Microzooplankton Grazing & Size Fraction Experiments

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Equipment

- 20L Carboy w/ Spigot, covered w/ black plastic
- Milk Crate
- 10,5,1 micron Filtering Rig
- 0.2 micron Filtering extension for rig
- 20L Carboy w/ Spigot
- Micrograzing Bottles
    - Clear 1L Nalgene Bottles w/ caps, both marked:
        - 100 A, B, & C
        - 75 A, B, & C
        - 50 A, B, & C
        - 25 A, B, & C
        - FSW A & B
    - Amber 1L Nalgene Bottles w/ caps, both marked:
        - Dark A & B
    - Clear 4L Nalgene Bottles w/ caps, both marked:
        - 1 each 75, 50, 25
    - 125ml amber, widemouth bottles & caps. The bottles are marked:
        - 100 A1, A2, B1, B2, C1, C2
        - 75 A1, A2, B1, B2, C1, C2
        - 50 A1, A2, B1, B2, C1, C2
        - 25 A1, A2, B1, B2, C1, C2
        - Dark A1, A2, B1, B2
        - FSW A1, A2, B1, B2
- Graduated cylinders
    - 1 2L
    - 2 1L
    - 2 500mL
- Materials to take 5 nutrient samples
- Incubation tank, in full light w/ flowthrough seawater
- 25% light screening for tank
- Clips to attach the screen to the tank
- Chlorophyll filtering manifolds & associated apparati (vaccuum pumps, waste flasks, etc.)
- 0.45 µ Filters (HAWP, Nitrocellulose)
- 20 µ Filters (Polycarbonate)
- 10 µ Filters (Polycarbonate)
- 8 µ Filters (Polycarbonate)
- 5 µ Filters (Polycarbonate)
- 3 µ Filters (Polycarbonate)
- GF/C Filters

- GF/F Filters
- 0.2 µ Filters (Polycarbonate)
- Numbered 20mL Scintillation vials, with good, teflon cone lined caps (use the chlorophyll vials)

Preparation

First of all, you will want to prepare ~15L of 0.2 µ gravity fed filtered sea water, stored in a clean 20L carboy. And, of course, all of the bottles, cylinders, etc. should be ready to go.

Sample Water

~15-18L sample water (usually collected from 50% light depth), stored in a covered 20L carboy.

Dilution & Initial Processing

Some things to remember during the whole process:

- Always mix water before pouring (gently invert) as the plankton can sink to the bottom.
- Always rinse a bottle once with whatever is going into it before filling (just a little water will do).

So, you'll want to have sample water distributed and diluted in the 1L bottles, they will be diluted to the percentage marked on each bottle with FSW (FSW is 0% sample water, and darks are 100%). To start, you can just fill the 100%s and darks with straight sample water & the FSWs with FSW. Each bottle gets 800mL. For the other dilutions, you use the big 4L bottles. Each one of THOSE ends up with 3L in them. So, for example, 75% gets 2,250mL Sample water & 750mL FSW. Once the big bottles are filled, use them to fill the smaller bottles, 75 into 75A, B, & C and so on. Once all of the 1L bottles are filled, you can move on to the little brown bottles.

The little brown bottles get filled to the very top, up to where there's a meniscal bulge sticking out and everything, then capped. They must be filled full for the volume written on them to be accurate. So, as you might imagine, the little bottles get filled from their corresponding 1L bottles, 25A into 25A1 & 25A2, 25B into 25B1 & 25B2, etc.

At some point in here, take your nutrients out of the 100% A, B, & C and the dark A & B

1L bottles.

Once all of this business is done, take all of the 1/2 empty 1L bottles, put them in the incubation tank, and cover it with the 50% screening. Make sure the screen isn't hanging in the water, as it might grow algae on it, which would change how much light it filters.

Then, take all of the little bottles in and filter them for chlorophyll analysis (see chlorophylls section) using 0.45 µ filters.

Size Fraction Experiment

With remaining sample water, filter a series of the following sized filters for chla. This is exactly like filtering 18 chlorophylls except you use a big range of filter sizes.

20 µ 10 µ 8 µ 5 µ 3 µ GF/C GF/F 0.45 µ (HAWP) 0.2 µ

Do duplicates of each filter type (for a total 18 filters). Each filter gets 100mL of the sample water sucked through it except for the 10 & 20 micron filters, which each get 200mL (or more as needed).

Incubation and Final Processing

The 1L bottles are incubated for 72 hours in the tank, then taken out and processed.

The processing of the bottles is exactly as above. The 1L bottles are used to fill the little widemouth bottles, which are then processed for chlorophyll analysis. Nutrients are taken from the same bottles as before.

For cleanup, everything gets rinsed once with nanopure water.