PROTOCOL (B)

Collecting unicellular Eukaryotes in the size-fraction >0.8 µm

The Standard Operating Procedure (SOP) for collecting unicellular Eukaryotes is based on protocols used during the Tara Oceans Expedition

1. **Collect 20-100L of seawater using Niskin bottles or 10% acid washed bucket from surface water (0-2m depth).** If possible, for each time zone, take the samples between 10am and 2pm, ideally at local noon. Filtration should start as soon as possible and no later than 2 hours after sampling, making sure to keep seawater in near-ambient conditions. Please make sure to gently homogenise the sample before filtration.

2. **Additional depths,** such as a deep chlorophyll maximum, an oxygen minimum zone, or a benthic boundary layer **may also be sampled for PROTOCOL B**. However, priority will be given to the analysis of surface s**a**mples.

3. **Record all OSD mandatory information about SAMPLING, EVENTS, SAMPLES and ENVIRONMENT** (see Table 2a in the OSD Handbook) **and handwrite them on OSD Logsheets**. Please use separate logsheets for PROTOCOLS A and B. Each logsheet can capture information for no more than 6 replicates.

- **Pre-filtration**: DO NOT pre-filter seawater.
  After filtration, occasional large organisms (e.g. copepods) should be carefully picked from the filter membrane and discarded. When there are too many large organisms (e.g. >5), filtration should be done again with a 200 µm pre-filtration. In any case, whenever large organisms are picked from the membrane filters, please leave a comment on the OSD Logsheet.

- **Filtration method:** Filtration should be done preferentially using a peristaltic pump.
  The flow rate will depend on the diameter of the filter. As a rule of thumb, adjust the rate to obtain a regular outflow occupying the full inner diameter of the tubing, i.e. not only dripping. The preferred filter type is a polycarbonate membrane with a pore size of 0.8 µm. The diameter of the membrane (e.g. 47 mm, 90 mm or 142 mm) is up to each OSD Site and should be selected based on the amount of material in the water and the intended filtration volume per filter, i.e. based on previous experience. (Alternatively, when the preferred filter type is not available, we recommend using different pore size polycarbonate membranes or Sterivex filters, within the range 0.2-1.2 µm.)

- **Filtration volume per replicate:** The filtration volume per replicate will vary, depending
  (a) on the diameter of the membrane filter (e.g. 47 mm, 90 mm or 142 mm) and
  (b) on the amount of material in the water (typically ranging from 5 L to 50 L).
  As an indication, that enough material is collected, filter membranes should be coloured.

- **Required number of replicates:** Filter a minimum of two (2) and up to six (6) replicates per sampling depth. The number of replicates will vary, depending
  (a) on the diameter of the membrane filter (e.g. 47 mm, 90 mm or 142 mm) and

(b) on the amount of material in the water.

Membranes should be replaced as soon as filtration appears to clog, thus adding replicates as you proceed with the filtration. You may filter for example 4x5 L, 2x10 L, 5x10 L, 5x20 L or 2x50 L. Priority is given to extracting enough material for sequencing, so having more than 2 replicates increases the chances of bio-arching your sample.

- **Required filtration time per replicate:** The maximum filtration time per replicate should be 60 minutes. Membranes should be replaced as soon as filtration appears to clog.
  As an indication that enough material was collected, filter membranes should be coloured.

  The SAMPLE Filtration time (min) must be recorded for each replicate on the OSD Logsheet.

- **Required storage:** Place each filter in a separate sample container. (e.g. 5-50 mL cryotubes) and store them immediately in liquid nitrogen or in a -80°C freezer.
  (A -20°C freezer can be used for temporary storage, while filtering several replicates.)

Please label your sample containers as follows
(this label = SAMPLE_Title on the logsheet):

<OSD_SiteID>_<Month>_<Year>_<SiteName>_<Protocol_Label>_<SampleNo>_<Depth>

OSD3_06_16_Helgoland_NE08_1_surface

OSD3_06_16_Helgoland_NE08_2_surface

Relevant Metadata about the Sampling Protocol for Eukaryotes

List of "mandatory" and "optional" information for this sampling protocol, together with example values. These need to be written by hand for each sample in the SAMPLE section of the OSD Logsheets

(Mandatory)	SAMPLE_Title	OSD3_06_15_Helgoland_NE08_1_surface
(Mandatory)	SAMPLE_Protocol_Label	NE08
(Mandatory)	SAMPLE_Depth (m)	2 (surface)
(Optional)	SAMPLE_Quantity	20 L
(Optional)	SAMPLE_Fitration_Time	60 min
(Optional)	SAMPLE_Container	5 mL cryotube
(Optional)	SAMPLE_Content	Particulate matter on a 47 mm 0.8 µm pore size polycarbonate membrane filter
(Optional)	SAMPLE_Size-Fraction_Upper-Threshold	no pre-filtration
(Optional)	SAMPLE_Size-Fraction_Lower-Threshold	0.8 µm
(Optional)	SAMPLE_Treatment_Chemicals	none
(Optional)	SAMPLE_Treatment_Storage	Liquid Nitrogen

Polycarbonate Membrane Filter for Sampling:

Code no ATTP04700, ISOPORE MEMBRANE, POLYCARBONATE, HYD POLYCARBONATE, HYDROPHILIC, 0.8 UM, 47 MM, WHITE, PLAIN - PKG 100 - FILTER MEMBRANES – POLYCARBONATE MEMBRANE (196 € for 100 filters)
http://www.millipore.com/catalogue/item/attp04700

Code no ATTP14250, ISOPORE MEMBRANE, POLYCARBONATE, HYD POLYCARBONATE, HYDROPHILIC, 0.8 UM, 142 MM, WHITE, PLAIN - PKG 50 - FILTER MEMBRANES – POLYCARBONATE MEMBRANE (410 € for 50 filers) –
http://www.millipore.com/catalogue/item/attp14250