## **EXPORTS**

## ***MOCNESS Tows***

MOCNESS – 200μm-mesh, 1 m2 nets, 10 nets (9 sampling).

Sample between 1000-1600 and 2100-0300.

Depths (m):

0-50

50-100

100-150

150-200

200-300

300-400

400-500

500-750

750-1000

## ***When tow comes up***

Rinse each net down with seawater so that any animals stuck in the net are dislodged and fall into the cod end. Pour the contents of each cod end into its corresponding white 5-gallon bucket (numbered to match the cod ends). Use a gentle spray of seawater hose to rinse the remaining plankton out of cod end. Cover with lid and bring to lab. For one paired day/night tow per Epoch, for the 3 shallowest depths (0-50m, 50-100m, and 100-150m) we will also add carbonated water to the tow to keep animals from clearing their guts.

## ***Sample Processing at Sea***

When splitting samples for many measurements (i.e. abundance, biomass, gut contents, gut fluorescence, etc.), it will be useful to have containers pre-labeled for each split. Pour the contents of the bucket into the plankton splitter. If there is too much volume for splitter to handle, then try to concentrate the water at the top of bucket onto 200μm mesh sieve and rinse any animals caught on sieve into splitter. Use a squirt bottle to remove the remaining plankton out of bucket. Split the sample in half. Pour split into a labeled container and place in refrigerator until you are ready to process further. Note: if there are any big jellies, crustaceans, or fish, take them out and preserve separately.

**Abundance:** First half gets preserved in formaldehyde for taxonomic ID. You may have to concentrate sample on a 53 µm or 200 µm mesh sieve if there is too much volume to fit in jar. Use about 10% (by volume) of the buffered formaldehyde to preserve sample. This will be about 20 mls of 37% buffered formaldehyde that goes into the 250 ml jar. Try to have the jars pre-labeled as much as possible. Note in lab book if there are any particularly large, abundant or otherwise interesting/unusual critters. [Use ~29 g borax / ½ gal of 37% formaldehyde (~1.5 g / 100 ml; 1 L = 35.2 oz; 1 gal = 3785.41 ml)]. Occasionally this sample will be split again for **gut fluorescence** or for **gut content** analysis (see below). Also, once during cruise we will **size fractionate and preserve** a whole day night pair. Each size fraction from each depth will be preserved separately in small vials (day/night x 9 depths x 5 size fractions = 90 samples)

**Biomass and ETS (and stable isotopes):** Second half gets split again, and each quarter poured through a set of nested sieves. Fill the tub with some seawater (set up near the sink). Place nested sieves in tub, the smallest sieve size on bottom, largest on top. So top to bottom is 5000-2000-1000-500-200 µm size. Be careful so that the water level in tub is lower than top of bottom-most sieve (200 µm). Pour the quarter sample through nested sieves. Rinse thoroughly with squirt bottle filled with seawater (may want to cut tip off slightly to get a better stream). Swish sieves around in basin at bit, will help separate by size fraction too.

Place Nitex filters onto filter apparatus, 1 for each size fraction and record the filter number on the datasheet. Add filter cups (tops). Rinse down mesh of each sieve swith seawater so material collects in bottom of sieve, and then rinse onto Nitex filters with seawater. When all material is onto Nitex, give a rinse with isotonic solution. This gets rid of the salt. (We have used DI or milli-Q water in the past).

Tips. You will likely need forceps to get plankton off of the 5000µm size sieve, and maybe even the next one. Use spatula if some material gets stuck in the filtration apparatus. [Nitex circles ~ 2.5” diameter; in one yard2 of mesh ((1yd x 36”/yd) / 2.5”/circle)2 ~ 196 circles/yd2; for Eddies: 1400 circles/196 circles/yd2 = 7.1 or 8 yards. Cut circles out, clean them if needed, number them, and finally weigh them.]

Once you have filtered each size class, place individual Nitex filters with zooplankton goo into Petri dish with label (size Fraction, FilterNum, etc.). Tape both ends of Petri dish with lab tape if needed and immediately freeze at -20°C. (put in Ziploc bags so keep samples from individual tows together). Take the samples home frozen, put into cooler with ice packs (dry ice better).

For **Electron Transport System (ETS)** samples, scrape biomass off of Nitex filters into cryovials or Falcon tubes, flash freeze in liquid N. Store at -80°C. Note: if freezing in Falcon tubes, use tongs to hold the tube in a small container of liquid N, *with cap off*. Once frozen, remove, cap and place in -80 freezer.

For **stable isotopes** (Brian Popp and Hilary Close), we do not need to do anything different; they will use our same biomass samples that we dry at home. They want one day/night pair from some point in the cruise, and at least 3 size fractions (1000-500-200) x 9 depths x d/n = 54 samples. They’ll pick most interesting pair after the cruise (will decide which best to analyze). Interested in separate species too. Note they are particularly interested in *Oithona* (as well as *Oithona* pellets, or any small size animal’s poop from mesopelagic).

| **epoch** | **1** | **1** | **1** | **1** | **2** | **2** | **2** | **2** | **3** | **3** | **3** | **3** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Day/night tow** |  |  |  |  |  |  |  |  |  |  |  |  |
| **Biomass** (& stable isotopes- B. Popp and H. Close)  Size fractionated | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| **Electron Transport System (ETS)** Size fractionated, flash frozen | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| **Abundance** Formalin preserved | **X** | **X** | **X** | **X** |  |  | **X** | **X** | **X** | **X** | **X** | **X** |
| **Abundance** Size fractionated |  |  |  |  | **X** | **X** |  |  |  |  |  |  |
| **Gut fluorescence** (0-50, 50-100, 100-150 only)  Size fractionated |  |  | **X** | **X** |  |  | **X** | **X** |  |  | **X** | **X** |
| **Gut contents (Ewelina)** One sample @ 3 depths: ML, mesopelagic, ‘deep’ | **X** | **X** |  |  |  |  |  |  | **X** | **X** |  |  |

## ***Biomass Sample Processing at home lab***

Wet weights. Take frozen samples out of freezer. Remove Nitex from dish and put onto blotter paper (e.g., large Kimwipes) to thaw, until not frozen (takes ~15 min or so). Transfer filters to a different blotter paper until they are not leaking so much (but not dry either). Weigh. The scale may drift a bit as the sample starts to dry out so be consistent about when you take your reading (give the scale a few seconds to settle, and then take reading). Note: Do in groups of about 20, so that samples don’t start to dry out.

Once wet weighed, put the Nitex circles onto aluminum foil and put the foil into the drying oven overnight (at ~60-65°C). Note, do not dry the Petri dishes because they may melt. To transport them back, you’ll need to then put back into respective Petri dishes. Then transport the dry samples in a cooler with some Drierite. Back at our lab, once dried, we weigh them again then scrape off the gunk. The gunk goes into the Petri dishes put in the freezer for later CHN.