and make sure to filter only for **15 min total!** 10 min filtering + 5min drying

Niskin



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Cleaning

Rinse everything with fresh water before driving back to station!

Big peristaltic pump + tripods:

- 5L (per line) freshwater + 2.5 L (per line) + 10% bleach (let it soak for 10 min) + 5 L MQ → use designated MQ carboy for big pump
- Clean tripod surface with MQ and wipe dry with KIM wipes
- Release pressure on tubing
- Disconnect tubing from tripod, make sure no liquid remains in the tubing!
- Clean carboy I+II & virus I+II 3x fresh water + 10% bleach (let it react for 10 min) + 3x MQ

Custom made pump (DOC):

- 2L 1%HCl + 2L MQ only! → green marked bottles
- Add 1%HCl to HP (200mL) +PM (500mL) +C-PG (200mL) bottle & let it soak overnight
- charge this pump immediately after use!

Small peristaltic pump (Jessika):

- 5L fresh water + 2L MQ pH2 only! → designated bottle and carboy
- Rinse carboys (blue + red) + Nalgene bottles (2L + 250mL) 3x freshwater + 3x MQ

Manyfold + tulips

- Rinse with 2L freshwater, dismount tulips and submerge them in 10% bleach bath & let them soak for 10 min (use the box we keep the tulips in) + rinse tulips & manyfold with MQ
- If possible, dismount tulips and soak them in freshwater (in their transportation box, followed by cleaning procedure described above)

Katadyn → 10L fresh water + 200mL 10% bleach + 1L MQ

Charge Anker! Rinse CTD immediately after deployment!

To prevent rust: clean when needed with vinegar + fresh water + MQ + bleach + MQ (remove O-rings for proper cleaning!)

Sample storage and shipping

Always prepare 6 cryoboxes for each sampling site containing the samples as follows:

I 4°C → DOC+CDOM/FDOM (makes sure the separators are in the box, glass vial break easily!)
II 4°C → FM20/FM5 (horizontally) + virus (S<0.2)
III -20°C → HLB + PPL cartridges
IV -20°C → MB20 + MB320 + MB033 + E20 + eDNA
V -20°C → NUT+PM
VI -80°C → SG + SG5 + S320 + S023 +HP + CP-G + FCP + FCG

Back at **EMBL** frozen samples can also be stored as follows:

I, II = 4°C
III, IV, V = -20°C > -80°C NUT & E20 stay at -20!
VI = LN_2> -80°C

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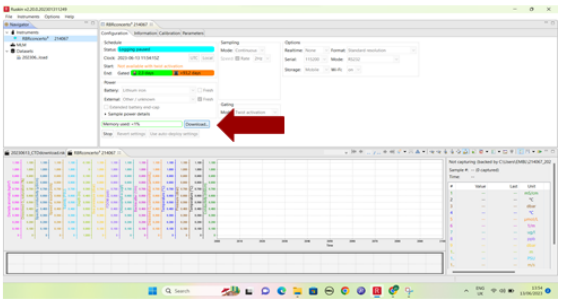
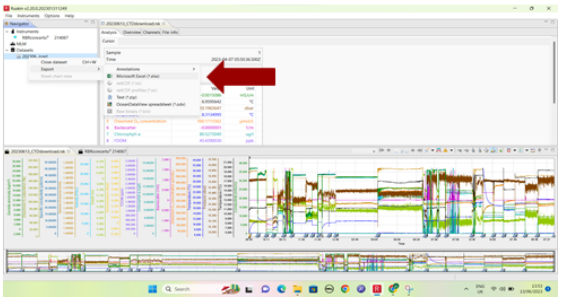
SW lead check-list

- Take a picture of the sampling site + site name and send in SW WhatsApp group
- In charge of the field communication with SW phone
- Update inventory of aliquots and consumables → make sure to order more before running out
- Instrument maintenance → **keep the van clean!**

- Download CTD data:
  - Plug in CTD and open RBR software
  - In the window opened klick → "Download" and save to CTD folder on the laptops in the SPL (server)
  - The new file created is saved as follows: 2023MMDD_*SamplingSite*



  - Now export the data as excel file and save in the same folder with the same name 2023MMDD_*SamplingSite*



1.4. Superficial sediments

1.4.1. Authors, affiliations and contact

Raffaele Siano (IFREMER, Raffaele.Siano@ifremer.fr), Tina Enders (EMBL), Thomas Haize (EMBL), Kiley Seitz (EMBL)

1.4.2. Introduction

Marine ecosystems are rich and complex. Sediments are one of the four Biomes of these ecosystems that the TREC expedition aims to study to the European scale to better understand the biogeochemical interrelationship with land and water This biome is considered as biodiversity and pollutant reservoir. The sediment protocols are inspired by European Marine Omics Biodiversity Observation Network (EMO BON). EMO BON is a European initiative to create a long term observatory. The sediment sampling strategy and protocols were developed as part of this initiative and with the aim of comparing future data across projects and international networks .

1.4.3. Sampling strategy

Superficial sediments are collected at ca. 120 sampling sites along the TREC land-sea transects, parallel to the water column and shallow waters sampling. The sampling areas represent pristine sites and a variety of human impacted sites (such as agricultural sites, cities, ports and touristic places) and river outputs. Muddy, sandy and mixed sediments but

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not hard, rocky sediments are sampled. Sediments are sampled at each site along a 40 metres transect, parallel to the water line, in 3 sampling areas. Each sampling area is an equilateral triangle of 1 metre side and subsamples are taken at each corner of the triangle. Three subsamples per sample area are pooled to take into consideration the local microscale variability to make a sediment sa. Sediments are sampled 2 hours before low tide or between 30cm to 1m of water depth, in case of no tide.

1.4.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• 3 poles • 1 triangle • 1 measuring tape • 10 corers • 3 trophies • 12 bodyboard stoppers • 4 rubber stoppers • 1 table • 2 basins • 1 scale • 1 cooler • 1 dry shipper and safety kit (glasses, gloves) • 1 camera • 1 whiteboard marker • 2 dry suits • 1 safety rope • 3 inox spatulas (sterile) (extra) • 6 wooden spatulas (extra) • Step systems combi 5000 probe (pH, Activity, EC,Moisture, Soil Temperature, Humidity ,Air Temperature, Air pressure) • GPS device	• 1 box of gloves for each size S, M, L • 12 falcon cryotube 50mL • 9 Whirl-Pak 2L (3 are extra) • 6 black Whirl-Pak 2L (3 are extra) • 3 Whirl-Pak 0.5L • 12 falcon cryotube 15mL • 3 Burned 100mL glass vial • 3 glass vial 100mL • 9 Nalgene bottles 250mL • 9 truncated 25ml syringes (sterile) (extra)	• MilliQ grade water (cleaning) • Liquid Nitrogen (samples storage) • DNA away (cleaning) • Sekusept (cleaning) • Ethanol 90% (cleaning) • Bleach 10% (cleaning) • MgCl 1M (microscopy) • PFA 4% (microscopy)

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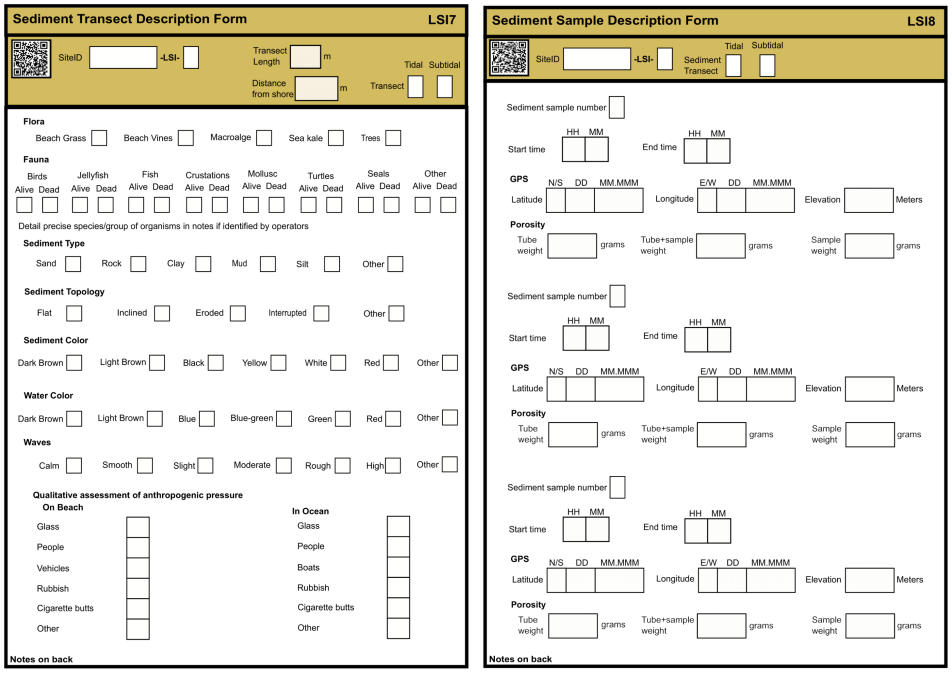
1.4.5. Metadata collection

Metadata collected by site includes:

- GPS coordinates
- Station number
- Flora present
- Fauna present
- Sediment type
- Sediment topology
- Sediment colour
- Waves
- Qualitative assessment of anthropogenic pressure
- Starting time sampling
- End time sampling
- Weight porosity
- Length transect
- Distance from shore
- Tidal or Subtidal transect

Logsheets for each sampling site includes all the metadata of the station site:



Superficial sediment log-sheet

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1.4.6. Environmental parameters measurement

In situ parameter :

In each sampling site some in situ parameters are recorded with different sensors from the Combi 5000 probe.

- Outside temperature
- Humidity
- Air pressure
- Geiger counter

1.4.7. Sampling protocols

Site preparation

Prepare the working area :

1. Set up the folding table on the shore
2. Keep all consumables, equipment and the dry shipper in the working area
    a. Unpack prelabeled tubes and bags
    b. Barcodes of each sample should be added to the checklist / log-sheet on the Sediment Sample Distribution form after samples are taken
3. Prepare and follow the TREC field safety guide (check surroundings, safety equipment, such as manual life jackets, dry suits, ropes, bodyboard, packraft etc...)

Transect assessment

Check whether sediment transect must be possible in the subtidal/intertidal zone. At low tide try to move at least 1-10 m from shoreline depending on the safety conditions at the site.

1. Using a field tape, measure off 40 metres (decrease down to 20 or 10 metres if necessary)
2. Mark each end of the transect with a pole
3. Identify the midpoint and mark with pole
4. Standing with back to the ocean start at the right most marker. This will be the location of triangle 1
5. Confirm that the triangle is suitable for sampling and place it on the line defined by the measuring tape

    Each triangle is equilateral with 1m side length. 1 core will be taken at each corner / point (with the centre of the triangle on the 40 m transect line previously selected) and labelled A, B and C. If this is not possible:

    i) Triangle can be rotated slightly left or right maintaining a 1m distance between subsamples.

    ii) If it is necessary to move the triangle more than 1 metre from original marker, the whole transect will need to be adjusted
6. Repeat step 5 for triangle 2 (middle marker) and triangle 3 (far left marker with back to ocean)

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Coring procedure

1. Record GPS location and take picture of the triangle
2. Put on gloves
3. Take 3 cores simultaneously at each triangle
    a. Take cores at each corner of an equilateral triangle
    b. 3 samplers place 12cm diameter cores simultaneously
    c. Push the core ¾ of the way down
    d. Reach underneath and slide PVC disk or spatula up against the bottom of the core
    e. Gently pull out the core
    f. Take back rapidly to shore
        i) If the sampler is stuck in the mud, pass the cores off to waiting partner
        ii) Get rescued
    g. Hand off to processing people

1.4.8. Samples processing transient storage, shipping conditions and Biobanking

Samples processing at normal site

1. Put on sterile gloves
2. Clean working area with Milli-Q water and DNA away
3. Slide core onto the plunger off of plexiglass plate or spatula
4. Push a little bit the core
5. Take picture of core from side view with sample name on the board (1 core only)
6. Remove the lid
7. Push the core until it reaches the top removing all remaining water
8. Process cores A, B and C simultaneously
    a. MetaG/T/B, Chlorophyll, Organic carbon, Organic Pollutants, Xenobiotics, Metals, and extra sub-samples are composed of a parts of cores A, B and C
    b. Microscopy / Meiofauna materials are sampled separately from each core (A, B and C)
    c. Ions, Granulometry and Porosity sub-samples are only taken from core A
9. Repeat core processing for remaining triangles (36 subsamples)
10. Store the samples in the processing van until arrival at the institutional stop / marine station where it can be transferred to final storage conditions before further transport.

Preservation for meta-barcoding, genomic, transcriptomic analyses, MetaBGT

CAUTION – This protocol is sensitive to DNA degradation and Contamination

<!> DO NOT touch or breathe just in front of the samples

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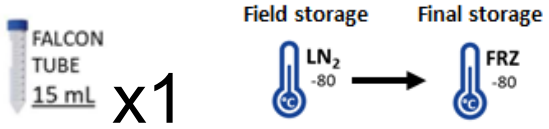
Step-by-Step

1. Into core A, B and C, insert 1 prepared sterile syringe (15 mL for normal sites or 60 mL for supersites) into the internal part of the core (avoid touching the core edges) up to 5 cm deep
2. Starting with core A, pull out the syringe gently tilting to keep material inside syringe
3. Open the falcon cryo tube, keep sterility of the tube (avoid contamination of the cap)
4. Eliminate sediment on the outside of the syringe by wiping it on core
5. Transfer to 50 mL cryo tube by gravity
6. Close the falcon cryo tube with cap
7. Repeat for core B and C in the same tube
8. Shake the tube strongly once to let the sediments deposit at the bottom of the tube
9. Repeat this operation two times (2x in total)
10. Place the tube in a cryo bag (Whirl-Pak 0.5 L), close the cryo bag and place in the dry shipper
11. Repeat this operation two times (2x) using new 50mL tubes for technical replicates and add to cryo bag (large Whirl-Pak 2 L) in dry shipper

Preservation for chlorophyll analysis, Chl

Contact : *Ifremer, Raffaele Siano* (raffaele.siano@ifremer.fr)



CAUTION – This protocol is sensitive to light and temperature degradation

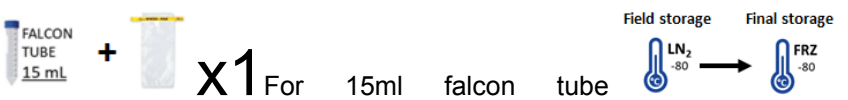
Step-by-Step

1. Use inox spatula / spoon or wooden tongue depressor
2. Collect 2mL from each core from the syringes holes (allow to have a mark at 5cm dept)
3. Place in the same 15mL tube to reach a total volume of 6mL
4. Place cryo bag (Whirl-Pak 0.5 L) and put in the Dry-shipper

Preservation for organic carbon measurement, OrgC

Contact : *Ifremer*, *Florian CARADEC (Florian.Caradec@ifremer.fr)*

ENS, Samuel Abiven (abiven@biotite.ens.fr)



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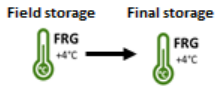


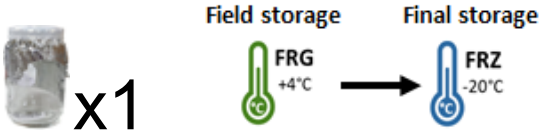
CAUTION – This protocol is sensitive to organic contaminations

Step-by-Step

1. Use inox spatula / spoon
2. Collect 2mL from each core from the syringes holes (allow to have a mark at 5cm dept)
3. Place in the same 15 mL tube to reach a total volume of 6mL
4. Place in 1st dark-coloured, large Whirl-Pak (2 L) and keep in cooler until final storage
5. Collect again 20mL from each core from the syringes holes (allow to have a mark at 5cm dept)
6. Place cryo bag (Whirl-Pak 0.5 L) and put in the Dry-shipper

Preservation for organic pollutants measurement, OrgPoll

Contact : *Ifremer, Nicolas Briant (Nicolas.Briant@ifremer.fr)* and *Javier Jimenez (Javier.Castro.Jimenez@ifremer.fr)*



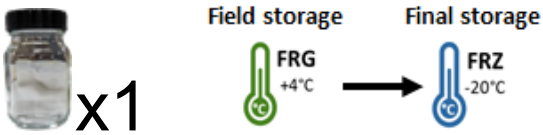
CAUTION – This protocol is sensitive to contaminations

Step-by-Step

1. Use inox spatula / spoon
2. Collect 30 mL from each core
3. Place into the same provided 100mL burned glass bottle
   a. Capped with sterile Aluminium foil
   b. And then with the final plastic cap
4. Place in dark-coloured, large Whirl-Pak (2 L) and keep in cooler until final storage

Preservation for xenobiotic analysis, Xeno

Contact: *EMBL, Michael Zimmerman* (michael.zimmerman@embl.de)



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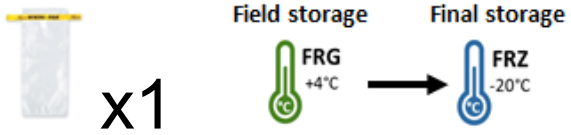
CAUTION – This protocol is sensitive to contaminations

Step-by-Step

1. Use inox spatula / spoon
2. Collect 30 mL from each core
3. Place into the same untreated 100mL glass vial
4. Place in the 1st dark-coloured, large Whirl-Pak (2 L) and keep in cooler until final storage

Preservation for inorganic pollutants / metals analysis

Contact: *EMBL, Michael Zimmerman* (michael.zimmerman@embl.de)



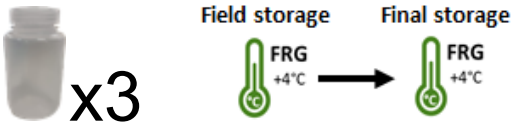
CAUTION – This protocol is sensitive to contaminations

Step-by-Step

1. Use plastic syringes used for DNA / wooden tongue depressor
2. Collect 50 to 100 g from each core used for DNA
3. Place in small Whirl-Pak (0.5 L)
4. Place in the 1st dark-coloured, large Whirl-Pak (2 L) and keep in cooler until final storage

Preservation of microscopy material / meiofauna, Meio

Contact: *Ifremer, Valentin Foulon* (valentin.foulon@enib.fr)



CAUTION – This protocol is sensitive to agitation, don't shake the bottles

Step-by-Step

1. Use plastic syringes used for DNA
2. Collect two syringes of the first 5 cm (as for DNA) from core A

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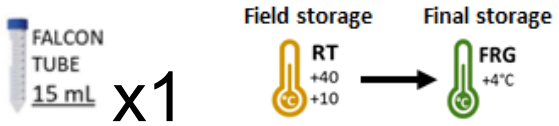
3. Place the sediment in 250 mL Nalgene bottle with 20 mL of $MgCl_2$ and gently invert (not shake!) to cover the sediments
4. Repeat for core B and C in a new bottle
5. Place in the 2nd dark-coloured, large Whirl-Pak (2 L) and keep in cooler until final storage
6. Keep in cooler
7. Back at van and without exposing bottles to light, add 20 mL of 4% formaldehyde in each
8. Place back in cooler until final storage

Preservation for granulometry measurement, Granu

Contact : *Ifremer, Axel EHRHOLD (axel.ehrhold@ifremer.fr) and Angelique Roubi (angelique.roubi@ifremer.fr)*

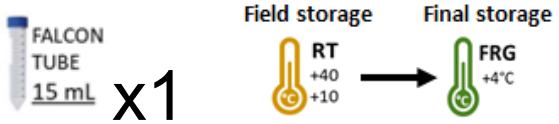


Step-by-Step

1. Use inox spatula / spoon
2. Collect 4mL from core A
3. Place in 15 mL cryo tube
4. Keep in cooler

Preservation for porosity measurement, Poro

Contact : *Ifremer, Raffaele Siano* (raffaele.siano@ifremer.fr)



Step-by-Step

1. Weigh 15mL cryo tubes
2. Use inox spatula / spoon
3. Collect 4mL from core A
4. Reweigh and record weight difference
5. Keep at RT

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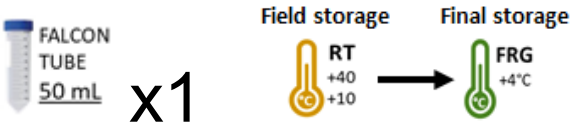
Preservation for ions analysis

Contact : Raffaele Siano (Raffaele.Siano@ifremer.fr)

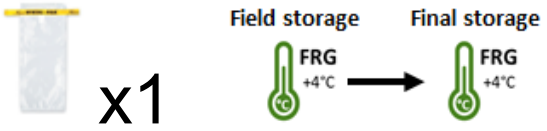


Step-by-Step

1. Use inox spatula / spoon
2. Collect (no more than) 20mL from core A
3. Place in 50 mL falcon tube
4. Keep at RT

Preservation of material for extra analysis

Contact : *Ifremer, Raffaele Siano* (raffaele.siano@ifremer.fr)



Step-by-Step

1. If there is extra material, fill up to 100g in a large Whirl-Pak (2 L)
2. Place in the 1st dark-coloured, large Whirl-Pak (2 L) and keep in cooler until final storage

Sample processing at Super site

1. Put on sterile gloves
2. Clean working area with Milli-Q water and 70% ethanol
3. Slide core onto the plunger off of plexiglass plate or spatula
4. Push a little bit the core
5. Take picture of core from side view with sample name on the board (1 core only)
6. Remove the lid
7. Push the core until it reaches the top removing all remaining water
8. Process cores A, B and C simultaneously
    a. MetaG/T/B and Organic Chemistry sub-samples are composed of equal parts of cores A, B and C, and 3 separate sub-samples of each core
    b. Chlorophyll, Organic Pollutants (Ifremer), Xenobiotics, Metals and Extra sub-samples are composed of equal parts of cores A, B and C.
    c. Microscopy / Meiofauna material are sampled separately from each core (A, B and C)

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d. Ions, Granulometry and Porosity sub-samples are only sampled from core A

Preservation for meta-barcoding, genomic, transcriptomic analyses, MetaBGT

Contact : *Ifremer, Raffaele Siano* (raffaele.siano@ifremer.fr)

CAUTION – This protocol is sensitive to DNA degradation and Contamination

<!> DO NOT touch or breathe just in front of the samples

Step-by-Step

1. In each core A, B and C, insert one prepared sterile syringes (15mL) into core A into the internal part of the core (avoid touching the core edges) up to 5cm
2. Sub-sample for MetaG/T/B for technical replicate on each core (3 per cores)
    a. Open the tube, but be careful to keep sterility of the tube (avoid contamination of the cap)
    b. Pull out the first syringe gently tilting to keep material in syringe
    c. Eliminate sediment on the outside of the syringe by wiping it on core
    d. Transfer to 50 mL cryo tube by gravity
    e. Place the syringes back in the sediment and repeat a second time in the same tube (only for syringe 15mL)
    f. Close the tube with cap
    g. Shake the tube strongly once to let the sediments deposit at the bottom of the tube
    h. Place the tube gently in a cryo bag and place in dry shipper
    i. Repeat this operation two times (x2) using new 50mL tubes for technical replicates and add to cryo bag in dry shipper (x3 sub-samples per core)
    j. Repeat for core B and C (x9 sub-samples per triangle)
3. Sub-samples composed of equal parts of cores A, B and C
    a. Starting with core A, pull out the syringe gently tilting to keep material in syringe
    b. Open the tube, keep sterility of the tube (avoid contamination of the cap)
    c. Eliminate sediment on the outside of the syringe by wiping it on core
    d. Transfer to 50 mL cryo tube (either by gravity or by pushing with the syringe piston)
    e. Repeat for core B and C in the same tube
    f. Close the tube with cap
    g. Shake the tube strongly once to let the sediments deposit at the bottom of the tube
    h. Place the tube gently in the cryo bag and directly in dry shipper
    i. Repeat this operation two times (x2) using new 50mL tube for technical replicates and add to cryobag in dry shipper

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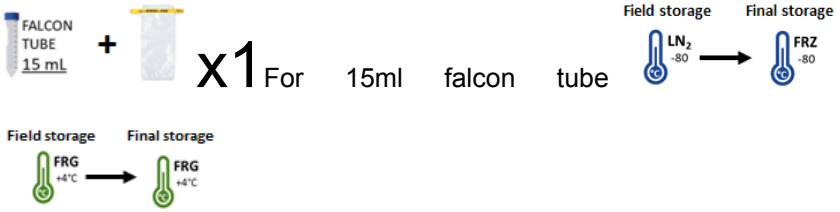
4. Repeat for each triangle (36 subsamples)

Preservation for organic carbon measurement, OrgC

Contact : *Ifremer, Florian CARADEC (Florian.Caradec@ifremer.fr)*

ENS, Samuel Abiven (abiven@biotite.ens.fr)



CAUTION – This protocol is sensitive to organic contaminations

Step-by-Step

1. Sub-sample for OrgC for technical replicate on each core (3 per cores)
    a) Use inox spatula / spoon
    b) Collect 6mL from each core
    c) Place in separate 15 mL tube
    d) Place in small Whirl-Pak and put in LN
    e) Collect again 20mL from each core
    f) Place in separate Whirl-Pak and keep in cooler until final storage
2. Sub-samples composed of equal parts of cores A, B and C
    a) Use inox spatula / spoon
    b) Collect 2mL from each core
    c) Place in the same 15 mL tube to reach a total volume of 6mL
    d) Place in in small Whirl-Pak and put in LN
    e) Collect again 20mL from each core
    f) Place in the same Whirl-Pak and keep in cooler until final storage

Preservation for other analyses - "super site"

The methods of other sub-samples analyses are the same as « normal site »; see previous section.

Cleaning Procedure

All material is firstly rinsed with tap water, then every box is cleaned properly to avoid any contamination.

The cores are immersed in a bucket filled with bleach for 3 to 5 minutes and rinsed 3 to 5 min in a bucket with MilliQ water. Then, they are placed in a box previously cleaned with DNA away. Bleach and MilliQ water are changed at every station.

When trophies are dry, they are cleaned with DNA away and put back into the box cleaned previously.

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Spatulas and syringes are cleaned following these steps:

- Wash with Sekusept
- Rinse with MilliQ
- Dry with Ethanol
- Wipe with DNA away
- Wrap into aluminium foil
- Autoclave

Analysis	**Field storage**	**Shipping storage**	**Long-term storage**	**Analysis institut/ Recipient's premises**	**Biobanking at EMBL SHUB**
MetaBGT	Dryshipper	Dryshipper	-80 °C	EMBL Heidelberg	-80 °C
Chl	Dryshipper	Dryshipper	-80 °C	IFREMER Brest	-20 °C
Org. C	Dryshipper	Dryshipper	-80 °C	IFREMER Brest	-20 °C
Extra Org.C	4 °C	4 °C	4 °C	ENS	4 °C
Organic pollutants	4 °C	-20 °C	-20 °C	IFREMER, Nantes	-20 °C
Xeno	4 °C	-20 °C	-20 °C	EMBL, Heidelberg	-20 °C
Microscopy	4 °C	4 °C	4 °C	IFREMER, Brest	4 °C
Inorg. C/Metals	4 °C	-20 °C	-20 °C	EMBL, Heidelbegr	-20 °C
Porosity	RT	4 °C	4 °C	IFREMER, Brest	4 °C
Granulometry	RT	4 °C	4 °C	IFREMER, Brest	4 °C
Ions	RT	-	-	EMBL, Heidelberg	-
Extra	4 °C	4 °C	4 °C	EMBL,Heidelberg	4 °C

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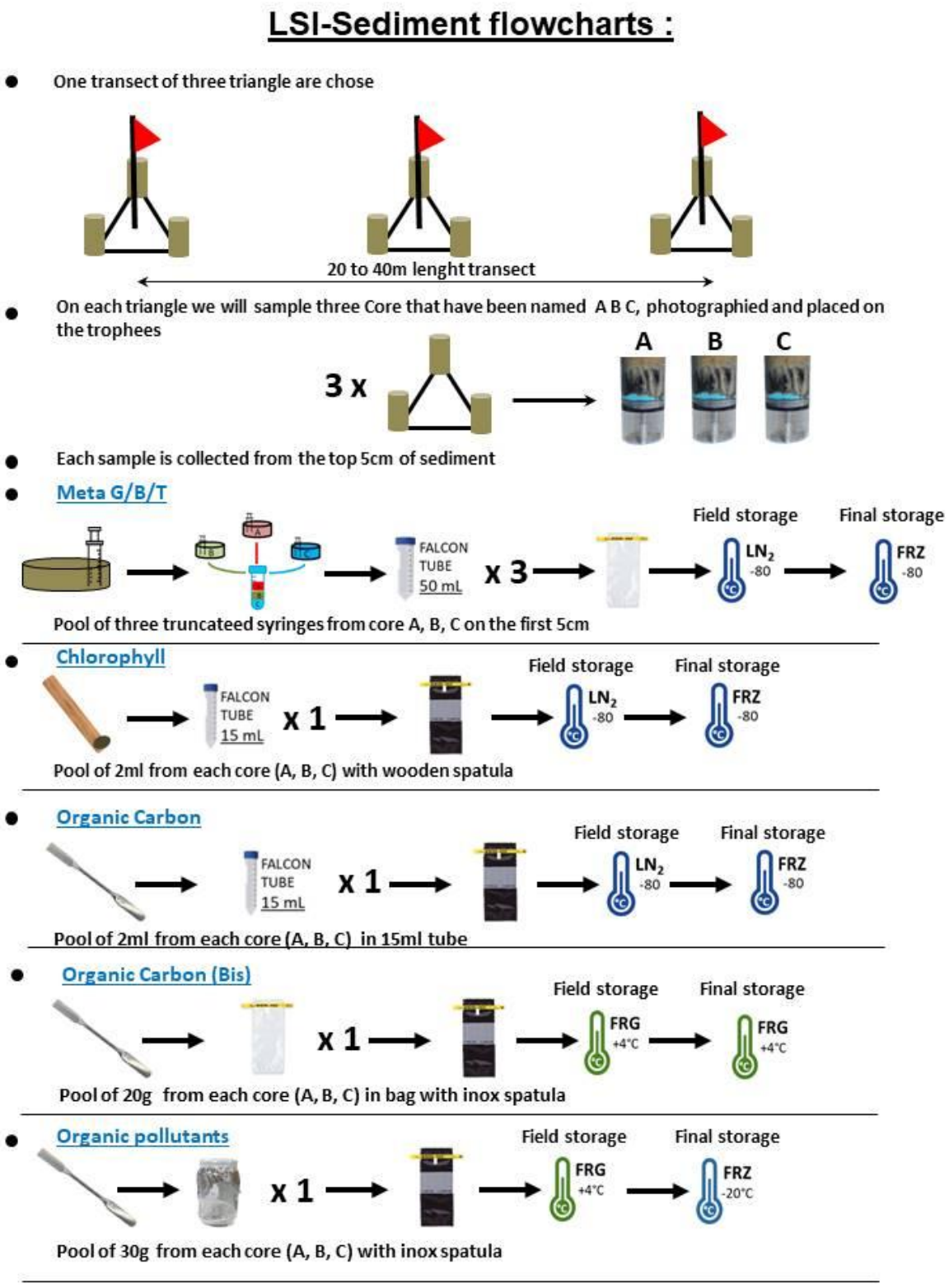
1.4.9. Flow charts



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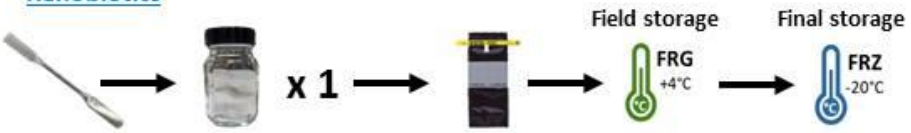
- **Xenobiotics**



Pool of 30g from each core (A, B, C) with inox spatula

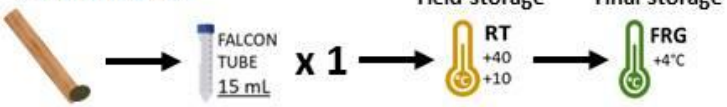
- **Metals**

Pool of 20g from each core (A, B, C) with wooden spatula

- **Microscopy / Meiofauna material**

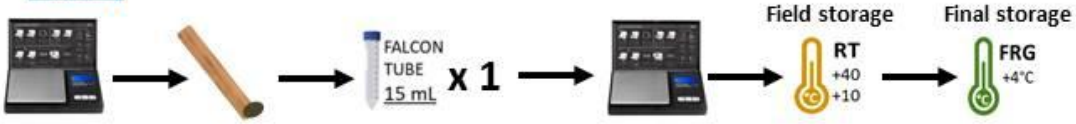
Pour 2 syringes from Core A in tube number 1, from Core B in tube number 2, from Core C in tube number 3

- **Granulometry**



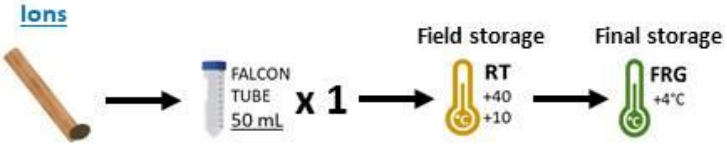
From Core A, collect 4ml with wooden spatula

- **Porosity**



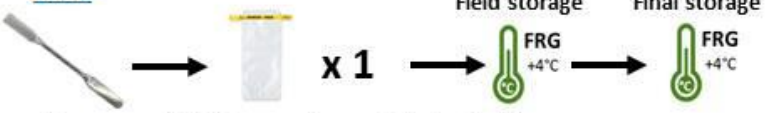
From Core A, collect 4ml with wooden spatula. Weight the tube before and after to add sediment

- **Ions**



From Core A, collect 20ml with wooden spatula

- **Extra**



Pour around 100g from each core into the first 5cm

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1.5 Sediment to unveil (sub)mesoscale processes shaping coastal marine biodiversity along land-sea gradients

1.5.1. Authors and affiliations

Castel Jade [Station Biologique, Roscoff (SBR), jade.castel@sb-roscoff.fr), Houbin Céline (SBR, houbin@sb-roscoff.fr), Thiébaut Eric (SBR, thiebaut@sb-roscoff.fr)], Paolo Fasciglione (SZN, paolo.fasciglione@szn.it), Daniela Zeppilli (Ifremer, daniela.zeppilli@ifremer.fr) Raffaele Siano (Ifremer, raffaele.siano@ifremer.fr)

1.5.2. Brief introduction to the protocols and analysis that will be carried out with the collected samples.

A seasonal sampling strategy is applied over 2 years (April 2023-January 2025) along land-to-sea transects in two contrasting systems, the Bay of Morlaix (Fig. 1) and the Gulf of Naples to disentangle the role of interacting local chemical and biological processes in shaping marine biodiversity. Sampling is conducted along each transect to measure environmental parameters, ecosystem processes and characterise macro and meiobenthic communities (see Macrobenthic & pelagic community analyses across the target LSGs in the section 2. Selected species and holobionts sampling and processing).

1.5.3. Sampling Strategy

Refer to section 1.2.3 for the sampling strategy.

1.5.4. Sampling tools and equipment

For Intertidal sediments (per station)

- 2 x corers 20 cm in diameter, 1 of which is treated with bleach 10% (metabarcode)
- 18 x 100µ polyethylene bags (40 x 60 cm) (morphology and metabarcoding macrofauna)
- 3 x 100µ polyethylene bags (20 x 30 cm) (sediment granulometry)
- 3 x 100µ polyethylene bags (10 x 15 cm) (sediment organic matter)
- 1 x round 1 mm sieving tank
- formalin solution at 3.5-4.5% of final concentration diluted with seawater, buffered with 0.1 g/ l of sodium tetraborate
- Ropes
- 1 x 30 l buckets

Note: Identification of the sample: each bag is prepared in advance with a label inside and noted with the indelible marker on the bag

For subtidal sediments (per station)

- 1 x Smith McIntyre grab

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- 2 x tills
- 1 x round 1 mm sieving tank
- 4 x 100µ polyethylene bags (40 x 60 cm) (morphology and metabarcoding macrofauna)
- 1 x 100µ polyethylene bags (20 x 30 cm) (sediment granulometry)
- 1 x 100µ polyethylene bags (10 x 15 cm) (sediment organic matter)
- Bleach
- formalin solution at 3.5-4.5% of final concentration diluted with seawater, buffer with 0.1 g/ l of sodium tetraborate
- Ropes
- 1 x 30 l buckets

Note: Identification of the sample: each bag is prepared in advance with a label inside and noted with the indelible marker on the bag

1.5.5. Metadata collection (log-sheets to guide the preparation of samples and to record metadata, if available)

For Roscoff, following metadata are noted in the file:

https://docs.google.com/spreadsheets/d/127JIfhlGHK8x5UeOeGVIcQq5lCtAk51n/edit?usp=drive_link&ouid=113218264493230036149&rtpof=true&sd=true

They include:

- Type of sample (morphology, metabarcoding)
- Sampling type (macrofauna, meiofauna)
- Sampling date
- Sampling location
- Tidal stage (intertidal, subtidal)
- Sampling device (Smith McIntyre grab, corer (20 cm))

1.5.6. Environmental parameters measurement

No environmental parameters are measured

1.5.7. Sampling protocol (step by step, detailed and simple)

Macrofauna morphology and metabarcoding subtidal sampling

Contact: Céline Houbin houbin@sb-roscoff.fr, Jade Castel (castel@sb-roscoff.fr) and Eric Thiébaut thiebaut@sb-roscoff.fr)

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Smith McIntyre grab | Round 1mm sieve | Bleach | Formalin 3 ,5% | Polyethylene bags

CAUTION

- This protocol is subject to contamination and uses toxic substances (Formalin is carcinogenic, mutagenic or toxic for reproduction)
- Personnel's protective equipment is mandatory (nitrile gloves, laboratory coat, full face mask with cartridge).

Step by step

All material for sampling metabarcoding must be bleached in a solution containing 500ml bleach 12.5% in 10 litres of water.

1. Clean all material (corer, sieve) with bleach for 20 minutes then rinse with seawater or fresh water.
2. For each site make 7 samples with the dredge (3 for morphology, 3 for metaB, 1 for granulometry and organic matter)
3. For morphology and metaB, sieve each sample separately in the round 1mm sieving tank.
4. For morphology, put the sieve residue in polyethylene bags and add formalin 3,5%. Store all bags in buckets in an appropriate room
5. For metaB, put the sieve residue in polyethylene bags. The samples processing should be done quickly
6. For granulometry and organic matter, put respectively 200g and 50g of raw sediment. Store samples at -20°C

Macrofauna morphology and metabarcoding intertidal sampling

Contact: Céline Houbin houbin@sb-roscoff.fr, Jade Castel (castel@sb-roscoff.fr) and Eric Thiébaut thiebaut@sb-roscoff.fr)

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20 cm diameter corer

round 1mm sieve

bleach

Formalin 3 ,5%

polyethylene bags

CAUTION

- This protocol is subject to contamination and uses toxic substances (Formalin is carcinogenic, mutagenic or toxic for reproduction)
- Personnel's protective equipment is mandatory (nitrile gloves, laboratory coat, full face mask with cartridge)

Step by step

The sampling protocol is adapted from the AQUAREF protocol (Garcia & al, 2014) and from Blanchet and Fouet in 2019.

All material for sampling metabarcoding must be bleached at least 20 minutes in a solution containing 500ml bleach 12.5% in 10 litres of water an. rinse with seawater or fresh water.

Prep for sampling:

Before collecting the first sample, a cotton bud is passed over the bench and sieve and placed in a 50mL falcon tube filled with DESS (see recipe at the end of the document) to produce a blank.

Sampling strategy:

For each station, about 2 to 3 m apart, 3 samples for the macrofauna morphology analysis and 3 samples for macrofauna metabarcoding are taken using a corer of 20 cm outside diameter (19.2 cm inside diameter) and 20 cm deep. For particle size analysis and organic matter measurements, for each replicate, 2 samples are taken with a small diameter corer (<5 cm) at 10 cm deep, in the immediate vicinity of each macrofauna sample. The monitoring points are accessed by sea in small boats.

For morphology:

1. Sieve gently under a light trickle of seawater onto a vacuum sieve with 1 mm circular mesh.
2. Place the sieve residue in 100µ polyethylene bags and fixed the same day in a formalin solution at 3.5-4.5% of final concentration, diluted with seawater, buffered with 0.1 g/ l of sodium tetraborate and homogenised.
3. Samples are kept until analysis in the laboratory in sealed and opaque containers with indelible labelling on the outside and inside (tracing paper, tape labeller, etc.) and placed in a ventilated room or provided for this use.

For metabarcoding:

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1. Sieve gently under a light trickle of seawater onto a vacuum sieve with 1 mm circular mesh.
2. Place the sieve recovered residue directly and the 3 samples for each sub-sampling are pooled for processing (see part 8.d).
3. Sediment samples (organic matter and particle size) are stored in the freezer (-20°C) before analysis

Macrofauna identification intertidal and subtidal sampling

Contact:Houbin Céline (SBR, houbin@sb-roscoff.fr), Paolo Fasciglione (SZN, paolo.fasciglione@szn.it)


1 mm sieve (circular mesh)


can of chemical waste


funnel


sorting bowl


Fine forceps


Pill boxes

Ethanol 70%


stereomicrosope


microscope

CAUTION

- This protocol is subject to contamination and uses toxic substances (Formalin is carcinogenic, mutagenic or toxic for reproduction)
- Personnel's protective equipment is mandatory (nitrile gloves, laboratory coat, full face mask with cartridge)

Step by step

1. Before handling, the samples are filtered over a can to remove formalin. They are then rinsed under running sea water for 24 hours to remove all traces of formalin.

   If long rinsing is not possible, the sorting should be carried out under laboratory hood.
2. The macrofauna is sorted manually. Organisms are identified at the lowest taxonomic level (species if possible) and counted.
3. filter samples over a can to remove formalin.
4. rinse samples under running seawater for 24h.
5. Sort manually the macrofauna.
6. put the macrofauna in pillboxes containing alcohol 70% by grouping it in large taxonomic groups (phylums, classes)
7. identify organisms at the lowest taxonomic level (species if possible) and count them.

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Sediment granulometry intertidal and subtidal

Contact:Houbin Céline (SBR, houbin@sb-roscoff.fr)




Bowl Oven Balance AFNOR sieves

List of sieves : 63µm, 80µm, 100µm, 125µm, 160µm, 200µm, 250µm, 315µm, 400µm, 500µm, 630µm, 800µm, 1mm, 1.25mm, 1.6mm, 2mm and 4mm

Step by step

1. Incubate the samples (once thawed) stay 48 hours in the oven at 60°C.
2. Weight the samples before being washed with fresh water on a 63µm mesh sieve.
3. Dry the samples again in the oven at 60°C for 48 hours, and are weighed again. This manipulation makes it possible to estimate the rate of pelites (particles of size less than 63µm) by the difference between the dry weight before washing and the dry weight after washing.
4. Sieve the sediments with an AFNOR sieve column comprising the following meshes: , 63µm, 80µm, 100µm, 125µm, 160µm, 200µm, 250µm, 315µm, 400µm, 500µm, 630µm, 800µm, 1mm, 1.25mm, 1.6mm, 2mm and 4mm. Particles less than 63 are collected at the bottom of the sieve column. For each particle size fraction the weighing is carried out with a precision balance of 0.01 g, the raw results are expressed as a weight percentage of the initial sediment sample.

Sediment organic matter intertidal and subtidal

Contact: Houbin Céline (SBR, houbin@sb-roscoff.fr)




Bowl Oven Oven Balance

Step by step

Measurements of organic matter content are carried out using the fire loss technique (Buchanan, 1984)

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1. Dry the sample 48 hours in the oven at 60°C
2. Weigh the sample
3. Burn sediment in a oven at 450°C during 4h
4. Weigh the sample
5. difference between the two measurements obtained (total dry weight – dry weight of residues) estimate the fraction of organic matter present in the sediment sample.

Intertidal meiofauna for diversity and abundance

Contacts: Daniella Zeppilli (Ifremer, Daniela.Zeppilli@ifremer.fr), Valentin Foulon (ENIB, valentin.foulon@enib.fr)

Sampling strategy:

Sample 3 core of sediment 3cm diameter/ 5cm depth for each "triangle" station (no mix cores).

Step by step

1. Sample one core of sediment (or two depending of the corer diameter)
2. Place each core in a pot containing 20mL of 6%MgCl2 solution for 10 minutes
3. The samples are fixed with 4% borax buffered formol (add 20mL)
4. Store at ambient temperature

1.5.8. Samples processing, transient storage, shipping conditions and Biobanking

Protocol code	Protocol name	Sample processing		Transient storage and T° at boat	Responsibles (PI) and biobanking
		sample	Chemical		
MACRO	Macrofauna morphology and metabarcoding subtidal sampling	Polyethylene bags	Formalin 3,5%	Room temperature or -20°C	Céline Houbin
MACRO	Macrofauna morphology and metabarcoding intertidal sampling	Polyethylene bags	Formalin 3,5%	Room temperature or -20°C	Céline Houbin
GRAN	Sediment granulometry intertidal and subtidal	?	?	?	Raffaele SIano
SOM	Sediment organic matter intertidal and subtidal	?	?	?	Raffaele Siano

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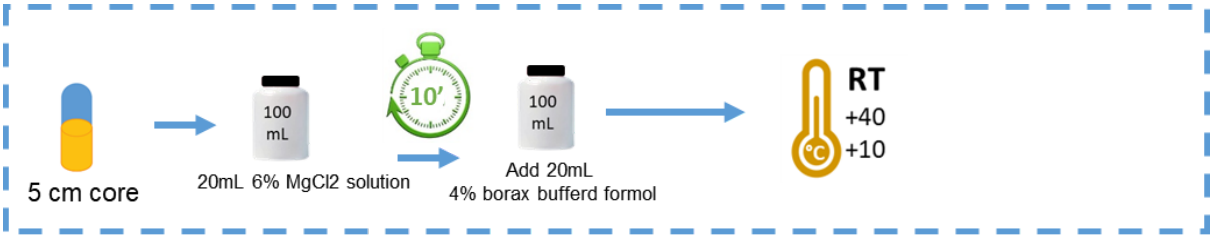
MEIO	Intertidal meiofauna for diversity and abundance	100 ml HDPE bottles	4% borax buffered formol	Room temperature	Daniella Zeppilli (Ifremer)

1.5.9. Flow charts (used to guide the processing of samples during stations)

Intertidal meiofauna for diversity and abundance



1.6. Paleocores

1.6.1. Authors, affiliations and contact

Raffaele Siano (IFREMER, Raffaele.Siano@ifremer.fr), Thomas Haize (EMBL, Thomas.haize@emb.de)

1.6.2. Introduction

Since the 19th century, Europe has been impacted by human development and industrialisation. The study of paleocores will combine a multiproxy paleo-ecological analysis of sediment cores of about metre length, to try to assess biodiversity changes in relation to man activities over the last 150-200 years. We are interested in different kinds of impacts from pre-industrial to modern times such as the presence of city and/or harbours, agriculture, aquaculture, industry and the impacts of the second World War in order to have a broad appreciation of human impact across Europe.

1.6.3. Sampling strategy

The sampling is done with sediment corers that allow to collect sediment cores of at least 80 cm. Five cores are collected in each sampling site, at one single station. Four core are sliced and used to study different parameters including, physical parameters, genomics and pollutants. sampling site are chosen precisely according to:

Sediment typology :

Sampling sites should have muddy unsuspended (no-dredged) sediments.

Ecosystem selection :

The sampling area should be close to human activities such as harbours, cities, bays and estuaries

History of the site :

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The sampling site should be the object of long-time researches and have an historical human perspectives, in order to link historical events to biological shifts

During 2023-2024, the paleocore study will cover between 14 and 16 sampling sites in 10 European countries.

Sites :

2023

- Roscoff (F)
- Lorient (F)
- Rade de Brest (F)
- Baie des Veys (F)
- Sète (F)
- La Tremblade (F)
- Bilbao (E)
- Sopot (Pl)

2024

- Rostock (D)
- Barcelone (E)
- Naples (It)
- Athens (Gr)
- Turku (Fin)
- Tallinn (Est)
- Stenungsund(S)
- Cork (Ir)

16 sites in 10 countries

In 2023, the sampling focused on France, Spain and Poland. In 2024, 8 countries will follow in the Mediterranean, North and Baltic Seas.

1.6.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• Sampling corer allowing the collection of 80-100 cm cores of 9 cm in diameter (different corers devices can be used) cazstoppers and tape - amount depends on number of cores • 1 whiteboard marker • 2 core racks • 1 safety helmet (supplied by the boat) • safety shoes • lifejacket • 4 core supports	• 400 rings • 400 blades • 100 2ml cryotubes • 100 falcon cryotubes 50mL • 250 7x5cm ziplock bags • 300 4ml tubes • 100 9x13cm ziplock bags • 60 100ml bottles • 100 100ml burned vial with aluminium foil and plastic cap • 400 plastic spatulas • rolls of paper towels	• MilliQ grade water (cleaning) • Liquid Nitrogen (samples storage) • DNA away (cleaning) • Ethanol 90% (cleaning) • MgCl 1M (microscopy) • PFA 4% (microscopy)

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• 1 table • 1 liquid nitrogen tank • 2 freezers • 1 cooler • 1 precision scale • 2 dispensers • 1 water pump • 100 inox spatulas (sterile)	• rolls of aluminium foil	

1.6.5. Metadata collection

- Location and time of sampling
- All historical information about human activities at the site

1.6.6. Environmental parameters measurement

There is no environmental parameters measurement data for the paleocores project

1.6.7. Sampling protocol

Site preparation

The location of the sampling site is chosen in advance according to the historical human impact on the sampling area and the existing geological information.

Post sampling laboratory preparation for core slicing

Thi “clean” room of about 20 m2 must have no potential biological or chemical contamination, limited air circulation (no window) and allow the operation of about 10 people. An electricity support and water access are needed

Put a tarpaulin on the floor

1. Set up the tables and workstations
2. Assemble the core supports
3. Put consumables on each workstation.

Core sampling

Once the sampling equipment has been installed on the boat, the boat sets off for the sampling site and the team puts on the safety equipment needed for the sampling to run smoothly.

1. Attach the corer to the winch for deployment.
2. Arm the corer
3. Deploy the corer

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Once the core is back on board:

4. Measure and take picture of the core
5. Remove the core from the corer
6. Add quickly a first polystyrene plug, then a second plastic plug and tape at the bottom to ensure a watertight seal
7. Remove water on top of the core with water pump
8. Add a plastic plug and tape on the top of the core
9. Name the core and place it in the rack

Repeat all steps until there are enough cores deemed of good quality.

1.6.8. Samples processing, transient storage, shipping conditions and Biobanking

After sampling on field is completed, cores must be slided and preserved for prospective various laboratory analyses. This step requires 4 teams of 2 to 3 people each (8-12 people in total) . The cores are opened one by one :

1. Put on sterile gloves
2. Remove the stopper at the top
3. Remove the water

<!> DO NOT touch the sediment layer

4. Put the core on the top of the support
5. Remove the tape and the bottom plug with 3 hammer blows

CAUTION – This is the most dangerous step, take care to not lose material and extract the sediment

6. Push the core on the support with the polystyrene foam
7. Slice each core following the protocols below

Core A

The core A is used to analyse :

- Granulometry
- Dating
- Metagenomic
- Metabarcoding (grouped under the name of DNA

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Cutting diagram for core A

1. Prepare the working area
   a. clean the table with ?
   b. Put on new sterile gloves.
2. Install all the consumables you need for the slice
3. Install one aluminium foil for each slice
4. Slice the core
   a. Take one sterile 1 cm ring and one sterile blade
   b. Remove the cap at the top of the core
   c. Put the ring at the top of the core
   d. Push the core until 1cm

<!> DO NOT Push to much

   e. Insert the blade between the core and the ring
   f. Slice the core and put the slice on the working area
   g. Distribute by procedure in the following order

A.1: Processing for granulometry analysis

Contact : *Ifremer, Axel EHRHOLD (axel.ehrhold@ifremer.fr) and Angelique Roubi (angelique.roubi@ifremer.fr)*

With new plastic spatula, put 2-3 mL in the corresponding cryo tube

1. Add the label
2. Put the cryo tube in the rack at room temperature

A.2: Processing for dating analysis

Contact : *CNRS,* Sabine Schmidt (sabine.schmidt@u-bordeaux.fr)

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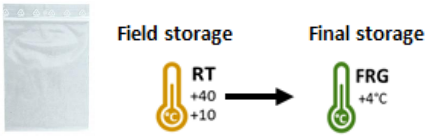
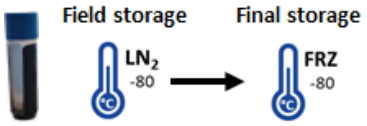


1. With the same spatula used for for the granulometry samples, add 15 to 25g in the ziplock bag
2. Remove air from the bag and close it
3. Weight the bag and write the weight on the log sheet
4. Add the label
5. Put the bag in the rack at room temperature

A.3: Processing for metaGenomic analysis

Contact : *University of Copenhagen, Antonio Fernandez Guerra (antonio.fernandez-guerra@sund.ku.dk)*



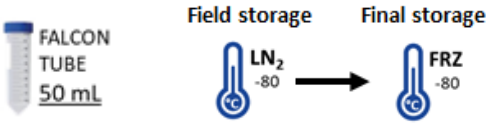
CAUTION – This protocol is sensitive to DNA degradation and Contamination

<!> DO NOT touch or breathe just in front of the samples

1. When the previous parameter are ended, return the half sterile petri-dish with sediment in
2. Pre-weight the tube and verify the right barcode
3. With new sterile plastic spatula, add at maximum 200mg of sediment in the tube
4. Weight the tube to be sure and make sure there is not more than 200mg therein.
5. Write the weight on the log sheet
6. Add the label
7. Flash-freeze the tube in the liquid nitrogen and put it in the rack, in the freezer (-20°C) until storage laboratory where samples should be stocked in -80°C Freezer

A.4: Processing for MetaBarcoding analysis

Contact : *Institut of oceanology PAN, Joanna Pawlowska (pawlowska@iopan.pl)*



CAUTION – This protocol is sensitive to DNA degradation and Contamination

<!> DO NOT touch or breathe just in front of the samples

1. With new sterile plastic spatula, add all the leftover sediment of that slice in the tube (between 20 to 40ml)

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2. Add the label
3. Flash-freeze the tube in the liquid nitrogen and put it in the rack, in the freezer (-20°c), until storage laboratory where samples should be stocked in -80°C Freezer

Between each slice :

1. Clean the working area with DNA-away
2. Change the aluminium foil
3. Prepare the next set of consumables
4. Change gloves if needed
5. Cut the next slice and repeat steps A.1 to A.4 until end of the core

Core B

The core B are used to analyse :

- Organic carbon
- Resurrection ecology
- Resting stage and pollens
- Dating
- Porosity

Cutting diagram for core B

1. Prepare the working area
   a. clean the table
   b. Put on new sterile gloves.
2. Install all the consumables needed for the slice
3. Install one aluminium foil for each slice
4. Slice the core
   a. Take one sterile ring and one sterile blade
   b. Remove the cap at the top of the core
   c. Put the ring at the top of the core
   d. Push the core until 1cm

<!> DO NOT Push to much

   e. Insert the blade between the core and the ring

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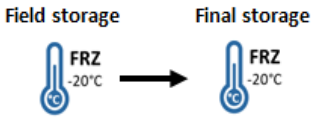
f. Slice the core and put the slice on the working area
g. Distribute by procedure in the following order

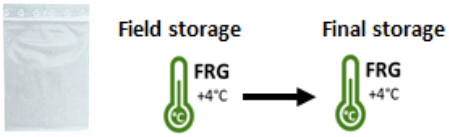
B.1: Processing for organic carbon analysis



Contact : *Ifremer, Florian CARADEC (Florian.Caradec@ifremer.fr)*

1. With new sterile wooden spatula, add 3mL of the sediment into the tube
2. Add the label
3. Put the tube into the freezer (-20°C)

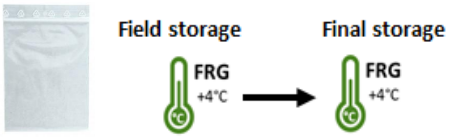
B.2: Processing for resurrection ecology analysis



Contact : *Turku University, Conny Sjoqvist (conny.sjoqvist@abo.fi)*

1. With same wooden spatula used for organic carbon, add around 40% of the slice into the ziplock bag (between 20 to 30gr)
2. Remove the air from the bag and close it
3. Add the label
4. Wrap the bag in aluminium foil,
5. Put the bag into a cooler (°4C)

B.3: Processing for analysis of resting stages and pollens



Contact : *Ifremer , Kenneth Mertens (kenneth.mertens@ifremer.fr)*

1. With same wooden spatula used for resurrection ecology, add around 40% of the slice into the bag (between 20 to 30gr)
2. Remove the air from the bag and close it
3. Add the label
4. Wrap the bag in aluminium foil,
5. Put the bag into into a cooler (°4C) freezer

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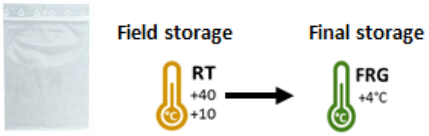
B.4: Processing for dating analysis



***<!> Sampling rates :* 0, 5, 10 cm and then every 10 cm**

Contact : *CNRS, Sabine Schmidt* (sabine.schmidt@u-bordeaux.fr)

1. With the same wooden spatula used for resting stages and pollens, add 15 to 25g in the ziplock bag
2. Remove air from the bag and close it
3. Weight the bag and write the weight on the log sheet
4. Add the label
5. Put the bag in the rack at room temperature

B.5: Processing for porosity analysis

Contact : *Ifremer, Florian CARADEC (Florian.Caradec@ifremer.fr)*

1. With the same wooden spatula used for dating, add 3mL of the sediment into the tube
2. Add the label
3. Put the tube into the rack at room temperature

Between each slice :

1. Clean the working area with Dna-Away
2. Change the aluminium foil
3. Prepare the next consumable
4. Change of glove if needed
5. Cut the next slice and repeat until end of the core

Core C

The core C are used for the following analyses :

- Morphological identification
- Dating

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Cutting diagram for core C

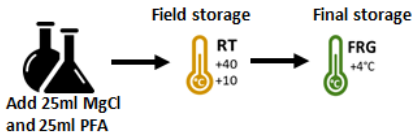
Prepare the working area

1. Clean the table with DNA-away
2. Put on new sterile gloves.
3. Install all the consumables you need for the slice
4. Install one aluminium foil for each slice
5. Slice the core
6. Take one sterile ring and one sterile blade
7. Remove the cap at the top of the core
8. Put the ring at the top of the core
9. Push the core until 1cm

<!> DO NOT Push to much

10. Insert the blade between the core and the ring
11. Slice the core and put the slice on the working area
12. Distribute by procedure in the following order

C.1: Processing for Morphological analyses



Contact : *Ifremer, Valentin Foulon (valentin.foulon@enib.fr)* and *Daniela Zeppilli (Daniela.Zeppilli@ifremer.fr)*

<!> Sampling rates : All first 20cm and them each 2 cm (even number)

1. Add 25mL of MgCl in the bottle
2. Add all the slice in the bottle
3. With wooden spatula, add all the slice into the tube
4. Stir gently the tube
5. Add 20mL of PFA (under hood with personnel safety equipment)
6. Put the bottle in box in ventilated area

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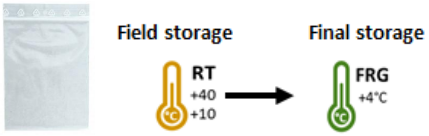
C.2: Processing for dating analyses



Contact : *CNRS, Sabine Schmidt (*sabine.schmidt@u-bordeaux.fr*)*

***<!> Sampling rates :* 0, 5, 10 cm and then every 10 cm**

1. With the same wooden spatula used for morphological parameter, add 15 to 25g in the ziplock bag
2. Remove air from the bag and close it
3. Weight the bag and write the weight on the log sheet
4. Add the label
5. Put the bag in the rack at room temperature

Between each slice **:**

6. Clean the working area with DNA-away
7. Change the aluminium foil
8. Prepare the next consumable
9. Change of gloves if needed
10. Cut the next slice and repeat previous procedures until end of the core

Core D

The core D are used to analyse :

- Pollutants
- Dating

Cutting diagram for core D

Prepare the working area

1. Clean the table with DNA-away
2. Put on new sterile gloves.
3. Install all the consumables you need for the slice

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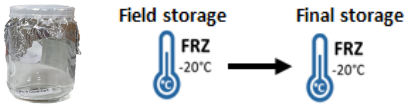
4. Install one aluminium foil for each slice
5. Slice the core
6. Take one sterile ring and one sterile blade
7. Remove the cap at the top of the core
8. Put the ring at the top of the core
9. Push the core until 1cm

<!> DO NOT Push to much

10. Insert the blade between the core and the ring
11. Slice the core and put the slice on the working area
12. Distribute by procedures in the following order

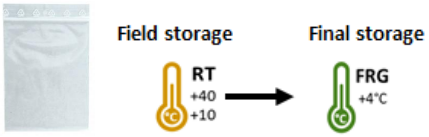
D.1 : Processing for pollutants measurements



Contact : *Ifremer, Nicolas Briant (Nicolas.Briant@ifremer.fr)* and *Javier Jimenez (Javier.Castro.Jimenez@ifremer.fr)*

1. Cut and remove the side of the slice with inox spatula
2. Remove the cap and carefully remove the aluminium foil without breaking it
3. Put on back the aluminium foil on the working area
4. Add 30-40g in the glass vial
5. Put back the aluminium foil on ? and carefully close the cap
6. Add the corresponding label
7. Put the glass vial in the freezer

D.2 : Processing for dating analysis



Contact : *CNRS, Sabine Schmidt* (sabine.schmidt@u-bordeaux.fr)

***<!> Sampling rates :* 0, 5, 10 cm and then every 10 cm**

1. With the same spatula used for pollutants, add 15 to 25g in the ziplock bag
2. Remove air from the bag and close it
3. Weight the bag and write the weight on the log sheet
4. Add the label
5. Put the bag in the rack at room temperature

Between each slice :

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6. Clean the working area with and change the aluminium foil
7. Prepare the next consumable
8. Change gloves if needed
9. Cut the next slice and repeat until end of the core

Core E

The core E are used to analyse :

- X-ray
- XRF

This core is kept whole until it reaches the analysis laboratory, where it is cut in half lengthways for analysis.

E.1 : Processing for X-ray analyses

Contact : *Ifremer, Axel EHRHOLD (axel.ehrhold@ifremer.fr) and Angelique Roubi (angelique.roubi@ifremer.fr)*

E.2 : Processing for XRF analyses

Contact : *Ifremer, Axel EHRHOLD (axel.ehrhold@ifremer.fr) and Angelique Roubi (angelique.roubi@ifremer.fr)*

Cleaning Procedure

All material is firstly rinsed with tap water to remove the sediment, then :

1. The rings are washed in the dishwasher and wiped with RNA free
2. The blades are washed by hand, dried and wiped with RNA free
3. The one meter cores are washed, bleached before and put in big trash bag
4. Inox spatulas are wraped in aluminium foil and burned at 300°c
5. Organic pollutant vials are burned before to be used with aluminium foil on top at 300°c.

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Analysis	Field storage	Shipping storage	Long-term storage	Analysis institut	Biobanking
Metagenomics analyses	Flash-Freeze Freezer (-20°C)	Dry ice	-80°C	University of Copenhagen	Ifremer
Metabarcoding analyses	Flash-Freeze Freezer (-20°C)	Dry ice	-80°C	Institute of oceanology PAN	Ifremer
Dating	Room temperature	Room temperature	4°C	CNRS	Ifremer
Granulometry	Room temperature	Room temperature	4°C	Ifremer	Ifremer
Organic carbon	Freezer (-20°C)	-20°C	-20°C in the dark	Ifremer, Brest	Ifremer
Resurrection ecology	4°C	4°C	4°C, in the dark	Turku University	Ifremer
Resting stages and pollens	4°C	4°C	4°C, in the dark	Ifremer, Concarneau	Ifremer
Porosity	4°C	4°C	4°C	Ifremer, Brest	Ifremer
Morphological analyses	Room temperature	4°C	4°C	Ifremer, Brest	Ifremer
Pollutants	Freezer (-20°C)	-20°C	-20°C	Ifremer, Nantes	Ifremer

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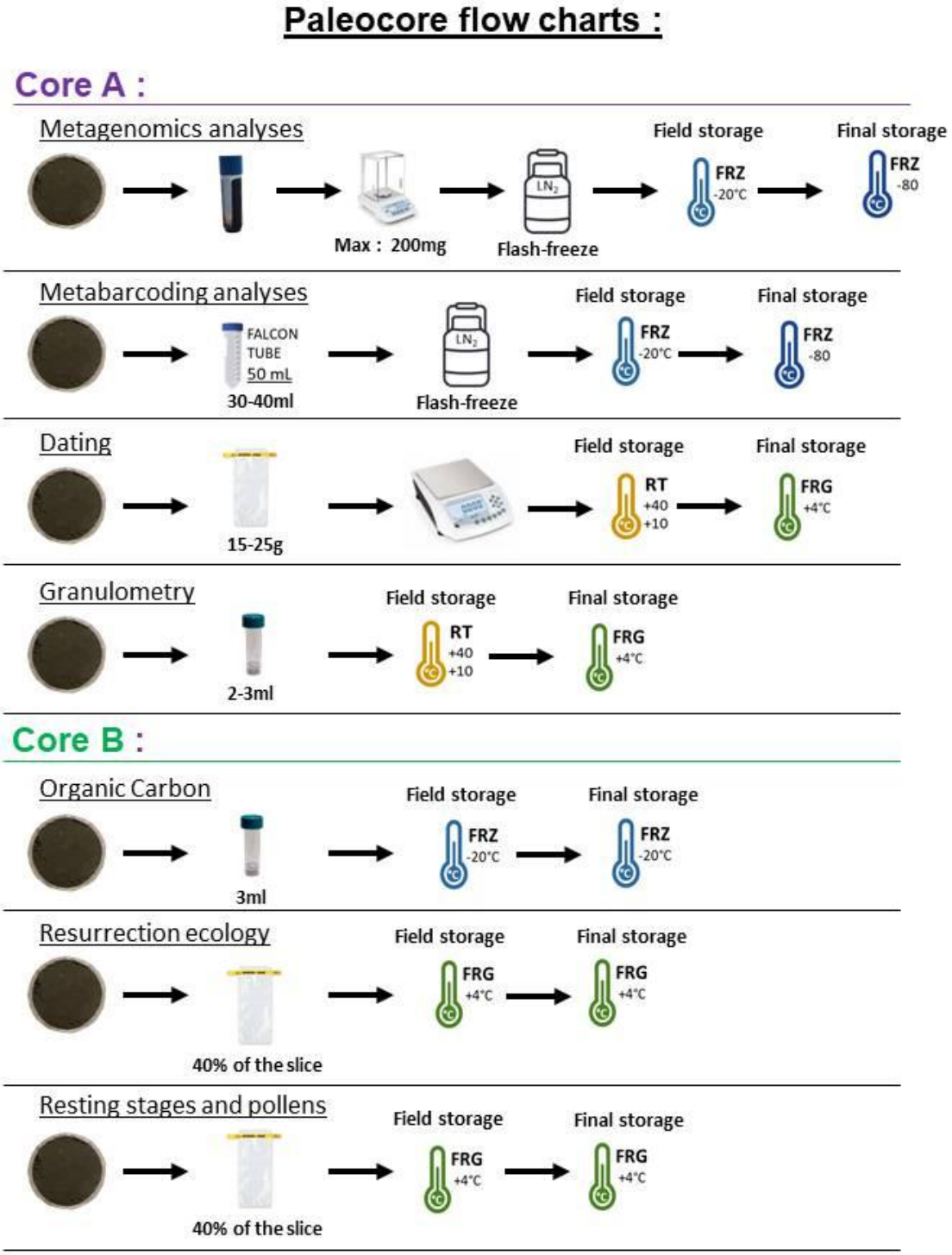
1.6.9. Flow charts



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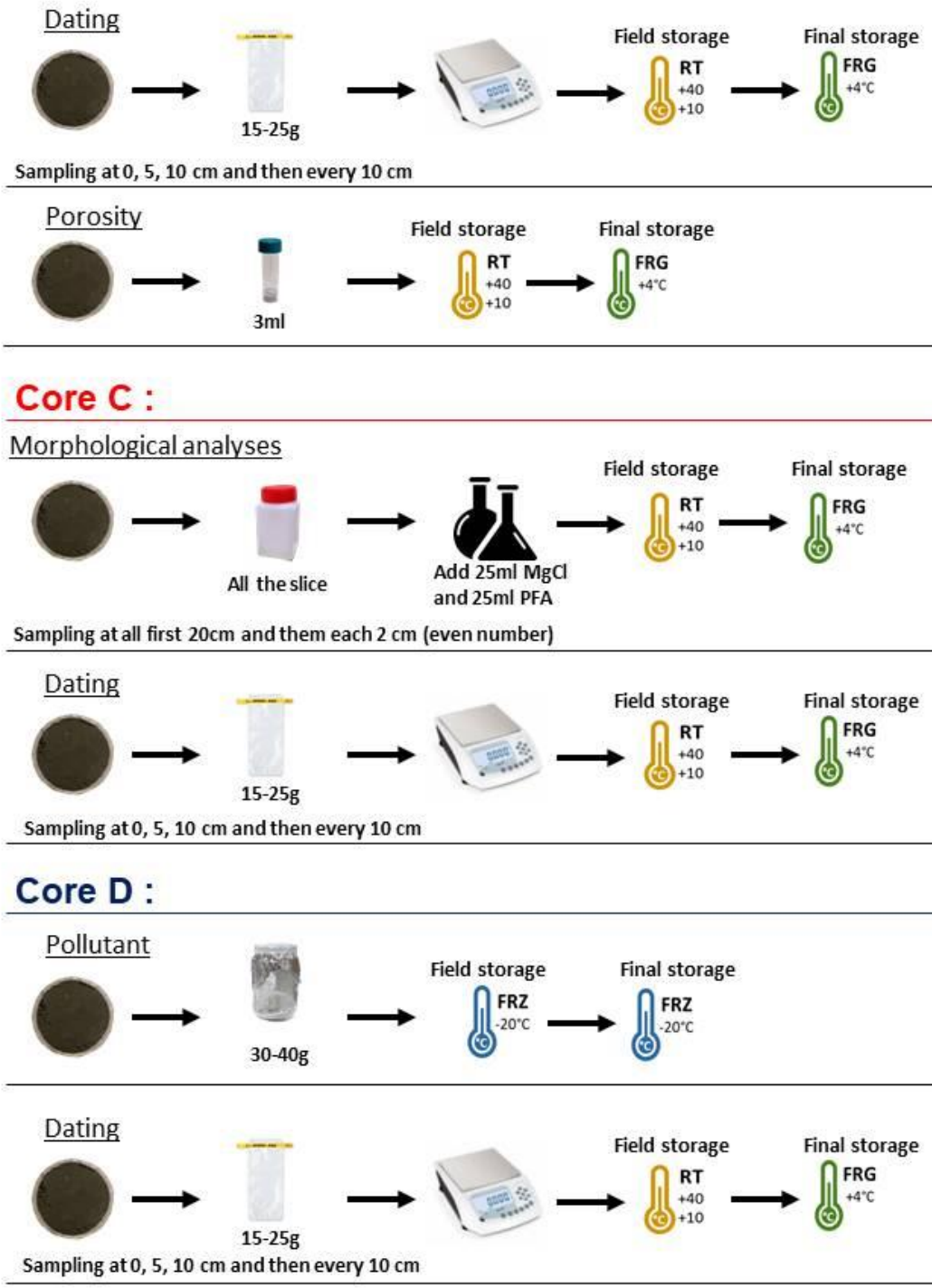

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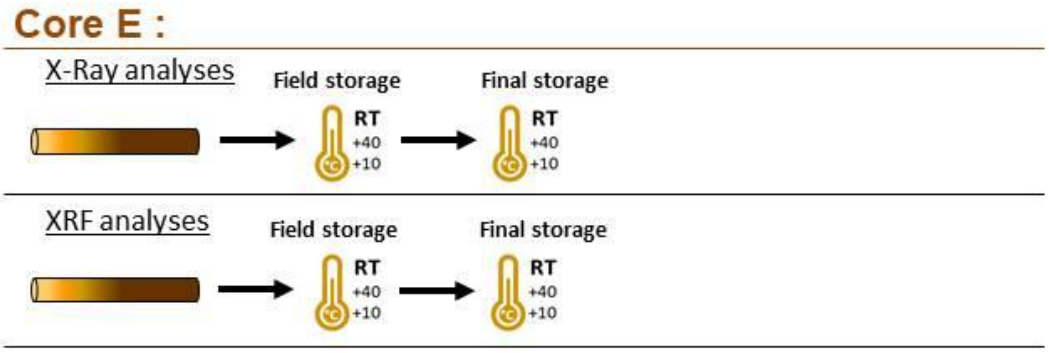

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2. Selected species and holobionts sampling and processing

2.1. Intertidal annelids

2.1.1. Authors, affiliations and contact

Rabea Klingberg (UGOE, rabea.klingberg@biologie.uni-goettingen.de), Christoph Bleidorn (UGOE, christoph.bleidorn@biologie.uni-goettingen.de), Conrad Helm (UGOE, conrad.helm@biologie.uni-goettingen.de)

2.1.2. Introduction

To unravel the taxonomic distribution patterns of the annelid macrofauna across the European coast, animals were sampled along defined transects covering the upper,- middle-, and lower- intertidal zones at sandy beaches and rocky shores. A subset of commonly found annelid focus taxa (seven at sandy beaches and four at rocky shores) will subsequently be analysed morphologically and genetically by barcoding and WGS.

2.1.3. Sampling Strategy

Different sampling strategies are used for sandy beaches, where marine worms are usually buried in the sand, and rocky shores, where animals are usually found between rocks, between algae covering the rocks or within rock pools.

Sampling along a transect, collecting sediment samples (sand)

Sandy beaches: upper zone: three plots, middle zone: five plots, lower zone: five plots, variations are possible depending on the habitat and the tidal range; one plot = 50 cm x 50 cm, 30 cm deep.

Sampling along a transect, collecting habitat samples (algae growth)

Rocky shores: one plot per zone (tide pool), collection amount of algae growth: 1/3 of a Kautex wide neck container (1 L) per zone (Fig. 2.2 -6c).

2.1.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• Spade • Bucket • Sampling quadrat for plot outline (50 cm x 50 cm) • Analyser sieve ø 400mm, mesh size: 1,7 mm	• Eppendorf DNA LoBind® Tubes 1.5mL • FalconTM Conical centrifuge Tubes 15mL • FalconTM Conical centrifuge Tubes 50mL	• DNA/RNA Shield • Ethanol (99%) • Magnesium chloride (7%)

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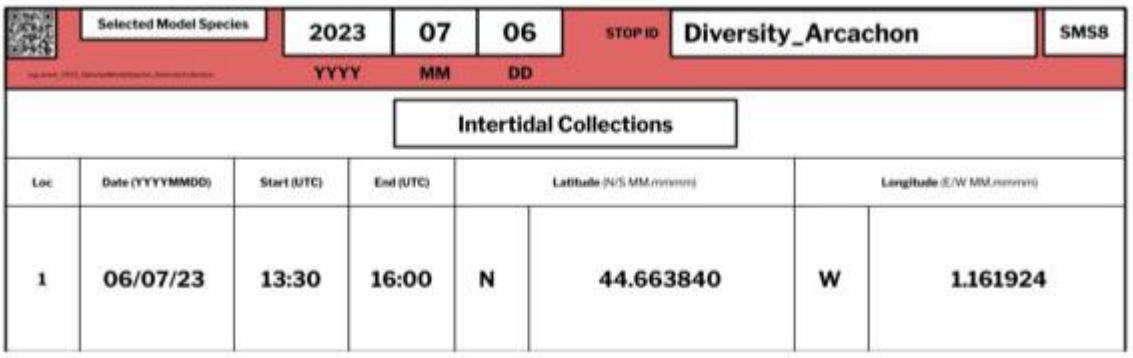
• Scraper • Kautex wide neck container (3 x 1 liter) • Sorting trays, white x 3 • Dissection scalpel • Spring-steel tweezers (blunt and pointed) • Tweezer fine needle-sharp (low carbon stainless steel)	• Nitrile gloves • Plastic pasteur pipettes 3.0mL • Cryoboxes 136 x 136 mm • Barcode strips	

2.1.5. Metadata collection

Following metadata are noted on log sheet ID (Fig. 2.2 -1):

- Name of the collection site
- Location ID
- Collection date
- Duration of collection
- GPS data from collection site



Selected Model Species	2023	07	06	STOP ID	Diversity_Arcachon	SMS8
	YYYY	MM	DD			

Intertidal Collections

Loc	Date (YYYYMMDD)	Start (UTC)	End (UTC)	Latitude (N/S MM.mmmm)		Longitude (E/W MM.mmmm)	
1	06/07/23	13:30	16:00	N	44.663840	W	1.161924

Figure 2.2 -1 Example of a Diversity sampling log sheet with metadata

Following metadata are noted on log sheet ID (Fig.2.2 -2):

- Name of the collection site
- Collection date
- Habitat type (sandy beach or rocky shore)
- Time of low tide and tidal range
- Overview of sampled taxa per zone

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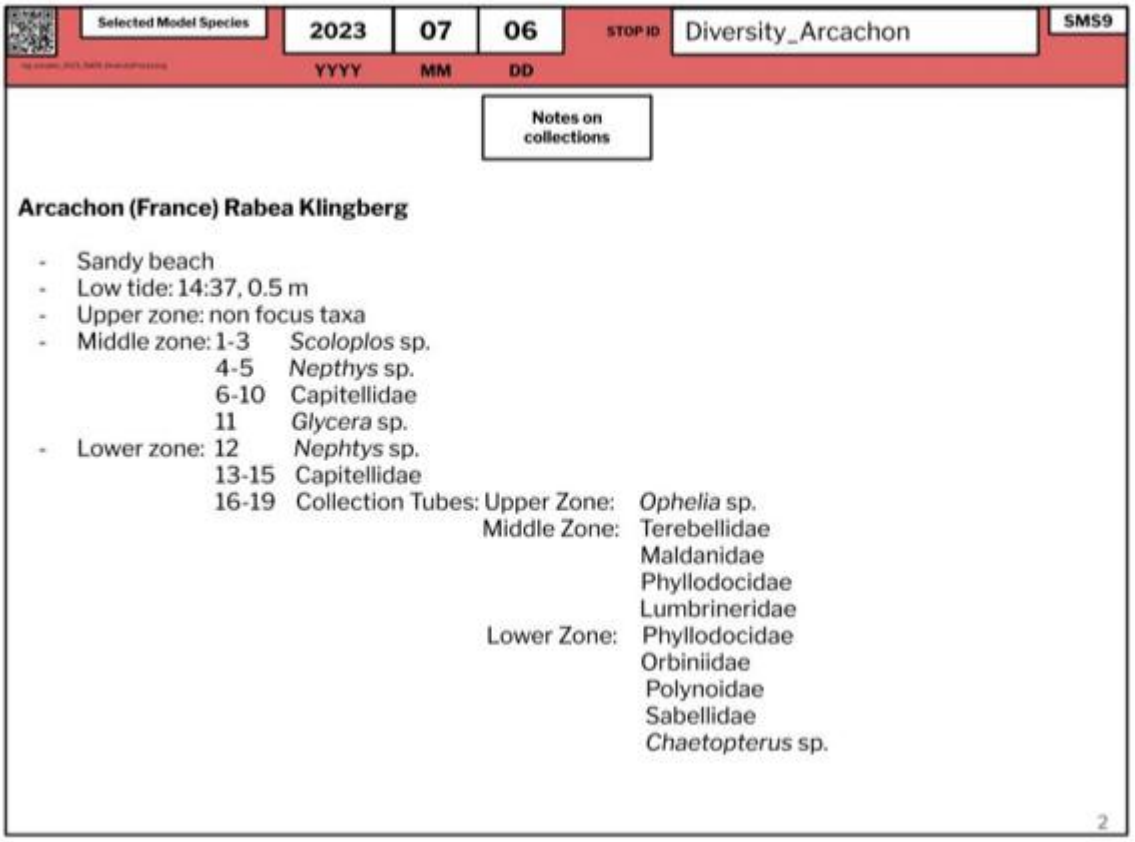

Figure 2.2 -2 Example of a Diversity-processing log sheet

2.1.6. Environmental parameters measurement

Tidal dates and tidal range are taken from tide charts which are online available (https://tides4fishing.com/).

2.1.7. Sampling protocol

Starting around 1.5 hours before low tide, the chosen habitat will be categorised visually according to the three different intertidal zones (upper, middle, lower) on the basis of specific bioindicators (rocky shores) or beach morphology (sandy beaches).

- sandy beaches: changes in sediment structure indicate the transition of the different intertidal zones (Fig. 2.2 -3).

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Figure 2.2 -3 Transition from upper zone to middle zone (arrow),

Upper zone with algae, Middle zone with different ribbed sediment structure, transition from middle to lower zone is determined by the individual habitat (beach) (Rabea Klingberg, in Aarhus, Denmark)

- rocky shores: changes in transition and colonisation of organisms, indicate the different intertidal zones, for example barnacles and limpets in the high-tight zone, hermit crabs, mussels and anemones in the mid-tight zone and benthic invertebrates in the low-tide zone (Fig. 2.2 -4).

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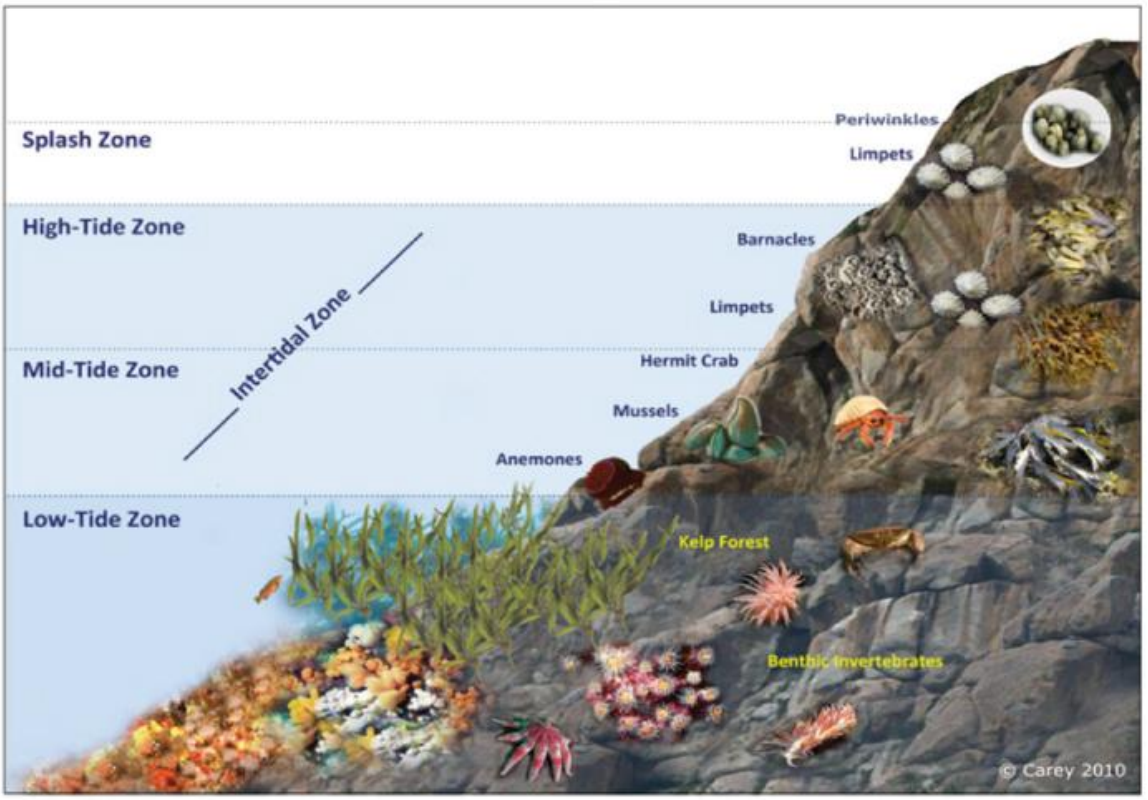

Figure 2.2 -4: Zonation of rocky shores with specific organisms in each zone
(Copyright - Claire Carey 2010)

Sampling on sandy beaches

Sampling starts from the high-water line along the transect down to the low-water line. First place the quadrant and start digging the first plot (Fig. 2.2 -5 and -6A).

- upper zone: three plots
- middle zone: five plots
- lower zone: five plots
- variations are possible depending on the habitat and the tidal range but will be noted on log sheets
- one plot = 50 cm x 50 cm, 30 cm deep

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Figure 2.2 -5: Working on a transect along the lower intertidal zone (Rabea Klingberg- sampling in Ferrol, Spain)

The sediment of each transect will be searched for annelids by hand and tweezers. Additionally, the sediment of one transect per zone will be sieved (Fig.2.2 -6B). All annelids from one zone will be stored in a labelled Kautex wide neck container (1L) (Fig.2.2 - 6C). Fill with a bit of water, so that the ground is covered, otherwise annelids will suffer oxygen starvation. Do not close the lid completely.

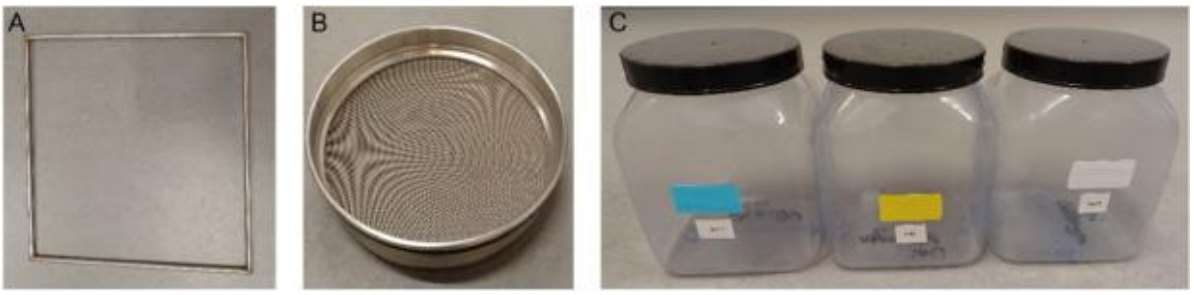

Figure 2.2 -6 :Sampling material. A: Quadrat 50 cm x 50 cm, B: Analyser sieve, mesh size 1,7 mm, C: Kautex wide neck container, each labelled for one sampling zone (Rabea Klingberg)

Sampling on rocky shores:

Sampling starts from the high-water line along the transect down to the low-water line. First chose a suitable tide pool (with enough algae growth and easy to reach) (Fig. 2.2 -7). Select one tide pool per zone, they do not have to be the same size. Use a scraper and carefully scrape off the algae (on European coasts this will be mostly *Corallina officinalis*). Store them

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in a Kautex wide neck container (1L). Fill the container up to 1/3 with algae holdfast, use almost no water to cover the sampled algae and do not close the lid completely.

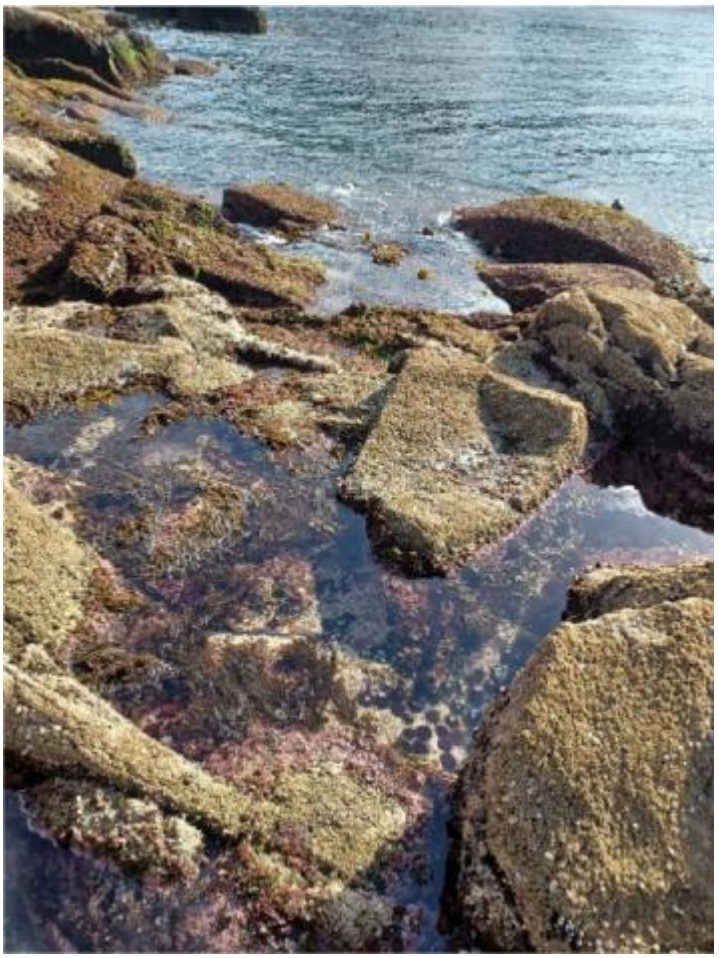

Figure 2.2 -7 Example of a tide pool with algae growth
(Rabea Klingberg - rocky shores in Ferrol, Spain)

2.1.8. Samples processing, transient storage, shipping conditions and Biobanking

Lab work for sandy beach samples:

Identification

Place the annelids from each zone in a respectively-labelled sorting tray. Sort out the annelids, place them in petri dishes, and clean them from sediment with a brush. For identification of the focus taxa use the binocular microscope.

Common focus taxa sandy beaches:

- *Scoloplos* sp.
- *Nephtys* sp.
- *Arenicola* sp.
- *Lanice* sp.
- Nereididae
- Capitellidae

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Fixation:

Up to 14 individuals of each focus taxon should be selected and rinsed with clean seawater

1. Put sample 1-2 into a 1.5 mL low-binding tube filled with 80% EtOH
2. Put sample 3-14 into a 1.5 mL low-binding tube filled with DNA/RNA Shield
3. All non-focus taxa of each zone will be collected and fixed with 80% EtOH in a 15 mL Falcon tube (Fig. 2.2 -9)

If the worms are to big, cut off the head or the tail and fix them, discard the rest of the worm. Label and barcode all tubes and place them into labelled cryoboxes. The barcodes come in matched pairs, one for the tube one for the log sheet (Fig. 2.2 -8).

Selected Model Species	2023	07	06	STOP ID	Diversity_Arcachon	SMS9
	YYYY	MM	DD			

#		ZONE	HEAD BARCODE	BODY BARCODE	WHOLE BODY BARCODE	Storage buffer	Storage box identifier
1	*Scoloplos* sp.	LOW ☐ MID ■ HIGH ☐			x	D/R Shield ☐ 80% EtOH ■	
2	*Scoloplos* sp.	LOW ☐ MID ■ HIGH ☐			x	D/R Shield ☐ 80% EtOH ■	
3	*Scoloplos* sp.	LOW ☐ MID ■ HIGH ☐			x	D/R Shield ■ 80% EtOH ☐	
4	*Nephtys* sp.	LOW ☐ MID ■ HIGH ☐	x			D/R Shield ☐ 80% EtOH ■	
5	*Nephtys* sp.	LOW ☐ MID ■ HIGH ☐	x			D/R Shield ☐ 80% EtOH ■	
6	Capitellidae	LOW ☐ MID ■ HIGH ☐			x	D/R Shield ☐ 80% EtOH ■	3

Figure 2.2 -8 Example of a log sheet, note: the x will be replaced by barcodes

Storage:

Samples are stored in the field at room temperature. Upon return to the institutes, the samples in DNA/RNA shield will be stored in -21°C (Fig. 2.2 -9).

Lab work for rocky shore samples:

Identification

Place the annelids from each zone in a respectively-labelled sorting tray. Cover the trey and wait until the annelids crawl out of the algae or place the algae growth in a beaker filled half with 7% MgCl2 and half with seawater. Wait for 10 minutes, shake out the algae in the beaker and pour the water through a sieve (100 µm). Sort out the annelids, rinse them with

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seawater, place them in petri dishes and clean them from sediment with a brush. For the identification of the focus taxa use the binocular microscope.

Focus taxa rocky shores:

- *Eulalia* sp.
- Nereididae
- *Fabricia* sp.
- Syllidae

Fixation:

Same as the fixation of the sandy beach samples (Fig. 2.2 -9)

Storage:

Same as the storage of the sandy beach samples (Fig. 2.2 -9)

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2.1.9. Flow charts

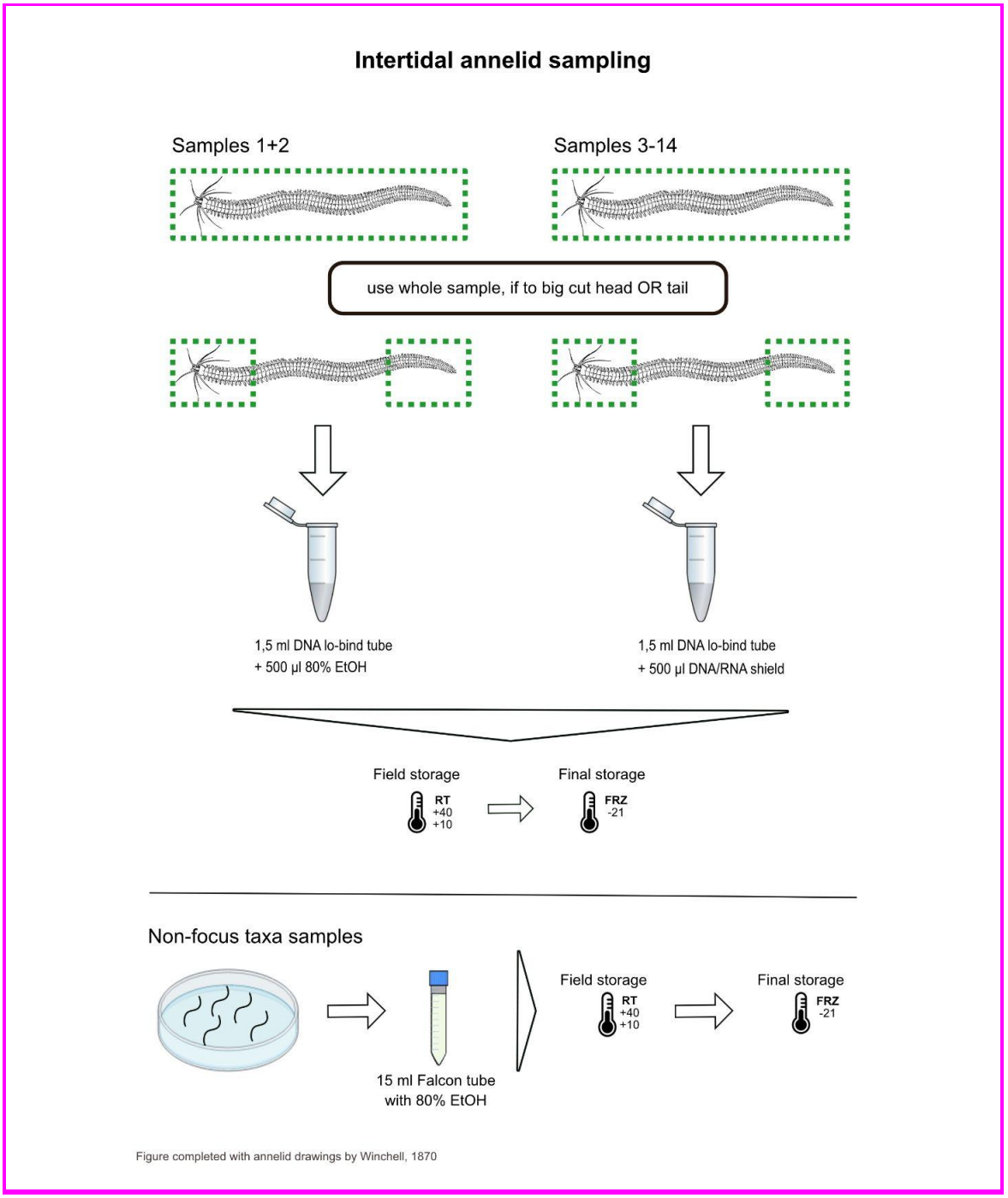

Figure 2.2 -9 Fixation of the intertidal annelid samples shown in flow charts

2.2. Subtidal Annelids and Associated Sediment and Water

2.2.1. Authors, affiliations and contact

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2.2.2. Introduction

Along the European coasts, we find many different species of annelids inhabiting various algal habitats. By investigating the population genetics and microbiome of these annelids with a focus on *Platynereis dumerilli*, we will gain insights to molecular changes that enable these organisms to adapt to changing environments.

Annelids, especially *Platynereis dumerilli*, will be isolated from different subtidal flora substrates retrieved by divers. The samples will be processed in methods that will permit downstream analysis such as but not limited to barcoding, whole genome sequencing, single cell sequencing, microbiome and metabolics.

2.2.3. Sampling strategy

At each site of at least 3m water depth(subtidal sampling), the following key sampling habitats are identified: Kelp forest (*Laminaria hyperborea* and *Saccharina latissima*) and seagrass meadows (*Zostera marina* and *Posidonia oceanica*). Once the sampling location and areas are selected, bioacoustics recorders are first deployed (see Bioacoustics chapter). Divers fill one sterilised Nalgene bottle with water from the sampling area and take photo documentation of the environment. Once completed, divers remove the kelp or seagrass from the substrate, while maintaining the rhizome integrity. Following, they place the collected material in cotton bags and bring them to the surface. As *Platynereis* atokes are known to inhabit various algal environment, the divers should retrieve cotton bags of other algal substrates as well (ie. Coralline algae, *Ulva* spp. etc). The cotton bags containing algal substrates will be placed in seawater until further processing. Once in the lab with access to the stereo microscopes, the contents of each bag will be taken out to be placed inside a white tray filled with seawater. Samplers will then sort through each tray and isolate annelids to separate petri dishes for photo documentation and processing for storage.

2.2.4. Sampling tools and equipment

Equipment	Consumables	Chemicals

• Reusable dissection kit including
  ○ dissection rubber pads x >5 pads
  ○ obsidian dissection scalpel (Fine Science Tools) vitrectomy scissors (Fine Science Tools)
  ○ minutien pins (Fine Science Tools)
• Fisherbrand™ Vacuum Pump x 2
	• Eppendorf DNA LoBind® Tubes 1.5mL x 5 per dissection
	• Nitril gloves (S,M,L) x 2 pairs per site per day
	• Eppendorf DNA LoBind® sterileTubes 1.5mL (x 60 per site
	• Durapore® Membrane Filter, 0,22 µm x 20 per site
	• KimWipes (KimWipes) x 1 box per site
		• DNA/RNA Shield x 1.5mL per tube (100mL per sampling site)
		• Ethanol (Ethanol 99%) OR Isopropyl Alcohol x 500mL (for sterilising)

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• Reusable Nalgene™ Bottle Top Filters -500mL x 2 per site • DURAN Glass1000mL bottle (for filtering sea water) x 2 • Cotton bags for algae collection and sorting x 15 per site • Zentis cups x 50 per site for short term partitioning of worms • forceps (various sizes) x 5 per site • Drying cloths (Drying cloths) x 2 per site • Large tote bag for carrying samples x 1 Dissection scope x 1 per site for us (reuseable at all sites) • Adaptor for phone camera to attach to dissection microscope to make photography easier	• Plastic pasteur pipettes 3.0mL x 10 per site • Disposable Stainless Steel Scalpels #24 x 2 per site • 50mL falcon tubes x 25 per site (on styrofoam tray) • Cryobox board 13x13x5 x 2 boxes per site • Barcode strips	

2.2.5. Metadata collection

The following metadata are noted on an excel database for each site:

- Name of the collection site
- Location ID
- Collection date
- GPS data from collection site
- Habitat type (sandy beach or rocky shore)
- Overview of sampled taxa per zone

2.2.6. Environmental parameters measurement

Temperature and light will be recorded by the HOBO sensors from bioacoustics protocol (see chapter 4).

2.2.7. Sampling protocol

Subtidal Documentation and Algal retrieval

a. Take photos and GPS points of selected sampling areas.

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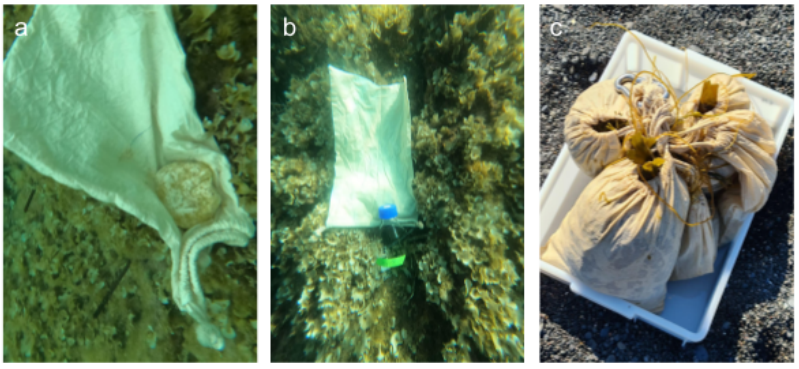
b. Open a 1 L polypropylene bottle to retrieve water samples from the sampling site.
c. Place the algal/ sea grass samples in cotton bags to bring up to shore.



Phillip Oel (a,b) Emily Savage (c-e)

Fig 2.4_1 a-c) Procedures for subtidal documentation of sampling area and substrate retrieval. d) Possible algal substrates for isolating annelids. e)Annelids can also be found in seagrass meadows.

2.2.8. Samples processing, transient storage, shipping conditions and Biobanking

Processing samples in the lab:

For each bag of algal or seagrass substrates, the contents are emptied into white trays which are filled with fresh sea water until further processing. Samplers examine the fond and seagrass blades for presence of annelid tube structure. Rhizomes are also closely examined, as they are the ideal dwellings for various polychaete species. After all the substrates have been examined and placed aside, the sea water will be examined for content. Any polychaetes and invertebrates identified are placed in a clean petri dish with sea water. Under stereomicroscope, the worms are sorted into different species and processed as per protocol while keeping apart worms from separate substrates.

Substrate Documentation

a. Document the entirety of the retrieved algal/seagrass substrate.
b. If substrate appears to have build up on the fond, rhizome or leaves, place it under stereo microscope
c. Take photos of the substrate under stereo microscope

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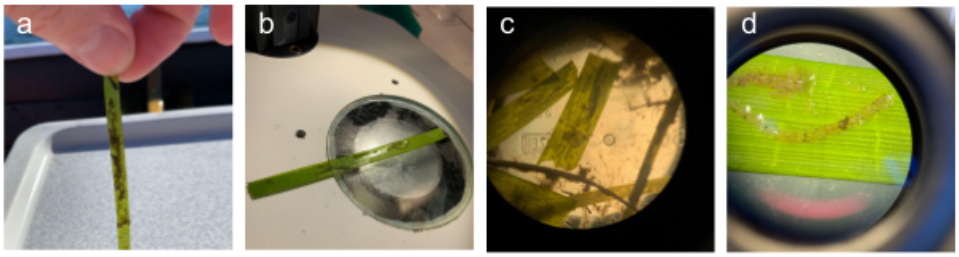

Phillip Oel (a-d)

Fig 2.4_ a-c) Procedures for substrate documentation. d) tubes formed by annelids dwelling on seagrass blade.

Sorting of the worms

a. Place the content of the cotton bags in a white tray
b. Comb through the algae, and place any worm collected into a 10 cm petri dish (use one dish for each kind of worm pre-identified)
c. Take photos to document all the diversity of specimens collected,, including through the dissection microscope lens Consider taking some videos too to document animal movements
d. When the diversity of annelids has reached a plateau, select up to 15 really good individuals per species/per kind, for snap-freezing & DNA shield preparation

Emily Savage (a-e)

Fig 2.4_3 a) Contents of substrates from a cotton bag in a white tray with seawater b)isolated worms in petri dish c-e) photo documentation of isolated organisms prior to processing

Worm processing

Head dissection (for the first 5 worms of the same kind)

a. Place a tiny speck of menthol crystal in the petri dish containing worms to dissect
b. Fix the anaesthetized worm with pins on rubber pad
c. If the worm has more than 4 cirri (tentacle looking structure) in the head region, trim the cirri from a single side of the head. *(optional)*
d. Isolate the head with a cut about the first segment and place it in a 1.5ml DNA lo-bind tube with a safe-lock cap.

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e. Barcode and flash freeze the tube.

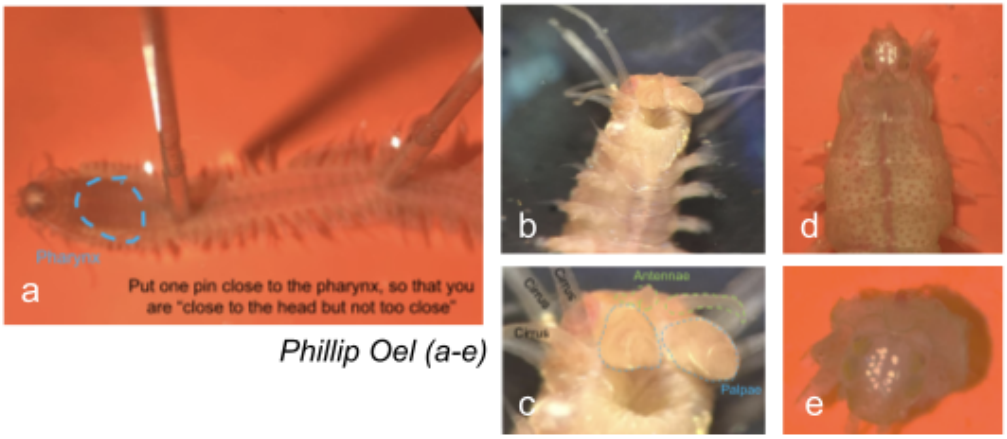

Fig 2.4_4 a) Fixing anesthetized worm with pins on rubber pad b,c) Presence of multiple cirri on worm head d,e) Head dissection with trimmed cirri from one side

Body processing (for the first 5 worms of the same kind)

a. Cut the remaining worm fragment into 3 pieces (two larger trunk pieces and one small tail piece for barcoding)
   - ➢ Trunk 1 will be placed in empty 1.5ml DNA lo-bind tube with a safe-lock cap
   - ➢ Trunk 2 and the tail piece will be placed in separate 1.5ml DNA lo-bind tubes with a safe-lock cap filled with 300µl of DNA/ RNA shield

b. Barcode and flash freeze the tube.

Worm processing (for the remaining >10 worms of the same kind)

a. Cut a tiny piece of the worm tail (around 1-2 mm) long and place it in a 1.5ml DNA lo-bind tubes with a safe-lock cap filled with 300µl of DNA/ RNA shield
b. Put the remainder of the worm in a 1.5ml DNA lo-bind tubes with a safe-lock cap
c. Barcode and flash freeze the tubes.

Transient storage

After flash freezing, the tubes will be kept in -20C or -80C until transfer to Dry Shipper for shipment back to EMBL Heidelberg.

Shipping condition

Samples can be shipped in dry shipper or containers filled with dry ice.

Biobanking

All samples will be kept at -80°C until further processing for sequencing or metabolic assays.

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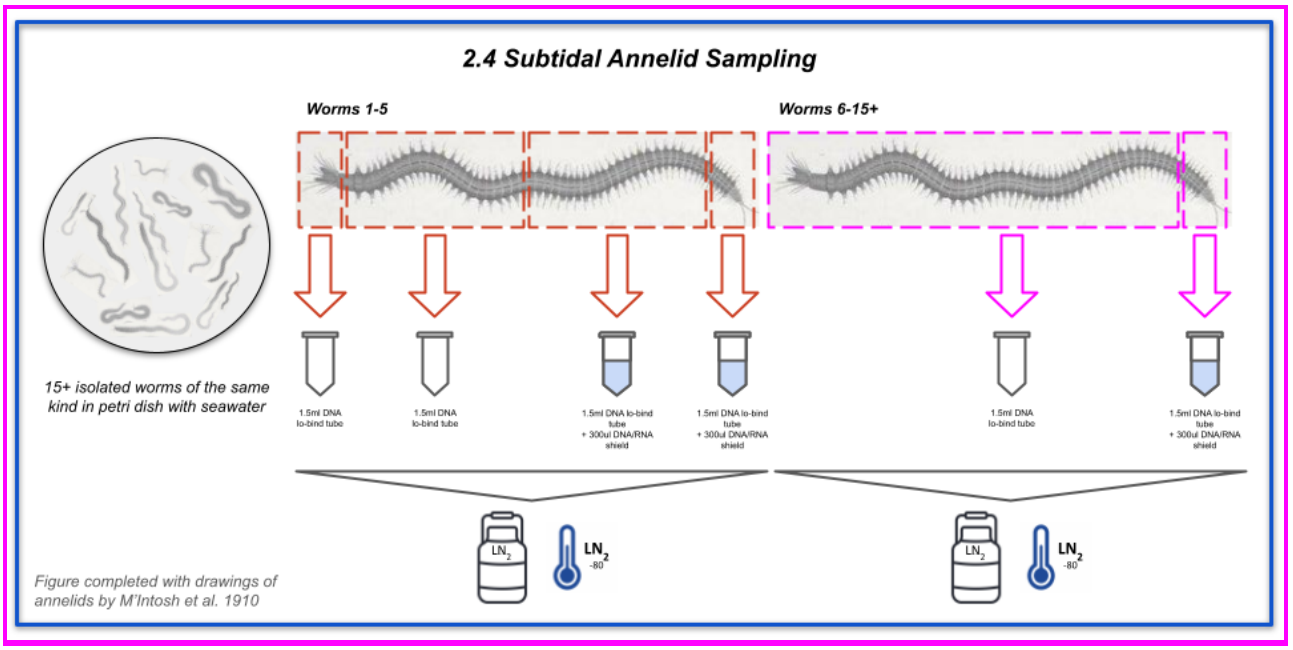
2.2.9. Flow charts



2.3. Sea grasses and associated sediment and water

Zostera marina sampling for population genomics and microbiome analysis

2.3.1. Authors, affiliations and contact

Sophie ARNAUD-HAOND, Ifremer (sophie.arnaud-haond@uumontpellier.fr), Jillian Petersen, University of Vienna (jillian.petersen@univie.ac.at)

2.3.2. Introduction

Samples of seagrasses, mostly *Zostera marina* but possibly also *Posidonia oceanica* in the Mediterranean, are collected following a nested sampling design (see sampling strategy) to study together the clonal and genetic diversity of the host plant, and the microbial diversity of associated communities.

Collected leaves, roots, rhizome and associated sediment will be used to produce i) whole genome sequencing (on leaves) to characterise the population genomics of the seagrass, and ii) metabarcode of 16S gene to unravel the diversity and composition of bacterial communities associated to the different tissues of the host and of associated sediment.

2.3.3. Sampling Strategy

Plants chosen for sampling should be selected haphazardly throughout the seagrass bed according to a nested strategy, as follows:

Quadrates depending on species:

- *Zostera marina*: large quadrates 30x20 m, small quadrates 3x2 m distance of 3 m max each

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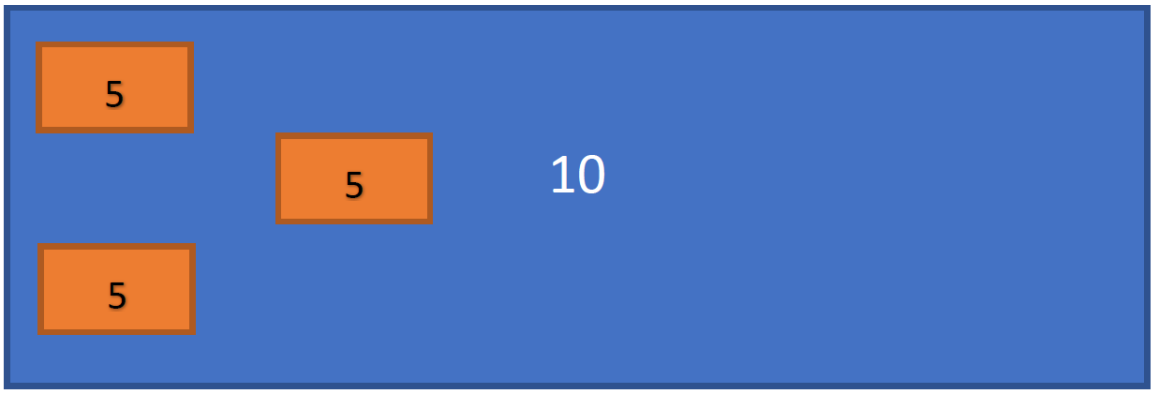
- *Posidonia oceanica*: large quadrates 80x20 m, small quadrates 4x2 m, distance of 4 m max each



In the small quadrates, 5 samples of shoot should be taken haphazardly, including some very close to each other, to be able to detect clonal replicates. Contrastingly in the remaining parts of the meadows, 10 samples should be chosen haphazardly avoiding to take plants very close in proximity to reduce the likelihood that samples are taken from the same genetic background/clonal lineage.

About 25 samples should be collected per site, two to three shoots may be necessary to obtain the necessary amount of leaf tissue for population genetics, particularly for *Z. marina*, yet this should be done only when the divers/samplers can ensure the shoots are attached through the rhizome and thus belong to the same genetic individual.

2.3.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
● For the sampling: ○ Rubber or boots to plant the quadrates at the beginning of the dive: 3 rubber or boot quadrates of 2x3 m, one decameter to trace the border of the large quadrate (QL), possibly buoys to identify the four corners of QL from distance ○ One Measuring tapes of at least 80m, ○ 3 to 7 measuring tapes of of 30 to 50 cm (alternatively, boot pre-prepared quadrates	● Sampling: ○ Ziplock bags (one per sample 160x200mm or more) labelled per sample (Stx, Q1/2/3/L, S1/2/…) ● 4 Serflex (also called colson) to attach bags together per quadrat ● Conditioning: ○ Cryotubes (3 per sample for leaves, rhizome and roots) ○ Storage boxes to group the cryotubes per station and	● Conditioning, to rinse forceps and blade between each sample and to clean space: ○ Ethanol 96° ○ MilliQ water ○ Absorbent & disposable paper ○ DNA exitus to clean surfaces or aluminium foil once deployed ● Preservation: liquid nitrogen

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of 3x2 or 4x2 with tent peg on the four corners) ○ Net bag to gather all groups of ziplock bags onece the sampling is complete ○ Disposable gloves to avoid manipulationg samples with naked hands (for microbiology application) ● For the conditioning: ○ scissors, dissection forceps & scalpel ○ Aluminium paper (or petri dishes) to work on a clean surface and wipe of change it between samples ○	destinations (leaves-Sète; roots & rhizome-Vianna) ○ Disposable gloves to manipulate samples. Pairs of gloves should be changed if there is a contact with a given sample to avoid cross contamination (tip: put two pairs of gloves of increasing size, it facilitates frequent changes so only the upper glove is removed and changed and it is easier to put on another glove than wet/humid hands)	

2.3.5. Metadata collection

Sample names are a combination of species name, station, site and quadrate:individual code including “Zm” for *Zostera marina*, and “Po” for *Posidonia oceanica*, followed by a three letter acronym for the sampling site, and the ?? , followed by “_R” for rhizomes and roots, and by “_L” for leaves. If using several shoots, make sure they are interconnected.

Examples:

Zm-Tha-St1-Q1_S1_R is the code for the rhizomes and root first sample of the first small quadrate in the first station (if several stations are sampled in the same harbour, adding a station in the name would help differentiating them while keeping the same acronym) of the Thau lagoon:

Example for the leaves of the 8th sample of the large quadrate of the first station in Ferrol:

Zm-Fer-St1-QL_S8_L

For each station, the GPS coordinates and depth of the meadows will be recorded. All related data on the status of the meadows will be useful for data interpretation as well as to use the results obtained in this axis for the interpretation of results of other axis of TREC.

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2.3.6. Environmental parameters measurement

Water temperature, salinity, depth and pH during the time of sampling.

2.3.7. Sampling protocol

1. Mark out the quadrats

   This underwater step takes the most time

   a. Unroll a long measuring tape to the maximum length of the large quadrat and fix it with a tent peg

   b. Place a second measuring tape perpendicularly to the first one, unrolled to the width of the quadrate, and fix with a tent peg.

   c. using the GPS, record the coordinates of the 4 quadrants.

   d. Lastly, prepare small quadrats of the desired size with boots, and fix them with camping pegs as well.

   Tip : For easier deployment, marking tools for the three small quadrats can be prepared prior sampling, by measuring out a square of visible tape of the appropriate dimensions, tied to four tent pegs.

2. Collect the samples

   Each sample is made of two to three interconnected shoots placed in individual bags. This ensures the shoots belong to the same clone/genet, and that enough material is collected for DNA extraction..

   a. In the small quadrates, 5 samples are collected haphazardly, including some very close to each other, to be able to detect clonal replicates.

   b. In the remaining part of the meadow, 10 samples are selected haphazardly, avoiding to take plants very close in proximity, to reduce the likelihood that samples originate from the same genetic background/clonal lineage.

This results in a total of 25 samples per site.

Tip : prepare ziplock bags with the name of the sample for each small (Q1,Q2,Q3) and large (QL) quadrates: Q1-S1 to Q1 S5, Q2 S1 to Q2 S5, Q3 S1 to Q3 S5, and QL S1 to QL S10 and attach them together by with a cable tie that goes through each bag making a ring. The bags can still be opened and closed underwater, and at the surface, the water will flow out.

2.3.8. Samples processing, transient storage, shipping conditions and Biobanking

Processing samples in the lab:

The same samples are used both for microbiology (roots & rhizomes) and for population genomics (internal leaves -as much as possible the younger ones, usually light green, rich in meristematic cells and protected from UV light), and are assigned the same individual sample codes (see above 2.5.5). Please view the flowchart in 2.4.9 to identify them.

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Microbiology studies:

Roots and rhizomes are extracted from the environment with gloved hands or with a shovel. If using a shovel, roots and rhizomes that were not in direct contact with the shovel should be chosen for sampling.

1. Break off segments of the roots and rhizome with sterile forceps and/or sterile scalpel.
2. Rinse pieces of root/rhizome with autoclaved MilliQ water (alternatively sterile-filtered seawater could be used) to remove sediment/debris.
3. Place the pieces in a 2 mL tube.
4. Freeze the tubes in liquid nitrogen.
5. Place the tubes in a storage box and then in a dry shipper.

Population genomics studies:

Use gloves for opening the shoots and the following steps

1. Extract the small, younger leaves and cut several of them from one shoot (or two to three, still interconnected ones) using a scalpel or a razor blade rinsed in between each sample. Young leaves are inside, they need to be extracted by peeling the shoot. It is important to have at least 2 or 4 for *P. oceanica* and 4 to 8 for *Z. marina* (depending on their size).
2. If needed, rinse the leaves with salted milliQ water (keep the salinity similar to the sea to avoid exploding cells)
3. Place them in a 2 mL tube.
4. Freeze the tubes in liquid nitrogen.
5. Place the tubes in a storage box and then in a dry shipper.

transient storage

The samples should be frozen at -80°C until transporting it on dry ice to the recipient research institutes.

2.3.9. Flow charts

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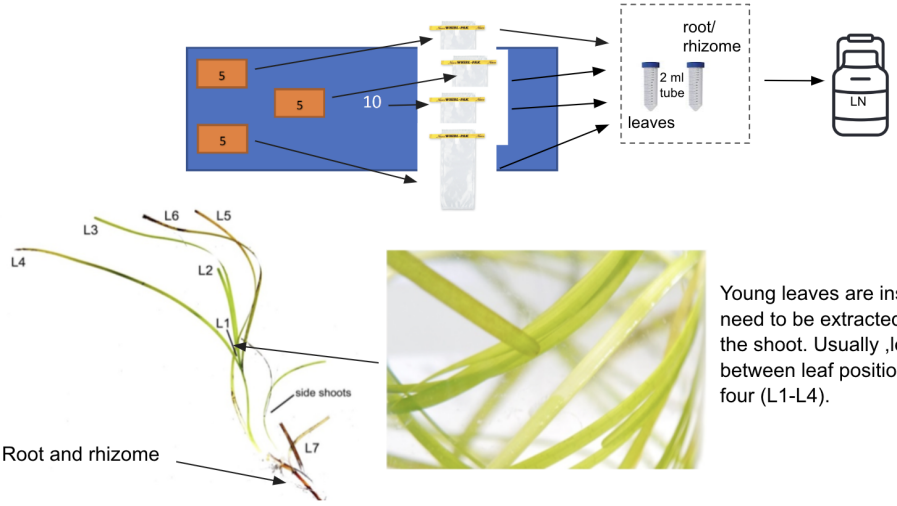
Zostera marina sampling in subtidal zone



Young leaves are inside, they need to be extracted by peeling the shoot. Usually ,located between leaf positions one and four (L1-L4).

Zostera marina representation adapted from Papazian, S., Parrot, D., Burýšková, B. *et al.* Surface chemical defence of the eelgrass *Zostera marina* against microbial foulers. *Sci Rep* 9, 3323 (2019). https://doi.org/10.1038/s41598-019-39212-3

2.4. Kelp forests/swabs

Sampling protocols for the kelp species *Laminaria hyperborea* and *Saccharina latissima.*

2.4.1. Authors, affiliation and contact

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Contact: susana.coelho@tuebingen.mpg.de, ljaugeon@sb-roscoff.fr

2.4.2. Introduction

The following process is applied to both *Laminaria hyperborea* and *Saccharina latissima* sporophytes in order to collect:

1. swabs of the algal biofilm for the study of microbiome (including virome) with metabarcoding.
2. seawater samples for microbiome and virome analysis.
3. samples of vegetative blade tissue for sequencing the host genome.
4. samples of fertile blade tissue (sorus) for spore release and gametophyte cultivation when sporophytes are fertile in the field.

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The final objective is to assess the relationship between the microbiome, kelp host species and sexual reproduction with the aim of linking sexual traits, host-microbe genomic interactions and ecological success.

2.4.3. Sampling Strategy

Sporophytes are collected individually, with each single sporophyte being placed in one net bag previously identified with a tag (see section below for the identification of the samples) and rinsed with surrounding seawater.

If sporophytes are not fertile: 15 sporophytes per species are sampled by divers. Collected sporophytes should be spaced at least by 0.5 m up to 1 m from each other.

If sporophytes are fertile: 30 sporophytes per species are sampled by divers, ideally following a transect of 15m, where two individuals are collected at every metre (0.5 m above and 0.5 m below the transect line as in Figure 2.5 -1). If this setup is not possible, 30 individuals spaced at least by 0.5 m from each other should be sampled instead.

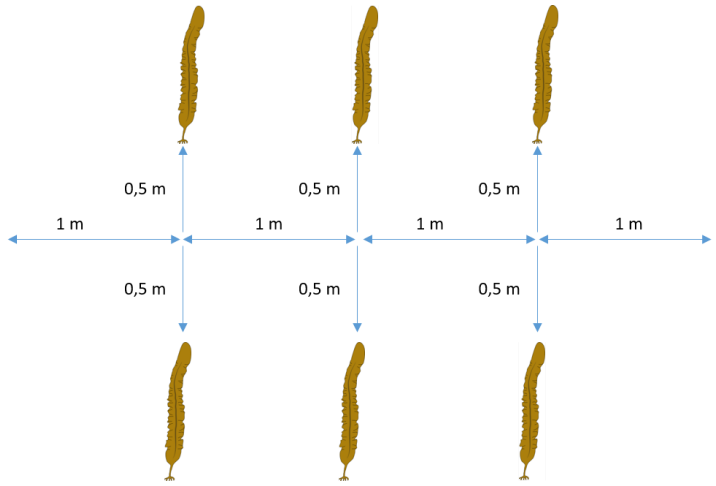

Figure 2.5 -1: best sporophytes sampling method: a transect of 15 m.

Each sporophyte is identified by its own ID code which reports:

- Species code: “Lh” for *Laminaria hyperborea*; “Sl” for *Saccharina latissima*.
- Population code: 3 letters from the name of the original population’s site (ex: “ROS” for Roscoff).
- Number of the concerned sporophyte. The first individual from one sampling campaign must follow the number of the last individual from the previous campaign on the same site.

For example, the 3rd *S. latissima* sporophyte which was sampled at Roscoff (ROS) is identified as “SL-ROS-3”. This code must be reported on the corresponding bags, tubes, sachets, etc.

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2.4.4. Sampling tools and equipment

Equipment	Consumables	Chemicals

Protocol 1: swabs
• dry shipper
 	
Protocol 2: seawater filtration
• Peristaltic pump and tubing (Vampire sampler; Buerkle).
• 5 L foldable jerry cans or similar
• dry shipper
 	
Protocol 3: vegetative host tissue sampling
• Scissors or cutter
• forceps
• Cooler or box with ice packs
• dry shipper
 	
Protocol 4: fertile host tissue sampling
• Scissors, cutter and perforating tool (2 cm diameter)
• Forceps
• Cooler or box with ice packs
	Protocol 1: swabs
	• Swabbing kits (one swab and one tube per individual)
	• Gloves
	Protocol 2: seawater filtration
	• 0.22 µm Sterivex filter units
	• Parafilm (and/or P10 tips)
	• Male luer lock caps
	• 50mL syringe
	• Gloves
	• Small plastic bags
	Protocol 3: vegetative host tissue sampling
	• Falcon tubes (ideally 5 mL tubes) or similar
	• Paper towel
	• Medium-size plastic bags
	• Medium-size petri dishes (for rinsing baths)
	Protocol 4: fertile host tissue sampling
	• Paper towel
	• 50 mL Falcon tubes (1 for each sampled individual) with :
	  ○ 45 mL of sterile filtered seawater
	  ○ 2 microscope slides
		Protocol 1: swabs
		• Liquid nitrogen
		
		Protocol 2: seawater filtration
		• Liquid nitrogen
		• Distilled water
		
		Protocol 3: vegetative host tissue sampling
		• Distilled water
		• Diluted bleach (final concentration 600 ppm sodium hypochlorite)
		• sterile filtered seawater
		• Liquid nitrogen
		• Ethanol 70%
		
		Protocol 4: fertile host tissue sampling
		• Distilled water
		• Ethanol 70%

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2.4.5. Metadata collection

At each sampling site the following information is recorded must be noted:

- Name of the site
- Date and hour
- Tide coefficient
- Corrected depth if subtidal
- GPS coordinates of the site
- Water temperature
- Names of the operators

No log-sheets to guide the preparation of samples and to record metadata available.

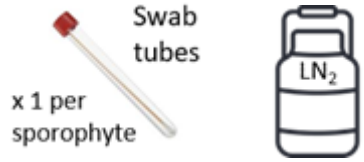
2.4.6. Environmental parameters measurement

Environmental parameters will be recorded by the HOBO sensors from bioacoustics protocol (see chapter 4).

2.4.7. Sampling protocols

After sampling, seawater samples and sporophytes are transported as soon as possible ashore or to the marine station. As much as possible, keep seawater samples and sporophytes in the dark, cool and slightly humid during transport . Swabs will be done first, as soon as possible, followed by seawater filtration and host tissues collection.

Microbiome composition of algal biofilms (swabs)



/!\ This protocol is subject to contamination, wear gloves.

1. Report the individual code of the sporophyte on the swab tube.
2. Swab a 5x5 cm area of kelp meristem for approx. 1 min. Begin swabbing the surface, using horizontal strokes. Flip the swab over and swab in a perpendicular direction using the same technique.

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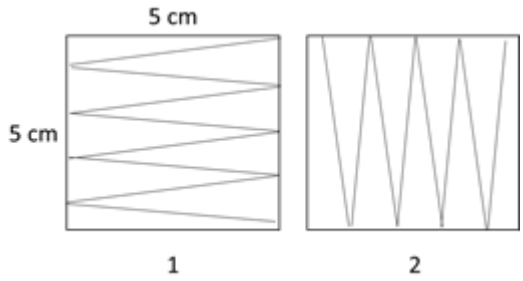
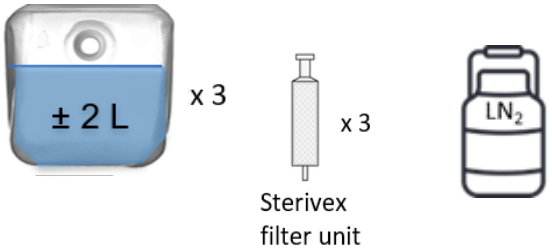


3. Put the swab into the tube.
4. Flash freeze in liquid nitrogen (dry shipper).
5. Store at -80°C. DNA foam swabs will then be extracted using standard DNA extraction protocols.

Seawater filtration for microbiome analysis



Caution

- This protocol is subject to contamination, wear gloves.
- This protocol has to be run twice if the several species are collected on two different sampling sites.
- Before each sampling campaign, all consumables not sold as DNA-free have to be immersed in 12.5 % commercial bleach (ca. 0.65 % hypochlorite) for at least 30 min, rinsed with miliQ water and placed under UV light for at least 15 min.

Step by step

1. Collect in triplicates 2 L of subsurface seawater with foldable jerricans
2. Pre-label one Sterivex filter unit per replicate.
3. Place a Sterivex unit at the end of the tubing system.
4. Filter 3* 2L of the seawater sample using a portable peristaltic pump (Vampire sampler; Buerkle). If the Sterivex unit clogs, note the filtered volume.
5. Once the seawater sample is filtered, remove the remaining liquid from the Sterivex unit using a sterilised syringe by pushing air.
6. Cap both ends of the Sterivex, one with a male luer lock cap and the other with parafilm or inserting a P10 tip into the inlet and burning it. Make sure the Sterivex unit is well sealed.
7. Repeat steps 3 to 6 for the other seawater sample replicates.
8. Label a small plastic bag and put the three Sterivex units inside.

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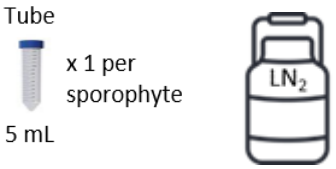
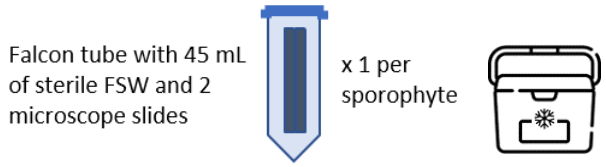
9. Flash freeze.
10. Store samples at -80°C.
11. Clean tubing system by running distilled water in it.

Vegetative host tissue sampling for further DNA extraction



1. Report the individual code of the sporophyte on the falcon tube.
2. Cut two 2x2cm pieces of the central blade, above but close to the meristem area with a clean scalpel or scissors (wash with Ethanol 70%)
3. If necessary, mechanically remove the epiphytes before rinsing.
4. Rinse the pieces successively with sterile filtered seawater, diluted bleach and fresh water. Each rinse lasts approx. 30 sec.
5. Dry the two pieces with a paper towel.
6. Put the two pieces in a 5 mL Falcon tube.
7. Flash freeze
8. Store at -80°C

Fertile host tissue sampling on fertile area of the blade (sorus) for fertile individuals



1. Report the individual code of the sporophyte on the Falcon tube (previously filled with 45mL of sterile filtered seawater).
2. Cut two disks of 2 cm diameter within the darkest parts of the sorus area with a sterile scalpel
3. Rinse them with fresh water and carefully clean them with paper towels.
4. Put the two pieces in the Falcon tube: one on each side of the microscope slides within the Falcon tube.
5. Put the Falcon tubes into a cooler or a box with ice packs and keep them in the dark during all the transport.

2.4.8. Samples processing, transient storage, shipping conditions and Biobanking

Swabs, sterivex filter units and pieces of vegetative host tissue are immediately flash frozen and stored at -80°C.

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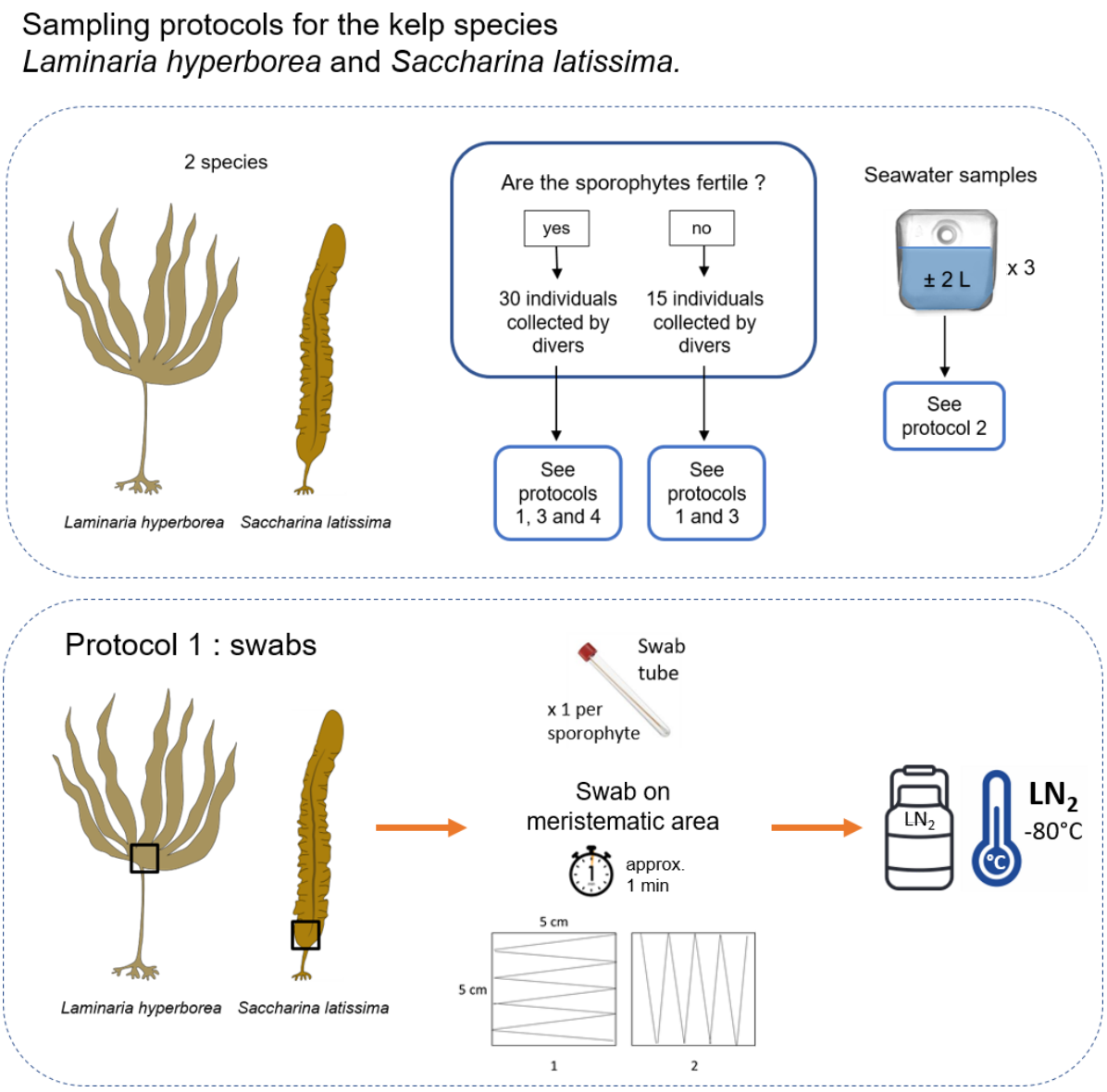
Falcon tubes containing the two disks of mature fronds are kept in dark in a box with ice packs during all transport.

2.4.9. Flow charts



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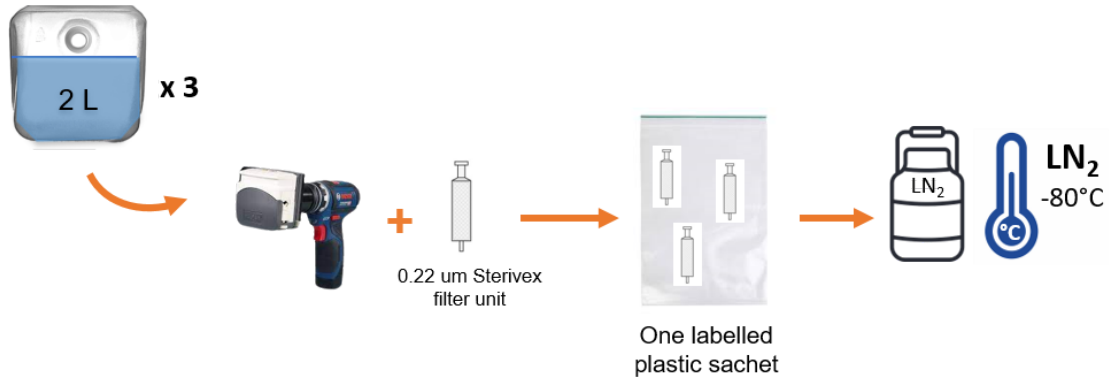
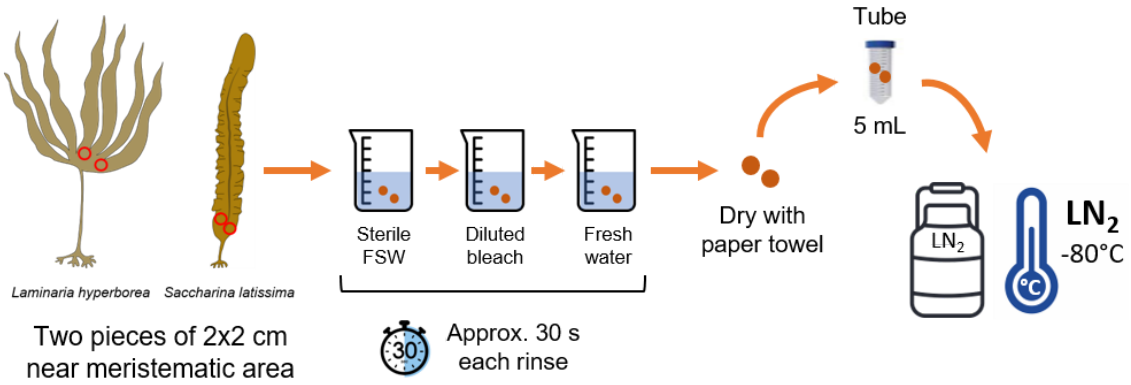
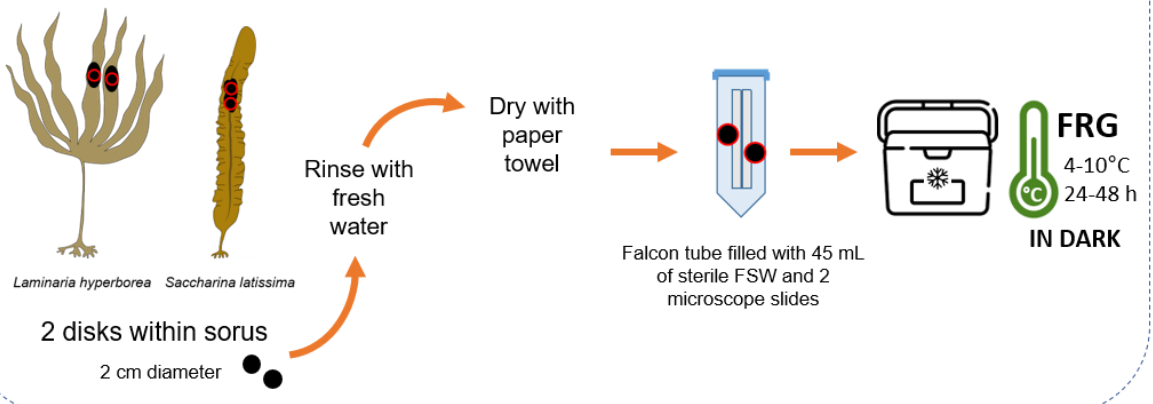
Protocol 2 : seawater filtration



Protocol 3 : vegetative host tissue sampling



Protocol 4 : fertile host tissue sampling



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2.5. Sea Anemone Tentacles (Anemonia viridis) for analysis of host and dinoflagellate symbionts

2.5.1. Authors, affiliations and contact

Elizabeth A. Hambleton, Division of Microbial Ecology (DOME), Centre for Microbiology and Environmental Systems Science (CMESS), University of Vienna.

(elizabeth.hambleton@univie.ac.at)

2.5.2. Introduction

The goal of this study is to assess the diversity and function of dinoflagellate algae, in symbiosis in anemone hosts or free-living in sediments, across evolutionary and environmental gradients throughout the Mediterranean basin, by measuring the responses of the holobiont to estuarine and other environmental conditions. This protocol describes the non-destructive sampling and the preservation of the photosymbiotic sea anemone *Anemonia viridis* by collection of tentacles. Tentacles frozen in the field will be used for:

- Identification of host and dinoflagellate symbiont with 18S/COI and LSU typing
- Lipidomics to measure symbiont-produced sterols, essential to the host
- Transcriptomics of host and symbiont
- Storage of voucher specimen for taxonomic archive keeping

2.5.3. Sampling Strategy

Sampling of the specimens will occur concomitantly with the TREC leg of 2024 on the locations that span the West and East Mediterranean: Mallorca, Villefranche, Naples, Split, Athens and Malta.

On scuba or snorkel, anemones will be located between 1 - 10 m depth on rocky coasts. Sampling will comprise ≥ 4 tentacles from 5 (min) - 10 (ideal/max) *Anemonia viridis* individual anemones per site.

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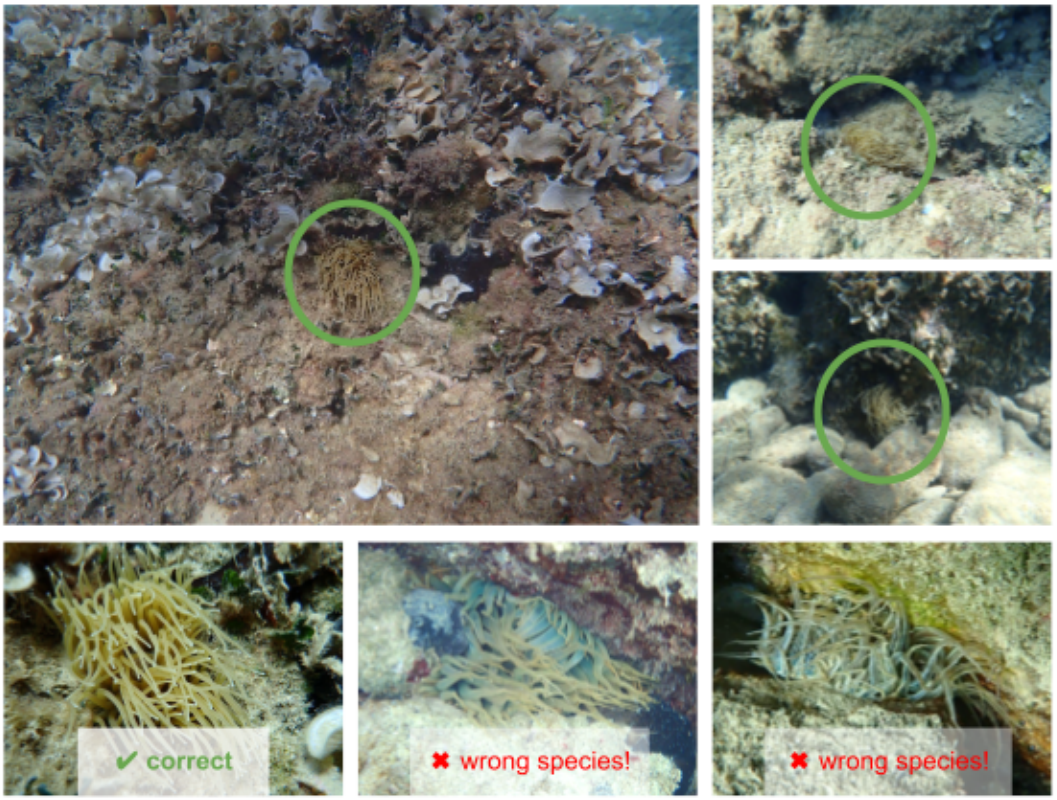
Organism identification



2.5.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• Scuba / snorkel gear • (optional: small plastic clips, e.g. food storage clips) • Forceps • Clean plastic disposable containers, for rinsing (e.g. plastic drinking cups, bowls etc) • Liquid nitrogen / dry shipper	• Latex gloves (one or two sizes larger than the sampling person usually wears) • 2 mL eppi tubes with SafeLock lids (e.g. Eppendorf DNA Lo-Bind tubes) •	• 70% Ethanol, for cleaning • Sterile seawater (e.g. filter-sterilized natural seawater = FNSW) • MilliQ water

2.5.5. Metadata collection

- Name/ID of the collection site
- GPS of collection site
- Date and time of collection (approx. ok, e.g. +/- 1 hour)
- Depth of collection

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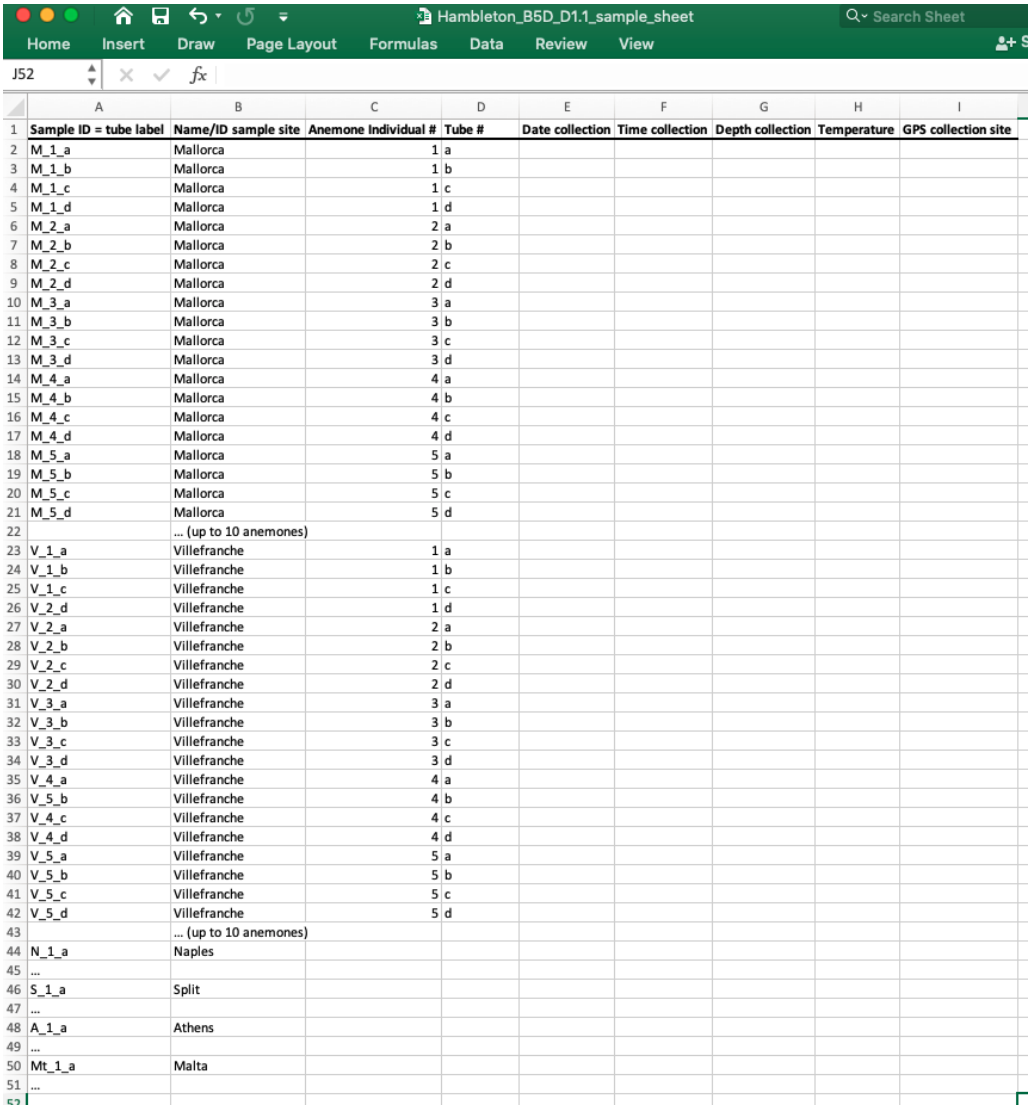
- Water temperature
- [Light level or photosynthetically active radiation (PAR), if available]

Sample sheet with sample ID / tube labels below:



Hambleton_B5D_D1.1_sample_sheet

Home Insert Draw Page Layout Formulas Data Review View

J52

	A	B	C	D	E	F	G	H	I
1	Sample ID = tube label	Name/ID sample site	Anemone Individual #	Tube #	Date collection	Time collection	Depth collection	Temperature	GPS collection site
2	M_1_a	Mallorca	1	a					
3	M_1_b	Mallorca	1	b					
4	M_1_c	Mallorca	1	c					
5	M_1_d	Mallorca	1	d					
6	M_2_a	Mallorca	2	a					
7	M_2_b	Mallorca	2	b					
8	M_2_c	Mallorca	2	c					
9	M_2_d	Mallorca	2	d					
10	M_3_a	Mallorca	3	a					
11	M_3_b	Mallorca	3	b					
12	M_3_c	Mallorca	3	c					
13	M_3_d	Mallorca	3	d					
14	M_4_a	Mallorca	4	a					
15	M_4_b	Mallorca	4	b					
16	M_4_c	Mallorca	4	c					
17	M_4_d	Mallorca	4	d					
18	M_5_a	Mallorca	5	a					
19	M_5_b	Mallorca	5	b					
20	M_5_c	Mallorca	5	c					
21	M_5_d	Mallorca	5	d					
22		... (up to 10 anemones)							
23	V_1_a	Villefranche	1	a					
24	V_1_b	Villefranche	1	b					
25	V_1_c	Villefranche	1	c					
26	V_2_d	Villefranche	1	d					
27	V_2_a	Villefranche	2	a					
28	V_2_b	Villefranche	2	b					
29	V_2_c	Villefranche	2	c					
30	V_2_d	Villefranche	2	d					
31	V_3_a	Villefranche	3	a					
32	V_3_b	Villefranche	3	b					
33	V_3_c	Villefranche	3	c					
34	V_3_d	Villefranche	3	d					
35	V_4_a	Villefranche	4	a					
36	V_5_b	Villefranche	4	b					
37	V_4_c	Villefranche	4	c					
38	V_4_d	Villefranche	4	d					
39	V_5_a	Villefranche	5	a					
40	V_5_b	Villefranche	5	b					
41	V_5_c	Villefranche	5	c					
42	V_5_d	Villefranche	5	d					
43		... (up to 10 anemones)							
44	N_1_a	Naples							
45	...								
46	S_1_a	Split							
47	...								
48	A_1_a	Athens							
49	...								
50	Mt_1_a	Malta							
51	...								

2.5.6. Environmental parameters measurement

Temperature, depth of sampling, time of sampling, photosynthetically active radiation (PAR), [ie. standard CTD measurements]

2.5.7. Sampling protocol

1. On scuba or snorkel, locate an anemone. Carefully check the species characteristics, ie. long and skinny light-brown tentacles, often tipped with purple or (occasionally) green tips, on rocky coasts typically between 1 - 10 m depth, and *no* white lines on tentacles or strong yellow colouring.
2. Record the relevant environmental parameters (see above).
3. From a diving pouch, put on one over-sized nitrile glove on the dominant hand (possible to go over an existing dive or neoprene glove).

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4. Using this gloved hand, gently but firmly rip tentacles from the anemone by pinching and pulling. Hold tightly but carefully; they typically stick to the glove's plastic. Carefully visually check that at least 4 tentacles have been collected. (NB. non-destructive sampling, as the tentacles can regenerate).
5. Quickly but carefully, invert the glove to take it off and trap the tentacles inside the glove. Close the glove by knotting it (or use optional plastic food-storage clips to securely close).
6. Repeat for a minimum of 5 anemones and an ideal / maximum of 10 anemones.

2.5.8. Samples processing, transient storage, shipping conditions and Biobanking

Processing and transient storage

1. Once on shore, keep samples covered and (ideally) floating in seawater in a container, so that they remain at ambient collection temperature.
2. Prepare per sample 2 clean rinsing cups: 1 with FNSW and 1 with MilliQ water. Volumes are in excess of tissue amount and approximate, e.g. from 10 mL to 100 mL.
3. Prepare per sample 4 labeled 2-mL eppi tubes (pre-labeled)
4. For each sample: open or cut the sealed glove and carefully invert, to release tentacles into the FNSW cup.
5. Using clean forceps, pick a tentacle, dip **quickly** in MilliQ water cup to rinse (approx 3-5 seconds), and then remove and let water drip off before placing in a 2-mL labelled eppi tube. Distribute tentacles so that 3 of the tubes contain 1 tentacle each; and the 4th tube contains all remaining tentacles.
6. Keep tubes on ice while processing all remaining samples.
7. Flash-freeze with liquid nitrogen and move to -70°C freezer box; or add to dry shipper. <!> caution of tube explosion!

Shipping conditions

Samples should be shipped deep-frozen, ie. either at -70°C or dry ice or dry shipper.

BioBanking

Of the 4 eppi tubes containing tentacles per each individual anemone, 1 will be kept deep-frozen as a voucher specimen.

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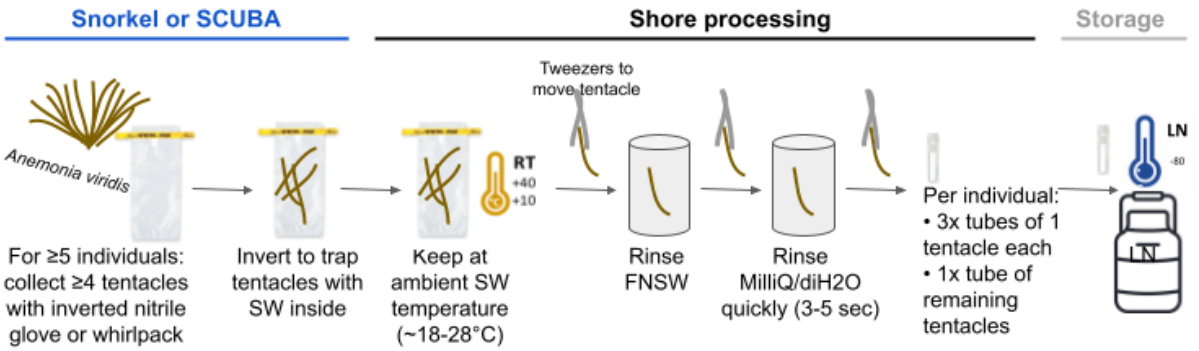
2.5.9. Flow charts



2.6. Lucinid clams (*Loripes orbiculatus*)

2.6.1. Authors, affiliations and contact

Jillian Petersen, University of Vienna (jillian.petersen@univie.ac.at), Hanin Alzubaidy, University of Vienna (hanin.alzubaidy@univie.ac.at)

2.6.2. Introduction

Lucinid clams live in the seagrass bed sediments around the roots of the seagrass. Their sulfur-oxidizing endosymbiont plays a vital role in detoxifying the sulfide, making the sediment suitable for seagrass to thrive. The symbiosis between the lucinid clam endosymbionts, the lucinid clam, the seagrass and its microbiome has been established millions of years ago. We are interested in understanding the function each of them play in this model, and how they influence each other and the surrounding environment. For that reason, we will be collecting the clam organs (gill, mantle, and foot) that will be preserved for metagenomics and functional analyses using FISH, EM techniques, and nano-SIMS.

2.6.3. Sampling Strategy

At the same site (seagrass bed) selected for the project 2.5 (seagrass sampling), sediment should be collected from the surrounding of the seagrass bed. The sediment is collected at 20-30 cm depth. One random location around the seagrass bed is sufficient. The ideal number of collected lucinid clams is around 10 clams at each site.

2.6.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• Cores or shovels to collect the sediment • Buckets to hold the collected sediments • Sample processing tools:	• Eppendorf tubes • Pipettes and tips • Razorblades	• RNAlater or DNA shield • PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid) • HEPES (4-(2-hydroxyethyl)-1-pip

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• kitchen sieves to sieve out the sediment • Callipers to measure the size of the clams • Forceps		erazineethanesulfonic acid) • EGTA (ethylene glycol-bis(2-aminoethyle ther)-N,N,N′,N′-tetraacetic acid) • $MgCl_2$ (magnesium chloride) • KOH • NaN_3 • Sucrose • ddH_2O • 25% Glutaraldehyde • NaCl • KCl • Na_2HPO_4 Dinatriumhydrogenphosphat • KH_2PO_4 • 70% ethanol

2.6.5. Metadata collection

- Clam size.
- Number of clams collected.
- Coordinates of the collection site.
- Record by drawing a scheme which part of the gill or clam's organ was used for each of the processing methods below.

2.6.6. Environmental parameters measurement

Salinity, water temperature and depth

2.6.7. Sampling protocol

***Loripes orbiculatus* or other lucinid species collection:**

1. At the same site selected for sampling for project 2.5 (seagrass), sampling for clams should occur.
2. Collect sediment from the surrounding of the seagrass bed (as close as possible to the seagrass bed). Make sure sediment is collected at 20-30 cm depth.

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2.6.8. Samples processing, transient storage, shipping conditions and Biobanking

Sample processing:

1. Back onshore, Sieve sediment over a sieve of appropriate mesh size - for most species, a kitchen sieve is sufficient.
2. Pick out lucinid clams by hand and keep in native seawater or sediment until further processing.
3. The ideal number of *Loripes orbiculatus* from each site processed is from 5-10 clams.
4. The size of each individual should be measured with callipers before dissection and saved in the (2.8. Lucinid clams metadata) google sheet.

Organ dissection:

1. Before dissection individuals should be measured with a calliper and their size recorded

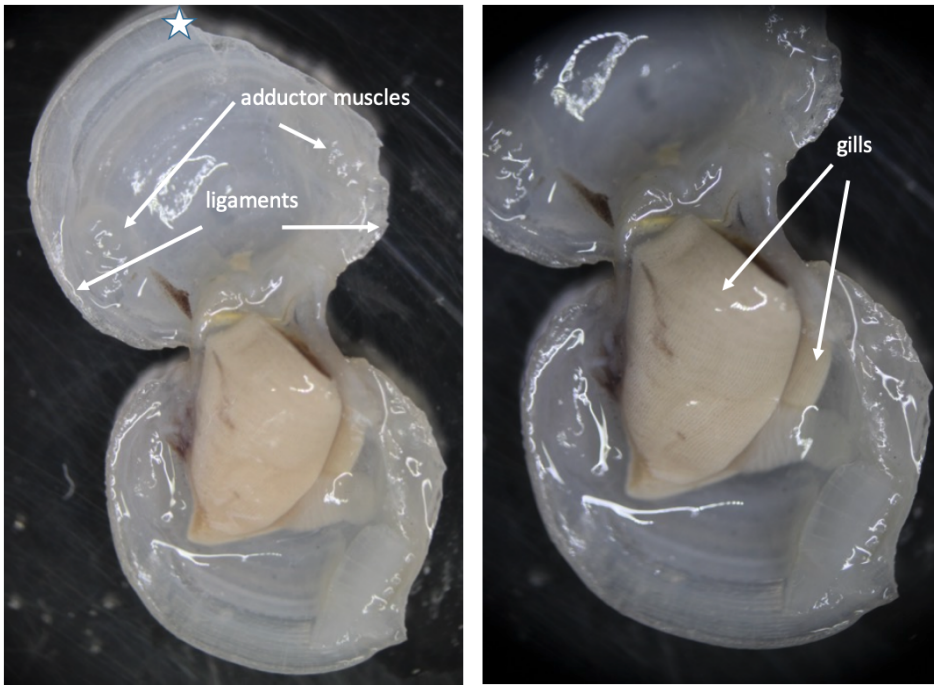

Figure 2.7-1: An open *Loripes lucinalis* clam shell. (a) highlighting the ligaments that hold the shells together. (b) the gills covering up the body part with all its organs. photo credit: Julia Polzin.

To open the clam use a scalpel and carefully cut the ligaments on each side of the clam:

2. Insert the scalpel in the middle- upper part of the shell (indicated by the star) Figure 2.7-1 (a). Then, carefully slide down with the scalpel to the ligaments

Once opened, the clam looks like the figure 2.7 -1 (b) :

-the whole body with all organs is covered by the gills from both sides. The body comprises all the organs, gonad, digestive system…

-the mantle is transparent and surrounds the body and the gills as you can see in Figure 2.7 -2.

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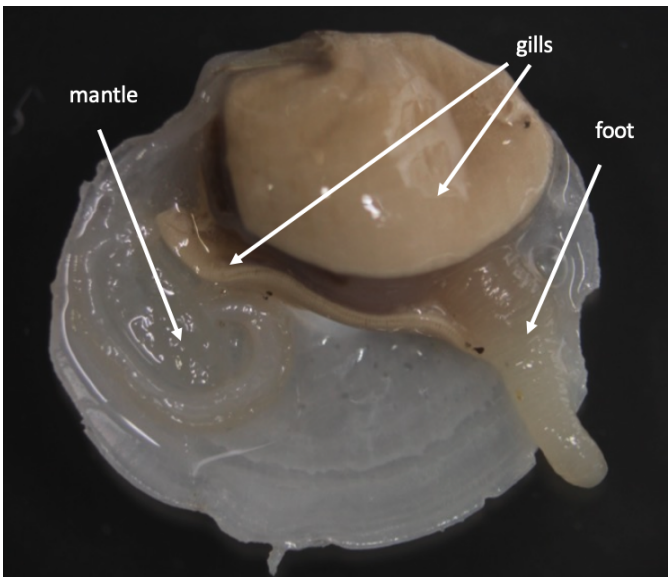

Figure 2.7 -2: a close look at the *Loripes lucinalis* clam showing the intact gills, foot, and mantle. photo credit: Julia Polzin.

3. Lift up the 1st gill with forceps and carefully cut it with some microscissors (or normal small scissors – dashed line) Figure 2.7 -3.

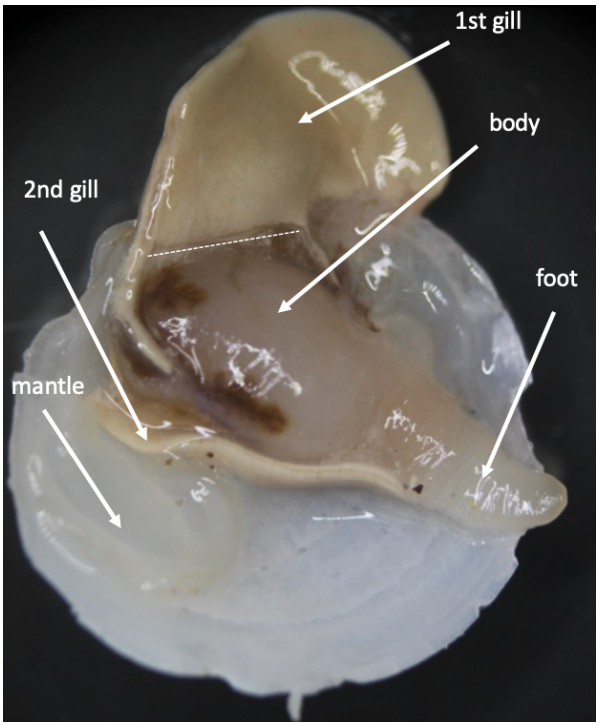

Figure 2.7 -3: a descriptive image of *Loripes lucinalis* clam organs. The dashed lines show the first gill cut up. photo credit: Julia Polzin.

4. After removing the 1st gill, (Figure 2.7 -4) turn around the whole body with the 2nd gill still attached.
5. Then dissect the 2nd gill as the 1st gill.
6. Dissect the body and the foot as illustrated below Figure 2.7 -5.

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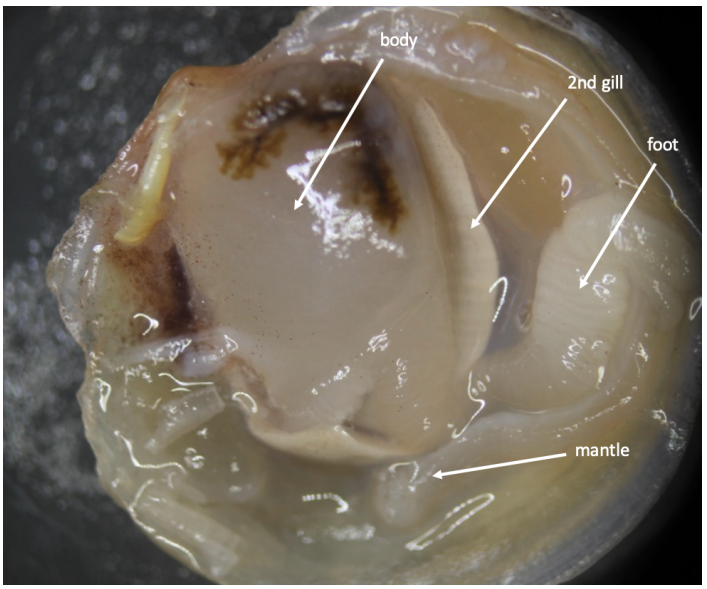

Figure 2.7 -4: Close up on the body after removing the 1st gill. photo credit: Julia Polzin

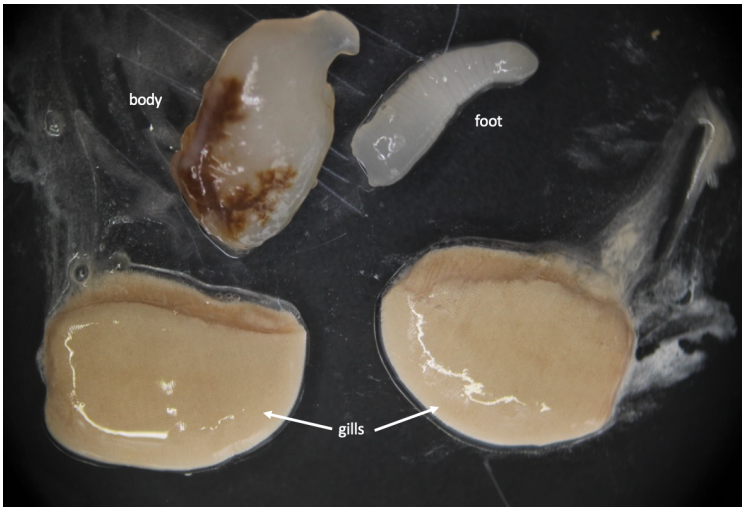

Figure 2.7 -5: *Loripes lucinalis* clam dissected organs. photo credit: Julia Polzin.

7. Record by drawing a scheme which part of the gill or animal was used for each of the processing methods below.

Gill fixation for EM:

The protocol is based on Montanaro J, Gruber D, Leisch N. 2016.

Improved ultrastructure of marine invertebrates using non-toxic buffers. PeerJ 4:e1860 https://doi.org/10.7717/peerj.1860)

1. Cut ~4mm wide pieces of the gill using a razor blade (e.g. 1-3 pieces per individual).
2. Transfer pieces (1 piece per tube) immediately into 2 mL tubes filled with fixative, fix the tissue at 4°C overnight.
3. After fixation, remove as much fixative as possible (do not let the sample fall dry) and fill the tube up with the washing solution.
4. After two washing steps, replace the washing solution with the storage buffer.

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5. Store the samples at 4°C in the fridge.

Preparing the fixative, washing solution, and storage buffer:

Preparing 100 mL **Fixative**:

1. Open a 10 mL ampule of 25% Glutaraldehyde and pour into container
2. Add 15 mL 10X PHEM stock solution
3. Add 9 g Sucrose
4. Fill up to 100 mL with ddH2O, close the container and shake slowly till the sucrose is dissolved.

Notes:

- EM Fixative should be as fresh as possible. Discard unused fixative solution at the latest 2 days after mixing.
- Fixative should be stored at 4°C when not in use.
- Fixative should be warmed to room temperature before using it to fix samples.

Preparing 100 mL PHEM **Washing** buffer

1. Add 15 mL 10X PHEM stock solution
2. Add 9 g Sucrose
3. Fill up to 100 mL with ddH2O, close the bottle and shake slowly till the sucrose is dissolved.

Preparing 100 mL PHEM **Storage** buffer

1. Add 15 mL 10X PHEM
2. Add 9 g Sucrose
3. Add 1 mL 1% NaN3 (TOXIC!) Fill up to 100 mL.

Preparing the 10x PHEM buffer stock

1. For 100 mL of a 10X stock solution, dissolve:
   a. 18.14 g (600 mM) PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid))
   b. 5.96 g (250 mM) HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
   c. 3.805 g (100 mM) EGTA (ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid)
   d. 0.41 g (20 mM) MgCl2 (magnesium chloride) in 80 mL of ddH2O.
2. The pH needs to be raised to ~7, using 10M KOH, for the components to go into solution. It takes ~15 mL 10M KOH to raise the pH of 100 mL 10X PHEM to 7.
3. Adjust the final pH to 7.4.
4. Fill up to 100 mL with ddH2O.

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Gill fixation for FISH in PFA:

1. Cut ~4 mm wide pieces of the gill using a razor blade (e.g. 1-3 pieces per individual).
2. Transfer pieces (1 piece per tube) immediately into 2 mL tubes filled with fixative, fix the tissue at 4°C overnight.
3. After fixation, remove as much fixative as possible (do not let the sample fall dry) and fill the tube up with the washing solution PBS washing solution (1 x PBS, pH 7.4, 0.01 M, 10 % sucrose).
4. Wait for 10 minutes and do the washing step 2 more times for 10 min each.
5. After discarding the last washing step, dehydrate the sample in 30%, 50% and 70% of ethanol for 10 min each.
6. Store the sample in 70% ethanol at 4°C until embedding.

Preparing the fixative, washing solution, and storage buffer:

Preparing **stock solution**:

1. For 1 L of 10 x PBS, pH 7.4, 0.1 M add:
   a. 80 g NaCl (M = 58.44 g mol-1)
   b. 2 g KCl (M = 74.55 g mol-1)
   c. {14.4 g Na2HPO4 Dinatriumhydrogenphosphat (M = 141.96 g mol-1) or 17.8 g Na2HPO4*2H2O Dinatriumhydrogenphosphat Dihydrat (M = 177.99 g mol-1) }
   d. 2.4 g KH2PO4 (M = 136.09 g mol-1)
2. dissolve in 800 mL MilliQ and adjust pH to 7.4
3. add MilliQ to 1 L and autoclave for long-term storage at room temperature

Preparing the working solution for the **fixative**:

For 1 L 2 x PBS, pH 7.4, 0.02 M, 20 % sucrose working solution add

a. 200 g sucrose to 200 mL of 10 x PBS stock solution
b. add MilliQ to 1 L

This will be used for to prepare the fixative

Preparing **washing solution**:

For 1 L 1 x PBS, pH 7.4, 0.01 M, 10 % sucrose, mix:

a. 500 mL of the sucrose working solution for the fixative (2 x PBS, pH 7.4, 0.02 M, 20 %)
b. 500 mL MilliQ

This will be used as a washing buffer after fixation

Preparing **fixative for FISH** For a final volume of 10mL with a final concentration of 0.01 M PBS pH 7.4, 4 % PFA, 10% sucrose:

a. add 5 mL of PBS 0.02 M (with 20 % sucrose)

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b. add 2 mL of PFA 20 %
c. add 3 mL of MilliQ

Gill preservation for nucleic acid extraction:

1. Cut a piece of the gill using a razor blade.
2. Place in a 2 mL cryotube and flash freeze it.
3. Store in 80°C until transporting it on dry ice to the lab.

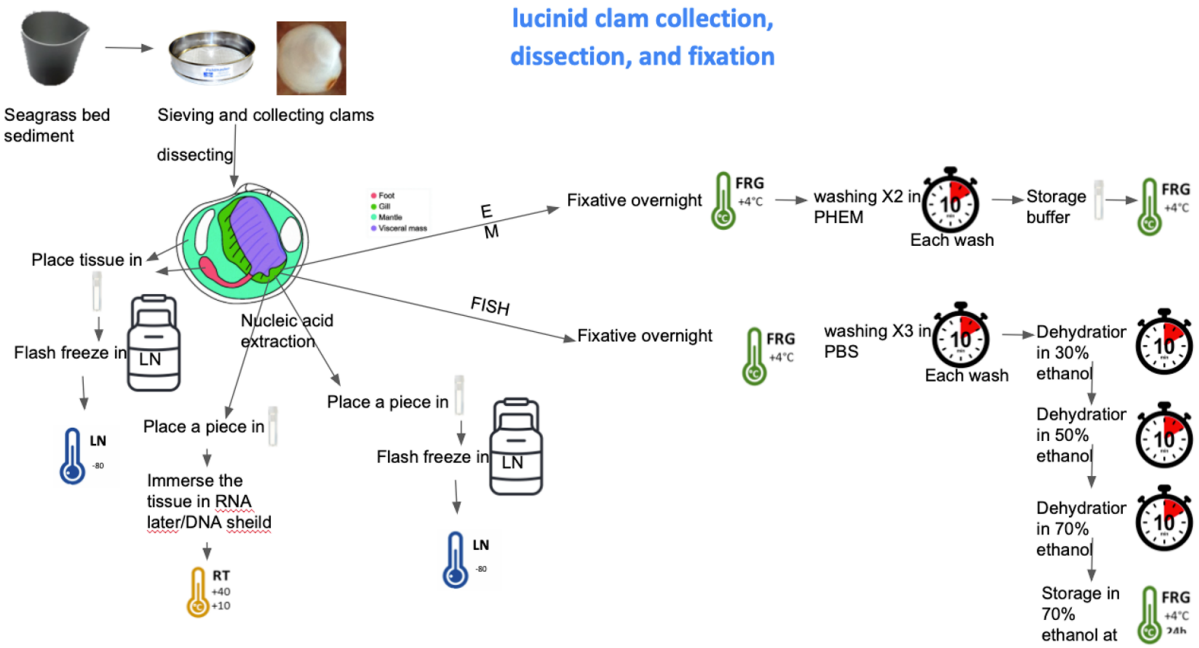
Gill preservation in RNAlater/DNA shield:

1. Cut a piece of the gill using a razor blade.
2. Place in a 2 mL tube and add RNAlater or DNA shield until it covers the tissue.
3. Store at room temperature until transporting it to the lab.

Remaining organs/tissues perseverance:

1. The rest of the tissues (foot, body,..) should be placed individually in 2 mL cryotube.
2. Flash freeze the tubes.
3. Store in 80°C until transporting it on dry ice to the lab.

2.6.9. Flow charts



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2.7. Marine Sponges and associated symbionts

2.7.1. Authors, affiliations and contact

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2.7.2. Brief introduction

The effects of environmental change and extreme events on the structure and functioning of sponges and associated microbiomes will be investigated with metabarcoding, metagenomics and metatrascriptomics.

This study will establish (1) whether and how taxonomic composition and functional profiles of sponge holobionts change across temperature gradients, (2) whether microbial composition changes more than functioning or vice versa, and (3) how climatic extremes alter holobiont structure and functioning, potentially triggering mass-mortality events.

2.7.3. Sampling Strategy

We will sample 3 sponge host holobionts (*Agelas oroides*, *Chondrosia reniformis, Dysidea avara*) across 3 sites within the Catalan coast (Banyuls-sur-mer, Medas Islands and Amposta). Sponge samples will be collected by scuba diving either from the coast or from a boat. In each site, a transect following a temperature gradient will be established. Within each transect, we will sample 5 locations, resulting in a total of 135 samples (3 sites x 5 locations per transect x 3 sponge species x 3 individuals of each sponge).

2.7.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• Scuba diving equipment • Boat (zodiac type) • Scalpel/Scissors •	• 2 mL microcentrifuge tubes • Pipettes and tips • Zip lock bags	RNAlater Ethanol 96% Glutaraldehyde 2.5%

2.7.5. Metadata collection

At each sampling site and location within each transect the following information will be recorded: Site and location identifier (unique name and/or attribute), GPS coordinates, individual identifier (unique code for each individual of each species), individual exposure (qualitative information, e.g., cave, direct sun,...), and environmental variables (temperature, depth).

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2.7.6. Environmental parameters measurement

Temperature, depth, in situ nutrient concentrations (dissolved nitrate, nitrite, ammonia, and phosphate).

2.7.7. Sampling protocol

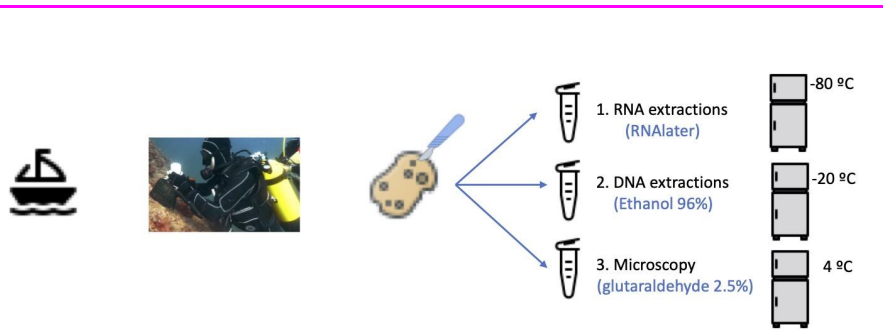
Sponges will be sampled by scuba diving, either from the beach or accessing the site with a boat. After collection under water, they will be transported to the beach/boat for performing 3 tissue cuts and placed in different tubes. They will be transported to the lab by car for preservation and sampling processing and DNA extraction.

2.7.8. Samples processing, transient storage, shipping conditions and Biobanking

Sponge tissue pieces will be cut with clean scalpel or scissors, depending on the tissue type. Pieces will include the sponge surface epithelium and the mesohyl, usually with a triangular cut for free-standing sponges, or slicing the entire tissue mass (from surface to rock) for encrusting sponges. These tissue samples will be placed into individual zip lock bags (or plastic containers) with surrounding water. Once on the surface, tissue samples will be divided in 3 pieces for different approaches, rinsed in filtered seawater, and placed in 2mL microcentrifuge tubes. These tubes will contain in advance: a) RNAlater (or flash-freeze) for RNA extractions; b) Ethanol 96% for DNA extraction; c) glutaraldehyde 2.5% for light and electron microscopy, and spicule identification.

All samples will be stored at 4°C overnight. RNAlater samples will be moved to -80°C freezer, ethanol samples to -20°C freezer, and glutaraldehyde will be kept in a 4°C fridge until future processing. If shipping is necessary, frozen samples will be transported on dry ice, and cold samples with ice packs.

2.7.9. Flow charts (used to guide the processing of samples during stations)



2.9. Microbiome of the microalga *Phaeocystis* in different life stages

2.9.1. Authors, affiliations and contact

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2.9.2. Introduction

Phaeocystis species are cosmopolitan microalgae living in polar, temperate and tropical waters. They are characterised by the ability to form blooms of colonies that release a large amount of dissolved organic matter (DOM) and sulfur compounds. This has an impact on fisheries and human activity. In addition to this solitary life, *Phaeocystis* species were described living in symbiosis within Acantharia (Radiolaria group) in different oceanic regions. Both free-living and symbiotic phases of *Phaeocystis* significantly contribute to primary production and biogeochemical fluxes in the ocean. However, it is not known whether prokaryotic cells can be a third partner impacting the biology and ecology of *Phaeocystis*. Unveiling the microbiome of this key marine phytoplankton taxa has the potential to improve our knowledge on plankton life strategies and is essential for the management and restoration of oceanic ecosystems (Peixoto and Voolstra 2023, Tara Ocean Foundation 2022). The overall objective of this study is to unveil the microbiome of the planktonic microalga *Phaeocystis* in different life stages (solitary, colonial, symbiotic) using molecular and imaging approaches. At the molecular level, we will identify putative partners using single colonies and single holobionts barcoding and transcriptomic analysis. The structure of the microscopic community will be studied through electron microscopy. This protocol describes the sampling of *Phaeocystis* and *Acantharia* (host) and their initial processing for subsequent molecular sequencing and ultrastructural imaging.

2.9.3. Sampling Strategy

Colonies of *Phaeocystis* and *Acantharia* (host) will be collected in the ocean at different sampling locations (e.g. TREC sites). We will sample at the same GPS point as all members of the water column sampling and if possible, try to connect with Tara sampling sites. Colonies grown in culture medium will also be isolated in parallel.

Plankton sampling

Plankton nets of 20 µm mesh-size and 150 µm mesh-size are used from a boat (2-5 min tow) at the subsurface. Once onboard, the content of the cod ends is filtered through a 500 µm metal sieve (for the 150 µm net) and 180 µm metal sieve for the 20µm plankton net. Organisms are isolated with a micropipette through the microscope or binocular and transferred in filtered seawater in an incubator (controlled temperature and light).

Isolation of cultured colonies

In the laboratory, colonies of *Phaeocystis* grown in culture medium K2 (Keller et al., 1987) are selected and isolated through a binocular and microscope. The colonies from the ocean are “cleaned” in sterile sea water produced with a filtering kit before being processed for freezing. Colonies can be isolated with a clean micropipette.

2.9.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• Plankton nets 150 and 20µm,	• 3-well concavity slides (x12 units in a box)	Liquid nitrogen

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• Sieve (500µm) • Thermos • Funnel • plastic beaker • binocular • AxioVert epifluorescent microscope • Pipetboy and Pipetman • -80°C freezer	• 1.5ml Eppendorf tubes • SteriCup to filter sea water (0.2µm) • Cryo boxes • Tips (20 to 200µm) • Plastic pipette (25ml) • Falcon tubes	

2.9.5. Metadata collection

For each sampling event, a log sheet is filled with GPS coordinates, external temperature and weather, sampler name and volume harvested through which nets.

2.9.6. Environmental parameters measurement

A CTD profile is made at each sampling site with chlorophyll a quantity (µg/l) and depth, profile, water temperature (°C), Sea pressure (dbar), salinity (PSU), depth (m), conductivity (mS/cm), PAR (Photosynthetic Active Radiation, µMol/m²/s).

2.9.7. Sampling protocol

1. Let the net dive into the water and avoid bubbles inside
2. Tow the net between 3 and 10 minutes depending on the water conditions
3. Pull up the net from the water and transfer the water from the Cod-end to a 5L recipient (used 500µm sieve if needed).
4. Transfer the water to a thermos or a Nalgene bottle (1L) and keep it in a cool box containing sea water from the sampling site (same temperature as the collected water)
5. Take at least one litre of seawater (non filtered) to prepare sterile sea water in the lab

2.9.8. Samples processing, transient storage, shipping conditions and Biobanking

Genomics (metabarcoding and transcriptomics).

Metabarcoding (DNA extraction from single cell or single colony, and PCR with 16S primers for microbiome or 18S to identify individual partners). Wear a lab coat, gloves. Decontaminate all the surface area, and equipment (rotor, racks, pipettes...) with Ethanol 70% and DNA away. Use filter tips. For each isolation (DNA or RNA), the microscopy images of the isolated organism are stored in a dedicated database.

1. Each pipetted cell is isolated and rinsed with filtered sea water in a three wells slides (3-well concavity slides (x12 units in a box));

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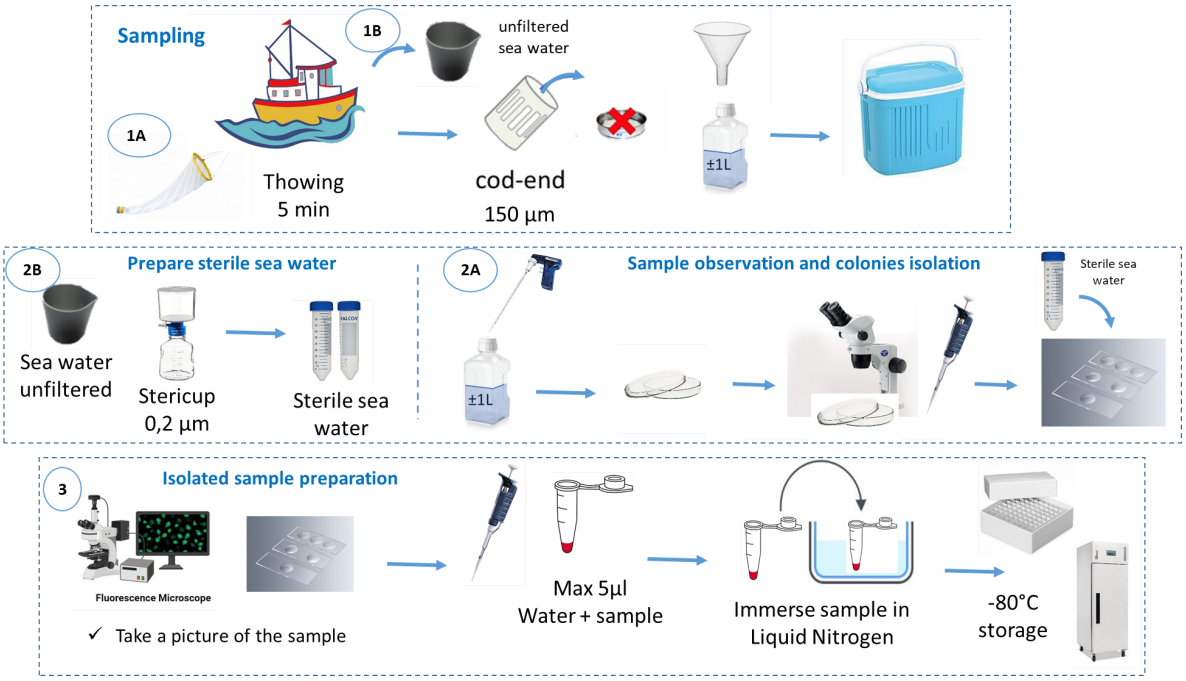
2. The rinsed/cleaned cell is then transferred from the slide well to a 1.5mL tube (no buffer) under the binocular;
3. The tubes can be kept on ice and flash frozen all at the same time at the end; do NOT let the tubes within liquid nitrogen for hours
4. Tubes are stored at -70°C in a dedicated box.

Electron Microscopy

1. After sampling and maintenance in controlled *ex situ* conditions, organisms (*Phaeocystis* colonies and *Acantharia*) are loaded onto a carrier for high pressure freezing .
2. Before freezing, pictures with an inverted microscope are taken to identify the organisms.
3. Samples are stored in liquid nitrogen and shipped in a dry shipper to keep cells vitrified.
4. In the lab, a freeze substitution with the frozen samples is conducted to have resin-embedded cells for electron microscopy.

2.9.9. Flow charts



1A = towing from the boat(2 to 5 min depending on weather and water conditions)

1B = collecting unfilter sea water at the sampling site (needed for the isolation of the cells)

2B = preparation of filtered sea water

2A = Sample isolation and observation under binocular and microscope

3 = Take picture of each isolated cell or colonies under AxioVert epifluorescent microscope and transfer the sample to a 1.5ml Eppendorf tube; freeze in liquid Nitrogen and store in a cryobox at -70°C

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2.10. Scleractinian coral holobionts (*Pocillopora spp* and *Porites spp*)

2.10.1. Authors, affiliations and contact

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2.10.2. Introduction

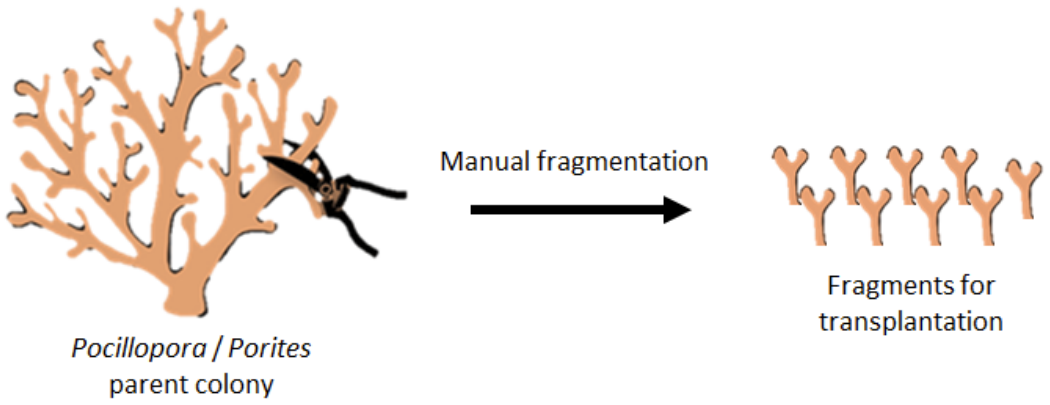
The objective of this study is to assess the relationships between the microbiome, the photosymbiont(s), and the host species of scleractinian corals, in relation to standing differences in the thermal and sedimentary environment. We aim to link host-symbiont genomic interactions to phenotypic plasticity and resilience to stress.

The following protocols are designed for scleractinian coral holobionts to collect:

1. tissue samples for genetic identification of the cnidarian host and microalgal photosymbiont(s) as well as gene expression (transcriptomes) and microbial community composition (metabarcoding).
2. seawater samples for microbiome analysis.
3. reef environmental parameters including sea surface temperature, turbidity, salinity, and photosynthetically active irradiance.

2.10.3. Sampling strategy

Pocillopora and *Porites* coral colonies are targeted for sampling at each site based on visible health indicators (e.g., avoidance of bleached or damaged colonies) as well as size (e.g., sufficiently large to produce 8-10 fragments of 2 cm length). After mechanical separation from the colony, coral fragments are to be treated individually - each fragment is being rinsed with surrounding seawater and placed in a single Whirl Pak bag marked with a unique identifier.



For example, the second fragment generated from the third *Pocillopora* parent colony sampled at the high sedimentation site that was subsequently back transplanted to that

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same site would be denoted as “POCH03F02B”. This code should be present on the corresponding bags, tubes, sachets, etc. used to transport and analyse this sample.

2.10.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
Coral colony sampling and transplantation ● Hammer and chisel AND/OR bone clippers ● Permanent marker ● Affixation surfaces (e.g., rebar coral mounting tables) ● -20°C freezer ● Large cooler *Water column and sediment associated microbiome* ● Peristaltic pump ● Plastic resealable carboys (two with a 50 L minimum capacity) ● 200 µm metallic sieve ● 20 µm metallic sieve ● Large cooler ● -80°C freezer	*Coral colony sampling and transplantation* ● Whirl-Pak sterile resealable bags (one bag per sample) ● Clear shipping tape ● Plastic cable ties (at least one per sample) ● Corning tubes 50 mL (one tube per sample) ● DNA/RNA Shield buffer (Zymo Research, Irvine, CA, USA) (10 mL per sample) ● Lysing Matrix A beads (MP Biomedicals, Santa Ana, CA, USA) (15 mL per sample) ● Nitrile gloves *Water column and sediment associated microbiome* ● 0.22 µm Durapore PVDF filters (Millipore, Billerica, MA, USA) ● 3 µm Durapore PVDF filters (Millipore, Billerica, MA, USA) ● Nitrile gloves	

2.10.5. Metadata collection

No log-sheets to guide the preparation of samples and to record metadata are available.

At each sampling site the following information should be recorded: Site identifier (unique name and/or attribute) and GPS coordinates.

Each fragment is identified by its own sample ID reports:

- Species code: “POC” for *Pocillopora*; “POR” for *Porites*

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- Origin site code: “H” high turbidity site ; “L” low turbidity site
- Parent colony ID: unique 2 digit code for each colony sampled ranging from colony 1 (C01) for the first colony sampled to colony XX (CXX) for the XXth colony sampled at each site.
- Fragment ID: unique 3 digit code for each fragment generated from the parent ranging from fragment 1 (F01) for the first fragment produced to fragment XX (FXX) for XXth fragment produced from each colony.
- Transplant status ID: “T” for out transplanted fragments ; “B” for back transplanted fragments.

2.10.6. Environmental parameters measurement

Temperature, photosynthetically active radiation (PAR), and conductivity should be continuously recorded at each transplant site using dedicated loggers (e.g., HOBO0431 U24-001 Conductivity Sensor respectively). Sediment deposition rates should be measured in triplicate at each study site using sediment traps.

2.10.7. Sampling protocols

After sampling, seawater samples and fragments marked for immediate molecular analysis should be transported ashore and processed as rapidly as possible. Fragments marked for transplantation and grow-out should be transported as rapidly as possible in a temperature controlled environment (e.g., sealed cooler) to their appropriate experimental site and affixed.

Protocol 1: Sampling coral colonies and transplantation

1. Photograph target *Pocillopora* and *Porites* colonies using a 20 cm quadrat for scale. Also record the depth, temperature, and location (GPS coordinates) of each colony sampled (see Section 2.10.6 for details).
2. Using a hammer and chisel or bone clippers, break off a number of branch tips (2 cm in length) from each colony, sufficient to meet downstream sampling requirements. Try to select branch tips with similar facing/orientation to the surface.
3. Fragments designated for immediate molecular molecular analyses should be quickly rinsed with filtered seawater and placed in sterile Whirl-Pak bags containing filtered seawater and pre-labeled with the sample ID. These samples should be placed in a temperature controlled cooler and taken ashore as soon as possible for processing as described in Section 2.10.8 below.
4. Fragments designated for out and back transplantation should be quickly rinsed with filtered seawater and placed in sterile Whirl-Pak bags containing filtered seawater and pre-labeled with the sample ID. These samples should be stored in a temperature controlled cooler and moved to their respective transplant site(s) as quickly as possible for affixation.
5. At each transplantation site, coral fragments should be attached to pre-installed permanent fixtures (e.g., stainless steel rods driven into the substrate) using plastic cable ties. Cable ties should be secured around the base of each fragment and fragments should be mounted in an upright position against the mounting surface by

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gently tightening the cable tie. Excess tie should be removed with scissors. Fragments from a given parent colony should be randomly distributed across the affixation surface.

6. Fragments should be allowed to grow out with minimal disturbance before subsequent scheduled harvesting of individual fragments for time series analyses. During this period, sediment traps should also be deployed at each site, their contents periodically dried and weighed, and the traps redeployed.

Protocol 2: Sampling of water column and sediment associated microbiome

1. Water samples should be collected at each sampling site from a consistent depth using a peristaltic or manual membrane pump and hose tubing. The hose tubing should be positioned below the surface at the selected depth and water pumped to a holding carboy (50 L minimum capacity) onshore or onboard a supporting sampling vessel.
2. To begin, set up the hose tubing, sieves, and pump such that the collected water will pass sequentially through the 200 µM, and 20 µM sieves before collection in the 50 L carboy. Then, water should be pumped through the hose tubing, 200 µM and 20 µM sieves and carboy in order to rinse the pumping system with the collection water.
3. Then, filter 50 L of water into the dedicated clean 50 L carboy. When possible, two replicates should be taken at each site. Samples of sediments (i.e. sand) should also be collected in duplicate at each site using two 10 mL cryovials. After filtering the 50 L sample, particulate matter collected on the 200 and 20 µM metal sieves should be individually collected onto 3 µM membranes using filtered seawater. These represent the >200 µM and 20 - 200 µM size fractions respectively.

2.10.8. Samples processing, transient storage, shipping conditions and Biobanking

1. Seawater and sediment samples should be transported ashore in dark, temperature controlled coolers. Sediment samples should be stored at −20 °C until processing.
2. Seawater samples should be further sequentially filtered through 3 and 0.22 µm Durapore PVDF filters (Millipore, Billerica, MA, USA). After this last filtration, all membranes should be carefully folded and placed within sterile tubes containing enough DNA/RNA Shield buffer to cover the folded filter. Tubes should be labelled by size fraction and stored at −20 °C until further analysis..
3. Coral fragments for molecular analyses should be removed from WhirlPak bags and preserved in 15 mL of Lysing Matrix A beads (MP Biomedicals, Santa Ana, CA, USA) along with 10 mL of DNA/RNA Shield buffer (Zymo Research, Irvine, CA, USA) and stored at −20 °C.

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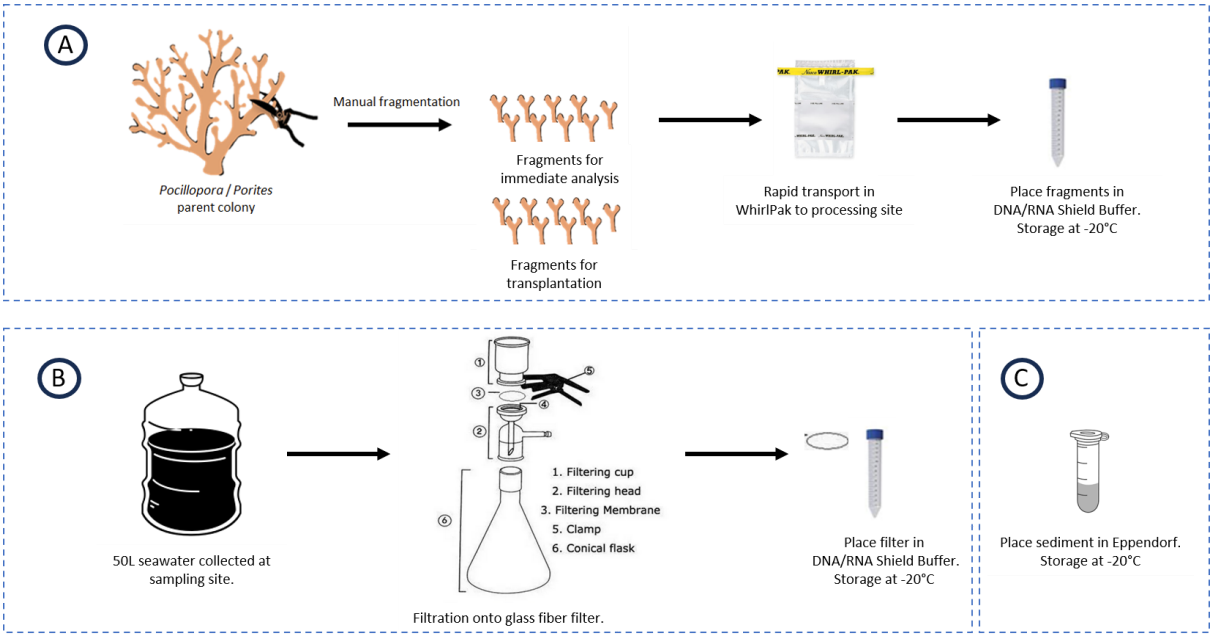
2.10.9. Flow charts



Processing and storage protocols for (A) coral, (B) seawater, and (C) sediment samples.

2.11. Oncholaimus nematode and Rimicaris shrimps

2.12.1. Authors, affiliations and contact

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2.12.2. Introduction

Objectives focus on 2 different hydrothermal models living in the Mid-Atlantic Ridge in geochemically contrasted vent fields. On the one hand, we aim to better understand the life cycle of *Rimicaris exoculata* and *Rimicaris chacei* shrimps and the establishment of the holobiont through symbiont metagenomic approaches. On the other hand, we aim to study intraspecific and holobiont diversity of *Oncholaimus dyvae* nematodes through metabarcoding approaches. The microbial diversity present in the habitats will also be investigated.

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Sampling will extend along the Mid-Atlantic Ridge from Lucky-strike site at 37.3°N to Puy des Folles site at 20.5°N during the MOMARSAT and BICOSE3 cruises, and will involve both adults and juveniles at various stages.

2.12.3. Sampling Strategy

Several sites will be sampled for these studies. While only the Lucky-Strike site is sampled for nematodes on the MOMARSAT cruise, 5 sites will be studied during the BICOSE3 cruise: TAG Active Mound, a new site "GRAPPE2", Snake-Pit with stations "The Moose", "The Beehive" and “The Lift”, the new site "MARK" and Puy des Folles.

Sampling will be carried out by submersible from depths of 1700 m to 4000 m. Dives with the Nautile will be carried out on BICOSE3 cruise and with the ROV Victor 6000 on MOMARSAT.

All individuals will be sampled and conditioned at each site.

Nematodes will be collected in both sediment samples and *Bathymodiolus* mussel byssus.

For shrimps, there are 3 juvenile stages for *Rimicaris exoculata* (stage A, stage B and sub-adult) versus 2 stages for *Rimicaris chacei* (stage A, sub-adult) to be sampled in addition to adults (Methou et al., 2020). Wherever possible, adult shrimp will be sampled with the Periscopette, which is lifted to the surface in PERISCOP at bottom pressure (Shillito et al., 2023, 2008), rather than with the suction sampler, as the shrimp are in much better health and potential bias is reduced.

For each habitat selected (dense aggregations, isolated individuals, nurseries, etc.), which may include a mixture of *Rimicaris exoculata* and *Rimicaris chacei*, and both adults and juveniles, the surrounding water will be sampled for free symbionts survey.

Chemistry measurements with CHEMINI Sulfides and CHEMINI Iron (Vuillemin et al., 2009) will be carried out in triplicate on these sampled habitats and water sampled in one point using the *In-Situ* Fluid Sampler (PIF) for complementary chemistry measurements (pH, methane, oxygen, organic matter, etc.).

For shrimps, a minimum of 10 individuals per stage, per species and per site is required for metagenomic studies, in order to have sufficient genetic material for sequencing. Additional individuals will be conditioned for complementary studies using FISH microscopy and electron microscopy (TEM/SEM).

Once on board, the shrimps are sorted by sampling tool and sampling bowl in a tank in cold storage (8°C°). After selecting the individuals of interest by species and stage, they are assigned a unique number and isolated in an individual can containing sterile seawater. Cephalothoracic cavity measurements are taken. Adults are sexed using the 2nd pleopod sexual morphotype.

Dissections are then carried out under sterile conditions. Each tube is then individually coded.

The metadata concerning the individual and the packaging are recorded in an Excel spreadsheet common to the cruise scientists. This data is then uploaded into Ifremer's MORSE software via LabCollector© to ensure sample traceability.

Samples are then stored on board at 4°C (SEM/TEM), -20°C (FISH) or -80°C (DNA). At the end of the mission, frozen samples are shipped in dry ice to the laboratory.

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2.12.4. Sampling tools and equipment

Protocol 1: seawater surrounding different populations

- Nautile's PLUME water sampler
- 2L Terumo medical transfer bag with quick connectors
- Sterile Masterflex tubing with male luer lock connectors
- 0.22 µm Sterivex filter units (Millipore)
- Peristaltic pump
- Parafilm
- Falcon 50 mL
- Beaker
- -80°C deep-freezer
- Gloves
- Ethanol 96°
- DNA away or DNA exitus

Protocol 2: *Rimicaris* shrimp

- Nautile's suction sampler or PERISCOP® with Periscopette device
- Laminar flow hood
- Chemical fume hood
- Binocular magnifier
- Dissecting tools : scalpel, micro-scissors, forceps, needle
- Petri dishes
- Ice pack
- 1.8 mL cryotubes
- 5 mL Eppendorf tube
- Cryotube boxes
- 125 ml plastic screw-top jars
- Icebox with crushed ice
- Plastic transfer pipet
- Liquid nitrogen
- -80°C deep-freezer
- Paper towel
- Gloves
- Sterile filtered sea-water
- Ethanol 96°
- DNA away or DNA exitus
- Formaldehyde 3%
- PBS1X / mix PBS2X/ethanol 50 :50
- Glutaraldehyde 2.5%
- Seawater with 0.043% sodium azide

Protocol 3: *Oncholaimus dyvae* nematode

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- The same than for protocol 2
- Microscope

2.12.5. Metadata collection

The metadata associated with the sampling performed by the submersible are downloaded via the ADELIE software. Once the navigation data have been corrected, they will be added to the SISMER database, which includes all cruise data from the French oceanographic fleet.

The metadata associated with the sample itself (species, life stage, sex, measurements, tissue dissected, fixative, conditioning) are recorded in a spreadsheet shared by all the scientists on the mission, part of which is then uploaded into LabCollector® and transmitted to Ifremer's MORSE software for sample traceability.

2.12.6. Environmental parameters measurement

For each shrimp and nematode habitat, temperature measurements will be taken prior to sampling for biology. Samples will also be taken using the *In-Situ* Fluid Sampler (PIF) to measure pH, alkalinity, iron (Fe^{2+} and total iron Fe_{tot}) and H_2S concentration. Major ions can also be measured. Sulfide and total dissolved iron Fe^{2+} concentrations will also be measured using CHEMINI sulfides and CHEMINI iron (Vuillemin et al., 2009).

2.12.7. Sampling protocol

Protocol 1: seawater surrounding different populations (shrimp or nematodes) for metabarcoding

Contact : Valérie Cueff-Gauchard, IFREMER institute (vcueff@ifremer.fr)

⚠ This protocol is subject to contamination, wear gloves.

Part 1: Preparation of « PLUME » bag

1) Decontaminate quick connectors with 96° ethanol, then with DNA away or DNA exitus and rinse rapidly in sterile milli-Q water
2) Take a sterile 2 L Terumo transfer bag and cut tube just before the junction that leads to 2 tubes
3) Fit the quick connector to the tube, moistening it with a little ethanol

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4) Mark bag with a clearly visible pen a number between 2 and 6 corresponding to the position of the bag on PLUME (position 1 for purge and 5 possible connections for transfer bags).
5) Install the transfer bags on the PLUME barrel on the submarine

Part 2: Sampling

6) Use the Nautile's nozzle to take a sample at the desired location by activating the Nautile's pump for 4 min. Note sampling temperature.
7) Purge, reversing the direction between each sample

Part 3: Samples processing - filtration

8) On arrival on board the submersible, remove the transfer bags of PLUME and store the transfer bags at 4°C until filtration.
9) Stand under a laminar flow hood and hang the transfer bags high up on the hood.
10) Connect a quick connector to a sterile Masterflex pipe line terminating in a male Luer-lock connector.
11) Connect the male Luer-lock connector to a Sterivex unit at the end of the tubing system
12) Install the assembly under the hood on a peristaltic pump
13) Connect the quick connector to transfer bag and filter the seawater sample using the peristaltic pump. If the Sterivex unit collapses, note the filtered volume and install a new Sterivex unit.
14) Collect the filtrate in sterile beaker for other applications such as viral diversity, which will then be filtered on a 0.02 µM filter.
15) Once the seawater sample is filtered, remove the remaining liquid from the Sterivex unit using a sterilized syringe by pushing air.
16) Cap both ends of the Sterivex with parafilm.
17) Label a 50 mL Falcon and put the Sterivex unit inside.
18) Store at -80°C.
19) Clean tubing system by running milli-Q water inside and autoclave it.

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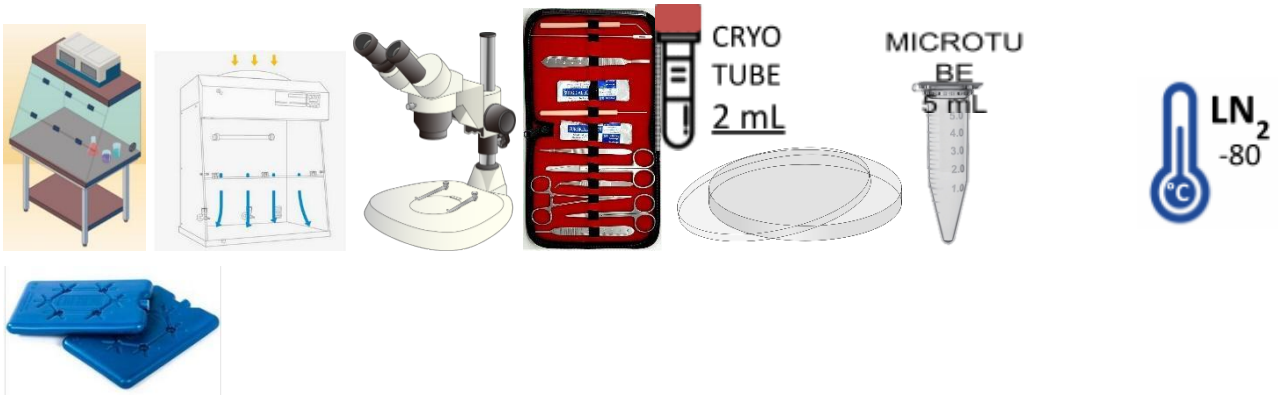
Protocol 2: *Rimicaris* shrimp for microbiome analysis

Contact : Valérie Cueff-Gauchard, IFREMER institute (vcueff@ifremer.fr)



⚠ This protocol is subject to contamination, wear gloves.

Part 1: Preparation of sampling and conditioning material

1) Clean suction sampler bowls thoroughly with hot water and washing-up liquid. Rinse well. Do not use alcohol, as this will damage the plastic.
2) Fill with fresh water filtered to 0.22 µM.
3) Close the lid and take them to the Nautile.
4) Prepare fixative and rinsing solutions in a fume hood:
    - Formaldehyde 3% from formaldehyde 10% methanol free (PolySciences) and seawater filtered at 0.22 µM,
    - Glutaraldehyde 2.5% from glutaraldehyde 25%, filtered sea-water and filtered milli-Q water,
    - Storage solution for SEM/TEM with sodium azide 0.043 g/100 ML filtered sea-water,
    - PBS1X from PBS10X and filtered milli-Q water
    - PBS2X/ethanol 50:50 mix from PBS10X, filtered milli-Q water and absolute ethanol
5) Filter all solutions at 0.22 µM
6) Fill 1.8 mL cryotubes in a fume hood with 2.5% glutaraldehyde or 3% formaldehyde
7) Prepare the frame to print the tube identification labels: cruise, dive number, sampling instrument, shrimp number, dissected tissue, conditioning, date

Part 2: Sampling

8) Once the area of interest has been identified, carry out the chemistry measurements with CHEMINI sulphides and CHEMINI iron, take the PIF samples, note the temperature and all starts and stops of measurments.
9) To suck up shrimp, use the Nautile slurp gun to sample into the suction sampler bowls or the Periscopette to then transfer shrimps to the PERISCOP.

Part 3: Samples processing – selection of collected animals

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10) Prepare the luminar flow hood to be sterile. Decontaminate the dissection tools by immersing them in an ethanol 96° bath followed with 5 min in DNA Exitus or DNA away and rinse them with sterile milli-Q water. Prepare sterile petri dishes.
11) On arrival on board either the PERISCOP in the lift, or the submersible, remove the Periscopette or bowls of suction sampler and transfer animals in an 8L clean plastic tray at 6°C in a cold chamber.
12) Select individuals of interest and transfer them individually into 125 ml screw jars filled with sterile seawater. Assign a number to the shrimp with a marker on the jar.
13) Identify the species: *Rimicaris exoculata* or *Rimicaris chacei* (Figures 1A and 1B for adults). Use a binocular magnifying glass to identify juveniles if necessary (Figure 2a or 2b) and identify life-stage (Methou et al., 2020).
14) Measure the cephalothoracic cavity length using a caliper and note information.
15) With forceps, remove Pleopod #2 for adults and place it in a 1.5 mL Eppendorf for observation under binocular magnifying and sex identification (Figure 1C). As juvenile shrimp are sexually immature, this identification is omitted for juveniles.
16)

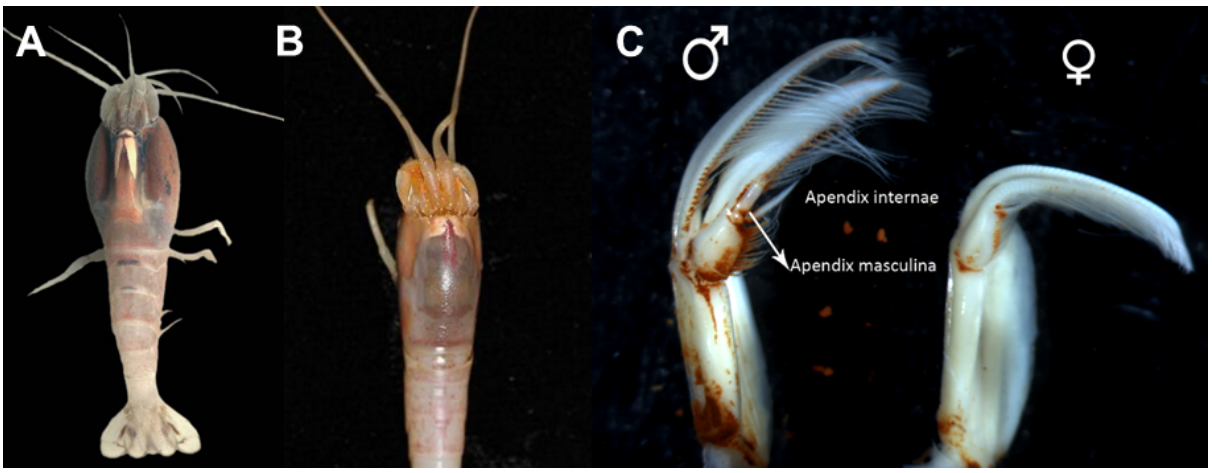

Figure 1 : pictures to identify species and sex. (A) Rimicaris exoculata adult (B) Rimicaris chacei adult (C) pleopod #2 male on left and female on right

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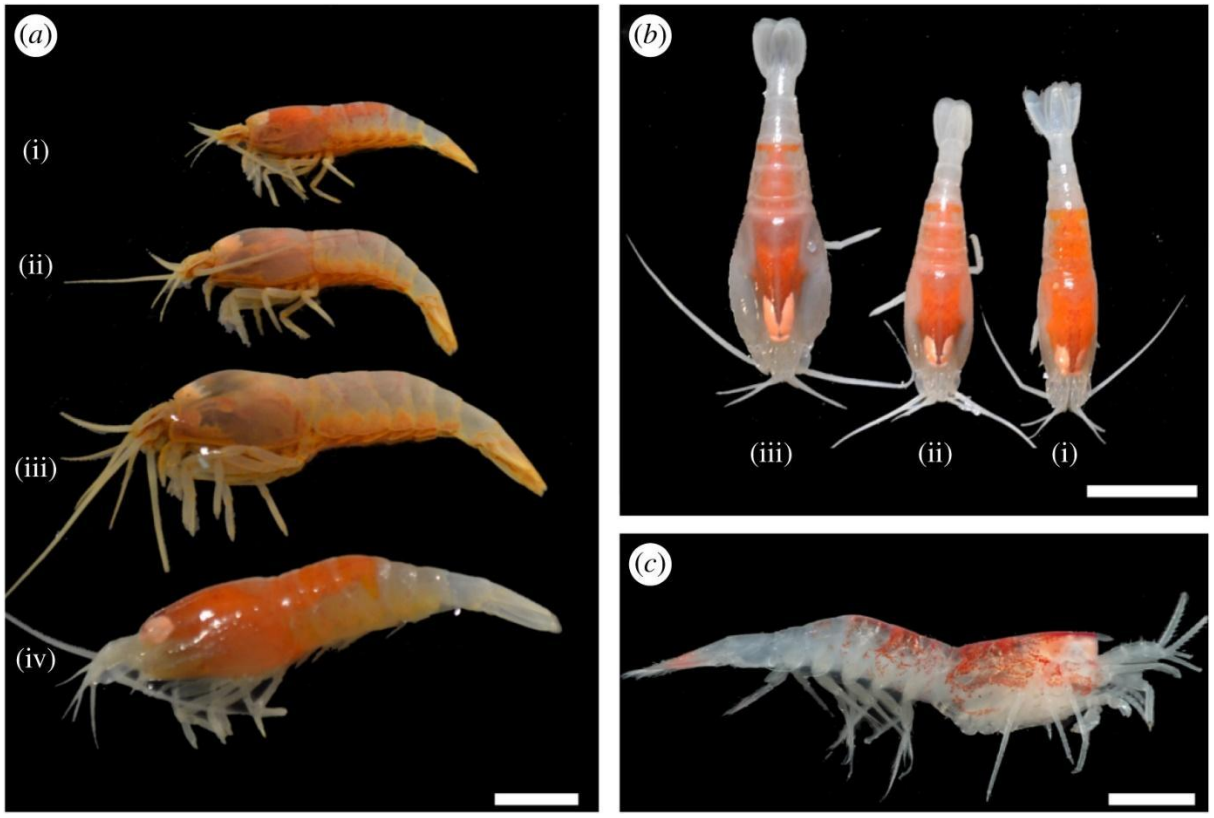

Figure 2: Juvenile stages of alvinocaridids from Mid-Atlantic Ridge vents according to the revised classification. (a) Different life stages of R. chacei: (i) stage A juvenile, (ii) stage B juvenile (or subadult) and (iii) small adult, compared with (iv) an early stage A juvenile of R. exoculata. Scale bar = 5 mm. (b) Different juvenile stages of R. exoculata: (i) stage A juvenile, (ii) stage B juvenile, (iv) stage C (or subadult). Scale bar = 10 mm. (c) A small individual of Mirocaris fortunata, morphologically similar to stage A juveniles of R. chacei and found in the same locations. Scale bar = 2 mm (Methou et al., 2020).

17) Once the shrimp have been selected and the number assigned, complete the label file to start printing and affix them to the tubes

Part 4: Samples processing – dissection of *Rimicaris* adults

18) Place 4 jars containing shrimp in an ice tray and transfer to laminar flow hood.
19) Place a petri dish on a cooling block and place a shrimp in it
20) Dissect shrimp into different parts (Figure 3):
    - Cephalothoracic cavity: split the 2 sides and dissect both branchiostegites, exopodites and scaphognathites. Pool together exopodite and scaphognathite per side.
    - Foregut
    - Midgut
    - Hepatopancreas
    - Gonads if present
    - Eggs if present, separe in 3 tubes
    - Telson
    - Muscle crumbled with scalpel and scissors

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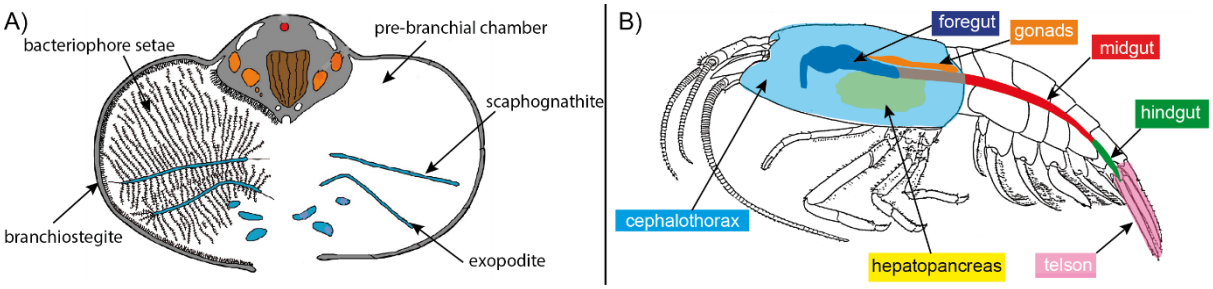

Figure 3 : Schematic drawing of the location of the various dissected tissues of the Rimicaris exoculata shrimp. (A) Schematic cross-section of the cephalothorax (adapted from Segonzac et al.(Segonzac et al., 1993)). (B) lateral view (adapted from Williams and Rona and Zbinden et al. (Williams and Rona, 1986; Zbinden et al., 2004)) on which the digestive tract and other internal tissues under cephalothorax are represented.

21) Rince tissues in sterile seawater and then placed in 1.8 mL cryotubes depending on the intended analysis:
    - Telson is placed in 96° ethanol and conserved at 4°C.
    - The muscle is dedicated to genetic analysis and flash frozen with liquid nitrogen before storage at -80°C.
    - There is always a half-cephalothorax dedicated to genetic analysis with same preservation than muscle.
    - For the other half cephalothorax and the other tissues, we will try to respect the following diagram per batch of 4 same species shrimps and preferentially 4 same sex :

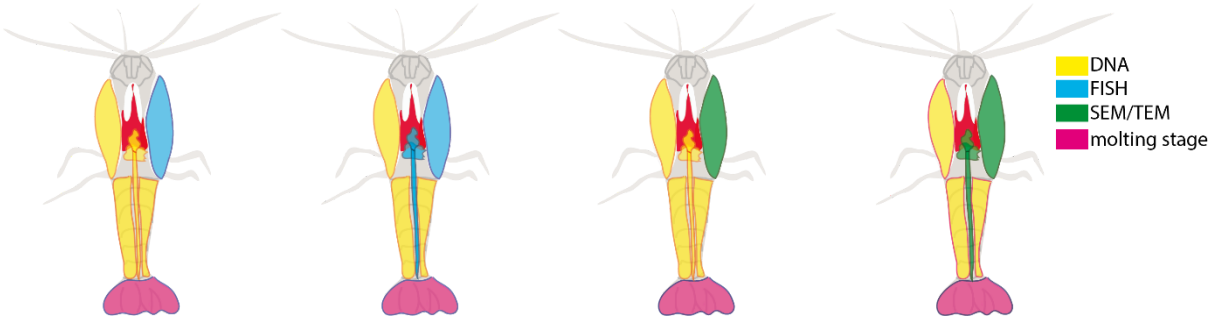

Figure 4 : conditioning diagram of the various tissues according to the planned analyses

Per batch of 4 shrimps of same species: 4 cephalothoraxes for DNA, 2 cephalothoraxes for FISH, 2 cephalothoraxes for SEM/TEM. For other tissues (digestive tract, gonads): 2 for DNA, 1 for FISH, 1 for SEM/TEM. If there are eggs, divide them into 3 parts and process for DNA, FISH and SEM/TEM.

22) Place the remaining shrimp tissues in a 5 mL Eppendorf tube, flash freeze in liquid nitrogen and store at -80°C.
23) Place dissecting tools in decontamination baths between each dissection.
24) Complete the metadata file with specimens data.

Part 4bis: Samples processing – dissections of *Rimicaris* juveniles

25) Place 3 jars containing shrimp in an ice tray and transfer to the laminar flow hood.
26) Place a petri dish on a cooling block and place a shrimp inside it
27) Dissect shrimp into different parts under binocular magnifying glass:

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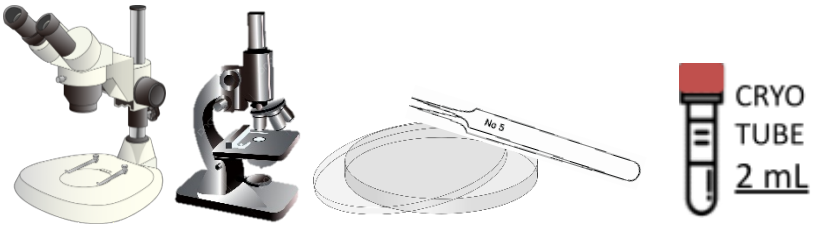
- Cephalothoracic cavity: separe the 2 sides but pool together branchiostegite, exopodite and scaphognathite per side.
- Foregut
- Midgut
- Hepatopancreas
- Muscle crumbled with scalpel and scissors

28) Rince tissues in sterile seawater and then placed in 1.8 mL cryotubes depending on the intended analysis:
    - The muscle is dedicated to genetic analysis and flash frozen with liquid nitrogen before storage at -80°C.
    - There is always a half-cephalothorax dedicated to genetic analysis with the same preservation as muscle.
    - For the other half cephalothorax, half for FISH and half for SEM/TEM
    - For the digestive tract, we need at least 10 specimens for genetic analyses per stage and species. If more specimens, mix between conditioning for FISH and for SEM/TEM.
29) store at -80°C.
30) Place dissecting tools in decontamination baths between each dissection.
31) Complete the metadata file with specimens data.

Part 5: Samples processing - fixations

32) During dissection, place the tissue directly into the fixative: formaldehyde 3% / SW for FISH and glutaraldehyde 2.5% /SW for SEM/TEM
33) For FISH, after 2h fixing in 3% formaldehyde / SW at 4°C, replace the fixative with PBS1X using a plastic transfer pipet and perform 2 successive rinses, then store at -20°C in ethanol / PBS 2X 50:50 in a cryobox. Use the chemical fume hood.
34) For SEM/TEM, fix for 4h to 15h at 4°C. Then replace fixative with sodium azide storage solution using a plastic transfer pipet. Use the chemical fume hood. Store in a cryobox at 4°C.

Protocol 3: *Oncholaimus dyvae* nematods for diversity analysis



Part 1: Preparation of sampling and conditioning material

1) Clean push core tubes thoroughly with hot water and washing-up liquid. Rinse well. Do not use alcohol, as this will damage the plastic.

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2) Clean biobox thoroughly with hot water and washing-up liquid. Rinse well. Clean interior surfaces with ethanol using a paper towel and ethanol. Fill with fresh water filtered to 0.22 µM. Close the lid
3) Prepare fixative and rinsing solutions in a fume hood:
    - Formaldehyde 3% from formaldehyde 10% methanol free (PolySciences) and seawater filtered at 0.22 µM
    - Glutaraldehyde 2.5% from glutaraldehyde 25%, filtered sea-water and filtered milli-Q water
    - Storage solution for SEM/TEM with sodium azide 0.043 g/100 ML filtered sea-water
    - PBS1X from PBS10X and filtered milli-Q water
    - PBS2X/ethanol 50:50 mix from PBS10X, filtered milli-Q water and absolute ethanol
4) Filter all solutions at 0.22 µM
5) Fill 1.8 mL cryotubes in a fume hood with 2.5% glutaraldehyde or 3% formaldehyde

Part 2: Sampling

6) Either the nematodes will be found in the byssus of mussels collected with the Nautile's forceps and placed in a biobox,
7) Or the nematodes will be found in the first few centimeters of sediment collected with a push core.

Part 3: Samples processing – selection of collected animals

Process core sediment

8) Place the 5 first cm of the core in a 4% formol solution (2x the volume of sediment).

Process isolated nematodes (opportunistic sampling from bioboxes and byssus): priority for FISH, Betain and DNA fixation.

9) FISH and 3D Analyses: Place X specimens in a 2mL cryotube in 3% formaldehyde solution, store for 2h, rinse fixative on a 20µm sieve with 1X PBS, replace sediment in absolute ethanol – 2X PBS (1:1) and store at -20°C.
10) Betain fixation for coupling Imaging/DNA analysis: Place X (opportunistic, possible multiple specimens in 1 tube) specimens in 2mL cryotube in 1mL of 10% Betain solution in filtered sea-water in, flash frozen in liquid nitrogen before storage at -80°C.
11) DNA fixation: place one specimen in a 2mL cryotube, flash freezing with liquid nitrogen before storage at -80°C.
12) SEM analyses: Place specimens in a 2mL cryotube in glutaraldehyde 2.5% for 16h at 4 °C52, then transferred to a sodium azide solution (0.065g in 150ml fltered sea water) and stored at 4°C.

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2.12.8. Samples processing, transient storage, shipping conditions and Biobanking

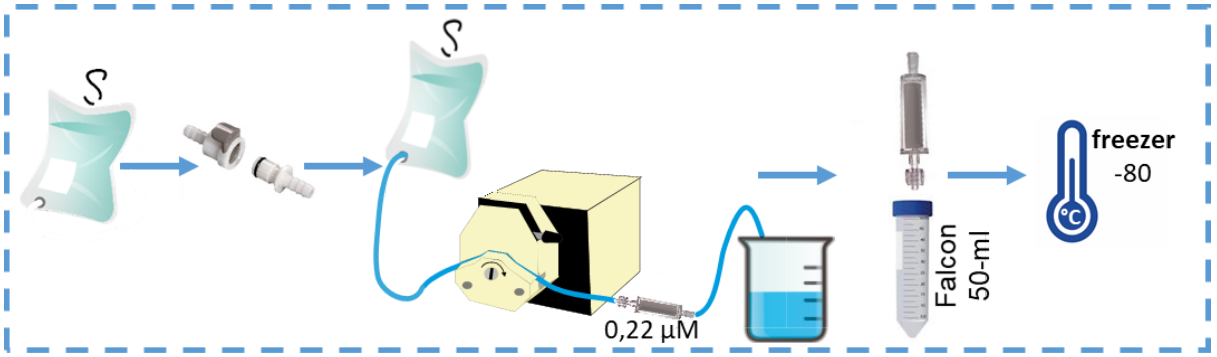
On board the vessel, samples for genetic are stored in deep-freezer -80°C until the cruise end. Samples for FISH microscopy are kept in -20°C freezer and samples for SEM/TEM in a fridge at 4°C.

At the end of the cruise, the frozen samples will be shipped in carbo ice and the 4°C samples will transit in refrigerated containers to the laboratory.

Upon arrival at the laboratory, the samples will be stored either in the -80°C freezer, or in the -20°C frozen room, or in the 4°C cold room. The storage spaces will be filled in the MORSE database for archiving monitoring.

2.12.9. Flow charts (used to guide the processing of samples during stations)

Protocol 1: seawater surrounding different populations



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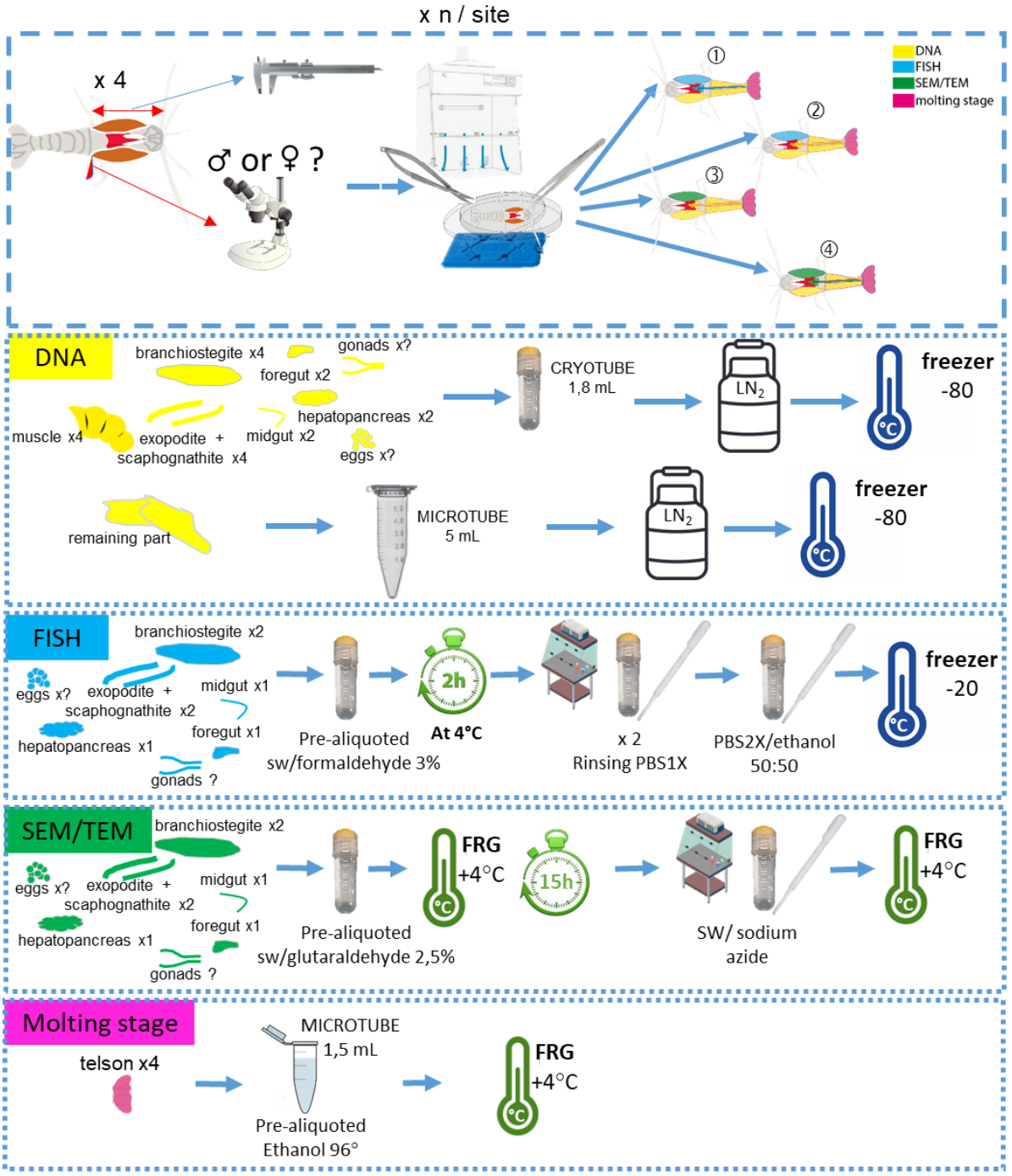
Protocol 2: *Rimicaris* adult shrimp



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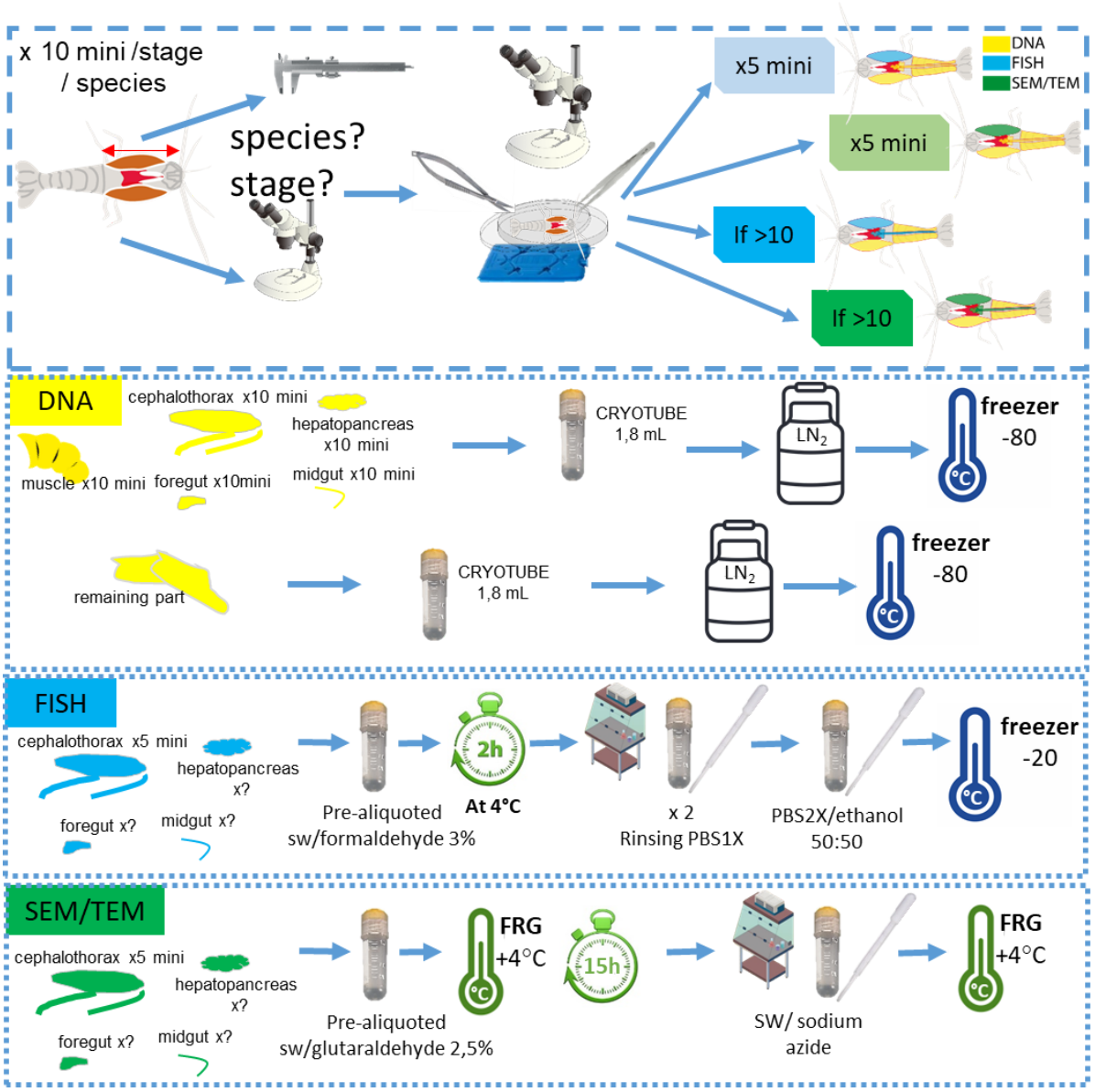
Protocol 2bis: *Rimicaris* juvenile shrimp



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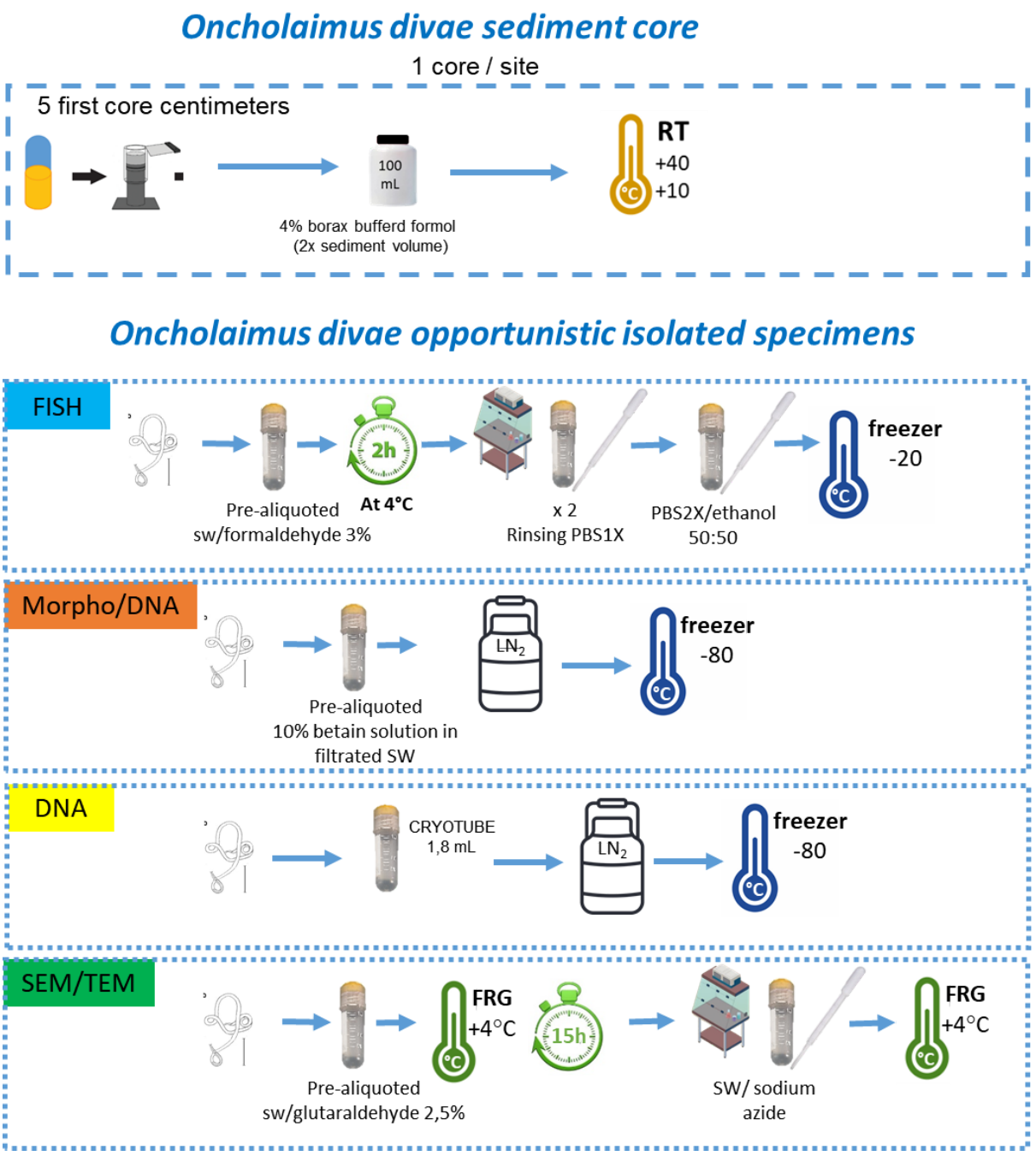
Protocol 3: *Oncholaimus dyvae* nematodes



Bibliography

Methou, P., Michel, L., Segonzac, M., Cambon-Bonavita, M.-A., Pradillon, F., 2020. Integrative taxonomy revisits the ontogeny and trophic niches of *Rimicaris* vent shrimps. Royal Society Open Science 7. https://doi.org/10.1098/rsos.200837

Segonzac, M., Desaintlaurent, M., Casanova, B., 1993. Enigma of the trophic adaptation of the shrimp Alvinocaridae in hydrothermal areas along the mid-atlantic ridge. Cahiers De Biologie Marine 34, 535–571.

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Shillito, B., Amand, L., Hamel, G., 2023. Update of the PERISCOP system for isobaric sampling of deep-sea fauna. Deep Sea Research Part I: Oceanographic Research Papers 193.

Shillito, B., Hamel, G., Duchi, C., Cottin, D., Sarrazin, J., Sarradin, P.M., Ravaux, J., Gaill, F., 2008. Live capture of megafauna from 2300m depth, using a newly designed Pressurized Recovery Device. Deep Sea Res. (I Oceanogr. Res. Pap.) 55, 881–889. https://doi.org/10.1016/j.dsr.2008.03.010

Vuillemin, R., Le Roux, D., Dorval, P., Bucas, K., Sudreau, J.P., Hamon, M., Le Gall, C., Sarradin, P.M., 2009. CHEMINI: A new in situ CHEmical MINIaturized analyzer. Deep Sea Research Part I: Oceanographic Research Papers 56, 1391–1399. https://doi.org/10.1016/j.dsr.2009.02.002

Williams, A.B., Rona, P.A., 1986. Two new caridean shrimps (bresiliidae) from a hydrothermal field on the Mid-Atlantic Ridge. Journal of Crustacean Biology 6, 446–462. https://doi.org/10.1163/193724086X00299

Zbinden, M., Le Bris, N., Gaill, F., Compere, P., 2004. Distribution of bacteria and associated minerals in the gill chamber of the vent shrimp *Rimicaris exoculata* and related biogeochemical processes. Marine Ecology-Progress Series 284, 237–251.

2.12. Deep-sea nematodes

2.13.1. Authors, affiliations and contact

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2.13.2. Introduction

To understand biodiversity patterns, functional roles of abyssal species in biogeochemical cycles, ecological networks and to try to anticipate the direct and indirect impacts of future mining activities, an integrative microscopy (3D morphological acquisition) and multi-omics approach will be performed on nematodes, the most abundant metazoans in deep-sea sediment, and their associated with microorganisms. Sediments will be sampled in different deep-sea habitats including hydrothermal inactive sediments (Mid-Atlantic Ridge), polymetallic nodules (Pacific Ocean) and other abyssal sediment systems.

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2.13.3. Sampling Strategy

Please refer to the sampling strategy reported at point 2.12.3. Possible opportunistic deep-sea sampling are forecasted.

2.13.4. Sampling tools and equipment

Nematodes sampling

- Nautile's suction sampler or multicore
- Laminar flow hood
- Chemical fume hood
- Binocular magnifier
- Dissecting tools : scalpel, micro-scissors, forceps, needle
- Petri dishes
- Ice pack
- 1.8 mL cryotubes
- 5 mL Eppendorf tube
- Cryotube boxes
- 125 ml plastic screw-top jars
- Icebox with crushed ice
- Plastic transfer pipet
- Liquid nitrogen
- -80°C deep-freezer
- Paper towel
- Gloves
- Sterile filtered sea-water
- Ethanol 96°
- DNA away or DNA exitus
- Formaldehyde 3%
- PBS1X / mix PBS2X/ethanol 50 :50
- Glutaraldehyde 2.5%
- Seawater with 0.043% sodium azide
- Microscope

2.13.5. Metadata collection

The metadata associated with the sampling performed by the submersible are downloaded via the ADELIE software. Once the navigation data have been corrected, they will be added to the SISMER database, which includes all cruise data from the French oceanographic fleet.

The metadata associated with the sample itself (species, life stage, sex, measurements, tissue dissected, fixative, conditioning) are recorded in a spreadsheet shared by all the

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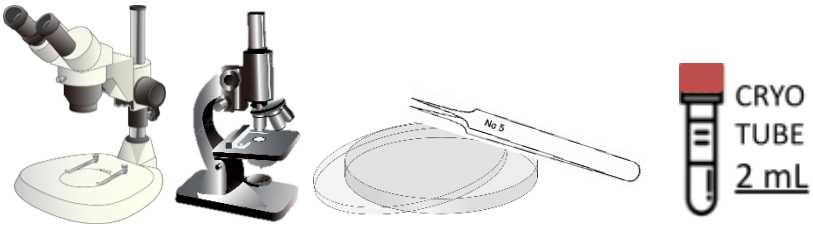
scientists on the mission, part of which is then uploaded into LabCollector® and transmitted to Ifremer's MORSE software for sample traceability.

2.13.6. Environmental parameters measurement

Depending on the cruise

2.13.7. Sampling protocol

Protocol 3: *Oncholaimus dyvae* nematods for diversity analysis



Part 1: Preparation of sampling and conditioning material

13) Clean push core tubes thoroughly with hot water and washing-up liquid. Rinse well. Do not use alcohol, as this will damage the plastic.
14) Clean biobox thoroughly with hot water and washing-up liquid. Rinse well. Clean interior surfaces with ethanol using a paper towel and ethanol. Fill with fresh water filtered to 0.22 µM. Close the lid
15) Prepare fixative and rinsing solutions in a fume hood:
    - Formaldehyde 3% from formaldehyde 10% methanol free (PolySciences) and seawater filtered at 0.22 µM
    - Glutaraldehyde 2.5% from glutaraldehyde 25%, filtered sea-water and filtered milli-Q water
    - Storage solution for SEM/TEM with sodium azide 0.043 g/100 ML filtered sea-water
    - PBS1X from PBS10X and filtered milli-Q water
    - PBS2X/ethanol 50:50 mix from PBS10X, filtered milli-Q water and absolute ethanol
16) Filter all solutions at 0.22 µM
17) Fill 1.8 mL cryotubes in a fume hood with 2.5% glutaraldehyde or 3% formaldehyde

Part 2: Sampling

18) Either the nematodes will be found in the byssus of mussels collected with the Nautile's forceps and placed in a biobox,
19) Or the nematodes will be found in the first few centimeters of sediment collected with a push core.

Part 3: Samples processing – selection of collected animals

Process core sediment

20) Place the 5 first cm of the core in a 4% formol solution (2x the volume of sediment).

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Process isolated nematodes (opportunistic sampling from bioboxes and byssus): priority for FISH, Betain and DNA fixation.

21) FISH and 3D Analyses: Place X specimens in a 2mL cryotube in 3% formaldehyde solution, store for 2h, rinse fixative on a 20µm sieve with 1X PBS, replace sediment in absolute ethanol – 2X PBS (1:1) and store at -20°C.
22) Betain fixation for coupling Imaging/DNA analysis: Place X (opportunistic, possible multiple specimens in 1 tube) specimens in 2mL cryotube in 1mL of 10% Betain solution in filtered sea-water in, flash frozen in liquid nitrogen before storage at -80°C.
23) DNA fixation: place one specimen in a 2mL cryotube, flash freezing with liquid nitrogen before storage at -80°C.
24) SEM analyses: Place specimens in a 2mL cryotube in glutaraldehyde 2.5% for 16h at 4 °C52, then transferred to a sodium azide solution (0.065g in 150ml fltered sea water) and stored at 4°C.

2.13.8. Samples processing, transient storage, shipping conditions and Biobanking

On board the vessel, samples for genetics are stored in -80°C until the cruise ends. Samples for FISH microscopy are kept in -20°C freezer and samples for SEM/TEM in a fridge at 4°C.

At the end of the cruise, the frozen samples will be shipped in carbo ice and the 4°C samples will transit in refrigerated containers to the laboratory.

Upon arrival at the laboratory, the samples will be stored either in the -80°C freezer, or in the -20°C frozen room, or in the 4°C cold room. The storage spaces will be filled in the MORSE database for archiving monitoring.

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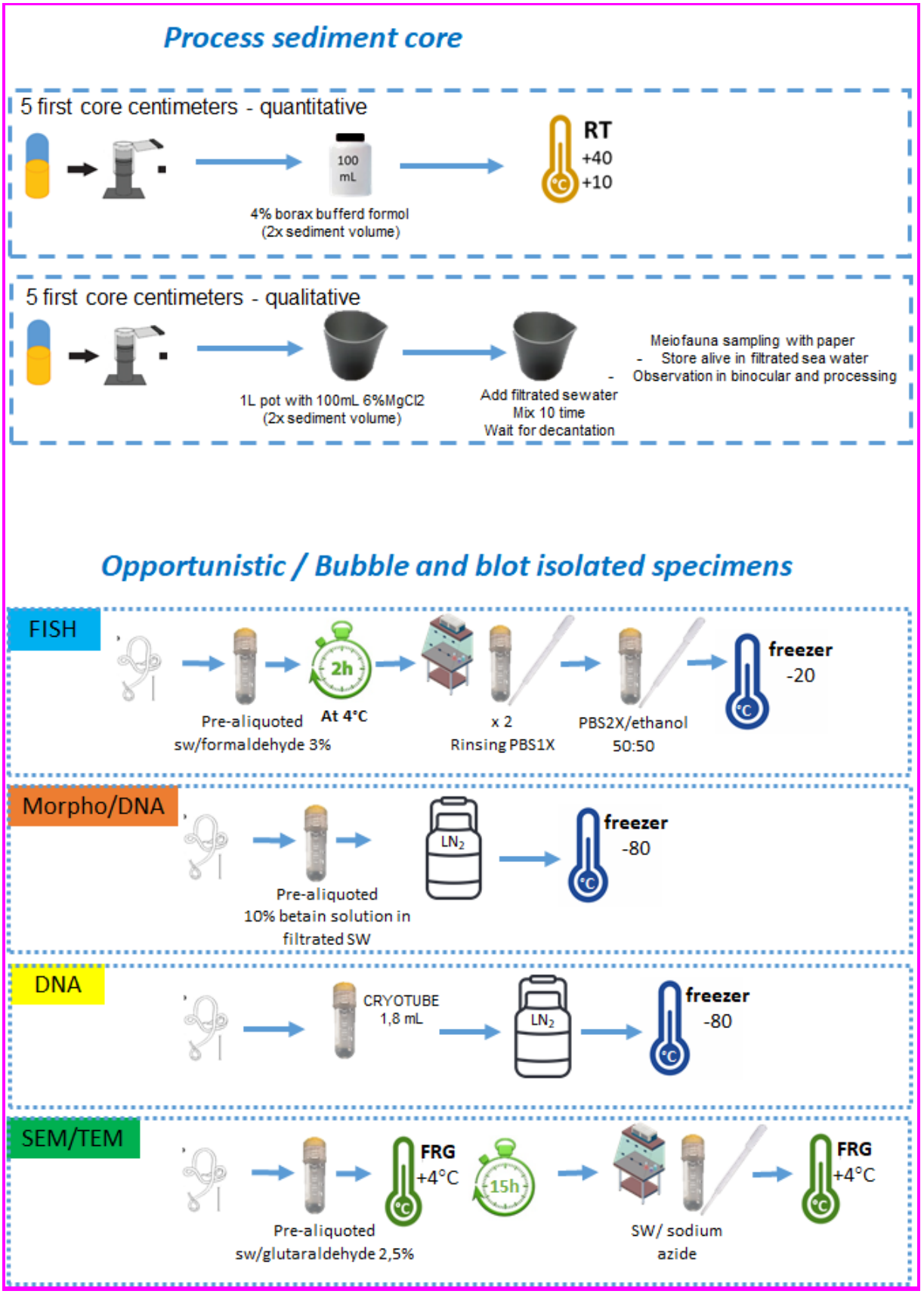
2.13.9. Flow charts (used to guide the processing of samples during stations)



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2.13. Oysters

2.14.1. Authors, affiliations and contact

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2.14.2. Introduction

Cupped oyster samples (Crassostrea gigas) samples are collected in the frame of the observatory systems ROME (Reseau d'Observatoires de Microbiologie Environnementale Integrée) (https://rome.ifremer.fr/) deployed by Ifremer in four estuarine ecosystems, where aquaculture farms are implemented

2.14.3. Sampling Strategy

15 specimen of cultivated adult oysters are collected monthly in each of the four sampling sites (Bay de Veys (Normandy), Brest Bay (Brittany), Marennes D'Olerons (New Aquitania), Thau lagoon (Occitania) of the ROME network. Oysters should be of commercila size (>8cm) and they have been present on site sicne at least 6 months.

2.14.4. Sampling tools and equipment

1. Knife
2. Cooler
3. Gloves
4. Meter
5. Plastic bag

2.14.5. Metadata collection

1. Oyster size

2.14.6. Environmental parameters measurement

Sea water temperature and salinity are collected in coincidence with the sampling

2.14.7. Sampling protocol

Oysters are collected from specific culture bags dedicated to the ROME projects. Once collected from the bags with gloves, oysters rinsed with seawater, put in a plastic bag and kept in the cooler. Samples should not be frozen

2.14.8. Samples processing, transient storage, shipping conditions and Biobanking

Samples are kept into the cooler until the laboratory, where the are stocked at 4°C. Samples are lnamed according to the ROME protocol nomenclature and biobanking criteria.

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2.14.9. Flow charts

2.14. Toxic dinoflagellate *Alexandrium minutum*

2.14.1. Authors, affiliations and contact

Le Gac Mickaël and Siano Raffaele, Ifremer, France

2.14.2. Introduction

In this study, we aim to determine the intraspecific diversity of the toxic dinoflagellate *Alexandrium minutum* using multiplex amplicon sequencing on seawater samples containing vegetative cells.

2.14.3. Sampling Strategy

Following initial *A. minutum* detection by the French Observation and Monitoring program for Phytoplankton and Hydrology in coastal waters (Rephy, https://bulletinrephytox.fr/accueil) water samples are collected as follows:

1. Subsurface concentrated water samples are obtained by towing 20µm plankton nets for ~30s. Samples are then prefiltered using 200µm nylon mesh and transferred to 500mL bottles. Samples are then filtered on 20µm polycarbonate filters. Filters are immediately flash frozen and stored at -70°C until DNA extraction. These samples are used to determine *A. minutum* population allele frequency using multiplex amplicon sequencing.
2. Additional subsurface water is sampled using a bucket. Samples are prefiltered using a 200µm nylon mesh, transferred to a 5L can and kept in the dark, in an icebox containing ice packs during transportation (<3h). Samples are then filtered on 20µm polycarbonate filters. Filters are immediately flash frozen and stored at -70°C until DNA extraction. These samples are used to determine *A. minutum* abundances using qPCR (Klouch et al. 2016).

2.14.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• 10L bucket • 5L cans • funnel • 500mL bottles • 200µm nylon mesh • 20µm plankton net • Icepaks • Coolers	• 20µm polycarbonate filters • Liquid nitrogen • Cryotubes • Tweezers	

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• Persistaltic pump with 47mm filter holders • vacuum pump with 47mm filter holders • Beakers		

2.14.5. Metadata collection

- Sample_name Date
- Sample_point_name
- Latitude
- Longitude
- Sampling_time Filtered_volume
-

2.14.6. Environmental parameters measurement

Not aplicable

2.14.7. Sampling protocol

On the Field

Water samples

1. Rinse water bucket, funnel, nylon mesh 3 times
2. Fill bucket with water
3. Place funnel and 200µm nylon mesh on top of a 500mL bottle
4. Transfer sample from cod end to 5l can through the 200µm nylon mesh (fill 5L can)
5. Place closed 5L can in cooler with ice packs

Concentrated water sample

1. Rinse cod end and plankton net without cod end 3 times
2. Screw cod end to plankton net
3. Cast plankton net and tow for a total of ~30s (if sampling from shore, several tows may be required to reach the 30s)
4. Fill bucket with water
5. Rinse inner side of plankton net using the bucket
6. Unscrew cod end
7. Place funnel and 200µm nylon mesh on top of a 500mL bottle
8. Transfer sample from cod end to 500mL bottle through the 200µm nylon mesh
9. Place closed 500mL bottle in cooler with ice packs

After sampling

Rinse water bucket, funnel, nylon mesh, cod end and plankton net without cod end 3 times.

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2.14.8. Samples processing, transient storage, shipping conditions and Biobanking

In the lab, wear a lab coat, gloves. Decontaminate all the surface area, and equipment with Ethanol 70% and DNA away.

In the lab

Water sample (wear clean gloves)

1. Rinse peristaltic pump filtration system using 70% ethanol during ~30sec
2. Rinse peristaltic pump filtration system using water sample during ~30sec
3. Use a DNA free tweezer to place a 47mm 20µm polycarbonate filter on filter holder
4. Filter 4-5 L of water sample
5. Record filtered volume
6. Use a DNA free tweezer to roll a 47mm 20µm polycarbonate filter, place it in a cryotube.
7. Flash freeze in liquid nitrogen and store at -70°c
8. Rinse peristaltic pump filtration system using 70% ethanol during ~30sec

Concentrated water sample

1. Rinse the vacuum pump filtration system using 200mL of 70% ethanol
2. Use a DNA free tweezer to place a 47mm 20µm polycarbonate filter on filter holder
3. Filter concentrated water sample
4. Use a DNA free tweezer to roll a 47mm 20µm polycarbonate filter, place it in a cryotube.
5. Flash freeze in liquid nitrogen and store at -70°c
6. Rinse peristaltic pump filtration system using 200mL of milliQ water
7. Rinse peristaltic pump filtration system using 200mL of 70% ethanol

After filtration

1. Rinse water bucket, funnel, nylon mesh, cans, cod end and plankton net 3 times with fresh water
2. Rinse cans with 70% ethanol

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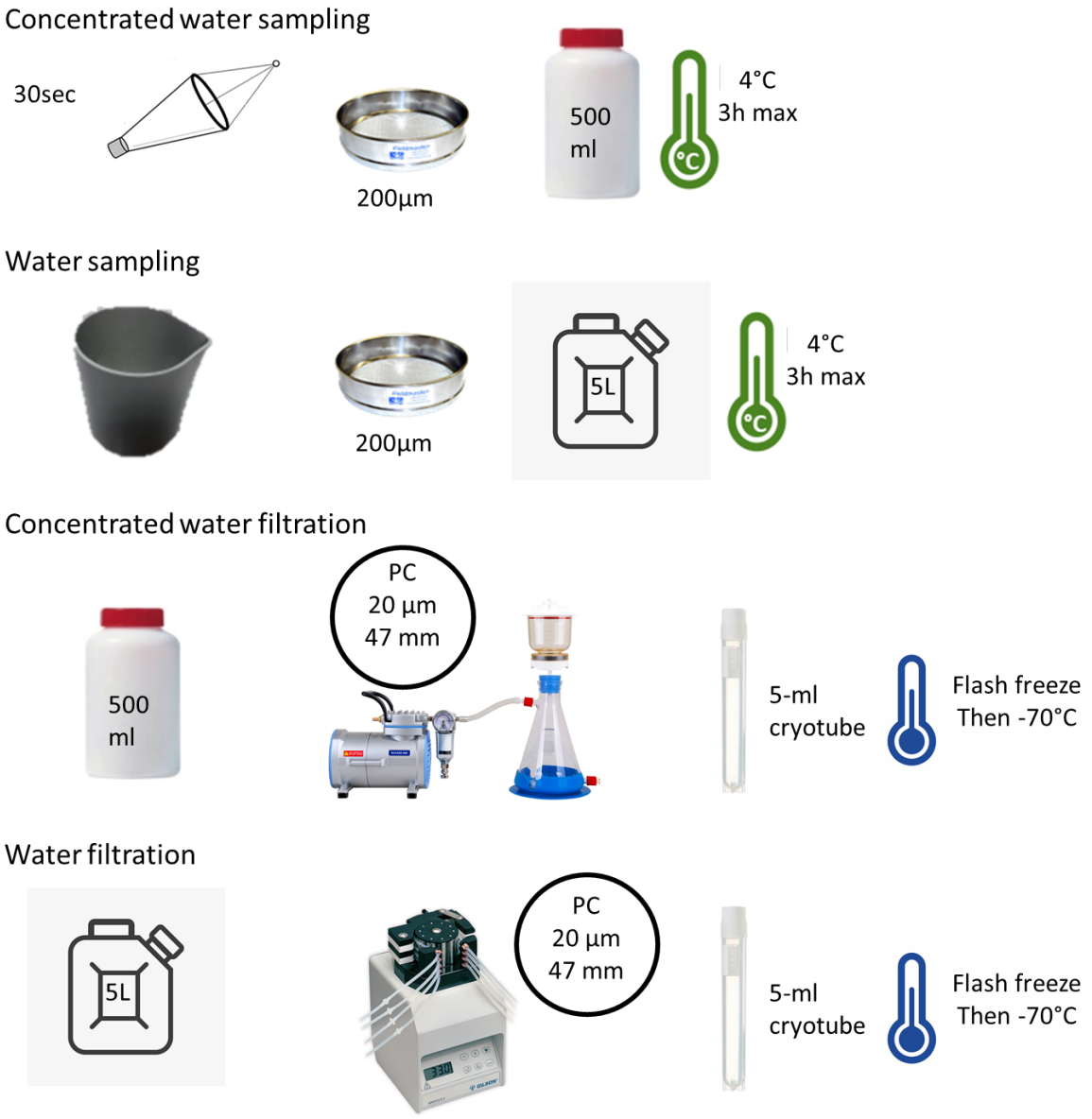
2.14.9. Flow charts (used to guide the processing of samples during stations)



2.15. Deep Water corals *Madrepora oculata* and *Desmophyllum pertusum*

2.15.1. Authors, affiliations and contact

Jeslin Claire, UMR BEEP, Université de Bretagne Occidentale, Meunier Valentine, UMR BEEP, Ifremer, Mathieu-Resuge Margaux, UMR BEEP, Ifremer, Michel N. Loïc, FOCUS, University of Liège, Menot Lénaïck, UMR BEEP, Ifremer

2.15.2. Introduction

The purpose of these protocols is to describe the sampling of specimens of two reef-building cold-water coral speciescorals (*Madrepora oculata and Desmophyllum pertusum*) to study their associated virome , as well as the trophic ecology of the corals as holobionts.

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These protocols may apply to coral-water coral specimens and seawater sampled *in-situ* or in experimental tanks.

To study the virome, we use sequencing and imaging technologies. To study the trophic ecology we analyse C/N isotopic ratios and lipid content. The protocols below present how specimens are collected from the field and experimental tanks, and how they are further processed in the laboratory for these analyses.

2.15.3. Sampling Strategy

In-situ, samples are collected across the range of their realised niches in the Lampaul submarine canyon (Bay of Biscay, NE Atlantic), including ecotones where the two species are associated. Coral colonies and seawater are sampled with the ROV Victor 6000. Coral colonies are collected with the arm of the ROV then stored in insulated and water-tight boxes. Seawater is pumped into 5L plastic bags. Once onboard biological and water samples are kept in a cold lab at 10°C until further processed.

In experimental tanks, samples are collected according to treatments (Control, Warming, Acidification, Warming+Acidification).

Whenever possible, coral specimens are sampled in triplicates, from three different colonies.

2.15.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
Polyp sampling • Cutting pliers *Mucus sampling for virome* • *Seawater filtration for microbiome and virome* • Filtration system including funnel, filter support, flask and vacuum pump • Flat nose pliers *Seawater filtration for trophic ecology* • Filtration system including funnel, filter support, flask and vacuum pump • Flat nose pliers	*Polyp sampling* • Cryotubes • Falcons tubes • Gloves *Mucus sampling for virome* • Cryotubes 2 mL • Gloves *Seawater filtration for microbiome and virome* • 0.22 µm isopore filter (GTTP) • 0.02 µm filters (Whatman Anodisc inorganic filter membrane) • Petri slides • 5 L glass bottle • 2 L glass bottle • Gloves	*Polyp sampling* • Ethanol • RNase decontamination solution • Distilled water *Mucus sampling for virome* • Formaldehyde 6% • Glutaraldehyde 25% *Seawater filtration for microbiome and virome* • Ethanol • RNase decontamination solution *Seawater filtration for trophic ecology* • Distilled water • Ethanol

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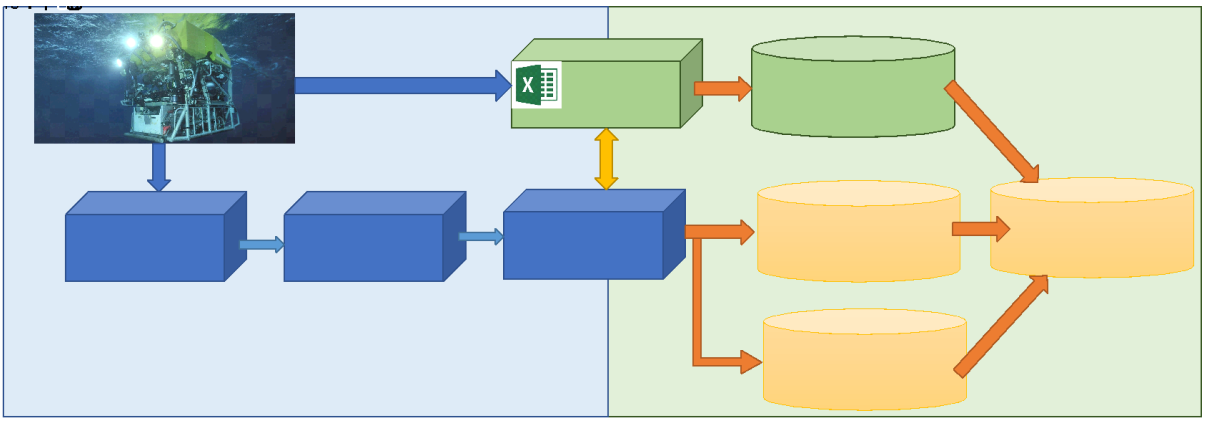
• Kettle	*Seawater filtration for trophic ecology* • GF/F filters burned at 450°C for 6h • 5 L bottle • Cryotubes • Gloves	

2.15.5. Metadata collection

Metada flow is illustrated on the figure below. During an ROV dive, sampling operations are annotated in Mimosa, a dive logbook software. At the end of a dive, the Adelie software formats the annotations made in Mimosa, the annotations are time coded and georeferenced. The output files from Adelie are uploaded into Sealog where sampling events are created that combine timestamp, geographic coordinates and depth with a sampling equipment.

Information regarding physical samples (sampling equipment, taxon, subsampling, fixative, preservation, data owner) are filled in a standardised spreadsheet. Each coral colony and each subsample from a colony is given a unique ID. These unique IDs are also filled in Sealog. This way, sampling metadata are associated with sample metadata.

On shore, the standardised spreadsheet is uploaded into a Labcollector database. Information regarding location of storage is added. The sealog offline database is uploaded into BIGOOD, an oracle database for biological and geological samples. Cruise metadata are uploaded into the “Catalogue des campagnes SISMER” and openly available after the cruise.



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2.15.6. Environmental parameters measurement

The CTD of the ROV provides data on Temperature, Salinity and Oxygen. Additional water samples are collected for pH, alkalinity and nutrients.

2.15.7. Sampling protocol

Polyp sampling for microbiome, virome, trophic ecology

1. Wear gloves, clean the cutting pliers with ethanol followed by RNase decontamination solution, rinse with distilled water.
2. Keep the coral colony in cold seawater as much as possible, hold the coral colony at the base where there is no living polyp.
3. Use the cutting pliers to sample a branch of 5-10 polyps
4. Place the sample in a cryotube or falcon, label the tube with a unique sample ID and store at -80°C.

Mucus sampling for virome

1. Wear gloves
2. Hold a coral branch above 2 mL cryotubes, collect 1 mL of mucus in each of three cryotubes, label each cryotube with a unique sample ID.
3. Store one cryotube at -80°C.
4. Add 1 mL formaldehyde 6% in a second tube, and store at 4°C.
5. Add 20 µL glutaraldehyde 25% in a third tube, and store at 4°C.

Seawater filtration for microbiome and virome

1. Wear gloves, clean 2 L and 5 L bottles, flat nose pliers, as well as funnel, filter support and flask from the filtration system with ethanol followed by RNase decontamination solution, rinse with distilled water.
2. Pour the seawater from the bag to the 5 L bottle
3. Set up the filtration system, use the flat nose pliers to place the 0.22 µm filter on top of the filter holder.
4. Pour the seawater from the 5 L bottle into the funnel of the filtration system until the 5 L have been processed, save 2 L of the filtrate in the 2 L bottle.
5. Remove the 0.22 µm filter with the flat nose pliers, fold it and put it in a cryotube, label the cryotube with a unique sample ID, and store the cryotube at -80°C.
6. Put the 0.02 µm on the filter holder with the flat nose pliers
7. Pour 500 mL of the filtrate from 2 L bottles into the funnel. When the volume has been processed, remove the 0.22 µm filter with the flat nose pliers, fold it and put it in a cryotube, label the cryotube with a unique sample ID, and store the cryotube at -80°C.
8. Repeat step 7 three times on three different 0.02 µm filters until all the 2 L filtrate have been processed.

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Seawater filtration for trophic ecology

1. Wear gloves, clean 5 L bottle, flat nose pliers, as well as funnel, filter support and flask from the filtration system with ethanol, rinse with distilled water.
2. Pour the seawater from the bag to the 5 L bottle.
3. Set up the filtration system, use the flat nose pliers to place the pre-burned GF/F filter on top of the filter holder.
4. Pour the seawater from the 5 L bottle into the funnel of the filtration system until all the 5 L have been processed.
5. For lipid analysis only (i.e. not for C/N stable isotope analysis), when filter is dry, pour 20 mL of boiling water in the funnel.
6. Remove the filter with the flat nose pliers, fold it and put it in a cryotube, label the cryotube with a unique sample ID, and store the cryotube at -80°C.

2.15.8. Samples processing, transient storage, shipping conditions and Biobanking

Upon arrival in Brest harbour, samples are transported to the lab in cool boxes with cooling packs within half an hour and permanently stored in -80°C freezers or 4°C fridge. For each sample, the precise location of storage (freezer/fridge ID and shelf) are filled in the Labcollector database.

2.16.9. Flow charts

3 Aerosols sampling and processing

3.1. Authors, affiliations and contact

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3.2. Introduction

We aim to understand the transport of airborne microbial communities between the ocean and land. To do so, we developed two protocols: one for regular stops along the TREC-Tara route, and another one for supersites. Our protocols include collecting bioaerosols on filters (both on Tara and on land), collecting aerosols for electron microscopy and elemental analysis (only on Tara), measuring the size distribution and concentration of aerosolized particles (both on Tara and on land), and sampling the sea surface microlayer (on Tara only).

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We will extract DNA from all bioaerosol filters and perform metabarcoding, and if possible, metagenomics. We will complement these datasets with aerosol particle size distribution and meteorological data.

3.3. Sampling Strategy

Regular stops:

- On land: we collect bioaerosols on filters at three different points along a perpendicular transect from the coast, while the land team samples the soil, and we have continuous aerosol size distribution and concentration measurements while sampling.
- On Tara: We collect bioaerosols for a 4-hour period at about 25 m above sea level, while other standard water protocols are performed (see sections 1.1 and 1.2). We also sample the sea surface microlayer (the first millimetre of the ocean) and have continuous aerosol size distribution and concentration measurements. Size- fractionated and electron microscopy samples are collected continuously from a height of approximately 28 m above sea level while at sea. These samples are collected for a maximum duration of 24 hours.

Supersites (focused on diel cycles):

- On land: we look for the highest possible suitable spot (e.g. avoiding human/animal traffic) and install a sampling station for the duration of the stop (usually 1-5 days). There we collect bioaerosols on filters every 4 hours.We also continuously measure aerosol size distribution and concentration with a particle counter.
- On Tara: we collect bioaerosols on filters and sample the sea surface microlayer every 4 hours (same times as on land), and have continuous aerosol size distribution and concentration measurements. Size-fractionated and electron microscopy samples are collected continuously from a height of approximately 28 m above sea level while at sea. These samples are collected for a maximum duration of 24 hours.

3.4. Sampling tools and equipment

Equipment	Chemicals	Consumables
• EDM264 (GRIMM Aerosol Technik Ainring GmbH & Co. KG): spectrometer that determines dust mass fractions and particle number size distribution. We have one on board Tara and one onland. • SASS® 3100 DRY AIR SAMPLER (Research International, Inc.) for the collection of biological	None for EDM264 Bioaerosols require: DNA away 70% Ethanol SML samples require: DNA away 70% Ethanol Glycerol Glutaraldehyde	None for EDM264 Bioaerosols require: Electret filters Nitrile gloves Whirl-pak bags 0.45-µm PVDF filters 47 mm dia. 0.8 µm PC filters Square aluminium foils 47 mm dia. 0.45 µm PC filters 70 mm dia. 0.45 µm PC

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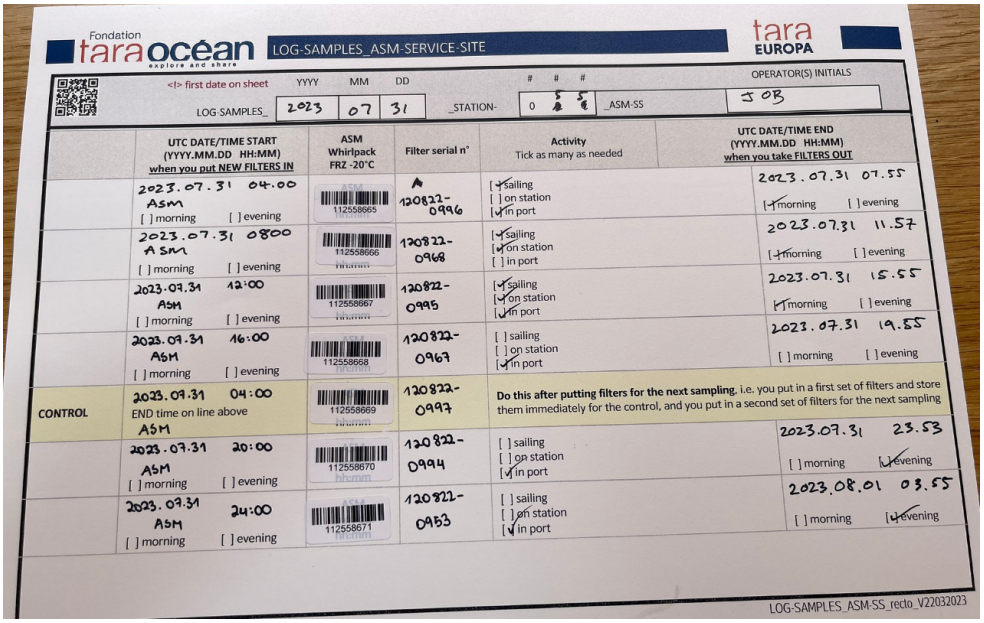
aerosols. We have three for the land team, two onboard Tara, and one for the land station of the supersites. • Custom-made sea surface microlayer sampler (30 x 30 cm ASTM MESH 16) • Peristaltic pump • Hard plastic (HDPE500) filtration manifold for six 47mm Swinnex Filter Holder DNA/RNA filtration unit and 6 Swinexx 47mm filter holders • DGI – Gravimetric Impactor (Dekati Ltd.) • ME 16 NT Diaphragm pump		filters SML samples require: 2 mL cryovials 5 mL cryovials 47 mm dia. 0.2 µm PC filters

3.5. Metadata collection

Tara log sheets:



Fondation tara océan explore and share — LOG-SAMPLES_ASM-SERVICE-SITE — tara EUROPA

<!> first date on sheet — YYYY MM DD: 2023 07 31 — _STATION-: 055 — _ASM-SS — OPERATOR(S) INITIALS: JOB

	UTC DATE/TIME START (YYYY.MM.DD HH:MM) when you put NEW FILTERS IN	ASM Whirlpack FRZ -20°C	Filter serial n°	Activity Tick as many as needed	UTC DATE/TIME END (YYYY.MM.DD HH:MM) when you take FILTERS OUT
	2023.07.31 04:00 ASM [ ] morning [ ] evening	112558665	120822-0996	[✓] sailing [ ] on station [✓] in port	2023.07.31 07.55 [✓] morning [ ] evening
	2023.07.31 0800 ASM [ ] morning [ ] evening	112558666	120822-0968	[✓] sailing [✓] on station [ ] in port	2023.07.31 11.57 [✓] morning [ ] evening
	2023.09.31 12:00 ASM [ ] morning [ ] evening	112558667	120822-0995	[✓] sailing [✓] on station [✓] in port	2023.07.31 15.55 [✓] morning [ ] evening
	2023.09.31 16:00 ASM [ ] morning [ ] evening	112558668	120822-0967	[ ] sailing [ ] on station [✓] in port	2023.07.31 19.55 [ ] morning [ ] evening
CONTROL	2023.09.31 04:00 END time on line above ASM	112558669	120822-0997	Do this after putting filters for the next sampling, i.e. you put in a first set of filters and store them immediately for the control, and you put in a second set of filters for the next sampling	
	2023.09.31 20:00 ASM [ ] morning [ ] evening	112558670	120822-0994	[ ] sailing [ ] on station [✓] in port	2023.07.31 23.53 [ ] morning [✓] evening
	2023.09.31 24:00 ASM [ ] morning [ ] evening	112558671	120822-0953	[ ] sailing [ ] on station [✓] in port	2023.08.01 03.55 [ ] morning [✓] evening

LOG-SAMPLES_ASM-SS_recto_V22032023

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Fondation tara océan explore and share

LOG_SML-SEA SURFACE MICROLAYER

tara EUROPA **B1**

YYYY	MM	DD	HH	MM	# # #	OPERATOR(S) INITIALS
					_STATION-	_SML

Water Collection — Number of dips: — Volume Collected (L)

Protocol	Quantity, Container Storage	Replicate 1	Replicate 2	Replicate 3	Comments
SML-FC	Cryotube (2ml) 1.5ml sample+30µl Glute25% LN2 (-80°C)	SML-FC-1	SML-FC-2		
SML-CP	Cryotube (5ml) 3ml sample+750µl Glycerol RT (-80°C)	SML-CS-1	SML-CS-2	SML-CS-3	
SML-320	Cryotube (2ml) Filter 3µm PC LN2 (-80°C)	SML-320-1	SML-320-2	SML-320-3	
SML-023	Cryotube (2ml) Filter 0.22µm PC LN2 (-80°C)	SML-023-1	SML-023-2	SML-023-3	

YYYY	MM	DD	HH	MM	# # #	OPERATOR(S) INITIALS
					_STATION-	_SML

Water Collection — Number of dips: — Volume Collected (L)

Protocol	Quantity, Container Storage	Replicate 1	Replicate 2	Replicate 3	Comments
SML-FC	Cryotube (2ml) 1.5ml sample+30µl Glute25% LN2 (-80°C)	SML-FC-1	SML-FC-2		
SML-CP	Cryotube (5ml) 3ml sample+750µl Glycerol RT (-80°C)	SML-CS-1	SML-CS-2	SML-CS-3	
SML-320	Cryotube (2ml) Filter 3µm PC LN2 (-80°C)	SML-320-1	SML-320-2	SML-320-3	
SML-023	Cryotube (2ml) Filter 0.22µm PC LN2 (-80°C)	SML-023-1	SML-023-2	SML-023-3	

Fondation tara océan explore and share

LOG_UDW-AEROSOLS

tara EUROPA **B1**

<i> first date on sheet | YYYY | MM | DD | # # # | OPERATOR(S) INITIALS

LOG-SAMPLES_ | | | | _STATION- | 0 0 0 | _UDW-AEROSOLS

LPM START 35:60:35	UTC DATE/TIME START (YYYY.MM.DD HH:MM) when you put NEW FILTERS IN	AI petri-slide RT >10°C	AS Cryo-2mL LN2 #2	AF Whirlpack FRZ -20°C	Activity Tick as many as needed	LPM END 35:60:35	UTC DATE/TIME END (YYYY.MM.DD HH:MM) when you take FILTERS OUT
1 [ ] 35:60:35	[ ] morning [ ] evening	AI mm-dd hh:mm	AS mm-dd hh:mm	AF mm-dd hh:mm	[ ] sailing [ ] on station [ ] in port	[ ] 35:60:35	[ ] morning [ ] evening
2 [ ] 35:60:35	[ ] morning [ ] evening	AI mm-dd hh:mm	AS mm-dd hh:mm	AF mm-dd hh:mm	[ ] sailing [ ] on station [ ] in port	[ ] 35:60:35	[ ] morning [ ] evening
3 [ ] 35:60:35	[ ] morning [ ] evening	AI mm-dd hh:mm	AS mm-dd hh:mm	AF mm-dd hh:mm	[ ] sailing [ ] on station [ ] in port	[ ] 35:60:35	[ ] morning [ ] evening
4 [ ] 35:60:35	[ ] morning [ ] evening	AI mm-dd hh:mm	AS mm-dd hh:mm	AF mm-dd hh:mm	[ ] sailing [ ] on station [ ] in port	[ ] 35:60:35	[ ] morning [ ] evening
CONTROL	END time on line above	AI mm-dd hh:mm	AS mm-dd hh:mm	AF mm-dd hh:mm	**Do this after putting filters for the next sampling**, i.e. you put in a first set of filters and store them immediately for the control, and you put in a second set of filters for the next sampling		
5 [ ] 35:60:35	[ ] morning [ ] evening	AI mm-dd hh:mm	AS mm-dd hh:mm	AF mm-dd hh:mm	[ ] sailing [ ] on station [ ] in port	[ ] 35:60:35	[ ] morning [ ] evening
6 [ ] 35:60:35	[ ] morning [ ] evening	AI mm-dd hh:mm	AS mm-dd hh:mm	AF mm-dd hh:mm	[ ] sailing [ ] on station [ ] in port	[ ] 35:60:35	[ ] morning [ ] evening

Land log sheets:

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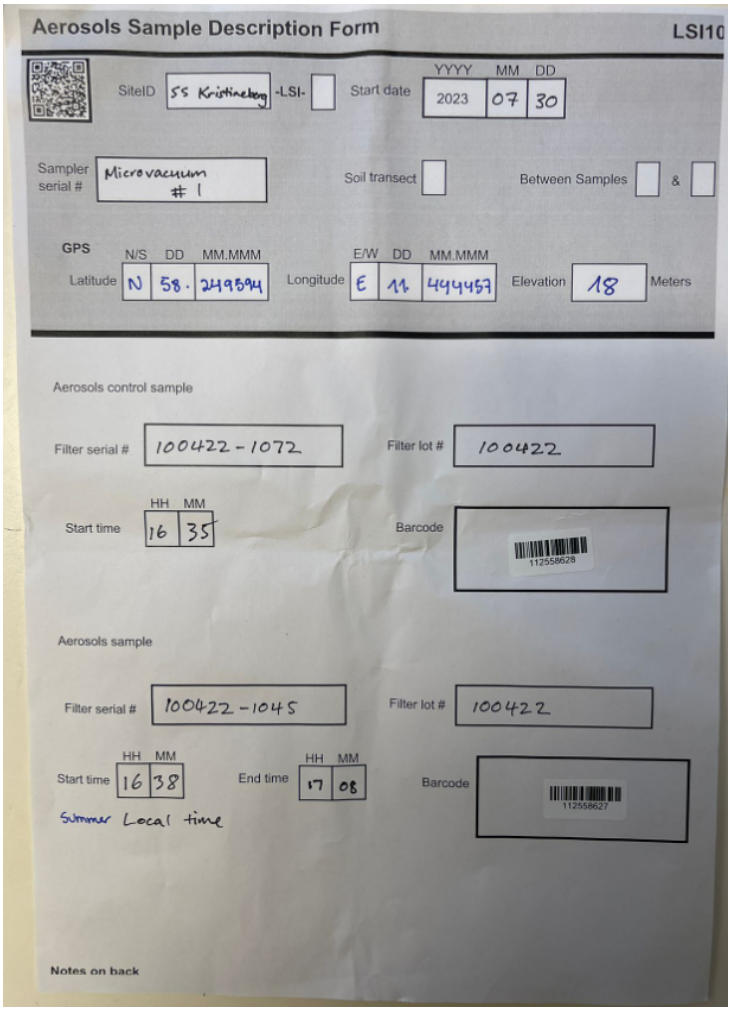

Aerosols Sample Description Form LSI10

SiteID SS Kristineberg -LSI- Start date YYYY MM DD 2023 07 30

Sampler serial # Microvacuum #1 Soil transect Between Samples &

GPS Latitude N/S DD MM.MMM N 58. 249594 Longitude E/W DD MM.MMM E 11 444457 Elevation 18 Meters

Aerosols control sample

Filter serial # 100422-1072 Filter lot # 100422

Start time HH MM 16 35 Barcode 112558628

Aerosols sample

Filter serial # 100422-1045 Filter lot # 100422

Start time HH MM 16 38 End time HH MM 17 08 Barcode 112558627

Summer Local time

Notes on back

3.6. Environmental parameters measurement

The EDM264 has an incorporated meteorological station that samples: rain, wind speed and direction, air temperature, and relative humidity.

3.7. Sampling protocols

Protocol AS - Genetics – metabarcoding

Contact: Michel Flores, Weizmann Institute (flores@weizmann.ac.il)

Put on gloves and wash your gloved hands with EtOH

Step-by-Step

1. The AS filter is in the YELLOW filter holder

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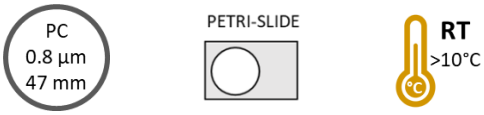
2. Open the filter holder and place the “o-ring” sideways in the black stage
3. Using tweezers, place a PVDF filter in the filter holder and close
4. Switch on vacuum pump and allow to filter air for a maximum of 24 h.
5. After sampling, open the filter holder and place the “o-ring” sideways in the black stage.
6. Fold the 47-mm-diameter 0.45-µm PVDF filter in two and again in two, and put it in the cryotube.
7. Place a new PVDF filter in the filter holder and close.
8. Store the cryotube in liquid nitrogen (Dewar #3) in the W-Lab

Protocol AI - Microscopy – SEM-EDX

Contact: Michel Flores, Weizmann Institute (flores@weizmann.ac.il)



Put on gloves and wash your gloved hands with EtOH

Step-by-Step

1. The AI filter is in the **BLUE** filter holder
2. Open the filter holder and place the “o-ring” sideways in the black stage.
3. Remove the 47-mm-diameter 0.8-µm polycarbonate membrane filter and place it in the petri slide, face up, on the white pad.
4. Place a new filter in the holder and close.
5. Store the petri slide in the “aerosols sample box” at room temperature (RT) in the F-Store

Protocol AF - Genetics – size-fractionated metabarcoding

Contact: Michel Flores, Weizmann Institute (flores@weizmann.ac.il)

Put on gloves and wash your gloved hands with EtOH

Step-by-Step

1. The five AF filters are in the **RED** “impactor” filter holder
2. Setup your working space in the fume hood with
    a. the box of 47mm filters,
    b. the box of 70 mm filters,
    c. the box of square aluminium foils,

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d. a marker pen,
e. the pre-labelled Whirl-Pak.

3. Place two square aluminium foils flat on the working surface, i.e. one for the first filter and one to pile-up the impactor stages (see next steps).
4. Open and slowly lift vertically the cap of the impactor, exposing the five stainless steel “stages” of the impactor. The stages are labelled in descending order from 4 (topmost) to 0 (bottom-most).
5. Take the filter on “stage 4” and put it on a clean aluminium foil, fold the filter in two and again in two. Fold each corner of the foil inwards to completely wrap the filter, flip it up-side down, write “4” on the foil, and put into the Whirl-Pak.
6. Put the “stage 4” filter holder on the second clean aluminium foil and place a new square aluminium foil on the working space for the next filter. Repeat for stages 3 to 0. The filters are therefore labelled in descending order from 4 (topmost) to 0 (bottom-most). Note that the filters on stages 4 to 1 are 47 mm in diameter, whereas the filter on stage 0 is 70 mm in diameter.
7. Place stickers on sterile bags and place in -20°C freezer.
8. Put new filters in the impactor, starting with the large filter on stage 0. The new filters for stages 1-4 are in an aluminium package (one package contains 4 filters). Place one filter on each stage as you reassemble the impactor.
9. Close the impactor.
10. Store the Whirlpack in the “aerosols sample box” at room temperature (RT)

Protocol EDM264

Contact: Michel Flores, (Weizmann Institute) (flores@weizmann.ac.il); James O'Brien, (ETHZ) (james.obrien@biol.ethz.ch)

Activating the Instrument - on the roof of the W-lab or on the land station

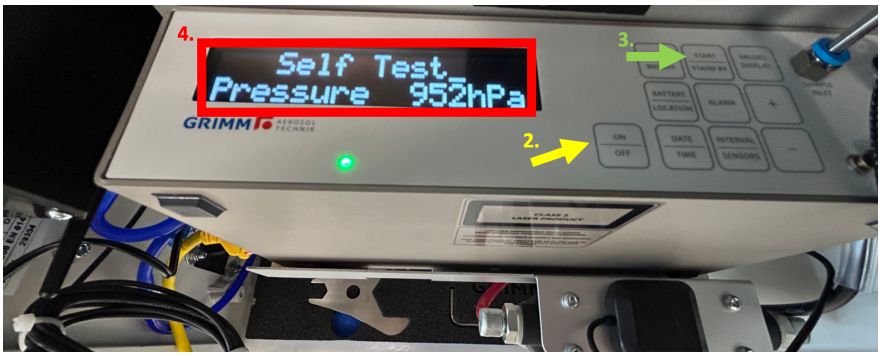

Figure 3 -1:

1. Open the door by turning black knobs to the right (open position).
2. Check to see if the EDM is on - this will be evident if the spectrometer screen is lit. If it is not, push the “ON” button until the spectrometer is active.
3. IF spectrometer is in “standby mode” push the “Start” button
4. The spectrometer will do a “SELF TEST”. Watch the test until the logger says “SELF TEST OK”; the EDM is now collecting data.

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5. Close the white door and lock the EDM by turning the black knobs to the left (closed position).

Monitoring the Instrument and backing-up collected data

Figure 3 -2:

1. Once a week, check that the EDM is operating as expected and that the data is being backed up.
2. To back up data, open the EDM door and place the spectrometer in “standby mode”.
3. Remove the SD card from the data logger and download data files using an SD card reader.
4. After downloading, place the SD card back into the data logger and re-activate the spectrometer by pushing the “Start” button.
5. Close the white door and lock the EDM by turning the black knobs to the left (closed position).

Protocol ASM - Genetics – metabarcoding

Contact: James O'Brien, ETHZ (james.obrien@biol.ethz.ch); Michel Flores, Weizmann Institute (flores@weizmann.ac.il), Shinichi Sunagawa, ETHZ (ssunagawa@ethz.ch)

Put on gloves and wash your gloves/hands with EtOH

Step-by-Step

1. Lower the SASS from the mast and take the rain cover off. Ensure SASS is turned off.
2. Using a Kimwipe with 70% ethanol, wipe the air inlet of the SASS and let it air dry for 2 minutes.
3. Carefully open the filter pouch. Touching only the filter frame, take the electret filter out of the bag and mount it on the SASS 3100 air inlet. Do not touch the filter with your hands, as it is sterile.

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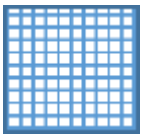
4. Place the filter on the SASS 3100 air inlet for ~5 seconds (negative control sample).
5. After time has elapsed, remove the filter from the air inlet by touching only the filter frame- be careful not to touch the filter with your hands.
6. Clean tweezers by wiping them with 70% ethanol and let them dry. After that, wipe them with a Kimwipe and DNA Away. Make sure they are dry before proceeding to the next step.
7. While holding the filter frame, gently push the filter in one corner using sterile tweezers. Grab the filter with the tweezers and place it in a clean pre-labelled Whirl-Pak bag. Be careful not to drop the filter as you push.
8. Close the Whirl-Pak bag by folding down on the metal tabs and make sure the package is as small as possible to make it easier to store.
9. Freeze a sealed Whirl-Pak bag in LN and store the samples in a -20°C freezer.
10. New filters are in the transparent box in the u-lab box.
11. Carefully open the filter pouch. Touching only the filter frame, take the next electret filter out of the bag and mount it on the SASS 3100 air inlet. Do not touch the filter with your hands, as it is sterile.
12. Place the filter on the SASS 3100 air inlet (ASM sample).
13. Turn the SASS ON/OFF switch all the way from OFF to ON (make sure not to end up in the middle position). The SASS is automatically programmed with a 5 min delay followed by 4 h of sampling, after which it will stop on its own.
14. Add the rain cover and hoist it up the backstay to filter air for 4 h.
15. After 4 h, lower the SASS from the backstay, turn the switch OFF and take the rain cover off.
16. Repeat the steps 6 to 9 for the ASM filter.

Protocol SML - Genetics- Surface MicroLayer – Deck

Contact: Michel Flores (Weizmann Institute; flores@weizmann.ac.il)



Collect up to 0.5 L of surface microlayer seawater using the steel SML-sampling screen and pre-filter the collected water through a 20 um mesh.

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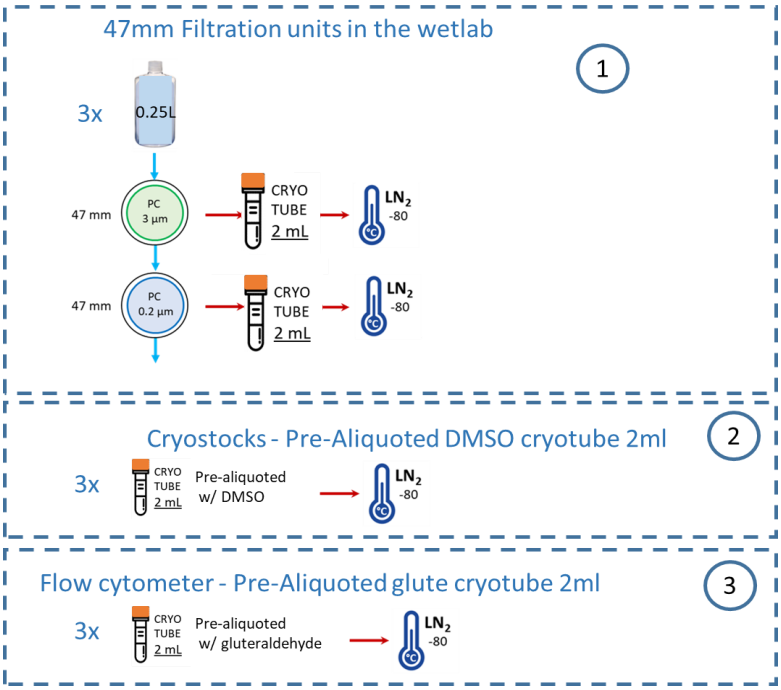

Figure 3 -3:

Step-by-step

1. Rinse the square stainless-steel screen with distilled water.
2. Find a position of the boat that is downwind, lower the screen to gently submerge it as little as possible to avoid disturbing the surface too greatly. Move the screen laterally, and draw back through the interface.
3. Tilt the screen over a stainless-steel funnel to recover the sample in a HDPE 1000 mL bottle fitted with a 20 µm mesh.
4. Repeat steps 2 and 3 until the bottle is filled.
5. Take two 2-mL cryotubes pre-aliquoted with glutaraldehyde and fill each with 1.5 mL. Put them in the fridge for 20 min and then drop them into LN2.
6. Fill 3 x 5 mL cryovials with 3 mL water samples for cryopreserving with pre-aliquoted glycerol.
7. Place three 3 µm and three 0.22 µm S-PAK filters into the swinnex 47mm filter holders in sequence in the wet lab and filter ~ 150 mL through each filter. Place each filter into a separate 2 mL cryotube. Drop cryotubes into LN2.
8. Rinse all reusable material with EtOH and distilled water.

Protocol Land-SASS - Genetics - metabarcoding

Contact: James O'Brien, ETHZ (james.obrien@biol.ethz.ch); Michel Flores, Weizmann Institute (flores@weizmann.ac.il), Shinichi Sunagawa, ETHZ (ssunagawa@ethz.ch)

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Put on gloves and wash your gloved hands with EtOH

Step-by-Step

1. Take the 3 tripods and the 3 SASS 3100s and place them perpendicular to the coast several tens of metres apart (either the 3 red stars or the 3 blue stars, relative to the soil sampling)

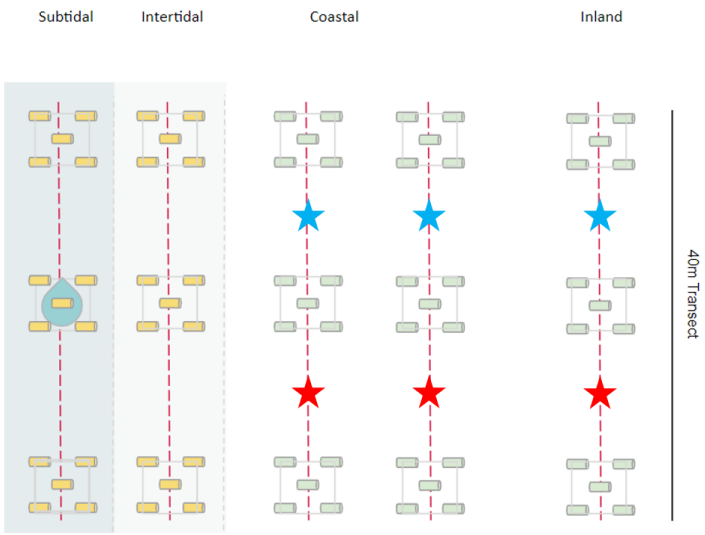

Figure 3 -4:

Do points 2 to 16 for each SASS:

2. Using a Kimwipe with 70% ethanol, wipe the air inlet of the SASS and let it air dry for half a minute.
3. Carefully open the filter pouch. Touching only the filter frame, take the electret filter out of the bag and mount it on the SASS 3100 air inlet. Do not touch the filter with your hands, as it is sterile.
4. Place the filter on the SASS 3100 air inlet for ~ 5 seconds (negative control sample).
5. Remove the filter from the air inlet by touching only the filter frame- be careful not to touch the filter with your hands.
6. Clean tweezers by wiping them with 70% ethanol and let them dry. After that, wipe them with a Kimwipe and DNA Away. Make sure they are dry before proceeding to the next step.
7. While holding the filter frame, gently push the filter in one corner using sterile tweezers. Grab the filter with the tweezers and place it in a clean pre-labelled Whirl-Pak bag. Be careful not to drop the filter as you push.
8. Close the Whirl-Pak bag by folding down on the metal tabs and make sure the package is

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as small as possible to make it easier to store.

9. Freeze a sealed Whirl-Pak bag in LN and store the samples in a -20°C freezer.
10. Carefully open the filter pouch. Touching only the filter frame, take the next electret filter out of the bag and mount it on the SASS 3100 air inlet. Do not touch the filter with your hands, as it is sterile.
11. Place the filter on the SASS 3100 air inlet (ASM sample).
12. Turn the SASS ON/OFF switch all the way from OFF to ON (make sure not to end up in the middle position). The SASS is automatically programmed with a 5 min delay followed by 4 h of sampling, after which it will stop on its own.
13. Add the rain cover and filter air for 4 h.
14. After 4 h, turn the switch OFF and take the rain cover off.
15. Repeat the steps 6 to 9 for the ASM filter.

3.8. Sampling processing, transient storage, shipping conditions and Biobanking

Transient storage

Land samples: All SASS 3100 filters are to be stored in liquid nitrogen. The EDM264 data should be backed up in an external harddrive every couple of weeks.

On Tara: For the AS and SML protocos, filters are stored in cryotubes in liquid nitrogen. The AF and ASM protocol filters are stored in the -20°C freezer. The AI filters are stored in a petrislide at room temperature. The EDM264 data should be backed up in an external harddrive every couple of weeks.

Shipping conditions

Land samples: All SASS3100 filtes need to be shipped in dry ice. The EDM264 data does not need to be shipped, it is sent by email to James O'Brien and Michel Flores.

On Tara: the AS, AF, SML, and ASM protocols samples need to be shipped in dry ice. The AI protocol filters can be shipped at room temperature.

Sampling processing

Land samples: The biomass from the SASS3100 filters will be extracted and send for sequencing. The EDM264 data will be used to estimate the amount of biomass in the air.

On Tara: the biomass from ASM, AF, AI, and SML fiters will be extracted and sequenced. The AI filters will be processed using a scanning electron microscope with an x-ray detector to obtain the particle-by-particle elemental composition. The EDM264 data will be used to estimate the amount of biomass in the air.

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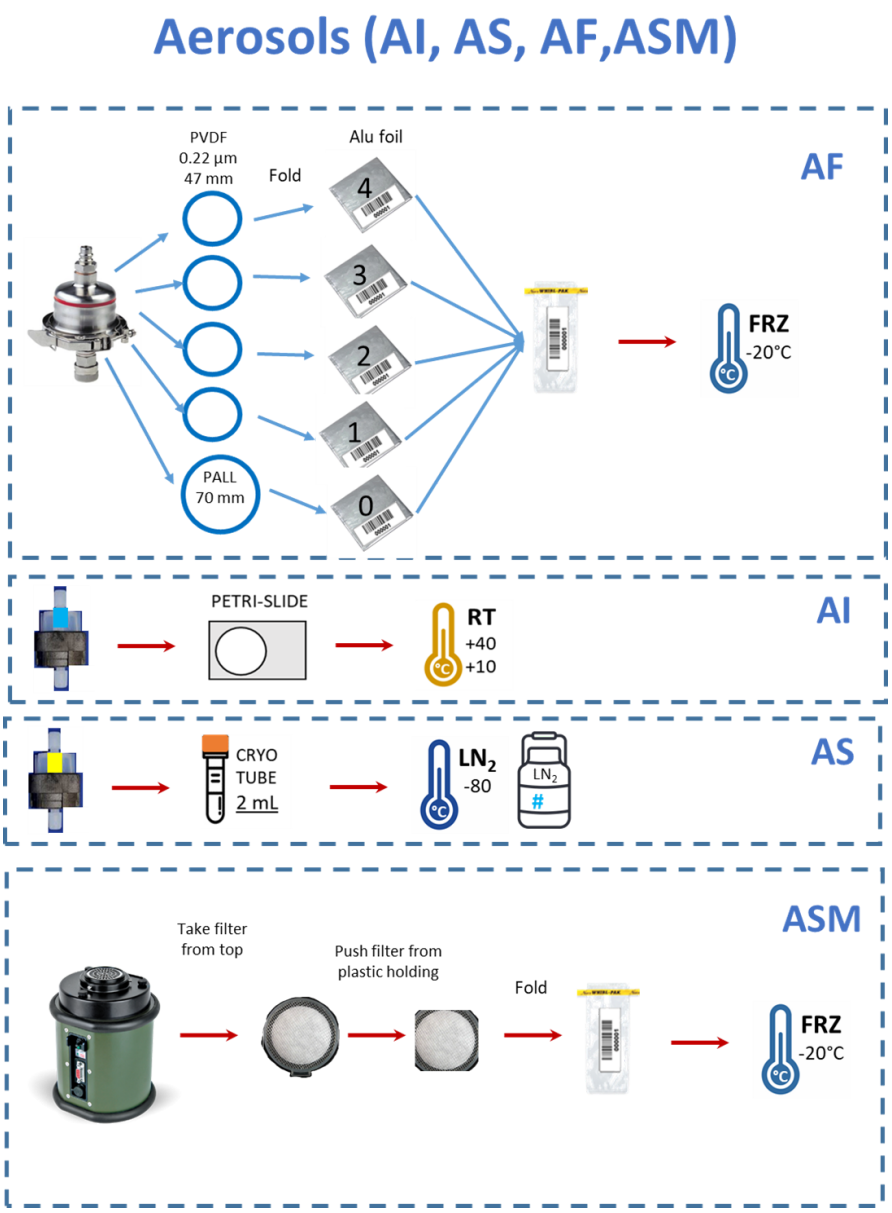
3.9. Flow charts



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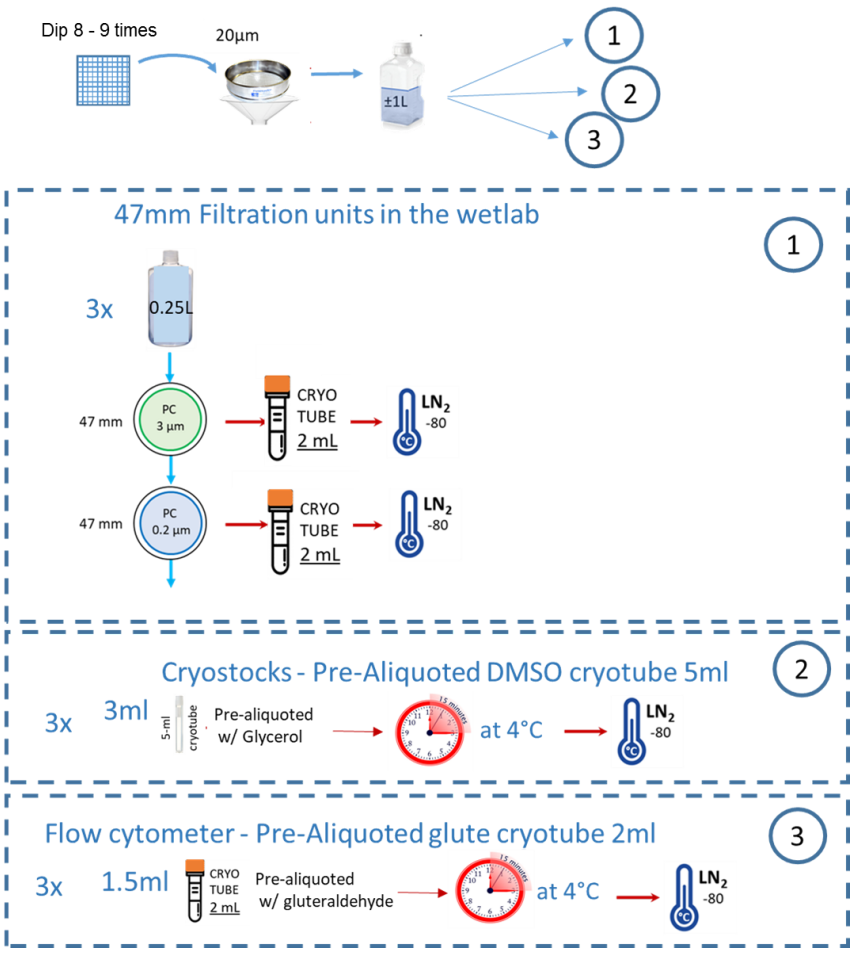
Sea surface microlayer (SML)



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4. Bioacoustics data acquisition

4.1. Authors, affiliations and contact

Lucia DI IORIO, CEFREM, CNRS UMR 5110, Université de Perpignan Via Domitia, 52, av. Paul Alduy, 66860 Perpignan, France

4.2. Introduction

Acoustic recorders are deployed by divers in key habitats (i.e., marine forests such as seagrass meadows and kelp forests) in conjunction with model species sampling, in particular *Platynereis dumerilii* and whenever possible, seagrass and kelp. Recorders are recovered after at least 48h. Data analysis occurs after recovering the data and consists of quantifying biological sounds (acoustic biodiversity, mass phenomena) and anthropogenic noise. Bioacoustic diversity and soundscape "quality" will be related to the biodiversity data investigated in model species.

4.3. Sampling Strategy

At each site with subtidal sampling (at least 3m water depth), three recorders are deployed for at least 48h. Recorder 1 and 2 in key habitats, where model species are also sampled, and Recorder 3 on sandy bottom, as control. The sandy bottom recorder should be at the same water depth as one of the other two key habitat recorders. Whenever possible, one recorder is deployed in seagrass and one in kelp. If one of the two habitats is not present, both key habitat-recorders are deployed either within the same habitat but at different positions, or in a different habitat (e.g., dominated by brown algae or sponges), where *Platynereis dumerilii* sampling occurs. To avoid acoustic "pollution" between recorders, they should be at least 100m apart from each other; the more, the better.

4.4. Sampling tools and equipment

Equipment	Consumables	Chemicals
• Acoustic recorders: 2 RTSYS LP 440 and 1 RTSYS LP 220 • Hydrophones: 3 Colmar GT 1516, SH: - 170 dB re dB re 1V/µPa • 4 512 GB SD cards per recorder • 1 external HDD for acoustic and environmental data storage • HOBO Pendant Temp/Light, 64kHz	Non-applicable	Non-applicable

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Sampling frequency: 128 kHz
Sampling cycle: continuous recordings
T° & light sensors: Sapling cycle: 1 measure every 15 min

4.5. Metadata collection

A log-sheet is available that has to be filled with the following information:

- Date and time
- Action: Programming, deployment, recovery
- Location (GPS)
- Habitat type: kelp, seagrass, control (sand), other (e.g; brown algae, sponges)
- Habitat condition: dense, spares/patchy, degraded
- Recorder ID: 1,2,3
- HOBO ID: 1,2 (associated to recorder)
- Other sampling: If Platy or seagrass sampling occurred at the recording sites
- Water depth
- Water temperature
- Operator

In the field, this information is added and merged with the Platynereis field log-sheet

4.6. Environmental parameters measurement

- Water depth
- Temperature (HOBO)
- Light (HOBO)

4.7. Sampling protocol

Before each deployment:

1. Program the two HOBO devices for temperature and light measurements and take screenshots of the programming. Before every new programming, the previous data has to be recovered and the HOBO sensor memories emptied.
2. Program the 3 recorders only if the SD cards have been retrieved for data download. Take screenshots of the status and programming page on the programming interface for each recorder.
3. Attach each recorder wrapped in their neoprene suits on the weighted grid using the heavy duty industrial cable ties. Make sure the writing RTSYS is on top to be able to activate and shut them down easily.
4. Clean the connections with the spray and connect the respective hydrophone cables (same colour code as recorder). Fix the hydrophones on the rope with the subsurface

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buoys that are attached to the grid. Marks indicate the position. Hydrophones need to be suspended between 0.5m (very shallow water < 4m, e.g., seagrass) and 1m (e.g., kelp) from the bottom.

5. Fix the HOBO sensors on the two long recorders (1 & 2) and make sure to note which HOBO (serial number) is attached to which recorder. The light sensor (green part of the HOBO) has to be oriented horizontally, facing the top
6. **Switch on the recorders** before deployment using the magnet on the last S of the writing RTSYS.

CAUTION The hydrophone is fragile and should not hit or be dragged on the floor.

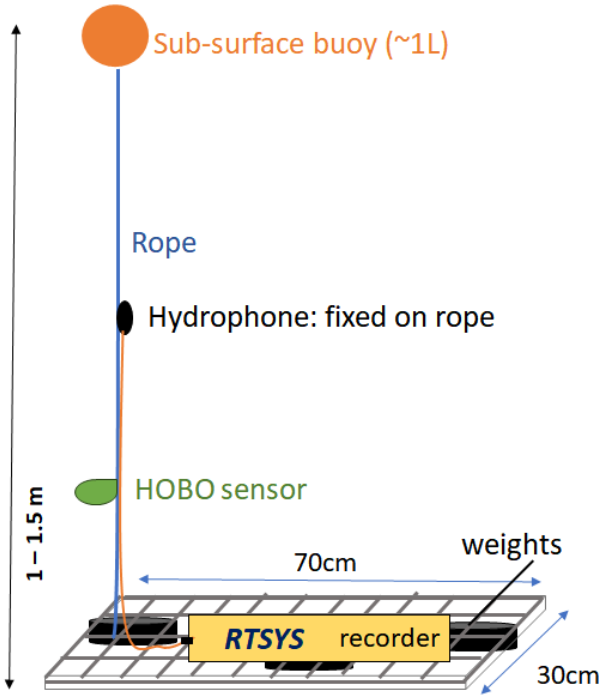

Figure 4 -1:

During deployment:

1. In shallow water depths (a few metres), the grid with the recorder can be lowered from a boat without a diver using a rope. This method is particularly suitable for the deployment on sandy bottoms, as there is no risk of damaging sensitive habitats. Otherwise, the recorders are deployed by a diver. Avoid surface buoys as they can be pulled, dragged and also caught into the boat's helix.
2. The grid with the recorder is not very heavy (~20kg) and can be easily carried by a diver using a parachute (30L or more). Recorders are always placed on a flat surface, a small patch of sand for example surrounded by the target habitat to avoid damages to the flora and fauna.
3. Once positioned, divers should **note the depth** and in areas with current or deeper waters they have to **send up a parachute** to the surface that indicates the position of the hydrophone.

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Attention! People on the boat must note the **exact GPS position** of the deployment site or of the surfacing parachute on the log sheet. It's best to pull a bit on the parachute so that the rope is straight and the position precise. This step is important as the recovery of the recorder will depend on this GPS position, otherwise we lose the recorder.

Attention! At each site, an operator should note which recorder (number on yellow part) is deployed at which GPS position!

The "control recorder" should be at the same depth as one of the two "habitat-recorders".

At recovery:

1. To recover the recorders, divers can use parachutes. Once on land, do not forget to turn off the recorders by holding the magnet ~10s on the last S until both the green and orange lights turn off.
2. Field log sheet data have to be transposed to the log sheet.

4.8. Samples processing, transient storage, shipping conditions and Biobanking

1. **Acoustic data** does not have to be copied on the external HDD after each deployment. Whenever possible, the recorders can be opened, the SD cards extracted and copied. Reprogramming is necessary if this occurs.
2. **Download the HOBO temp/light data after each deployment** using the base station and software because the memory saturates quickly. Copy the data into the right folder on the HDD (serial number of the HOBO) and name it with the name of the sampling site. Once downloaded, the memory can be emptied and the HOBO reprogrammed for the next deployment.

4.9. Flow charts (used to guide the processing of samples during stations)

No flow chart as not really a stepwise process for sample processing

Conclusion

References

If required

Annexes

If required

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