BIOMARKS SAMPLING PROTOCOLS

MOLECULAR

DNA & RNA subsurface and DCM

I- Introduction

Analyze the diversity of marine protists across different size fractions.

II – Major Peculiarities

For each depth and size fraction:
*x 30-50L for metagenomics purposes
8x10-20L for PCR based approaches
Samples for 142mm filters RNA and DNA : [3-2000µ] followed by [0.8-3µ]Subsurface and DCM ; [3-20µ] followed by [0.2-3µ] Subsurface and DCM ;
Samples for 47mm filters RNA and DNA : [3-20µ] Subsurface and DCM ; [0.8-3µ] or [0.6-3] Subsurface and DCM.

Timing:
For a particular depth, once the metagenomics filtration is started, you can set up and start the 4x20L filtration and run both in parallel.
2 persons full day on it.
Numbers of replicates are indicative, do as many as you can.

III – Material and Instruments

Peristaltic pump (I/P 2 channels)
Peristaltic pump (L/S 4 channels)
4x Filtration units 142 mm
Handle vacuum pump
8 in line Filtration units 47 mm
Tubing and connectors
Decontaminated tweezers
PC filters 142mm 3µ
PC filters 142mm 0.8µ
Dacron separator
PC filters 47mm 3µ
PC filters 47mm 0.8µ or 0.6µ
Cryovials 10mL and 2mL and racks
EtOH 70% and distilled water squizzes.

IV – Chemicals and buffers

RNA Later or Liquid Nitrogen

V – Material preparation

Set up the pump, tubing and filter.

VI – Sampling and conditioning

Metagenomics and PCR approaches :

- Put the PC filters 142mm 3µm and 0.8µm on a 124 mm Dacron separator in the 142mm holders ;

- For the first filtration, measure the flow-rate of the pump and let filter until there is too pressure. It's useful to know how much seawater you can filter in one time.

Filtrate in parallel both fraction series with the 2 pump heads.

For the [3-2000µ] and [0.8-3µ] fractions :

- Collect SW with Niskin bottles, sieve it onto 2000µm before to fill up 50L carboy;
- Filter the <2000µm SW onto a 3 µm polycarbonate filter 142mm followed by a 0.8 µm polycarbonate filter 142mm (in line setup of the filter holders).
- Once filtration is done, collect the filter with clean tweezers, transfer the two filters in separate 10mL cryovials and flash freeze the tubes in liquid nitrogen ;
- If there is no liquid nitrogen on the boat, cover the filter with RNA Later (ca 5 ml) and store the tubes in -20°C ;

For the [3-20µ] and [0.2-3µ] fractions :

- Collect SW with Niskin bottles, sieve it onto 20µm before to fill up 50L carboy;
- Filter the <20µm SW onto a 3 µm polycarbonate filter 142mm followed by a 0.2 µm polycarbonate filter 142mm (in line setup of the filter holders). You can use many 0.2 µm filters and pool them in the same tube if there is a lot of pressure.
- Once filtration is done, collect the filter with clean tweezers, transfer the two filters in separate 10mL cryovials and flash freeze the tubes in liquid nitrogen ;
- If there is no liquid nitrogen on the boat, cover the filter with RNA Later (ca 5 ml) and store the tubes in -20°C ;

- Label the tubes accordingly to the size fractions: Station_S or D _DNA or RNA_ [size fraction]_date_ Vfiltered.
- Do as many replicates as you can.

Once the 50L filtration is started, you can set up and start the 4x20L filtration and run both in parallel.

- Put the PC filters 47mm 3µm and 0.8µm in line setup of the filter holders;
- Collect SW with Niskin bottles, sieve it onto 20µm before to fill up 4x20L carboys.
- Filter the <20µm SW onto a 3 µm polycarbonate filter 47mm followed by a 0.8 µm polycarbonate filter 47mm. Use the four channels of the pump separately, with four independent set up.
- Once filtration is done, collect the filter with clean tweezers, transfer the two filters in separate 2ml cryovials and flash freeze the tubes in liquid nitrogen ;
- If there is no liquid nitrogen on the boat, cover the filter with RNA Later (ca 1 ml) and store the tubes in -20°C ;
- If required to be able to filter larger volume you can change the 3µm filter and pool them in one single cryovial. The 0.8µm filter should handle 20L without problems.
- Label the tubes accordingly to the size fractions: Station_S or D_DNA or RNA_ [size fraction]_date_ Vfiltered.

Repeat the procedure described above and label the tubes Station_S or D _DNA or RNA_ [size fraction]_date_ Vfiltered.

From 20µm plankton nets :

***Prepare before station*:**

- Bongo nets 20 µm + their pairs of cod-ends + flow-meters
- Sieve 180µm
- Sieve 2mm
- Funnel
- One 5L Nalgen bottle filled with 3L of filtered seawater
- Four 2L Nalgen bottles
- RNAlater.
- Polycarbonate membranes 47mm 10µm

Size-fraction 20–2000µm

On deck:

1. Tow the 20µm Bongo-net system (equipped with a flow-meter) for 15 min in sub-surface waters (**horizontal towing**) ; and later from DCM waters to the surface (**oblique towing**). Each towing will be repeated twice. Note the flow-meter numbers before and after towing.
2. Carefully rinse each net from outside with filtered-seawater.
3. Recover the two Cod-Ends and poor their content into the 5L Nalgen bottle pre-filled with 3L of filtered seawater (final volume should reach 4L). **!! Use a 2mm sieve fixed into a large funnel to transfer/filtter the net sample into the bottle !! Work on outside the filtration cabinet.**
4. Gently mix the plankton sample and split into 4 equal 1L parts. Use the 4 2L-Nalgen bottles marked ***A1****(RNA)*, ***A2****(RNA),* ***B1****(DNA)* ***B2****(DNA)*. Make sure that no aggregates a formed during the process.

Inside the wet-lab –with plastic gloves!

5. Quickly filter 2X 500ml from ***Bottles A1 et A2(RNA)*** through 47mm 10 µm polycarbonate membranes (using the water-jet pumping system in the wet-lab). Use as many filters as necessary for each 500ml split. Use two pairs of ethanol cleaned tweezers to recover the membranes and put them into a 5ml cryovial tube. Flash freeze the tubes in liquid nitrogen ; If there is no liquid nitrogen on the boat, cover the filter with RNA Later (ca 1 ml) and store the tubes in -20°C ;
6. As in **5.**, but from ***Bottles B1 and B2 (DNA)***.

Size-fraction 20–180µm, same process except for:

3. **!! Use a 180µm sieve fixed into a large funnel to transfer/filter the net sample into the 4L Nalgen bottle !!**

VII – Storage and shipping conditions

After having flash freezed the tubes in liquid nitrogen and store the tube whatever possible at -80°C or at -20°C. Ship on dry ice.

VIII – Chemicals and buffers preparation

IX – Disposal of trials and waste

None

X - Bibliography

XI - Annexes

Extracellular DNA

I- Introduction

Estimate the quantity of extracellular DNA in sea water and analyze its diversity through molecular markers. This parameter potentially set the baseline for diversity studies of environmental protistan communities.

II – Major Peculiarities

Work only on 0.2 µm filtered SW
Use the water filtered previously at <0.8µm.

III – Material and Instruments

Peristaltic pump (I/P 2 channels)
Filtration units 142 mm
Handle vacuum pump
4 x 20 L carboys (2 of which are contaminated with CTAB)
4 x 1 L Bottles (2 of which are contaminated with CTAB)
0.2 µm 142 mm PC filters
0.2 µm 47 mm PC filters
2 x 47 mm filtration units (1 of which are contaminated with CTAB)
2x 142 mm filtration units (1 of which are contaminated with CTAB)
50 ml Falcon tubes
2 ml and 10mL cryovials
DNA & RNA free (flamed) tweezers to fold the filters

IV – Chemicals and buffers

CTAB 0.5%

V – Material preparation

VI – Sampling and conditioning

For the entire procedure: Collect 100L from the SW filtered onto 0.8µm for DNA and RNA ("waste" water of the previous filtrations). Filter those 100L dispensed in 20L carboys onto 0.2µm pore size filter 142 mm.

* *For molecular Biology*
  - Collect 20L of seawater samples previously filtered onto 0.2 µm pore-size filters.
  - Add to 20 L of sample 400 ml of 0.5% CTAB solution pH 8.0 to precipitate the extracellular DNA. Mix and let sit for 1 h at room temperature
  - Filtrate the CTAB-treated seawater through 0.2 µm, 142 mm, polycarbonate (PC) filters by vacuum (or peristaltic depending of material available). The CTAB precipitate should be captured on the PC filter.
  - Remove the filter and place into a 10 ml sterile cryovial .
* *For quantification*
  - Collect 1 L of seawater samples previously filtered onto 0.2 µm pore-size filters.
  - Add to 1 L of sample 20 ml of 0.5% CTAB solution pH 8.0 to precipitate the extracellular DNA. Mix and let sit for 1 h at room temperature.

- Filtrate the CTAB-treated seawater through 0.2 µm, 47 mm, polycarbonate (PC) filters by vacuum. The CTAB precipitate should be captured on the PC filter.
- Remove the filter and place into a 2 ml Eppendorf sterile tube.

* *For contribution of Viral DNA to Total DNA pool*

- Collect 35-40 ml of seawater previously filtered onto 0.2 µm pore-size filter into a 50 ml Falcon sterile tube.

**** Prepare 3 replicates (at least 2 if not enough time) for each type of analysis, i.e.*
3 x 142 mm filters in 10 ml cryovials
3 x 47 mm filters in 2 ml eppendorfs
3 x 50 ml falcons filled with 35 ml of filtered SW

VII – Storage and shipping conditions

Store samples (i.e. tubes) at -20°C and ship on dry ice.

VIII – Chemicals and buffers preparation

Preparation of CTAB solution pH 8.0

Cetyltrimethylammonium bromide (CTAB)	5 gr.
TRIS (FW =121.1)	60.55 gr.
EDTA (FW=372.2)	18.60 gr.
Q-water	1 liter qsp

Store at 4°C

To prepare on site before the sampling.

IX – Disposal of trials and waste

X - Bibliography

XI - Annexes

Girus DNA

I- Introduction
Girus large filtration is for DNA extraction. All of Girus samples are filters. Two sets of filters are required, for different treatments. (a) is for direct preparation of DNA directly and (b) for a further fractionation step in the laboratory before DNA isolation.

II – Major Peculiarities
If possible use 20µm prefiltered sea-water.
Two types of storage conditions

III – Material and Instruments
GF/A 150 mm/1.6µm Filters
Millipore Express Plus membrane 142mm/0.22µm
200µm sieve or mesh
20µm sieve or mesh
Filtration holder
Peristaltic pump (I/P 2 channels)
Tubing
2 Tweezer
50ml falcon

IV – Chemicals and buffers

V – Material preparation
100L from SS and 100L from DCM. The pumping is done first through a GF/A filter then through a 0.22µm Express Plus filter. Make sure that the filter is centrally positioned and that the inlet & outlet tubes are securely fixed. It may be necessary to put on adjustable metal collars on joints that are under pumping pressure to avoid loss of the sample. The filter holders should be set up in series to optimise sample processing times. Check the position of the valve between the two filter holders. Gently turn on the pump until some water is in contact with the filter, then turn up the flow rate to 13. It may be necessary to remove trapped air by raising the bleed valve lever before a high flow rate can be maintained.

VI – Sampling and conditioning
(a) Direct preparation of DNA - Sub-surface and DCM
Pump 100L of seawater through the filter that has been mounted in the stainless steel filter holder (>1 filter may be necessary to permit passage of this volume). On removal from the holder, these filters should rolled up. Use two pairs of long tweezers, a first pair to hold the filter in the middle and the other to wind the filter around the first pair. Put it into a 50ml Falcon tube directly using the first pair of tweezers and screw the cap down tightly. When >1 filter is produced from one station/depth, it may be possible to store >1 filter per tube by tightly rolling it up and putting it into the same 50ml Falcon tube.

(b) DNA isolation with further fractionation step in the laboratory - Sub-surface and DCM
Pump 100L of seawater through the filter that has been mounted in the stainless steel filter holder as above (>1 filter may be necessary to permit passage of this volume). On removal from the holder,

these filters should rolled up. Use two pairs of long tweezers, a first pair to hold the filter in the middle and the other to wind the filter around the first pair. Put it into a 50ml Falcon tube directly using the first pair of tweezers. When >1 filter is produced from one station/depth, it may be possible to store >1 filter per tube by tightly rolling it up and putting it into the same 50ml Falcon tube. Add 10ml of 1.6µm- filtered seawater taken from the same sampling location. Screw on the cap tightly and encircle in with parafilm.

VII – Storage and shipping conditions

(a) All the filters will be stored in 50ml empty falcon for each membrane and have to be frozen in liquid nitrogen and subsequently transferred to -80°C or directly to -20°C.

(b) Just keep the Millipore Express Plus membrane 142mm/0.22µm in 50ml empty falcon for each membrane and have to be stored at 4°C

VIII – Chemicals and buffers preparation

IX – Disposal of trials and waste

X - Bibliography

XI - Annexes