Sample collection from the field for downstream molecular analysis - microbial eukaryote-focused

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Abstract

This protocol describes steps and considerations for working in the field to collect samples to be used for molecular work targetting marine (or freshwater) microbial eukaryotes. See downstream protocols for DNA/RNA extraction and library prep (pending) for 18S rRNA gene tag sequencing.

Many steps in this protocol can be modified to fit a users' specific needs or environment type. Here, we strive to make recommendations that consider the delicate nature of single-celled eukaryotes and collect samples in the field as cleanly as possible. We use this approach for RNA and DNA-based sequencing - both for tag sequencing and metatranscriptomics.

This protocol is based off of the field work we conduct at the San Pedro Ocean Time-series (SPOT) station. We go out to the SPOT station once a month. Once we arrive at the SPOT station, we try to sample at the same time of day each month.

Timelapse video for sampling SPOT: https://youtu.be/1US3h_qD00w

Materials required:

(1) 20 L carboys (volume amount flexible)

(2) Covers for carboys (we use pizza bags, black trash bags, or coolers)

(3) Ice packs

(4) Tubing for Niskin

(5) Vacuum pump filtration system (see time-lapse video)

- trap flask

- pump

- extra tubing & rubber/silicon stopper (to fit filter towers)

(6) Filter towers that fit in vacuum pump filtration system

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(7) In-line filters, 47 mm

(8) Nitex mesh, cut for 47 mm - 200 um & 80 um

(9) RNase-spray

(10) Absorbent pads (for reducing water spillage & covering countertops)

(11) Liquid nitrogen - make sure to check regulations on transporting liquid nitrogen and storing it on-site/shipboard

(12) 15 mL falcon tubes - RNase/DNase-free

(13) RNA later or Lysis buffer (e.g. RLT buffer from this protocol)

(14) Filters for collecting material, we use 0.7um GF/F filters*

*Our reasoning to use this filter size is that the 0.7um nominal pore size will collect the protistan fraction of the community, but it is a woven filter style, so more water can be filtered through without clogging.

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Before start

Materials required:

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Protocol

Set-up on site

Step 1.

My set up requires counter space, easy access to a sink/drain, power outlet (for pump), and is ideally inside (or as much out of direct sunlight as possible). If you are sampling on a ship or will be dumping anything (even seawater) down the drain or nearby, please consult with whomever necessary to ask about what can be put down the drain (or overboard).

Line your countertop with absorbant pads. This acts to reduce spilled water from spreading all over and is a clean place to set materials before use.

Set-up (pre-filtration)

Step 2.

In order to exclude multi-cellular genetic material or metazoa onto our filters, we introduce a prefilter of both 200um and 80um. The two sizes allow us to keep the filtration process gentle and exclude as much unwanted material as possible. We often skip the 200um filter if we are sampling in an oligotrophic environment (e.g. North Pacific Subtropical Gyre), as the lower biomass is less likely to clog up the 80um filter.

Nitex mesh prefilters are pre-cut to fit into the 47mm diameter in-line filters. Pre-filters acid washed and MQ rinsed, then placed in the in-line filters before heading out to the field (reducing contamination to the pre-filter directly). From the 'source' of the water (i.e. spigot of carboy or niskin), the water will be filtered through the 200um, 80um, and then placed into the sample carboy.

Prepping for field work

Step 3.

Ideally, prep for field work in a lab.

Make sure all carboys, filter towers, tubing, forceps, or any 'washable' material is rinsed thoroughly with acid (HCl 5%). If an item can be autoclaved it is a good idea to do that as well. I often rinse all materials with DEPC water to inactivate RNase enzymes. Of course, work with gloves and store all materials inside clean bags or containers to reduce any contamination while traveling to field work site.

*If this is your first time performing this type of sampling, it is a good idea to stage your set up at your home lab to test everything out and check for all materials.

See this timelapse video as an example: https://youtu.be/1US3h_qD00w

Collection from Niskin bottle (or carboy)

Step 4.

Following water collection (at whichever site or depth), immediately connect silicon tubing with in-line filters to spigot or opening.

Allow water to flow through tubing into sample collection vessel (in this case carboys). Rinse collection vessel/carboy 3 times with a small amount of sample water.

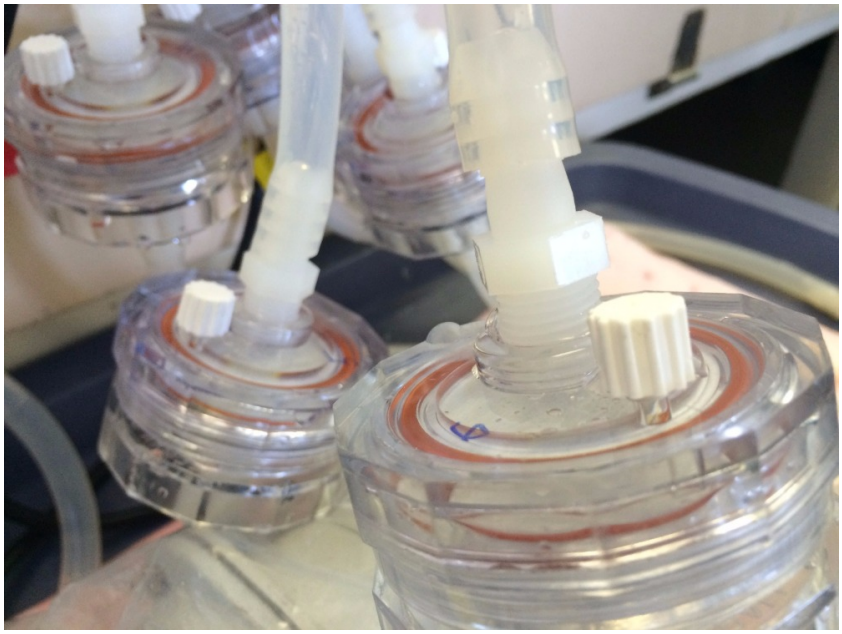
***Place carboy in pizza bag or dark trash bag to reduce exposure to any sunlight (pictured below):

Collection from Niskin bottle (or carboy)

Step 5.

During this step, we often place in-line filters (with 200um and 80um nitex mesh) to filter seawater as it comes out the niskin (two in one, combining initial collection with prefiltration). Alternatively, you can fill the carboy (after rinsing) with whole seawater and perform an additional filtration step by emptying that whole seawater into an additional carboy via the in-line filters (step-wise).

Pictured: Close of in-line filters



Start the clock for filtration to freeze

Step 6.

Immediately, bring carboy inside, place back into pizza bag or darkened trash bags.

You want to reduce any impact your surrounding environment (temperature/light) has on the community you've captured in the bottle. So keep carboys in the dark and if needed, place ice packs. We sample as deep as 900m, so it is very important that we keep these samples in complete darkness and chill the containers as much as we can.

For any transcriptomic data, I keep the filtration to freeze time <30 minutes. If needed I will increase the number of replicates being sampled. Meaning I increase the filters in use on a vacuum manifold or peristaltic pump to the max, increasing the volume I can collect within 30 minutes (across a # of filters).

Carboys are now inside and filled with sample water (pre-filtered).

Filtration

Step 7.

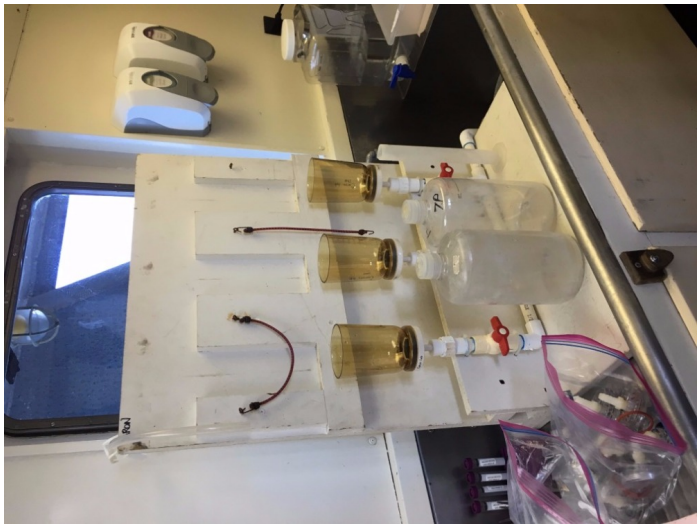
Rinse 2L bottles three times with sample water

Filtration

Step 8.

Fill with 2L and start filling filtration tower (this can be done by pouring or setting up a filtration rig). See picture and video below:

Filtration rig:



https://www.youtube.com/watch?v=1US3h_qD00w

Filtration

Step 9.

I use 2L bottles because I need to keep track of how much I filter. This can be done in two ways:

1. Use graduated cylinders or 2L (or any other size) bottle to continually pour, or set up above filter towers as in the video. So you can keep track of INPUT water volume.
2. Measure the amount of water being collected in your trap flask. Keeping track of the OUTPUT water volume.

Regardless, keep track of how much water is being filtered onto EACH filter.

Freeze

Step 10.

Once the appropriate amount of water has been filtered onto each filter, stop filtration. Quickly use forceps to gently fold filter and place into 15 mL falcon tube (conical tube). Make sure filter is covered with RNALater or lysis buffer (i.e. DNA/RNA lysis buffer for downstream extraction) and place into liquid nitrogen for storage.

Extraction from filter

Step 11.

See other Caron lab protocols for extracting DNA and/or RNA from these filters and other steps!